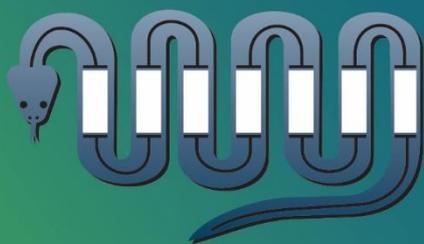




PROGRAM AND ABSTRACTS



53rd Brazilian
Congress of
Pharmacology
and Experimental
Therapeutics

November 16-19, 2021



ONLINE
EVENT

Dear fellow SBFTE Members, Colleagues, and Friends,

It is my great pleasure to welcome you to the 53rd Brazilian Congress of Pharmacology and Experimental Therapeutics. The human suffering and loss of life from COVID-19 forced us to make our annual meeting online for the second consecutive year. Therefore, despite being unable to keep to the original plan of holding an in-person congress on the beautiful island of Florianopolis, we have kept the theme “Pharmacology connecting Islands: Post-Pandemic Challenges and Opportunities”, which covers a significant part of the program.

The Scientific Program Committee and SBFTE board of directors have organized what I am confident will be a unique and memorable annual meeting. Although we will miss aspects of the face-to-face meeting, there are still many remarkable things to look forward to in our current event.

The 53rd Congress will gather national and international leaders at the frontier of pharmacological and biomedical knowledge, addressing the most recent advances in basic science, medicinal chemistry, nanomedicine, and pharmacometrics. A magnificent overview of the latest research progress in sensitive areas of pharmacology, including drug discovery and development, will be carried out. Areas such as natural products, cardiovascular pharmacology, neuropharmacology, and putative innovations in treating infection, cancer, inflammation, and pain, will all be approached and shared in numerous conferences, lectures, symposia, and round tables. Fourteen poster discussion sessions and a record of twelve courses will address educational, scientific dissemination and the popularization of Pharmacology, encouraging the maximum as possible of formal and informal interaction among all participants.

One of the hallmarks of our event will be the symposium entitled “Pharmacology and Covid-19 in Latin American”, with the distinguished participation of representatives from Chile, Argentina, Cuba and Brazil.

The Rocha e Silva and Sergio Ferreira Memorial Lectures, the most significant events of the annual Society’s meetings, will bring presentations by Gilberto de Nucci (UNICAMP) and Mauro Teixeira (UFMG), respectively. They are outstanding pharmacologists and clinicians developing and testing new medications and treating patients suffering from Covid-19 and other disorders, which have not gone away and, in many cases, have only been further aggravated during the pandemic.

Another highlight of the SBFTE annual meeting is the José Ribeiro do Valle (JRV) Award Symposium, which is a fantastic opportunity to congratulate the five young pharmacologists selected for the 2021 JRV Award. It is indeed a great initiative that motivates early-career scientists interested in developing the skills necessary for a fruitful and lifelong biomedical career. These are indeed the future of Pharmacology! I am already looking forward to announcing the Jose Ribeiro do Valle Award winners in our closing ceremony.

I also would like to draw your attention to the Elisaldo Carlini and Ivan Izquierdo tribute rooms. In these spaces, you will find seminal interviews, essential articles, and videos of these two great scientists. Ivan Izquierdo and Elisaldo Carlini have left an incredible and inspiring legacy to the field of Pharmacology in Brazil and abroad.

Finally, but not less importantly, on behalf of the SBFTE’s board of directors, I would like to express my gratitude to the CNPq and FAPERJ for providing the financial support for the 53rd Congress, despite the significant budget cuts imposed on them. We want to thank the partnership of Biolab and Aché Pharmaceuticals for supporting the JRV Award and Senior Pharmacologist Award, respectively. Also, we are extremely grateful to all our sponsors for their financial support.

I wish you all an exciting and fruitful experience in the 53rd Brazilian Congress of Pharmacology and Experimental Therapeutics.

Respectfully yours,

Marco Aurélio Martins

President of SBFTE

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E-Poster presenters must attend the Room Session scheduled (Nov 17 from 17h50 to 20h20 and Nov 18 from 16h45 to 19h15 within 20min in advance) when posters will be viewed by Poster Evaluators.

o E-Poster Session 1: <https://padlet.com/SBFTE/y1vhayhlj0lnah7>

o E-Poster Session 2: <https://padlet.com/SBFTE/ti14ul6axberdg9m>

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The course certification will be given for the participants with at least 2 classes attendance within at least 30min.

Abstracts

Abstracts presented at the poster session will be available at SBFTTE website (<http://www.sbftte.org.br>).

Keynote Speaker – Mauricio Rocha e Silva Lecture: Opening Lecture



Gilberto De Nucci studied Medicine at Ribeirão Preto Medical School, University of São Paulo (USP) and graduated in 1981, after which he began his post-graduate studies at the Royal College of surgeons, where he obtained his PhD in 1986 under the supervision of Prof. Salvador Moncada and Y.S. Bakhle. Prof. De Nucci then did post-doctoral training at the William Harvey Research Institute of St. Bartholomew's Hospital Medical College under the supervision of Sir John Vane, Nobel Laureate in Medicine or Physiology. Back in Brazil, Prof. De Nucci was awarded a position at the Department of Pharmacology at the Faculty of Medical Sciences of the State University of Campinas (UNICAMP), where he established a research laboratory in basic and clinical pharmacology. Prof. De Nucci also became Professor of Pharmacology in the Department of Pharmacology at the Biomedical Institute at the University of São Paulo (USP), São Paulo. In 2013, he obtained a degree in Medicinal Chemistry from the Faculty of Pharmacy, University Federico II, Naples. Prof. De Nucci was a pioneer in the implementation of bioavailability and bioequivalence studies in Brazil that subsequently lead to creation of the Brazilian Generic Medicines Law (9787/99). Many of the professionals who work in Clinical Pharmacology in Brazil did part of their academic training under Prof. De Nucci's supervision at UNICAMP and USP. Prof. De Nucci has received numerous research grants from FAPESP and CNPq. In basic pharmacology, his main contributions have been in cardiovascular pharmacology, especially in the biology of endothelin, nitric oxide and the recently described 6-nitrodopamine. Over the years, Prof. De Nucci has supervised 58 MSc and 61 PhD students, as well as various post-doctoral students from Brazil and abroad, many of whom occupy positions in Brazilian universities and pharmaceutical industries and Universities and Research Institute abroad. Prof. Prof. De Nucci is a member of the Brazilian National Academy of Medicine (since 2003), the Brazilian National Academy of Pharmacy (since 2009) and the Brazilian National Academy of Sciences (since 2011). He is also a member of the Brazilian Pharmacology and Experimental Therapeutics Society (SBFTe) and is a Fellow member of the British Pharmacological Society (BPS). Prof. De Nucci has published more than 420 papers indexed in Pubmed with 15632 citations and *h*-factor of 57. Prof. De Nucci's strong interest in research and development has resulted in 28 patents registered in the National Industrial Property Institute (INPI) and 35 patents registered in the World Intellectual Property Organization (WIPO). Prof De Nucci is the editor and only author of the booktext "Tratado de Farmacologia Clínica", released in 2021, and finally he makes up the list of the 800 most influential Brazilian scientists in the world.

Rocha e Silva Memorial Lecture Invited Speakers History



- 1984 *The role of endothelial cells and relaxation of vascular smooth muscle by acetylcholine and bradikinin.* Robert Furchgott (04/07)
- 1987 *Caracterização do fator de relaxamento arterial.* Salvador Moncada
- 1989 *Asma: uma doença inflamatória.* Boris Vargaftig
- 1991 *A morada Perigosa: morte e a vida da Leishmania nos fagolisossomas.* Michel Rabinovich
- 1993 *Structure, dynamics, and functions of atrial natriuretic factor receptor.* Tomas Maack

- 1995 *Receptores para Bradicinina.* Domenico Regoli
- 1997 *Disfunções na produção de fatores vasoativos em doenças cardiovasculares* Paul Vanhoutte
- 1999 *Purinergic signaling*-Geoffrey Burnstock
- 2001 *Mecanismos celulares da Asma Brônquica.* Bernardo Boris Vargaftig
- 2003 *Pharmacology adventures down a long and winding Road.* John Wallace (University of Calgary)
- 2005 *Inflammation: my wanderings along Mauricio Rocha e Silva's trail.* Roderick John Flower (University of London, England)
- 2007 *Can we develop anti-inflammatory drugs for infectious diseases?* Mauro Martins Teixeira (UFMG)
- 2009 *Understanding peripheral analgesics.* Sérgio Henrique Ferreira (USP)
- 2011 *Bradykinin revisited 62 years after its discovery.* João Batista Calixto (UFSC)
- 2012 *Discovery of nitric oxide and cyclic GMP in cell signaling and their role in drug development.* Ferid Murad (Nobel Prize Laureate, George Washington University, USA)
- 2014 *Resolution pharmacology: A new approach to anti-inflammatory therapy.* Mauro Perretti (The William Harvey Research Institute, UK)
- 2016 *The joy of discovery. My life in Pharmacology.* Salvador Moncada (University of Manchester, UK)
- 2018 *Functional selectivity and spatio-temporal propagation of GPCR signaling; from structural determinants to better drugs.* Michel Bouvier (University of Montreal, Canada)

Senior Pharmacologist Award History



2017 First Senior Pharmacologist Award *Recipient*: Prof. Dr. João B. Calixto (UFSC, Cienp)

2019 Second Senior Pharmacologist Award *Recipient*: Prof. Dr. Fernando de Queiroz Cunha (USP-RP)

2021 Third Senior Pharmacologist Award *Recipient*: Prof. Dr. Mauro Martins Teixeira (UFMG)

The rules can be seen at:

<http://www.sbfte.org.br/edital-para-o-premio-farmacologista-senior-uma-realizacao-sbfte-e-laboratorio-ache/>

Third Senior Pharmacologist Award Recipient

Keynote Speaker: Sergio Ferreira Lecture / Closing Lecture



Professor Mauro Martins Teixeira holds a medical degree from the UFMG Medical School (1990) and a PhD in Immunopharmacology from the University of London (1994). He is a Full Professor of the Department of Biochemistry and Immunology at Federal University of Minas Gerais, researcher 1A of CNPq. Professor Teixeira's contribution to the field of pharmacology as a discipline is outstanding and this is reflected both in his significant publications, citations and participation in the Editorial Boards of Pharmacology Journals worldwide. This has led to significant world-wide recognition, including being invited as a

Senior Editor at the British Journal of Pharmacology and membership of the editorial board of the Journals: Pharmacology and Therapeutics (2005-2015), Inflammation Research (2012- present), Frontiers in Immunology (2012-present), Frontiers in Pharmacology (2012-present). Significantly, he is part of the scientific committee of the IUPHAR WCP 2023 - World Congress of Basic & Clinical Pharmacology. Professor Teixeira's initial work focused on mechanisms of leukocyte migration, especially eosinophils, and how these could be modulated pharmacologically, in particular by cyclic AMP elevating agents. Significantly, these studies were among the first to investigate the role of chemokines *in vivo* and contributed to the development of PDE4 inhibitors for the treatment of inflammatory disease. Upon his return to Brazil, Professor Teixeira diligently worked at UFMG and has created one of the most prolific centers for the study of inflammation in Brazil. Professor Teixeira is Coordinator of the INCT in Dengue and the Pronex Network in Dengue of CNPq. Important work conducted in Brazil included the role of chemokines in infection and chronic inflammation, the relevance of the microbiota for inflammation, the relevance of inflammation for obesity. In the last few years, Professor Teixeira has conducted several vaccines (including against Dengue, COVID-19 and Influenza) and drug trials. With this expertise, he conducted an investigator-initiated clinical trial in collaboration with SynAct Pharma investigating the effects of the pro-resolving molecule, AP-1189, a melanocortin agonist, in patients with moderate COVID-19 pneumonia and needing respiratory care. Professor Teixeira is a member of the Brazilian Academy of Sciences (since 2009), of the National Order of Scientific and Technological Merit, and of the World Academy of Sciences (TWAS, since 2012) and received the Medal of The National Order of Scientific Merit in 2009. He is currently Vice-President of the Brazilian Academy of Sciences (Regional CO-MG, since 2013). He is currently Previous Vice-President (2012-2016) and current member of the Board of the Immunopharmacology Section of the International Union of Pharmacological Societies (IUPHAR), President of the Immunopharmacology Committee of the International Union of Immunological Societies (IUIS, since 2018), and President of the Brazilian Society of Inflammation (2018-2021). He was member of the Scientific Committee of the Brazilian Health Regulatory Agency (Anvisa, CCVISA, 2014-2019)), member of Board of Directors of the Brazilian Society of Pharmacology and Experimental Therapeutics (SBFTE) from 2002 to 2011 and President of the Society from 2012-2014.

José Ribeiro do Valle Award – First Place Winner History



- 1998: Maria Martha Campos (UFSC; Adviser: João Batista Calixto)
- 1999: José Eduardo da Silva Santos (UFSC; Adviser: Jamil Assreuy)
- 2000: Ana Paula V. Dantas (USP-SP; Adviser: Maria Helena Catelli de Carvalho)
- 2001: Liliam Fernandes (USP-SP; Adviser: Maria Helena Catelli de Carvalho)
- 2002: Isaias Gleizer (USP-SP; Adviser: Cristoforo Scavone)
- 2003: Juliano Ferreira (UFSC; Adviser: João Batista Calixto)
- 2004: João Alfredo de Moraes (UERJ; Adviser: Thereza Christina Barja-Fidalgo)
- 2005: Tiago Chiavegatti (Unifesp-EPM; Adviser: Rosely O. Godinho)
- 2006: Ana Letícia G. Cabral Maragno (USP-RP; Adviser: Marcelo Damário Gomes)
- 2007: Maria Fernanda de Paula Werner (UFSC; Adviser: Giles A. Rae)
- 2008: Ana Luiza Andrade de Paula Lopes (Unifesp-EPM; Adviser: Rosely O. Godinho)
- 2009: Silvio Manfredo Vieira (USP-RP; Adviser: Fernando de Q. Cunha)
- 2010: Vanessa Olzon Zambelli (Instituto Butantan; Adviser: Yara Cury)
- 2011: Tatiana Paula Teixeira Ferreira (Fiocruz; Adviser: Patrícia Machado Rodrigues e Silva)
- 2012: Maíra Assunção Bicca (UFSC; Adviser: João Batista Calixto)
- 2013: Jaqueline Raymondi Silva (USP-RP; Adviser: Fernando de Q. Cunha)
- 2014: Jhimmy Talbot (USP-RP; Adviser: Fernando de Q. Cunha)
- 2015: Daniele Maria Ferreira (UFPR; Adviser: Maria Fernanda de Paula Werner)
- 2016: Gabriela S Kinker (USP, Adviser: Pedro Augusto Carlos Magno Fernandes)
- 2017: Fernando Olinto Carreño (UFRGS, Adviser: Teresa C. Dalla Costa)
- 2018: Bruna da Silva Soley (UFPR, Adviser: Daniela de Almeida Cabrini)
- 2019: Douglas da Silva Prado (USP-RP) Adviser: José Carlos Alves Filho



José Ribeiro do Valle Award – 2021 Finalists



Roberta Giusti Schran
 BSc in Veterinary Medicine (UFFS) (2013-2018)
 MSc in Pharmacology (UFSC) (2019-2020)
 PhD in Pharmacology (UFSC)
 Adviser: Juliano Ferreira



Rianne Remus Pulcinelli
 BSc in Pharmacy (UFRGS) (2011-2016)
 MSc in Biological Sciences: Pharmacology and Therapeutics (UFRGS) (2016-2018)
 PhD in Biological Sciences: Pharmacology and Therapeutics (UFRGS)
 Adviser: Rosane Gomez



Naiara Ayako Satori
 BSc in Biotechnology (Unifesp-EPM) (2016-2019)
 MSc in Pharmacology (Unifesp-EPM)
 Adviser: Rosely O. Godinho



Anderson Romério Azevedo Cerqueira
 BSc in Biomedicine (UESC) (2009-2015)
 MSc in Pharmacology (USP) (2015-2018)
 PhD in Pharmacology (USP)
 Adviser: Soraia Katia Pereira Costa



Matheus Leite de Medeiros
 BSc in Biomedicine (Unip) (2011-2016)
 PhD in Pharmacology (Unicamp)
 Adviser: Edson Antunes



SBFTE Jovem, founded in 2013, is a Committee of the Brazilian Society of Pharmacology and Experimental Therapeutics (SBFTE). The Committee is composed of young Pharmacologist's members of SBFTE, working in association with the SBFTE Board of Directors. Our mission is to create a permanent political-scientific forum dedicated to undergraduate, graduate students, Post-Docs, as well as young investigators and Junior faculty members of SBFTE to discuss scientific topics related to Pharmacology in order to promote the development of early-career investigators,

stimulating the participation, insertion and collaboration of our members into the activities of the Society. This year, SBFTE Young will promote an activity during the 53rd Brazilian Congress of Pharmacology and Experimental Therapeutics. *Beyond the academy* is a roundtable to open a discussion about opportunities to Brazilian early-career scientists regarding innovation, new challenges in science, how to engage into carriers in the industry or any other relevant opportunities that are beyond scholar-driven carriers. This section is scheduled for November 19th, 2021 from 10:10 am to 12:10 pm.

SBFTE Young Committee

João Alfredo de Moraes (UFRJ, Coordinator)

Jamyllle Nunes de Sousa Ferro (UFAL, Vice-Coordinator)

Jessika Cristina Bridi (ICB-USP)

João Agostinho Machado Neto (USP-SP)

Sanseray Cruz Machado (USP-SP)

16/11/2021 (Tuesday)

08h00-09h00	Courses					
Cr1	Cr2	Cr3	Cr4	Cr5	Cr6	
Class 1	Class 1	Class 1	Class 1	Class 1	Class 1	
09h00-11h00	Meeting of SBFTE Directory Board and Deliberative Council (Council and Directory Board Members only) (Google Meet)					
12h00-13h25	Lunch					
13h25-14h25	Courses					
Cr7	Cr8	Cr9	Cr10	Cr11		
Class 1	Class 1	Class 1	Class 1	Class 1	Class 1	
14h30-16h00	Roundtable 1					
16h15-17h30	Meeting of SBFTE Permanent Forum of Postgraduation in Pharmacology (only for Heads of Postgraduation Courses in Pharmacology, Deliberative Council and Society Board) (Google Meet)					
18h00-18h30	Opening Session					
18h30-19h30	Opening Lecture					
	C1					
	<i>Rocha e Silva Memorial Lecture</i>					

17/11/2021 (Wednesday)

08h00-09h00	Courses					
Cr1	Cr2	Cr3	Cr4	Cr5	Cr6	
Class 2	Class 2	Class 2	Class 2	Class 2	Class 2	
09h05-10h05	Lectures					
	C2			C3		
10h05-10h15	Coffee-break					
10h15-12h15	Symposia/Oral Communication					
	S1		S2		S3	
12h20-13h20	Lunch					
12h20-13h20	Technical Lectures					
13h25-14h25	Courses					
Cr7	Cr8	Cr9	Cr10	Cr11		
Class 2	Class 2	Class 2	Class 2	Class 2	Class 2	
14h30-16h30	Symposia/Oral communication					
	S4		S5		S6	
16h35-16h45	Coffee-break					
16h45-17h45	Lectures					
	C4			C5		
17h50-20h20	E-Poster Session 1					

18/11/2021 (Thursday)

08h00-09h00	Courses					
CR1	CR2	CR3	Cr4	Cr5	Cr6	
Class 3	Class 3	Class 3	Class 3	Class 3	Class 3	
09h05-10h05	Lectures					
	C6			C7		

10h05-10h15	Coffee-break			
10h15-12h15	Symposia/Oral Communication			
	S7	S8	S9	
12h20-13h20	Lunch / SBFTE Jovem Assembly (Google Meet)			
12h20-13h20	Technical Lectures			
13h25-14h25	Courses			
	Cr7	Cr8	Cr9	Cr10
	Class 3	Class 3	Class 3	Class 3
14h30-16h30	Symposia/Oral Communication			
	S10			

José Ribeiro do Valle Award

16h35-16h45	Coffee-break
16h45-19h15	E-Poster Session 2

19/11/2021 (Friday)

08h00-09h00	Meeting of the North-Northeast and Central West Region Pharmacology Network (Google Meet)			
09h05-10h05	Lectures			
	C8		C9	
10h10-12h10	Symposia/Oral Communication			
	S11	S12	MR2 (SBFTEJovem)	
12h10-13h10	Lunch			
13h15-14h45	SBFTE Assembly (Google Meet)			
15h00-16h00	Closing lecture			
	C10			
	Senior Pharmacologist Award Recipient – A SBFTE Ache Initiative <i>Sergio Ferreira Lecture</i>			
16h05-16h35	Closing Session			
	Awards and Prize Announcements Closing Ceremony			

08h00-09h00	Courses
Cr1	<p>Systematic Review and Meta-Analysis for Pharmacologists (Presented in Portuguese) Chair: Cilene Lino de Oliveira (UFSC)</p> <ul style="list-style-type: none"> Class 1: <i>Systematic reviews: The qualitative synthesis</i> Vanessa Beijamini-Harres (UFES)
Cr2	<p>General Principles and Therapeutic Applications of Extracellular Vesicles (Presented in Portuguese) Chair: Ionara Rodrigues Siqueira (UFRGS)</p> <ul style="list-style-type: none"> Class 1: <i>General principles of Extracellular Vesicles: Origin, cargo, release and uptake</i> Laura Reck Cechinel (UFRGS)
Cr3	<p>Neuropharmacological Potential of Medicinal Plants in Post-Pandemic Times: New Approaches to Plant Use in Mental Health (Presented in Portuguese) Chair: Marcia Maria de Souza (Univali)</p> <ul style="list-style-type: none"> Class 1: <i>Anti-inflammatory activity of ayahuasca and its potential against neuroinflammation from COVID-19: therapeutic implications in neurological and psychiatric diseases.</i> Rafael Mariano Bittencourt (Unisul)
Cr4	<p>Ion Channels: Biophysical, Biological and Pharmacological Aspects. (Presented in Portuguese) Chair: Cristiano Gonçalves Ponte (IFRJ)</p> <ul style="list-style-type: none"> Class 1: <i>Ion Channels: Biophysical and electrophysiological aspects.</i> Ricardo Mauricio Xavier Leão (USP-RP)
Cr5	<p>Didactic Tools and Methodologies of Active Learning in the Teaching of Pharmacology (Presented in Portuguese) Chair: François G. Noël (UFRJ)</p> <ul style="list-style-type: none"> Class 1: <i>Methodologies and technological resources for biomedical education of digital native learners</i> Camilo Lellis-Santos (Unifesp)
Cr6	<p>Improving Standards for Reproducibility in Basic Pharmacology (Presented in Portuguese) Chair: Roberto Andreatini (UFPR)</p> <ul style="list-style-type: none"> Class 1: <i>Developing a structure for confirmatory experiments in basic Biomedical Science</i> Olavo B Amaral (UFRJ)
09h00-11h00	Meeting of SBFTE Directory Board and Deliberative Council (Council and Directory Board Members only) (Google Meet)
12h00-13h25	Lunch
12h20	Screeener Game Interview (Presented in Portuguese)
13h25-14h25	Courses
Cr7	<p>Scientific Dissemination and Popularization in Pharmacology: Lessons from the Past, Current Challenges and Opportunities in the Post-Pandemic World (Presented in Portuguese) Chair: Luisa Mota da Silva (Univali)</p> <ul style="list-style-type: none"> Class 1: <i>Lessons from the past about scientific dissemination and popularization in Pharmacology</i> Luisa Mota da Silva (Univali)
Cr8	<p>Pandemic' Stress as a Risk Factor for Neuropsychiatric Disorders: Insights from Translational Evidences (Presented in Portuguese) Chair: Fábio Cardoso Cruz (Unifesp)</p> <ul style="list-style-type: none"> Class 1: <i>Stress as a risk factor for anxiety and depression</i> Cristiane Aparecida Favoretto (Unifesp)

Cr9	<p><i>Tenebrio molitor</i> Larvae Model and its use in Pharmacology Teaching and Research (Presented in Portuguese) Chair: Elizabeth Soares Fernandes (IPPPP) <ul style="list-style-type: none"> Class 1: <i>Use of animal models in Pharmacology: most used animal species versus alternative animal models</i> Maria Christina Werneck de Avellar (Unifesp-EPM) </p>
Cr10	<p>Evaluation of Clinical Trials for Vaccine Approval (Presented in Portuguese) Chair: Mauricio Schuler Nin (FURG) <ul style="list-style-type: none"> Class 1: <i>Evaluation of Efficacy and Safety of Vaccines against COVID-19</i> Gustavo Mendes Lima Santos (ANVISA) </p>
Cr11	<p>G-Protein Coupled Receptors (GPCRs) and their Signal Transduction Pathways (Presented in Portuguese) Chair: Bagnólia Araújo Costa (UFPB) <ul style="list-style-type: none"> Class 1: <i>GPCRs & G-proteins: structure and functions</i> Claudio M. Costa Neto (USP-RP) </p>
14h30-16h00	<p>Roundtable 1 Impact of the Pandemic on Brazilian Science and Graduation Courses (Presented in Portuguese) Chair: Rui Daniel Schroder Prediger (UFSC, Coordinator SBFTE Permanent Forum of Postgraduation in Pharmacology) <ul style="list-style-type: none"> Adelina Martha dos Reis (UFMG, Coordinator CBII-CAPES) Luiz Eugenio Araujo de Moraes Mello (Unifesp-EPM, FAPESP Scientific Director) Jerson Lima da Silva (UFRJ, President FAPERJ) Rafael Roesler (UFRGS, FAPERGS Scientific Director) </p>
16h15-17h30	<p>Meeting of SBFTE Permanent Forum of Postgraduation in Pharmacology (only for Heads of Postgraduation Courses in Pharmacology, Deliberative Council and Society Board) (Google Meet)</p>
18h00-18h30	Opening Session
18h30-19h30	Opening Lecture
C1	<p><i>Rocha e Silva Memorial Lecture</i> 6-NitroDopamine, the New kidDo in town Gilberto de Nucci (Unicamp) Presented by: Marco Aurélio Martins (Fiocruz)</p>

08h00-09h00	Courses
Cr1	<p>Systematic Review and Meta-Analysis for Pharmacologists (Revisão Sistemática e Meta-Análise para Farmacologistas) Chair: Cilene Lino de Oliveira (UFSC)</p> <ul style="list-style-type: none"> • Class 2: <i>Meta-analysis: The quantitative synthesis.</i> (Presented in English) Sarah McCann (Quest Center BIH, Berlin, Germany)
Cr2	<p>General Principles and Therapeutic Applications of Extracellular Vesicles (Presented in Portuguese) Chair: Ionara Rodrigues Siqueira (UFRGS)</p> <ul style="list-style-type: none"> • Class 2: <i>Isolation and Analysis of Extracellular Vesicles - Potential Clinical Relevance</i> Rafael Soares Lindoso (UFRJ)
Cr3	<p>Neuropharmacological potential of Medicinal Plants in Post-Pandemic Times: New Approaches to Plant Use in Mental Health (Presented in Portuguese) Chair: Marcia Maria de Souza (Univali)</p> <ul style="list-style-type: none"> • Class 2 <i>Plants native to South Brazil as source of new antidepressant prototypes.</i> Stela Maris Kuze Rates (UFRGS)
Cr4	<p>Ion Channels: Biophysical, Biological and Pharmacological aspects. (Presented in Portuguese) Chair: Cristiano Gonçalves Ponte (IFRJ)</p> <ul style="list-style-type: none"> • Class 2: <i>Ionotropic Receptors (LGIC).</i> Newton Gonçalves de Castro (UFRJ)
Cr5	<p>Didactic Tools and Methodologies of Active Learning in the Teaching of Pharmacology (Presented in Portuguese) Chair: François G. Noël (UFRJ)</p> <ul style="list-style-type: none"> • Class 2: <i>SCREENER, an educational game for teaching the Drug Discovery and Development process</i> François G. Noël (UFRJ)
Cr6	<p>Improving Standards for Reproducibility in Basic Pharmacology (Presented in Portuguese) Chair: Roberto Andreatini (UFPR)</p> <ul style="list-style-type: none"> • Class 2: Open access to science facilitating the discussion about reproducibility Plinio Casarotto (Helsinki University, Finland)
09h05-10h05	Lectures
C2	<p>Using Data Science For Outbreak Preparedness Helder Nakaya (USP-SP) Presented by: Fabiola Taufic Mónica (Unicamp)</p>
C3	<p>Kinases as Potential Therapeutic Targets for Anti-Coronaviral Therapy Stefan Laufer (Eberhard-Karls-University Tübingen, Germany) Presented by: Lidia Moreira Lima (UFRJ)</p>
10h05-10h15	Coffee-break
10h15-12h15	Symposia/Oral Communication
S1	<p>Frontiers of Antidepressant Research: Approaches for Discovery Chair: Cilene Lino de Oliveira (UFSC)</p> <ul style="list-style-type: none"> • <i>Translational neuropsychopharmacology: Bench to bedside</i> Gregers Wegener (Aarhus University, Denmark) • <i>Multi laboratory studies for antidepressants discovery</i> Roberto Andreatini (UFPR) • <i>Evidence synthesis for antidepressants discovery</i> Alexandra Bannach-Brown (Quest Center BIH, Germany) • Oral Communication 1: 03.021 <i>CB2 receptors' spontaneous activity is relevant for the adverse effects of chronic unpredictable stress in mice treated with antidepressant.</i> Araújo MR²; Aguiar RP¹; Füsse EJ³, Scarante FF²; Oliveira RMMW¹; Guimarães FS², Campos AC² ¹Dpt of Pharmacology and Therapeutics, State Univ of Maringá, Maringá, Brazil ²Dpt

	<p>of Pharmacology - Ribeirão Preto Medical School, Univ of São Paulo- Ribeirão Preto, Brazil ³Mental Health Graduate Program, Ribeirão Preto Medical School, Univ of São Paulo Ribeirão Preto, Brazil</p> <ul style="list-style-type: none"> • Oral Communication 2: 03.012 <i>Studies using the forced swim test: an interim meta-analysis.</i> Martins, T^{1,2}, Lino de Oliveira, C^{1 2}. ¹UFSC Florianópolis, PPG in Pharmacology, Brazil; ²UFSC Florianópolis, Dpt of Physiological Sciences, Brazil
S2	<p>Transient Receptor Potential (TRP) Channels as Targets for Pain Control Chair: Gabriela Trevisan dos Santos (UFSM)</p> <ul style="list-style-type: none"> • <i>Transient receptor potential (TRP) channels as targets of natural products in pain models</i> Maria Fernanda de Paula Werner (UFPR) • <i>Peripheral Nerve Resident Macrophages and Schwann Cell TRPA1 in Cancer Pain</i> Romina Nassini (Firenze University, Italy) • <i>Neuropathic pain observed in multiple sclerosis is mediated by TRPA1 activation</i> Gabriela Trevisan dos Santos (UFSM) • Oral Communication 1: 01.016 <i>Effect of extracellular vesicles derived from breast tumor cells on human neutrophils polarization.</i> Amorim CS¹, Docasar CL¹, Renovato-Martins M², Barja-Fidalgo C³, Moraes JA¹ ¹UFRJ Redox Biology Lab, Inst de Ciências Biomédicas, Brazil ²UFF Dpt of Cell and Molecular Biology, Brazil ³UERJ Dpt of Cell Biology, Brazil • Oral Communication 2: 02.014 <i>Is the pineal gland able to detect brain damage? N-acetylserotonin (NAS), a neuroprotective darkness hormone.</i> Sousa KS, Quiles CL, Muxel SM, Ferreira ZS, Markus RP. USP São Paulo, Dpt Physiology, Lab Chronopharmacology, Brazil
S3	<p>Application of Nanotechnology in the Treatment of Inflammatory Disorders Chair: Patrícia Machado Rodrigues e Silva Martins (Fiocruz)</p> <ul style="list-style-type: none"> • <i>Lipid-core nanocapsules as a promising strategy to treat inflammatory disorders</i> Silvia Staniscuaski Guterres (UFRGS) • <i>Nanosystems as vectors for cell and gene therapies</i> Patrícia Rieken Macedo Rocco (UFRJ) • <i>Therapeutic administration of gold nanoparticles accelerates silica-induced pulmonary fibrosis in mice</i> Patrícia Machado Rodrigues e Silva Martins (Fiocruz) • Oral Communication 1: 10.006 <i>Co-encapsulation of 5-fluorouracil in multiple nanoemulsions containing short chain triglycerides improves drug cytotoxicity against colorectal cancer cells.</i> Fukumori C, Branco PC, Lopes LB. ICB-USP – Dpt of Pharmacology, Brazil • Oral Communication 2: 08.018 <i>Controlled release of JME-173 from nanocapsules improves lipopolysaccharide-induced lung inflammation in mice.</i> Coutinho DS¹, Bernardi A¹, Guterres SS², Pohlmann AR³, Silva PMR¹, Martins MA¹ ¹Lab of Inflammation, Oswaldo Cruz Foundation, Rio de Janeiro, Brazil ²Pharmaceutical Sciences Post-Graduation Program, Federal Univ of Rio Grande do Sul, Porto Alegre, Brazil ³Dpt of Organic Chemistry, Federal Univ of Rio Grande do Sul, Porto Alegre, Brazil
12h20-13h20	Lunch
12h20-13h20	<p>Technical Lectures</p> <p>New technologies to accelerate drug discovery – the impact of qualified data Gabriel Kaetan Baio Ferreira (CAS, a Division of the American Chemical Chemical Society)</p> <p>Como facilitar e potencializar o ensino, as tomadas de decisões clínicas e ainda poupar tempo ao realizar: Revisão da Prescrição e da Farmacoterapia e Conciliação Medicamentosa Gladys Marques (Wolters Kluwer – Health Learning, Research & Practice)</p>
13h25-14h25	Courses
Cr7	<p>Scientific Dissemination and Popularization in Pharmacology: Lessons from the Past, Current Challenges and Opportunities in the Post-Pandemic World (Presented in Portuguese) Chair: Luisa Mota da Silva (Univali)</p>

	<ul style="list-style-type: none"> • Class 2: <i>Current challenges in scientific dissemination and popularization</i> Hermógenes David de Oliveira (UFC)
Cr8	<p>Pandemic' Stress as a Risk Factor for Neuropsychiatric Disorders: Insights from Translational Evidences (Presented in Portuguese) Chair: Fábio Cardoso Cruz (Unifesp)</p> <ul style="list-style-type: none"> • Class 2: <i>Impact of stress on drug abuse and addiction</i> Paula Cristina Bianchi (Unifesp)
Cr9	<p><i>Tenebrio molitor</i> Larvae Model and its use in Pharmacology Teaching and Research (Presented in Portuguese) Chair: Elizabeth Soares Fernandes (IPPPP)</p> <ul style="list-style-type: none"> • Class 2: <i>Overall physiological similarity between insects with humans and rodents</i> Daniele Maria-Ferreira (IPPPP)
Cr10	<p>Evaluation of Clinical Trials for Vaccine Approval (Presented in Portuguese) Chair: Mauricio Schuler Nin (FURG)</p> <ul style="list-style-type: none"> • Class 2: <i>Evaluation of Quality and Biotechnology of Vaccines against COVID-19</i> Gustavo Mendes Lima Santos (ANVISA)
Cr11	<p>G-Protein Coupled Receptors (GPCRs) and their Signal Transduction Pathways (Presented in Portuguese) Chair: Bagnólia Araújo Costa (UFPB)</p> <ul style="list-style-type: none"> • Class 2: <i>Adenylyl cyclase signal transduction pathway: pathophysiological role</i> Bagnólia Araújo Costa (UFPB)
14h30-16h30	Symposia/Oral communication
S4	<p>Sperm-Associated Proteins as Drug Targets for Male Contraception Chair: Erick José Ramo da Silva (UNESP-Botucatu)</p> <ul style="list-style-type: none"> • <i>Independent validation of TSSK1 and TSSK2 as targets for male contraception</i> Pablo E Visconti (University of Massachusetts Amherst, USA) • <i>New insights about mammalian sperm acrosomal exocytosis</i> Mariano G Buffone (IBYME-CONICET, Argentina) • <i>Molecular pathways that control sperm capacitation</i> Dario Krapf (IBMC-CONICET, Argentina) • Oral Communication 1: 07.006 <i>New insights into the bladder dysfunction caused by long-term methylglyoxal intake: reversal by metformin.</i> Oliveira AL, Medeiros ML, Oliveira MG, Mónica FZ, Antunes E Dept de Farmacologia, Faculdade de Ciências Médicas, Univ de Campinas (UNICAMP), Campinas (São Paulo), Brasil • Oral Communication 2: 07.010 <i>Intrauterine and lactational exposure of male rats to supraphysiological doses of manganese: short-term reproductive and development toxicity.</i> Silva APG¹, Correia MH², da Silva LN¹, Santiago MSA¹, Perobelli JE¹. ¹Dpt de Ciências do Mar, PPG Interdisciplinar em Ciências da Saúde ^{1,2}Unifesp, Brasil
S5	<p>Pharmacology and Covid19 in Latin America Chair: Guilherme Suarez-Kurtz (INCa, Iuphar Deliberative Council Member)</p> <ul style="list-style-type: none"> • <i>The Chilean Society of Pharmacology (SOFARCHI) and its contribution to the fight against SARS-CoV-2 in Chile</i> Javier Andrés Bravo Vivallo (President Chilean Pharmacological Society, Chile) • <i>Impact of COVID-19 in Argentine</i> Ventura Simonovich (Vice-President Argentinian Association of Experimental Pharmacology, Argentina) • <i>Impact of COVID-19 in Cuba</i> Sonsire Fernandez (Instituto Finlay de Vacunas, Cuba) • <i>Impact of COVID-19 in Brazil</i> Mauro M. Teixeira (UFMG)
S6	<p>Cannabinoids and Cannabinoid-like Molecules Acting on the CNS Chair: Maíra Assunção Bicca (Johns Hopkins University, USA)</p> <ul style="list-style-type: none"> • <i>Mechanisms of the Cannabidiol-induced anxiolytic effects</i> Francisco Silveira Guimarães (USP-RP)

- *Cannabis is for the elderly: THC and CBD as therapeutic tools for age-related diseases*
Francisney Pinto Nascimento (UNILA)
- *Terpenoids, cannabimimetic ligands, as therapeutic tools for neuroimmunological disorders*
Rafael Cypriano Dutra (UFSC)
- *Oral Communication 1: 05.010 The CB2 receptor agonist beta-caryophyllene attenuates oxaliplatin-induced peripheral neuropathy in a tumor-bearing mice model.* Agnes JP¹, Neves RN¹, Gonçalves RM¹, Delgobo M¹, Silva RC², Ferreira AR², Senna EL², Zanotto-Filho A¹ ¹UFSC Florianópolis, PPG Pharmacology, Dpt of Pharmacology, Brazil; ²UFSC Florianópolis, PPG Pharmacy, Dpt of Pharmaceutical Sciences, Brazil
- *Oral Communication 2: 03.011 The role of anandamide in the anxiety-like behavior in female rats.* Salemme BW, Raymundi AM, Sohn JMB, Stern CAJ. UFPR Curitiba, Dpt of Pharmacology, Brazil

16h35-16h45	Coffee-break
16h45-17h45	Lectures
C4	What Does Basic Neuroscience Research Predict about the Future of Pain Management? Allan Basbaum (UCSF, USA) Presented by: Gabriela Trevisan dos Santos (UFSM)
17h50-20h20	E-Poster Session 1
	<p>Room 01: 01. Cellular and Molecular Pharmacology (01.001 a 01.007; 01.017 a 01.019)</p> <p>Room 02: 02. Neuropharmacology (02.001 a 02.007)</p> <p>Room 03: 02. Neuropharmacology (02.019 a 02.027)</p> <p>Room 04: 03. Psychopharmacology (03.007 a 03.014)</p> <p>Room 05: 04. Inflammation and Immunopharmacology (04.001 a 04.005) 05. Pain and Nociception Pharmacology (05.001 a 05.005)</p> <p>Room 06: 04. Inflammation and Immunopharmacology (04.013 a 04.015) 05. Pain and Nociception Pharmacology 05.009 e 05.010; 05. 012; 05. 014 e 05. 015</p> <p>Room 07: 04. Inflammation and Immunopharmacology (04.027 a 04.037)</p> <p>Room 08: 06. Cardiovascular and Renal Pharmacology (06.007 a 06.016)</p> <p>Room 09: 06. Cardiovascular and Renal Pharmacology (06.030 a 06.039)</p> <p>Room 10: 07. Endocrine, Reproductive and Urinary Pharmacology (07.003 a 07.012) 13. Pharmacology Education and Technology (13.001)</p> <p>Room 11: 09. Natural Products and Toxinology (09.001 a 09.008)</p> <p>Room 12: 10. Cancer Pharmacology (10.001 a 10.009)</p> <p>Room 13: 11. Clinical Pharmacology, Pharmacokinetics, Pharmacogenomics and Toxicology (11.004 a 11.006) 12. Drug Discovery and Development (12.007 a 12.014)</p>

08h00-09h00	Courses
CR1	<p>Systematic Review and Meta-Analysis for Pharmacologists (Presented in Portuguese) Chair: Cilene Lino de Oliveira (UFSC)</p> <ul style="list-style-type: none"> Class 3: <i>Risk of bias: Tools to the assessment of research quality.</i> Cilene Lino de Oliveira (UFSC)
CR2	<p>General Principles and Therapeutic Applications of Extracellular Vesicles (Presented in Portuguese) Chair: Ionara Rodrigues Siqueira (UFRGS)</p> <ul style="list-style-type: none"> Class 3: <i>Potential Clinical Relevance of Extracellular Vesicles: Therapy and Biomarkers</i> Ionara Rodrigues Siqueira (UFRGS)
CR3	<p>Neuropharmacological potential of Medicinal Plants in Post-Pandemic Times: New Approaches to Plant Use in Mental Health (Presented in Portuguese) Chair: Marcia Maria de Souza (Univali)</p> <ul style="list-style-type: none"> Class 3: <i>Pain in times of COVID and the development of new herbal medicines: Case of a University x Pharmaceutical Company partnership</i> Tania Mari Bellé Brezolin (Univali)
Cr4	<p>Ion Channels: Biophysical, Biological and Pharmacological aspects (Presented in Portuguese) Chair: Cristiano Gonçalves Ponte (IFRJ)</p> <ul style="list-style-type: none"> Class 3: <i>Voltage-activated Potassium Channels as Pharmacological Targets</i> Cristiano Gonçalves Ponte (IFRJ)
Cr5	<p>Didactic Tools and Methodologies of Active Learning in the Teaching of Pharmacology (Presented in Portuguese) Chair: François G. Noël (UFRJ)</p> <ul style="list-style-type: none"> Class 3: <i>The software "Basic pharmacology of the autonomic nervous system by computer simulation" by Zyngier, Garcia & Zyngier (1995)</i> André Sampaio Pupo (UNESP-Botucatu)
Cr6	<p>Improving Standards for Reproducibility in Basic Pharmacology (Presented in Portuguese) Chair: Roberto Andreatini (UFPR)</p> <ul style="list-style-type: none"> Class 3: <i>Multicenter preclinical studies</i> Roberto Andreatini (UFPR)
09h05-10h05	Lectures
C6	<p>The role of ion channels in neutrophil function Markus Sperandio (Ludwig-Maximilians-Universität München, Germany) Presented by: Hugo Caire de Castro Faria Neto (Fiocruz)</p>
C7	<p>Compositions of Cannabis Compounds for the Treatment of Medical Conditions: Covid-19 and Glioblastoma Hinanit Koltai (ARO, Volcani Center, Israel) Presented by: Fabricio Alano Pamplona (Unila)</p>
10h05-10h15	Coffee-break
10h15-12h15	Symposia/Oral Communication
S7	<p>Natural and Synthetic Molecules as Antiviral drugs Chair: Bagnólia Araújo Costa (UFPB)</p> <ul style="list-style-type: none"> <i>Anti-Zika and anti-dengue virus activity of the citrus flavonoid naringenin</i> Juliano Bordignon (Fiocruz-PR) <i>Medicinal plants from Indian Ocean are a promising source of antiviral phytochemicals against emerging mosquito-borne flavivirus at doses devoid of toxicity in Zebrafish</i> Chaker El Kalamouni (University of Reunion Island, France) <i>Action of Antivirals on Arboviruses and SarsCov-2</i> Paulo Ricardo da Silva Sanchez (Unesp-São José do Rio Preto)

	<ul style="list-style-type: none"> • Oral Communication 1: 04.036 <i>Effect of H1N1 viral infection on lung fibrosis induced by silica particles in mice</i>. Ferreira TPT¹, Arantes, ACS¹, Jannini-Sá YAP¹, Hogaboam CM², Martins MA¹, Silva PMR¹ ¹Lab of Inflammation, Oswaldo Cruz Inst-Fiocruz, RJ, Brazil, ²Cedars Sinai Medical Center, LA, USA • Oral Communication 2: 06.015 <i>Serum from COVID-19 patients decreases endothelial cell antioxidant defense via downregulation of the Nrf2 transcriptional factor</i>. Rodrigues D¹, Machado MR¹, Costa RM^{1,2}, Tostes RC¹, ¹Dpt of Pharmacology, Ribeirão Preto Medical School, Univ of São Paulo, Brazil, ²Academic Unit of Health Sciences, Federal Univ of Jatai, Brazil
S8	<p>Beyond the Classical Renin-Angiotensin System: What's next? Chair: Jamil Assrey (UFSC) / Daniella Bonaventura (UFMG)</p> <ul style="list-style-type: none"> • <i>Renin-Angiotensin System in the Central Nervous System: What have we learned so far?</i> Aline Silva de Miranda (UFMG) • <i>Circulating Angiotensin-(1-7) is reduced in Alzheimer's Disease patients and correlates with white matter abnormalities: Results from a pilot study</i> Ana Cristina Simões e Silva (UFMG) • <i>Perivascular adipose tissue as a source and target of Renin-Angiotensin system</i> Luciana Venturini Rossoni (USP-SP) • <i>Understanding the RAS puzzle: lessons from COVID-19</i> Robson Augusto Souza dos Santos (UFMG)
S9	<p>Pharmacometrics Applications in Drug Development and Dosing Optimization for Clinical Practice Chair: Bibiana Verlindo de Araujo (UFRGS)</p> <ul style="list-style-type: none"> • <i>Population pharmacokinetics applications: how the modeling and simulation can be used in research and clinical practice</i> Bibiana Verlindo de Araujo (UFRGS) • <i>Physiologically-based pharmacokinetic (PBPK) modelling to predict drug disposition after bariatric surgery</i> Natália Valadares de Moraes (Unesp-Araraquara) • <i>QSP modelling approaches for immune related disease: why, when and how?</i> Zinnia Patricia Parra Guillén (University of Navarra, Spain) • Oral Communication 1: 11.003 <i>Population pharmacokinetic modelling of tobramycin lung and epithelial lining fluid disposition due to biofilm-forming Pseudomonas aeruginosa infection</i>. Dias BB¹, Carreño F², Helfer VH¹, Garzela PM¹, Barreto F³, Araújo BV¹, Dalla Costa T¹. ¹UFRGS PPG Pharmaceutical Sciences Porto Alegre, Brazil; ²Univ of North Carolina at Chapel Hill, US; ³Federal Lab of Animal and Plant Health and Inspection, Porto Alegre, Brazil
12h20-13h20	Lunch / SBFTE Jovem Assembly (Google Meet)
12h20-13h20	<p>Publishing in the BJP: Guidelines for Reproducibility and Transparency Chair: Amrita Ahluwalia (Queen Mary University of London, UK)</p> <ul style="list-style-type: none"> • Amrita Ahluwalia (Queen Mary University of London, UK) • Clare Stanford (University College London, UK) • Mauro M. Teixeira (UFMG)
12h20-13h20	<p>Research and New Technologies at Aché Laboratórios Farmacêuticos: a Brazilian pharmaceutical industry case of successful innovation (presented in Portuguese) Romulo Reis (Aché Laboratórios Farmacêuticos)</p>
12h20-13h20	<p>Editora Atheneu Lançamento Livro-texto de Farmacologia - Casos clínicos e atividades</p>
13h25-14h25	Courses
Cr7	<p>Scientific Dissemination and Popularization in Pharmacology: Lessons from the Past, Current Challenges and Opportunities in the Post-Pandemic World (Presented in Portuguese) Chair: Luisa Mota da Silva (Univali)</p>

	<ul style="list-style-type: none"> Class 3: <i>Opportunities in scientific dissemination and popularization in pharmacology field in the post-pandemic world</i> Alexandra Acco (UFPR)
Cr8	<p>Pandemic' Stress as a Risk Factor for Neuropsychiatric Disorders: Insights from Translational Evidences (Presented in Portuguese) Chair: Fábio Cardoso Cruz (Unifesp)</p> <ul style="list-style-type: none"> Class 3: <i>Stress and schizofrenia</i> Cássio Morais Loss (Unifesp)
Cr9	<p><i>Tenebrio molitor</i> Larvae Model and its use in Pharmacology Teaching and Research (Presented in Portuguese) Chair: Elizabeth Soares Fernandes (IPPPP)</p> <ul style="list-style-type: none"> Class 3: <i>Use of Tenebrio molitor to study and investigate novel therapies</i> Elizabeth Soares Fernandes (IPPPP)
Cr10	<p>Evaluation of Clinical Trials for Vaccine Approval (Presented in Portuguese) Chair: Mauricio Schuler Nin (FURG)</p> <ul style="list-style-type: none"> Class 3: <i>Evaluation of Pharmacovigilance Plan of Vaccines Against COVID-19</i> Gustavo Mendes Lima Santos (ANVISA)
Cr11	<p>G-Protein Coupled Receptors (GPCRs) and their Signal Transduction Pathways (Presented in Portuguese) Chair: Bagnólia Araújo Costa (UFPB)</p> <ul style="list-style-type: none"> Class 3: <i>Phospholipase C beta signal transduction pathway pathophysiological role</i> Bagnólia Araújo Costa (UFPB)
14h30-16h30	Symposia/Oral Communication
S10	<p>Prêmio José Ribeiro do Valle Chair: Marco Aurelio Martins (Fiocruz) <i>Roberta Giusti Schran</i></p> <ul style="list-style-type: none"> 05.011 <i>Nociceptive effect of TLR2 on a mice model of postoperative pain</i> Schran RG, Ferreira MDA, Silva AMD, Martins F, Ferreira J Department of Pharmacology, Federal University of Santa Catarina, Florianópolis, SC, Brazil <p><i>Rianne Remus Pulcinelli</i></p> <ul style="list-style-type: none"> 02.018 <i>Taurine restores extracellular GABA levels in the nucleus accumbens reduced by alcohol withdrawal and decreases voluntary alcohol intake in withdrawal rats.</i> Pulcinelli RR¹, Caletti G¹, Izolan LR¹, Eller S², Nin MS³, Oliveira TF², Gomez R¹. ¹UFRGS Porto Alegre, PPG Farmacologia e Terapêutica (PPGFT), Brazil; ²UFCSA Porto Alegre, Dpt de Farmacociências, Brazil; ³FURG Rio Grande, Dpt de Ciências Biológicas, Brazil <p><i>Naiara Ayako Satori</i></p> <ul style="list-style-type: none"> 08.011 <i>Relaxation of airway smooth muscle induced by classical phosphodiesterase (PDE) inhibitors involves inhibition of ecto-PDE</i> Satori NA¹, Pacini ESA¹, Jackson EK², Godinho RO¹. ¹Division of Cellular Pharmacology, Dpt of Pharmacology, Escola Paulista de Medicina, Univ Federal de São Paulo (EPM/Unifesp), São Paulo, Brazil ²Dpt of Pharmacology and Chemical Biology, Univ of Pittsburgh School of Medicine, Pittsburgh, PA, USA <p><i>Anderson Romério Azevedo Cerqueira</i></p> <ul style="list-style-type: none"> 04.017 <i>Antioxidant effect of mitochondrial H2S donor (AP39) in topical treatment for burn injury</i> Cerqueira ARA¹, Teixeira SA¹, Coavoy-Sanchez SA¹, Oliveira JP¹, Wood ME², Whiteman M³, Muscará MN¹, Lopes LB¹, Costa SKP¹. ¹USP São Paulo, Inst of Biomedical Sciences, Dpt of Pharmacology, Brazil; Univ of Exeter Exeter, ²Dpt of Biosciences, ³College of Medicine and Health, UK <p><i>Matheus Leite de Medeiros</i></p> <ul style="list-style-type: none"> 04.016 <i>Methylglyoxal aggravates lipopolysaccharide-induced mouse lung inflammation.</i> Medeiros ML, Oliveira AL, Oliveira MG, Mônica FZ, Antunes, E. Dept de Farmacologia, Faculdade de Ciências Médicas, Univ de Campinas, Campinas, Brasil
16h35-16h45	Coffee-break
16h45-19h15	E-Poster Session 2
	Room 14:

01. Cellular and Molecular Pharmacology (01.008 a 01.016; 01.020)
Room 15:
02. Neuropharmacology (02.008 a 02.017)
Room 16:
03. Psychopharmacology (03.001 a 03.006; 03.023 a 03.026)
Room 17:
03. Psychopharmacology (03.015 a 03.022)
Room 18:
04. Inflammation and Immunopharmacology (04.006 a 04.012)
05. Pain and Nociception Pharmacology (05.006 a 05.008)
Room 19:
04. Inflammation and Immunopharmacology (04.018 a 04.026)
Room 20:
06. Cardiovascular and Renal Pharmacology (06.001 a 06.006; 06.017 a 06.019)
Room 21:
06. Cardiovascular and Renal Pharmacology (06.020 a 06.029)
Room 22:
07. Endocrine, Reproductive and Urinary Pharmacology (07.001 a 07.002)
08. Respiratory and Gastrointestinal Pharmacology (08.001 a 08.007)
13. Pharmacology Education and Technology (13.002)
Room 23:
08. Respiratory and Gastrointestinal Pharmacology (08.008 a 08.010; 08.012 a 08.018)
Room 24:
09. Natural Products and Toxinology (09.009 a 09.017)
Room 25:
11. Clinical Pharmacology, Pharmacokinetics, Pharmacogenomics and Toxicology (11.001 a 11.003)
12. Drug Discovery and Development (12.001 a 12.006)
Room 26:
14. Pharmacology: Other (14.001 a 14.010)

08h00-09h00	Meeting of the North-Northeast and Central West Region Pharmacology Network (Google Meet)
09h05-10h05	Lectures
C8	Potassium Channels in Breast Cancer Development and Treatment Peter Ruth (University of Tübingen, Germany) Presented by: Eliezer Jesus de Lacerda Barreiro (UFRJ)
C9	Resolution (of Inflammation): Women do it better! Amrita Ahluwalia (The William Harvey Research Institute, UK) Presented by: Patrícia Machado Rodrigues e Silva Martins (Fiocruz)
10h10-12h10	Symposia/Oral Communication
S11	Specialized Pro-Resolution Lipid Mediators: Novel Non-Opioid Analgesics and Anti-Inflammatory Perspectives Chair: Daniela de Almeida Cabrini (UFPR) <ul style="list-style-type: none"> <i>Effects of pro-resolution lipid mediators in experimental diabetes-associated complications</i> Joice Maria da Cunha (UFPR) <i>Analgesic potential of Maresin 2 and Resolvin D5 in models of orofacial pain</i> Juliana Geremias Chichorro (UFPR) <i>Annexin A1 attenuates cardiac diastolic dysfunction in mice with arthritis</i> Jianmin Chen (Queen Mary University of London, UK) <i>Neuronal and non-neuronal effects of specialized pro-resolution lipids regulating pain, inflammation and neuroimmune interactions</i> Waldiceu A. Verri Jr (UEL)
S12	Targeting Inflammation in COVID-19 Chair: Thiago M Cunha (USP-SP) <ul style="list-style-type: none"> <i>Anti-inflammation and Covid therapy</i> Ivan Marazzi (Icahn School of Medicine at Mount Sinai, USA) <i>Platelets and infectious disease.</i> Patricia T Bozza (Fiocruz) <i>NETs and Covid-19</i> Fernando de Q. Cunha (USP-SP) Oral Communication 1: 06.038 <i>SARS-CoV-2-induced endothelial cell damage by mitochondrial DNA release and TLR9 activation.</i> Costa TJ¹, Potje SR¹, Fraga-Silva² TFC, Silva-Neto JA¹, Barros PB¹, Rodrigues D¹, Machado MR¹, Martins RB³, Santos-Eichler RA⁴, Bonato VLD², Arruda E³, Bomfim GF⁵, Tostes RC¹. ¹Dpt of Pharmacology, Ribeirão Preto Medical School, Univ of São Paulo – USP, Brazil ²Dpt of Biochemistry and Immunology, Ribeirão Preto Medical School, Univ of São Paulo – USP, Brazil ³Virology Research Center, Ribeirão Preto Medical School, Univ of São Paulo USP, Brazil ⁴Dpt of Pharmacology, Inst of Biomedical Science, Univ of São Paulo – USP, Brazil ⁵Inst of Biological and Health Sciences, Federal Univ of Mato Grosso – UFMT, Brazil Oral Communication 2: 01.006 <i>Investigation of the neuroprotective effects of cannabidiol and artesunate in an in vitro model of SARS-CoV-2 neuroinfection.</i> Pires-dos-Santos I¹, Costa KCM¹, Scomparin DS¹, Scarante FF¹, Martins-Júnior RB², Arruda-Neto E², Campos AC¹, Ferreira RR¹. ¹Dpt of Pharmacology, Medical School of Ribeirão Preto, Univ of São Paulo, Ribeirão Preto, Brazil; ²Dpt of Cell and Molecular Biology, Medical School of Ribeirão Preto, Univ of São Paulo, Ribeirão Preto, Brazil

MR2 (SBFTEJovem)	<p>Beyond the Academy (Presented in Portuguese) Chair: João Alfredo de Moraes (SBFTE Jovem, UFRJ)</p> <ul style="list-style-type: none"> • <i>Msl: Assignments and perspectives</i> Pedro Barcellos de Souza (Janssen-Cilag Farmacêutica) • <i>Forensics: Science for justice</i> Carina Maria Bello de Carvalho (SR/DPF/RS) • <i>Empowering Patients: Deconstructing the passive subject to build the active subject, protagonist of patient journey</i> Alessandra de Souza (Farmale)
12h10-13h10	Lunch
13h15-14h45	SBFTE Assembly (Google Meet)
15h00-16h00	Closing lecture
C10	<p><i>Sergio Ferreira Lecture</i> From Recruitment to resolution: an inflammatory tale of opportunities Mauro M. Teixeira (UFMG) Chair: Marco M. Teixeira (UFMG) Introduced by: Fernando de Q. Cunha (USP-SP)</p>
16h05-16h35	Closing Session
	Awards and Prize Announcements Closing Ceremony

Room 1:**01. Cellular and Molecular Pharmacology**

01.001 **Vascular reactivity, angiotensin receptor and intracellular signaling in *Oxyrhopus guibei* (false coral snake), and *Crotalus durissis terrificus* (rattlesnake).** da Silva ILMS, Marinho JL, Breno MC Butantan Inst

01.002 **Endothelial oxidative stress and antioxidant profile during schistosomiasis.** Monteiro MMLV, Valença SS, Silva CLM UFRJ, Inst of Biomedical Sciences, Pharmacology and Inflammation Research Program

01.003 **The P2X7 receptor agonist ATP induces prostate cancer cells adhesion to human endothelial cells** Rocha MA, Cardoso TC, Silva CLM Lab. de Farmacologia Bioquímica e Molecular, Inst de Ciências Biomédicas, CCS, UFRJ

01.004 **Gene expression of healing markers in human keratinocytes HaCat treated with a biomembrane derived from *Calotropis procera* (BioMemCpLP)** Duarte RS¹; Nunes, MO²; Rabelo LMA¹; Ferreira KQ²; Rangel GFP²; Alencar NMN²; Ramos MV³. ¹UFC, Dpt of Physiology and Pharmacology, Brazil; ²UFC, Fortaleza, PPG Pharmacology, Fortaleza, Brazil; ³UFC, Dpt of Biochemistry and Molecular Biology, Fortaleza, Brazil

01.005 **Acetylcholine alpha 7 nicotinic receptors in microglia: association of their microglial activity with increased intracellular calcium.** Bello Santos VG, Gonçalves de Castro N. ICB-UFRJ

01.006 **Investigation of the neuroprotective effects of cannabidiol and artesunate in an *in vitro* model of SARS-CoV-2 neuroinfection.** Pires-dos-Santos I¹, Costa KCM¹, Scomparin DS¹, Scarante FF¹, Martins-Júnior RB², Arruda-Neto E², Campos AC¹, Ferreira RR¹. ¹Dpt of Pharmacology, Medical School of Ribeirão Preto, Univ of São Paulo, Ribeirão Preto, Brazil; ²Dpt of Cell and Molecular Biology, Medical School of Ribeirão Preto, Univ of São Paulo, Ribeirão Preto, Brazil

01.007 ***In vitro* antineoplastic, anti-CD34 and anti-Ki67 effects of simvastatin in human acute lymphoblastic/lymphocytic leukemia and lymphoma cell lines.** Rotta TD, Nicolosi JS, Souza VB, Schenka AA FCM-Unicamp, Dpt of Pharmacology, Brazil

01.017 **MR/GR heterodimerization may impair glucocorticoids effects in LPS-induced inflammation depending on *in vitro* brain cellular composition.** Duque EA, Munhoz CD. ICB-USP, Dpt of Pharmacology, Brazil

01.018 **HDAC6/8 inhibitor, LASSBio 1911: a new alternative to sensitize prostate cancer cells to anticancer agents** Guerra FS¹, Rodrigues DA², Manssour Fraga CA², Dias Fernandes P¹ ¹Univ Federal do Rio de Janeiro, Inst de Ciências Biomédicas; ¹Lab de Farmacologia da Dor e da Inflamação e ²Lab de Avaliação e Síntese de Substâncias Bioativas (LASSBio). Rio de Janeiro, Brasil

01.019 **Analysis of intracellular peptides action on gene expression and their interaction with microRNAs related to energy metabolism and obesity.** Gewehr MCF¹ Eichler RAS¹ Carvalho EA² Oliveira V² Tersariol ILS³ Ferro ES¹ ¹Dpt of Pharmacology, Univ of São Paulo, Brazil ²Dpt of Biophysics, Federal Univ of São Paulo, Brazil ³Dpt of Biochemistry, Federal Univ of São Paulo, São Paulo, Brazil

Room 2:**02. Neuropharmacology**

02.001 **Evaluation of PTEN modulation on LPS-driven neuroinflammation.** Nagy GS¹, Kawamoto EMI². ICB-USP São Paulo, Dpt of Pharmacology, Brazil ¹Lab of Molecular and Functional Neurobiology, Dpt of Pharmacology, ²Inst of Biomedical Sciences, Univ of São Paulo, São Paulo, Brazil

02.002 **Respiratory anatomofunctional changes following apocynin treatment in a Parkinson's disease model.** Medeiros POS, Nascimento ALF, Pedrão LFAT, Oliveira LM, Takakura AC, Falquetto B. USP Inst of Biomedical Science, Dpt of Pharmacology, Brazil

02.003 **Sex differences in stress-related disorders: role of neurosteroids in PTSD, depression and anxiety.** Gazzi G, Almeida FB, Barros HMT UFCSPA, PPG Health Sciences, Brazil

02.004 **BEHAVSOFT® – Software for Scoring Animal Behavior Patent: BR 294091919042-3, May 2019.** Fiore R; Costa PA; Marostega F; Freese L; Nin MS; Barros HMT. Federal Univ of Health Sciences of Porto Alegre Neuropsychopharmacology Lab, Brazil

02.005 **Parkinson's disease treatment is not associated with changes on peripheral biomarkers of Fe metabolism.** Santos BN¹, Maes M², Bonifácio KL², Matsumoto AK², Brinholi FF², Melo LB², Moreira EG², Barbosa DS², Farias CC¹. ¹UFES, Vila Velha, Coordination of Biomedicine, Brazil; ²UEL, Graduation Program in Health Sciences, Londrina, Brazil

02.006 **Reduced adult hippocampal neurogenesis on Nitric Oxide knockout mice are not modified by escitalopram treatment.** Freire JB, Fernandes GG, Costa KMC, Scomparin DS, Guimarães FS, Campos AC Dpt of Pharmacology, Ribeirão Preto Medical School, Univ of São Paulo, Brazil

02.007 **Evaluation of the anticonvulsive potential of fatty acid amides from olive oil.** Monteiro VHMB¹, Silva WLG¹, Ferreira IM², Fujishima MAT¹, Oliveira FR¹. ¹UNIFAP Macapá, Dpt de Ciências Biológicas e da Saúde - Curso de Farmácia, Brasil; ²UNIFAP, PPG Ciências Farmacêuticas, Macapá, Brasil

Room 3

02. Neuropharmacology

02.019 **Neurochemical inter-hemispheric asymmetries and depression: a scoping review of the preclinical literature.** Almeida FB¹, Fiore RL¹, Gomez R², Nin MS^{1, 2}, Barros HMT¹. ¹PPG-CS-UFCSPA; ²PPG-FT-UFRGS

02.020 **Obesity impairs Na,K-ATPase activity in the cerebellum of female mice**

Lima GM¹, Leite JA², Ribeiro MR¹, De-Sá-Lima L¹, Donato J³, Tavares-de-Lima W¹, Scavone C¹. ¹USP São Paulo, Dept of Pharmacology, Brazil; ²UFG Goiânia, Dept of Pharmacology, Brazil; ³USP São Paulo, Dept of Physiology and Biophysics, Brazil

02.021 **Long-lasting behavioral consequences of social isolation and ethanol consumption during adolescence: effects of physical exercise.** Righi T, Zaniboni C, Favoretto CA, Morais I, Bertagna NB, Palombo P, Vrechi T, Engi S, Pereira GJDS, Cruz F Unifesp São Paulo, Dpt of Pharmacology, Brazil

02.022 **Stress susceptibility and response to fluoxetine in the learned helplessness model: involvement of the P2X7-NLRP3 inflammasome pathway.** Vieira L¹, Roncalho AL², Chiavegatto S³, Lisboa SF¹, Joca SRL^{1,4}. ¹BioMolecular Science Dpt, School of Pharmaceutical Sciences of Ribeirão Preto, ²Univ of São Paulo (USP), School of Medicine of Ribeirão Preto (USP), ³Pharmacology-Biomedical Sciences Inst and Psychiatry-Medical School (USP), ⁴Dpt of Biomedicine, Aarhus Univ, Denmark

02.023 **Andrographolide mitigates the cognitive impairment induced by an idiopathic alzheimer's disease model in rats.** Souza LC, Ramos DC, Andrade MK, Vital MABF. UFPR Curitiba, Dpt of Pharmacology, Brazil

02.024 **Phosphodiesterase-4 inhibitor Roflumilast improves anxiety-like behavior in type-1 diabetes mellitus likely through its protective function.** Waltrick APF¹, Chaves YC¹, Da Silva ACF¹, Prickaerts J², Oliveira RMMW³, Zanoveli JM¹. ¹UFPR Curitiba, Dpt of Pharmacology, Brazil; ²Maastricht Univ, School for Mental Health and Neuroscience, the Netherlands; ³UEM Maringá, Dpt of Pharmacology and Therapeutics, Brazil

02.025 **Pi3ky participates in the amnesic effect induced by scopolamine in mice.** Silveira DS, Scarante FF, Costa KCM, Guimarães FS, Campos AC Dpt of Pharmacology, Ribeirão Preto School of Medicine, Univ of São Paulo, Ribeirão Preto, Brazil

02.026 **Low-cost experimental apparatus for cigarette smoke exposure in rats.** Izolan LR¹, Bandiera S², Pulcinelli RR², Zilli GAL², Almeida FB³, Nin MS^{3,4}, Marques D¹, Leal MB^{1,2}, Gomez R^{1,2}. ¹UFRGS, PPG em Neurociências, Porto Alegre, Brazil; ²UFRGS, PPG em Farmacologia e Terapêutica, Porto Alegre, Brazil; ³UFCSPA, PPG em Ciências da Saúde, Porto Alegre, Brazil; ⁴FURG, Dept de Farmacologia, Rio Grande, Brazil

02.027 **Mineralocorticoid modulation in the infralimbic subregion of the medial prefrontal cortex (IL-mPFC) prevents the stress-induced impairment of aversive memory extinction.** Albernaz-Mariano, KA, Munhoz, CD USP-São Paulo, Dpt. de Farmacologia-ICB, PPG em Farmacologia, Brazil

Room 4

03. Psychopharmacology

03.007 **S-Ketamine effects on female mice tested in the Marble Burying Test.** Ayub JGM, Tosta CL, Beijamini V. UFES Vitória, PPG Pharmaceutical Sciences, Health Sciences Centre, Brazil

03.008 **Behavioral and biochemical effects of acute triazophos intoxication in young and adult rats** Vidigal, APP, Dos Santos, JG, Rodrigues, JVF, Minassa, VS, Batista, TJ, Sampaio, KN, Beijamini, V ¹UFES Vitória, PPG Pharmaceutical Sciences, Health Sciences Centre, Brazil

03.009 **Behavioral effects induced by the cannabidiol analogs HU-556 and HU-502.** Colodete, DAE¹; Silva, NR¹; Fogaça, MF¹; Pedrazzi, JF²; Del Bel, EA³; Mechoulam, R⁴; Gomes, FV¹; Guimarães, FS¹ ¹FMRP-USP, Dpt of Pharmacology, Brazil ²FMRP USP, Dpt of Neurosciences and Behavioral Sciences, Brazil ³FORP-USP, Dpt of Physiology, Brazil ⁴Hebrew Univ, Jerusalem, Israel

03.010 **Involvement of cannabinoid type 2 receptors in aversive memory processing in mice evaluated in the contextual fear conditioning paradigm.** Werworn LFM¹, Coelho AA², Galani, LC¹, Lisboa SFS^{1,2}. ¹FCFRP-USP Ribeirão Preto, Dpt of BioMolecular Sciences, Brazil; ²FMRP-USP Ribeirão Preto, Dpt of Pharmacology, Brazil

03.011 **The role of anandamide in the anxiety-like behavior in female rats.** Salemme BW, Raymundi AM, Sohn JMB, Stern CAJ. UFPR Curitiba, Dpt of Pharmacology, Brazil

03.012 **Studies using the forced swim test: an interim meta-analysis.** Martins, T^{1,2}, Lino de Oliveira, C^{1 2}. ¹UFSC Florianópolis, PPG in Pharmacology, Brazil; ²UFSC Florianópolis, Dpt of Physiological Sciences, Brazil

03.013 **Functional inactivation of basolateral amygdala does not alter context-induced reinstatement of alcohol-seeking in rats.** Tavares GEB, Bianchi PC, Yokoyama TS, Palombo P, Cruz FC. Unifesp, São Paulo, Pharmacology Dpt, Brazil

03.014 **TRPV1 antagonism associated with FAAH inhibition attenuated the impaired fear extinction recall induced by social isolation in mice.** Coelho AA^{1,2}, Werworn LFM¹, Lisboa SFS¹. ¹FCFRP-USP Ribeirão Preto, Dpt of BioMolecular Sciences, Brazil; ²FMRP-USP, Dpt of Pharmacology, Brazil

Room 5

04. Inflammation and Immunopharmacology

04.001 **Investigation of the anti-inflammatory mechanism of action of stigmasterol** Morgan LV¹, Petry F¹, Scatolin M¹, Oliveira PV², Alves BO¹, Zilli GAL¹, Volfe CRB¹, Oltramari AR¹, Oliveira D², Scapinello J¹, Müller LG¹. ¹Unochapecó Chapecó, Brazil; ²UFSC Florianópolis, Dpt of Chemical and Food Engineering, Brazil

04.002 **Effects of α -Klotho protein in TNF- α signaling through TNFR1 in mice.** Araujo de Souza G, Scavone C, Kinoshita PF. USP São Paulo, Dpt of Pharmacology, Brazil

04.003 **The acute inflammatory response is attenuated in elastase-2 knockout mice.** Dantas P, Mestriner F, Silva-Jr E, Becari C. USP Ribeirão Preto, Dpt of Surgery and Anatomy, Brazil ²UFRN Natal, Dpt of Biophysics and Pharmacology, Brazil

04.004 **Influence of COX-1 and COX-2 pathways on hemorrhage and tissue ischemia events during skeletal muscle degeneration and regeneration induced by a snake venom.** Correia MR¹, Han SW², Escalante T³, Moreira V¹. ¹Unifesp, Dpt of Pharmacology, Brazil; ²Unifesp. Dpt of Biophysics, Brazil; ³Universidad de Costa Rica, Inst Clodomiro Picado, SJCR

04.005 **Anti-inflammatory effect of the lipid-transferring protein, MCLTP1, isolated from noni seeds *Morinda citrifolia* L. (Rubiaceae) in mice burns model.**

Rabelo LMA¹, Kurita BM¹, Macedo FS¹, Rangel GFP¹, Souza TFG¹, Duarte RS¹, Costa AS², Oliveira HD², Alencar NMN¹. ¹UFC, Dpt of Physiology and Pharmacology, Biochemical Pharmacology Lab, Drug Research and Development Nucleus, Brazil; ²UFC, Dpt of Biochemistry, Medicinal Chemistry Lab, Drug Research and Development Nucleus, Brazil

05. Pain and Nociception Pharmacology

05.001 **Role of TRPA1 expressed in bone tissue and the antinociceptive effect of the TRPA1 antagonist repeated administration in a breast cancer pain model.** Mazzochi N¹, Almeida AS², Pereira GC², Brum ES³, Silva CR⁴, Antoniazzi CTD², Ardisson-Araújo D³, Oliveira SM³, Trevisan G². ¹Medical Graduate Student, Health Sciences Center, Federal Univ of Santa Maria, Santa Maria, Brazil ²Pharmacology Postgraduate Program, Health Sciences Center, Federal Univ of Santa Maria, Santa Maria, Brazil ³Biological Science: Toxicological Biochemistry Postgraduate Program, Biological Sciences, Federal Univ of Santa Maria, Santa Maria, Brazil ⁴Genetics and Biochemistry Postgraduate Program, Biotechnology Inst, Federal Univ of Uberlândia, Uberlândia, Brazil

05.002 **Evaluation of antinociceptive and anti-inflammatory activities of substituted synthetic chalcones LC24, LC31 and LC41 *in vivo*.** Melo EDN¹, Botinhão MC¹, Reis JVR¹, Souza ROMA², Leal ICR², Raimundo JM¹, Bonavita AGC¹, Muzitano MF¹, Carmo PL¹. ¹UFRJ Macaé, Brasil; ²UFRJ Rio de Janeiro, Brasil

05.003 **Evaluation of Vitex polygama extract and fractions effects on vincristine-induced peripheral neuropathic pain in mice.** Ramos IFO, Melo EDN, Santos IS, Carmo PL, Muzitano MF, Bonavita AGC. UFRJ - Campus Macaé, Bioactive Products Pharmacology Lab, Brazil

05.004 **Nonclinical investigation of the analgesic efficacy of gabapentin infiltration in a mice surgical pain model.** Martins F¹, Ferreira M², Schran R², Ferreira J². ¹Undergraduate student in Pharmacy, Federal Univ of Santa Catarina, Florianópolis, Brazil ²PPG in Pharmacology, Federal Univ of Santa Catarina, Florianópolis, Brazil

05.005 **Participation of the transient potential receptor ankyrin 1 (TRPA1) in facial mechanical allodynia induced by chronic administration of corticosterone.** Silva NAR¹, Dalenogare DP¹, Pereira GC¹, Bochi GV¹, Trevisan G¹. ¹UFMS Santa Maria, Dpt of Physiology and Pharmacology, Brazil

Room 6

04. Inflammation and Immunopharmacology

04.013 **Role of RAS in glucose metabolism and autophagy in metabolically active tissues from type 1 diabetic and obese mice.** Guimarães JPT^{1,2,3}, Menikdiwela KR², Ramalho T³, Queiroz LAD¹, Kalupahana NS^{2,4}, Jancar S³, Ramalingam L², Moustaid-Moussa N², Martins JO¹. ¹Lab of Immunoendocrinology, Dpt of Clinical and Toxicological Analyses. School of Pharmaceutical Sciences of Univ of São Paulo (FCF-USP), São Paulo, Brazil; ²Lab of Nutrigenomics, Inflammation and Obesity Research, Dpt of Nutritional Sciences, Texas Tech Univ (TTU), Lubbock, Texas, USA; ³Lab of Immunopharmacology, Dpt of Immunology, Inst of Biomedical Sciences, Univ of São Paulo (ICB-USP), São Paulo, Brazil; ⁴Dpt of Physiology, Univ of Peradeniya, Peradeniya, Sri Lanka

04.014 **Investigating mechanisms behind angiotensin, leukotrienes and insulin signaling in type 1 diabetes.** Guimarães JPT², Queiroz LAD¹, Jancar S², Moustaid-Moussa N³, Martins JO¹. ¹Lab of Immunoendocrinology, Dpt of Clinical and Toxicological Analyses. School of Pharmaceutical Sciences of Univ of São Paulo (FCF-USP), São Paulo, Brazil ²Lab of Immunopharmacology, Dpt of Immunology, Inst of Biomedical Sciences, Univ of São Paulo (ICB-USP), São Paulo, Brazil ³Lab of Nutrigenomics, Inflammation and Obesity Research, Dpt of Nutritional Sciences, Texas Tech Univ (TTU), Lubbock, Texas, USA

04.015 **Phenotypic and functional characterization of T lymphocytes of alloxan and streptozotocin induced diabetes.** Queiroz LAD¹, Guimarães JPT¹, Assis JB², Souza ESA¹, Milhomem AC³, Sunahara KKS⁴, Sá-Nunes A², Martins JO¹. ¹USP-FCF São Paulo, ²Dpt of Clinical and Toxicological Analyses, Brazil; USP-ICB São Paulo, Dpt of Immunology, Brazil; ³UFG-IPTSP Goiania, Dpt of Microbiology, Immunology, Brazil; USP-FM São Paulo, ⁴Dpt of Sciences-Experimental Physiopathology, Brazil

05. Pain and Nociception Pharmacology

05.009 **The neuroinflammatory process and TRPA1 role in two multiple sclerosis mouse models.** Dalenogare PD¹, Trevisan G¹, Araújo DSM², Landini L², Titiz M², De Logu F², Nassini R², Geppetti P². ¹UFSC Santa Maria, PPG Pharmacology, Brazil; ²UNIFI Firenze, Dpt Health Sciences, Italy

05.010 **The CB2 receptor agonist beta-caryophyllene attenuates oxaliplatin-induced peripheral neuropathy in a tumor-bearing mice model.** Agnes JP¹, Neves RN¹, Gonçalves RM¹, Delgobo M¹, Silva RC², Ferreira AR², Senna EL², Zanotto-Filho A¹. ¹UFSC Florianópolis, PPG Pharmacology, Dpt of Pharmacology, Brazil; ²UFSC Florianópolis, PPG Pharmacy, Dpt of Pharmaceutical Sciences, Brazil

05.012 **Benefits of hydrogen sulfide-releasing non-steroidal anti-inflammatory drug, ATB-352, on postoperative pain and gastric safety: role of endocannabinoid system** Dallazen JL¹, Santos LG¹, Teixeira SA¹, Muscará MN¹, Wallace JL^{2,3}, Costa SKP¹; ¹Dpt of Pharmacology, Inst of Biomedical Sciences, Univ of São Paulo; ²Antibe Therapeutics, Inc., Toronto, Canada; ³Dpt of Physiology and Pharmacology, Univ of Calgary

05.014 **Succinate/sucnr1 signaling in non-NaV1.8+ nociceptors drive paclitaxel-induced neuropathic pain.** Gomes FIF, Kusuda R, Silva CEA, Guimarães RM, Silva NR, Carmo BR, Mendes AS, Paiva IM, Oliveira AER, Lopes AHP, Alves-Filho JCF, Cunha FQ, Cunha TM Dpt of Pharmacology, Ribeirão Preto Medical School, Univ of São Paulo

05.015 **Aldehyde dehydrogenase-2 is a critical enzyme involved in 4-hydroxy-2-nonenal-induced pain: role of protein kinase C ϵ .** Martins BB¹, Zambelli VO^{1,2}, ¹Lab. Especial de Dor e Sinalização, Butantan Inst, Brazil, ²Dep. de Anestesia, Univ de Stanford, EUA

Room 7

04. Inflammation and Immunopharmacology

04.027 **Dissecting the mechanisms involved in differential responses and outcomes of identical mice to sepsis.** Cebinelli GCM, Nascimento DCB, Damasceno LEA, Tavares AC, Donate PB, Cunha TM, Farias JC, Cunha FQ. Center for Research in Inflammatory Diseases (CRID) - Dpt of Pharmacology, Ribeirão Preto Medical School Univ of São Paulo São Paulo, Brazil

04.028 **Treatment with the flavonoid quercetin accelerates resolution of lung changes caused by silica particles in mice.** Guimarães FV¹; Ferreira TPT¹; Arantes ACS¹; Jannini-Sá YAP¹; Silva CD¹; Moraes JA²; Carvalho VF¹; Martins MA¹; Silva PMR¹; ¹Lab de Inflamação, Inst Oswaldo Cruz/Fiocruz; ²Labi Redox, UFRJ; RJ - Brazil

04.029 **Therapeutic treatment with gold nanoparticles suppresses lung fibrosis in silica-challenged mice.** Ribeiro NBS¹, Janinni-Sá YAP¹, Arantes ACS¹, Chaves AS¹, Pelajo-Machado M², Silva-Aguiar RP³, Peruchetti DB³ Caruso-Neves C³, Santos-Oliveira R⁴, Martins MA¹, Silva PMR¹ ¹Lab of Inflammation; ²Lab of Pathology, Oswaldo Cruz Inst; ³Lab of Biochemistry and Cellular Signalling, UFRJ; ⁴Inst of Nuclear Engineering, RJ, Brazil

04.030 **STING is an intrinsic checkpoint inhibitor that restrains the TH17 Cell Pathogenic Program.** Damasceno LEA¹, Cebinelli GCM¹, Oliveira SC², Cunha TM¹, Cunha FQ¹, Alves-Filho JC¹. ¹Center for Research on Inflammatory Diseases, Dpt of Pharmacology, Ribeirão Preto Medical School, Univ of São Paulo, Ribeirão Preto, Brazil; ²Inst of Biological Sciences, Dpt of Biochemistry and Immunology, Federal Univ of Minas Gerais, Belo Horizonte, Brazil

04.031 **Lipopolysaccharide (LPS)-induced inflammation on proximal and distal epididymis differentially affects sperm motility parameters.** De Andrade AD, da Silva AA, Kushima H, Silva EJR. Unesp, Dpt of Biophysics and Pharmacology, Botucatu, Brazil

04.032 **Glucagon prevents airway hyperreactivity, inflammation and remodeling induced by ovalbumin in a murine model of asthma.** Insuela DBR¹, Azevedo CT¹, Coutinho DS¹, Magalhães NS¹, Ferrero MR¹, Ferreira TPT¹, Cascabulho CM², Pons AH², Martins PMRS¹, Martins MA¹, Carvalho VF¹ ¹Lab of Inflammation, Oswaldo

Cruz Inst, Oswaldo Cruz Foundation (Fiocruz), Rio de Janeiro, Brazil; ²Lab of Innovations in Therapies, Education and Bioproducts, Oswaldo Cruz Inst, Oswaldo Cruz Foundation (Fiocruz), Rio de Janeiro, Brazil

04.033 **Low birth weight induced by maternal food restriction decreases lipogenesis activity, in mesenteric adipocytes.** Andreoti S^{1,2}, Reis GB², Komino ACM², Silva FF², Gil NL¹, Azevedo GA¹, Ramos APA¹, Balbino AM¹, Sertié RAL², Lima FB², Landgraf RG¹, Landgraf MA³. ¹Dpt of Pharmaceuticals Sciences, Univ Federal de São Paulo - campus Diadema, Diadema, Brazil; ²Dpt of Physiology, Univ de São Paulo, São Paulo, Brazil; ³Univ Paulista - campus Rangel, Santos, Brazil

04.034 **Nebulized gold nanoparticles down-regulate inflammation and lung remodeling in a murine model of steroid-resistant asthma** Cotias, AC¹, Serra, MF¹, Pimentel, AS¹, Lanzetti, M², Hickmann, J³, Arante, ACS¹; Silva, PMR¹; Cordeiro, RSB¹, Barreto, E⁴, Martins, MA¹ ¹Lab of Inflammation, Oswaldo Cruz Inst, Fiocruz, Brazil; ²Lab of Cell Biology, Federal Univ of Alagoas, Maceió, AL, Brazil; ³Inst de Física, Univ Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil; ⁴Univ Federal do Rio de Janeiro (UFRJ), Inst de Ciências Biomédicas, Rio de Janeiro, Brazil

04.035 **Role of toll-like receptor (TLR) 3 in the fibrosis triggered by silica particles in the lungs of mice.** Jannini-Sá, YAP¹, Ferreira TPT¹, Ribeiro, NBS¹, Guimarães, FV¹, Souza LM¹, Alves-Filho, JC², Hogaboam, CM³, Martins, MA¹, Silva PMR¹. ¹Lab of Inflammation - Oswaldo Cruz Inst, Brazil, ²Lab of Inflammation and Pain FMRP-USP, Brazil, ³Cedars-Sinai Medical Center Medicine Dpt Los Angeles, CA, USA

04.036 **Effect of H1N1 viral infection on lung fibrosis induced by silica particles in mice.** Ferreira TPT¹, Arantes, ACS¹, Jannini-Sá YAP¹, Hogaboam CM², Martins MA¹, Silva PMR¹ ¹Lab of Inflammation, Oswaldo Cruz Inst-Fiocruz, RJ, Brazil, ²Cedars Sinai Medical Center, LA, USA

04.037 **Lantana canescens (Kunth) inhibits inflammatory responses in murine models** Lencina JS¹; Moslaves ISB¹; Muller JAI¹; Alves FM³ Silva DB²; Toffoli-Kadri MC¹ ¹Lab of Pharmacology and Inflammation, FAFAN-Federal Univ of Mato Grosso do Sul, Campo Grande, Brazil ²Lab of Natural Products and Mass Spectrometry, FAFAN-Federal Univ of Mato Grosso do Sul, Campo Grande, Mato Grosso do Sul, Brazil ³Lab of Botany, INBIO/Federal Univ of Mato Grosso do Sul, Campo Grande, Brazil

Room 8

06. Cardiovascular and Renal Pharmacology

06.007 **Evaluation of the cardiovascular effects of new acilhydrazone derivatives in the acute phase of myocardial infarction.** Carlos-Nascimento B¹, da Silva JS¹, Beltrame F^{1,2}, Montagnoli TL¹, Rocha BS¹, Maia RC¹, Barreiro EJ¹, Zapata-Sudo G.^{1,2} ¹ICB-UFRJ, Rio de Janeiro, Brazil ²ICES-UFRJ, Rio de Janeiro, Brazil

06.008 **Cilostazol exerts positive effects on cardiovascular system of Wistar rats exposed to doxorubicin.** Autran LJ¹, Brazão SC¹, Lima GF¹, Mendes ABA², Lopes RO¹, Motta NAV¹, Brito FCF¹. ¹ Lab de Farmacologia Experimental - LAFE, Univ Federal Fluminense. ²Grupo de Pesquisa, Inovação e Desenvolvimento em Endocrinologia Experimental, Univ Federal do Rio de Janeiro, RJ

06.009 **Adjuvant-induced arthritis causes contractile dysfunction in rat aorta by a mechanism related to Nitric Oxide.** Araújo TS¹, Spadella MA², Tirapelli CR³, Pinheiro JCD⁴, Chies AB¹. ¹FAMEMA Marília, Dpt of Pharmacology, Brazil; ²FAMEMA Marília, Dpt of Human Embriology, Brazil; ³USP Ribeirão Preto, Dpt of Cardiovascular Pharmacology, Brazil; ⁴FAMEMA Marília, Dpt of Pharmacology, Brazil

06.010 **Preventive effects of kefir in the behavioral and renal changes induced by chronic unpredictable stress in mice.** Silva AO¹, Ribeiro JM¹, Amorim GE¹, Pereira-Junior AA¹, Ângelo ML¹, Torres LHL¹, Paula FBA², Leitão SGR³, Dias MVS⁴, Ceron CS⁵. ¹Unifal Alfenas, Dpt of Food and Drugs; ²Unifal Alfenas, Dpt of clinical and toxicological analysis; ³Unifal Alfenas, Dpt of Physiological Sciences; ⁴Unifal Alfenas, Nature science institute; UFOP Ouro Preto, Dpt of biological Sciences

06.011 **Nox5 contributes to vascular hyperreactivity associated with pre-eclampsia.** Barbosa NC¹, Machado MR², Alves JV², Oliveira Neto JT², Silva JF², Cavalli RC³, Passaglia RCAT², Costa RM². ¹UFJ, Jataí Academic Unit of Health Sciences, Federal Univ of Jataí, Brazil ²FMRP USP Ribeirão Preto, Dpt of Pharmacology,

Ribeirão Preto Medical School, Univ of São Paulo, Brazil ³FMRP USP Ribeirão Preto, Dpt of Gynecology and Obstetrics, Ribeirão Preto Medical School, Univ of São Paulo, Brazil

06.012 **Chronic ethanol consumption induces oxidative stress and NLRP3 activation in thoracic aorta via mineralocorticoid receptor activation.** Dourado TMH, Assis VO, Awata WMC, Tirapelli CR Lab. of Pharmacology, EERP-USP, Ribeirão Preto, Brazil

06.013 **Phenotypical and pharmacological differences evoked by *in vitro* aging of LLC-PK1 proximal tubule renal cells.** Barros GMO¹, Silva AAC², Quintas, LEM³, Inst of Biomedical Sciences, Federal Univ of Rio de Janeiro, Brazil

06.014 **Blood pressure effects in male and female rats due to antidepressant use: A systematic review and pilot meta-analysis.** Santos TM, Linder AE. UFSC - PPG Pharmacology – Florianópolis, Brazil

06.015 **Serum from COVID-19 patients decreases endothelial cell antioxidant defense via downregulation of the Nrf2 transcriptional factor.** Rodrigues D¹, Machado MR¹, Costa RM^{1,2}, Tostes RC¹, ¹Dpt of Pharmacology, Ribeirão Preto Medical School, Univ of São Paulo, Brazil, ²Academic Unit of Health Sciences, Federal Univ of Jatai, Brazil

06.016 **Evaluation of endothelial function in vascular reactivity and antioxidant systems in mice with chronic kidney disease.** Garlet TC¹, Moecke DMP¹, Martins GHC², Probst JJ², Hahmeyer MLS³, Dafre AL², da Silva-Santos JE³, Hizume-Kunzler DC¹. ¹UFSC Florianópolis, Dpt de Fisioterapia, Brazil; ²UFSC Florianópolis, Dpt de Neurociências, Brazil; ³UFSC Florianópolis, Dpt de Farmacologia, Brazil

Room 9

06. Cardiovascular and Renal Pharmacology

06.030 **PDE3 inhibition in an experimental model of sepsis.** Oliveira JG¹, Sordi R¹, Amarantes ELA¹, Oliveira MRP², Fernandes D¹ ¹UFSC Florianópolis, Dpt of Pharmacology, Brazil; ²UEPG Ponta Grossa, Dpt of Structural Biology, Molecular, and Genetic, Brazil

06.031 **Evaluation of the role of gastroenteric xanthine oxidoreductase in the hypotensive effect of sodium nitrite.** Medeiros CFA, Neves EMN, Santana IV, Lopes JMS, Nogueira RC, Tanus-Santos JE Lab of Cardiovascular Pharmacology, Univ of São Paulo, Ribeirão Preto, Brazil

06.032 **NONO2P, a new nitric oxide donor, induce vasorelaxation in superior mesenteric artery from rats.** Moraes RA^{1,2}, Araújo FA^{1,2}, Jesus RLC¹ Silva LB¹, Meira CS², Capinan Filho JWS², Soares, MBP², Sá DS³, Silva CDS³, Silva DF^{1,2} ¹Lab of Cardiovascular Physiology and Pharmacology, Bioregulation Dpt, Federal Univ of Bahia (UFBA), Salvador, Bahia, Brazil ²Gonçalo Moniz Inst, Fiocruz, Salvador, BA, Brazil ³Federal Inst of Bahia, IFBA, Salvador, BA, Brazil

06.033 **Bariatric surgery impairs the nitrate-nitrite-NO pathway and prevents the beneficial cardiovascular effects of nitrate-rich beetroot extract supplementation.** Sanches-Lopes JM, Barros AC, Tanus-Santos JE Dpt of Pharmacology, Ribeirão Preto Medical School, Univ of São Paulo, Ribeirão Preto, Brazil

06.034 **Acute chikungunya infection induces vascular dysfunction related to changes in ROS/NF-κB/iNOS/NO pathways.** ¹Dpt of Pharmacology, Ribeirão Preto Medical School, Univ of São Paulo, Ribeirão Preto, Brazil ²Dpt of Cellular and Molecular Biology and Pathogenic Bioagents, Ribeirão Preto Medical School, Univ of São Paulo, Ribeirão Preto, Brazil ³Special Academic Unit of Health Sciences – Federal Univ of Goiás, Jatai, Brazil

06.035 **Temporal changes of renal and hepatic protein SUMOylation in hemorrhagic shock.** Oliveira FRMB, Soares ES, Ramos HP, Cimarosti HI, Sordi R. UFSC, Dpt of Pharmacology, PPG Pharmacology

06.036 **Post-occlusive reactive hyperemia in the skeletal muscle of rats at model of adjuvant-induced arthritis.** Santos WP, Souza-Silva E, Dornelles FN, Tonussi CR. UFSC Florianópolis, PPG Pharmacology, Brazil

06.037 **Acute kidney injury in Wistar rats experimentally envenomated with *Bothrops jararacussu* venom.** Romanelli MA¹, Soeiro PA¹, da Silva RC¹, Taveira-da-Silva R², Melo PA¹, Lara LS¹ ¹PPG em Farmacologia e Química Medicinal, Inst de Ciências Biomédicas, Univ Federal do Rio de Janeiro, Brazil ²Inst de Biofísica Carlos Chagas Filho, Univ Federal do Rio de Janeiro, Rio de Janeiro, Brazil

06.038 **SARS-CoV-2-induced endothelial cell damage by mitochondrial DNA release and TLR9 activation.** Costa TJ¹, Potje SR¹, Fraga-Silva² TFC, Silva-Neto JA¹, Barros PB¹, Rodrigues D¹, Machado MR¹, Martins RB³, Santos-Eichler RA⁴, Bonato VLD², Arruda E³, Bomfim GF⁵, Tostes RC¹. ¹Dpt of Pharmacology, Ribeirão Preto Medical School, Univ of São Paulo, Brazil ²Dpt of Biochemistry and Immunology, Ribeirão Preto Medical School, Univ of São Paulo, Brazil ³Virology Research Center, Ribeirão Preto Medical School, Univ of São Paulo, Brazil ⁴Dpt of Pharmacology, Inst of Biomedical Science, Univ of São Paulo, Brazil ⁵Inst of Biological and Health Sciences, Federal Univ of Mato Grosso, Brazil

06.039 **Effect of Paroxetine for the treatment of sepsis.** Galant LS¹, Borges V¹, Rodrigues FC¹, Kanashiro A¹, Monteiro V¹, Cebinelli GM¹, Duarte DA², Costa-Neto C², Pupo AS³, Cunha FQ¹ ¹Faculdade de Medicina de Ribeirão Preto, Univ de São Paulo – USP – Dpt. de Farmacologia Ribeirão Preto (SP) - Brasil. ²Faculdade de Medicina de Ribeirão Preto, Univ de São Paulo, Dpt de Bioquímica, Ribeirão Preto, Brasil. ³Univ Estadual Paulista, Botucatu – Dept de Farmacologia, Inst de Biociências SP- Brasil

Room 10

07. Endocrine, Reproductive and Urinary Pharmacology

07.003 **Food supplementation with *Spirulina platensis* prevents strength training-induced oxidative stress on rat uterus.** Lacerda-Júnior FF², Ferreira PB², Diniz AFA², Silva, MCC², Silva AS³, Silva, BA^{1,2}. ¹DCF-CCS-UFPB, ²PPgPNSB-CCS-UFPB, ³PAPGEF-UFPB

07.004 **Analysis of phenotypic and metabolic changes in neurolysin knockout animals in a diet-induced obesity model.** Caprioli B, Gewehr MCF, Eichler RAS, Ferro ES Dept de Farmacologia, Inst de Ciências Biomédicas, Univ de São Paulo

07.005 **Cinnamaldehyde action on muscular glucose uptake and insulin resistance.** Roriz RNS¹, Sulis PM², Padilla DPR², Padilla DPR², Alencar NMN¹, Silva FRMB², Frederico MJS¹. ¹UFC Fortaleza, Dpt de Fisiologia e Farmacologia, Brasil; ²UFSC Florianópolis, Dpt de Bioquímica, Brasil

07.006 **New insights into the bladder dysfunction caused by long-term methylglyoxal intake: reversal by metformin.** Oliveira AL, Medeiros ML, Oliveira MG, Mônica FZ, Antunes E Dept de Farmacologia, Faculdade de Ciências Médicas, Univ de Campinas, Campinas, Brasil

07.007 **Are the anti-inflammatory and metabolic effects of mometasone furoate dependent on glucocorticoid receptor activation?** Zimath PL^{1,2}, Almeida M², Rafacho A^{1,2}. ¹UFSC Florianópolis, PPG Pharmacology, Brazil; ²UFSC Florianópolis, Lab. of Investigation of Chronic Disease, Brazil

07.008 **Targeting the new H₂S-releasing ketoprofen derivative for visceral pain and cystitis: A potential therapeutic approach.** Santos LG¹, Teixeira SA¹, DE Oliveira MG², Dallazen JL¹, Oliveira JP¹, Mônica FT², Wallace J^{3,4}, Muscará MN¹, Antunes E², Costa SKP¹. ¹USP, Dpt of Pharmacology, Brazil; ²UNICAMP, Dpt of Pharmacology, Brazil; ³Antibe Therapeutics, Inc., Toronto, Canada; ⁴Univ of Calgary, Dpt of Physiology and Pharmacology, Canada

07.009 **Are muscarinic receptors and EGF involved in sperm activity?** Gontijo LS¹, Moreira TJ¹, Corrêa-Ramos TL¹, Ribas JAS¹, Porto CS², Maróstica, E¹. ¹Lab of Experimental Pharmacology, UFF-Niterói, RJ; ²Experimental Endocrinology, Unifesp-SP, Brazil

07.010 **Intrauterine and lactational exposure of male rats to supraphysiological doses of manganese: short-term reproductive and development toxicity.** Silva APG¹, Correia MH², da Silva LN¹, Santiago MSA¹, Perobelli JE¹. ¹Dpt de Ciências do Mar, PPG Interdisciplinar em Ciências da Saúde ^{1,2}Unifesp, Brasil

07.011 ***Spirulina platensis* reverses damage to erectile function *in vivo* and oxidative stress in obese rats fed a hypercaloric diet.** Diniz AFA¹, Souza ILL³, Ferreira ES¹, Ferreira PB¹, Barros BC¹, Lacerda-Júnior FF¹, Silva MCC¹, Silva BA^{1,2} ¹UFPB-PPgPNSB, ²DCF-CCS-UFPB, ³UERR-DCM

07.012 **NADPH oxidase but not uncoupled eNOS is the source of ros in murine experimental cystitis.** Oliveira MG¹, Mónica FZ¹, Passos GR¹, Victorio JA², Davel AP², Silva FH³, Antunes E¹ ¹Dpt of Translation Medicine (Pharmacology Area), Faculty of Medical Sciences, Univ of Campinas. ²Dpt of Structural and Functional Biology, Inst of Biology, Univ of Campinas. ³Hematology and Hemotherapy Center, Univ of Campinas

13. Pharmacology Education and Technology

13.001 **A patent landscape of pregnancy treatments.** Pereira KV¹, Pacheco CO², Haas SE^{1 2}. ¹PPG in Pharmaceutical Sciences, UFSM, Santa Maria, Brazil ²PPG- Unipampa in Pharmaceutical Sciences, Uruguaiiana, Brazil

Room 11

09. Natural Products and Toxinology

09.001 **Short and Long-term effects of *Salacia impressifolia* on management of hyperglycemia for glucose homeostasis.** Furtado IP¹, Da Luz G², Altenhofen D², Ruani AP³, Pizzolatti MG³, Silva FRMB², Frederico MJS¹. ¹UFC Fortaleza, Dpt de Fisiologia e Farmacologia, Brasil; ²UFSC Florianópolis, Dpt de Bioquímica, Brasil; ³UFSC Florianópolis, Dpt de Química, Brasil

09.002 **Amazonian guaraná and açai: conjugated extract improves the healing of *Eisenia fetida* submitted to tail-amputation.** Bonotto NCA¹, Felin FD¹, Maia-Ribeiro EA², Barbisan F³, Felin CD⁴, Teixeira CF¹, Roggia I¹, Turra BO¹, da Cruz IBM¹.¹UFSM Santa Maria, Dpt of Morphology, Brazil;² FUnATI Manaus, Brazil; ³UFSM Santa Maria, Dpt of Patology, Brazil; ⁴UFN Santa Maria, Dpt of Medicina, Brazil

09.003 **Methyl cinnamate suppress migration and induce cell cycle arrest at S phase in NIH 3T3 fibroblasts.** Barros A, Ferreira E, Aquino F, Silva J, Carmo J, Barreto E UFAL, Lab of Cell Biology

09.004 **Methyl cinnamate inhibits migration and inflammatory response in A549 human epithelial cells.** Ferreira E, Barros A, Nascimento L, Silva J, Carmo J, Barreto E. UFAL, Lab of Cell Biology

09.005 **Blockade of calcium channels in the vasorelaxant effect of (E)-N-(4-metoxifenetil)-3-(tiofen-2-il)acrilamid in Wistar rats.** Moura TMC¹, Silva ARLFC², Pessoa RF², Fernandes JM¹, Silva LAA³, Rodrigues LC⁴, Cavalcante FA^{2,5} ¹PIBIC-UFPB, João Pessoa, Brazil; ²PPgPNSB-UFPB João Pessoa, Brazil; ³PPgDITM-UFPB João Pessoa, Brazil, ⁴UFPB João Pessoa, Dpt of Biotechnology, Brazil, ⁵UFPB João Pessoa, Dpt of Physiology and Pathology, Brazil

09.006 **Screening of oleanolic acid and its derivatives for monoamine oxidase inhibitory activity.** Cabral IB¹, Martins JLR¹, Pedrino GR¹, Costa EA¹, Fajemiroye JO¹. ¹UFG, Dpt de Farmacologia, PPG Ciências Biológicas, Brasil

09.007 **Counteracting action of *Coutarea hexandra* (Rubiaceae) stem bark extract on the systemic toxicity induced by *Lachesis muta muta* (Viperidae: Crotalinae) venom in rats.** Torres AGL¹, Moraes AM¹, Moraes-Santos LS¹, Sales-silva MS¹, Santarém CL², Nogueira RM², Giuffrida R², Silva EO², Gerez JR³, Santos MG⁴, Silva-junior NJ⁵, Pilon GD⁶, Oshima-franco Y⁶. Floriano RS¹ ¹Lab of Toxinology and Cardiovascular Research, PPG in Health Sciences, Univ of Western São Paulo, Presidente Prudente, Brazil; ²PPG in Animal Science, Univ of Western São Paulo, Presidente Prudente, Brazil; ³Dpt of Histology, State Univ of Londrina, Londrina, Brazil; ⁴PPG in Environmental Sciences, Tocantins Federal Univ, Palmas, Brazil; ⁵PPG in Environmental Sciences and Health, School of Medical, Pharmaceutical and Biomedical Sciences, Pontifical Catholic Univ of Goiás, Goiânia, Brazil; ⁶PPG in Pharmaceutical Sciences, Univ of Sorocaba, Sorocaba, Brazil

09.008 **Antinociceptive activity of gamma terpinene in rats with chemotherapy-induced peripheral neuropathy: Possible involvement of opioid system and KATP channels.** Reis Filho AC, Acha BT, Pinheiro Neto FR, Bandeira SRM, Gomes LS, Lopes EM, Almeida, FRC. Medicinal Plants Research Center, Federal Univ of Piau, Teresina, Brazil

Room 12

10. Cancer Pharmacology

10.001 **Simulation of pharmacokinetic parameters of an antitumor prototype LOE420 with the aid of the Certara SimCyp® program.** Manso MP¹; Pereira JVM¹; Carvalho GGC¹; Vieira-Neto JB¹; Sales SLA¹; Costa PMS¹; Guimarães CJ^{1,2}; Miranda-Furtado CL¹; Pessoa C¹. ¹UFC Fortaleza, Dpt of Physiology and Pharmacology. ²Amazonas State Foundation Center of Oncology Control

10.002 **Simulation of tissue partition coefficients (Kp) of LOE420 antitumor prototype, with the aid of the Certara SimCyp® program.** Pereira JVM¹; Manso MP¹; Carvalho GGC; Vieira-Neto JB¹; Sales SL¹; Costa PMS¹; Guimarães CJ²; Miranda-Furtado CL¹; Pessoa C¹ ¹UFC Fortaleza, Dpt of Physiology and Pharmacology, Brazil ²Amazonas State Foundation Center of Oncology Control, Manaus, Brazil

10.003 **Evaluation of the antineoplastic activity of pentacyclic triterpene Friedelin in mice bearing Ehrlich's ascites carcinoma.** Silva ELES, Santos DLF, Almeida JH, Souza TPM¹, Silva LMP, Barreto E, Ferro JNS. Federal Univ of Alagoas (UFAL), Maceió, AL, Brazil

10.004 **Antiproliferative effects of telocinobufagin on human colorectal adenocarcinoma.** Godoy TM¹, Godoy TM¹, Lopes BJ², Castelo-Branco MTL², Moraes JA³, Quintas LEM¹ ¹Lab of Pharmacology and Molecular Biochemistry, ICB - Federal Univ of Rio de Janeiro, Brazil ²Lab of Immunology, ICB - HUCFF - Federal Univ of Rio de Janeiro, Brazil ³LABIO-Redox, ICB - Federal Univ of Rio de Janeiro, Brazil

10.005 **CD39 inhibition enhance temozolomide effect in non-sensibile glioma cells.** Scheffel TB¹, Merino BM¹, Kist LW², Bogo MR², Rockenbach L¹, Morrone FB¹. ¹PUCRS, Porto Alegre, Lab de Farmacologia Aplicada, Escola de Ciências da Saúde e da Vida, Brazil ²PUCRS, Porto Alegre, Lab de Biologia Genômica e Molecular, Escola de Ciências da Saúde e da Vida, Brazil

10.006 **Co-encapsulation of 5-fluorouracil in multiple nanoemulsions containing short chain triglycerides improves drug cytotoxicity against colorectal cancer cells.** Fukumori C, Branco PC, Lopes LB. ICB-USP - Dpt of Pharmacology, Brazil

10.007 **Effects of soluble polysaccharide fraction in the cytochrome P450-inflammation-cancer triad.** Stipp MC¹, Kulik JD², Galindo CM¹, Corso CR³, Adami ER¹, Nardin JM⁴, Ioshii S⁴, Winnischofer SMB², Sasaki GL², Cadena SMSC², Acco A¹ ¹Dpt of Pharmacology, Federal Univ of Paraná, Curitiba, PR, Brazil ²Dpt of Biochemistry and Molecular Biology, Federal Univ of Paraná, Curitiba, Brazil ³Pelé Pequeno Principe Research Inst ⁴Erasto Gaertner Hospital, Curitiba, Brazil

10.008 **Thioredoxin reductase-1 levels are associated with NRF2 pathway activation and tumor recurrence in non-small cell lung cancer.** Delgobo M¹, Gonçalves RM¹, Delazeri MA², Falchetti M¹, Zandoná A², Nascimento Das Neves R¹, Almeida Lima K¹, Fagundes AC¹, Isidro Fracasso J³, Baroni Macedo G³, Priori L³, Pens Gelain D⁴, Forcelli CM², Fonseca Moreira JC⁴, Zanotto-Filho A¹. ¹UFSC Florianópolis, Dpt of Pharmacology, Brazil; ²UPF, Passo Fundo, Dpto of Medicine, Brazil; ³HSVP, Passo Fundo, Brazil; ⁴UFRGS, Porto Alegre, Dpt of Biochemistry, Brazil

10.009 **Extracellular vesicles released by adipose tissue from obese subjects can induce epithelial-mesenchymal transition in breast cancer cells.** Ramos-Andrade I¹, De Jesus ME¹, Moraes JA^{1,2}, Renovato Martins M³, Barja-Fidalgo C¹ ¹UERJ Rio de Janeiro, Dpt of Cell Biology, Brazil; ²UFRJ Rio de Janeiro, Dpt of Basic and Clinical Pharmacology, Brazil; ³UFF Niterói, Dpt of Molecular and Cell Biology, Brazil

Room 13

11. Clinical Pharmacology, Pharmacokinetics, Pharmacogenomics and Toxicology

11.004 **Population pharmacokinetic model of Ceftaroline distribution to muscle and subcutaneous tissue of healthy subjects and cerebrospinal fluid of neurosurgical patients.** Helfer VE¹, Zeitlinger M², Zavaski A³, Verlindo de Araújo B¹, Dalla Costa T¹. ¹UFRGS, PPG Pharmaceutical Sciences, Brazil; ²Medical Univ of Vienna, Dpt of Clinical Pharmacology, Austria; ³Hospital de Clínicas de Porto Alegre, Brazil

11.005 **Pharmacogenetic testing in psychiatry and cardiology: strategies for obtaining robust scientific data, and for building overview of systematic reviews**

Lara DVD¹, Melo DOD², Silva RAM², Xavier DDS², Tonin SA², Gonçalves TS¹, Santos PCJL¹. ¹Unifesp São Paulo, Dpt of Pharmacology, Brazil; ²Unifesp Diadema, Dpt of Pharmaceutical Sciences, Brazil

11.006 **Transgenerational reproductive effects (F2) in male offspring mediated by F0 generation exposure to Benzo(A)Pyrene from juvenile period to peripuberty in rats.** Jorge BC¹, Reis ACC¹, Stein J¹, Paschoalini BR¹, Nogueira JB¹, Moreira SS¹, Manoel BM¹, Arena AC^{1,2}. ¹Dpt of Structural and Functional Biology, PPG Pharmacology and Biotechnology, Inst of Biosciences of Botucatu, Univ. Estadual Paulista-Botucatu, Brazil ²Center of Toxicological Assistance, Inst of Biosciences of Botucatu, Univ. Estadual Paulista-Botucatu, São Paulo, Brazil

12. Drug Discovery and Development

12.007 **Targeting myeloperoxidase ameliorates edema in a gouty arthritis model: discovery of inhibitors with biological activity through virtual screening.** Matos IDA¹, Dallazen JLD², Costa SKP², Meotti FCM¹. ¹Dpt of Biochemistry, Inst of Chemistry, Univ of São Paulo ²Dpt of Pharmacology, Inst of Biomedical Sciences, Univ of São Paulo

12.008 **Effects of naturally occurring and synthetic indole molecule in the open field and conditioned place preference paradigms.** Heidrich N¹, Bif TF¹, Feddern CF^{2,3}, da Silva IA², Fiore RL², Fonseca AR^{2,3}, Almeida FB¹, Fernandes PR¹, Freese L¹, Barros HMT^{1,2}. ¹UFCSA Porto Alegre, PPG Health Sciences, Brazil; ²UFCSA Porto Alegre, Behavioral Neurosciences Lab, Brazil; ³UFRGS Porto Alegre, Dpt of Pharmacology, Brazil

12.009 **Positive screening for new antiepileptic drugs induced by kainic acid and maximal electroshock in Wistar pups.** Feuerharmel F1, Silva RB2, Schneider PH2, Schneider JMFM1, Barros HMT1; ¹Federal Univ of Health Sciences of Porto Alegre, Porto Alegre, Brazil; ²Inst of Chemistry, Federal Univ of Rio Grande do Sul, Porto Alegre, Brazil

12.010 **Fenretinide-loaded microemulsions reduce cell migration, spheroid growth and breast cancer development in a chemically induced carcinogenesis model.** Salata GC, Malago ID, Costa SKP, Marçal-Pessoa AF, Lopes LB Dpt of Pharmacology, Inst of Biomedical Sciences, Univ of São Paulo

12.011 **Anticholinesterase-antimuscarinics: study of dual agents aiming at application for Alzheimer's disease.** Guimarães MJR¹, Neves GA¹, Romeiro LAS², Castro NG¹. ¹Univ Federal do Rio de Janeiro, Inst de Ciências Biomédicas, Brazil ²Univ de Brasília, Dept de Farmácia, Brazil

12.012 **N-octadecanoyl-5-hydroxytryptamide reduces neuroinflammatory responses induced by Aβ1-42 in microglial cells.** Giorno TBS¹, Lima FA², Brand ALM², Oliveira CM², Rezende CM², Fernandes PD¹. ¹UFRJ, Inst of Biomedical Sciences, Brazil; ²UFRJ, Chemistry Inst, Brazil

12.013 **Preparation and characterization of capsules with a standardized dry extract from leaves of *M. ilicifolia* to be used in a clinical trial: Scale up from laboratory to industrial scale.** Meirelles GC^{1,2}, Bianchi SE², Bassani VL², Siqueira IR^{1,3}. ¹UFRGS, Porto Alegre, Dpt of Pharmacology, Brazil; ²UFRGS, Porto Alegre, PPG Pharmaceutical Sciences, Brazil, ³UFRGS, Porto Alegre, PPG Pharmacology and Therapeutics, Brazil

12.014 **Impact of *Achyrocline satureioides* on clinical outcomes in viral respiratory infections, such as COVID-19: preliminary results of an open randomized clinical trial.** Bastos CIM¹, Cechinel LR¹, Dani C¹, Rasia FB¹, Neves AHS¹, Possa LR², Meirelles GC², Bassani VL³, Siqueira IR^{1,3}. ¹UFRGS, Porto Alegre, PPG em Farmacologia e Terapêutica, Brazil ²UFRGS, Porto Alegre, Dpt de Farmacologia, Brazil ³UFRGS, Porto Alegre, PPG em Ciências Farmacêuticas, Brazil

Room 14

01. Cellular and Molecular Pharmacology

01.008 **Different neural cells have distinct mitochondrial function against hypoxia, a possible implication to psychiatric disorders.** Silva LFS, Rosenstock TR Univ of São Paulo – Inst of Biological Sciences

01.009 **Expression of HCC markers in the liver of rats exposed to dexamethasone in utero and subjected to the excess of fructose intake in adult life.** Almeida LS¹, Campos CV¹, Teixeira CJ², Sodré FS², Anê GF¹, Bordin S². ¹Dpt of Pharmacology, Faculty of Medical Sciences, State Univ of Campinas, Brazil ²Dpt of Physiology and Biophysics, Inst of Biomedical Sciences, Univ of São Paulo, São Paulo, Brazil

01.010 **Effects of chronic administration of ouabain in rats on high salt diet.** Feijó PRO, Panice MS, Lara LS, Quintas LEM UFRJ, Inst of Biomedical Science, Brazil

01.011 **Dietary supplementation with chia oil alters the white adipose tissue remodeling in obese mice.** Assis-Ferreira A, de Brito NM, Simões RL, Saldanha-Gama R, Barja-Fidalgo C, da Silva SV. ¹UERJ Dpt of Cell Biology, Rio de Janeiro, Brazil

01.012 **Endothelial P2Y2 receptor is involved in metastatic prostate cancer cell adhesion and its effect is inhibited by atorvastatin.** Cardoso TC, Rocha MA, Silva CLM UFRJ-ICB Lab. Farmacologia Bioquímica e Molecular

01.013 **Activation of kinases downstream of angiotensin II type 1 receptor is potentiated in biological systems with high levels of O-glucNAcylated proteins.** Silva-Neto JA¹, Duarte DA², Simões SC², Mestriner FL¹, Pedersoli CA¹, Costa TJ¹, Bressan AF¹, Abrão EP¹, Silva JF¹, Costa-Neto CM², Tostes RC¹ ¹Univ de São Paulo. Faculdade de Medicina de Ribeirão Preto – Dept de Farmacologia, ²Univ de São Paulo. Faculdade de Medicina de Ribeirão Preto – Dept Bioquímica e Imunologia

01.014 **Endothelial P2Y2 receptors signaling in primed cells contribute to mesenteric inflammation in schistosomiasis.** Oliveira NF¹, Tamura AS², Coutinho-Silva R², Savio LEB², Silva CLM¹. ¹Inst de Ciências Biomédicas - Lab de Farmacologia Bioquímica e Molecular, UFRJ; ²Inst de Biofísica Carlos Chagas Filho - Lab de Imunofisiologia, UFRJ

01.015 **Binding of the seminal plasma protein SVS2 to EPPIN: implications to sperm function.** Mariani NAP, Santos NCM, Santos GVM, Camargo IA, Kushima H, Silva EJR. PPG Pharmacology and Biotechnology, Dpt of Biophysics and Pharmacology, Inst of Biosciences, São Paulo State Univ, Botucatu, Brazil

01.016 **Effect of extracellular vesicles derived from breast tumor cells on human neutrophils polarization.** Amorim CS¹, Docasar CL¹, Renovato-Martins M², Barja-Fidalgo C³, Moraes JA¹ ¹UFRJ Redox Biology Lab, Inst de Ciências Biomédicas, Brazil ²UFF Dpt of Cell and Molecular Biology, Brazil ³UERJ Dpt of Cell Biology, Brazil

01.020 **Comparative effects of N-acetylprocainamide and procainamide on maximum rate of rise and half decay time of the cardiac action potential: evidence for class IA antiarrhythmic mechanisms for N-acetylprocainamide.** Sigler W¹, Oliveira AC². ¹Faculty Oswaldo Cruz, Brazil; ²USP São Paulo, Dpt of Pharmacology, Inst of Biomedical Sciences, Brazil

Room 15

02. Neuropharmacology

02.008 **CB2 receptor blockade reverses a schizophrenia-related memory deficit.** Andrade BS¹, Nunes LED¹, Cunha GNB¹, Cunha NF¹, Ferreira BK², Cardoso F², Ferreira GC², Castro NG¹, Neves GA¹. ¹Lab of Molecular Pharmacology, Inst of Biomedical Sciences, Federal Univ of Rio de Janeiro, Brazil; ²Lab of Bioenergetics and Inborn Errors of Metabolism, Inst of Biochemistry, Federal Univ of Rio de Janeiro, Brazil

02.009 **Inhibitory effect of *Spirulina platensis* on acetylcholinesterase activity.** Tavares J^{1,3}, Bezerra JR^{1,3}, Souza TN^{1,3}, Oliveira AV^{1,3}, Andrade, GM^{1,2,3} ¹Dpt of Physiology and Pharmacology; ²Dpt of Clinical Medicine, Center for Research and Drug Development (NPDM), ³Federal Univ of Ceara, Brazil

02.010 **Cannabidiol induces distinct changes on fear memory and emotional processes that are dependent on the type of treatment: a preclinical approach in a model of type-1 diabetes mellitus.** Chaves YC, Raymundi AM, Waltrick APF, Stern CAJ, Zanoveli JM. Dpt of Pharmacology, Federal Univ of Paraná, Curitiba, Parana, Brazil

02.011 **Effects of sexual dimorphism and mk-801 administration during neurodevelopment on c57bl/6j mice behavior.** Ferreira GN¹, LEITE AL², Loss CM³, Abílio VC⁴. ^{1,2}Unifesp São Paulo, Dpt of Pharmacology, Brazil; ^{3,4}National Inst for Translational Medicine, ⁴National Council of Scientific and Technological Development, Ribeirão Preto, Brazil

02.012 **Effect of cigarette smoke on amino acid content of cerebrospinal fluid of male rats.** Zilli GAL¹, Izolan LR², Bandiera S¹, Pulcinelli RR¹, Marques D², Fontella FU³, Almeida RF⁴, Leal MB^{1,2}, Gomez R^{1,2} ¹PPG Pharmacology and Therapeutics, UFRGS, Porto Alegre, Brazil ²PPG Neurosciences, UFRGS, Porto Alegre, Brazil ³PPG Biochemistry, UFRGS, Porto Alegre, Brazil ⁴Dpt of Biological Sciences, PPG Biological Sciences, UFOP, Ouro Preto, Brazil

02.013 **Uliginosin B interacts with the purinergic system.** Silva CPM¹, Andrejew R², Marangon CG¹, VON Poser GL¹, Batastini AMO², Fraga CAM³, Rates SMK¹. ¹UFRGS Porto Alegre, PPG Pharmaceutical Sciences, Brazil; UFRGS Porto Alegre, Dpt of Biochemistry, Brazil; ²UFRJ Rio de Janeiro, LASSBIO, Brazil

02.014 **Is the pineal gland able to detect brain damage? N-acetylserotonin (NAS), a neuroprotective darkness hormone.** Sousa KS, Quiles CL, Muxel SM, Ferreira ZS, Markus RP. USP São Paulo, Dpt Physiology, Lab Chronopharmacology, Brazil

02.015 **G15 induces tau clearance and autophagy in 2D and 3D cultures of SH-SY5Y overexpressing tau.** Nishino MS¹, Costa AJ², Pereira GJ², Smaili SS², Stilhano RS³, Ureshino RP¹. ¹Unifesp-Diadema, PPG Chemical Biology, Brazil; ²Unifesp São Paulo, Dept. of Pharmacology, Brazil; ³FCMSCSP, Dept of Physiological Sciences, Brazil

02.016 **Antidepressants response evaluation in Wistar Hannover rats submitted to forced swim test and learned helplessness paradigm.** Silveira KM¹, Sartim AG¹, Vieira L¹, Lisboa SF¹, Wegener G², Joca SRL^{1,3}. ¹FCFRP-USP, Dpt of Biomolecular Sciences, Ribeirão Preto, Brazil ²Aarhus Univ, Translational Neuropsychiatry Unit, Dpt of Clinical Medicine, Aarhus, Denmark. ³Aarhus Univ, Dpt of Biomedicine, Denmark

02.017 **Analysis of autophagy and SUMOylation expression profile in YAC128 mice, an animal model of Huntington's disease.** Soares ES¹, Camargo A², Kietzer K³, Rodrigues ALS^{2,4}, Prediger RD^{1,2}, BROCARDI PS², CIMAROSTI HI^{1,2}. ¹UFSC Florianopolis, PPG Pharmacology, Brazil; ²UFSC Florianopolis, PPG Neurosciences, Brazil; ³UEPA Belem, Dpt of Morphological and Physiological Sciences, Brazil; ⁴UFSC Florianopolis, PPG Biochemistry, Brazil

Room 16

03. Psychopharmacology

03.001 **Delayed fear extinction deficit induced by acute stress: modulatory effects on the medial-prefrontal cortex-basolateral amygdala connectivity and the role of norepinephrine signaling.** Juliano VAL¹, Munhoz CD¹, Novaes LS¹. ¹ICB-USP São Paulo, Dpt of Pharmacology, Brazil

03.002 **Toll-like receptor 4 (TLR4) knockout mice do not develop depressive-like behavior after repeated restraint stress but have worse anxiety behavior.** Cunha LC¹, Lisboa SFS². ¹Ribeirão Preto Medical School; ²School of Pharmaceutical Sciences of Ribeirão Preto, Univ of São Paulo, USP

03.003 **Investigation of the involvement of cannabinoid receptors in locomotor responses induced by methylphenidate and cocaine in male and female mice.** Neves LS¹, Gobira PH¹, Joca, SRL¹, Lisboa, SF¹. ¹FCFRP-USP, Dpt of Biomolecular Sciences, Brazil ²Aarhus Univ, Dpt of Biomedicine, Aarhus, Denmark

03.004 **Brain development during adolescence and the impact on psychiatric disorders and drug abuse: a scoping review.** Lamarca LD¹, Poian LR², Chiavegatto S^{2,3} ¹Sch. Pharm. Sci. – Univ. of São Paulo, São Paulo, Brazil ²Pharmacol Biomed. Sci. Inst. – Univ. of São Paulo, São Paulo, Brazil ³Psychiatry – Sch. of Med – Univ. of São Paulo, São Paulo, Brazil

03.005 **What is the caffeine intake profile of the students from PUC Minas?** Almeida, LG ^{1,2}; Cardoso, MCBS ¹; Fonseca, MLA ¹; Ribeiro, AF ³ ¹Undergraduate in Biology, PUC-MG; ²Scholarship from the Tutorial Education Program, PET-Biology PUC-MG; ³PUC-MG

03.006 **Antagonism of TRPV1 receptors did not facilitate the impaired fear extinction in iNOS KO mice.** Ferreira BF¹, Silveira JRCA¹, Guimarães FS¹, Resstel LBM¹, Lisboa SF². ¹USP, Dpt of Pharmacology, Medical School of Ribeirão Preto, Brazil; ²USP, Dpt of BioMolecular Sciences, School of Pharmaceutical Sciences of Ribeirão Preto, Brazil

03.023 **Glucocorticoid and noradrenaline signaling in the stress-induced dendritic remodeling in the amygdala and anxiety-like behavior in rats.** Novaes LS¹, Bueno-de Camargo LM¹, Almeida, AS¹, dos Santos, NB¹, Goosens KA², Munhoz CD¹. ¹Dpt of Pharmacology, São Paulo. ²Dpt of Psychiatry, Icahn School of Medicine at Mount Sinai, New York

03.024 **Tolerance to the antidepressant-like effect of ketamine after sub chronic administration: the role of oxygen and nitrogen reactive species in the brain mice.** Contó MB¹, Camarini R¹. ¹USP São Paulo, Dpt of Pharmacology, Brazil

03.025 **The Impact of social isolation in alcohol use and mental health during the COVID-19 pandemic in Brazil** Freese, L¹; Nin, MS^{1,2}; Almeida, FB¹; Heidrich, N¹; Constant, HMRM¹; Izolan, LR³; Bortolon, CB⁴; Gomez, R³; Barros, HMT¹. ¹UFCSA, PPG-Ciências da Saúde, Neuropsychopharmacology Lab, RS, Brazil; ²FURG, Dpto. de Farmacologia, Brazil; ³UFRGS, PPG-Farmacologia e Terapêutica, Brazil; ⁴Unifesp, PPG-Psiquiatria e Psicologia Médica, Brazil

03.026 **Association between hypnotics/sedatives use and social isolation during the COVID-19 pandemic in Brazil.** Nin MS¹, Freese L², Almeida FB², Heidrich N², Constant HMRM², Izolan LR³, Bortolon CB⁴, Gomez R³, Barros HMT². ¹FURG, Dpto. de Farmacologia, Brazil; ²UFCSA, Dpto. Farmacociências, Neuropsychopharmacology Lab, Brazil; ³UFRGS, PPG-Farmacologia e Terapêutica, Brazil; ⁴Unifesp, PPG-Psiquiatria e Psicologia Médica, Brazil

Room 17

03. Psychopharmacology

03.015 **Mitochondrial inhibition during neurodevelopment leads to schizophrenia-like-phenotype, an outcome related to a decreased mitochondrial biogenesis.** Santos AS¹, Garcia TV², Simões JGT², Henrique E², Ramos AC³, Rosenstock TR^{1,4}. ¹Dpt of Pharmacology, Inst of Biomedical Science, Univ of São Paulo, São Paulo, Brazil; ²Dpt of Physiological Science, Santa Casa de São Paulo School of Medical Science, São Paulo, Brazil; ³Dpt of Bioscience, Federal Univ of São Paulo, Santos, Brazil; ⁴Inst of Cancer and Genomic Sciences, Inst of Biomedical Research, College of Medical and Dental Sciences, Univ of Birmingham, United Kingdom

03.016 **Antidepressant treatment and behavior responses in *Drosophila melanogaster*: a systematic review.** Eckert FB^{1,4}, Triches FF^{1,4}, Costa JEM^{1,4}, Marino-Neto J², De Toni DC³, Lino de Oliveira C⁴. ¹UFSC Florianópolis, PPG Pharmacology, Brazil; ²UFSC Florianópolis, IEB, Brazil; ³UFSC Florianópolis, Dpt of Cellular Biology, Embryology and Genetics, Brazil; ⁴UFSC Florianópolis, Dpt of Physiological Sciences, Brazil

03.017 **Effect of folic acid on anxiety-like behavior modulation of rats.** Bonancea AM¹, Estrada VB¹, Silva KGN¹, Miguel MVO¹, Pelosi GG¹. ¹UEL Londrina, Dpt of Physiological Sciences, Brazil

03.018 **Long-term consequences of maternal separation stress on ethanol intake in male and female mice.** Bertagna NB¹, Favoretto CA¹, Rodolpho BT¹, Loss CM¹, Palombo P¹, Yokoyama TS¹, Righi T¹, Anesio A¹, Miguel TT², Cruz FC¹. ¹Unifesp-EPM Dpt of Pharmacology, São Paulo, Brazil; ²UFU Uberlândia, Dpt of Pharmacology, Brazil

03.019 **Impact of the schizophrenia associated nuclear distribution element genes on nematode *Caenorhabditis elegans* monoamines levels and behavior: effects of typical and atypical antipsychotics.** Nani JV¹, Campeiro JD¹, Monte GG¹, Mori MA², Hayashi MAF¹ ¹Dpt of Pharmacology, Escola Paulista de Medicina (EPM), Univ Federal de São Paulo (Unifesp), Brazil ²Dpt of Biochemistry and Tissue Biology, Inst of Biology, Univ Estadual de Campinas, Campinas, Brazil

03.020 **Behavioral effects of the combined treatment with cannabidiol and antidepressants in stressed mice.** Scarante FF¹, Araújo MR¹, Campos AC¹. ¹Dpt of Pharmacology, Ribeirão Preto Medical School, Univ of São Paulo, Brazil

03.021 **CB2 receptors' spontaneous activity is relevant for the adverse effects of chronic unpredictable stress in mice treated with antidepressant.** Araújo MR²; Aguiar RP¹; Füsse EJ³, Scarante FF²; Oliveira RMMW¹; Guimarães FS², Campos AC² ¹Dpt of Pharmacology and Therapeutics, State Univ of Maringá, Maringá, Brazil ²Dpt of Pharmacology - Ribeirão Preto Medical School, Univ of São Paulo- Ribeirão Preto, Brazil ³Mental Health Graduate Program, Ribeirão Preto Medical School, Univ of São Paulo Ribeirão Preto, Brazil

03.022 **Zebrafish exposure to valproic acid as a translational platform for Autism Spectrum Disorders research and drug discovery.** Costa KCM¹, Brigante TAV¹, Fernandes GG¹, Ferreira RR¹, Scomparin DS¹, Scarante FF¹, Pires-dos-Santos I¹, Oliveira DP², Campos AC¹. ¹FMRP-USP, Dpt of Pharmacology, Ribeirão Preto, Brazil; ²FCFRP-USP, Dpt of Clinical, Toxicological and Bromatological Analysis, Ribeirão Preto, Brazil

Room 18

04. Inflammation and Immunopharmacology

04.006 **Role of hyperglycemia and inflammation in bone marrow derived macrophages in Type 1 Diabetes.** Sousa ESA, Queiroz LAD, Martins JO. Lab of Immunoendocrinology, Dpt of Clinical and Toxicological Analyses, School of Pharmaceutical Sciences of Univ São Paulo (FCF-USP), São Paulo, Brazil

04.007 **Gamma-Terpinene improves pruritus and atopic dermatitis-like lesions in animal models.** Bandeira SRM¹; Lima CMB¹, Reis-Filho AC¹; Oliveira LSA¹; Gonçalves RLG¹; Rezende DC¹; Nunes DB¹; Pinheiro-Neto FR¹; Trindade GNC¹; Barros RO², Ramos RM², Medeiros MGF¹, Almeida FRC¹, Oliveira FA¹ ¹Center for Research on Medicinal Plants, Federal Univ of Piauí, Teresina, Brazil ²Research Lab in Information Systems, Information Dpt, Environment, Health and Food Production, Federal Inst of Education, Science and Technology of Piauí, Teresina, Brazil

04.008 **Investigation of the effect of fluoxetine on sickness behavior in zebrafish (*Danio rerio*).** Petry F¹, Ultramari AR¹, Da Costa CAM¹, Kuhn KZ¹, Garbinato CLL¹, Aguiar GPS¹, Kreutz LC², Oliveira JV³, Siebel AM¹, Muller LG¹. ¹Unochapecó Chapecó, Brazil; ²UPF Passo Fundo, Brazil; ³UFSC Florianópolis, Brazil

04.009 **Investigation of the neurogenic component in the irritative effect of dithranol in mice.** Silva AMD, Ferreira J UFSC Florianópolis, Dpt of Pharmacology, Brazil

04.010 **Renina is involved in increasing osteopontin expression in the jaws of diabetic mice with periodontitis.** Ribeiro BS^{1,2}, Balera VGB^{1,2}, Frasnelli SCT¹, Soares VL³, Santos CF⁴, Oliveira SHP¹ ¹Dept. Basic Science; São Paulo State Univ, School of Dentistry, Araçatuba, Brazil ²Multicenter PPG in Physiological Sciences, SBFis ³Dpt of Biological Science, Bauru School of Dentistry, Univ of São Paulo, Brazil ⁴Dpt of Stomatology, Bauru School of Dentistry, Univ of São Paulo, São Paulo, Brazil

04.011 **Role of TLR-4 in a model of COPD exacerbation induced by H1N1 virus infection** Almeida MD¹, Ferrero MR¹, Garcia CC², Martins MA¹. ¹Lab of Inflammation, Oswaldo Cruz Inst, Fiocruz, Rio de Janeiro, Brazil; ²Lab of respiratory virus and Measles, Oswaldo Cruz Inst, Fiocruz, Rio de Janeiro, Brazil

04.012 **Variation in the chemical composition and anti-inflammatory activity of the essential oil of *Schinus terebinthifolius* Raddi leaves in different Brazilian states.** Marangoni JM¹, dos Santos SM², Oliveira-Júnior PC¹, Vieira AR², da Silva ME², Narcizo LL¹, Cardoso CAL³, Formagio ASN^{1,2} ¹Faculty of Biological and Environmental Sciences, Federal Univ of Grande Dourados, Brazil ²Faculty of Health Sciences, Federal Univ of Grande Dourados, Brazil ³State Univ of Mato Grosso do Sul, Univ City of Dourados, Brazil

05. Pain and Nociception Pharmacology

05.006 **Mechanism of action of the antinociceptive and anti-inflammatory activities of a synthetic substituted 4-dimethylaminochalcone in acute pain models.** dos Santos IS, Melo EDN, Reis JVR, Muzitano MF, Bonavita AGC, do Carmo PL, Raimundo JM. UFRJ-Macaé, Bioactive Products Pharmacology Lab, Brazil

05.007 **Aldehyde dehydrogenase-2 activation alleviates neuropathic pain by controlling neuroinflammation in the spinal cord.** Alcantara QA¹, Neto BS¹, Mochly-Rosen D², Cury Y¹, Zambelli VO¹. ¹Lab of Pain and Signaling, Butantan Inst, Brazil; ²Chemistry and Systems Biology, Stanford Univ, USA

05.008 **Isopulegol presents antinociceptive activity in a cancer pain model: *in vivo* study and *in silico* predictions.** ¹Dias WA, ¹Pimentel VD, ¹Sales SCS, ¹Acha BT, ²Silva JN, ²Paulo Ferreira PMP, ¹Almeida FRC ¹Lab of Pain Pharmacology, Medicinal Plants Research Center; ²Lab of Experimental Cancerology, Dpt of Biophysics and Physiology, Federal Univ of Piauí, Teresina, Brazil

Room 19

04. Inflammation and Immunopharmacology

04.018 **Effects of tyrosine kinase inhibitor in experimental cerebral malaria.** Moraes BPT^{1,2}, Rodrigues SO^{1,2}, Soares GMV¹, Abreu VHP¹, Maron-Gutierrez T², Batista CN², Bozza PT², Castro Faria Neto HC², Silva AR², Albuquerque CFG¹. ¹Lab de Imunofarmacologia, Univ Federal do Estado do Rio de Janeiro, Rio de Janeiro, Brasil; ²Lab de Imunofarmacologia, Fundação Oswaldo Cruz, Rio de Janeiro, Brasil.

04.019 **Role of TLR4 regulation and activation in the hypothalamus-pituitary-adrenal axis hyperactivity in diabetic animals.** Magalhães NS¹, Chaves AS¹, Torres RC², Gonçalves-de-Albuquerque CF³, Castro-Faria-Neto HC⁴, Martins PMRS¹, Martins MA¹, Carvalho VF¹ ¹Lab of Inflammation, Oswaldo Cruz Inst, Oswaldo Cruz Foundation, Rio de Janeiro, Brazil; ²Carlos Chagas Filho Biophysics Inst – Federal Univ of Rio de Janeiro, Rio de Janeiro, Brazil; ³Federal Univ of the State of Rio de Janeiro, Rio de Janeiro, Brazil; ⁴Lab Immunopharmacology, Oswaldo Cruz Inst, Oswaldo Cruz Foundation, Rio de Janeiro, Brazil

04.020 **PI3K-γ inhibition attenuates the irinotecan-associated intestinal mucositis in mice.** Cajado AG¹, Rangel GFP¹, Nobre LMS¹, Quintela LCS², Paguada ALP³, Ferreira LMM⁴, Alves APNN², Wong DVT², Lima-Júnior RCP^{1,2,3,4}. ¹UFC Ceará, Dpt of Physiology and Pharmacology, Brazil; ²UFC Ceará, Dpt of Pathology, Brazil; ³UFC Ceará, Dpt of Pharmaceutical Sciences, Brazil; ⁴UFC Ceará, Dpt of Medicine, Brazil

04.021 **Effect of physical exercise on a preclinical model of nonalcoholic fatty liver disease.** Rodrigues KL¹, Silva VV¹, Pereira ENGS¹, Silveiras RR¹, Araujo BP¹, Flores EEI¹, Ramos, IP, ²Daliry A¹. ¹Lab of Cardiovascular Investigation, Oswaldo Cruz Inst, Fiocruz, Rio de Janeiro, Brazil; ²National Center of Structural Biology and Bio-imaging, Federal Univ of Rio de Janeiro, Rio de Janeiro, Brazil

04.022 **Effect of Mo-CBP4, a purified chitin-binding protein from *Moringa oleifera* seeds, in irinotecan-induced intestinal mucositis in mice.** Ferreira KQ¹, Carmo LD¹, Rangel GFP¹, Nunes MO¹, Duarte RS¹, Rabelo LMA¹, Lopes TDP², Sousa DOB², Alencar NMN¹. ¹Drug Research and Development Center, Dpt of Physiology and Pharmacology, Federal Univ of Ceará, Fortaleza, Brazil; ²Dpt of Biochemistry and Molecular Biology, Federal Univ of Ceará, Fortaleza, Brazil

04.023 **Captopril reduces hypothalamus-pituitary-adrenal axis hyperactivation in diabetic mice.** Chaves AS¹, Magalhães NS¹, Cardoso CF¹, Martins PMRS¹, Martins MA¹, Carvalho VF¹. ¹Oswaldo Cruz Foundation, Oswaldo Cruz Inst, Lab of Inflammation, Rio de Janeiro, Brazil

04.024 **Experimental Alzheimer's disease: Kinin-B2 receptor signaling in immune response and neuroinflammation in mice.** Viero FT¹, Mello CF¹, Pillat MM², Ulrich H³. ¹Pharmacology, Federal Univ of Santa Maria, Santa Maria, Brazil; ²Microbiology and Parasitology, Federal Univ of Santa Maria, Santa Maria, Brazil; Dep. of Biochemistry, Inst of Chemistry, Univ of São Paulo, Brazil

04.025 **The reduction of inflammation and oxidative stress by a novel laticifer protein sub-fraction II of *Calotropis procera* decreases mucositis induced by irinotecan.** Rangel GFP¹, Carmo LD¹, Rabelo LMA¹,

Duarte RS¹, Costa ADC¹, Macedo FS¹, CAJADO AG¹, Souza TFG¹, Ramos MV², Alencar NMN¹. ¹UFC Fortaleza, Dpt de Fisiologia e Farmacologia, Brasil; ²UFC Fortaleza, Dpt de Bioquímica e Biologia Molecular, Brasil

04.026 **Low birth weight induced by maternal malnutrition prejudices phagocytic activity of alveolar macrophages and increases susceptibility to infections.** Azevedo GA¹, Gil NL¹, Balbino AM¹, Silva MM¹, Landgraf MA², Landgraf RG¹. ¹Dpt of Pharmaceuticals Sciences, Univ Federal de São Paulo-Diadema, Diadema, Brazil; ²Univ Paulista-Rangel, Santos, Brazil

Room 20

06. Cardiovascular and Renal Pharmacology

06.001 **Hypertensive aged rats showed altered vasomotion in testis.** Machado NR, Colli LG, Rodrigues SF. USP, Dpt of Pharmacology, Inst of Biomedical Sciences

06.002 **Characterization of caffeine effect on cardiovascular changes induced by adenosine.** Albino LB, Oliveira JG, Fernandes D. UFSC Florianópolis, Dpt of Pharmacology, Brazil

06.003 **Wedelolactone as a possible treatment for cardiotoxicity induced by *Bothrops jararacussu* venom.** Albernaz LCS¹, Pinto HMC¹, Romanelli MA², Lara LS², Melo PA², Gonzalez SR¹. ¹UFRJ, Campus Macaé, Brazil; ²UFRJ, Inst of Biomedical Sciences, PPG Biological Sciences (Pharmacology and Medicinal Chemistry), Brazil

06.004 **Wedelolactone as a possible treatment for acute kidney injury induced by *Bothrops jararacussu* venom.** Pinto HMC¹, Albernaz LCS¹, Santos MARF², Souza PDN², Lara LS², Melo PA², Gonzalez SR¹. ¹Univ Federal do Rio de Janeiro – Campus Macaé, Brazil ²PPG em Ciências Biológicas (Farmacologia e Química Medicinal), Inst de Ciências Biomédicas, Univ Federal do Rio de Janeiro, Brazil

06.005 **Acute toxicity and molecular docking of ruthenium complexes FOR811A and FOR811B.** Oliveira JPH¹, Nogueira PMM¹, Alves RS¹, Santos PN², Rocha DG², Nogueira-Júnior FA², Lopes LGF³, pod³, de Sousa EHS³, Braz HLB⁴, Olivier DS⁵, Monteiro SMN⁶, Monteiro HSA⁶, Silveira JAM⁶, Jorge RJB⁶. ¹UFC Fortaleza, Dpt de Farmácia, Brasil; ²UFC Fortaleza, PPG Farmacologia, Brasil; ³UFC Fortaleza, Dpt de Química Orgânica e Inorgânica, Brasil; ⁴UFC Fortaleza, PPG Ciências Morfofuncionais, Brasil; ⁵UFT Palmas, Campus Araguaína, Brasil; ⁶UFC Fortaleza, Dpt de Fisiologia e Farmacologia, Brasil

06.006 **Antihypertensive action of new N-acylhydrazonic derivatives.** Rocha BS^{1,2}, Da Silva JS^{1,2}, Pedreira JGB¹, Barreiro EJ¹, Zapata-Sudo G^{1,2}. ¹ICB-UFRJ, Rio de Janeiro, Brazil; ²UFRJ, PPG in Medicine - Cardiology, ICES, Rio de Janeiro, Brazil

06.017 **Salt-inducible Kinase (SIK): A pharmacological target of salt-sensitive hypertension.** Gomes DS¹, Visniauskas B², Prieto MC², Lara LS¹. ¹UFRJ, Inst de Ciências Biomédicas, Programa de Pesquisa em Farmacologia e Inflamação, Brazil ²Tulane Univ School of Medicine, Dpt of Physiology and Renal Hypertension Center of Excellence, USA

06.018 **Endothelium dysfunction in gestational hypertension in rats induced by the reduced uteroplacental perfusion pressure model.** Santos-Silva, ML; Souza-Paula, E and Dias-Júnior, CA Dpt of Pharmacology, São Paulo State Univ, Botucatu, Brazil

06.019 **H1-Receptors may mediate the vasodilator activity of a natural substance extracted from piper rivinoides.** Barenco TS¹, Souza PDN¹, Marques AM², Ramalho TC³, Nascimento JHM¹, Ponte CG⁴. ¹UFRJ, IBCCF, Brazil; ²Fiocruz, Dpt of Chemistry of Natural Products, Brazil; ³UFLA, Dpt of Chemistry, Brazil; ⁴IFRJ, NCBA, Brazil

Room 21

06. Cardiovascular and Renal Pharmacology

06.020 **Type 1 Collagen (COL-1) proteolysis by Matrix Metalloproteinase (MMP)-2 may contribute to FAK activation and increased vascular smooth muscle cells proliferation in aorta of acute hypertensive rats.** Neves VGO¹, Blascke de Mello MM¹; Silva PHL¹; Pernomian L¹; Parente JM¹; Falchetti F¹; Castro MM¹. ¹Dpt of Pharmacology, Ribeirão Preto Medical School, Univ São Paulo

06.021 **Analysis of the action of H₂S from the perivascular adipose tissue in different vascular vessels of hypertensive pregnant rats.** Paula ES, Santos-Silva MLSS, Bozoni FT, Dias Junior CAC São Paulo State Univ, Botucatu, Brazil

06.022 **Platelet activity from Antiphospholipid Syndrome (APS) patients is enhanced: possible role of the ADP signaling pathway.** Leonardi G¹, Lescano CH¹, dos Santos APR², Jacinto BC², Mazetto BM², Orsi FA³, Mónica FZ¹. ¹Dpt of Pharmacology, Faculty of Medical Sciences, Univ of Campinas, Campinas, Brazil; ²Faculty of Medical Sciences, Univ of Campinas, Campinas, Brazil; ³Lab of Haemostasis, Hematology and Hemotherapy Center, Univ of Campinas, Campinas, Brazil

06.023 **Chronic ethanol consumption induces loss of the anticontractile effect of perivascular adipose tissue: role for Angiotensin II.** ^{1,2}Awata, WMC; ^{1,2}Sousa, AH; ²Tirapelli CR ¹FMRP-USP – PPG em Farmacologia, ²EERP-USP Lab de Farmacologia

06.024 **BMP9 / ALK1 / BR-SMAD signaling pathway regulates cardiac remodeling of the offspring of dexamethasone-treated mothers.** Sodr  FSS¹, Pereira GA¹, Amaral AG¹, Murata GM¹, Castelo-Branco RC¹, Campos CV², Almeida LS², Teixeira CJ¹, Couto GK¹, Rossoni LV¹, Anh  GF², Bordin S¹. ¹Dpt of Physiology and Biophysics, Inst of Biomedical Sciences, Univ of S o Paulo, S o Paulo, Brazil ²Dpt of Pharmacology, Faculty of Medical Sciences, State Univ of Campinas, Brazil

06.025 **Cardiac dysfunction in sepsis: the involvement of intercalated discs.** Hahmeyer MLS, Assreuy J, da Silva-Santos JE. UFSC Florian polis, Dpt of Pharmacology, Brazil

06.026 **TNF-alpha inhibition reverses endothelial dysfunction and renovascular hypertension-induced ROS formation.** Vitorino TR^{1,2}, Mantovani B¹, Bon cio GF¹, Batista RIM³, Tanus-Santos JE³, Rizzi E¹ ¹Biotechnology Unit, Univ of Riber o Preto, S o Paulo, Brazil ²Dpt of Pharmacology, School of Medical Sciences, Univ of Campinas, S o Paulo, Brazil ³Dpt of Pharmacology, Ribeir o Preto Medical School, Univ of S o Paulo, S o Paulo, Brazil

06.027 **Proteolytic action of Matrix Metalloproteinase (MMP)-2 on sarcoplasmic reticulum calcium ATPase (SERCA) and the morphofunctional vascular alterations of hypertension.** Mello MMB¹; Pernomian L¹; Parente JM¹; Neves VGO¹; Silva PHL¹; Castro MM¹ ¹Dpt of Pharmacology, Ribeir o Preto Medical School, Univ of S o Paulo, Brazil

06.028 **NLRP3 inflammasome mediates testosterone-induced cardiac dysfunction.** ¹Dpt of Pharmacology, Ribeir o Preto Medical School, Univ of S o Paulo, Ribeir o Preto, Brazil ²Special Academic Unit of Health Sciences, Federal Univ of Goias, Jatai, Brazil ³Dpt of Physiology, Ribeir o Preto Medical School, Univ of S o Paulo, Ribeir o Preto, Brazil

06.029 **Resistin contributes to PVAT dysfunction in a rheumatoid arthritis experimental model.** Fedoce AG, Veras PF, Silva JF, Cunha FQ, Tostes RC. USP-FMRP

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07. Endocrine, Reproductive and Urinary Pharmacology

07.001 **Experimental model of insulin resistance in Swiss mice female and male**

¹Freire GA; ³Mack JM; ²Castro AJG, ²Luz G; ²D Altenhofen D; ²Mendes CP; ²Rieg CE; ²Heim JBA; ⁴Yunes RA; ³Santos ARS; ²Silva FRMBS, ¹Frederico MJS. ¹Univ Federal do Cear , N cleo de Pesquisa e Desenvolvimento de Medicamentos, Fortaleza, Brasil; ²Univ Federal de Santa Catarina, Dept de Bioqu mica, Florian polis, Brazil ³Univ Federal de Santa Catarina, Dept de Ci ncias Fisiol gicas, Florian polis, Brazil ⁴Univ Federal de Santa Catarina, Dept de Qu mica, Florian polis, Brazil

07.002 **Hypoglycemic effect of *Lippia origanoides* Kunth hydroalcoholic extract in an experimental model of alloxane-induced diabetes.** Pereira YLG, Diniz LA, Miranda VC, J ia-Mello V, Hamoy M Federal Univ of Par , Inst of Biological Sciences, Brazil

08. Respiratory and Gastrointestinal Pharmacology

08.001 **Intestinal health assessment of patients diagnosed with celiac disease.** Marques CRS¹, Couto PEA¹, Costa LATJ², Santos, AT³; Aragao, KS¹ ¹Centro Universitário Estácio de Ceará ²Univ de Fortaleza ³Univ Federal do Ceará

08.002 **Histamine H4R antagonist (LINS01007) reduces development of colon inflammation, in DSS-induced colitis in mice.** Lippi BK¹, Balbino AM¹, Fernandes GAB¹, Landgraf MA², Fernandes JPS¹, Landgraf RG¹. ¹Dpt of Pharmaceutical Sciences, Univ Federal de São Paulo, Campus Diadema, São Paulo, Brazil; ²Univ Paulista, Campus Rangel, Santos, São Paulo, Brazil

08.003 **Mechanism of action of tracheal hyper-reactivity in a model of asthma exacerbated by obesity.** Martins AMO¹, Ferreira SRD², Araújo MPA¹, Figueiredo IAD², Pessoa RF², Cavalcante FA^{2,3}, Vasconcelos LHC^{2,3} ¹UFPB, ²UFPB - PPgPNSB, ³UFPB - DFP

08.004 **Carvacrol attenuates cigarette smoke-induced acute lung injury.** Holanda-Pereira, AK¹, Santos, CA¹, Pereira-Gonçalves, A², Oliveira-Melo, P³, Felix, RGS¹, Moura, MJN¹, Borges, CS¹, Silva, GM¹, Lima, CC², Kennedy-Feitosa, E¹ ¹Univ Federal Rural do Semi-Árido, Mossoró, Brazil; ²Inst Superior de Ciências Biomédicas, Univ Estadual do Ceará, Fortaleza, Brazil; ³Inst do Coração, Univ de São Paulo (USP), São Paulo, Brazil

08.005 **Anti-inflammatory activity of eugenol in acute lung inflammation induced by cigarette smoke in mice.** Barbosa MCO¹, Araújo BVS¹, Holanda-Pereira AK¹, Santos CCA¹, Oliveira-Melo P², Silva AGG¹, Borges CS¹, Silva GM¹, Kennedy-Feitosa E¹ ¹Univ Federal Rural do Semi-Árido, Mossoró, Brazil; ²Inst do Coração, Univ de São Paulo, São Paulo, Brazil

08.006 **Consumption of *Spirulina platensis* prevents deleterious effects on the contractile reactivity of the ileum of Wistar rats fed a hypercaloric diet.** Francelino DMC¹, Diniz AFA², Souza PPS¹, Claudino BFO¹, Duvirgens MV¹, Lacerda-Júnior FF², Barros BC², Ferreira PB², Silva BA^{1,2} ¹DCF-CCS-UFPB, ²PPgPNSB-CCS-UFPB

08.007 **Food supplementation with *Spirulina platensis* prevents obesity and deleterious effects on ileal histomorphometry induced by hypercaloric diet.** Claudino BFO¹, Diniz AFA², Duvirgens MV¹, Souza PPS¹, Francelino DMC¹, Ferreira PB², Lacerda-Júnior FF², Alves AF³, Silva BA^{2,4} ¹CCS-UFPB, ²PPgPNSB-CCS-UFPB, ³DFP-CCS-UFPB, ⁴DCF-CCS-UFPB

13. Pharmacology Education and Technology

13.002 ***As aventuras de Farmarquinhos: teaching through gamification.*** Farias RHC, Meneses JRL, Bayerlein MJ, Lorga ACM, Antonini HK, Pereira MLL, Carlos CP, Rodrigues AC USP, Inst of Biomedical Sciences, Brazil

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08. Respiratory and Gastrointestinal Pharmacology

08.008 **A comparative approach to evaluate *in vitro* contractile and relaxing responses of the gastrointestinal smooth muscle of mice.** Oliveira SM, da Silva-Santos JE. UFSC Florianópolis, PPG Pharmacology, Brazil

08.009 **Acid exposure impairs the duodenal mucosal integrity - A translational study.** Sousa MKA¹, Saraiva LGM², Borges IC³, Costa Filho HB¹, Sales TMAL², Freire PRP¹, Ribeiro TA⁴, Souza MC⁴, Lederhos QR⁴, Araújo GAC³, Paula SM², Souza MHL². ¹Dpt of Pharmacology, Federal Univ of Ceará. ²Dpt of Medical Sciences, Federal Univ of Ceará. ³Federal Univ of Ceará. ⁴Estácio Univ Center of Ceará

08.010 **Diet-Induced Nonalcoholic Steatohepatitis (Nash) animal model for preclinical pathogenesis and therapy research.** Araujo BP, Pereira ENGDS, Martins CSM, Silveiras RR, Rodrigues KL, Flores EEI, Daliry A. Lab of Cardiovascular Investigation, Oswaldo Cruz Inst, Oswaldo Cruz Foundation, Rio de Janeiro, Brazil

08.012 The treatment with virgin coconut oil (*Cocos nucifera* L.) improves the murinometric p. arameters and tracheal reactivity of obese-asthmatic Wistar rats

Pessoa RF¹, Figueiredo IAD¹, Ferreira SRD¹, Martins AMO², Araújo MPA^{2,2}, Vasconcelos LHC³, Cavalcante, FA^{1,3}. ¹UFPB-PPgPNSB, ²UFPB-PIBIC/CNPq, ³UFPB-DFP

08.013 Role of the Pepsin in the Pulmonary Inflammatory Dysfunction induced by gastroesophageal reflux in mice. Sales TMAL¹, Sousa MKA², Costa-Filho HB², Gadelha KKL², Dias-Júnior GJ², Paula SM¹, Ribeiro TA³, Lederhos QR³, Magalhães PJC², Soares PMG², Sifrim D⁴, Souza MHL¹. ¹UFC, Fortaleza, Dpt of Medicine, PPG Medical Sciences, Brazil; ²UFC, Fortaleza, Dpt of physiology and pharmacology, PPG pharmacology, Brazil; ³Estacio Univ Center of Ceará, Fortaleza, Brazil; ⁴Queen Mary Univ of London, London, United Kingdom

08.014 Impair in mucosal integrity associated with colitis correlates with microscopic inflammatory damage: a translational study. Costa-Filho HB¹, Lopes AKM², Sales TMAL², Paula SM¹, Sousa MKA¹, O4.0¹, Souza MC³, Silva LMG³, Araújo GAC², Soares PMG⁴, Barbosa ALR⁵, Souza MHL¹. ¹UFC Fortaleza, Dpt of Physiology and Pharmacology, Brazil; ²UFC Fortaleza, Dpt of Medicine, Brazil; ³Estácio Univ Center of Ceará, Fortaleza, Brazil; ⁴UFC Fortaleza, Dpt of Morphology, Brazil; ⁵UFPI Parnaíba, Dpt of Physiotherapy, Brazil

08.015 Experimental gastroprotective potential of the dry hydroalcoholic extract from flowers of *Tagetes erecta* L., a useful medicinal plant in gastrointestinal diseases. Silva TFQ¹, Meurer MC¹, Felisbino F¹, Muller FB¹, Somensi LB^{1,2}, Cury BJ¹, Jerônimo DT¹, Venzon L¹, França TC¹, Mariott M¹, Santos AC¹, Boeing T¹, Cruz AB¹, Souza P¹, Silva LM¹ ¹PPG in Pharmaceutical Sciences, Chemical Pharmaceutical Research Nucleus, Univ of Vale do Itajaí, Itajaí, Brazil ²PPG in Development and Society, Alto Vale do Rio do Peixe Univ, Caçador, Brazil

08.016 Evaluation of the geraniol gastric healing mode in rodents. Venzon L¹, Meurer MC¹, França TCS¹, Longo B¹, Mariott M¹, Somensi LB², Mariano LNB¹, Boeing T¹, Cazarin CA¹, Pereira LN¹, da Silva LM¹. ¹Univali Itajaí, Pharmaceutical Sciences Graduate Program, Brazil; ²Uniarp Caçador, PPG in Development and Society, Brazil

08.017 Experimental model of obesity-induced exacerbated asthma: an analysis of *in vivo* and *in vitro* dysfunctions on airways of Wistar rats. Ferreira SRD¹, Pessoa RF¹, Figueiredo IAD¹, Martins AMO², Araújo MPA², Alves JLB³, Alves AF⁴, Vasconcelos LHC⁴, Cavalcante, FA^{1,4}. ¹UFPB PPgPNSB, João Pessoa, Brazil; ²UFPB PIBIC/CNPq, João Pessoa, Brazil; ³UFPB Dpt of Nutrition and PPgCN, João Pessoa, Brazil; ⁴UFPB - Dpt of Physiology and Pathology, Brazil

08.018 Controlled release of JME-173 from nanocapsules improves lipopolysaccharide-induced lung inflammation in mice. Coutinho DS¹, Bernardi A¹, Guterres SS², Pohlmann AR³, Silva PMR¹, Martins MA¹ ¹Lab of Inflammation, Oswaldo Cruz Foundation, Rio de Janeiro, Brazil ²Pharmaceutical Sciences Post-Graduation Program, Federal Univ of Rio Grande do Sul, Porto Alegre, Brazil ³Dpt of Organic Chemistry, Federal Univ of Rio Grande do Sul, Porto Alegre, Brazil

Room 24

09. Natural Products and Toxinology

09.009 Evaluation of the anti-inflammatory activity of the ethanolic extract from the roots of *Eriosema campestre* on RAW 264.7 cells stimulated by bacterial lipopolysaccharide Ottoni MHF¹, Barra A², Fernandes-Braga W³, Santos MG¹, Pereira WF¹, Klein A², Melo GEBA¹. ¹UFVJM Diamantina, Integrated Center of Postgraduate and Research in Health, Brazil; ²UFMG Belo Horizonte, Inst of Biological Sciences, Dpt of Pharmacology, Brazil; ³UFMG Belo Horizonte, Inst of Biological Sciences, Dpt of Biochemistry and Immunology, Brazil

09.010 Chemical characterization and gastroprotective effect of *Lonchocarpus sericeus* on ethanol-induced gastric ulcers in mice. FREIRE GP¹, Almeida-Filho LCP², Nunes PIG¹, Silva AVL³, Lima RP³, Ribeiro PRV⁴, Brito ES⁴, Carvalho AFFU², Santos FA³. ¹UFC Fortaleza, PPG Medical Sciences, Brazil; ²UFC Fortaleza, PPG Biochemistry; ³UFC Fortaleza, PPG Pharmacology, Brazil; ⁴Embrapa Tropical Agroindustry, Brazil

09.011 Linalool antinociceptive activity evaluation in acid-induced nociceptive-like behavior in fish. Rodrigues P, Barbosa LB, Ferrari FT, Bianchini AE, Baldisserotto B, Heinzmann BBM Post-Graduation

Program in Pharmacology, Federal Univ of Santa Maria, Santa Maria, RS, Brazil Pharmacy School, Federal Univ of Santa Maria, Santa Maria, RS, Brazil

09.012 **Treatment with pyridoxamine protects kidney endothelial dysfunction caused by diet-induced metabolic syndrome.** Silveiras, RR¹, Pereira ENGDS¹, Flores, EEI¹, Rodrigues, KL¹, Silva, AR², Gonçalves-de-Albuquerque, CF^{2 3} and Daliry, A¹. ¹Lab of Cardiovascular Investigation, Oswaldo Cruz Inst, Oswaldo Cruz Foundation, Rio de Janeiro, Brazil ²Lab of Immunopharmacology, Oswaldo Cruz Inst, Oswaldo Cruz Foundation, Rio de Janeiro, Brazil ³Lab of Immunopharmacology, Federal Univ of the State of Rio de Janeiro, Rio de Janeiro, Brazil

09.013 **Antagonism of *Apis mellifera* venom activities by *Eclipta prostrata* extract and wedelolactone.** Nogueira-Souza PD, Rocha-Junior JRS, Pinheiro AN, Cesar MO, Strauch MA, Monteiro-Machado M, Patrão-Neto FC, Melo PA CCS-ICB- UFRJ Lab de Farmacologia das Toxinas

09.014 **Evaluation of the effect of *Hesperozygis ringens* (Benth.) Epling extract on the total antioxidant capacity of silver catfish infected by *Aeromonas hydrophila*.** Rosa IA¹, Bressan CA¹, Ferrari FT², Pavanato MA¹, Baldisserotto B¹, Heinzmann BM¹². ¹UFSM Santa Maria, Dpt of Physiology and Pharmacology, Brazil; ²UFSM, Santa Maria, Dpt of Industrial Pharmacy, Brazil

09.015 **Alpha, beta-amyrin improves glucose uptake through membrane GLUT4 expression of TNF α -induced insulin resistance in 3T3-L1 cells.** Lima RP¹, Oliveira FTB², Viana AFSC¹, Silva RAC², Nunes PIG², Silva AVL¹, Freire GP², Carvalho AA³, Chaves MH³, Santos FA¹. ¹UFC Fortaleza, PPG Pharmacology, Brazil; ²UFC Fortaleza, PPG Medical Sciences, Brazil; ³UPI Teresina, Dpt of Organic Chemistry, Brazil

09.016 **Effect of alpha,beta-amyrin, a triterpenoid mixture from *Protium heptaphyllum* on insulin resistance in skeletal muscle of high fat diet-induced obese mice.** Nunes PIG¹, Oliveira FTB¹, Lima RP², Viana AFSC², Silva RAC¹, Freire GP¹, Silva AVL², Carvalho AA³, Chaves MH³, Santos FA². ¹UFC Fortaleza, PPG Medical Sciences, Brazil; ²UFC Fortaleza, PPG Pharmacology, Brazil; ³UFPI Teresina, Dpt of Organic Chemistry, Brazil

09.017 **Thymic epithelial cells are sensible to the triterpene friedelin.** Porto FL¹, LINS MP^{1,2}, Barreto EO^{1,2}, Smaniotto S^{1,2}, Reis MDS^{1,2}. ¹Lab of Cell Biology, Inst of Biological and Health Sciences, Federal Univ of Alagoas, Maceió, Brazil; ²Brazilian National Inst of Science and Technology on Neuroimmunomodulation, Oswaldo Cruz Inst, Oswaldo Cruz Foundation, Rio de Janeiro, Brazil

Room 25

11. Clinical Pharmacology, Pharmacokinetics, Pharmacogenomics and Toxicology

11.001 **Evaluation of the treatment effectiveness of second-generation direct-action antivirals for Hepatitis C.** Silva-Neto MR, Ziolkowski MI, Santos RB, Mocellin LP, Haas SE Unipampa Uruguiana, Pharmacology Lab

11.002 **Predictions of complex drug-drug-disease interactions with carvedilol using physiologically based pharmacokinetics (PBPK).** Micheletto AL¹, Yamamoto PA², DE Moraes NV¹. ¹Unesp, School of Pharmaceutical Sciences, Dpt of Drugs and Medicines, Araraquara, Brazil ²USP, School of Pharmaceutical Sciences of Ribeirão Preto, PPG Toxicology, Ribeirão Preto, Brazil

11.003 **Population pharmacokinetic modelling of tobramycin lung and epithelial lining fluid disposition due to biofilm-forming *Pseudomonas aeruginosa* infection.** Dias BB¹, Carreño F², Helfer VH¹, Garzela PM¹, Barreto F³, Araújo BV¹, Dalla Costa T¹. ¹UFRGS PPG Pharmaceutical Sciences Porto Alegre, Brazil; ²Univ of North Carolina at Chapel Hill, US; ³Federal Lab of Animal and Plant Health and Inspection, Porto Alegre, Brazil

12. Drug Discovery and Development

12.001 **Synthesis of galectin-3 inhibitors with applications in neurobehavioral disorders.** Leite FT, Campo VL. Centro Universitário Barão de Mauá, Brazil

12.002 **Cytotoxic effects of lipid nanoparticles for sustained release of fenretinide and *in vivo* localization in the mammary tissue.** Malagó ID, Salata GC, Lopes LB. USP São Paulo, Inst of Biomedical Sciences, Dpt of Pharmacology, Brazil

12.003 **Analysis of the anti-inflammatory potential of novel of new phenylbenzohydrazides.** Souza, ACN¹ Paiva JPB¹, Carvalho PR¹, Branco LOP¹, Lima EC², Fernandes, PD¹ ¹Federal Univ of Rio de Janeiro, Inst of Biomedical Science, Lab of Pain and Inflammation. Rio de Janeiro, Brazil ²Federal Univ of Rio de Janeiro - Macaé, Dpt of Chemistry, Lab of Catalysis and Synthesis of Bioactive Substances. Macaé, Brazil

12.004 **LQFM 247, a synthetic derivative of anandamide, has antioxidant activity *in vitro*.** Jesus FSD¹, Farias ERA¹, Souza RRLS¹, Pereira RM¹, Campos HM¹, Port's NMS², Orellana AMM², Marques TR³, Scavone C², Menegatti R³, Ghedini PC¹, Leite JA¹. ¹Dpt of Pharmacology, Inst of Biological Sciences, Univ Federal de Goiás, Goiânia, Brazil ²Dpt of Pharmacology, Inst of Biomedical Sciences, Univ of São Paulo. ³Lab de Química Farmacêutica Medicinal, Faculdade de Farmácia

12.005 **Effect of the mimetic peptide Ac9-22 derived from Annexin A1 on skeletal muscular function after myotoxicity induced by a bothropic venom.** Alecrim NN¹; Damico MV ¹; Icimoto MY²; Escalante T³; Moreira V¹ ¹EPM-Unifesp São Paulo, Dpt of Pharmacology, Brazil ²EPM-Unifesp São Paulo, Dpt of Biophysics, Brazil ³Universidad Costa Rica, Inst Clodomiro Picado, San José, Costa Rica

12.006 **Pre-clinical evaluation of the anti-inflammatory effects of novel capsaicin-curcumin hybrid molecules.** Paiva JPB¹, Carvalho PR¹, Etienne R², Viegas Júnior CV², Fernandes P¹ ¹Federal Univ of Rio de Janeiro, Inst of Biomedical Science, Lab of Pain and Inflammation, PPG in Pharmacology and Medicinal Chemistry. Rio de Janeiro, Brazil ²Federal Univ of Alfenas, Medicinal Chemistry Research Lab. Minas Gerais, Brazil

Room 26

14. Pharmacology: Other

14.001 **Use of medications for the treatment of Covid-19 in Brazil: A cross-sectional online survey.** Alves GMS, Botinhão MC, Bonavita AGC, Carmo PL, Gonzalez SR, Raimundo JM. UFRJ-Campus Macaé

14.002 **Use of medicines for COVID-19 prevention in Brazil: a cross-sectional study.** Botinhão MC, Alves GMS, Gonzalez SR, Bonavita AGC, Montani JR, Carmo PL. UFRJ-Macaé

14.003 **Cytotoxicity of new indole molecules in rat glioma cells.** Amorim, I, Heidrich N, Steinmetz A, Almeida FB, Freese L, Barros HMT ¹UFCSA, Neuropsychopharmacology Lab, Brazil; ²UFCSA, Genotoxicity Lab, RS, Brazil

14.004 **Standardization and evaluation of changes induced by primary dysmenorrhea in Wistar rats.** Souza PPS¹, Lacerda-Júnior FF² Barros BC², Diniz AFA², Ferreira PB², Costa BA³ ¹DCF-CCS-UFPB; ²PPgPNSB-CCS-UFPB; ³DCF-UFPB

14.005 **Transcriptional analysis of TRPA1, TRPV1, TRPV4, TRPM8 in human systems.** Kudsi SQ¹, Piccoli BC², Araújo DA², Trevisan G¹. ¹UFMS Santa Maria, PPG Pharmacology, Brazil; ²UFMS Santa Maria, PPG Biochemistry Toxicology, Brazil

14.006 **Chronic ethanol consumption causes oxidative stress in the thymus via mineralocorticoid receptor activation.** Assis VO Dourado TMH, Tirapelli CR. EERP-USP Ribeirão Preto, Lab of Pharmacology, Brazil

14.007 **Influence of periprostatic adipose tissue from obese mice on prostate smooth muscle contraction and human epithelial cell viability.** Passos GR, Oliveira MGD, Ghezzi AC, Antunes E, Mónica FZ. Dpt of Translation Medicine, Faculty of Medical Sciences, Univ of Campinas

14.008 **Investigation of pleiotropic effects of simvastatin in a non-alcoholic fatty liver disease model.** Pereira ENGS, Martins CSM, Araujo BP, Rodrigues KL, Silveiras RR, Flores EEI and Daliry A. Lab of Cardiovascular Investigation, Oswaldo Cruz Inst, Oswaldo Cruz Foundation, Rio de Janeiro, Brazil

14.009 **Standardization of rodent occlusion trauma model and effectiveness of ATB-352, a H₂S-releasing non-steroidal anti-inflammatory.** Oliveira, JP¹, Santos LGK¹, Teixeira, SA¹, Contin, I², Wallace, J³, Muscará,

MN¹, Costa, SKP¹. ¹ICB-USP Dpt of Pharmacology, Brazil; ²Dpt of Prosthesis of Dentistry Faculty-USP, Brazil; ³Dpt of Physiology and Pharmacology, Univ of Calgary, Canada

14.010 *Spirulina platensis* modulates the NO and COX pathways and prevents the increase in uterine contractility promoted by strength training. Barros BC¹, Lacerda-Júnior FF¹, Diniz AFA¹, SouzaPPS², Ferreira PB¹, Silva BA^{1,3,4} - ¹PPgPNSB-UFPB,²PIBIC-UFPB, ³IPeFarM-UFPB, ⁴DCF-UFPB

Courses

Cr1 – Systematic Review and Meta-Analysis for Pharmacologists

Systematic Reviews: The qualitative Synthesis. Vanessa Beijamini. Department of Pharmaceutical Sciences, Pharmaceutical Sciences Graduate Program, Health Sciences Center, Federal University of Espirito Santo, Vitoria, Brazil.

The article reviews are beneficial to the scientific community in providing an overview and summarizing a body of literature evidence. The most known type of review is the traditional or narrative review, in which the authors summarize or critique a group of studies without describing, necessarily, a precise method of how the studies were selected. Systematic reviews differ from narrative ones by notating a search method, presenting explicit inclusion and exclusion criteria, performing qualitative analysis and, when appropriated, quantitative analysis (meta-analysis) of the selected studies. Systematic reviews may identify knowledge gaps or, for instance, factors influencing treatment efficacy or the best experimental models of a disease. A great vantage of systematic reviews is to update and replicate the search easily. The main steps of a systematic review are: define a specific research question, search for all available evidence, select the studies, extract relevant characteristics of those studies, and assess studies quality. The research question usually defines the animal species or population studied (P), the intervention or exposure method (I), the comparison (C), and the outcome (O) for a disease of interest/health problem. For example, “what is the effect of systemic ketamine versus the control group on anxiety/fear-related behaviors in rodent tests for anxiety.” Performing a well-designed and comprehensive search reduces the risk of bias. Thus, it is recommended to search in more than one database, incorporate synonyms and different spellings to the search string, and specify the inclusion and exclusion criteria previously. At least two independent researchers usually perform the selection/extraction of data. The reasons for removing citations should be reported to increase transparency. Typically, study characteristics extracted are species/strain, sex, age, model, disease model, details of intervention (drug, dose), comparison groups, and outcomes. Finally, presenting data extracted in tables facilitates the insights and analysis in similarities and differences between the selected studies (qualitative synthesis).

Risk of bias: Tools to the assessment of research quality. Lino de Oliveira, C. Department of Physiological Sciences, Pharmacology Graduate Program, Biological Sciences Center, Federal University of Santa Catarina, Florianópolis, Brazil.

Systematic reviews and meta-analyses (SRMA) are powerful tools that synthesize literature qualitatively and quantitatively. Syntheses of abundant literature are useful to inform decision making in Pharmacology and Therapeutics. Planning an SRMA involves a long list of methodological and analytical decisions, which a reviewer should take beforehand to minimize sources of bias. Despite the efforts to make transparent and pre-established protocols, some amount of bias is inevitable. Although unavoidable, biases should be estimated to inform about the extent to which an SRMA provides reliable conclusions. There is a myriad of tools to evaluate the quality of reviews allowing for the identification of high-quality SRMA. Among these tools are AMSTAR-1, R-AMSTAR, or ROBIS, developed to assess the SRMAs synthesizing randomized trials, later adapted to evaluate SRMAs of non-randomization studies (e.g., AMSTAR 2). The reliability of an SRMA also depends on the quality of experiments and the validity of data in the primary studies. There are tools such as RoB-Cochrane (1 and 2), ROBINS-I, QUADAS, and RoB-Syrclle to evaluate the risk of bias of the studies included in an SRMA. These tools were created to estimate the risk of biases in different primary studies such as randomized trials, non-randomization trials, diagnostic accuracy studies, preclinical studies in laboratory animals. A tool like RoB-Syrclle, for example, may assess if the risk of selection, performance, detection, attrition, reporting, or other biases is high, low, or unclear for each primary study (in laboratory animals). A transparent report of risk of bias assessment will help the reader appraise the reliability of the conclusions presented in an SRMA.

Cr2 – General Principles and Therapeutic Applications of Extracellular Vesicles.

General Principles of Extracellular Vesicles: origin, cargo, release and uptake. Laura Reck Cechinel Universidade Federal do Rio Grande do Sul

The role of extracellular vesicles (EVs) in the development of novel diagnostic and therapeutic strategies for several diseases has attracted interest. EVs is a collective term including various subtypes of cell-released bilayer membrane-enclosed vesicles that can carry a variety of biologically active molecules and deliver to local or distant targets to execute defined biological functions. The heterogeneity of EVs subpopulations makes this research field complex and challenging. EVs subpopulations can be classified according to their size, origin, composition and density. In addition to morphological differences, EV

subtypes play different roles that are probably dependent on their contents. It is important that researchers have a broad and supportive base to differentiate well-established biological features of EV from speculative assumptions and hypotheses. Therefore, the goal of this class will be to review key concepts of (1) *EVs origin*, such as different biogenesis pathways, for example, endosomal pathway, formation of outward buds from the plasma membrane to release microvesicles and membrane disintegration, among other; (2) *EVs cargo*: in this context it will be important to discuss different EVs cargo such as proteins, lipids, RNAs, etc. Since EVs isolated from different fluids may be traced back to their cells of origin according to their content; although a selective content packaging mechanism remains unclear, RNA molecules (such as microRNAs) and proteins may be selectively sorted to EVs. (3) *Release and uptake of EVs* which might depend on unique features provided by a combination of specific stimulus and surface molecules that can be recognized by the target cell/tissue. The nature of this communication remains a central question in the field and will be discussed in this class. However, it is possible that certain cells attract specific EVs, whereas others reject them, passively or actively.

Isolation and Analysis of Extracellular Vesicles - Potential Clinical Relevance. Rafael Soares Lindoso
Instituto de Biofísica Carlos Chagas Filho – UFRJ

The extracellular vesicles (EVs) are a heterogeneous group of nanosized structures that can be secreted by several cell types. In addition, the EVs can be isolated from different body fluids like blood, urine and saliva. Studies on the potential use of EVs and their translation to the clinic require an optimized process for isolation and a precise characterization as quality control of the samples. This course section will present the techniques that can be applied to promote EV isolation (ultracentrifugation, precipitation, chromatography, immune-magnetic beads, ultrafiltration and microfluidics), pointing to the different aspects to consider as the source, sample availability and costs. Moreover, the course will approach the importance of characterizing the EVs used in therapy or as biomarkers. The characterization requires a combination of techniques that can show size distribution, morphology and the presence of specific markers that will be presented during the course (e.g. electron microscopy, DLS, NTA and immune characterization). Financial support: CNPq, FAPERJ e INCT-REGENERA

Potential Clinical Relevance of Extracellular Vesicles: Therapy and Biomarkers. Ionara Rodrigues Siqueira.
Department of Pharmacology, Federal University of Rio Grande do Sul

Growing evidence shows that extracellular vesicles are involved in biological and pathological processes. Besides, several biomedical and therapeutic applications of extracellular vesicles have been raised, such as biomarkers in order (1) to improve clinical staging of diseases; to predict treatment outcomes and/or evaluate responses of patients to treatments, monitoring regression or progression of disease; (2) to amplify therapeutic approaches including their potential beneficial cargoes, such as mRNAs, miRNAs and proteins, in addition to drug delivery by loading cargoes into extracellular vesicles that target recipient cells and tissues. Although multiple questions remain unanswered, such as if sufficient amounts of effectors cargo are delivered; the possibility of providing customized EVs; and their specificity and selectivity for target tissues and cells, EVs are promising strategies in the management of several diseases. Financial support: CNPq, CAPES

Cr3 – Neuropharmacological Potential of Medicinal Plants in Post-Pandemic Times: New Approaches to Plant Use in Mental Health

Anti-inflammatory activity of ayahuasca and its potential against neuroinflammation from COVID-19: therapeutic implications in neurological and psychiatric diseases. Rafael Mariano Bittencourt (Unisul)

Ayahuasca is a decoction with psychoactive properties, used for millennia for therapeutic and religious purposes by indigenous groups and the population of amazonian countries. It is essentially constituted by β -carbolines and tryptamines, and it has therapeutic effects on behavioral disorders due to the inhibition of the monoamine oxidase enzyme and the activation of 5-hydroxytryptamine receptors, demonstrated through preclinical and clinical studies. It was recently observed that the pharmacological response presented by ayahuasca is linked to its anti-inflammatory action, attributed mainly to dimethyltryptamines (N, N-dimethyltryptamine and 5-methoxy-N, N-dimethyltryptamine), which act as endogenous systemic regulators of inflammation and immune homeostasis, also through sigma-1 receptors. Therefore, since neuroinflammation is among the main pathophysiological mechanisms related to the development of neurological and psychiatric diseases, it is suggested that ayahuasca is a promising and very safe therapeutic strategy since extremely high doses are required to reach toxicity. However, even so, additional studies are needed to confirm such evidence, as well as the complete elucidation of the mechanisms involved.

Plants native to South Brazil as source of new antidepressant prototypes. Stela Maris Kuze Rates (Faculty of Pharmacy – UFRGS - Brazil)

In spite of the great number of antidepressants available in the market for the treatment of major depression, the rate of refractory patients to the treatment is still significant, ranging from 30-35%, which is itself an evidence of the need for the development of better antidepressants. Many medicinal plants and chemical substances isolated from them has been evaluated in animal models and clinical trials. Some of them have been shown to inhibit the expression of cytokines, while others seem to act directly on monoamines, modifying their expression, metabolism, reuptake, or effect on the target. Others exhibit antioxidant effects that can reduce neuronal alterations and damage. Saffron, turmeric (or its principle, curcumin), and St. John's are currently the principal species of interest for potential use as antidepressant agents, with positive effects in clinical trials against both a placebo and standard treatments. Other studies have reported antidepressant-like properties of different *Valeriana* species, which may represent a new approach to the therapeutic use of this genus, since *V. officinalis* has been mainly used for alleviating insomnia and anxiety symptoms. In this class, we will focus on preclinical studies by our group investigating the antidepressant like effects of phloroglucinol derivatives and terpenes isolated from species of *Hypericum* and *Valeriana* natives to South Brazil, respectively. These compounds acts on monoaminergic neurotransmission and on inflammation and oxidative markers. Uliginosin B (ULI), which is a dimeric acylphloroglucinol derivative described in approximately 20 *Hypericum* species native to South America, increased hippocampal GSH, MCP-1 and IL-10 levels. The diene valepotriates from *V. glechomifolia* have antidepressant-like effect mediated by dopaminergic (D₁ and D₂ receptors) and noradrenergic (α 2 receptor) neurotransmission, which represents a dual-action different from most of the available antidepressants. Furthermore, diene valepotriates restored both normal behavior and prefrontal NMDA-dependent long-term potentiation as well as the expression of cortical pro-inflammatory cytokines in LPS-injected mice. Our results are in accordance with accumulating evidences pointing to anti-inflammatory effects of antidepressants and further support the inflammatory hypothesis of depression. Financial Support: INCT (INOVAR), CNPq and CAPES-COFECUB.

Pain in times of COVID and the development of new herbal medicines: Case of a University x Pharmaceutical Company partnership. Tania Mari Bellé Bresolin. Universidade do Vale do Itajaí (UNIVALI), Pharmaceutical Sciences Graduation Program (PPGCF), Pharmacy Course, Health Sciences School.

Aleurites moluccanus (L.) Willd., Euphorbiaceae, popularly known as “noqueira-da-Índia” or “Indian walnut”, is an Asian tree, introduced in Brazil in the 50s, especially in the South and Southeast regions, aiming the extraction of almond oil, used for varnishes, soap, candles, fuels and lubricants, and in tanning. Based on the popular use of the leaves as an antirheumatic, researchers from Univali started a partnership project with Eurofarma (São Paulo, SP), a national pharmaceutical industry, in 1997. The project, although it has not yet reached the final stage of clinical studies and registration in ANVISA, represents a relevant experience to be analyzed in light of the difficulties and overcomes during the stages that make up the chain of establishment of a new herbal medicine in Brazil. Tablets were developed based on the standardized dry extract of *A. moluccanus*, on pilot scale, with approval in the stages of non-clinical studies (mouse and minipigs) and in the clinical stage-phase I. In addition, formulations such as topical cream and oral suspension were developed and evaluated in nonclinical studies. The extract was quantified in flavonoid swertisin, one of the major markers, related with the antinociceptive and anti-inflammatory effect of the extract. These activities were comparable to non-selective non-steroidal anti-inflammatory drugs, but with superior tolerability/safety profile. The potential therapeutic indications to this new phytomedicine are: postoperative pain, low back pain, osteoarthritis (chondropathies), rheumatoid arthritis, among others. The overcoming of challenges related to the standardization of the extract on industrial scale, under good manufacturing practices (GMP) and the stability aspects of the formulations are addressed. Therefore, this Project provides an insight into the setbacks and overcoming experienced by researchers from Brazilian Community University with a focus on research and development of new herbal medicine. Financial support: This work received financial support from the CNPq (Edital Verde Amarelo, 2002), MCT/FINEP Transversal Action – ICT-Business Cooperation (2005), CT-Biotechnology/CT-Saúde/MCT/CNPq/MS/SCTIE/DECIT-Edital BIOINOVA No. 20 (2007) and from Eurofarma (São Paulo, SP)

Cr4 – Ion Channels: Biophysical, Biological and Pharmacological Aspects.

Ion Channels: Biophysical and electrophysiological aspects. Ricardo Mauricio Xavier Leão (USP-RP)

In this lecture we will talk about the biophysical and physiological aspects of ion channels, the basic biophysics of membrane bioelectrogenesis, the physiological role of ion channels, basic pharmacology and molecular structure. Research supported by FAPESP

Ionotropic Receptors (LGIC). Newton G. Castro. Instituto de Ciências Biomédicas, Universidade Federal do Rio de Janeiro, Brasil.

Ionotropic receptors, aka ligand-gated ion channels (LGIC), are multi-subunit transmembrane proteins that allow passive ion flow across the cell membrane upon binding of a (usually) small-molecule agonist. These receptors are best known for their role in fast synaptic transmission, but they are essential to many other types of fast and slow chemical signaling, including sensory transduction and immune cell activation and regulation. I present an overview of the main families of LGIC, covering their distinct structural motifs and main functional and pharmacological properties. The lecture emphasizes the relevance of each LGIC family as a drug target, showing recent advances in the characterization of their multiple binding sites, mechanisms of modulation and possible applications. The targets discussed include nicotinic acetylcholine, GABA_A, NMDA, AMPA and kainate (glutamate), P2X (ATP) and TRP receptor-channels. Examples from the literature and from the Molecular Pharmacology Lab at UFRJ illustrate different experimental approaches to study drug-receptor interaction for selected LGICs. These include data from manual high-content or automated high-throughput patch-clamp assays as well as alternative, non-electrophysiological methods. I expect to draw attention of the students and non-specialist researchers to the continuing interest in LGICs both for drug discovery and basic science ventures, in spite of the methodological challenges they present. N. G. Castro is supported by a CNPq fellowship.

Voltage-activated Potassium Channels as Pharmacological Targets. Cristiano Gonçalves Ponte Núcleo de Ciências Biomédicas Aplicadas. Instituto Federal do Rio de Janeiro (IFRJ)

Voltage-activated ion channels (VGICs) are transcribed by a superfamily of genes and are involved in cellular functions such as signal transduction by controlling membrane potential, increasing intracellular calcium and in homeostasis, controlling cell volume. About half of these channels are permeable to potassium ions and are divided into subfamilies according to protein homology and also by functional parameters. They have different kinetic behaviors and a large number of pharmacological modulators of natural products, synthetic compounds and peptide toxins. Many voltage activated potassium channels (VAPC) are identified as pharmacological targets, but they still lack selective and potent modulators. In this module we will give some examples of VAPCs that are studied in cardiovascular disease, cancer, immunosuppression and urinary incontinence. Identifying the target VAPCs and presenting different strategies for identifying and developing new modulators with pharmacological potential for the clinical treatment of these pathologies will be some of the topics covered in this module. Support: IFRJ, Faperj and CNPq.

Cr5 – Didactic Tools and Methodologies of Active Learning in the Teaching of Pharmacology

Methodologies and technological resources for biomedical education of digital native learners. Camilo Lellis-Santos Instituto de Ciências Ambientais, Químicas e Farmacêuticas – Universidade Federal de São Paulo

Students of the Z generation are already in the higher education classrooms. The didactic strategies should address their culture in order to increase engagement, motivation, and academic performance. Courses for biomedical education, including pharmacology education, can benefit from the inclusion of the technology-assisted teaching and learning. In this course, we will present the active learning methodology designed to include educational technology as medium to foster the interest of digital natives in learning. Examples of smartphone-assisted pedagogical practices will be shared. And the ultimate innovative resources to create a technological environment in the classroom will be presented and used for reflections on student's learning and achievement.

SCREENER, an educational game for teaching the Drug Discovery and Development process. François Noël (UFRJ)

Although the use of games as a strategy for educational purpose is an important current trend, there is practically no option available for training people on the Drug Discovery and Development (DDD) process. In order to fill this gap, we designed "SCREENER", a science pedagogy game that is intended to be educational but also sufficiently challenging and interesting in order to ensure the player engagement. Our main target audience is students of post-graduate programs of pharmacology, medicinal chemistry, toxicology, pharmacy and medicine. We decided to perform the first version in Portuguese in order to avoid any language barrier that could difficult the wide use of this game. Here we discuss the creation of SCREENER, a mixed of board and card game, and present its components with some examples of cards and resources as well as the dynamics of the game. SCREENER mimics the process of discovering and developing drugs from the validation of a target to the registration of the new drug with the regulatory agency and can be played individually (self-learning) or with the help of a monitor, assisting up to six players (or teams). Briefly, 29 task cards categorized in four major areas (efficacy, safety, pharmacokinetics and pharmaceutical development) have to be purchased sequentially. Classical characteristics of games have been incorporated such as decision making and challenge. In-deep

information on the tasks and technical terms are available through QR codes. The vagaries of the DDD are mimicked by the Bonus/Setback cards that have to be read when a player roll the number 6 on the dice. Finally, we will discuss the student's survey performed in May 2021 at the Post-graduation Program in Pharmacology and Medicinal Chemistry of the Federal University of Rio de Janeiro, after the first use of this game in the regular discipline on DDD offered to our students since 2015. Financial support: CNPq

The software “Basic pharmacology of the autonomic nervous system by computer simulation” by Zyngier, Garcia & Zyngier (1995). André Sampaio Pupo (UNESP-Botucatu)

The replacement of practical classes that employ animals by software that simulates the effects of drugs is an old concern in Brazilian pharmacology. Since the mid-1990s we already have available an elegant software developed by Zyngier, Garcia & Zyngier (Department of Pharmacology, University of São Paulo / SP), which allows the animated simulation of the cardiovascular effects of drugs that affect the sympathetic and parasympathetic nervous systems. This software replaces with great convenience the classic practical class to demonstrate the effects of these drugs on the blood pressure of an anesthetized dog. In the mini-course, a version of the Zyngier, Garcia & Zyngier software adapted to work in current operating systems will be presented and examples of its use will be given.

Cr6 – Improving Standards for Reproducibility in Basic Pharmacology

Developing a structure for confirmatory experiments in basic biomedical Science. Olavo B Amaral (UFRJ) The Brazilian Reproducibility Initiative is a systematic, multicenter effort to replicate 60 experiments from the last two decades of Brazilian biomedical science. The project focuses on a set of common methods, repeating each experiment in 3 different laboratories from a network of over 60 labs that integrate the project. Although the results are only expected in 2022, the project has served as a testing ground for setting up a preregistered, confirmatory framework for experiments in the wet lab life sciences, an area where most projects are typically exploratory. In this lecture, we will focus on the challenges of implementing such a framework. As not all protocols can be specified in advance, setting up confirmatory experiments involves registering decision rules for experiments to inform one another, as well as criteria for determining whether they are methodologically valid. Ideally, confirmatory experiments should also try to incorporate heterogeneity, using different labs and methods in order to assess robustness by synthesizing data. Such measures are costly and cannot be expected to be used in every experiment, and most research projects are likely to remain predominantly exploratory. Nevertheless, moving on to a more confirmatory framework at some point of the research process is vital for arriving at robust conclusions on issues deemed important by the scientific community.

Open access to science facilitating the discussion about reproducibility. Plinio Casarotto, PhD Neuroscience Center - HiLife, University of Helsinki, Finland. Journal for Reproducibility in Neuroscience. Finnish Reproducibility Network

The replicability of results is a cornerstone of science, a feature not shared by any other human activity. However, the current journal-based peer-reviewed publication of studies is set in a manner that ‘novelty pays’. Scientists are rewarded if they publish high-impact discoveries in high-impact journals. Institutions, in order to keep their reputation, and the money flowing in, want those scientists publishing well; and journals, to keep their impact factors, flow of articles and subscriptions or fees demand ever more novelty. This feeds a cycle of fast-paced, ill-curated results that unsurprisingly reflects in poor replicability, and therefore reliability of the scientific discoveries. Another contributing factor is the “file-drawer effect”: a significant amount of valuable data is never translated into a manuscript, peer-reviewed and published as a scientific article because this material is an attempt to replicate (successfully or not) previously published studies. There is little reward for successful replications and publishing material with controversial results can face harsh opposition. Consequently, a huge amount of helpful data remains inaccessible to researchers. In the absence of a facilitatory infrastructure that does not discriminate against replications, we decided to provide an Open Access outlet that actively encourages replication work. This led us to launch the Journal for Reproducibility in Neuroscience - JRepNeurosci (ISSN: 2670-3815). JRepNeurosci was set up in early 2020, and it is exclusively dedicated to publishing results of replication studies, being full manuscripts or single experiments. The articles are judged about the soundness of the findings, i.e. if the experiments were properly designed, conducted, analyzed and the results are clear and reliable. All the data should be freely available, respecting the FAIR principles (Findable, Accessible, Interoperable and Reusable) in open repositories. All the reviews (but not the referee's identity) are openly published as a supplement to the main article, and authors can opt for a double-blinded peer-review to prevent implicit biases. Following these principles, the JRepNeurosci is open access and no article processing fees are requested from the authors, a model known as diamond/platinum-OA. Our goal is to become a laboratory where principles of open science to the publication process are evaluated, and a platform where

researchers, regardless of their budget and location, can freely share and obtain information about the reproducibility of models and experiments in the field of basic and applied neuroscience.

Multicenter preclinical studies. Roberto Andreatini. Departamento de Farmacologia, Universidade Federal do Paraná

In recent years, there is a growing concern in preclinical research with the lack of reproducibility of findings and their poor translation to clinical. Several proposals have been posed such as an increasing rigor in methodological procedures (e.g. randomization, sample-size calculations, blinding, etc.) and adherence to structured guidelines such as ARRIVE and PPRECISE. Meta-analysis of preclinical studies is also proposed for increasing translation. Another potential source of poor reproducibility and translation of preclinical data would be the search for high homogeneity of procedures and subjects of studies, using, for example, inbred strains. This high standardization is important to reach high internal validity. In this line, single-center (single-laboratory) studies may have more control over a wide range of variables that results in higher homogeneity. Specifically, in pharmacology, single-center studies with a small sample size are fundamental to screen potential drug candidates. On the other hand, single-center studies may have some problems, as, for example, they do not predict adequately the effect size. The multicenter (or multi-laboratory) studies, similar to multicenter clinical trials, will increase heterogeneity in the sample, which may decrease internal validity but result in increased external validity (generalization) of the data. This increase in heterogeneity gives more representative study samples. Thus, they have a higher translational value than single-center studies. Moreover, the multicenter studies permit the detection of some center effect, avoiding that unknown biases from one center could lead to a potential false result. The sequence from a positive result on preclinical single-center studies to confirmatory larger sample-size studies is analogous to drug clinical development, that small sample-size rigorous studies (Phase II) will be followed by multicenter studies with a larger sample size (Phase III). Thus, preclinical multicenter studies can permit the conduction of adequate confirmatory studies with a higher translational perspective, improving the preclinical base for clinical studies. Financial support: CNPq

Cr7 – **Scientific Dissemination and Popularization in Pharmacology: Lessons from the Past, Current Challenges and Opportunities in the Post-Pandemic World**

Lessons from the past about scientific dissemination and popularization in pharmacology Luisa Mota da Silva - Graduate program in pharmaceutical sciences, University of Vale do Itajaí (UNIVALI)

Among the many effects and consequences, for good and bad, that the COVID-19 pandemic brought to our society is the highlight of the importance of scientific dissemination and popularization. Especially in pre-pandemic Brazil, the population knew even less about science, which could not be different since they have not been educated for it. Moreover, people got used to the stereotype of scientists vastly different from what we are. And rejecting this ridiculous vision, we do not take our place of speech and remain silent when faced with pseudoscientific absurdities. But we were called to our duty with the case of phosphoethanolamine as a cure for cancer and we decided to speak given the seriousness of that situation. Unfortunately, at that moment we found that our voice was incredibly low and almost no one was listening. Then came many other contexts that suddenly forced us to speak more and more audible and understandable, and that is how we took our place against the anti-vaccine movement and so many other anti-science movements. It was in this scenario, where, in a way, our efforts to popularize science grew, that the new coronavirus pandemic mercilessly emerged and with it the infodemic. Unluckily, there was no time to overcome the Cassandra syndrome that collapsed Brazilian science, especially the health sciences, including pharmacology. We still lacked credibility and saw many lives being claimed by fighting the pandemic in a way not based on scientific knowledge, including the use of drugs without scientific validation as early treatment. The current numbers of the pandemic in Brazil do not lie, the paths taken were not illuminated by the light of science, but we scientists are increasingly engaged in calling the population to the path guided by science and we will not remain silent because we understand that the attacks on science they are also attacks on democracy and life. Financial support: UNIVALI; CNPq.

Current challenges in scientific dissemination and popularization Hermógenes David de Oliveira: Núcleo de Pesquisa e Desenvolvimento de Medicamentos, Departamento de Bioquímica e Biologia Molecular, Universidade Federal do Ceará

The dissemination of scientific knowledge has been expanded and disrupted by the internet and digitally networked technologies. This activity is an essential tool for expanding the frontiers of knowledge. It fuels a virtuous circle that allows researchers to reach society and citizens to reap the rewards, in cultural, and more concrete terms, of society's investment in research. However, the pandemic has taught us that publishing our scientific results in high-quality journals is not enough to demonstrate the impact of our research on society. Therefore, researchers around the globe agree that it is vital to find new ways of

reaching and involving audiences beyond their usual primary dissemination targets. Additionally, there are concerns about the misinformed public regarding scientific consensus on vaccine safety, evolution, or climate change, to cite few examples. Beyond these concerns, the democracy score has declined in several countries during the last decade, making this issue more complex and problematic. A big question can be raised based on the facts mentioned above: What are the main challenges in science dissemination and popularization in the social media era? The first challenge to overcome is improving the science literacy for the population, enabling everyone to understand better the world where they live, enlarge their critical capacity and citizen participation, and exercise better surveillance upon their own lives and society. Secondly, it is imperative to become science dissemination and popularization activities a central feature of academic routines, expand financial support, and promote more events and conferences for students and researchers devoted to this topic. Moreover, it is essential to use innovative science dissemination practices, then, means dissemination beyond traditional academic publishing (e.g., academic journals, books, or monographs) and meetings (conferences and workshops) to achieve more widespread research uptake and understanding. Prioritizing rapid and reliable information is a way to fight against misinformation. In a global pandemic, we could perceive that clear communication galvanized public responses, reduced anxiety, and improved well-being. Ultimately, it is necessary to go beyond scattered science dissemination practices to a more organizational model of action: to define objectives, map potential target audience(s), target messages, specify the mode of communication/engagement, and create a dissemination plan for universities and research institutes, for example. In conclusion, public science dissemination, not just academic publications, are crucial to today's researchers. Assessing, identifying, and overcoming the challenges of this practice will give us the evidence for the most effective strategies to reach the audience. Supported by: UFC

Opportunities in scientific dissemination and popularization in pharmacology field in the post-pandemic world. Alexandra Acco Department of Pharmacology, Federal University of Paraná (UFPR)

The current COVID-19 pandemic has created a need for rapid knowledge and solutions in response to the disease, and approximates the general public to biomedical and health sciences. The pandemic highlighted the difference between scientific communication and scientific dissemination, which is the diffusion of scientific and technological information to the general public. Many scientists around the world have expanded their scientific divulgation, driven by the opportunity to explain scientific results to the lay public. Scientists have established open science even with closed borders. However, the pandemic accelerated the spread of fake news, infodemic, information overload and cyberchondria. Information overload during the COVID-19 pandemic has posed a set of challenges that were not encountered before: false news, conspiracy theories and magical cures are shared at an alarming rate. Thus, some areas of science, such as health, informatics and basic sciences, have proven to be essential in combating infodemic and promoting quality of life. Pharmacology (non-clinical and clinical) and Therapeutics are inserted in this context and foresee opportunities for the science dissemination in the post-pandemic period, through: a) establishing and strengthening national and international networks for cooperation and scientific dissemination; b) expanding the reach of science in various media and formats (TV, internet, podcast, social networks, scientific journals, pre-print repositories, e-book); c) conducting multidisciplinary (teachers, researchers and journalists) courses and training in science dissemination; d) developing public and private policies to encourage science communicators; e) promoting integration with journalists, communicators and press vehicles; and f) establishing an academic culture of science dissemination in the training of young pharmacologists. Thus, scientific dissemination will be a counterpoint to infodemic, which has caused more harm than benefit; it can guide attitudes of authorities and society; and it can accelerate the discovery of efficient solutions to health issues. Financial support: UFPR; CNPq.

Cr8 – Pandemic' Stress as a Risk Factor for Neuropsychiatric Disorders: Insights from Translational Evidences

Stress as a risk factor for anxiety and depression. Cristiane Aparecida Favoretto. Departamento de Farmacologia, Universidade Federal de São Paulo, São Paulo, Brasil

The term "stress" is defined as a set of organism's response to stimuli that may disturb homeostasis. Physiologically, exposure to stress results in catecholamines (adrenaline and norepinephrine) release by autonomic nervous system, and activation of hypothalamic-pituitary-adrenal (HPA) axis. In addition, HPA axis activity may be modulated by several neurotransmission systems, such as the monoaminergic (noradrenergic, serotonergic and dopaminergic). Studies indicate that repeated exposure to stress affects HPA axis function, as well as brain monoamine levels, mechanisms that possibly underlies the increased vulnerability to stress-induced neuropsychiatric disorders, such as anxiety and depression. Anxiety disorders affects approximately 3.6% of global population and is characterized by negative affect, fear sensation,

excessive concern, and physiological events, such as sweating, nausea, hormonal changes and tachycardia. Also, more than 300 million people around the world are diagnosed with major depressive disorder, which symptoms include persistent negative mood, anhedonia, fatigue, social deficits, and represents one of the leading causes of disability and death. The main source of stress in humans arises from social contexts. In the same way, the main type of stress that we have been exposed during the coronavirus pandemic is caused by social situations (social isolation, changes in family and work relationships, grief, etc.). Thus, animal models that use social disturbances as stressful stimuli have been established, enabling the study of behavioral and neurochemical consequences of exposure to social stress. Maternal deprivation/separation, social defeat and social isolation represents examples of commonly used animal models of social stress, especially in rodents. In this class, we will approach an overview on physiology of stress, behavioral and neurobiological consequences of exposure to social stress, and how this implicates in increased vulnerability to anxiety and depression. Financial support: FAPESP, CAPES, CNPq

Impact of stress on substance use disorders (SUD) or addiction. Paula Cristina Bianchi Departamento de Farmacologia, Universidade Federal de São Paulo (UNIFESP), São Paulo – SP, Brazil

Addiction is defined as a chronic, relapsing disorder characterized by compulsive drug seeking, continued use despite harmful consequences, and long-lasting changes in the brain. Addiction is the most severe form of a full spectrum of substance use disorders, and is a medical illness caused by repeated misuse of a substance or substances of abuse. Stress has long been known to increase vulnerability to addiction. The last decade has led to a dramatic increase in understanding the underlying mechanisms for this association. Behavioral, neurobiological correlates, and molecular and cellular changes associated with chronic stress and addiction has been identified in human and non-human animals. The outbreak of the 2019 novel coronavirus (COVID-19) has had a devastating global impact. One ensuing consequence of COVID-19 is the substantial negative economic impact, which has resulted in loss of employment and income for millions of people worldwide. Given the devastating consequences of COVID-19, increases in psychological symptoms and disorders, including depression, anxiety, stress, worry, and substance use, among others, have been observed and a recent report emphasizes the importance of considering substance use problems in the context of COVID-19. Specifically, COVID-19 is hypothesized to interfere with substance use disorder treatment, causing an increase in withdrawal symptoms and relapse. A recent study realized in the United States showed that 29% of survey respondents reported increased alcohol use since the start of the COVID-19 pandemic. In addition, the researchers observed that the odds of reporting increased alcohol use were higher in persons with symptoms of anxiety and depression. Although the literature concerning substance use in the context of COVID-19 is only nascent, past research from other large-scale disasters suggests that, in general, increases in substance use are observed following disaster exposure. In this class, we will approach an overview on neurobiology of addiction and some aspects of behavioral, molecular and cellular changes associated with chronic stress and addiction. Financial support: FAPESP, CAPES, CNPQ

Stress and schizophrenia. Cássio Morais Loss. Departamento de Farmacologia, Universidade Federal de São Paulo (UNIFESP); Instituto Nacional de Ciência e Tecnologia Translacional em Medicina (INCT-TM)

Schizophrenia is a psychiatric neurodevelopmental disorder with a lifetime prevalence of just under 1%. It stands out as one of the most debilitating psychiatric disorders because it impairs brain functioning in multiple ways, triggering the expression of positive, negative and cognitive symptoms. Negative and cognitive symptoms are more enduring and can precede the first psychotic episode by years. This period, defined as the prodromal phase, occurs during adolescence/early adulthood in which the expression of “limited intermittent psychotic symptoms” and “attenuated psychotic symptoms” are also observed. Individuals identified to present these symptoms are classified as at clinically high-risk (CHR). During this CHR state, there are several pro-schizophrenia events (risk factors) that can increase the risk of conversion to a full-blown manifestation of schizophrenia. Due the current “2019 novel coronavirus” (COVID-19) pandemic state, the risk factors related to stressful events deserve special attention. Stress, as well as some related behaviors (such social isolation and drug abuse), are suggestive of a high risk of conversion to schizophrenia in CHR individuals. Likewise, pro-schizophrenia effects of stress are also observed in schizophrenic patients, who present worsening of symptoms after experiencing stressful events. In this class I will discuss some preclinical and clinical findings around the topic “stress and schizophrenia”, with special focus on the prodromal period. Finally, as the prodromal phase is the most compelling ‘window of opportunity’ for preventing or at least slowing schizophrenia progression, I will also approach some evidence around pharmacological strategies focused in treat schizophrenia during this period. Financial support: INCT-TM, Capes, CNPq, FAPESP

Cr9 – *Tenebrio molitor* Larvae Model and its use in Pharmacology Teaching and Research

Use of animal models in Pharmacology: most used animal species versus alternative animal models. Maria Christina Werneck de Avellar (Unifesp-EPM)

Advances in biomedical sciences have greatly rely on research data with mammalian models, being rodents the most used group of species. The species chosen, the number of animals and experimental protocol used are dependent on the goals/type of the study. The strategy of the 3Rs (i.e., reduction, refinement and replacement) is being applied in a practical context. In parallel, there is a growing literature highlighting experimental models with various alternative organisms (such as fishes, flies, worms, insects, among others) that allow real time monitoring of the effect of a tested compound. There are examples showing that the findings in these alternative models could help to reduce/refine subsequent planning/development of preclinical/clinical studies for drug and chemical testing. These topics will be addressed during this talk.

Overall physiological similarity between insects with humans and rodents. Daniele Maria-Ferreira, Lara Luisa Valerio de Melo Braga, Fernanda da Silva Platner, Elizabeth Soares Fernandes Instituto de Pesquisa Pelé Pequeno Príncipe and Faculdades Pequeno Príncipe, Curitiba, PR, Brazil

Model organisms are often used in biomedical research. Among insects, *Drosophila melanogaster*, *Caenorhabditis elegans* and *Galleria mellonella* are often used. However, new experimental models still appear and in recent years, and an increasing number of insect species has been suggested as model organisms in life sciences research, including *Tenebrio molitor*. This has occurred more frequently due to the ease of acquisition, low cost of rearing, and the possibility of extrapolating research studies to vertebrates. Furthermore, insects have several similarities at the physiological level with mammals. In this sense, this lecture will discuss the overall physiological similarity between insects with humans and rodents, which enables the development of experiments aimed at reducing or even replacing rodents in research and education. Financial support: Instituto de Pesquisa Pelé Pequeno Príncipe

Use of *Tenebrio molitor* to study and investigate novel therapies. Elizabeth Soares Fernandes, Fernanda da Silva Platner, Gisele Simão, Daniele Maria-Ferreira. Instituto de Pesquisa Pelé Pequeno Príncipe and Faculdades Pequeno Príncipe, Curitiba, PR, Brazil

Animal models have largely been used in Pharmacology research and education. Although rodent models have been the most used ones, alternative animal models such as the *Tenebrio molitor* larvae model represent useful tools to investigate and learn of the mechanisms of disease as well as of novel therapies. In fact, insects such as *T. molitor* share common features to those of mammalians in regard to their physiology and ability to respond to harmful stimuli. Therefore, the larvae model offers ground to develop translational research and new educational tools with application in Pharmacology and correlated areas. This lecture will discuss the potential use of *T. molitor* in drug discovery, in particular, in the study of novel antimicrobials, anti-inflammatory and antioxidants. Financial support: CNPq and Instituto de Pesquisa Pelé Pequeno Príncipe

Cr10 – Evaluation of Clinical Trials for Vaccine Approval Gustavo Mendes Lima Santos (ANVISA)

The COVID-19 pandemic required from regulatory agencies around the world innovative strategies for the rapid approval of vaccines against COVID-19, without dispensing with the minimum criteria of quality, safety and efficacy. In Brazil, Anvisa is responsible for the regulatory assessment that authorizes the use of vaccines. The classes will cover the main topics related to regulatory approval in Brazil of vaccines against COVID-19. Three main topics will be discussed. In the first, non-clinical and clinical studies conducted to demonstrate efficacy and safety will be discussed and criteria for evaluation and approval by Anvisa will be presented. The primary and secondary outcomes, as well as the main safety aspects observed in the studies are part of the criteria for approving a vaccine package insert. At the second meeting, data on the biotechnological development of vaccines will be discussed, as well as tests for quality control of batches and their respective validations. At the last meeting, the topics considered by the agency for the approval of risk minimization plans in the use of vaccines will be presented, in addition to the pharmacovigilance plan, which make up the monitoring proposals. The agency's regulatory review will be discussed so that the public can understand the rationale for approving COVID-19 vaccines, based on a risk/benefit ratio

Cr11 – G-Protein Coupled Receptors (GPCRs) and their Signal Transduction Pathways

This course aims to present the news in the structure and function of G-protein coupled receptors (GPCRs) with an emphasis on their two main effector systems, Adenylyl Cyclase and Phospholipase C beta. We will discuss the main physiological roles of the signal transduction pathways, Adenylyl Cyclase and Phospholipase C, highlighting their relevance for understanding the mechanisms involved in the treatment

of some pathological processes. The course will work with synchronous classes, which will enable the interaction of participants with teachers.

Lectures

C1 – 6-NitroDopamine, the New kidDo in town. Gilberto de Nucci. De Nucci, G(Unicamp)

The 6-nitrodopamine (6-ND) is a novel cardiovascular mediator detected by Liquid Chromatography In Tandem Mass Spectrometry (LC/MS-MS). This new mediator is released from endothelium of human umbilical cord vessels, rat vas deferens and by the tortoise aorta endothelium. In the human umbilical arteries and veins 6-nitrodopamine modulates vascular reactivity by acting as a dopamine D2-antagonist. The endothelium intact release was inhibited by the pre-treatment with NO synthesis inhibitor L-NAME (100 μ M). In contrast to dopamine, noradrenaline and adrenaline, 6-ND did not contract HUCV, even in presence of L-NAME or ODQ. 6-ND (10 μ M) produced a rightward shift of the concentration-response curves to dopamine (pA₂: 5.96 in HUA and 5.72 in HUV). Contractions induced by noradrenaline and adrenaline were not affected by pre-incubation with 6-ND (10 μ M). These findings may rewrite understanding of the role of catecholamines in cardiovascular control. In the rat vas deferens, basal releases of 6-ND and noradrenaline were detected from the rat isolated vas deferens. 6-ND release was reduced by tissue incubation with L-NAME and from the vas deferens obtained from L-NAME-treated rats. SCH-23390 caused leftward shifts on concentration-response curves to 6-ND without affecting dopamine- or EFS-induced rat isolated epididymal vas deferens (RIEVD) contractions. Haloperidol, PG-01037 and sonepiprazole caused significant rightward shifts on concentration-response curves to dopamine but had no effect on either the 6-ND or EFS-induced RIEVD contractions. The tricyclic compounds desipramine, clomipramine, amitriptyline, cyclobenzaprine and carbamazepine induced rightward shifts on 6-ND concentration-response curve but did not reduce the noradrenaline, dopamine and adrenaline contractile responses. The finding that 6-ND is released in substantially greater amounts than noradrenaline (20-fold approximately) generates the exciting possibility of its involvement in the ejaculatory process. Funding: FAPESP - Process number 2021/02373-5

C3 – Kinases as Potential Therapeutic Targets for Anti-Coronaviral Therapy. Thanigaimalai Pillaiyar & Stefan Laufer. Institute of Pharmacy, Pharmaceutical/Medicinal Chemistry and Tuebingen Center for Academic Drug Discovery, Eberhard Karls University Tübingen, Auf der Morgenstelle 8, 72076 Tübingen, Germany.

The global coronavirus disease-19 (COVID-19) has affected more than 140 million and killed more than 3 million people worldwide as of 20 April 2021. The novel human severe respiratory syndrome coronavirus-2 (SARS-CoV-2) has been identified as an etiological agent for COVID-19. Several kinases have been proposed as possible mediators of multiple viral infections, including life-threatening-coronaviruses like SARS-CoV-1, Middle East syndrome coronavirus (MERS-CoV), and SARS-CoV-2. Viral infections hijack abundant cell signaling pathways, resulting in drastic phosphorylation rewiring on the host and viral proteins. Some kinases play a significant role throughout the viral infection cycle (entry, replication, assembly, and egress), and several of them are involved in the virus-induced hyperinflammatory response that leads to cytokine storm, acute respiratory distress syndrome (ARDS), organ injury, and death. Here, we highlight kinases that are associated with coronavirus infections and their inhibitors with antiviral and potentially anti-inflammatory, cytokine-suppressive, or anti-fibrotic activity.

Technical Lecture – New technologies to accelerate drug discovery – the impact of qualified data. Gabriel Kaetan Baio Ferreira (CAS, a Division of the American Chemical Chemical Society)

When pandemics strike, drug discovery and repurposing become critical for faster development of therapies. However, assembling all of the critical information and connections around new proteins, new viruses, targets, pathways, and clinical information can be challenging. A comprehensive analysis of qualified and well-structured scientific data is key to develop new technologies to accelerate innovation and streamline the investigative process for drug discovery and repurposing. This presentation will show how CAS leverages their unique connections across the world's science for novel knowledge graphs that identify top clinical candidates to repurposing and to accelerate drug discovery.

C4 – What does basic neuroscience research predict about the future of pain management? Allan Basbaum, PhD, FRS, Department of Anatomy, University California San Francisco

Local anesthetics block all voltage-gated Na channels and are the most effective analgesic, producing temporary relief in many peripheral injury-induced chronic pain conditions. Unfortunately, the side effects of systemic lidocaine limit its use. As the NaV1.7 subtype of Na channel is expressed almost exclusively in sensory neurons and that NaV1.7 loss of function underlies a condition of congenital indifference to pain, efforts continue to develop selective NaV1.7 antagonists. The molecular heterogeneity of nociceptors also points to the utility of selectively targeting specific molecular entities (e.g., TRPV1, TRPM8). Gene

therapy approaches that use a virus to increase sensory neuron expression of a novel receptor are also being developed. When engaged by a novel ligand, these receptors inhibit sensory neuronal activity and pain. As the novel ligand can only act at the expressed receptor, therapeutic window is greatly increased. Another promising approach attempts to alter the chemical milieu of the injury, for example, using antibodies directed against nerve growth factor. Of course, opioids are highly effective analgesics, but have a poor therapeutic window, with many serious adverse side effects. One effort is developing so-called biased opioid receptor agonists. These opioids exert an analgesic action through one downstream pathway, and ideally do not engage beta arrestin-mediated downstream effects that are implicated in respiratory depression, constipation and dependence. Unclear, however, is the extent to which existing biased agonists are, in fact, partial agonists. Also of interest are recent chemogenetic approaches, in which a novel receptor is targeted to “pain” circuits. The receptor is engaged by a novel ligand, resulting in selective inhibition of the pain processing circuit. And depending on the limited engagement of the novel ligand with endogenous receptors, the therapeutic window for pain control can be significantly increased. Lastly, our laboratory has taken a very different direction. Our therapeutic approach considers that neuropathic pain is not a symptom of nerve damage, but rather represents a “disease” of the nervous system, comparable to epileptic conditions that result from reduced cortical GABAergic inhibition. To date, we have demonstrated that spinal cord transplants of embryonic progenitors of GABAergic interneurons can reduce the mechanical allodynia and ongoing pain that occurs in peripheral nerve injury and chemotherapy models of neuropathic pain. These and other basic science-influenced approaches to novel pain management will be discussed.

C7 – Compositions of cannabis compounds for the treatment of medical conditions: Covid-19 and Glioblastoma. Hinanit Koltai, PhD Senior Research Scientist. ARO, Volcani Institute. Rishon LeZion, Israel
Cannabis sativa is widely used for medical treatments. Cannabis produces hundreds of different compounds, and the optimal combinations of molecules with best activity for the treatment of medical conditions are unknown. We have examined the anti-inflammatory activity of cannabis on immune response markers associated with coronavirus disease 2019 (COVID-19) inflammation. A high CBD extract fraction (FCBD) from a high CBD strain substantially reduced dose dependently inflammation-associated markers in an alveolar epithelial cell line. Treatments with FCBD and a FCBD formulation using phytocannabinoid standards (FCBD:std) reduced in these cells expression of angiotensin I converting enzyme 2 (ACE2), which is the COVID-19 virus (SARS-CoV-2) receptor. However, treatment with FCBD induced macrophage polarization, phagocytosis and expression of inflammation markers in vitro. FCBD:std, while maintaining anti-inflammatory activity in alveolar epithelial cells, led to reduced phagocytosis and pro-inflammatory markers secretion in macrophages in comparison to FCBD. The phytocannabinoid formulation may show superior activity versus the cannabis-derived fraction for reduction of lung inflammation, yet there is a need of caution proposing cannabis as treatment for COVID-19. Glioblastoma multiforme (GBM) is highly invasive and lethal subtype of glioma brain tumors. Individual phytocannabinoids have been shown to trigger GBM cell death. We identified fractions from a high THC cannabis strain that substantially reduced human GBM cell viability and their ability to migrate. The fractions also reduced the ability of GBM cells to form neuro-spheres in 2D and 3D models. These neuro-spheres are associated with the resistance of GBM to chemotherapy. Hence, these results suggest that the cannabis treatment may also have potential for reducing the development of GBM resistance to current therapies. Yet, clinical trials are needed in order to determine the effectiveness of the fractions and combinations of cannabis compounds against GBM.

C8 – Potassium Channels in Breast Cancer Development and Treatment. Peter Ruth, Department of Pharmacology, Toxicology and Clinical Pharmacy, Institute of Pharmacy, University of Tuebingen, Germany. Several tumor entities have been reported to overexpress K⁺ channels. The KCa3.1 K⁺ channel reportedly contributes to the proliferation of breast tumor cells and may serve pro-tumor functions in the microenvironment. The putative interaction of KCa3.1 with major anti-cancer treatment strategies, which are based on cytotoxic drugs or radiotherapy, remains largely unexplored. We employed KCa3.1-proficient and -deficient breast cancer cells derived from breast cancer-prone mice, pharmacological KCa3.1 inhibition, and a syngeneic orthotopic mouse model to study the relevance of functional KCa3.1 for therapy response. KCa3.1 activation by ionizing radiation in breast tumor cells enhanced radioresistance, probably via an involvement of the channel in ionizing radiation stimulated Ca²⁺ signals and DNA repair pathways. Consistently, KCa3.1 knockout increased survival time of wildtype mice upon syngeneic orthotopic transplantation of breast tumors followed by fractionated radiotherapy. Combined, our results imply that KCa3.1 confers resistance to radio- but not to chemotherapy. Apart from KCa3.1, also the voltage- and Ca²⁺-activated K⁺ channel with large conductance (BK channel) promote malignant phenotypes of breast tumour cells. Auxiliary subunits such as LRRC26 may be required to permit activation

of BK currents at a depolarized resting membrane potential occurring in tumour cells. Anti-tumour effects of BK loss were investigated in breast tumour-bearing transgenic BK knockout (KO) mice, and in a syngeneic transplantation model of breast cancer. The therapeutic relevance of BK channels in the context of endocrine treatment was assessed in human breast cancer cell lines expressing either low or high levels of BK and LRRC26, as well as in BK-negative cells. BK promoted breast cancer onset and overall survival in preclinical models. Conversely, lack of BK or knockdown of LRCC26 attenuated proliferation of murine breast cancer cells. At low concentrations, tamoxifen and its active metabolites stimulated proliferation of BK-positive breast cancer cells, independent of transcriptional activity controlled by the oestrogen receptor. Finally, tamoxifen increased the relative survival time of BK KO but not of wild-type tumour cell recipient mice. Thus, breast cancer initiation, progression, and tamoxifen sensitivity depend on BK channels.

Symposia

S1 – Frontiers of Antidepressant Research: Approaches for Discovery

Multi laboratory studies for antidepressants discovery. Roberto Andreatini. Departamento de Farmacologia, Universidade Federal do Paraná

Multicenter (or multi-laboratory) preclinical study has been proposed to increase the reproducibility and translational value of a preclinical research finding. In antidepressant drug discovery, single-center (or single-laboratory) studies are important to screen several putative antidepressant-like drugs, indicating the most promising drug candidates to be evaluated in a confirmatory multicenter study. As single-center studies, multicenter studies also present rigorous methodology (e.g. randomization, sample size calculations, blinding, predetermined primary and secondary outcomes, inclusion of male and female animals, etc.) but they also introduce additional steps as an independent administrative center (i.e., a center that does not perform experiments) and a protocol brochure (with detailed methodological procedures and standardized data recording). Moreover, independent data monitoring and analysis could be implemented. The administrative center can also handle logistic issues (e.g. test drug distribution). Ethical evaluation should be requested from the local ethical board by each laboratory. Multicenter studies can also detect an unrecognized bias in one center that may influence the general results (“center effect”). On the other hand, multicenter studies may increase data variability due to heterogeneity of subjects (e.g. gene-environment interaction), small differences in procedures (e.g. standard vivarium care), etc., requesting a more robust result to detect a positive effect. This increase in sample heterogeneity would lead to a more representative sample, which will enhance its external validity. The selection of the centers involved in a multicenter study may require some characteristics of laboratories (e.g. previous high-quality studies with antidepressant-like drugs, experience with the model selected, etc.) and a preliminary laboratory accreditation could be done by a consortium formed to conduct confirmatory studies for evaluation of antidepressant-like drugs. In conclusion, multicenter studies can contribute to improving preclinical data translation to clinical development of new antidepressant drugs. However, currently, it is still a hypothesis that needs to be corroborated. Financial support: CNPq

S2 – Transient Receptor Potential (TRP) Channels as Targets for Pain Control

Transient receptor potential (TRP) channels as targets of natural products in pain models. Maria Fernanda de Paula Werner, Pharmacology Department (UFPR)

Pain is an agonizing but essential sensation that prepares the body for potentially tissue damage while guaranteeing its protection. However, pain remains a universal problem throughout medicine and may represent an unmet clinical need. Thus, novel effective therapeutic agents are necessary to minimize acute and chronic pain, along to the identification of safer analgesic molecular targets with diminished side effects. Transient receptor potential (TRP) channels are a well-documented group of non-selective cation channels expressed throughout the somatosensory nervous system, that integrates multiple pain producing stimuli. Members of the TRP family channels, such as TRPV (TRP vanilloid) and TRPA (Ankyrin), have gained attention as potential therapeutic targets for treating several pathological conditions, including painful states. The use of natural products, especially those derived from medicinal plants, is a traditional form of providing pain relief. Considering that natural products have undoubtedly played an important role in the TRPs family study, we have been employed the ethnopharmacological approach as strategy for study pharmacological properties of medicinal plants in *in vivo* mice models of acute and chronic pain, using different experimental methodologies. Our group showed that *Acmella oleracea* (L.) R.K. Jansen (*jambu*), a medicinal plant used by some communities from Amazon region to treat toothache, promotes analgesic and/or anesthetic effects due to the presence of alkylamides (mainly spilanthol), through the modulation of TRPV1 and TRPA1 channels. Moreover, we also investigated the mechanisms underlying the

Petasistes hybridus (butterbur), that is used for migraine prophylaxis. The bioactive constituent of butterbur extract are sesquiterpenes (petasins), which seems to be responsible for the periorbital antiallodynic effects, through the modulation of TRPV4 and TRPA1 trigeminal signaling. Here we are going to discuss the importance of the data presented above to develop innovative products that are beneficial for human health. CEUA/BIO – UFPR 544, 970, 1107, 1299. Financial Support: CNPq (476653/2010-0; 313982/2020-1) and Capes (Finance Code 001).

Peripheral Nerve Resident Macrophages and Schwann Cell TRPA1 in Cancer Pain. Francesco De Logu¹, Matilde Marini¹, Lorenzo Landini¹, Daniel Souza Monteiro de Araujo¹, Niccolò Bartalucci², Gabriela Trevisan³, Gennaro Bruno^{1,4}, Martina Marangoni¹, Brian L. Schmidt⁵, Nigel W. Bunnett⁶, Pierangelo Geppetti¹ and Romina Nassini¹. ¹Department of Health Sciences, Clinical Pharmacology Unit, University of Florence, Italy, ²Department of Experimental and Clinical Medicine, University of Florence, ³Graduated Program in Pharmacology, Federal University of Santa Maria (UFSM), ⁴Division of Pediatric Oncology/Hematology, Meyer University Children's Hospital, ⁵Department of Oral and Maxillofacial Surgery, Bluestone Center for Clinical Research, New York University College of Dentistry, New York, ⁶Department of Molecular Pathobiology, New York University College of Dentistry, New York

Pain is a common and devastating symptom of cancer, which afflicts 70-90% of cancer patients and can diminish the quality of life more than the cancer itself. However, cancer pain remains incompletely understood and poorly managed, thus representing a major unmet medical need. A series of cytokines, chemokines, and their receptors have been proposed to contribute to signal cancer pain and although cytokines may directly or indirectly attract inflammatory and immune cells, the implication of macrophages in cancer pain remains unknown. The role of macrophages in neuropathic pain associated with nerve injury has been extensively investigated, and distinct macrophages-dependent proalgesic pathways have been identified in the central and peripheral nervous systems. Thus, neuropathic pain may be promoted by macrophages, which, recruited on demand after neural injury, rapidly invade the damaged peripheral nerves as mature and activated macrophages to promote neuroinflammation and persistent pain. The Transient receptor potential ankyrin 1 (TRPA1), a proalgesic ion channel expressed in a subset of primary sensory neurons, is uniquely sensitive to oxidative stress byproducts. In a mouse model of neuropathic pain, TRPA1 expressed in Schwann cells was proposed to maintain mechanical allodynia elicited by oxidative stress generated by macrophages recruited at the site of nerve injury. However, the mechanism by which TRPA1 mediates mechanical allodynia associated with tumor growth is unknown. Our study aims at identifying a role for macrophages in mechanical allodynia in murine models of cancer-induced pain. We proposed that neuroinflammation and mechanical allodynia are maintained by a feed-forward mechanism which requires the continuous interaction between Schwann cell TRPA1 and expanded macrophages throughout the entire sciatic nerve trunk. Thus, macrophages, oxidative stress, and Schwann cell TRPA1 signal mechanical allodynia in mouse tumor models and may represent potential targets for the treatment of cancer pain. Financial Support European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme (Grant Agreement No. 835286; to PG), Associazione Italiana per la Ricerca sul Cancro (AIRC, IG2016-ID19247 and IG2020-ID24503) and Fondazione Cassa di Risparmio di Firenze, Italy (to RN), NIH (NS102722, DE026806, DK118971, DE029951, to NWB, BLS), and Department of Defense (W81XWH1810431, NWB, BLS).

Neuropathic pain observed in multiple sclerosis is mediated by TRPA1 activation. *Gabriela Trevisan, Universidade Federal de Santa Maria.*

Central neuropathic pain (NCD) is a common symptom in multiple sclerosis (MS), this type of pain is often difficult to treat, thus it is associated with poor quality of life for patients. The neuroinflammation process and mitochondrial dysfunction in MS lesions generate reactive species. Transient potential receptor ankyrin (TRPA1) is identified as one of the main mechanisms contributing to neuropathic pain signaling and can be activated by products of oxidative stress. Initially, we performed experiments to observe the involvement of the transient receptor ankyrin 1 potential (TRPA1) in neuropathic pain observed in two models of multiple sclerosis (MS) in mice. Until the time of the publications arising from this project, there were still no data regarding the role of the TRPA1 receptor in neuropathic pain observed in MS models. Models of progressive multiple sclerosis (PMS) and relapsing-remitting multiple sclerosis (RRMS) in mice were used. From the results obtained we demonstrate that these models cause nociception (mechanical and cold allodynia, and heat hyperalgesia) which is reduced by the administration of gabapentin, a drug used to control neuropathic pain. Also, nociception in these models depends on TRPA1 receptor activation, as TRPA1 receptor antagonists (HC-030031, A967079, propyphenazone and dipyrone) were able to reduce nociceptive behaviors. Treatment with the antisense oligonucleotide for TRPA1 also reduced nociception in both MS models. We did not observe changes in TRPA1 channel expression in the RRMS model, but there was an increase for the mRNA of this receptor in the spinal

cord in the PMS model. There is also an increase in the production of TRPA1 receptor agonists (4-hydroxynonenal and hydrogen peroxide) and in the activity of superoxide dismutase and NADPH oxidase enzymes in the spinal cord after induction of PMS and RRMS models. Furthermore, treatment with an antioxidant compound (α -lipoic acid) or an NADPH oxidase inhibitor (apocynin) was able to reduce nociception in both models. Thus, the TRPA1 receptor may be a potential target for the treatment of neuropathic pain in multiple sclerosis with the results that have been obtained and published so far. Apoio Financeiro: CNPq, CAPES, L'ORÉAL - ABC - UNESCO Para Mulheres na Ciência.

S3 – Application of Nanotechnology in the Treatment of Inflammatory Disorders

Lipid-core nanocapsules as a promising strategy to treat inflammatory disorders. Silvia S. Guterres. UFRGS, Brazil

Our research group developed lipid-core nanocapsules (LNC) which differ from the conventional nanocapsules by their higher rigidity. So, the aim of this presentation is to illustrate the applications of LCN to treat inflammatory disorders. Acute lung injury and acute respiratory distress syndrome are severe clinical conditions. We developed LNC to improve the anti-inflammatory effects of α -bisabolol. LNC reduced airway hyperreactivity, neutrophil infiltration, myeloperoxidase activity, chemokine levels and tissue lung injury. We also evaluated the properties of nanoencapsulated resveratrol. The posttreatment improved lung function and diminished pulmonary inflammation. The nanoencapsulation enhanced the antioxidant catalase level with a decrease in the oxidative biomarker in lungs. The co-existence with rhinitis limits the control of asthma. To enable the treatment of asthma via nasal administration, we developed budesonide-loaded nanocapsules. The formulation showed improved efficacy in terms of reduction of immune cell influx, production of eotaxin-1 and arrest of airway remodeling. The interventional therapeutic treatment in the long-term asthma model showed results more compelling than the results obtained in the short-term model. Rheumatoid arthritis is the most common autoimmune disease. In this work, methotrexate-loaded LNC were evaluated. The formulation was effective in the control of inflammation, achieving these effects at doses 75% lower than conventional administration. Also, the formulation was effective on activated human synovial cells and in cellular conditions resistant to the drug. The electronegative low-density lipoprotein plays an important role in atherogenesis. In this work, a formulation of multiwall polymeric nanocapsules with a functionalized surface covered with 2C7 scFv anti-LDL(-) has been developed. This formulation inhibited the progression of atherosclerotic lesions. In conclusion, LNC proved to be promising to treat inflammatory disorders, showing the ability to improve bioavailability and efficacy of different treatments. The great versatility also deserves to be highlighted, as the nanocapsules can be administered by different routes and in several dosage forms. Acknowledgements: INCT Nanofarma, Pronex-Fapegs, CNPq.

Nanosystems as vectors for cell and gene therapies. Patrícia Rieken Macedo Rocco, Universidade Federal do Rio de Janeiro

Nanoparticles are one of the most widely studied classes of drug delivery systems. They have a wide range of unique properties and capabilities and can be used to improve drug administration. Therefore, exhaustive efforts have focused on developing advanced nanotherapeutic delivery systems for improved efficacy, enhanced patient compliance, and optimal treatment safety. Recently, nanomedicine has made great progress in targeting nanoparticles to individual organs. With the aim of reducing systemic toxicity induced by various medications, a range of nanocarriers have been developed as a promising strategy. During this presentation, we will discuss new perspectives in the therapeutic use of nanocarriers for chronic lung diseases, including asthma and occupational lung disease (silicosis), highlighting some of recent preclinical studies in gene and cell therapy, as well as drug delivery, addressing the many pros and cons of this innovative technology. Despite long-standing efforts to enhance care for chronic asthma, symptomatic treatments remain the only option to manage this highly prevalent and debilitating disease. We demonstrated that key pathology of allergic asthma can be almost completely resolved in a therapeutic manner by inhaled gene therapy (single dose of thymulin-expressing plasmids delivered via nanoparticles engineered to have a unique ability to penetrate the airway mucus barrier). Silicosis is a pneumoconiosis caused by inhaled crystalline silica microparticles, which trigger inflammatory responses and granuloma formation in pulmonary parenchyma, thus affecting lung function. Mesenchymal stromal cells (MSCs) were incubated with magnetic nanoparticles, and we observed that magnetic targeting technique prolong MSC retention in the lungs, enhancing their beneficial effects on experimentally induced silicosis. Intratracheal administration of Nintedanib nanosuspensions led to beneficial effects in experimental silicosis, decreasing lung fibrosis and improving lung function. Supported by: CNPq, FAPERJ, MS/DECIT, CAPES

Therapeutic administration of gold nanoparticles (AUNPs) accelerates resolution of silica-induced pulmonary fibrosis in mice. Patrícia Machado Rodrigues e Silva – Laboratório de Inflamação – Instituto Oswaldo Cruz/FIOCRUZ.

Silicosis is an occupational lung disease developed as a result of crystalline silica particle inhalation. At the moment, there is no treatment available for the disease. Evidence exists that gold nanoparticles (AuNPs) have a marked anti-inflammatory activity, which indicates them as a potential therapeutic option. To investigate the effect of AuNPs on lung fibrosis experimental silicosis, Swiss-Webster mice were therapeutically treated with AuNPs, 24 h after the last administration. Inhibition of lung function decrease (resistance and elastance) as well as airways hyper-reactivity were reduced in silicotic mice. Total content of lung collagen showed similar levels in AuNP-treated and untreated silicotic mice. Interestingly, alterations in the lung tissue morphology was noted under condition of AuNP therapy, including changes in the granuloma pattern (disruption and disorganization) with adjacent mononuclear cells and granulocytes being detected. In parallel, an enlargement of the alveolar spaces and the presence of cellular plugs inside the bronchioles were detected, supporting the idea that AuNPs improve silicotic lung functionality and the clearance of inflammatory cells. Fifteen days after the last administration of AuNPs, silicotic mice still exhibited improvement of the lung function and granuloma deconstruction, though at this time point, the total content of lung collagen was at lower levels when compared to the untreated ones. Additionally, transmission electron microscopy revealed restoration of the ultrastructure of lung epithelial, endothelial cells and extracellular matrix, supporting the suppressive effect of AuNPs in the silicotic lungs. Altogether, our results show the therapeutic treatment with AuNPs has the ability to reverse the fibrotic response associated to silica particle inhalation in mice, in a time-dependent manner, indicating that this seems to be a promising approach in the discovering of an effective treatment to silicosis. Financial support: FIOCRUZ, FAPERJ, CNPQ, CAPES.

S4 – Sperm-Associated Proteins as Drug Targets for Male Contraception

Independent validation of TSSK1 and TSSK2 as targets for male contraception. Nayyab S., Gervasi MG, Tourzani DA, Teves ME, Cui W, Salicioni AM, Visconti PE

Given the importance of phosphorylation in cell signaling and differentiation, it is not surprising that protein kinases are involved in spermatogenesis and in regulation of sperm function. Members of the testis-specific serine kinase (TSSK) family are almost exclusively expressed post-meiotically in germ cells and remain present in mature sperm. The predicted importance of TSSKs has been confirmed using knock-out (KO) mice models. Male, but not female, TSSK6 (aka SSTK) KO mice and TSSK1/TSSK2 double KO are sterile without exhibiting somatic abnormalities. Despite these findings, the double *tssk1/tssk2* KO mouse model is silent regarding the individual contribution of these kinases to the overall phenotype and therefore, *Tssk1* and *Tssk2* cannot be considered validated contraceptive targets yet. In this work, we used Crispr/Cas9 technology to eliminate exclusively *tssk1* or exclusively *tssk2*. After multiple rounds of breeding, our results indicate that, each of these homozygous mice models present a male sterility phenotype. Both *Tssk1*^{-/-} and *Tssk2*^{-/-} have lower sperm number and present defects in mitochondria organization. In both KO models, sperm failed to hyperactivate and to fertilize in vitro. On the other hand, contrary to sperm from *Tssk1*^{-/-}, sperm from *Tssk2*^{-/-} present defects in axoneme formation. Overall, our data validate the use of each of these kinases as targets for male contraception. Saman Nayyab has a fellowship from the Male Contraceptive Initiative (MCI).

Molecular pathways that control sperm capacitation. Krapf D

Protein kinase A (PKA) is a highly promiscuous Ser/Thr kinase involved in the regulation of several cellular activities. Thus, specificity of its activity relies on more than target recognition. On one hand, PKA needs to localize at specific subcellular places known as discrete PKA signalosomes. A-Kinase anchoring proteins (AKAPs) form scaffolding assemblies that play a pivotal role in PKA regulation by restricting its activity to specific microdomains. Because one of the first signaling events observed during mammalian sperm capacitation is PKA activation, understanding how PKA activity is restricted in space and time is crucial to decipher the critical steps of sperm capacitation. Here, we demonstrate that the anchoring of PKA to AKAP is not only necessary but also actively regulated during sperm capacitation. Moreover, we also show that cAMP, the ubiquitous agonist of PKA, is not the only activator of PKA. Conformational changes induced by binding of cAMP could also be performed by post translational modifications in a cAMP independent manner, triggering PKA activity. How this cAMP independent activation of PKA impacts on sperm capacitation as well as its broader implications are discussed.

S5 - Pharmacology and Covid19 in Latin America

The Chilean Society of Pharmacology (SOFARCHI) and its contribution to the fight against SARS-CoV-2 in Chile. Javier A. Bravo. ¹President, Sociedad de Farmacología de Chile (SOFARCHI), Chile. ²Instituto de Química, Facultad de Ciencias, Pontificia Universidad Católica de Valparaíso, Valparaíso, Chile.

The SARS-CoV-2 coronavirus has inflicted a severe strain on every public health system worldwide. In Chile, since the first identified case in March 2020 up until August 2021, there have been a total of 1,620,389 confirmed cases, with 35,806 deaths. Health authorities permanently promote the use of face masks, social distancing, and vaccination. In Chile, vaccination efforts began in December 2020, when the Pfizer-BioNTech vaccine was approved for emergency use by Chile's Public Health Institute (ISP). The ISP is in charge of revising and authorizing every pharmaceutical principle used in Chile, whether these are simple synthetic molecules, novel biological compounds, and also vaccines. In relation to the latter, the Chilean Society of Pharmacology (SOFARCHI) has a long-standing relationship with ISP, in which our opinion as external experts is strongly taken into account. For example, when the ISP receives an application for a novel pharmaceutical principle (i.e.: mRNA vaccine), the Institute asks SOFARCHI to review such application. This information is then revised by a member of SOFARCHI, which has the academic the appropriate academic expertise to provide a report to ISP. This SOFARCHI report will suggest to accept or reject the application made by the laboratory. In the case of vaccines against SARS-CoV-2, SOFARCHI has evaluated the application of five vaccines approved in Chile. In these reports, the scientific quality of the provided information, from preclinical and clinical studies is taken into account. Then the report is submitted back to ISP, which then calls a meeting of experts to discuss the information provided by SOFARCHI, and thus approve or reject the emergency use of the afore mentioned vaccines. Therefore, through our interaction with health authorities, SOFARCHI has been able to contribute to Chile's vaccination process, but in addition our participation has added visibility to biological sciences, which gives a somewhat unexpected value in the fight against SARS-CoV-2, and also against fake news and false promises. Financial support: SOFARCHI

S6 – Cannabinoids and Cannabinoid-like Molecules Acting on the CNS

Mechanisms of the cannabidiol-induced anxiolytic effects. Guimarães, Francisco S. Department of Pharmacology, Medical School of Ribeirão Preto, University of São Paulo, Brazil.

Cannabidiol (CBD) is a major non-psychotomimetic compound present in the *Cannabis sativa* plant. Pre-clinical studies show that CBD induces anxiolytic-like effects in models associated with several anxiety and stress-related disorders. In humans, anxiolytic effects of CBD have been demonstrated in healthy subjects and patients with social anxiety. The acute anxiolytic effects of CBD have been associated with the facilitation of post-synaptic serotonin-1A (5HT1A)-mediated neurotransmission in brain sites related to defensive responses. After prolonged administration, however, other mechanisms, such as the facilitation of CB1 and CB2 receptor-mediated neurotransmission, seem to be responsible for these effects. Through this facilitation, which probably involves an indirect increase in the levels of the endocannabinoid anandamide due to the inhibition of its metabolism, CBD attenuates the chronic stress-induced neuroplastic changes such as the decrease in hippocampal neurogenesis and synaptic remodeling. Other mechanisms, such as anti-inflammatory, could also be involved in the anti-stress effects of CBD. Together, these findings suggest that CBD could be useful in treating anxiety disorders through several mechanisms. Financial support: FAPESP (2017/24304-0) and National Institute of Science and Translational Medicine, CNPq (465458/2014-9)

Cannabis is for the elderly: THC and CBD as therapeutic tools for age-related diseases. Francisney Pinto do Nascimento. Universidade Federal da Integração Latino-Americana – UNILA – Foz do Iguaçu, PR, Brazil. A close relationship between aging and the onset of various diseases has been demonstrated, especially in neurological, psychiatric and rheumatological diseases. We know an inverse relationship between aging and tone of the endocannabinoid system (ECS). Endocannabinoids have several physiological functions, especially helping to maintain biochemical homeostasis. In this way, cannabinoids regulate functions such as memory, mood, pain, inflammation, immune system, neuronal nutrition and many others. So, as we age, we produce less endocannabinoids and we also produce fewer cannabinoid receptors. That is, elderly people have low levels of endocannabinoids and the low levels of these molecules make these people more susceptible to various diseases. This inverse relationship between aging and SEC activity can be considered part of a syndrome called Clinical Endocannabinoid Deficiency. Thus, people with low levels of anandamide - the main endocannabinoid - are more likely to have central nervous system and inflammatory diseases. An article from 2017 showed that low doses of THC, the main active ingredient in cannabis, improved memory in old rats but not in young ones. The study demonstrated that this phenomenon occurred because THC induced hippocampal neurogenesis and also induced increased

expression of cannabinoid receptors. In addition, we know that cannabinoids have effects on inflammation and oxidative stress, processes that are closely related to aging. Thus, the use of phytocannabinoids has great potential for to prevent and treat aging diseases. In this panel, I will address this window of opportunity for cannabinoid research and present clinical results from our research group that evaluated the effects of phytocannabinoids on Parkinson's, Alzheimer's, and Fibromyalgia. Financial support: UNILA and ABRACE (Associação Brasileira de Apoio Cannabis Esperança).

Terpenoids, cannabimimetic ligands, as therapeutic tools for neuroimmunological disorders. Rafael Cypriano Dutra. Associate Professor of Universidade Federal de Santa Catarina (UFSC) Founder and Principal Investigator (PI) of Laboratory of Autoimmunity and Immunopharmacology (LAIF), Department of Health Sciences, Campus Araranguá, Universidade Federal de Santa Catarina, Araranguá, SC, Brazil.

Cannabis sativa has a very long history of use for medical purposes. However, this use declined early in the twentieth century due to the growing evidence of marijuana's addictive potential. Additionally, recent progress in cannabinoid pharmacology, together with the discovery of the endocannabinoid system (ECS), which includes cannabinoid receptors, endogenous ligands, and enzymes responsible for the synthesis and degradation of endocannabinoids, as well as cannabimimetic ligands beyond the *Cannabis* plant, has renewed interest in cannabis- and phytocannabinoids-based medicines. This Symposium is intended to provide a fascinating exploration into the latest evidence of therapeutic potentials of cannabimimetic ligands, with special focus on terpenes, in neuroimmunological disorders. Financial Support: CNPq; CAPES; FAPESC; INCT-INOVAMED; PPG NEURO/UFSC.

S7 - Natural and Synthetic Molecules as Antiviral drugs

Anti-Zika and anti-dengue virus activity of the citrus flavonoid naringenin. Juliano Bordignon. Instituto Carlos Chagas, Fiocruz, PR, Brazil

Arboviruses like dengue and Zika represent important health problems in tropical and sub-tropical regions throughout the world. Dengue causes successive epidemics in Brazil since the 1980's and Zika caused an important epidemic in 2015 associated with cases of Guillain-Barré syndrome in adults and neurological defects in newborns. To date, no antiviral treatment has become available to control DENV or ZIKV replication, and only a partially protective vaccine is available for DENV. Thus, the development of alternative therapies for both DENV and ZIKV are of utmost relevance to threat infected patients avoiding mortality and morbidity associated with both infections. Among the natural compounds recognized for their medical properties, flavonoids, which can be found in fruits and vegetables, have been found to possess biological activity against a variety of viruses. Here, the *in vitro* anti-DENV and anti-ZIKV activity of the citrus flavonoid naringenin (NAR) was demonstrated. This flavonoid seems to impair both DENV and ZIKV replication in human cell lines, like Huh7.5 and A549, but also, in primary human cells, like monocyte derived-dendritic cells and monocytes. Additionally, the anti-viral activity of the compound was observed against the four DENV-serotypes and the Asiatic- and African-lineage of ZIKV. Also, using subgenomic DENV replicon systems (RepDV-1 and RepDV-3) the ability of naringenin to inhibit DENV replication was confirmed. Moreover, a molecular docking analysis indicated that NAR potentially interacts with the protease domain of the NS2B-NS3 protein of ZIKV which could explain the anti-ZIKV activity. Thus, although further *in vivo* validation of the data is still required, the citrus flavonoid naringenin appears as a potential candidate molecule for the development of treatments against DENV and ZIKV. Financial support: CNPq and Fiocruz.

Medicinal plants from Indian Ocean are a promising source of antiviral phytochemicals against emerging mosquito-borne flavivirus at doses devoid of toxicity in Zebrafish. Juliano Haddad¹, Nicolas Diotel², Claudia Nunes Duarte dos Santos³, Philippe Desprès¹ and Chaker El Kalamouni¹ ¹Université de La Réunion, UM134 Processus Infectieux Insulaire Tropical (PIMIT), INSERM U1187, CNRS UMR 9192, IRD UMR 249. Plateforme Technologique CYROI, 97490 Sainte Clotilde, France. ²Université de La Réunion, INSERM, UMR 1188 Diabète athéromatose Thérapies Réunion Océan Indien (DéTROI), Plateforme Technologique CYROI, 94791 Sainte Clotilde, La Réunion, France. ³Laboratório de Virologia Molecular, Instituto Carlos Chagas, ICC/FIOCRUZ/PR, Curitiba, Parana, Brazil.

Dengue virus (DENV) and Zika virus (ZIKV) belong to the *flavivirus* genus of *Flaviviridae* family. Dengue fever, caused by 4 virus serotypes, is the most prevalent mosquito-borne viral infection in tropical and subtropical countries including the southwest of Indian Ocean region. Epidemics of emerging mosquito-transmitted ZIKV have been recently recorded in the Americas, Asia and the Pacific. ZIKV infection has been associated to severe neurological disorders in humans. A worldwide research effort has been undertaken to identify safe and effective compounds to prevent or treat dengue and Zika diseases. Medicinal plants may be a source of natural antiviral drugs which mostly target viral entry. We have studied the antiviral activity of several indigenous or endemic medicinal plants from Mascarene archipelago

in Indian Ocean against ZIKV and DENV infection. Here, we reported that thymohydroquinone dimethyl ether (THQ), geraniin, isolated from *Ayapana triplinervis* and *Phyllanthus phillyreifolius* medicinal plants, respectively, inhibit ZIKV and DENV entry in human cells at non cytotoxic concentrations. At the antiviral effective concentration, both THQ and geraniin injection in zebrafish does not lead to any signs of stress and does not impact fish survival, demonstrating the absence of acute toxicity. We conclude that THQ and geraniin are a new potent antiviral phytochemicals against ZIKV and DENV. Our data highlight the potential use of medicinal plants from Indian Ocean as a source of natural and safe antiviral substances against medically important mosquito-borne viruses such as dengue virus.

S8 – Beyond the Classical Renin-Angiotensin System: What's next?

Renin-Angiotensin system in the central nervous system: What have we learned so far? Aline Silva de Miranda. Departamento de Morfologia, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brasil. A growing body of evidence has implicated the involvement of Renin Angiotensin System (RAS) components in the pathophysiology of neuropsychiatric and neurodegenerative disease, like Alzheimer's and Parkinson's diseases, cerebrovascular conditions, anxiety and depression. RAS is classically conceived as a hormonal system mainly responsible for blood pressure control and hydroelectrolyte balance. Inhibition of the classical branch/axis of the RAS, formed by angiotensin converting enzyme (ACE)-Angiotensin II-AT1/AT2 receptors, alongside activation of the alternative RAS axis, comprising ACE2-Angiotensin-(1-7)-Mas receptor, can increase cerebral blood flow and decrease reactive oxygen species production, cytokines and chemokine release and the local activation of immune cells, ultimately resulting in neuroprotection. It is worth mentioning that the majority of the evidence relies on pre-clinical studies, mostly with genetic-modified animals. Very few data are available regarding the ACE2/Ang-(1-7)/Mas receptor axis in human central nervous system. Further clinical studies are highly needed to determine whether the administration of ACE2 activators and/or Mas receptor agonists may exert beneficial effects in neuropsychiatric conditions. Financial Support: FAPEMIG; CNPQ; CAPES; 2016 NARSAD Young Investigator Grant Awardee from the Brain and Behavior Research Foundation; 2019 "For women in Science"- L'Oreal Brazil-UNESCO-Brazilian Academy of Science (ABC).

Circulating Angiotensin-(1-7) is Reduced in Alzheimer's Disease Patients and Correlates with White Matter Abnormalities: Results from a Pilot Study. Victor Teatini Ribeiro¹, Thiago Macedo e Cordeiro¹, Roberta da Silva Filha¹, Lucas Giandoni Perez¹, Paulo Caramelli², Antônio Lúcio Teixeira³, Leonardo Cruz de Souza^{1,2}, Ana Cristina Simões e Silva¹. ¹Laboratório Interdisciplinar de Investigação Médica, Faculdade de Medicina, Universidade Federal de Minas Gerais (UFMG), Belo Horizonte, MG, Brazil, ²Departamento de Clínica Médica, Faculdade de Medicina, Universidade Federal de Minas Gerais (UFMG), Belo Horizonte, MG, Brazil, ³Neuropsychiatry Program & Immuno-Psychiatry Lab, Department of Psychiatry and Behavioral Sciences, University of Texas Health Science Center at Houston, Houston, TX, United States of America

Introduction: Alzheimer's disease (AD) is the leading cause of dementia worldwide. Despite the extensive research, its pathophysiology remains largely unelucidated. Currently, more attention is being given to the disease's vascular and inflammatory aspects. In this context, the renin-angiotensin system (RAS) emerges as a credible player in AD pathogenesis. RAS has multiple physiological functions, conducted by its two opposing axes: the classical, led by Angiotensin II (Ang II), and the alternative, driven by Angiotensin-(1-7) [Ang-(1-7)]. These peptides were shown to interact with AD pathology in animal studies, but evidence from humans is scarce. Only 20 studies dosed RAS molecules in AD patients' bloodstream, none of which assessed both axes simultaneously. Therefore, we conducted a cross-sectional, case-control exploratory study to compare plasma levels of Ang II and Ang-(1-7) in AD patients vs. age-matched controls. Within each group, we searched for correlations between RAS biomarkers and measures from magnetic resonance imaging (MRI). Methods: We evaluated patients with AD (n=14) and aged-matched controls (n=14). Plasma Ang II and Ang-(1-7) were dosed using ELISA. Brain MRI was performed in a 3 Tesla scan, and a three-dimensional T1-weighted volumetric sequence was obtained. Images were then processed by FreeSurfer to calculate: 1) white matter hypointensities (WMH) volume; 2) volumes of hippocampus, medial temporal cortex, and precuneus. Statistical analyses used non-parametrical tests (Mann-Whitney and Spearman). Results: Ang-(1-7) levels in plasma were significantly lower in the AD patients than in controls [median (25th – 75th percentiles)]: AD [101.5 (62.43 – 126.4)] vs. controls [209.3 (72 – 419.1)], $p = 0.014$ (Fig. 1A). There was no significant difference in circulating Ang II. In the AD patients, but not in controls, there was a positive and significant correlation between Ang-(1-7) values and WMH volumes in AD (Spearman's $\rho = 0.56$, $p = 0.038$). Ang-(1-7) did not correlate with cortical volumes in AD or in controls. Ang II did not correlate with any MRI variable in none of the groups. Conclusions: If confirmed, our results strengthen the hypothesis that RAS alternative axis is downregulated in AD, and points to a possible interaction between Ang-(1-7) and cerebrovascular lesions in AD.

Perivascular adipose tissue as a source and target of Renin-Angiotensin system. Luciana Venturini Rossoni. Department of Physiology and Biophysics, Institute of Biomedical Science, University of Sao Paulo, Sao Paulo, Brazil

Perivascular adipose tissue (PVAT) is an endocrine tissue that anatomically encompasses the majority of vessels and releases vasoactive substances that are capable of attenuate vascular tonus. However, PVAT dysfunction is associated with vascular damage in cardiometabolic diseases. Interestingly, renin-angiotensin system (RAS) components are expressed in the PVAT of the aorta and mesenteric arteries of healthy rats, and angiotensin 1-7 is considered one putative vasodilator factor that is released from PVAT. Previous studies have shown that the activation of the RAS is involved in the pathophysiology of several cardiovascular diseases, including heart failure (HF). Although HF-induced endothelial dysfunction is associated with RAS activation, no data have correlated this syndrome with PVAT dysfunction. Thus, our group investigate whether the hyperactivation of the RAS in PVAT participates in the vascular dysfunction observed in rats with HF after myocardial infarction surgery. In the present speech we will focus on data that shows that PVAT is dysfunctional in the thoracic aortas of HF post myocardial infarction surgery in rats. Through the overactivation of ACE1/angiotensin II/AT1R and AT2R axes, which causes oxidative stress and, consequently, reduces NO bioavailability, the anticontractile effect of PVAT is impaired in the thoracic aorta of HF rats. These data highlight the importance of the PVAT phenotype and dysfunction to the pathophysiology of the vascular disease observed in HF and provide new perspectives for the treatment of this syndrome. This work was supported by: “Fundação de Amparo à Pesquisa do Estado de São Paulo – FAPESP” and “Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPq”.

Understanding the RAS puzzle: lessons from COVID-19. Robson Augusto Souza dos Santos (UFMG)

The renin-angiotensin system (RAS) plays an important role in a series of physiological processes. The formation of angiotensins is rather complex, involving many enzymes. One of its key components, the angiotensin-converting enzyme 2 (ACE2), which forms angiotensin-(1-7) from angiotensin II, has been identified as the entry point of the SARS-CoV-2 virus into the host cells. Considering that the internalization of ACE2 due to its interaction with SARS-CoV-2, could lead to a functional deficiency of this enzyme, many studies have aimed to study the potential RAS dysregulation in COVID-19. In my presentation I will discuss the alterations of the regulatory RAS axes due to SARS-CoV-2 infection on the basis of our recent studies and of a series of recent clinical and experimental data, which, aimed to quantify the levels and activity of RAS components. The possible links between the unbalanced RAS function and the pathophysiological characteristics of COVID-19 will be discussed.

S9 – Pharmacometrics Applications in Drug Development and Dosing Optimization for Clinical Practice

Physiologically based pharmacokinetic (PBPK) modelling to predict drug disposition after bariatric surgery. Natalia Valadares de Moraes. School of Pharmaceutical Sciences, São Paulo State University (UNESP)

Physiologically based pharmacokinetic (PBPK) models have been developed to predict the effect of bariatric surgery on pharmacokinetics. These models are based on changes in the ‘Advanced Dissolution Absorption and Metabolism’ (ADAM) model implemented into Simcyp® simulator. The model is based on modifications in the morbidly obese population template such as: a) higher gastric pH; b) reduced gastric emptying time; c) bypass in the duodenum and proximal jejunum; d) delay in the bile release to the distal jejunum; e) reduced CYP3A4/5 abundance; and f) reduced concomitant fluid intake with administered dose. However, current PBPK models to predict drug disposition post-bariatric surgery are limited by the limited clinical drug data in this special population. Our research group is conducting two clinical trials to investigate pharmacokinetics in healthy volunteers, obese and post-Roux-en-Y gastric bypass (RYGB). The optimization of the post-RYGB model to better predict drug disposition will be presented. Financial support: FAPESP 18/06569-9

QSP modelling approaches for immune related disease: why, when and how? Zinnia P Parra Guillén^{1,2}

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Over the past decade, quantitative systems pharmacology (QSP) has emerged as a novel field aiming to place drug systems properties in the context of health and disease by integrating systems biology and pharmacometric concepts. In this regard, QSP models represent the current understanding of the physiology and physiopathology of the system (components, interactions, pathways, cells and organs) and leverage relevant mechanistic characteristic of the system and drug properties in a quantitative manner. QSP models can be applied across all phases of drug development and used for a wide range of applications including target or biomarker identification, combination strategies or clinical trial simulations among others. These models can be of especial interest for complex diseases where multiple biological components and interactions are involved, as they might be difficult to tackle and project with simpler

model representations. However, QSP models also require longer developing times, larger amount of data across different entities, and are challenging in terms of model exploration and validation. Therefore, a clear benefit is needed to select this approach over classical empirical or semi-mechanistic models. The goal of this presentation is to provide some practical recommendations regarding why, when and how to build these models. To do so, models for immune-related diseases such as viral hepatitis B or inflammatory bowel disease will be provided as examples

S11 – Specialized Pro-Resolution Lipid Mediators: Novel Non-Opioid Analgesics and Anti-Inflammatory Perspectives

Effects of pro-resolution lipid mediators in experimental diabetes-associated complications. Joice Maria da Cunha. Federal University of Parana

Diabetes is a metabolic disease characterized by hyperglycemia, which is the main cause for chronic complications development. Diabetic neuropathy is the most prevalent and costly, which often manifests as pain in the distal limbs in a pattern known as the glove and boot in about 10 to 26% of patients. Studies suggest that the onset of microvascular complications, like neuropathy, are related to the development of anxiety and depression states. The pathophysiology of these diabetic complications is multifactorial and may be linked in some way. Current pharmacological treatment of patients diagnosed with diabetic neuropathic pain, depression, and anxiety has been unsatisfactory in relieving the symptoms besides being able to interact pharmacologically with hypoglycemic drugs, which commonly leads to poor treatment adherence and efficacy. Then, these facts highlight the need for alternative therapies for the treatment of complications and comorbidities associated with diabetes. In this sense, specialized pro-resolution lipid mediators (SPMs) have stood out for presenting a broad beneficial profile. SPMs are biosynthesized from both omega-6 and omega-3 polyunsaturated fatty acids. In this lecture will be presented the effects of acute and intermittent treatment with fish oil (an omega-3 polyunsaturated fatty acid), with its major constituent fatty acids, eicosapentaenoic (EPA) and docosahexaenoic acids (DHA), or with the SPMs protectin and resolvin D5 over some diabetic complications, such as mechanical allodynia and depressive- and anxiety-like behaviors in streptozotocin-induced diabetic rats. The possible mechanisms of action of these compounds will be also addressed. Financial Support: CAPES and CNPq/Fundação Araucária (PRONEX 02/16; Grant# 014/2017)

Analgesic potential of Maresin 2 and Resolvin D5 in models of orofacial pain. Raphael Vieira Lopes¹, Darciane Favero Baggio¹; Luiz Eduardo Nunes Ferreira²; Camila Rodrigues Ferraz³, Telma Saraiva-Santos³, Mariana Marques Bertozzi³, Tiago Henrique Zaninelli³, Waldiceu Aparecido Verri Jr³; Juliana Geremias Chichorro¹ ¹Department of Pharmacology, Biological Sciences Sector, Federal University of Parana; Curitiba, PR, Brazil; ²Laboratory of Inflammation and Immunology, University of Guarulhos, Guarulhos, SP, Brazil ³Laboratory of Pain, Inflammation, Neuropath and Cancer, Department of Pathology, State University of Londrina, Londrina, PR, Brazil.

Trigeminal neuralgia (TN) is characterized by recurrent unilateral brief electric shock-like pain attacks, limited to the distribution of one or more divisions of the trigeminal nerve. Carbamazepine, the first line treatment, presents poor pharmacokinetic profile and limiting side effects. TN has been modeled in rodents through the chronic constriction injury of the infraorbital nerve (CCI-ION). In the past few years, potent antinociceptive effects of specialized proresolving mediators (SPMs), including resolvins and maresins have been reported. Herein it was investigated the effect of resolvin D5 (RvD5) in heat and mechanical hyperalgesia in male and female rats, and the effect of maresin 2 (MaR-2) in heat and mechanical hyperalgesia in male rats and mice, all subjected to CCI-ION. All procedures were approved by CEUA/BIO-UFR # 1118). After CCI-ION, RvD5 (3 e 10 ng) or MaR-2 (10 ng) were injected in 4 alternate days via the subarachnoid route, to reach the subnucleus caudalis (Sp5C). Facial heat and mechanical hyperalgesia were assessed daily, followed by animals' euthanasia and tissues' extraction. RvD5 (10 ng) caused a significant and persistent reduction of heat and mechanical hyperalgesia in female and male rats, and reduced the levels of interleukin-6 (IL-6) in the Sp5C of male rats. MaR-2 caused significant and long-lasting reduction of heat and mechanical hyperalgesia in rats and mice and reduced the positive neurons for c-Fos and CGRP in the trigeminal ganglion of mice. These results provide the first demonstration of the analgesic potential of RvD5 and MaR-2 in trigeminal neuropathic pain. Financial support: This study was granted by PRONEX (CNPq/Fundação Araucária-PR). Lopes and Baggio are recipients of CAPES scholarship. Chichorro and Verri Jr are recipients of CNPq research productivity fellowship.

Annexin A1 attenuates cardiac diastolic dysfunction in mice with arthritis. Jianmin Chen, Lucy V Norling, Marina De Paula Silva, Mauro Perretti, Dianne Cooper. William Harvey Research Institute, Barts and The London School of Medicine, Queen Mary University of London, London, UK, EC1M6BQ

Rheumatoid arthritis (RA) carries a 2-fold increased incidence of heart failure with preserved ejection fraction, accompanied by diastolic dysfunction, which can lead to death. This is a major unmet clinical need, as the causes of diastolic dysfunction are unknown and not addressed by current medications. The lack of well-characterized animal models of cardiomyopathy in arthritis hampers the study of these mechanisms. We monitored the progeny of KRN mice crossed with the NOD colony, and noted that K/BxN F1 mice exhibited fully developed arthritis with normal cardiac function at 4 weeks (echocardiography), however by week-8 all mice displayed left ventricular diastolic dysfunction with preserved ejection fraction. This dysfunction was associated with cardiac hypertrophy, inflammation and fibrosis. Daily treatment of K/BxN F1 mice with human recombinant AnxA1 (AnxA1; an endogenous agonist for the pro-resolving receptor FPR2; 1 µg/mouse) from week-4 to week-8 halted progression of the diastolic dysfunction. AnxA1 treatment reduced cardiac transcripts of pro-inflammatory cytokines and pro-fibrotic markers. Cellular analyses in the K/BxN F1 hearts revealed that AnxA1 i) decreased activated T cells; ii) increased MHC II^{low} macrophage infiltration and iii) reduced fibroblast numbers. When given from week-8 to week-15, hrAnxA1 reversed established diastolic dysfunction and attenuated cardiac remodelling. With either protocol, no evident effects on joint disease were observed. In summary, we describe a murine model of inflammatory arthritis that recapitulates the cardiomyopathy of RA. Treatment with hrAnxA1 after disease onset corrected the diastolic dysfunction through modulation of both fibroblast and inflammatory cell phenotype within the heart. Funding: This work was supported by funding from the Barts Charity (grant number: MRC0209) to DC and JC. MP was funded by the Medical Research Council (MR/P026362/1) and Versus Arthritis UK (21274). LVN acknowledges the support of Versus Arthritis Senior Fellowship (22235) and Barts Charity Project Grant (MGU0443).

Neuronal and non-neuronal effects of specialized pro-resolution lipids regulating pain, inflammation and neuroimmune interactions. Waldiceu A. Verri Jr, PhD. Associate Professor. Department of Pathology. Londrina State University, Brazil

Specialized pro-resolution lipid mediators (SPMs) orchestrate the resolution of inflammation. Nevertheless, their actions go beyond resolution since they also act as anti-inflammatory and analgesic molecules. Focusing on nociceptor sensory neurons, some SPMs can silence these neurons, which has as consequence the reduction of pain. However, it is becoming clear that silencing nociceptor sensory neurons has other functions and is part of the mechanisms of action of SPMs. The silencing of neurons by SPMs reduces the release of neuropeptides, thus, reducing the effect of such molecules in the tissues innervated by nociceptor sensory neurons. One consequence is the reduction of leukocyte recruitment, but it also has a functional impact on leukocytes. We will use examples of SPMs to discuss how these lipids can shape inflammation and pain, and how they affect neuroimmune communication and the fate of inflammatory responses. **Funding:** CAPES (finance code 001); CNPq (307186/2017-2; 427946/2018-2); Programa de Apoio a Grupos de Excelência (PRONEX) grant supported by SETI/Fundação Araucária and MCTI/CNPq, and Governo do Estado do Paraná (agreement 014/2017, protocol 46.843); PPSUS grant funded by Decit/SCTIE/MS intermediated by CNPq with support of Fundação Araucária and SESA-PR (agreement 041/2017, protocol 48.095); and MCTI/FINEP/CT-INFRA-PROINFRA 02/2014 under the reference number 0119/16 and agreements 01.12.0294.00 and 01.13.0049.00. We thank the Core Facility CMLP-UEL.

S12 – Targeting Inflammation in COVID-19

Anti-inflammation and Covid therapy. Ivan Marazzi (*Icahn School of Medicine at Mount Sinai, USA*)

Inhibition of topoisomerase 1 through the FDA-approved molecule topotecan suppresses SARS-CoV-2-infection associated lethal inflammation in hamster and mouse models without compromising antiviral immune responses

Roundtables

MR2 – Beyond the Academy

Empowering Patients: Deconstructing the passive subject to build the active subject, protagonist of patient journey. Alessandra de Souza Farmale/ALEMDII

Patient empowerment is an educational process promoted by health professionals with the specific objective of making the patient an active and conscious subject in their journey with chronic disease. Since chronic disease requires permanent treatment, the patient must cultivate habits and attitudes that promote awareness of self-care, feel responsible for the treatment plan, as well health professionals to improve the relationship and communication with patients. The empowered patient is an individual with the knowledge, skills, attitudes, and self-knowledge necessary to effectively take responsibility for decisions about their journey with the disease. They are more informed, involved patients, and become responsible subjects with a treatment plan, interacting more effectively with health professionals. This construction of

the empowered patient goes through the deconstruction of the paradigm and conceptions of the current model, still focused on the disease, where the patient is a passive subject in the treatment plan.

01. Cellular and Molecular Pharmacology

01.001 **Vascular reactivity, angiotensin receptor and intracellular signaling in *Oxyrhopus guibei* (false coral snake), and *Crotalus durissis terrificus* (rattlesnake).** da Silva ILMS, Marinho JL, Breno MC Butantan Inst

Introduction: Angiotensin II (AngII) has an important role in the mammalian cardiovascular system by interacting with specific AT1 and AT2 receptors. We have characterized a functional angiotensin receptor (AT) in the tissues (aorta and heart) of the snake *Bothrops jararaca* (BJ-Viperidae family) that has a low affinity to the classical selective antagonists: losartan (AT1) and PD123319 (AT2) (Breno et al. Eur.J.Pharmacol, 417: 27,2001; Esteves et al. Life Sci. 91: 944, 2012). An AngII receptor pharmacologically distinct from the classical AT1 and AT2 receptors was also detected in vascular tissue of two other snake species, *Oxyrhopus guibei* (Og) and *Crotalus durissis terrificus* (Cdt) belong to Colubridae and Viperidae families, respectively. The aim is to investigate some intracellular signaling pathways activated by the AngII receptor in the aorta of Og and Cdt snakes as well as the role of the endothelium-derived factors on the AngII response, and the biological activity of some AngII analogues in this vascular tissue.

Method: Adult male and female snakes (100-350g/IBAMA: 38-02001005104/2008) were captured in the wild and were kept under controlled environmental conditions (12h light/dark, 21-27°C). Snakes were anesthetized (sodium pentobarbital, 30 mg/kg) and euthanized. Aortic rings were removed free of connective tissues and suspended in an organ chamber containing a nutritive solution (37°C, 95% O₂-5% CO₂, 1g tension). Isometric tension induced by cumulative concentration-effect curves to AngII (human) were obtained in the absence (control) and in presence of Cheleretrine or GF109203X (PKC inhibitors) in aorta of Cdt. The cumulative concentration-response curve to AngII was compared in intact (E+) and denuded-endothelium (E-) aorta from Og and Cdt, and AngII analogues ([Asp1, Val5] AngII and [Asn1, Val5] AngII) cumulative curves were obtained also in the aorta of both snake species. In addition, the AngII response was evaluated in the aorta distally from the heart (liver region) to verify the presence of a functional AngII receptor in this region of Cdt. **Results:** Cheleretrine (10⁻⁵M n=8) did not modify AngII-effect in Cdt aorta, but GF109203X (10⁻⁵M n=4) produced an AngII maximum-effect reduction (65%). The endothelium removal did not modify the AngII vasoconstrictor-effect pD₂ 5.9 (E+) to 6.0 (E-) n=3 (Og) and pD₂ 6.4 (E+) to 6.7 (E-) n=7 (Cdt). The analogue [Asp1, Val5] Ang II was as potent as the human AngII (pD₂ 6.0 to 6.1 n=2 - Cdt), differently from [Asn1, Val5] AngII which was less active in Og (pD₂ 5.9 to 3.9 n=2) and Cdt (pD₂ 6.0 to 5.4 n=1). The vasoconstriction response to Ang II was also observed distally from the heart in Cdt (pD₂ 6.4 to 5.6 liver region, n=4). **Conclusions:** These results indicate that any PKC isoform is involve in the Ang II-response and the Ang II receptor is also present in aorta distally from the heart in Cdt. Endothelium-derived factors did not collaborate to Ang II response in Og and Cdt, and [Asn1, Val5] AngII was the less potent analogue in both snakes, like we observed in the *Bothrops jararaca* snake (aorta and heart). Our data contribute to the knowledge of the renin-angiotensin system in vertebrates, and provide insight into the understanding of snake AngII receptor. Financial Support: Fundação Butantan, CNPq. **License number of ethics committee:** 1691061115 / 9738030616

01.002 **Endothelial oxidative stress and antioxidant profile during schistosomiasis.** Monteiro MMLV, Valença SS, Silva CLM UFRJ, Inst of Biomedical Sciences, Pharmacology and Inflammation Research Program

Introduction: Schistosomiasis is a neglected tropical disease caused by *Schistosoma mansoni*. Intravascular parasites establish a chronic infection sustained by host immune responses and mesenteric endothelial cells acquire a pro-inflammatory phenotype (Oliveira et al., 2011, Plos One, 6: e23547). Endothelial dysfunction could be secondary to cell stress caused by an increased reactive oxygen species (ROS) production such as superoxide anion. The aim of the present work was to evaluate ROS formation, lipid peroxidation and antioxidant enzymes expression in mesenteric endothelial cells (MEC) from control and *S. mansoni*-infected mice. **Methods:** CEUA A02/21-048-16. Seventy-five day-old male Swiss mice were divided in two groups (control and *S. mansoni*-infected mice). Mesenteric vessels were removed, minced and plated with DMEM enriched with 20% fetal bovine serum, 1% antibiotics at 37°C, 5% CO₂ until confluence. All assays used confluent MEC (1st passage). Flow cytometry for cell culture phenotypic characterization used antibody for PECAM-1 (CD31). ROS quantification (superoxide anion) adapted from Choi *et al.*, 2006 (J. Immunoassay Immunochem, 27: 31) used blue nitrotriazolium (NBT). Lipid peroxidation rate as malondialdehyde (MDA), thiobarbituric acid-reactive species (TBARs) assay was used. Total protein extracts were harvested from MEC and the antioxidant enzymes levels were analyzed by Western blotting for superoxide dismutase (SOD1), glutathione peroxidase (GPx1), nuclear factor erythroid 2-related factor 2 (Nrf2), oxidative stress marker nitrotyrosine (PNK) and β -actin expressions. Statistical analysis was performed by using Student's t test expressed as means \pm SEM and $P < 0.05$ was considered significant. **Results:** MEC from both groups showed immunoreactivity to CD31+ (> 97%). MEC from the infected group

produced twice ROS levels as compared to control (0.06 ± 0.005 a.u. vs. 0.028 ± 0.005 a.u., $n=5$ *** $P < 0.001$). Moreover, the same pattern was observed in TBARS levels (7.1 ± 0.5 ; vs. 3.7 ± 0.51 $\mu\text{mol}/\text{mg}$ protein, $n=3$, $P < 0.05$). Antioxidant enzymes expressions did not change between groups GPx1 (1.2 ± 0.04 vs. 1.3 ± 0.04 a.u., $n=3$, $P < 0.05$); SOD1 (0.99 ± 0.028 vs. 0.95 ± 0.054 a.u., $n=3$, $P < 0.05$) and Nrf2 (1.0 ± 0.3 vs. 0.96 ± 0.07 a.u., $n=1$). The reaction between superoxide anion ($\text{O}_2^{\cdot-}$) and nitric oxide (NO) generates peroxynitrite (ONOO^-) causing the nitrosylation of tyrosine residues (PNK). However, despite the increased levels of superoxide anion in the infected group, we observed a reduced (1.44 ± 0.13 a.u.) level in relation to control (1.84 ± 0.05 a.u., $n=3$, * $P < 0.05$). The smallest production of ONOO^- in *S. mansoni*-infected mice could result from the reduced NOS expression and NO production in this group (Oliveira et al., 2011, Plos One, 6(8): e23547). **Conclusion:** The mesenteric endothelial cells phenotype during schistosomiasis is characterized by an increased superoxide anion production and lipid peroxidation, a hallmark of endothelial dysfunction. These alterations may contribute to mesenteric inflammation. **Financial Support:** CNPq. **License number of ethics committee:** CEUA A02/21-048-16

01.003 **The P2X7 receptor agonist ATP induces prostate cancer cells adhesion to human endothelial cells**
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Introduction: Prostate cancer is a malign tumor type that is characterized by 1,28 million cases worldwide. It is the second malignant neoplasia most common in men (Bray *et al.*, 2018, Cancer J Clin., 68: 394–424). Among all the complications, metastasis is the most severe. In endothelial dysfunction, the endothelial cells that coat the vascular lumen change from a quiescent phenotype to an activated one by contributing cell adhesion and transmigration processes (Strassheim *et al.*, 2020, Int. J. Mol. Sci., 21: 6855). ATP is one of the major constituents of the tumor microenvironment through the modulation of P2 purinergic receptors. The P2X₇ receptor (P2X₇R) is activated by high concentrations (0.5-1 millimolar) of ATP and is characterized by a pro-inflammatory action (Eltzschig *et al.*, 2012, N Engl J Med. 367: 2322-2333). In this condition, vascular permeability increases and it occurs an induction of expression of adhesion molecules favoring the endothelium-tumor interaction. We hypothesize that P2X₇R contributes to DU-145 tumor cell adhesion and metastatic niche formation and that inhibition of this step can reduce the metastatic process. In this study, we intend to characterize the effect of P2X₇R on prostate cell adhesion to the endothelial monolayer, and to investigate the effect of atorvastatin on purinergic signaling in this model. **Methods:** EA.hy926 human endothelial cells (EC) and DU-145 human prostate cancer cells were cultured in DMEM and RPMI media enriched with 10% fetal bovine serum and antibiotics (37°C, 5% CO₂). The ECs (96 wells) were pretreated with purinergic agonist ATP (500 μM , 10 min) or basal, in the absence and presence of endothelial NTPDase inhibitor ARL 67156 100 μM , selective P2X₇R antagonist (A74003 25 nM), both added 30 min before, or atorvastatin (added 24h before). Then calcein-labeled cancer cells were added and co-incubated for 30 min. The wells were washed and photographed for cell counting in a fluorescence microscope (200X). Experiments were performed in triplicate. Data were expressed as mean and SEM. The differences between groups were analyzed by one-way ANOVA followed by the Neuman-Keuls test, considering $P < 0.05$. **Results:** ECs were treated with ATP 500 μM which induced adhesion of DU-145 cells to EC (32.9 ± 1.9 vs baseline 14.3 ± 0.7 , $P < 0.001$). The pretreatment of EC with the selective antagonist of P2X₇R (A74003) inhibited the effect of ATP (15.07 ± 1.1 , $P < 0.001$), corroborating that the effect is mediated by P2X₇R. Moreover, this pro-adhesive effect was not altered by pretreatment with ARL 67156, suggesting that it is not degraded into ATP metabolites during the experiment. In parallel, the pretreatment of EC with atorvastatin 1 μM inhibited ATP-induced adhesion. Statins are used clinically to reduce LDL cholesterol, but they also have pleiotropic effects, including endothelial barrier protection, so they have been studied as possible inhibitors of this process (Bedi et al., 2016, Schmiedeberg's Arch Pharmacol, 386: 695-712). **Conclusion:** Preliminary data suggest that activation of P2X₇R stimulates the DU-145 cells adhesion to ECs suggesting the contribution of this pathway to the metastatic process in prostate cancer. This effect was inhibited by atorvastatin indicating a possible supporting approach in anti-tumor therapy. **Acknowledgements:** CNPq. **License number of ethics committee:** N/A

01.004 **Gene expression of healing markers in human keratinocytes HaCat treated with a biomembrane derived from *Calotropis procera* (BioMemCpLP)** Duarte RS¹; Nunes, MO²; Rabelo LMA¹; Ferreira KQ²; Rangel GFP²; Alencar NMN²; Ramos MV³. ¹UFC, Dpt of Physiology and Pharmacology, Brazil; ²UFC, Fortaleza, PPG Pharmacology, Fortaleza, Brazil; ³UFC, Dpt of Biochemistry and Molecular Biology, Fortaleza, Brazil
The biomembrane composed of proteins isolated from the latex of *Calotropis procera* and PVA as a vehicle (BioMemCpLP) is a dressing that has demonstrated a healing action in pre-clinical and clinical trials. Keratinocytes are responsible for maintaining a barrier against water loss and microbial attack, therefore, after an injury to the skin, these cells proliferate, migrate and adhere in a process of re-

epithelialization. This work aimed to investigate the mechanisms involved in the healing activity of BioMemCpLP on HaCaT human keratinocytes. To assess cell proliferation and migration, a scratch test was performed in groups with and without the cell division inhibitor (mitomycin). Cell adhesion was assessed by staining the cells with crystal violet in direct contact with the biomembrane. The gene expression of E-cadherin, β -catenin and P63 was analyzed by Q-PCR. In this study, plant proteins were able to stimulate migration, reducing the scratch area at a concentration of 1.56 $\mu\text{g}/\text{mL}$ after 24 and 48 hours of incubation, a result also observed with mitomycin. The contact of cells with BioMemCpLP for 24 hours promoted cell adhesion observed by the increased intensity of the purple color of crystal violet. These bioactive proteins stimulated keratinocytes to express greater amounts of E-cadherin, β -catenin and P63 at a dose of 3.12 $\mu\text{g}/\text{mL}$. The synthesis of E-cadherin plays a fundamental role in the intercellular adhesion of the epidermis and in the activation of signals related to the structure and architecture of the cytoskeleton. Furthermore, the increase in β -catenin contributes to the activation of signaling pathways that allow the proliferation, survival, apoptosis, differentiation, adhesion and motility of these cells. Transcription factor P63 is also related to cell proliferation, being considered a marker of epidermal differentiation during stratification. Therefore, the results suggest that BioMemCpLP directly stimulates keratinocytes, helping to resolve the healing process. Acknowledgments: CNPq. Keywords: keratinocytes, biomembrane, *Calotropis procera*. License number of ethics committee: N/A

01.005 Acetylcholine alpha 7 nicotinic receptors in microglia: association of their microglial activity with increased intracellular calcium. Bello Santos VG, Gonçalves de Castro N. ICB-UFRJ

Introduction: Inflammatory responses to stimuli are essential body defenses against foreign threats. In the past few years, a number of studies demonstrated the functional expression of nicotinic acetylcholine receptors of the $\alpha 7$ type ($\alpha 7$ nAChRs) in macrophagic immune cells, both in the central nervous system (microglia) and in the periphery, where they have an essential role in the control of inflammation by the inhibition of pro-inflammatory cytokine release. While the $\alpha 7$ nAChR is well characterized as a calcium-permeable ligand-gated ion channel in neurons, published whole-cell patch clamp experiments suggest that ion channel activity is absent in macrophages and microglia. This divergence may indicate an uniqueness in the receptor's location (possibly intracellular) and function (possibly metabotropic) in these cell types. Therefore, we aimed at elucidating the $\alpha 7$ receptor transduction mechanism, which is critical to understanding the physiological role of these receptors in the immune response. **Methods:** We have used the RAW 264.7 cell line and mouse primary microglial cell culture (approved animal use protocol A6/19-001-16 CEUA-UFRJ). A fluorescence assay of alpha-bungarotoxin-rhodamine (α BGT-Rh) binding was used to identify $\alpha 7$ nAChRs in these cells. We performed whole-cell patch-clamp recordings and ratiometric (F340/F380) calcium microfluorimetry with fura 2-AM, to show agonist-evoked transmembrane currents and variations in intracellular Ca^{2+} concentration. **Results:** Cell imaging showed that α BGT-Rh (500 nM) bound to the receptors and methyllycaconitine (MLA) inhibited its binding (ANOVA and Tukey's test, $P < 0.005$), which proved that the labeling was specific and, therefore, microglia and RAW 264.7 were expressing nAChRs- $\alpha 7$. However, RAW 264.7 cells didn't show calcium transients in response to rapid infusion of the nicotinic agonists (1 mM choline or 1 mM nicotine), with or without the selective $\alpha 7$ potentiator (3 microM PNU-120596). In similar standard conditions, microglia showed no nicotinic calcium responses, and none of the two cell types showed nicotinic ionic currents. In contrast, $>90\%$ of the cells responded to stimulation with adenosine triphosphate (1 mM ATP), showing both ionic currents and calcium transients. It is already known that microglial inflammatory responses are highly temperature-sensitive, so we also performed microfluorimetry assays with temperature control. At 37°C , microglial cultures responded to $\alpha 7$ agonist (1mM nicotine with 3 microM PNU-120596) applied by a heated perfusion system with robust intracellular Ca^{2+} increases, while a selective $\alpha 7$ antagonist (MLA 10 nM) inhibited these responses. Extracellular Ca^{2+} restriction attenuated the $\alpha 7$ nicotinic calcium response in microglial cells, so the increase of intracellular Ca^{2+} was partially dependent on Ca^{2+} influx. **Conclusion:** These results suggest that the activation of $\alpha 7$ nAChRs triggers calcium signaling, which at least in part depends on extracellular calcium. This receptor activity is strongly dependent on experimental conditions and is compatible with an ionotropic transduction mechanism. License number of ethics committee: CAPES, CNPQ e FAPERJ.

01.006 Investigation of the neuroprotective effects of cannabidiol and artesunate in an *in vitro* model of SARS-CoV-2 neuroinfection. Pires-dos-Santos I¹, Costa KCM¹, Scomparin DS¹, Scarante FF¹, Martins-Júnior RB², Arruda-Neto E², Campos AC¹, Ferreira RR¹. ¹Dpt of Pharmacology, Medical School of Ribeirão Preto, Univ of São Paulo, Ribeirão Preto, Brazil; ²Dpt of Cell and Molecular Biology, Medical School of Ribeirão Preto, Univ of São Paulo, Ribeirão Preto, Brazil

At the end of 2019, the first cases of a new respiratory disease were reported. Rapidly the cause of the problem was identified by researchers – a new type of coronavirus, SARS-CoV-2, an RNA virus that has become a major public health concern, and the new disease was named COVID-19. The disease quickly spread worldwide, triggering the COVID-19 pandemics. Despite causing mostly respiratory symptoms, COVID-19 can also cause long-term neurological symptoms such as headache, dizziness, stroke, cognitive and neuropsychiatric symptoms. In 2015, our group demonstrated that the combination of Cannabidiol, the main non-psychotomimetic compound of *Cannabis sp.*, with artesunate, an antimalarial drug with anti-inflammatory properties, prevented the long-term neurological and psychiatric effects of severe cerebral malaria. Therefore, the present study was designed to determine if the neuroprotective effects of Cannabidiol and artesunate can prevent the deleterious effects of SARS-CoV-2 neuroinfection in an *in vitro* model. The human glioblastoma cell line SH-SY5Y was incubated with different concentrations of cannabidiol or artesunate, at concentrations of 100, 300, 1000, and 10000 nM, for 24 hours and underwent the MTT viability assay. This test was utilized as an initial screening of the drug concentrations for the following experiments. Then, the cells were incubated for 2h with 1 MOI of SARS-CoV-2. After the end of the experiments, we performed the proliferation assay (BrdU) and qPCR for the detection/determination of SARS-CoV2 replication (cells and supernatant). In the MTT assay, CBD (3 and 10 μ M) increased the viability of human neural-like cells in presence of SARS-CoV-2, while Artesunate did not alter the cell viability. CBD (at the concentration of 1000 and 3000 nM) increased cell proliferation and changed the neuronal dendritic morphology of SH-SY5Y. At the concentrations of 300 and 1000 nM, Artesunate facilitated the replication rate of SARS-CoV-2 replication in cell lysate and cell supernatant (300 nM). Our results suggest a possible neuroprotective effect of cannabidiol, but not artesunate, in an *in vitro* model of SARS-CoV-2 neuroinfection. Our results are still preliminary, and more experiments are needed to confirm and better elucidate the mechanism behind these results. Financial Support: CAPES, FAPESP, CNPq. License number of ethics committee: N/A

01.007 *In vitro* antineoplastic, anti-CD34 and anti-Ki67 effects of simvastatin in human acute lymphoblastic/lymphocytic leukemia and lymphoma cell lines. Rotta TD, Nicolosi JS, Souza VB, Schenka AA FCM-Unicamp, Dpt of Pharmacology, Brazil

Introduction. Acute lymphoblastic leukemias (ALL) are malignant disorders of immature B and T cells that occur characteristically in children, generally under the age of 6, representing 80% of childhood leukemias. Although therapeutic regimens for the pediatric age group have evolved in recent decades, in adulthood, the cure rate still remains close to 40%. The prognosis for children with ALL has substantially improved with the use of multi-agent therapy over the last few decades. But, unfortunately, two opposing challenges remain in childhood ALL treatment: relapse and toxicity. One of toxicities from ALL therapy consists in avascular necrosis (AVN). A recent research suggested over 70% of children with this cancer develop AVN. While the cause of AVN is multi-factorial, significant high-risk factors include high cholesterol and treatment with corticosteroids. Statins widely used as a cholesterol-lowering drug, especially simvastatin, have demonstrated cytostatic properties against several types of cancer cell lines. In previous studies, our group has confirmed some the anticancer and anti-CSC effects *in vivo*. However, so far, only a few studies have evaluated these effects in human lymphomas and lymphoblastic leukemias, none of them investigating the advantages of a possible association with classical chemotherapy agents. In this context, we evaluated cytotoxic, cellular proliferation and anti-CD34 effects of simvastatin isolated or combined with daunorubicin on MOLT-4, REH and Daudi cell lines. **Methods:** Acute lymphoblastic leukemia ALL (MOLT-4), Acute lymphocytic leukemia; non-T; non-B (REH) and Burkitt's lymphoma (Daudi) cell lines (5×10^4 /well/each cell) were exposed to simvastatin (SNV) and daunorubicin (DN) (0.0001-100 μ g/mL) in 96-well microtiter plate, for 72 hours. At the end of treatment protocols, the cells were assessed for viability using MTT assays. The absorbance was read in a SpectraMax 340PC 384 microplate reader (Molecular Device, 1311 Orleans Drive Sunnyvale, CA 94089) at 570 nm. The results were expressed as IC₅₀ and compared to those of a control drug (daunorubicin). IC₅₀ values were reported as mean \pm standard deviation (SD) of two independent experiments, each performed in quintuplicate. Then, each cell line was treated with IC₅₀ values of each drug alone, and the combination with IC₂₅ values (IC₂₅Daunorubicin+IC₂₅Simvastatin). The effect of these drugs on protein expression of Ki-67 (cell proliferation marker) and CD34 (expressed in acute leukemias) was determined by immunocytochemistry, respectively. One-way analysis of variance (ANOVA) was used for comparing groups and differences were assessed by a Tukey's post hoc test. **Results:** The results demonstrated that simvastatin exhibited cytotoxicity effect at a low dose on MOLT-4, REH and Daudi cell lines. The IC₅₀ values of simvastatin (SNV) and daunorubicin (DN) was determined as 0.308 ± 0.390 and 0.002 ± 0.001 on MOLT-4; 0.735 ± 0.517 and 0.002 ± 0.002 on REH and 2.233 ± 2.922 and 0.041 ± 0.024 on Daudi, at 72 h. The morphological alterations were evaluated, and it was observed that these leukemic cell lines showed similar morphology,

with proliferation of round lymphoid cells, moderate pleomorphism, hyperchromatic nuclei with irregular contours and sparse eosinophilic cytoplasm. There were no significant morphological changes after treatment. Immunocytochemistry was used to assess the expression of CD34 and Ki67 after the treatments using IC_{50} values (SNV, DN) and IC_{25} (SNV+DN). REH cells had significant decreased ($p < 0.0003$) when compared to the untreated cells (used as control). Ki67 marker had also high positivity on MOLT4 control with $p < 0.0031$, but CD34 marker showed no statistically significant expression with the analyzed variables ($p > 0.05$). And the treatments had no effect on the expression of both markers on Daudi cell line. **Conclusion:** Our study revealed anticancer effects of simvastatin on MOLT-4, REH and Daudi cell lines. We were able to confirm the anticancer effects of simvastatin, however, their relationship with anti-CD34 and anti-proliferation effects was shown only on REH cells. Such effects do not seem to be affected by all the type of leukemia. Furthermore, there was no significant benefit when simvastatin was **associated to the reference drug (daunorubicin)**. **Support:** FAEPEX-UNICAMP, FAPESP. **Keywords:** Acute lymphocytic leukemia, Simvastatin, Daunorubicin, anti-neoplastic drugs. **License number of ethics committee:** N/A

01.008 **Different neural cells have distinct mitochondrial function against hypoxia, a possible implication to psychiatric disorders.** Silva LFS, Rosenstock TR Univ of São Paulo – Inst of Biological Sciences **Background:** The relationship between hypoxia and psychiatric illnesses is known mainly due to the association of placental vasoconstriction and changes in neurodevelopment. Because hypoxia modifies energy production and mitochondrial metabolism and dynamics, our goal was to evaluate mitochondrial function in primary culture of astrocytes and neurons after chemical and neonatal hypoxia. The cobalt chloride ($CoCl_2$ - 800 μM and 2 mM for 24 hours) mimics hypoxia in cells (chemical hypoxia), and neonatal hypoxia was achieved using as model the Spontaneously Hypertensive Rats (SHR). **Methods:** Cells were loaded with i) Fluo-4-AM (10 μM) to verify cytosolic calcium levels, ii) TMRE (500 nM) to analyze mitochondrial membrane potential, and iii) H_2DCF -DA (20 μM) and MitoSox (5 μM) to investigate redox homeostasis. Real-time PCR were performed to verify the expression of genes related to mitochondrial metabolism and biogenesis. The levels of high-energy compounds were also investigated. All the experimental procedures were performed according to the ethical principles and experimentation of the University of São Paulo (CEUA: 7646061120). **Results:** Astrocytes and neurons after chemical or neonatal hypoxia presented disturbances in Ca^{2+} handling, depolarized mitochondria, and alterations in redox system with an increase in reactive oxygen species (ROS) and superoxide levels concomitant with a disturbance in *Nfe2l2* expression. In addition, both hypoxias promoted alterations in ATP, Pyruvate and Lactate levels, and NAD^+ / $NADH$ ratio in both cell's types. Interestingly, the neonatal hypoxia showed increased expression of genes related to mitochondrial content, as *Pgc1a*, *Nrf1*, *Tfam*, *MtCo1* and *Tom-20* in astrocytes, in neurons the *MtCo1* were downregulated. **Conclusion:** Altogether, our data suggest that hypoxia can induce mitochondrial deregulation and decrease energy metabolism in both neurons and astrocytes, despite only astrocytes react to hypoxia increasing mitochondrial DNA content. This mechanism may be related to differences in adaptation to hypoxia, which can ultimately lead to different cellular responses, such as modification of neurotransmission. Financial support: FAPESP: 2018/13814-0 **License number of ethics committee:** University of São Paulo (CEUA: 7646061120)

01.009 **Expression of HCC markers in the liver of rats exposed to dexamethasone in utero and subjected to the excess of fructose intake in adult life.** Almeida LS¹, Campos CV¹, Teixeira CJ², Sodré FS², Anê GF¹, Bordin S². ¹Dpt of Pharmacology, Faculty of Medical Sciences, State Univ of Campinas, Brazil ²Dpt of Physiology and Biophysics, Inst of Biomedical Sciences, Univ of São Paulo, São Paulo, Brazil **Introduction:** *In utero* glucocorticoids exposure associated with fructose consumption during adult life can exacerbate metabolic disorders, particularly in the liver. Hepatocellular Carcinoma (HCC) has been recently reported in patients with Nonalcoholic Fatty Liver Disease (NAFLD) without evidence of underlying cirrhosis. In the present study, we investigated alterations in hepatic lipid metabolism and the expression of HCC markers in rats exposed to dexamethasone *in utero* and subjected to excess of fructose intake in adult life. **Methods:** Pregnant wistar rats were randomized into two groups during the third gestational period: one was treated with dexamethasone (0.1mg/kg/day in drinking water), and the other was kept untreated. Adult offspring born to control or treated mothers (CTL and DEX) were assigned to receive 10% fructose in the drinking water (fructose and DEX-fructose) for eight weeks. Biochemical parameters were measured using commercial kits. Gene expression was analyzed by both real time RT-PCR and western blotting. The results were expressed as mean \pm standard error of the mean and analyzed by two-way ANOVA followed by Tukey post-test ($p \leq 0.05$). **Results:** Offspring subjected to high fructose intake displayed increased plasma triglycerides level (CTL-fructose 63% higher than CTL; $p = 0.04$; DEX-fructose 73% higher than CTL; $p = 0.01$) and DEX-fructose rats displayed increased cholesterol level (28% higher than CTL; $p = 0.007$) and decreased HDL (32% lesser than CTL; $p = 0.01$), accompanied by an increase in the mesenteric

fat (57% higher than CTL; $p=0.01$). In the liver, DEX-fructose rats exhibited increased expression of Apolipoprotein B (Apo-B) and Alpha Fetoprotein (AFP) compared to the CTL group ($p<0.0001$ and $p=0.009$, respectively) along with increased expression of Proliferative Nuclear Cell Antigen (PCNA) ($p=0.04$ vs. CTL) and Hepatocyte Growth Factor (HGF) ($p=0.03$ vs. CTL). In addition, DEX-fructose rats showed reduced expression of Insulin-like Growth Factor I (IGF-I) compared to the CTL group ($p=0.04$). **Conclusions:** The present data confirm previous results showing that *in utero* exposure to excess of glucocorticoids associated with high fructose consumption later in life exacerbates metabolic disorders, leading to hepatic steatosis and hypercholesterolemia. These features are associated with increased AFP, PCNA and HGF, and decreased IGF-I expression. High levels of AFP, PCNA and HGF, and low levels of IGF-I are classical findings of HCC. Further investigations are required to establish the functional relevance of our current findings. **Financial Support:** CNPq (Grants 140333/2020-7), FAPESP (Grants 2013/07607-8 and 2019/03196-0). **License number of ethics committee:** CEUA/UNICAMP (5530-1/2020).

01.010 **Effects of chronic administration of ouabain in rats on high salt diet.** Feijó PRO, Panice MS, Lara LS, Quintas LEM UFRJ, Inst of Biomedical Science, Brazil

Introduction: The main risk factor for the development of systemic arterial hypertension (SAH) is the excessive intake of sodium. One of the hypotheses to justify this pressure effect of sodium is its possible role in the hypothalamus and adrenal glands, stimulating the secretion of the endogenous cardiogenic steroid ouabain (OUA), whose molecular target is the Na^+/K^+ -ATPase (NKA). An experimental model of OUA-induced hypertension in rats was developed to elucidate the relevance of OUA in SAH. However, the literature presents controversial results in relation to OUA-evoked pressure effect and lacks results of the long-term OUA administration in kidney, heart and liver. Thus, the objective of this work was to evaluate the blood pressure and tissue effects in rats submitted to OUA chronic administration and high salt diet (HSD). **Methods:** Male Wistar rats received OUA subcutaneous administration (30 $\mu\text{g}/\text{kg}/\text{day}$) or vehicle (control group: C) and normosodic (NS: Na^+ 0.5%) or hypersodic (HS: Na^+ diet 3.12%) for 14, and 35 days. Ultrasonography of animals was performed on days 0, 14, and 35. The animals were placed in metabolic cages for urine collection and assessment of renal parameters. On the last day of administration, systolic blood pressure (SBP) was measured by carotid cannulation. After euthanasia, blood was collected. Kidneys and hearts were dissected to prepare homogenates to assess enzyme activity and NKA expression. All tissues were submitted to immunohistological evaluation. The results were expressed as mean \pm SEM and statistically analyzed using the One-way ANOVA test (significance: $p<0.05$). Protocol 046/19 of the Ethics Committee for the Use of Animals of UFRJ. **Results:** There was no significant change in SBP after OUA administration. The pre-administration and OUA administration for 14 days did not produce ultrasound changes of renal sagittal or transverse length. After 14 days of administration there was an increase in water intake in OUA+HS (74 ± 7.0 mL, $n=5$), compared to the OUA+NS group (20 ± 2.9 mL, $n=4$; $p<0.0003$) and C+NS (19 ± 0.6 mL, $n=3$; $p=0.001$), and the C+HS (50 ± 11.6 mL, $n=3$; $p=0.0327$) compared to the OUA+NS group. Proportionally, there was an increase in urinary volume in the OUA+HS (52.5 ± 8.4 mL, $n=4$) compared to the OUA+NS (6.5 ± 1.4 mL, $n=4$; $p=0.0008$) and C+NS groups (7.3 ± 0.4 mL, $n=3$; $p=0.0062$) and in the C+HS (36.5 ± 9.8 mL, $n=3$; $p=0.0156$) compared to OUA+NS group. After 14 days, the blood count revealed a decrease in globular volume in the C+HS ($39.4 \pm 1.1\%$, $n=5$) compared to the C+NS group ($n=4$; $45.3 \pm 0.5\%$, $n=5$; $p=0.0024$) and urinalysis revealed an increase in the pH of the C+HS (7.9 ± 0.2 , $n=4$) compared to the C+NS (6.6 ± 0.2 , $n=4$; $p=0.0102$). After 14 days, renal cortex NKA enzymatic activity of the OUA+HS ($11,30 \pm 2,1$, $n=5$; $p=0,046$) was lower compared to the C+NS group ($17,56 \pm 1,6$, $n=5$). The NKA activity of other groups did not show significant differences (C+HS: $12,65 \pm 0,4$, $n=5$; O+NS: $13,39 \pm 0,6$, $n=5$). **Conclusion:** Our preliminary results show that association of OUA with or without HSD in rats do not elevate SBP up to 35 days. The decreased NKA activity is suggested to be compensatory to excessive salt intake, which would maintain normal blood pressure in rats. The decrease in globular volume may be related to the increase in water intake and the increase in urine pH to the hypersodic diet. **Financial Support:** CAPES, CNPq, FAPERJ. **License number of ethics committee:** Protocol 046/19 of the Ethics Committee for the Use of Animals of UFRJ.

01.011 **Dietary supplementation with chia oil alters the white adipose tissue remodeling in obese mice.** Assis-Ferreira A, de Brito NM, Simões RL, Saldanha-Gama R, Barja-Fidalgo C, da Silva SV. ¹UERJ Dpt of Cell Biology, Rio de Janeiro, Brazil

Introduction: How white adipose tissue (WAT) expands, and remodels directly impacts the development of metabolic disorders associated with obesity. Our group had demonstrated that dietary supplementation with chia seed oil (*Salvia hispanica* L.) improves insulin sensitivity in the skeletal muscle and induces browning of subcutaneous adipose tissue (SAT) during obesity. **OBJECTIVE:** To investigate the role of dietary supplementation with chia oil in WAT remodeling from obese mice. **Methods:** Thirty-days-old male

C57BL/6 mice were fed a regular chow diet (10% Kcal from fat - C-group) or a high-fat diet (45% Kcal from fat - H-group) for eight weeks. H group was supplemented with 1.5% (v/v) of chia seed oil (HC group) after eight weeks on a high-fat diet for a further six weeks. **Results:** Chia oil supplementation reduced body fat mass (C: 10,26g \pm 1,26; H: 21,61g \pm 2,91; HC: 19,54g \pm 2,13) and increased body lean mass in obese mice (C: 72,67g \pm 0,70; H: 64,92g \pm 1,91; HC: 66,35g \pm 1,16). The body composition alterations were accompanied by reducing fat pad mass of visceral adipose tissue (VAT) (C: 478,1g \pm 47,26; H: 1545g \pm 104,5; HC: 1427g \pm 131,6) without changes in subcutaneous adipose tissue (SAT) (C: 258,1g \pm 16,85; H: 941,5g \pm 68,97; HC: 874,9g \pm 99,2) from obese mice. The supplementation with chia oil also improved glucose tolerance and insulin sensitivity. Besides, it reduced the IL-10 (C: 654pg/mL \pm 75,29; H: 442,7pg/mL \pm 106,1; HC: 392,6pg/mL \pm 43,79) levels and increased TNF- α (C: 399,9pg/mL \pm 96,55; H: 628,6pg/mL \pm 95,5; HC: 1449pg/mL \pm 368,9) levels in supernatants of VAT from obese mice, and increased the IL-10 (C: 449pg/mL \pm 92,27; H: 346pg/mL \pm 39,81; HC: 568,3pg/mL \pm 143) production by SAT. Mesenchymal cells isolated of stromal vascular fractions (SVF-MCs) of VAT and SAT from obese mice had higher proliferative capacity in basal conditions than control and, chia oil supplementation seems to inhibit this effect in MCs of obese SAT. We also observed that obesity increases lipid accumulation in MCs from obese VAT and chia oil treatment seems to increment this effect. No significant alterations were observed in adipogenic differentiation in MCs from SAT. Ultimately, preliminary data showed that chia oil supplementation appears to induce the browning process of both WAT compartments from HC mice. Statistical analysis was performed using the software GraphPad Prism version 5.00 for Windows. A two-tailed Mann-Whitney U test was used for nonparametric analysis between groups, and The Kruskal-Wallis with Dunn's test was used for the nonparametric ANOVA between groups. Data are presented as mean \pm s.e.m. **Conclusion:** So far, we have concluded that chia oil supplementation during obesity acts differentially in the WAT morphofunctional remodeling, which seems to contribute to improving the associated metabolic dysfunction. Financial support: CAPES; CNPq. **License number of ethics committee:** CUEA protocol n $^{\circ}$ 024 / 2017

01.012 **Endothelial P2Y₂ receptor is involved in metastatic prostate cancer cell adhesion and its effect is inhibited by atorvastatin.** Cardoso TC, Rocha MA, Silva CLM UFRJ-ICB Lab. Farmacologia Bioquímica e Molecular

Objectives/background: Prostate cancer is the second most prevalent type of cancer in men worldwide (Bray *et al.*, Lancet Oncol. 13: 790-801, 2012), and metastasis is the main cause of cancer-related death. Understanding the molecular mechanisms underlying tumor metastasis is crucial to control this fatal disease (Maishi and Hida, Cancer Sci. 108(10): 1921-1926, 2017). ATP is released at high levels from malignant cells acting as potent prometastatic factor (Ferrari *et al.*, Trends Pharmacol Sci. 38(3): 277-290, 2017) and is known to modulate a variety of processes linked to endothelial cell (EC) activation. P2Y₂ receptor (P2Y₂R) activation by ATP or UTP markedly induces ICAM-1 and VCAM-1 expression in ECs, a hallmark of endothelial dysfunction which may play a role in cancer cell adhesion (Jin *et al.*, Breast Cancer Res. 26;16(5): R77, 2014). The non-lipid modifiable effects of statins termed as pleiotropic effects improves endothelial dysfunction and it has been considered for drug repositioning purposes (Bedi, *et al.*, Naunyn Schmiedebergs Arch Pharmacol. 389(7): 695-712, 2016). Thus, our aim was to characterize the UTP effect on prostate cancer cells adhesion to EC and the putative inhibitory effect of atorvastatin.

Methods and Results: EC EA.hy926 and tumor prostate cancer cells DU-145 were obtained from BCRJ. The endothelial cell line EA.hy926 and prostate DU-145 cells were maintained in DMEM and RPMI (supplemented with 10% FBS and antibiotics), respectively (37 °C and 5% CO₂). EA.hy926 cells were incubated for 4 h with DMEM (basal) and UTP (100 μ M) in the absence or presence of the antagonist suramin (100 μ M) or atorvastatin (added 24 h before). After that, calcein-labeled DU-145 cells (5 \times 10³ cells/well) were added for 30 min following washing. Four fields per well were randomly chosen and analyzed by fluorescence microscopy (200X magnification). The P2Y₂R agonist UTP (100 μ M) stimulated DU-145 cell adhesion to ECs and suramin (100 μ M) blocked its effect. The P2Y₂ receptor agonists ATP (100 μ M) and the selective P2Y₂ agonist 2thioUTP (100 nM) also induced the adhesion of DU-145 cells with an effect similar to UTP. The effect of 2thioUTP was also blocked by 100 μ M suramin corroborating that the P2Y₂R are mediating the tumor cell adhesion. Moreover, the EC pretreatment with atorvastatin 1 μ M (24 h) prevented the adhesion of DU-145 cells to EC monolayer induced by UTP (100 μ M). **Conclusion:** Our data indicate the role of purinergic signaling mediated by P2Y₂R on the adhesion of metastatic prostate cancer cells to endothelial cells and the inhibition by atorvastatin of this process. These data suggest that atorvastatin could have a beneficial role to reduce endothelial prostate cancer cell adhesion *in vivo*. **Acknowledgements:** CNPq. **License number of ethics committee:** N/A

01.013 **Activation of kinases downstream of angiotensin II type 1 receptor is potentiated in biological systems with high levels of O-GlcNAcylated proteins.** Silva-Neto JA¹, Duarte DA², Simões SC², Mestriner FL¹, Pedersoli CA¹, Costa TJ¹, Bressan AF¹, Abrão EP¹, Silva JF¹, Costa-Neto CM², Tostes RC¹ ¹Univ de São Paulo. Faculdade de Medicina de Ribeirão Preto – Dept de Farmacologia, ²Univ de São Paulo. Faculdade de Medicina de Ribeirão Preto – Dept Bioquímica e Imunologia

Introduction: The angiotensin II (ANG II) type I receptor (AT1R) is a seven transmembrane-spanning receptor implicated in volume maintenance, blood pressure, outflow of autonomic neurotransmitters, and other cardiovascular parameters. AT1R is a key therapeutic target in many cardiovascular diseases. Following AT1R activation, a variety of intracellular pathways, including G-proteins, beta-arrestins, and several kinases are activated. Cardiovascular risk conditions, such as diabetes and hypertension are linked to overactivation of ANG II signaling as well as to high levels of intracellular O-GlcNAcylated proteins. O-GlcNAc is a posttranslational modification of intracellular proteins where N-acetylglucosamine is attached to serine or threonine residues, that can also be targeted by phosphorylation. O-GlcNAc modifies protein function in many organs, including the kidney, blood vessels and heart. Following both premises, this study investigated whether high O-GlcNAcylated-protein levels impact kinases activity downstream of AT1R, counteracting beta-arrestin activity. **Methods:** Two experimental approaches were used: first, HEK293T cells stably expressing a human clone of AT1R were treated with thiamet-G (TMG 1 μ M for 16 h) to pharmacologically increase O-GlcNAc-proteins. The AT1R was activated by ANG II or TRV27, a beta-arrestin-biased agonist, and their potency (pEC_{50}) and efficacy (E_{max}) were determined. Phosphorylation of P42/44-MAP-kinase was determined by immunoblotting; real-time calcium mobilization with the FLUO-4 fluorescent probe; beta-arrestin recruitment and receptor internalization by bioluminescence energy resonance transfer; and receptor density by an ELISA assay. Another set of experiments were performed in isolated resistance mesenteric arteries of a spontaneously diabetic mouse strain [C57BLKS/6JLepR-/- (+), protocols approved by the CEUA-FMRP (72/20)]. Isometric vascular contractile responses to ANG II were evaluated in the presence of vehicle or TRV27, and pEC_{50} , E_{max} , desensitization ratio and AT1R tachyphylaxis were determined. **Results:** After enrichment of O-GlcNAc-proteins, HEK293T cells showed increased P42/44 phosphorylation in response to ANG II and TRV27 (100 nM), compared to non-treated cells. YM-254890 (1 μ M), a G α_q inhibitor, did not reverse increased P42/44 phosphorylation in TMG-treated cells. The basal content of P42/44, PKC, GRK2 kinases, or beta-arrestin was not modified by TMG treatment. ANG II efficacy for calcium mobilization increased in TMG-treated cells; the potency or AT1R density were not altered. The pattern of beta-arrestin recruitment and AT1R internalization in response to ANG II or TRV27 was maintained after TMG treatment. Isolated arteries of diabetic mice exhibited higher E_{max} , but no changes in pEC_{50} to ANG II compared to arteries from non-diabetic counterparts. Tachyphylaxis and desensitization were higher in diabetic mice, but the effect of TRV27 (1 nM) was similar in both groups. **Conclusion:** These data direct us to conclude that increased O-GlcNAcylation of proteins enhances AT1R-stimulated kinases activity, but does not change beta-arrestin activation. Increased O-GlcNAcylation-driven AT1R overactivation may increase cardiovascular risk factors in diabetes. Financial Support: University of São Paulo, CAPES and FAPESP **License number of ethics committee:** CEUA FMRP 72 /2020

01.014 **Endothelial P2Y2 receptors signaling in primed cells contribute to mesenteric inflammation in schistosomiasis.** Oliveira NF¹, Tamura AS², Coutinho-Silva R², Savio LEB², Silva CLM¹. ¹Inst de Ciências Biomédicas - Lab de Farmacologia Bioquímica e Molecular, UFRJ; ²Inst de Biofísica Carlos Chagas Filho - Lab de Imunofisiologia, UFRJ

Introduction: Schistosomiasis is a chronic intravascular disease caused by *Schistosoma mansoni*. Parasite and egg antigens induce a primed pro-inflammatory endothelial cell (EC) phenotype and nucleotides release. In turn, ATP triggers purinergic signaling and modulates host immune responses. The P2Y2 receptors (P2Y2R) are equally activated by both ATP and UTP and has been studied in some vascular injury models (Burnstock G., Front. Pharmacol. (8): 661, 2017) but their role during schistosomiasis is unknown. Therefore, the objective of this work was to investigate the role of endothelial P2Y2R to leukocyte adhesion during schistosomal inflammation in mice. **Methods:** Newborn (7-10 days old) male Swiss mice were infected with *S. mansoni* and animals were used in compliance with ethical standards (CEUA A01/21-048-16). Primary cultures of EC were obtained from control and infected mice (52-63 days p.i.) kept in DMEM enriched with 20% FBS and plated to leukocyte adhesion assay. Mononuclear cells were isolated from peripheral blood using Ficoll gradient. Confluent EC were stimulated with UTP or vehicle (basal) for 5h, in the absence or presence of the specific pre-treatment (added 30 min before) and then co-incubated with mononuclear cells for 30 min. Cells were washed and then four fields/well were randomly chosen and imaged using an optical microscopy (400x magnification) to determine the number of adherent cells. **Results:** UTP (1-300 μ M) increased leukocyte adhesion to EC in a concentration-

dependent manner with the maximal effect observed at 100 μM in both groups. In the control group, the EC treatment with UTP 100 μM increased leukocyte adhesion from 2.6 ± 0.3 to 6.5 ± 0.3 cells/field ($n = 36$, $P < 0.001$), but the effect was higher in the infected group (12.4 ± 0.6 cells/field, $n = 36$, $P < 0.001$). The P2Y2R antagonist suramin 50 μM blocked the agonist effect, while ectonucleotidases inhibitor (ARL67156, 100 μM) did not alter UTP effect. Both data suggest a P2Y2R-mediated effect. Western blotting data confirmed the presence of P2Y2R and showed similar levels of protein expression. In both groups, phospholipase C inhibition (U73122, 1 μM), intracellular Ca^{2+} chelation (BAPTA-AM, 3 μM), and Src inhibition (SU6656, 5 μM) impaired the agonist effect. However, in the infected group, both U73122 and BAPTA not only blocked the UTP (100 μM) effect, but also decreased the basal leukocyte adhesion suggesting that the EC has a pro-adhesive phenotype ($P < 0.001$). **Conclusion:** The mesenteric endothelial P2Y2R increases leukocyte adhesion. However, the downstream endothelial Ca^{2+} signaling is enhanced during schistosomiasis. These data unveil the role of endothelial P2Y2R signaling to mesenteric inflammation during schistosomiasis. **Acknowledgements:** CNPq, CAPES. **License number of ethics committee:** CEUA A01/21-048-16

01.015 Binding of the seminal plasma protein SVS2 to EPPIN: implications to sperm function. Mariani NAP, Santos NCM, Santos GVM, Camargo IA, Kushima H, Silva EJR. PPG Pharmacology and Biotechnology, Dpt of Biophysics and Pharmacology, Inst of Biosciences, São Paulo State Univ, Botucatu, Brazil

Introduction: Seminal plasma proteins play essential roles in the modulation of male fertility. The major seminal plasma protein in mice is the seminal vesicle secretory protein SVS2, which is a homolog to human semenogelin-1 (SEMG1). SVS2 binds to ejaculated spermatozoa and inhibits sperm capacitation-associated events, such as hyperactivation and acrosome reaction. We previously showed that SVS2 was coimmunoprecipitated with the sperm-binding protein EPPIN in mouse spermatozoa. Thus, we hypothesize that SVS2 modulates mouse sperm function by interacting with EPPIN. Herein, we aimed to investigate EPPIN/SVS2 interaction profile. **Methods:** We performed a protein-protein interaction assay using the AlphaScreen technology, a bead-based platform to evaluate biomolecular interactions. We used full-length recombinant GST-tagged mouse EPPIN (mEPPIN; P22-T134, lacking the signal peptide) conjugated to anti-GST acceptor beads and full-length recombinant 6xHis-tagged mouse SVS2 (mSVS2; G35-G375, lacking the signal peptide) conjugated to Ni^{2+} chelate donor beads. In additional experiments, we replaced mSVS2 with its truncated constructs: C-terminal truncation containing SVS2 unique Cys residue (mSVS2-CT; Q32-V118; C98) or N-terminal truncation lacking SVS2 Cys residue and containing its two 31-aminoacid long repeats (mSVS2-NT; R98-G375). **Results:** We showed that mSVS2 binds to mEPPIN in a concentration-dependent and saturable manner (10 nM EPPIN + 0.3 to 100 nM SVS2: $\text{EC}_{50} = 24$ nM, 95% CI: 20.6-27.9; 30 nM SVS2 + 0.1 to 10 nM EPPIN: $\text{EC}_{50} = 0.6$ nM, 95% CI: 0.4-0.8). AlphaScreen assays performed with recombinant mSVS2 truncations showed that mSVS2-CT displayed low EPPIN-binding capacity (<80% reduction in the specific signal, $p < 0.05$). In parallel, we observed no changes in EPPIN binding between mSVS2-NT and full-length mSVS2, indicating that residues downstream SVS2 Cys residue are required to bind EPPIN. Interestingly, we also observed that human SEMG1 binds mEPPIN in a concentration-dependent manner. **Conclusions:** Our findings show that SVS2 sequence R98-G375 contains the binding pocket required for its interaction with EPPIN. Moreover, the interaction between mouse EPPIN and human SEMG1 suggests that EPPIN functions were conserved as a binding site for seminal plasma proteins on spermatozoa during speciation events. Further studies will be required to further pinpoint SVS2 residues critical to binding EPPIN and the physiological relevance of EPPIN/SVS2 interaction in events associated with sperm function. **Financial Support:** FAPESP/CAPES/CNPq. **Approval by Animal Research Ethical Committee:** #5219150420 (Institute of Biosciences, São Paulo State University, Botucatu-SP, Brazil). **License number of ethics committee:** #5219150420 (Institute of Biosciences, São Paulo State University, Botucatu-SP, Brazil)

01.016 Effect of extracellular vesicles derived from breast tumor cells on human neutrophils polarization. Amorim CS¹, Docasar CL¹, Renovato-Martins M², Barja-Fidalgo C³, Moraes JA¹ ¹UFRJ Redox Biology Lab, Inst de Ciências Biomédicas, Brazil ²UFF Dpt of Cell and Molecular Biology, Brazil ³UERJ Dpt of Cell Biology, Brazil

Breast cancer is the second most common type of cancer in the world and the first most common among women. It is well established the relationship between cancer and inflammation, and neutrophil infiltration has been described in tumors since 1863¹. Inflammation is a protective mechanism, as it aims to eliminate the causative agent of a lesion with subsequent tissue repair². Immune system cells, including neutrophils, are recruited by the tumor microenvironment as a site of chronic inflammation and begin to favor tumor growth. The neutrophils present in the tumor site are called tumor-associated neutrophils (TAN) and can present two phenotypes: N1 (antitumor) or N2 (pro-tumor)³. As an *in vitro* polarization

strategy for TAN, we use extracellular vesicles (EVs) derived from breast tumor cells (MDA-MB-231) compared to the EVs derived from non-tumor epithelial cells (MCF10). Our aim was to investigate the role of EVs derived from MDA-MB-231 in the polarization of neutrophils to N2 phenotype and to elucidate the mechanisms involved in this effect. Neutrophils were isolated in a ficoll gradient from peripheral blood of healthy donors and were treated with EVs for different times at 37°C and 5% CO₂. We investigated EVs effects on neutrophils: chemotactic capacity (1h), production of neutrophilic extracellular traps (NET, evaluation of extracellular DNA after 3h), apoptosis protection (morphology and Annexin+/PI- for 20h), intracellular reactive oxygen species production (probes DCF-DA and DAF-DA after 3h), cytokines secretion (ELISA, evaluation after 3h), as well as the specific labeling for CD95 (N1) and CD184 (N2) (flow cytometry after 3h). The viability of tumor cells treated with polarized neutrophil was observed by MTT, and pro-caspase-3 expression was observed via western blotting after 24h of treatment. We observed that MDA EVs showed chemotactic capacity when compared to MCF10 EVs. Interestingly, treatment of tumor EVs with annexin-V reduced their chemotactic effect. Neutrophils treated with tumoral EVs produced more extracellular DNA than non-tumor EVs, suggesting NET production. On the other hand, annexin V inhibited tumor EVs effect in the release of extracellular DNA. Neutrophils treated with MDA EVs had their half-life increased, and they were able to produce more intracellular ROS than the other experimental groups. We also observed that neutrophils treated with tumor EV had an increase in the secretion of IL-8 and VEGF. Corroborating these data, we observed that MDA EVs induced an increase in the N2 marker CD184. Finally, neutrophils treated with MDA EVs were able to increase tumor cell viability, probably through the inhibition of pro-caspase-3 cleaved. Altogether, our results show that MDA EVs can induce an N2-like phenotype, and annexin-V acts as an important agent counter-regulating this effect. 1. HANAHAN, D.; WEINBERG, R.A. Hallmarks of Cancer: The Next generation. *Cell* 144: 646-674, 2011. 2. MANTOVANI, A. et al., Neutrophils in the activation and regulation of innate and adaptive immunity. *Nature Reviews – Immunology*, 2011. 3. FRIDLINDER, Z.G. et al, Polarization of tumor-associated neutrophil phenotype by TGF-β: “N1” versus “N2” TAN. *Cancer Cell*, 2009. Financial support: FAPERJ. License number of ethics committee: CAAE 38257914.7.0000.5259

01.017 **MR/GR heterodimerization may impair glucocorticoids effects in LPS-induced inflammation depending on *in vitro* brain cellular composition.** Duque EA, Munhoz CD. ICB-USP, Dpt of Pharmacology, Brazil

Introduction: Although the anti-inflammatory and immunosuppressive actions of glucocorticoids (GCs) secreted by the adrenal glands during stress are well established, evidence suggests that GCs can potentiate some aspects of inflammation in the brain. Receptor heterodimerization may differentially modulate GCs-related gene expression and we hypothesized that mineralocorticoid (MR) and glucocorticoid (GR) receptors heterodimerization could play a crucial role in the pro-inflammatory GCs effects (Mifsud et al. *PNAS*, 113: 11336, 2016). Furthermore, the later would be region and cell-type-dependent in the brain (Sorrells et al. *J. Neur.*, 33: 7877, 2013). In this study, we verified whether corticosterone (CORT) pre-treatment followed by LPS could differently induce MR/GR heterodimers and gene expression of classical GR targets (Gilz and Fkbp5) in mixed and astrocyte-enriched primary cortical cultures. **Methods:** Primary mixed cortical cultures were obtained from newborn rats (P1-P4) as described previously (Ahlemeyer et al., *J Neur. Meth.*, 149: 110, 2005). Astrocyte-enriched cortical cultures were obtained by shaking mixed cortical cultures on 14^oDIV (days-in-vitro) as described previously (Ni et al., *Curr Protoc Toxicol.*, Chapt 12: Unit 12, 2010). Cultures were maintained in DMEM High Glucose media supplemented with 10% Fetal Bovine Serum (FBS), 0,5% penicillin/streptomycin. Then it was changed to 10% FBS charcoal-stripped on 5^oDIV, and to 2% FBS charcoal-stripped during the experiments on 7^oDIV after culturing or separation. Cultures were treated with ethanol (1%, 24 h) or CORT (1 μM, 24 h) followed by saline or LPS (10 μg/mL, 1 h). For MR/GR heterodimerization analysis, we immunoprecipitated GR as described previously (Kaboord et al., *Meth Mol Biol.*, 424: 349, 2008) and performed western blot assay for MR protein within the immunoprecipitated GR samples. Real-time RT-PCR of two GR known gene targets was done to assess proper GR functionality. Primer sequences: Gilz 5'-CAGGCCATGGATCTAGTGAA-3'(sense) and 5'-AGCGTCTTCAGGAGCGTATT-3'(antisense); Fkbp5, 5'-ATGTACTIONGCTCCCTTGAAG-3'(sense) and 5'-GAACCAATGCTGAGCTTATG-3'(antisense). Both western blot and PCR assays were done as described previously (Munhoz et al., *J. Neur.*, 26: 3813, 2006). **Results:** CORT pre-treatment followed by LPS increased MR/GR heterodimers (CORT+LPS- 216,9±40,8%) when compared to control ethanol and CORT alone groups (EtOH-100% and CORT-66,9±4,3%, p<0.001) in mixed cortical cultures, but not in astrocyte-enriched cultures. Real-time RT-PCR assay showed that expression of GR classical target genes was impaired in mixed cortical cultures (Gilz: CORT 0,69±0.49 fold and CORT+LPS-1,36±0.72 fold; Fkbp5: CORT- 2,73±0.86 fold and CORT+LPS-3,58±0.89 fold) but typically expressed in astrocyte-enriched cultures (Gilz: CORT-3,8±1,3 fold vs EtOH and CORT+LPS-4,1±0.9 fold vs LPS-0,3±0,1 fold, both p<0.001; Fkbp5:

CORT-10,7±3,2 fold vs EtOH, $p < 0.05$, and CORT+LPS-7,1±3 fold vs LPS 0,7±0,2 fold, $p < 0.001$). **Conclusion:** MR/GR heterodimers were only increased when cultures were submitted to CORT pre-treatment followed by LPS in mixed but not in astrocyte-enriched cortical cultures. In addition, GR classical target genes expression was impaired in mixed but not in astrocyte-enriched cortical cultures. These data suggest that GCs exposure previously to an inflammatory stimulus can differently regulate gene expression depending on cellular composition, and this could be due to GCs receptors heterodimerization. **Financial support:** CNPq and FAPESP. This study was financed in part by CAPES-Finance Code 001 **License number of ethics committee:** CEUA-ICB 84/2016

01.018 HDAC6/8 inhibitor, LASSBio 1911: a new alternative to sensitize prostate cancer cells to anticancer agents Guerra FS¹, Rodrigues DA², Manssour Fraga CA², Dias Fernandes P¹ ¹Univ Federal do Rio de Janeiro, Inst de Ciências Biomédicas: ¹Lab de Farmacologia da Dor e da Inflamação e ²Lab de Avaliação e Síntese de Substâncias Bioativas (LASSBio). Rio de Janeiro, Brasil

Introduction: WNT signaling pathway has potential importance in the development of prostate tumors and is also important in the prostate tumor microenvironment, in which WNT proteins secreted by the tumor cells promote resistance to therapy as well WNT- β -catenin signals promote self-renewal or expansion (Murillo-Garzón, V. & Kypta, R. Nat Rev Urol. 2017 Nov;14(11): 683-696). Histone deacetylase (HDAC) inhibitors have an effect on cell cycle progression and can act to promote self-renewal of several lineages of tumor cells (Bora-Singhal et al., Sci Rep. 2020 Mar 13;10(1): 4722). We hypothesized that HDAC inhibitors regulate downstream Wnt signaling and proliferation of prostate tumor cells. The aim of this work was to investigate whether doxorubicin-induced apoptosis can be potentiated by the HDAC inhibitor LASSBio 1911, and whether the changes in Wnt signaling pathway are involved in LASSBio 1911/doxorubicin-induced apoptosis. **Methods:** We treated PC3 and DU145 cells, both human prostate cancer cell lines, with 0.05, 0.1, 0.5, 1, 5 and 10 μ M of LASSBio-1911 and/or doxorubicin, examined the cell viability by MTT assay and the membrane integrity examined by LDH activity assay. The intracellular generation of reactive oxygen species (ROS) was assayed with the 2'-7'-dichlorofluorescein diacetate (DCFH-DA) method. The apoptosis was detected by using Annexin V/PI staining and examined by DNA degradation. Western blot was used to confirm changes in the expression of Wnt pathway. **Partial Results:** Doxorubicin-induced cell death and apoptosis was enhanced by LASSBio-1911 about 3 times at all the concentrations in both prostate cancer cell lines tested. Also, increased the production of ROS at the concentrations of 5 and 10 μ M in the DU145 cell line, when compared with the single treatment of LASSBio or Doxorubicin. Promoted down-regulation of survival and resistance proteins, AKT and STAT3, observed by western blotting. **Partial Conclusions:** LASSBio 1911 treatment promoted an increase in doxorubicin-induced cell death through formation of reactive oxygen species, indicating the formation of mitochondrial damage that will be evaluated later. Also, down-regulation of survival and resistance proteins can indicate an involvement of these pathways with the Wnt-pathway. These findings suggest a theoretical basis for the therapeutic application of combined treatment of LASSBio/doxorubicin for prostate cancer and helps to elucidate the regulation of the Wnt signaling pathway by HDAC6/8. **Financial Support:** CNPq, CAPES, Faperj **License number of ethics committee:** N/A

01.019 Analysis of intracellular peptides action on gene expression and their interaction with microRNAs related to energy metabolism and obesity. Gewehr MCF¹ Eichler RAS¹ Carvalho EA² Oliveira V² Tersariol ILS³ Ferro ES¹ ¹Dpt of Pharmacology, Univ of São Paulo, Brazil ²Dpt of Biophysics, Federal Univ of São Paulo, Brazil ³Dpt of Biochemistry, Federal Univ of São Paulo, São Paulo, Brazil

Introduction: The constant increase in the obese and overweight population creates opportunities for new treatments to improve the quality of life of these people. In a previous study, we showed that C57BL6 thimet oligopeptidase (THOP1-/-) knockout mice were resistant to obesity and associated diseases and had alterations in the composition of the intracellular peptides on adipose tissue. In the present study, we chose seven peptides that were altered and synthesized to evaluate their actions on gene expression and interaction with microRNAs in a 3T3-L1 cell model. **Methods:** The seven peptides were chemically synthesized without (May1-May7) or with a cell-permeable peptide (cpp;YGRKRRQR) covalently linked to its C-terminus (May1C-May7C) to allow its cellular internalization. In addition, random sequences of these peptides were also synthesized and served as controls in the tests performed (May1-scb - May7-scb. May1C-scb - May7C-scb). The possible roles of the May peptides were investigated in the expression of the PPAR- α , PPAR- γ , FAS, LPL, and FABP4 genes in a well-characterized adipocyte cell model, 3T3L1. Differentiated 3T3L1 adipocytes were exposed to concentrations of 0.1 or 1 μ M of the peptides. The indicated peptide concentration was added at times 0, 30, and 60 min and at the end of 120 min from the first addition of peptides for total RNA extraction using TRIzol® and analyzed by real-time PCR (RT-PCR). The interaction of peptides and microRNAs was evaluated by the circular dichroism (CD) method.

The CD spectra of the precursor and mature miR-143 and miR-222, the May peptides (5 and 6), and the mixture of each miR with each peptide were measured using a Chirascan™CD Applied Photophysics spectrometer. The readings were taken at room temperature, using a 1 mm high transparency quartz cuvette. Samples were recorded at wavelengths of 300-190 nm, with a total of four scans for each.

Results: The results obtained demonstrated that May peptides act differently, modulating the expression of genes involved in obesity and energy metabolism that change according to the concentration and whether the peptide has the capability to permeate into the cell or not. We observed that intracellular peptides act both on the plasma membrane and inside the cells (where the effects of intracellular peptides were even more evident). Furthermore, through the data obtained from the CD, we can observe a direct interaction of intracellular peptides with specific microRNAs, as demonstrated here, occurring for miR143 and miR222. **Conclusions:** In conclusion, May peptides acting modulating gene expression and may be related to the interaction of peptides with microRNAs seen in the CD spectra. With these results presented, we can already indicate a connection between degradation and protein synthesis through intracellular peptides. That means the proteasomal degradation of proteins does not represent the end of protein function. Also, we can have a new action of intracellular peptides through interaction with microRNAs. **Financial support.** FAPESP. **License number of ethics committee:** N/A

01.020 Comparative effects of N-acetylprocainamide and procainamide on maximum rate of rise and half decay time of the cardiac action potential: evidence for class IA antiarrhythmic mechanisms for N-acetylprocainamide. Sigler W¹, Oliveira AC². ¹Faculty Oswaldo Cruz, Brazil; ²USP São Paulo, Dpt of Pharmacology, Inst of Biomedical Sciences, Brazil

Introduction: Procainamide (PA), is classified as a IA antiarrhythmic drug, because blocks sodium channels in a lower concentration, decreasing the maximum depolarizing rate of the cardiac action potential (V_{max}) and blocks cardiac potassium channels, in a higher concentration, increasing the half-decay time (HDT) of the cardiac action potential. PA is metabolized, *in vivo*, to an active metabolite: N-acetylprocainamide (NAPA), that is generally considered a pure class III antiarrhythmic drug, as found in pharmacological textbooks (Goodman & Gilman, 2018; Golan et al., 2017). This is because an initial study demonstrated NAPA to increase the HDT of the cardiac action potential without affecting the V_{max} (Dangman, KH, J. Pharmacol. Exp. Ther., 217,851, 1981). However, only a single animal species, the dog, was used, and it is well known that the pharmacology of cardiac antiarrhythmic drugs is prone to animal species variation. Indeed, it is our former experience that, differently from the dog, NAPA is able to block sodium channels in mouse cardiac myocytes (Sigler, W. Pflug. Arch., 443, 227, 2002). This is not expected from a pure class III antiarrhythmic drug that, by definition, should only block cardiac potassium channels. These contradictory findings led us to thoroughly restudy the cardiac pharmacology of NAPA, in a species hitherto not studied (guinea pig) and taking care to also concomitantly study PA, used as a reference, a paradigm of a class IA antiarrhythmic drug. **Methods:** An electrophysiological/pharmacological study was performed, *in vitro*, using glass microelectrodes to record the intracellular cardiac action potential. The preparation was the isolated right papillaris magnus of guinea pig's heart. The effects of NAPA and PA on the V_{max} and HDT of the cardiac action potential, in three different concentrations (mM): 0.8; 3.5 and 7.0, were recorded. ANOVA followed by Tukey test was used for statistical analyses, at a 5% level of significance. **Results:** The V_{max} (V/s) values (X±SE) were: control: 142 ±8; 0.8 mM: NAPA=123±13; PA=102±10*; 3.5 mM: NAPA=71±6*; PA=33±4*; 7.0 mM: NAPA=50 ±6*; PA=44±4*. The HDT (msec) values (X±SE) were: control: 163±7; 0.8 mM: NAPA=200±15; PA=148±15; 3.5 mM: NAPA=215±7; PA=378±59*; 7.0 mM: PA=511±52*; NAPA=393 ±22*. Each subgroup encompassed 5 to 8 different cells. The asterisks indicate subgroups significantly different (p<0.05) from the respective controls. **Conclusions:** Both NAPA and PA affected the V_{max} in lower concentrations than those affected the HDT, NAPA being less potent in either case. Assuming that modifications in V_{max} or HDT imply modifications, respectively, in cardiac sodium or potassium channels, it can be concluded that although NAPA and PA are both able to affect these channels, they have a larger affinity for cardiac sodium than for potassium channels. This is a typical class IA antiarrhythmic behavior. Therefore, probably due to species variation the concept that NAPA is a pure class III antiarrhythmic drug is not as general as previously envisaged by others and should be revised. **Financial support:** FAPESP doctoral fellowship (WS); FAPESP and CNPq grants (ACO); Approved by the local animal research ethical committee. **License number of ethics committee:** Animal research ethical committee of the Institute of Biomedical Sciences, USP, São Paulo

02. Neuropharmacology

02.001 Evaluation of PTEN modulation on LPS-driven neuroinflammation. Nagy GS¹, Kawamoto EMI². ICB-USP São Paulo, Dpt of Pharmacology, Brazil ¹Lab of Molecular and Functional Neurobiology, Dpt of Pharmacology, ²Inst of Biomedical Sciences, Univ of São Paulo, São Paulo, Brazil

Introduction: Neuroinflammation is essential to protect the brain against several insults like infections¹. However, this process can be harmful depending on the intensity, possibly leading to brain degeneration^{1,2}. Neuroinflammation occurs when the microglia are activated through the recognition pathogen associated molecular patterns (PAMPs) or danger associated molecular pattern (DAMPs)³. The recognition of these patterns by specific receptors on the microglia activate intracellular cascades that induces the nuclear translocation of the nuclear factor kappa B (NF-KB) and consequently leads to expression of pro-inflammatory mediators like the enzyme inducible nitric oxide synthase (iNOS)³. In addition, the inflammatory process can interfere with the glutamatergic signaling, therefore contributing to neurodegeneration. The PI3K pathways can be activated during the inflammation process exerting both proinflammatory or anti-inflammatory effects depending on the context⁴. Phosphatase and Tensin Homolog deleted on chromosome ten (PTEN) antagonize PI3K pathways, possibly modulating inflammatory process⁵. However, literature is divergent about the role of PTEN in inflammation, suggesting that this role may vary from cell type, tissue, and the inflammatory stimulus^{6,7}. Although PTEN plays a large role in the brain, mainly related to the synaptic function⁸, its role in neuroinflammation remains underexplored. Here we show that PTEN can modulate the neuroinflammation by dampening the nuclear translocation of NF-KB and consequently inhibiting the augment in the expression of iNOS driven by LPS. Aim: To investigate the role of PTEN in modulating neuroinflammation and glutamatergic signaling in a model of LPS-driven inflammation. **Methods:** A *PTEN* transgenic lineage of mice (enolase Cre-LoxP *PTEN* +/-), lineage of *PTEN* conditional knockout mice that present *PTEN* deletion only in neurons, were used. Animals were donated by Dr Simonetta Camandola, NIA, NIH, USA. Crossing: enolase Cre-LoxP *PTEN* +/- animals were crossed. Wild type (WT) animals express *PTEN* normally (*PTEN* +/+) and heterozygous (HT) mice (*PTEN* +/-) do not express one of *PTEN* alleles in neurons. The offspring were genotyped based on micés provider protocol. A 2 cm fragment of the animal's tail was used to extract the DNA. The DNA was used to perform 2 PCR assays, one to verify the presence of Flox sequence and the other to verify the presence of Cre recombinase. The animals were separated in 4 groups (n=5/6): one group *PTEN* +/+ received intraperitoneal (ip) injection of LPS (250 µg/kg) dissolved in sterile saline (WTL), one group *PTEN* +/+ that received ip injection of sterile saline (WTS), one group *PTEN* +/- that received ip injection of LPS (250 µg/kg) dissolved in sterile saline (HTL) and one group *PTEN* +/- that receive ip injection of sterile saline (HTS). Four hours after the treatment, mice were euthanized, the cortex was removed, and cytosolic and nuclear proteins were obtained by protein extract assay. With cytosolic and nuclear extracts Western Blotting (WB) assays were performed based on the Laemmli method. The primary antibodies used nitric oxide synthase inducible (NOSi) (dilution 1: 300), p65 (dilution 1: 200), subunit GluNR1 of N-methyl-D-aspartate (NMDA) receptor (dilution 1: 200), and subunit GluA1 of α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic (AMPA) receptor (dilution 1: 200). Statistical analysis: GraphPad Prism 8 was used. Comparison between the 4 groups was performed with two-way ANOVA, followed by Tukey's test and p values were considered significant for p<0.05. **Results:** WB to iNOS: the treatment with LPS increased the expression of iNOS in wild-type animals (p=0.0069), but not in *PTEN* +/- animals (p=0.9812). Also, among the groups treated with saline, the animals with partial deletion of *PTEN* expressed more NOSi in relation to controls (p=0.0031). WB to cytosolic p65: there were no significant differences in the expression of IL-1 beta between the groups (WTS vs WTL p=0.9994; WTS vs HTS p>0.9999; WTS vs HTL p>0.9999; WTL vs HTS p=0.9963; WTL vs HTL p=0.9911; HTS vs HTL p>0.9999). WB to nuclear p65: Among the groups treated with LPS, the wild-type one expressed more p65 in the nucleus than the *PTEN* +/- (p=0.0398). Quantification of p65 nuclear/p65 cytosolic (NF-KB nuclear translocation): Among the groups treated with LPS, the nuclear translocation of p65 was increased in wild-type group in relation to the *PTEN* +/- group (p=0.0239). WB to GluNR1: there were no significant differences in the expression of GluNR1 between the groups (WTS vs WTL p=0.3144; WTS vs HTS p=0.4437; WTS vs HTL p=0.9459; WTL vs HTS p=0.9879; WTL vs HTL p=0.6017; HTS vs HTL p=0.7649). WB to GluA1: there were no significant differences in the expression of GluA1 between the groups (WTS vs WTL p=0.1530; WTS vs HTS p=0.1965; WTS vs HTL p=0.1512; WTL vs HTS p=0.9945; WTL vs HTL p=0.9997; HTS vs HTL p=0.9984). **Conclusion:** Our results suggest that animals with partial deletion of *PTEN* present a basal inflammatory state, once the HTS group had increased iNOS expression compared to the WTS group. By contrast, *PTEN* +/- animals seem to not respond to the inflammatory stimulus with LPS by innate immune pathways, as no differences were observed in iNOS expression between HTS and HTL groups. This dysfunctional inflammatory response possibly depends on the decreased activity of the NF-KB transcription factor, as the HTL group expressed less p65 on the nucleus than WTL. The dual immunologic properties of the PI3K/PTEN pathway may explain this phenomenon⁴. In turn, the glutamatergic response to LPS does not seem to be altered by *PTEN* insufficiency, suggesting an interaction of *PTEN* with immune innate pathways only. This work was supported by a FAPESP fellowship (2020/08005-5) to Gabriela Nagy and a grant from FAPESP

(2016/07427-8, 2019/12974-6). **References:** 1. Kielian T. *J Neurochem.* v. 130(1), p. 1, 2014; 2. DiSabato DJ. *J Neurochem.* v. 139, p. 136. 2016.; 3. Haroon E. *Neuropsychopharmacol.* v. 42(1), p. 193, 2017.; 4. Thell S. *J Immunol.* v. 193, p. 1717, 2014.; 5. Garcia-Junco-Clemente P. *Commun Integr Biol.* v. 7(2), p. e28358, 2014.; 6. Wang L. *Nat Med.* v. 20(5), p. 484, 2014.; 7. Huang SY. *J Neuroinflamm.* v. 12(1), p. 59, 2015.; 8. Spina Nagy G. *Neurosci Lett.* v. 759, p. 136015, 2021. **License number of ethics committee:** This work was approved by the Animal Research Ethical Committee (CEUA) (128/2017/CEUA).

02.002 Respiratory anatomofunctional changes following apocynin treatment in a Parkinson's disease model. Medeiros POS, Nascimento ALF, Pedrão LFAT, Oliveira LM, Takakura AC, Falquetto B. USP Inst of Biomedical Science, Dpt of Pharmacology, Brazil

Introduction: Parkinson's disease (PD) is a progressive neurodegenerative disease characterized by the loss of dopaminergic neurons in the Substantia Nigra (SN). There are several motor and non-motor symptoms, such as respiratory problems. It's also known that oxidative stress (OS) is directly related to the neurodegeneration and also developed in respiratory regions, noted in 6-hydroxydopamine (6-OHDA) animal models, causing a high loss in respiratory function, which may be due to increased enzymatic activity of NOX (NADPH oxidase). **Objective:** Evaluate the effects of the NOX non-specific inhibitor, apocynin (APO), preventing the neurodegeneration of respiratory nuclei and the respiratory deficits in 6-OHDA animals. **Methods:** Wistar male rats (n=19) were set up in four experimental groups: I) Vehicle, II) 6-OHDA, III) Vehicle treated with APO and IV) 6-OHDA treated with APO. Vehicle or 6-OHDA (24 µg/µl) were injected bilaterally into the striatum to produce the PD model. At 20 days after surgery, groups III and IV were treated with APO (50 mg/ml/kg, water intake, for 20 days). At 40 days after surgery, respiratory parameters were recorded by whole body plethysmography and the immunohistochemistry was performed in the brains. **Results:** 6-OHDA reduced TH⁺ neurons in SN and APO treatment did not reverse it (I: 100±19, II: 21±10, III: 92±23, IV: 21±5 % neurons; p<0.0001). At normoxia, 6-OHDA animals showed reduced respiratory frequency (f_R) (I: 93.8±15.5, II: 66.4±3.4, III: 93.8±15.5, IV: 92.3±8.7 bpm; p=0.0003) and ventilation (V_E) (I: 226.6±104.1, II: 103.9±22.6, III: 226.6±104.1, IV: 204.5±52.6 ml/min/kg; p=0.0401) with an increased in inspiratory time (Ti) (I: 270.9±39.2, II: 395.6±117.4, III: 270.9±39.2, IV: 262.6±51.6 ms; p=0.0001). APO was able to prevent all resting respiratory changes. During hypercapnia, 6-OHDA rats showed reduced f_R (I: 148.9±18.2, II: 124.8±10.9, III: 148.9±18.2, IV: 155.0±20.1 bpm; p=0.0010) and V_E (I: 601.5±180.5, II: 340.8±78.3, III: 601.5±180.5, IV: 644.5±170.9 ml/min/kg; p=0.0014) with changes in Ti and expiratory time (Te) (Ti: I: 201.4±20.8, II: 235.3±21.0, III: 201.4±20.8, IV: 190.5±37.6 ms; p=0.0019; and Te: I: 211.2±34.0, II: 254.2±26.4, III: 211.2±34, IV: 206.8±24.7 ms; p=0.0019). APO was able to prevent all hypercapnia-induced respiratory changes. We also observed that treatment with APO prevented neurodegeneration of respiratory nuclei in 6-OHDA by quantifying neurons labeled with the NK1 receptor of the rostral ventral respiratory group (I: 81.7±2.8, II: 68.4±7.5, III: 79.3±5.0, IV: 79.3±8.0 integrated density/area; p=0.0389) and the pre-Bötzinger complex (I: 98.8±5.5, II: 79.8±8.4, III: 94.9±3.9, IV: 97.8±17.1, integrated density/area; p=0.0353), and the Phox2b marker of the commissural subnucleus of nucleus of solitary tract (I: 952±256, II: 327±86, III: 761±343, IV: 720±286 neurons; p=0.0302), intermediate (I: 2229±510, II: 1144±312, III: 1,621±501, IV: 1936±614 neurons; p=0.0327) and retrotrapezoid nucleus (I: 293±35, II: 185±59, III: 337±58, IV: 261±46 neurons; p=0.0388). **Conclusion:** Treatment with APO improved respiratory function and prevented the neurodegeneration in respiratory nuclei in PD animal models. **Financial Support:** FAPESP 2019/00065-1 and CNPq/PIBIC 2020. **License number of ethics committee:** CEUA nº: 2740200319

02.003 Sex differences in stress-related disorders: role of neurosteroids in PTSD, depression and anxiety. Gazzi G, Almeida FB, Barros HMT UFCSPA, PPG Health Sciences, Brazil

Introduction: Women have a higher prevalence of certain psychiatric illnesses than men, in addition to several female-specific disorders such as premenstrual syndrome, premenstrual dysphoric disorder, and postpartum depression. The reasons for the sex differences might rest in distinct vulnerability to a variety of stress-related illnesses, but the underlying neurobiological mechanisms and the differences in treatment responses are still not well understood. An overview of both the biomarker role and the therapeutic potential of brain neurosteroids, considering sex differences in psychiatric disorders is the objective of this review. **Methods:** we conducted a systematic scoping review to map the literature on neurosteroids as biomarkers and treatments in stress related disorders in males and females. We are searching four different databases (PubMed, EMBASE, PsycInfo and LILACS) for relevant papers using the following search string with operational Boolean operators: (PTSD OR depression OR anxiety) AND neurosteroids AND (sex OR (Male AND female) OR (men AND women)) NOT review?. **Results:** The search yielded 175 articles to be screened for eligibility, being that 35 of them were reviews and were discarded. The results about neurosteroids and sex hormone actions in brain areas after stress and in post-traumatic stress disorder

(PTSD), depression, and anxiety were categorized for males and females. **Conclusion:** There are few neurobiological studies in animal models that assess male and female subjects at the same time, while human neuroimaging studies often do not model sex as a variable of interest. The sex differences are frequently attributed to the actions of sex hormones. We summarize our current understanding of neurosteroids-related neurobiological mechanisms that underlie sex-related differences in behavior and discuss implications for diagnosis and treatment of stress-related psychiatric disorders. **License number of ethics committee:** N/A

02.004 **BEHAVSOFT® – Software for Scoring Animal Behavior Patent: BR 294091919042-3, May 2019.** Fiore R; Costa PA; Marostega F; Freese L; Nin MS; Barros HMT. Federal Univ of Health Sciences of Porto Alegre Neuropsychopharmacology Lab, Brazil

Introduction: The study of behavioral manifestations expressed by animals and their analysis, as they make it possible to draw parallels between the behavior of animals and the modeled species, is widely used in preclinical research in the field of psychobiology. Animal models, especially rodents, provide over the years numerous important inferences from the observation of their behaviors, which can be used for different types of studies, such as drug addiction, depression, anxiety, stress, as well as for the development of pharmaceuticals. The tests used in these models have a scoring system for some complex and exploratory behavior of rodents, and are commonly filmed for further evaluation. Parameters such as locomotion, cleaning and risk assessment or residence time can be computed by automated computational modules, but they are highly subjective and subtle, which created the need to develop software capable of performing the analysis more precisely and with the possibility of automatic database creation. **Objective:** To develop and present a more up-to-date prototype of software capable of scoring more efficiently and more automatically the animal's behaviors. **Methods:** BEHAVSOFT® is Windows® software written in C# programming language that requires Windows 7 or later versions, 2.2 MB and 64 RAM memory. Time precision is 0.1 sec, being able to play video files like .mp4 and .mov. The validation of BEHAVSOFT® was performed by three investigators following a blind observation protocol and confirmed by statistical analysis. **Results:** the software was developed and registered by the Behavioral Neuroscience research group of the Laboratory of Neuropsychopharmacology at UFCSPA (INPI nº BR512019000979-7). In addition to the functions described, the program also allows the observer to customize the keys of each test, pause and resume analysis at any time, record both the frequency and duration and latency of each event in a sequential and chronological order. The analyzes obtained can be exported to spreadsheets and all files can be merged into an Excel® file, facilitating statistical analysis and graphing. The program has already been tested and is being validated by the research group, being referenced in scientific publications in which behavioral analyzes were performed with the aid of the software (ALMEIDA et al., 2018; COSTA et al., 2015; FERNANDES, 2019; HEIDRICH, 2020). In addition, the software was also used in research by groups from other universities (DOS SANTOS, 2017). Currently, the research group is looking to include software optimization for macbook and updates like "tracking" module, increase the time counter of each slot from seconds to milliseconds, add the option to return/undo actions. **Financial Support -** UFCSPA, CNPQ, CAPES. **References:** GOMEZ-MARIN, A. Nat. Neurosci, v. 17, no. 11, p. 1455. 2014. ALMEIDA, F.B. Physiol. Behav, v. 194, p. 246. 2018. COSTA, P.A. et al. Psychopharmacology, v. 232, no. 19, p. 3623. 2015. DOS SANTOS, L.D. Doctoral Thesis—Porto Alegre: UFRGS, 2017. FERNANDES, P.R. Master's Dissertation—Porto Alegre: UFCSPA, 2019. HEIDRICH, N. Master's Dissertation—Porto Alegre: UFCSPA, 2020. **License number of ethics committee:** N/A

02.005 **Parkinson's disease treatment is not associated with changes on peripheral biomarkers of Fe metabolism.** Santos BN¹, Maes M², Bonifácio KL², Matsumoto AK², Brinholi FF², Melo LB², Moreira EG², Barbosa DS², Farias CC¹. ¹UFES, Vila Velha, Coordination of Biomedicine, Brazil; ²UEL, Graduation Program in Health Sciences, Londrina, Brazil

Introduction: Parkinson's disease (PD) is important age-related and motor dysfunction. Studies discuss blood biomarkers for diagnosis and prognosis of PD, like iron (Fe) metabolism, immune-inflammatory and oxidative stress. Nowadays, pramipexole, levodopa, amantadine, biperiden, entacapone are medications generally used. They decrease the motor synthons, but can't stop the disease's evolution. This study was to evaluate the influence of current medications on blood biomarkers of iron metabolism on PD. **Methods:** approved by Ethics Committee on Research Involving Human Subjects of the State University of Londrina (UEL). 56 patients with PD in stages 1-3 of the Hoehn and Yahr Scale were recruited from the neurology ambulatory at Clinical Hospital, UEL, as well as 56 healthy individuals. PD patients were being treated with levodopa and carbidopa (n=13), levodopa and benserazide (n=37), pramipexole (n=30), amantadine (n=12), biperiden (n=7) and/or entacapone (n=5). Soluble transferrin receptor (sTfR) was quantified by ELISA, Fe and total iron binding capacity (TIBC) were measured on Dimension®, ferritin and transferrin

(Tf) were measured on Architect i2000SR. **Results:** we found significant multivariate effects of diagnosis ($df=5/79$, $p<0,001$) and sex ($df=5/79$, $p=0,0018$) on the 5 iron variables, i.e Fe, Tf, TIBC, Ln sTfR, Ln ferritin. The estimated marginal mean (SE) values of the significant biomarkers. The results show the estimated marginal mean (SE) values of the significant biomarkers: in controls, Tf was 2.36 (0.54) g/mL, sTfR 0.76 (0.03) mg/mL and ferritin 126.4 (19.5) ng/mL; in PD, Tf was 2.22 (0.04) g/mL, sTfR 0.84 (0.02) mg/mL and ferritin 189.0 (15.7) ng/mL. Thus, Tf was significantly lower while sTfR and ferritin levels were significantly higher in PD than in controls. Other putative predictors (entered separately together with diagnosis and sex yielding 3 predictors) showed that these were not significant and additionally did not change the impact of diagnosis and sex on the Fe metabolism biomarkers, including use of medications, levodopa ($df=5/72$, $p=0.905$), levodopa + benserazide ($df=5/72$, $p=0.967$), amantadine ($df=5/72$, $p=0.535$), pramipexole ($df=2/72$, $p=0.561$), biperiden ($df=2/72$, $p=0.199$) and entacapone ($df=2/72$, $p=0.187$). **Conclusion:** The major finding is the significant association between PD diagnosis and three Fe metabolism variables (Tf, sTfR and ferritin levels) and that the principal medications used by PD patients have no influence on the blood biomarkers linked to iron metabolism. **References:** FARIAS, C. C. et al. Parkinson's Disease is Accompanied by Intertwined Alterations in Iron Metabolism and Activated Immune-inflammatory and Oxidative Stress Pathways. *CNS & Neurological Disorders*, v. 16, n. 4, 2017. **License number of ethics committee:** The study was approved by the Ethics Committee on Research Involving Human Subjects of the UEL. CAAE: 26325814.6.0000.5231.

02.006 Reduced adult hippocampal neurogenesis on Nitric Oxide knockout mice are not modified by escitalopram treatment. Freire JB, Fernandes GG, Costa KMC, Scomparin DS, Guimarães FS, Campos AC Dpt of Pharmacology, Ribeirão Preto Medical School, Univ of São Paulo, Brazil

Introduction: Inducible nitric oxide synthase (iNOS) is one of the three isoforms of the enzyme family nitric oxide synthase (NOS). iNOS is involved in a wide range of signaling pathways, and is expressed in several types of cells and tissues, mainly in macrophages, induced by cytokines or other biomolecules, such as lipopolysaccharide (LPS). In the context of central nervous system, iNOS can be found in microglia and other glial cells, and plays an important role in neuroinflammation, since it is upregulated during the immune responses in the brain, by proinflammatory cytokines, inducing an oxidative and inflammatory environment. Due to its function, iNOS can lead to impairments in some physiological functions, such as neurogenesis, in imbalanced brain conditions caused by stress or exacerbated immune response, for example. Neurogenesis in adults consists in an important process of division, maturation and migration from neural stem cells (NSC), and occurs in two niches of the adult brain, the subventricular zone (SVZ) of the lateral ventricles and the subgranular zone (SGZ) of the dentate gyrus of the hippocampus. The process of adult neurogenesis can be affected and diminished by the effects of chronic stress, wherein iNOS might be involved. Here we focus on the adult hippocampal neurogenesis, in the SGZ, to investigate if the basal reduced neurogenesis in iNOS knockout mice is reversed by the administration of escitalopram.

Methods: WT or iNOS KO mice were randomly divided to be treated with either vehicle or escitalopram (10mg/kg) during non-stressed or stressed (CUS 21 days) conditions. After 23 days the animals were euthanized and perfused and their brains were removed to assess neurogenesis on hippocampus. All data were analyzed by three-way ANOVA, and all experiments were preapproved by CEUA protocol number: 187/2017. **Results:** Our results demonstrated that iNOS KO mice had a reduced number DCX expressing cells (type IIb or III) that was unaffected by either CUS or escitalopram treatment. Moreover, we observed that our manipulations had no effect on GFAP+SOX2+ (type I). Although, iNOS KO mice presented an augmented cells expressing SOX2+, these was not affected by any of our manipulations. **Conclusion:** Our results suggests that iNOS plays an important role in neurogenesis and may be involved in the survival and differentiation of newborn neurons in the adult brain. Financial support: CAPES, CNPq and FAPESP. **References:** Altman, J.; Das, G. D. *J Comp Neurol.* 124(3): 319-35. 1965. Anacker, C. et al. *Nature.* 559(7712): 98-102. 2018.; Calabrese, V. et al. *Nat Rev Neurosci.* 8(10): 766-75. 2007.; Carreira B. P. et al. *Front Cell Neurosci.* 28;8: 343. 2014.; Cinelli, M. A. et al. *Med Res Rev.* 40(1): 158-189. 2019.; Ekdahl, et al. *Proc Natl Acad Sci U S A.* 11;100(23): 13632-7. 2003.; Kempermann, G. et al. *Cold Spring Harb Perspect Biol.* 1;7(9): a018812. 2015.; Peng, Y. et al. *J Neuroinflammation.* 6;9: 75. 2012.; Plümpe, T. et al. *BMC Neurosci.* 15;7: 77. 2006.; Steiner, B. et al. *Glia.* 54(8): 805-14. 2006.; Urban, N.; Guillemot, F. *Front Cell Neurosci.* 27;8: 396. 2014.; **License number of ethics committee:** CEUA protocol number: 187/2017

02.007 Evaluation of the anticonvulsive potential of fatty acid amides from olive oil. Monteiro VHMB¹, Silva WLG¹, Ferreira IM², Fujishima MAT¹, Oliveira FR¹. ¹UNIFAP Macapá, Dpt de Ciências Biológicas e da Saúde - Curso de Farmácia, Brasil; ²UNIFAP, PPG Ciências Farmacêuticas, Macapá, Brasil

Introduction: Seizure, characterized by involuntary contractions and in many cases accompanying loss of consciousness, affects approximately 9% of the world population, and many suffer from the refractory or

drug-resistant form of the disease, there is evidence that endocannabinoids can pharmacologically modulate the action against seizures and epileptic disorders, therefore, this study involves the use of fatty acid amides (FAA) analogous to endocannabinoids and their effect on seizures. **Methods:** Fatty acids, present in extra virgin olive oil with low acidity, commercially obtained (Andorinha Portugal®, L0206423) were used to produce FAA through the enzymatic catalysis process with Lipase of *Pseudomonas fluorescens* and characterized by gas chromatography coupled to mass spectrometry (GC-MS). Swiss mice (*Mus musculus*) weighing 30-40g were used, kept in the Toxicology Laboratory of the Federal University of Amapá, in a controlled environment (23-25°C and 12h light-dark cycle), with food and water *ad libitum*. (CEUA/UFPA No. 4335290521). Animals were divided into 3 groups (Saline, Diazepam and FAA) (n=10 per group). FAA were tested in a chemical induction of seizures with pentylenetetrazole (PTZ). FAA group were pre-treated for 3 days with a dose of 200 mg/kg intraperitoneally (ip) of FAA, daily, Diazepam group received 5 mg/kg ip in a single dose 30 minutes before experiment. All groups were administered with 60mg/kg of PTZ (ip) in a single dose. Behavioral parameters of the seizures (latency of myoclonic and tonic-clonic seizures and seizure duration) were evaluated. **Results:** GC-MS analysis showed the fatty acids were converted to FAA, with the majority identified being oleyl ethanolamide (70%) followed by palmitoyl ethanolamida (22%). FAA increased latency of myoclonic ($136,60 \pm 56,31$ s) and tonic-clonic ($224 \pm 104,46$ s) while the saline + PTZ group had latencies of $59,00 \pm 28,90$ s and $94,75 \pm 20,72$ s, respectively. FAA group had shorter seizures attack, during about 13s against saline + PTZ group with 46s, average. Diazepam group showed no signs of seizures. **Conclusion:** FAA produced from olive oil fatty acids were able to improve behavioral parameters of pentylenetetrazol-induced seizures in mice. **Financial Support:** Federal University of Amapá. **Keywords:** Fatty acid amides, Pentylenetetrazole, Anticonvulsant. **License number of ethics committee:** CEUA N°4335290521

02.008 **CB2 receptor blockade reverses a schizophrenia-related memory deficit.** Andrade BS¹, Nunes LED¹, Cunha GNB¹, Cunha NF¹, Ferreira BK², Cardoso F², Ferreira GC², Castro NG¹, Neves GA¹. ¹Lab of Molecular Pharmacology, Inst of Biomedical Sciences, Federal Univ of Rio de Janeiro, Brazil; ²Lab of Bioenergetics and Inborn Errors of Metabolism, Inst of Biochemistry, Federal Univ of Rio de Janeiro, Brazil

Cognitive deficits play a central role in schizophrenia. Among those deficits, working memory (WM) is one of the most commonly affected domains (Sharma & Antonova, 2003). Current pharmacotherapy has no impact on such impairments, highlighting the need for novel therapeutic targets (Miyamoto et al., 2012). Several studies showed that the endocannabinoid system is altered in the brain of schizophrenia patients (Fakhoury, 2017). Also, genetic variations related to the functioning of the CB2 receptor (CB2R) were associated with schizophrenia, and the *knockout* of this receptor in mice leads to a WM improvement (Li & Kim, 2017). In this way, previous data from our group showed that the CB2R antagonist AM630 inhibited the impairment induced by the NMDA antagonist MK-801 in the spontaneous alternation task. Thus, this study aims to further characterize the effects of CB2R blockade in memory impairments related to schizophrenia. Male adult Swiss mice WM and reference memory (RM) were assessed using an 8-arm radial maze task (CEUA CCS-UFRJ 144/19). Animals were trained to search for a reward at the end of seven maze arms, while the unbaited arm was used to evaluate reference memory. Each entry in the unbaited arm represents a reference memory error (RME). Each entry in a previously visited arm represents a working memory error (WME). The number of entrances until the first WME represents working memory capacity (WMC). Deficits in mice memory were induced by MK-801 (0.15 mg/kg i.p.). Blockade of CB2R was achieved by pretreatment with AM630 (0.3 or 1.0 mg/kg i.p.). MK-801 reduced WMC from 4.4 ± 0.6 arms (control) to 3.7 ± 0.4 arms ($p = 0.044$). It also increased the number of WME from 4.1 ± 0.9 to 14.4 ± 3.3 ($p = 0.001$). Control group made 1.1 ± 0.2 RME while animals treated with MK-801 showed a worst performance (2.7 ± 0.7 RME, $p = 0.042$). CB2R antagonist did not change mice performance in the radial maze *per se* ($p > 0.05$). Pretreatment with AM630 0.3 mg/kg successfully reduced the number RME in mice exposed to MK-801 (1.2 ± 0.3 , $p = 0,020$) without improving WMC or WME deficits ($p > 0.05$). Finally, MK-801 increased the number of arm entrances (49.9 ± 3.8 vs. 22.9 ± 2.3 in the control group, $p = 0,001$) while AM630 did not change mice locomotion ($p > 0.05$). After the behavioral test, mice brains were collected to assess reduced glutathione (GSH) levels. AM630 increased GSH levels in the prefrontal cortex, but not in the hippocampus, in both control and MK-801 groups ($p = 0.005$). In summary, our results show that the CB2R antagonist AM630 inhibits MK-801-induced deficit in RM but not in WM. This memory improvement is associate with an increase in the levels of an antioxidant marker. Further experiments to confirm these observations as well as to understand the involved mechanisms are underway. **References:** LI, Y. & KIM, J. Neuroscience. v.363, p.11, 2017. MIYAMOTO, S et al. Mol Psychiatry. v.17, p.1206, 2012. FAKHOURY, M. Mol Neurobiol. v.54, p.768, 2017. SHARMA, T. & ANTONOVA, L. Psychiatr Clin North Am. V.26, P.25, 2003. **License number of ethics committee:** CEUA CCS-UFRJ 144/19

02.009 **Inhibitory effect of *Spirulina platensis* on acetylcholinesterase activity.** Tavares J^{1,3}, Bezerra JR^{1,3}, Souza TN^{1,3}, Oliveira AV^{1,3}, Andrade, GM^{1,2,3} ¹Dpt of Physiology and Pharmacology; ²Dpt of Clinical Medicine, Center for Research and Drug Development (NPDM), ³Federal Univ of Ceara, Brazil

Introduction: Acetyl-cholinesterase (AChE) and butyryl-cholinesterase (BuChE) are attractive therapeutic targets in the treatment of Alzheimer's Disease (AD) since inhibition of these enzymes can be used to restore synaptic concentrations of acetylcholine. *Spirulina platensis* (SPI) is a cyanobacterium that contains flavonoids, polyphenols and has been gaining increasing attention because of its nutritional value and anti-inflammatory, antioxidant and neuroprotective properties. The aim of this study was to evaluate the effect of SPI on AChE activity *in vitro* and *ex vivo* and to determine its kinetic parameters. **Methods:** The inhibitory effect of SPI on AChE activity was tested on pure AChE from electric eel *in vitro* at concentrations of 125-3,000 $\mu\text{g}\cdot\text{mL}^{-1}$, and using brain homogenates from mice (125-3,000 $\mu\text{g}\cdot\text{mL}^{-1}$) using the colorimetric assay described by Ellman's. Kinetics of inhibition of AChE activity were determined using different concentrations of acetylthiocholine (1-15 mM) with or without SPI at the 50% inhibitory concentration of AChE activity (IC_{50}). Michaelis constant (K_m) and maximum velocity (V_{max}) values were obtained by non-linear regression (Michaelis-Menten plot) and used to analyze the type of inhibition exhibited by SPI on AChE activity. The study was approved by local ethics committee (registration number: 3598100320). **Results:** SPI significantly inhibited cerebral AChE activity, with inhibition percentage of 76.19 ± 0.85 , 70.22 ± 0.63 and $67.25 \pm 0.66\%$ at 3,000, 2000 and 1000 $\mu\text{g}\cdot\text{mL}^{-1}$, respectively. SPI also inhibited pure AChE activity with inhibition percentage of 80.95 ± 2.81 and $45.93 \pm 2.42\%$, at concentrations of 3,000 and 2,000 $\mu\text{g}\cdot\text{mL}^{-1}$. Cerebral and pure AChE presented IC_{50} of 840 $\mu\text{g}\cdot\text{mL}^{-1}$ and 496 $\mu\text{g}\cdot\text{mL}^{-1}$ respectively. SPI caused a significant increase in K_m and a decrease in V_{max} , suggesting a reversible and competitive inhibition, in addition to reducing hydrolysis efficiency, which is represented by the enzyme's ability to convert the substrate into a product, considering its affinity for the substrate. **Conclusion:** Our study revealed that spirulina can be a rich source of cholinesterase inhibitors and therefore may play a role in AD treatment. Funded by FUNCAP, CNPq and CAPES. **License number of ethics committee:** The study was approved by local ethics committee (registration number: 3598100320)

02.010 **Cannabidiol induces distinct changes on fear memory and emotional processes that are dependent on the type of treatment: a preclinical approach in a model of type-1 diabetes mellitus.** Chaves YC, Raymundi AM, Waltrick APF, Stern CAJ, Zanoveli JM. Dpt of Pharmacology, Federal Univ of Paraná, Curitiba, Parana, Brazil

Introduction: Evidence indicates a higher prevalence of post-traumatic stress disorder (PTSD) in type-1 diabetes *mellitus* (T1DM) patients. Based on the severity of these diseases along with the impact on the quality of life, the search for new therapies that may interfere in the consolidation process of the fear memory becomes urgent. Cannabidiol (CBD), a non-psychotomimetic component of *Cannabis sativa* plant, presents a great potential to treat PTSD, in addition to improving several pathological aspects associated with diabetes. Thus, we investigated whether a single injection or a more prolonged treatment (1 week) with CBD would interfere - quickly and lastingly - in the consolidation process of contextual fear memory, as well as in its generalization. Moreover, whether the effect of CBD would be related preferentially to a short- or long-term memory. **Methods:** T1DM was induced with one injection of streptozotocin (60 mg/kg; i.p). Four weeks later, animals were exposed to a contextual fear conditioning (CFC) session. Immediately after, all animals received one single injection of CBD (0, 10, 30, or 60 mg/kg; i.p.). Twenty-four hours later, the conditioned fear response (same context of CFC - test A1) was evaluated and, in the next day, the generalization of this fear response (neutral context - test B1; Experiment 1). After this, part of these animals was subjected to a continuous 7-day treatment with CBD (Experiment 2). After 1 week, to assess the effect of treatments (acute or prolonged) on the persistence of this fear memory, all animals (both experiments) were submitted again to the same tests (Test A2 and B2) with 1-day of an interval between them. To investigate the CBD effects on short-term memory (Experiment 3), an independent group of animals were submitted to the CFC session and immediately after they received a single injection of CBD. Two hours later, the tests A1 and B1 were performed sequentially with a 30-minute interval between them. All experiments were carried out by the University Ethics Committee under number 1390. **Results:** Our data showed that a single injection of CBD was able to impair only the generalization of the fear memory, not being this effect persistent. Differently from the acute treatment (Experiment 1), a more prolonged treatment with CBD (Experiment 2) impaired the persistence of the conditioned fear memory (test A2). This effect can be related preferentially to the emotional aspects than memory processes; once these chronically-treated animals demonstrated a clear anxiolytic-like effect when submitted, in the next day, to the elevated plus-maze test. Interestingly, our data showed that CBD acts preferentially on processes related to long-term memory. **Conclusion:** Our finding demonstrated that the CBD, depending on the type of treatment - if acute or prolonged, acts differently in T1DM animals on distinct fear-related

memory processes and/or emotional processes. Thus, more studies are needed to better understand how CBD acts within and outside the temporal window of memory labilization, as well as the best type of treatment - whether acute or prolonged, depending on the required result. **Acknowledgments:** We thank CNPq for the financial support. **License number of ethics committee:** All experiments were carried out by the University Ethics Committee under number 1390

02.011 Effects of sexual dimorphism and mk-801 administration during neurodevelopment on c57bl/6j mice behavior. Ferreira GN¹, LEITE AL², Loss CM³, Abílio VC⁴. ¹²Unifesp São Paulo, Dpt of Pharmacology, Brazil; ³⁴National Inst for Translational Medicine, ⁴National Council of Scientific and Technological Development, Ribeirão Preto, Brazil

Schizophrenia (SCZ) is associated to insults during neurodevelopment. Its etiology is related not only to genetic and epigenetic but also to environmental factors. SCZ manifests itself through a range of symptoms classified into positive (e.g. delusions and hallucinations), negative (e.g social withdrawal, anhedonia and emotional impairments) and cognitive (e.g attentional and memory deficits). Its diagnosis occurs after the emergence of psychosis, typically in late adolescence / early adulthood. However, the first-episode psychosis is preceded by a prodromal phase, characterized by the emergence of attenuated negative and cognitive deficits. Although sexual dimorphism on SCZ development and progression is well established, there are only few studies investigating the influence of sex on animal models of SCZ. Here, we investigated if MK-801 administration during neurodevelopment (an animal model of SCZ) affects male and female mice behaviour distinctly. In our study we used males and females C57BL/6J mice treated with MK-801 on post-natal day 7 to 10 (twice a day, i.p., 0.25 mg/kg). To control the litter-litter-variation we used 4 litters (total of 24 animals) distributing the treatment with MK-801 or control on both sex in a same litter. The behavioral tasks locomotor activity, social investigation and prepulse inhibition of startle (PPI) was carried out at a peripubertal phase (days 31 to 33) and during adulthood (days 122 to 124). During the peripubertal period, the MK-801 group (regardless of sex) had a shorter locomotion time in the open field than the control groups, in addition to a lower frequency but longer time of exploration of the cages (social and non-social) in the social investigation test. Regarding social preference, there was no difference between groups. Regarding PPI, the animals in the MK-801 group presented a deficit which was more accentuated in females than males. Regarding the tests performed during adulthood, the MK-801 group (regardless of sex) had a higher number of stops, shorter mobile time and decrease distance between stops, in addition to a lower frequency of exploration of the cages (social and non-social) than the control group. In addition, the females (regardless of the treatment) traveled a greater distance in the open-field, performed fewer stops in the arena and also during locomotion. On the PPI, there was no effect of sex, but the MK-801 group presented a deficit in the task. In conclusion, our results suggest that the treatment with MK-801 during neurodevelopment alters C57BL/6J mice behavior in both peripubertal and adult periods, and that some of these effects are influenced by sex. **Acknowledgements:** This work was supported by grants of Coordenação de Aperfeiçoamento de Pessoal Nível Superior (CAPES), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq). **License number of ethics committee:** 7103180518

02.012 Effect of cigarette smoke on amino acid content of cerebrospinal fluid of male rats. Zilli GAL¹, Izolan LR², Bandiera S ¹, Pulcinelli RR¹, Marques D², Fontella FU³, Almeida RF⁴, Leal MB^{1,2}, Gomez R^{1,2} ¹PPG Pharmacology and Therapeutics, UFRGS, Porto Alegre, Brazil ²PPG Neurosciences, UFRGS, Porto Alegre, Brazil ³PPG Biochemistry, UFRGS, Porto Alegre, Brazil ⁴Dpt of Biological Sciences, PPG Biological Sciences, UFOP, Ouro Preto, Brazil

Introduction: Studies exploring the tobacco effects on the central nervous system often use isolated nicotine administration. However, other compounds in cigarette smoke may play psychoactive effects and change neurotransmission. Thus, this study aimed to evaluate the effects of chronic cigarette smoke exposure on some behaviors and changes in the amino acid content of the cerebrospinal fluid (CSF) in rats. **Method:** Adult male Wistar rats were exposed to cigarette smoke (2 h, 2x/day) or environmental air, for 30 days, in a whole-body cigarette smoke exposure apparatus. On day 26, after the 3rd cigarette, rats were anesthetized, and the CSF was collected by direct puncture of the cisterna magna for amino acid analysis by high-performance liquid chromatography. On day 30, rats were tested in the open field test, and different experiments were performed on other behavioral assays as locomotor activity cage, elevated plus-maze, and light-dark box. All behaviors were video recorded, and an observer trained and blinded for the experimental groups analyzed them. **Results:** Cigarette smoke exposure increased glutamate (P=0.022) and aspartic acid (P=0.021), and decreased leucine (P=0.004), isoleucine (P=0.0006), ornithine (P=0.017), phenylalanine (P<0.0001), tryptophan (P=0.009) levels in the CSF of rats. Furthermore, the smoke exposure increased the locomotion in the open field test and locomotion activity cage (P < 0.05), and

decreased anxiety-like behaviors in rats ($P < 0.001$). **Conclusions:** Our results showed that cigarette smoke exposure presents a psychostimulant and an anxiolytic-like effect in rats. Elevated glutamate and aspartic acid levels, both excitatory amino acids, may contribute to the stimulatory effect showed by cigarette exposed rats, while changes on other amino acids may contribute to the anxiolytic-like effects. Most of these amino acids are precursors of different neurotransmitters, like dopamine, norepinephrine, and serotonin, and may contribute to reward and drug addiction in smokers. **Financial Support:** CNPq, CAPES, Propeq-UFRGS **License number of ethics committee:** CEUA-UFRGS # 29773

02.013 **Uliginisin B interacts with the purinergic system.** Silva CPM¹, Andrejew R², Marangon CG¹, VON Poser GL¹, Batastini AMO², Fraga CAM³, Rates SMK¹. ¹UFRGS Porto Alegre, PPG Pharmaceutical Sciences, Brazil; UFRGS Porto Alegre, Dpt of Biochemistry, Brazil; ²UFRJ Rio de Janeiro, LASSBIO, Brazil

Introduction: Studies have shown that uliginisin B (ULI), a dimeric phloroglucinol isolated from *Hypericum polyanthemum* [1], a plant species native to South Brazil, has antidepressant-like effect in rodents, which seems to be at least partially due to the inhibition of synaptosomal uptake of dopamine in the striatum [1] [2]. In addition, ULI (10 mg/kg, p.o) increased Na⁺K⁺-ATPase activity in cerebral cortex of mice [3]. Stolz et al. [2] demonstrated that the treatment of mice with ULI (15 mg/kg, i.p) induces antinociception and increases AMP and ATP hydrolysis in spinal cord and cerebral cortex synaptosomes, respectively. In this study, we aimed to evaluate the effect of ULI on the hydrolysis of ATP in striatal synaptosomes and its interaction with the 5'-ecto-nucleotidase enzyme. **Methods:** Synaptosomes were isolated from striatum of male Wistar rats and the hydrolysis of ATP, ADP and AMP was performed according to Silva et al. and Nagy et al. [4] [5]. The synaptosomal preparation was incubated with ULI at 0.1 μ M, 1 μ M, 10 μ M and 100 μ M. The interaction with ecto-5'-nucleotidase was assessed by molecular docking (GOLD program) using the homology model of the human enzyme deposited in the PDB (Protein Databank) under the code 4H2I. **Results:** ULI 0.1 μ M, 1 μ M, 10 μ M and 100 μ M decreased by 14%, 8%, 21% and 70% the hydrolysis of ATP, respectively. The best-fit value of the estimated IC₅₀ by non-linear regression was 127 μ M. The hydrolysis of ADP decreased by 70% at ULI 100 μ M only. The hydrolysis of AMP was not affected. Docking study suggests that it ULI is capable of binding to the active site of the enzyme ecto-5'-nucleotidase, in the same region as the co-crystallized AMPCP ligand, interacting with some of the same amino acid residues, with a Score of 43.20. **Conclusions:** ULI decreased the hydrolysis of striatal ATP and ADP nucleotides, suggesting an inhibition of the activity of NTPDases enzymes. Possibly it also interacts with the enzyme ecto-5'-nucleotidase, through binding with its active sites. **References:** [1] STEIN et al., Behav. Brain Res., v. 228, p. 66, 2012. [2] STOLZ et al., Evidence-Based Comp. and Alt. Med., v. 2016, p.8, 2016. [3] STEIN, et al., Rev. Brasileira de Farmacognosia, vol.26 p. 611, 2016. [4] da SILVA, et. al, Neurochem. Res., p. 1249, 2003. [5] NAGY et al., Jornal of Neurochemistry, v. 43, p 1114, 1984. **Acknowledgements:** PhD scholarship from CAPES. PROEX 0477/2017. FAPERGS – Pq Gaúcho 2017. INCT-INOVAR. **License number of ethics committee:** Approval Committee on Animal Research Ethics CEUA/UFRGS: Letter n^o 33550

02.014 **Is the pineal gland able to detect brain damage? N-acetylserotonin (NAS), a neuroprotective darkness hormone.** Sousa KS, Quiles CL, Muxel SM, Ferreira ZS, Markus RP. USP São Paulo, Dpt Physiology, Lab Chronopharmacology, Brazil

Introduction: The pineal gland is a player of acute immune responses. Danger- or pathogen-associated molecular patterns (DAMPs & PAMPs) activate the immune-pineal axis by suppressing nighttime pineal melatonin (MEL) synthesis and inducing or enhancing MEL synthesis by macrophage/microglia (Markus et al. Br. J. Pharmacol. 175: 3239,2018). High ATP signalizes cell death via P2X7 receptors (a low-affinity ATP receptor). Indeed, high extracellular ATP is a DAMP, once its results from damaged or dead cells. However, the mechanisms that lead to the suppression of nocturnal melatonin surge by ATP and PAMPs are different. PAMPs impair the synthesis of the melatonin precursor NAS, while activation of P2X7R inhibits the transcription of the gene and the expression of the enzyme acetyl-serotonin methyltransferase (ASMT), which converts NAS into MEL (Souza-Teodoro et al. J. Pineal. Res. 60: 242,2016). Thus, dying cells in the central nervous system increase ATP levels and promote a switch in the hormone that signalizes nighttime. We tested this hypothesis by evaluating the effect of intracerebral injections of ATP on the MEL and NAS content in the pineal gland, cortex, cerebellum, and blood. **Methods:** All drugs were injected into a lateral ventricle (5mL). The data were analyzed by ANOVA or Student "t"-test. NAS and MEL content was measured by HPLC/ELISA and gene expression by qPCR. The agonists, ATP (0.3-3.0 μ g) and BzATP (15.0-50.0ng), were injected in Wistar rats 30min before darkness (ZT11.5). The selective P2X7 (A438079; 1.7ng) and P2Y1 (MRS2179; 2.7ng) antagonists were injected in ZT10.5. Animals were euthanized at ZT18. Another group of rats are treated with ATP (0.3-3.0 μ g) in the light period (ZT04) and euthanized at ZT09.5. **Results:** ATP induced a dose-dependent increase in NAS while MEL content followed a bell-

shaped curve (maximal increase with 1.0µg, n=24). ATP potentiation of NAS/MEL synthesis was inhibited with MRS2179 (n=14). BzATP, a P2X7 agonist, induced an increase in NAS and a decrease in MEL (n=16), which was blocked by A438079. No NAS or melatonin pineal synthesis was observed during the daytime, in the presence or absence of ATP. The changes in pineal activity were translated into a strong nocturnal elevation in plasma NAS (n=13). In the cortex, MEL content increased even when the pineal MEL content decreased (n=14) due to a dose-dependent increase in *Asmt*, even with the reduction of *Aa-nat* transcription, strongly suggesting that NAS present in the cerebrospinal fluid (CSF) is converted into MEL. In the cerebellum, the transcription of *Asmt* was reduced, that of *Cyp1b1* was increased, resulting in no change in MEL content (n=14). **Conclusion:** For the first time, a dissociation between NAS and melatonin synthesis induced by an endogenous DAMP is reported. Indeed, a high increase in ATP CSF level, which signalizes cell death, provides a fine-tuning control of pineal activity and extra-pineal modulation of MEL content, reinforcing the idea that the pineal gland is a regular player in successful defense response. Moreover, it points to a new role for NAS, which might be converted into the darkness hormone in pathophysiological conditions that affect the brain. **Support:** FAPESP 2019/03348-4; CNPq 480097/2013-5, 140274/2018-9; CAPES. **License number of ethics committee:** 324/2018

02.015 **G15 induces tau clearance and autophagy in 2D and 3D cultures of SH-SY5Y overexpressing tau.** Nishino MS¹, Costa AJ², Pereira GJ², Smaili SS², Stilhano RS³, Ureshino RP¹. ¹Unifesp-Diadema, PPG Chemical Biology, Brazil; ²Unifesp São Paulo, Dept. of Pharmacology, Brazil; ³FCMSCSP, Dept of Physiological Sciences, Brazil

Introduction: Ageing related neurodegenerative diseases have been gaining attention due to the increased life expectancy of the overall population (1). Tauopathies are a group of neurodegenerative diseases characterized by deposition of hyperphosphorylated tau protein (2). In diseases with protein aggregation, degradation systems such as autophagy, which is a recycling mechanism of intracellular components, play important roles (3). Modulation of autophagy would be an important therapeutic strategy for those diseases (4). Autophagy flux is a highly regulated process which draws in several signaling pathways (5, 6). This process can be induced by starvation, stress, or pharmaceutical approach (5, 6). In this last category, estrogens present a potential modulation of autophagy once there is a crosstalk between the autophagic and the estrogen signaling pathways (7). Estrogen plays its functions through the activation of its classical receptors, estrogen receptor α (Er α) and estrogen receptor β (Er β), or through G-protein coupled estrogen receptor (GPER) (8, 9). The objective is to study the effect of G15 (GPER antagonist) in the autophagy modulation and tau clearance in 2D and 3D cultures of SH-SY5Y overexpressing tau. **Methods:** SH-SY5Y cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM)/F12 media supplemented with 10% fetal bovine serum. Cells were transduced with Tet-On conditional expression systems carrying human tau gene. For the 2D assays, cells were plated in culture dishes and treated with G15 (100nM). For the 3D assays, cells were seeded onto micromolded agarose gels to induce spheroids formation. These were treated with doxycycline, to activate the tet system, and with G15 for 15 days. Every two days pictures were taken for volume quantification. Cell viability were measured through trypan blue. All methods were approved by CEP no. 9432090818. **Results:** G15 was able to induce autophagy in SH-SY5Y cells and in cells with tau overexpression at the concentration of 100nM. It was also able to induce tau clearance in 24 hours treatments. The treated groups had a spherical shape, meanwhile the control groups did not present a regular form. The viability assays showed that the treated groups had higher viability compared to the control with no treatment. The total number of cells were higher in the treated groups, which might be an indirect evidence of cell proliferation. **Conclusions:** Here we show that G15 was able to induce autophagy both in SH-SY5Y and in the cells overexpressing human tau. It also promotes tau clearance. In the 3D model, G15 lead to cell survival, showing a possible neuroprotective role *in vitro*. **Acknowledgments:** This work was supported by grants from Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) project no. 2018/16719-8. **References:** 1. WHO, October 2011 2. Orr, ME, Trends Pharmacol Sci, vol 37, p637, 2017 3. Fujikake N, Front Neurosci, 2018 4. Budini M, Front Mol Neurosci, 2017 5. He C, Annu Rev Genet, p67, 2009 6. Russel CR, Cell Res, vol 24, p42, 2014 7. Xiang J, Autophagy, p197, 2019 8. Mangelsdorf DJ, Cell, vol 86, p835, 1995 9. Prossnitz ER, Mol Cell Endocrinol, vol 308, p32, 2009. **License number of ethics committee:** 9432090818

02.016 **Antidepressants response evaluation in Wistar Hannover rats submitted to forced swim test and learned helplessness paradigm.** Silveira KM^{1 2}, Sartim AG¹, Vieira L¹, Lisboa SF¹, Wegener G², Joca SRL^{1,3}. ¹FCFRP-USP, Dpt of Biomolecular Sciences, Ribeirão Preto, Brazil ²Aarhus Univ, Translational Neuropsychiatry Unit, Dpt of Clinical Medicine, Aarhus, Denmark. ³Aarhus Univ, Dpt of Biomedicine, Denmark **Introduction:** The Wistar Hannover (HNR) rat strain is commonly used for general toxicity studies but rarely used to investigate the neurobiology of depression and treatment response. Recently at the

University of São Paulo, the outbred Wistar strain was replaced by HNR. Therefore, we aimed to characterize the behavior and response to antidepressants of these rats in two animal models: forced swim test (FST) and learned helplessness paradigm (LH). **Methods and Results:** Male Wistar HNR rats were submitted to one of two protocols of FST, with or without pre-swim. In the pre-swimming group, a first swim session (pre-test-PT; 15min) was followed by the test session (5min) after 24h. Antidepressant (imipramine-IMI (5, 10, 15, 20mg/kg), fluoxetine-FLX (5-10mg/kg), or escitalopram-ESC (3-10mg/kg)) was administered at 0, 5h, and 23h after PT. Antidepressants did not change the immobility time in the test of animals submitted to the PT. In an independent experiment, IMI, FLX, ESC (all at 20mg/kg), or vehicle was given once daily for seven days (subchronic treatment), or vehicle daily for six days, and a single injection of antidepressant or S-ketamine on the 7th day (acute treatment). Animals in this experiment were only exposed to a single swim session (10min). IMI (20mg/kg, subchronic) and S-ketamine (10mg/kg, acute) decreased the immobility time in the test. No locomotor change was observed in the open field test. For the LH experiments, rats were also submitted to two different protocols, traditional and adapted. In the traditional, a PT session (40 min, randomized inescapable footshocks, 0.6 mA) was followed seven days later by a test session (30min, randomized escapable footshocks, 0.4mA, preceded by a warning tone). After the PT, the animals were treated subchronically with FLX (20mg/kg) or vehicle. No effect was observed in the test session. In the adapted model, rats were subjected to a PT session and 24 hours later to the test section 1 (30min, randomized escapable footshocks, 0.6mA) and then classified as resilient or susceptible according to their number of escape failures (resilient: 0-10; susceptible: 15-30 failures). Susceptible animals were subchronically treated with vehicle, FLX, or IMI (both at 20mg/kg) and submitted to a second test session (same as 1). Again, no antidepressant reversed the susceptible phenotype (Animal Research Ethical Committee: 19.1.582.60.3/19.1.248.60.6). **Conclusion:** We showed that the HNR rats are less sensitive to conventional antidepressants in FST and LH. Only acute S-ketamine or subchronic imipramine treatment in the FST was effective. No effect was observed in the LH. Furthermore, the selective serotonin reuptake inhibitors FLX and ESC did not affect behavior in the different tested protocols. Although more studies are needed, the low sensitivity to conventional antidepressants, a common characteristic in animal models of treatment-resistant depression-TRD, suggests HNR strain could be a valuable tool in investigating new drugs to use in TRD. **Financial support:** CNPq, CAPES **License number of ethics committee:** Animal Research Ethical Committee: 19.1.582.60.3 / 19.1.248.60.6

02.017 Analysis of autophagy and SUMOylation expression profile in YAC128 mice, an animal model of Huntington's disease. Soares ES¹, Camargo A², Kietzer K³, Rodrigues ALS^{2,4}, Prediger RD^{1,2}, BROCARDI PS², CIMAROSTI HI^{1,2}. ¹UFSC Florianopolis, PPG Pharmacology, Brazil; ²UFSC Florianopolis, PPG Neurosciences, Brazil; ³UEPA Belem, Dpt of Morphological and Physiological Sciences, Brazil; ⁴UFSC Florianopolis, PPG Biochemistry, Brazil

Introduction: Huntington's disease (HD) is a genetic neurodegenerative disease caused by a mutation in the huntingtin (HTT) gene, which expresses a HTT protein more susceptible to aggregation, accumulation and neurotoxicity. Both men and women have the same risk of inheriting the disease, marked by motor, cognitive and psychiatric symptoms. HD-mediated neurodegeneration is increased by deficits in autophagy, an essential process for cell survival. Recent studies have shown that SUMO (small ubiquitin-like protein) conjugation (SUMOylation) to target proteins could modulate key autophagy proteins, such as beclin-1 and sequestosome-1 (SQSTM1). Among the models to study HD, there are the YAC128 mice, which express the human HTT protein containing a glutamine repeat expansion. Therefore, the aim of this study was to investigate the putative role of SUMOylation and autophagy-related proteins in HD using this genetic animal model. **Methods:** Male and female YAC128 transgenic mice, both aged between 3 and 6 months old (m.o.), were used (CEUA-UFSC: #4502210318). Following cervical dislocation, animals had their brains collected to dissect the pre-frontal cortex (PFC), striatum (STR) and hippocampus (HP). The immunoccontents of beclin-1 and SQSTM1 were analysed by Western blotting, while the only SUMO conjugating enzyme, Ubc9, and the pathology-associated SUMO isoform, SUMO-2/3, were analysed by dot blotting. **Results:** Levels of beclin-1 were increased in the PFC of 6 m.o. WT and YAC128 male and female mice, but decreased in the STR of these same animals, when compared to WT 3 m.o. mice. In addition, beclin-1 was reduced in the HP (3 and 6 m.o. YAC128 female and male mice), in comparison to WT animals of both ages. Ubc9 was decreased in the PFC and in the HP of 6 m.o. YAC128 male, compared to WT 6 m.o. mice, as well as in the HP of 3 and 6 m.o. YAC128 female mice, when compared to their respective WT groups. SUMO-2/3 was decreased in the PFC of 6 m.o. YAC128 female and in the STR of 3 m.o. YAC128 male mice, compared to WT 6 m.o. and WT 3 m.o., respectively. Otherwise, SUMO-2/3 was increased in the HP of 3 m.o. WT and YAC128 female mice, in comparison to male WT and YAC128 3 m.o. No differences in SQSTM1 were observed. **Conclusion:** These findings demonstrate a temporal-dependent decreased expression of beclin-1 in the STR and HP of YAC128 animals, regardless

of gender, disturbing the normal functioning of autophagy. Moreover, the hippocampal Ubc9 decrease suggests impairment in the SUMOylation pathway, marked by a hippocampal increase of SUMO-2/3, normally present in pathological processes. **Financial Support:** CAPES and CNPq. **Acknowledgements:** We are thankful to MSc. Eduarda Zampieri Centeno for technical assistance with dot blotting and some crucial pilot experiments. **License number of ethics committee:** CEUA-UFSC: 4502210318

02.018 Taurine restores extracellular GABA levels in the nucleus accumbens reduced by alcohol withdrawal and decreases voluntary alcohol intake in withdrawal rats. Pulcinelli RR¹, Caletti G¹, Izolan LR¹, Eller S², Nin MS³, Oliveira TF², Gomez R¹. ¹UFRGS Porto Alegre, PPG Farmacologia e Terapêutica (PPGFT), Brazil; ²UFCSA Porto Alegre, Dpt de Farmacociências, Brazil; ³FURG Rio Grande, Dpt de Ciências Biológicas, Brazil.

Introduction: Taurine is an abundant amino acid in the brain and shows a neuromodulatory effect on GABAergic and glutamatergic systems, both related to neuroadaptive changes in progression from occasional alcohol intake to dependence. Previously, we found that chronic taurine administration increases voluntary alcohol intake in rats. Here we investigated the effect of repeated taurine administration during alcohol withdrawal and re-exposure, as well as *In Vivo* extracellular GABA levels in the nucleus accumbens (NAcc) of rats. **Methods:** Adult male Wistar rats were allowed to choose from two bottles containing 20% alcohol and vehicle solution (AL group) or two bottles containing vehicle solution (CT group), 24 h/day, for four weeks. On day 22nd, half of the AL rats had their alcohol bottle substituted for another containing vehicle solution (WH group). Then CT, AL, and WH groups were subdivided to receive 100 mg/kg taurine or saline i.p. (CTS; CTT; ALS; ALT, WHS, WHT; n=6/group), once a day, for five days. Before starting this treatment (day 20th), a stereotaxic surgery was conducted to insert a guide cannula in the NAcc for the microanalysis study. After seven days from the stereotaxic surgery, microdialysis was performed along 2.5 h, with samples collected every 30 min. The perfusates were analyzed by a UFLC system coupled to a triple quadrupole mass spectrometer to determine GABA efflux in the NAcc. Later, the rats from WH groups received an additional taurine/saline administration and were re-exposed to the voluntary alcohol intake model and allowed to drink for 24 h. Daily alcohol voluntary intake was monitored throughout the experiment. **Results:** Taurine treatment (WHT group) prevented the lower baseline GABA levels in the NAcc of rats found in the WHS group compared with CTS and ALS groups. Moreover, taurine decreased GABA levels in alcohol-exposed rats (ALT group) compared to the ALS group. Replaying previous studies, taurine increased twice as much alcohol intake in the ALT group on days 4th and 5th of the treatment. However, taurine during withdrawal prevented the increased alcohol consumption seen in the WHS group, decreasing by 64% the alcohol intake of the WHT group during re-exposure (P = 0.010). Pearson test indicated an inverse correlation (r = -0.51; P = 0.0216) between the GABA levels and alcohol consumption considering AL and WH groups. **Conclusion:** Taurine produces an alcohol anti-addictive effect dependent on the abstinence condition. This effect may be related to the restoration of extracellular GABA levels in NAcc impaired by alcohol withdrawal that, indirectly, modulates alcohol-related reward pathways. Taurine treatment during alcohol withdrawal could be beneficial as an adjuvant therapy to prevent alcohol relapse in abstinent alcohol-dependent. **Financial Support:** CNPq, CAPES, Propeq-UFRGS Approval by Animal Research Ethical Committee: CEUA-UFRGS # 36606 **License number of ethics committee:** CEUA-UFRGS # 36606

02.019 Neurochemical inter-hemispheric asymmetries and depression: a scoping review of the preclinical literature. Almeida FB¹, Fiore RL¹, Gomez R², Nin MS^{1, 2}, Barros HMT¹. ¹PPG-CS-UFCSA; ²PPG-FT-UFRGS

Introduction- Many clinical studies have demonstrated an asymmetry in cortical function of depressed individuals, but the relationship of this functional asymmetry with the neurobiological mechanisms of depression is not clear. Preclinical studies may disclose the hemisphere-specific role of neurochemical markers in depressive-like behaviors and antidepressant action. Thus, we conducted a systematic scoping review to map the preclinical literature on depression that investigated hemisphere-specific levels of neurochemical markers related to animal models of depression and antidepressant treatment. **Methods-** We searched four different databases (PubMed, EMBASE, PsycInfo and LILACS) for relevant papers that were subsequently screened for eligibility, and a final number of 23 articles were included. **Results-** Our results show that animal models of depression used were based on stress, congenitally depressed rat lines or pharmacological induced. Several different antidepressants were used as pharmacotherapies, but other classes of substances such as neurosteroids and hypertensives were also administered. The array of neurochemical markers assessed was highly heterogeneous, with dopamine, serotonin, brain-derived neurotrophic factor (BDNF) and subunits of the GABA_A receptor appearing with the higher frequencies. For each neurochemical marker, studies were of very different experimental designs to derive generalizable conclusions, but potential implications of the findings are discussed. **Conclusion-** Interhemispheric

differences in the expression/levels of neurochemical markers were often hemisphere-specific and cannot be extrapolated to the whole brain. Potential issues with the design of neurochemical analysis, as well as of publication/reporting bias in the field are raised, and recommendations for further studies based on the present findings are made. Acknowledgements of scholarships: FBA- doctoral/CAPES; RLF- scientific initiation/FAPERGS; RG-researcher 2/CNPQ; HMTB-researcher 1B/CNPQ. **License number of ethics committee:** N/A

02.020 Obesity impairs Na,K-ATPase activity in the cerebellum of female mice

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Introduction: Obesity is a chronic disease characterized by excessive accumulation of adipose tissue and systemic inflammation. Na,K-ATPase is an electrogenic transmembrane protein, and its activity is impaired during metabolic-related disorders due to oxidative stress. The central nervous system (CNS) is permeable to cytokines from periphery and the cerebellum has been identified as prone to develop neuroinflammation. However, studies lack data referring to the effects of obesity on Na-K-ATPase activity in the CNS. We aimed to evaluate the involvement of obesity in the neuroinflammatory response in Na,K-ATPase in the cerebellum of female mice. **Methods:** Female Balb/c mice were given high fat (Ob) or control (Con) diet for 10 weeks. Weekly changes in body weight (BW), body weight gain (BWG), fat mass (FM), fat mass percentage (%FM), fat mass gain (FMG), muscle mass (MM), muscle mass percentage (%MM) and muscle mass gain (MMG) were measured using the Bruker Minispec Live Mice Analyzer (Bruker Optics, Inc). After 10 weeks of experimental protocol, mice were euthanized and cerebellar, and blood samples were collected. Serum levels of cytokines (TNF- α , IL-1 β and IL-17) were quantified using the Milliplex® MAP kit (Luminex). Na,K-ATPase (NKA), Glutathione-S-Transferase (GST), Glutathione Peroxidase (GPx), and Glutathione Reductase (GR) activities were measured in cerebellar tissue homogenates. NKA subunits (α 1, α 2, and α 3-NKA), nuclear factor erythroid 2-related factor (Nrf2), extracellular signal-regulated kinase (ERK), phosphorylated-extracellular signal-regulated kinase (p-ERK), 4-hydroxynonenal (4-HNE) and tubulin protein expressions were determined in the cerebellar homogenates by western blotting. Statistical analysis was performed using the Students t-test. Data were expressed as mean \pm SEM. The research was approved by the Committee on Ethics in Animal Use (5288160218). **Results:** The increase in BW, BWG, FM, %FM, FMG, MM, %MM and MMG were similar between control and obese groups in the first nine weeks. After the 10th week, although no significant changes ($p > 0.05$) were observed in MM, %MM and MMG, Ob mice have shown a significant increase in BW (Ob: 23.3 ± 0.5 vs Con: 20.8 ± 0.6 , $n=5/\text{group}$, $g^*p < 0.05$), BWG (Ob: 5.5 ± 0.0 vs Con: 3.6 ± 0.3 , $n=5/\text{group}$, $g^{**}p < 0.01$), FM (Ob: 3.1 ± 0.2 vs Con: 1.8 ± 0.1 , $n=5/\text{group}$, $g^{**}p < 0.01$), %FM (Ob: 13.5 ± 0.9 vs Con: 8.9 ± 0.7 , $n=5/\text{group}$, $\%g^{**}p < 0.01$), FMG (Ob: 1.4 ± 0.2 vs Con: 0.2 ± 0.0 , $n=5/\text{group}$, $g^{**}p < 0.01$) than those of the Con mice. Serum levels of IL-1 β and IL-17 were no different ($p > 0.05$) between mice fed a high fat or control diet. On the other hand, a significant increase ($*p < 0.05$) in TNF- α levels was observed between Ob mice (6.8 ± 1.0 , $n=11/\text{group}$, pg/mL) and Con mice (3.9 ± 0.6 , $n=11/\text{group}$, pg/mL).

An increased activity of GST (Ob: 3.0 ± 0.1 vs Con: 2.5 ± 0.0 , $n=10/\text{group}$, U/mg prot $**p < 0.01$), GPx (Ob: 0.9 ± 0.0 vs Con: 0.8 ± 0.0 , $n=10/\text{group}$, U/mg prot $*p < 0.05$) and GR (Ob: 0.8 ± 0.0 vs Con: 0.7 ± 0.0 , $n=10/\text{group}$, U/mg prot $*p < 0.05$) were observed in the cerebellum of Ob mice, compared to the Con group. Nevertheless, Ob mice have shown a reduction in α -2,3 NKA activity (Ob: 0.09 ± 0.00 vs Con: 0.12 ± 0.01 , $n=5/\text{group}$, nmol/mg prot $*p < 0.05$), but not in α -1 NKA or NKA total activities ($p > 0.05$). Western blotting analysis of cerebellar tissue homogenates suggested there's no significant changes ($p > 0.05$) in NKA subunits (α 1, α 2 and α 3-NKA) expression observed between groups. Nonetheless, it was observed a significant increase in 4-HNE (Ob: 1.1 ± 0.0 vs Con: 0.9 ± 0.0 , $n=8/\text{group}$, A.U $*p < 0.05$) and p-ERK (Ob: 1.9 ± 0.1 vs Con: 0.7 ± 0.0 , $n=8/\text{group}$, A.U $****p < 0.0001$) expressions, without changes in total ERK ($p > 0.05$). On the other hand, Ob mice have shown a reduction in Nrf2 cytosolic expression (Ob: 0.8 ± 0.0 vs Con: 1.1 ± 0.1 , $n=7/\text{group}$, A.U $*p < 0.05$), followed by its increased nuclear expression (Ob: 1.4 ± 0.1 vs Con: 1.0 ± 0.0 , $n=10/\text{group}$, A.U $*p < 0.05$), when compared to Con mice. **Conclusion:** Our results may suggest that obesity leads to a diminished Na,K-ATPase activity in the cerebellum of female mice. It might be related to oxidative stress and systemic inflammation. Financial Support: FAPESP, CAPES (Finance Code 001), CNPQ. **License number of ethics committee:** The research was approved by the Committee on Ethics in Animal Use (5288160218).

02.021 Long-lasting behavioral consequences of social isolation and ethanol consumption during adolescence: effects of physical exercise.

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Introduction: Social isolation and ethanol consumption may influence brain development in adolescence and may cause long-lasting behavioral consequences. Thus, the present study aimed to assess whether social isolation and ethanol consumption during adolescence could cause memory impairment and changes in anxiety - and depression-like behaviors in adulthood. Further, if physical exercise could revert such deficits. **Experiment.** **Methods:** Male Swiss mice were allocated into eight experimental groups: isolated-ethanol-sedentary (ESi) (n=6), isolated-ethanol-exercise (EEi) (n=7), isolated-water-sedentary (ASi) (n=6), isolated-water-exercise (AEi) (n=8), grouped-ethanol-sedentary (ESa) (n=8), grouped-ethanol-exercise (EEa) (n=8), grouped- water-sedentary (AAs) (n=7), grouped-water-exercise (AEa) (n=8). Animals from "ethanol" groups underwent the intermittent ethanol access protocol for 4 weeks, and from the "exercise" group underwent the forced physical exercise protocol (treadmill) for 4 weeks. **Results:** It was observed an increase in ethanol consumption (from the third week) by isolated groups (7.8 ± 1.7 g/kg) compared to grouped animals (6.4 ± 1 g/kg) ($p < 0.05$). Moreover, the animals in the EEi group showed a decrease in the time spent in the open arms ($p = 0.0563$), as well as in the number of unprotected heads dipping ($p = 0.0091$) when compared to the other groups ($p = 0.0091$). Regarding the memory test, it was observed that the animals from the EEi group did not recognize the new object, in the tests performed 1 and 24 hours after the training, presenting short- and long-term memory impairments. For the depressive-type behavior, the ESi group showed an increase in the immobility time when compared to the other groups ($p < 0.05$). **Conclusion:** Social isolation, during adolescence, leads to long-lasting neurobiological plasticity, and increases alcohol consumption. **Financial Support:** FAPESP n° 2018/15505-4; CAPES. **License number of ethics committee:** CEUA N° 8253041219

02.022 **Stress susceptibility and response to fluoxetine in the learned helplessness model: involvement of the P2X7-NLRP3 inflammasome pathway.** Vieira L¹, Roncalho AL², Chiavegatto S³, Lisboa SF¹, Joca SRL^{1,4}. ¹BioMolecular Science Dpt, School of Pharmaceutical Sciences of Ribeirão Preto, ²Univ of São Paulo (USP), School of Medicine of Ribeirão Preto (USP), ³Pharmacology-Biomedical Sciences Inst and Psychiatry-Medical School (USP), ⁴Dpt of Biomedicine, Aarhus Univ, Denmark

Introduction: How individuals deal with stressful events can determine resilience or susceptibility to the development of stress-related psychiatric disorders, such as major depressive disorder. Stress exposure increases ATP release in the brain, which activates purinergic P2X7 receptors. In microglia, P2X7R activation is linked to NLRP3 inflammasome activation. In rodents, P2X7 antagonists induce antidepressant-like effects and modulate the NLRP3 pathway. Antidepressant drugs attenuate behavioral consequences induced by chronic stress in rodents, but their effects on the P2X7-NLRP3 inflammasome pathway are poorly understood. Therefore, this study investigated if 1.the expression of P2X7-NLRP3 inflammasome genes is associated with stress susceptibility and antidepressant response, and 2. if the NLRP3 inflammasome inhibition induces an antidepressant effect. **Methods:** Male Wistar Hannover rats (WHR) (n=80) were submitted to learned helplessness (LH) pre-test (PT) session (40 cycles shocks, 0.8 mA, 10 s duration, 30-90 s interval) or were placed in the shuttle box, with no footshocks (non-stressed rats). Rats were treated once daily, for seven days, with fluoxetine-FLX (10mg/kg/10ml) or saline. 1h after injection on the 7th day, in the test session (T; 30 cycles shocks, 0.6 mA, 10 s duration, 30-90 s interval, preceded by a 60db beep for 5s), the number of escape failures, avoidance, intertrial crossings were quantified. After T, rats were classified into resilient/susceptible (vehicle-treated group) or responsive/non-responsive (FLX-treated group). P2X7 and NLRP3 gene expression were quantified by qPCR in the prefrontal cortex (PFC), dorsal and ventral hippocampus (DH and VH). Independent groups of WHR were submitted to the same protocol but treated daily with MCC950 (10, 20, and 30mg/kg) or saline or a combination of FLX (10 mg/Kg) and MCC950 (20 mg/Kg). (Ethics Committee: Prot. No.18.1371.60.1). **Results:** Helpless behavior was observed in 65% of animals (susceptibles n=11; resilient n=6). P2X7 and NLRP3 expression in the DH, but not in the VH or PFC, was reduced in animals that responded to FLX treatment (responders, n=7) compared to non-responders (n=9). MCC950 did not induce an antidepressant-like effect by itself, but in combination with an ineffective dose of FLX, it decreased the number of failures and increased of flight, with no locomotor change (crossings) (Kruskal Wallis test, $p \leq 0,05$). **Conclusion:** Our data show that response to the effect of FLX in the LH model is associated with decreased P2X7 and NLRP3 expression in the DH, which did not occur in non-responders' rats, and that combined FLX and MCC950 attenuated helplessness behavior, suggest that treatment-resistant depression could be associated with failure to dampen P2X7-NLRP3 activation in the brain and that adjunct treatment with NLRP3 inhibitors could be an alternative in this case. **Financial support:** FAPESP fellowships (2017/24304-0 to JOCA SRL and LISBOA SF, 2017/19731-6 to LISBOA SF, 2019/04616-2 to VIEIRA L and 2017/06100-8 to CHIAVEGATTO S). **License number of ethics committee:** No.18.1371.60.1

02.023 Andrographolide mitigates the cognitive impairment induced by an idiopathic alzheimer's disease model in rats. Souza LC, Ramos DC, Andrade MK, Vital MABF. UFPR Curitiba, Dpt of Pharmacology, Brazil Alzheimer's disease (AD) is a progressive neurodegenerative disorder and the most common form of dementia, it is clinically manifested by a gradual memory loss and decline in cognitive function [1]. Intracerebroventricular injection of streptozotocin (icv-STZ) is used as a model of Sporadic AD (SAD) in rodents, the icv-STZ model shows many aspects of SAD abnormalities (i.e., oxidative stress, neuroinflammation, neuronal loss, decreased neurogenesis and amyloid angiopathy) resulting in cognitive impairment [2]. Andrographolide (ANDRO), a natural diterpene lactone, has been explored for its numerous bioactivities including anti-inflammatory and antioxidant in humans and animals. Recently, studies revealed that ANDRO also has several pharmacological actions on the central nervous system, including anti-dementia, anti-neuroinflammation, pro-neurogenic, and neuroprotective actions in rodents [3]. The aim of the present study was to investigate the role of ANDRO on the cognitive impairment induced by the icv-STZ model. Male Wistar rats underwent stereotaxical surgery for the icv-STZ (3 mg/kg, dissolved in saline), and were treated intraperitoneally with ANDRO (2 mg/kg, dissolved in saline with dimethyl sulfoxide (2%, v/v)) 3 times/week throughout 4 weeks. In the last 4 days of treatment the rats were subjected to the Y-maze test (Spatial version) (n=10-11 per group); object localization (OLT, n=8-9 per group) and object recognition (ORT, n=10-12 per group) tests; and the open field test (OFT, n=10-11 per group). All tests were approved by the Animals Use Ethics Committee of UFPR (CEUA #1315) and conducted like previously described in the literature. One-way ANOVA showed a significant difference between the groups in all the tests (Y-maze [F(1, 38)=5.8; p<0.05]; ORT [F(3, 30)=2.9; p<0.05]; OLT [F(3, 38)=3.7; p<0.05]; OFT [F(3, 38)=3.9]). The icv-STZ disturbed the spatial and recognition memory, the Tukey post-hoc test revealed a significant decrease in the time spent on the novel arm in the Y-maze, p<0.05, and in the discrimination index in the ORT, p<0.05, of STZ+vehicle group in relation to SHAM+vehicle group (control); the STZ+ANDRO group showed a similar performance to control. This suggests that ANDRO was able to partially protect the rats against icv-STZ cognitive impairment. However, in the ORL and OFT post-hoc showed only a decrease in the STZ+ANDRO group discrimination index and number of lines crossed, respectively, in relation to control. Surprisingly, in contrast to the anterior tests, ANDRO worsened the spatial memory of the rats in the ORL. In relation to the OFT, ANDRO decreased the exploration behavior, which could indicate a motor or motivational impairment. In summary, ANDRO exhibits a pro-cognitive like effect in the icv-STZ rat model. Nevertheless, it also showed a contradictory anti-cognitive like effect in one of the three cognitive tests. More tests will be needed to have a broader view of the role of ANDRO. The authors report no conflicts of interest. This work was supported by grants from CNPq and CAPES, which had no further role in the study. VITAL MABF is a recipient of a CNPq fellowship. [1] CORREIA, SC et al. Age Res Rev, v. 10, p. 264, 2011. [2] SILVA, TGS et al. Heliyon, v. 6, 2020. [3] ZHANG, JJ et al. Int Jour of Neuropsych, v. 22, p. 585, 2019. **License number of ethics committee:** Animals Use Ethics Committee of UFPR (CEUA-BIO – UFPR) - CEUA #1315

02.024 Phosphodiesterase-4 inhibitor Roflumilast improves anxiety-like behavior in type-1 diabetes mellitus likely through its protective function. Waltrick APF¹, Chaves YC¹, Da Silva ACF¹, Prickaerts J², Oliveira RMMW³, Zanolini JM¹. ¹UFPR Curitiba, Dpt of Pharmacology, Brazil; ²Maastricht Univ, School for Mental Health and Neuroscience, the Netherlands; ³UEM Maringá, Dpt of Pharmacology and Therapeutics, Brazil **Introduction:** Type-1 diabetes *mellitus* (T1DM) is highly related to the development of neuropsychiatric diseases, such as anxiety disorders. It is known that cAMP responsive element-binding protein (CREB) pathway regulates crucial cell stages (e.g., proliferation, differentiation, and survival), and in the adult brain, it participates in neuronal plasticity and prevention of neuroinflammation, as well as of the mediation of learning/memory processes and emotions. Curiously, evidence demonstrates that this pathway is damaged in brain areas involved in mediating emotional responses, such as the hippocampus and prefrontal cortex from diabetic animals. Thus, a way to re-establish this pathway is increasing intracellular cAMP levels by using drugs able to inhibit the enzyme phosphodiesterase 4 (PDE-4), responsible for cAMP hydrolysis. Therefore, inhibitors of PDE-4 (iPDE-4) like roflumilast have been identified as an important option for the treatment of some cognitive disorders, as they can help increase neuroplasticity and to decrease damage processes like neuroinflammation. **Aim and Methods:** The present study aimed to evaluate the effect of roflumilast on parameters related to anxiety-like responses through behavioral tests in animals with experimental T1DM. Male *Wistar* rats received one injection of streptozotocin (60 mg/kg; ip; day 0) to induce T1DM, while non-diabetic animals (nDBT) received the citrate buffer. Seven days after the induction of experimental T1DM, these animals received roflumilast (i.p.; 0, 0.01, 0.1 mg/kg) for 21 days, while nDBT animals received only its vehicle (i.p.) during the same time. On the 28th day after T1DM induction, the animals were submitted to the light-dark transition test (LDTT). On the following day, the animals underwent the open field test (OFT) and, on the 30th day, the elevated plus maze test

(EPMT) was performed. Soon after, the animals were euthanized and had the prefrontal cortex and hippocampus removed for ELISA analysis of the pro-inflammatory cytokine interleukin-1 β (IL-1 β). All protocols were approved by the Ethics Committee on Animal Use of the Biological Sciences Sector of the Federal University of Paraná (n $^{\circ}$ 1204). **Results:** The treatment with the iPDE-4 roflumilast did not alter the locomotor activity in the OFT and the EPMT and it was not able to decrease the anxiety parameters in the EPMT. However, in the LDTT and the ethological measurements of the OFT, we observed a clear anxiolytic-like effect promoted by roflumilast. Importantly, the treatment decreased the glycemia of treated-T1DM animals and decreased IL-1 β levels in these two brain areas evaluated. **Conclusion:** Our data confirm the neuroprotective characteristic of roflumilast. Furthermore, indicate that this drug presents the potential to minimize anxiety-like behavior in an animal model of T1DM, and may even improve the diabetic condition itself. **License number of ethics committee:** All protocols were approved by the Ethics Committee on Animal Use of the Biological Sciences Sector of the Federal University of Paraná (n $^{\circ}$ 1204).

02.025 Pi3K γ participates in the amnesic effect induced by scopolamine in mice. Silveira DS, Scarante FF, Costa KCM, Guimarães FS, Campos AC Dpt of Pharmacology, Ribeirão Preto School of Medicine, Univ of São Paulo, Ribeirão Preto, Brazil

Scopolamine (SCO), an antagonist of muscarinic receptors, induces anterograde amnesia in animal models, but the exact mechanism associated with this action remains unclear. The PI3K is a family of kinase proteins that play a role in regulating several intracellular processes and classified in 3 different classes. The PI3K γ is the member of class Ib and can control immune responses and central nervous system functions. Therefore, this study hypothesizes that the PI3K γ protein and its downstream signaling pathways are involved in the anterograde amnesia induced by SCO. Male and female mice (10-12 weeks old) knock-out (KO) for the PI3K γ protein and their wild-type (WT) littermates were used. The mnemonic function of the animals was addressed with the novel object recognition test (NOR). SCO (1 and 2 mg/kg) was administered before the acquisition step of the NOR protocol. A separate group of WT mice received an intra-hippocampal treatment with the selective PI3K γ antagonist AS605240 (Vehicle, 3 nM, 10 nM, and 30 nM) in the acquisition phase of the NOR protocol and this pharmacological intrahippocampal inhibition of PI3K γ (at the highest concentration tested) was paired with SCO treatment as well. Both in male and female mice, the treatment with SCO (1 mg/kg) induced a significant decrease in the discrimination index between the novel and familiar objects in the NOR test in WT mice, but this effect was absent in PI3K γ KO mice. None of the concentrations of AS605240 injected into the hippocampus altered the behavioral response of mice in the NOR test. The highest ineffective concentration tested (30nM), therefore, was administered before SCO treatment, however, AS605240 was not able to prevent the anterograde amnesia induced by SCO. The expression of proteins associated with the PI3K γ pathway (mTOR and the MAP kinase-associated protein Erk) in the hippocampal formation was analyzed. Compared to their WT littermates, the PI3K KO mice presented altered hippocampal levels of Erk but not mTOR. Our results support that the PI3K γ protein and its signaling pathways are involved in the anterograde amnesia induced by SCO in mice. **License number of ethics committee:** Ceua n $^{\circ}$ 159/2016

02.026 Low-cost experimental apparatus for cigarette smoke exposure in rats. Izolan LR¹, Bandiera S², Pulcinelli RR², Zilli GAL², Almeida FB³, Nin MS^{3,4}, Marques D¹, Leal MB^{1,2}, Gomez R^{1,2}. ¹UFRGS, PPG em Neurociências, Porto Alegre, Brazil; ²UFRGS, PPG em Farmacologia e Terapêutica, Porto Alegre, Brazil; ³UFCSA, PPG em Ciências da Saúde, Porto Alegre, Brazil; ⁴FURG, Dept de Farmacologia, Rio Grande, Brazil

Background: Cigarette smoking is the main cause of preventable death and accounts for 5 million people die each year. Studies have been conducted to investigate the deleterious effect of this drug of abuse in humans and animals. However, results from most studies are limited to non-invasive methods or isolated nicotine administration. Some few whole-body cigarette smoke exposure automatized systems are very complex and expensive. Thus, our objective here was to describe a low-cost, feasible, and safe apparatus for whole-body cigarette smoke exposure in rodents. **Method:** We designed and built a whole-body cigarette smoke exposure system. The main elements were an exposure chamber, a vacuum pump, and a chimney. In this closed system, environmental air or cigarette smoke moves unidirectionally from the chamber to the chimney compelled by the vacuum pump (~10 L/min) and exhausters connected in the system. The cigarette burns outside the exposure chamber in a glass T-tube, with the airflow adjusted by the vacuum pump. Each chamber (50 x 30 x 30 cm) was built to allows the exposure of up to 8 rats simultaneously with one cigarette burning along 10 min and a 10 min break between each one. Our protocol was designed to repeat the procedure until 6 cigarettes burned with a 2 h interval, twice a day (9 - 11 AM and 1-3 PM), along 4 to 5 weeks. The carbon monoxide concentration was monitored (Bacharach Monoxor II, PA, New Kensington, USA) during the cigarette burning and during intervals.

Cotinine plasma levels were evaluated by high-performance liquid chromatography and different experiments were performed in adult Wistar rats. Results: Our whole-body cigarette smoke exposure system costs less than 67-fold compared to an automated smoking machine. It can be easily built, without specialized knowledge, and does not require expensive manutention. Because it is a sealed system, it is safe for the staff. Carbon monoxide level reached a maximum peak of 380 ppm into the chamber during the exposition and decreased to less than 15 ppm in the intervals. Cotinine plasma levels reached 100.64 ± 17.01 ng/mL in cigarette exposed rats and were significantly higher than the control non-exposed rats. **Conclusions:** Our cigarette smoke exposure method is cheap, safe, easy to build and maintain. Chronic cigarette smoke exposure reproduces cotinine levels similar to active human smokers, without toxic carbon monoxide concentration. This apparatus can be used to mimic acute and chronic exposure to cigarette smoke and investigate the role of other compounds than nicotine in animals. **License number of ethics committee:** CEUA-UFRGS # 19566

02.027 Mineralocorticoid modulation in the infralimbic subregion of the medial prefrontal cortex (IL-mPFC) prevents the stress-induced impairment of aversive memory extinction. Albernaz-Mariano, KA¹, Munhoz, CD¹ ¹USP-São Paulo, Dpt. de Farmacologia-ICB, PPG em Farmacologia, Brazil

Individuals deal with aversive situations and return to a normal lifestyle when adversity ends. Nevertheless, in certain circumstances, traumas may be preceded by memory distortions in stress-related disorders. Aversive memory extinction impairment is strictly associated with the symptoms of post-traumatic stress disorder. Glucocorticoid hormones are released during stress targeting mineralocorticoid (MR) and glucocorticoid (GR) receptors. The MRs are present in essential regions related to cognition, emotions, and initial stress processing, such as the medial prefrontal cortex (mPFC). However, while most studies attempt to elucidate the stress-induced deleterious actions of glucocorticoids via GR, the need to understand the relationship between stress, mPFC, memory, and how MR-mediated intracellular signaling influences this relationship and modulates memory extinction is evident. Male adult Wistar rats ($n = 8-11$ per group) (CEUA protocol number 85/2016 ICB/USP) were bilaterally implanted with guide-cannula into the infralimbic subregion of the mPFC (AP = +2.5; ML = 3.1; DV = -5, angle for 35°), and randomly divided into control (nST) and stressed (ST) groups. The subjects of each group received an intra-mPFC injection of vehicle (PBS + 2% chloroform), spironolactone (10 ng/1µL; MR antagonist), or CORT118335 (10 ng/1µL; MR antagonist + GR agonist) and were exposed to 2h of restraint stress. After 10 days, the animals were submitted to the contextual aversive conditioning (footshock, 1 mA for 1 second) followed by the aversive memory extinction protocol (6 re-exposures in the conditioned environment). We observed that the stressed animals showed a high concentration of corticosterone during acute restraint stress (two-way ANOVA: experimental group [$F_{(3, 137)} = 10.412$; $p < 0.0001$], collection period [$F_{(4, 137)} = 28.578$; $p < 0.0001$] and there was an interaction between both [$F_{(12, 137)} = 1.848$; $p < 0.05$]). The spironolactone decreased corticosterone 60 minutes after the acute restraint stress started ($p < 0.05$, Tukey's test). The CORT118335 increased corticosterone during all stress ($p < 0.05$, Tukey's test). In the retrieval test, all experimental groups showed an increase in freezing time ($p < 0.05$, Tukey's test). However, no difference between groups (control x ST) was observed ($p > 0.05$, Tukey's test), suggesting that all groups acquired the aversive contextual memory, evoking in the context of re-exposure. During the aversive memory extinction test, the stress impaired memory extinction (persistence of freezing) (Student's t-test [$t_{(39)} = 5.683$; $p < 0.0001$]), that was prevents by intra-mPFC injection of spironolactone stress ($p < 0.05$, Tukey's test) (two-way ANOVA: stress [$F_{(1, 47)} = 9.555$; $p = 0.0033$], not for the treatment [$F_{(1, 47)} = 1.596$; $p = 0.2126$], despite an interaction between factors [$F_{(1, 47)} = 14.733$; $p = 0.0004$]), but not CORT118335 ($p > 0.05$, Tukey's test) (two-way ANOVA: stress [$F_{(1, 58)} = 16.166$; $p < 0.001$], but not for the treatment [$F_{(1, 58)} = 0.315$; $p = 0.577$] or interaction between both [$F_{(1, 58)} = 2.992$; $p = 0.089$]). Our results suggest that the infralimbic MR activation during the previous restraint stress impairs the late aversive memory extinction. Financial Support: USP, CAPES, CNPq, FAPESP. **License number of ethics committee:** (CEUA protocol number 85/2016 ICB/USP)

03. Psychopharmacology

03.001 Delayed fear extinction deficit induced by acute stress: modulatory effects on the medial-prefrontal cortex-basolateral amygdala connectivity and the role of norepinephrine signaling. Juliano VAL¹, Munhoz CD¹, Novaes LS¹. ¹ICB-USP São Paulo, Dpt of Pharmacology, Brazil

Introduction: The basolateral amygdala (BLA) and medial prefrontal cortex (mPFC, infralimbic (IL), and prelimbic (PL) subdivisions), which share reciprocal neural connections, are key brain structures for fear memory formation and extinction (Lingawi NW Psychopharmacology. v.236; p.303; 2019). Data from our group demonstrate that 2h acute restraint stress promoted a fear extinction deficit in rats 10 days later. Here we analyzed the expression of the activity-related genes (FosB and cFos) in the BLA and mPFC 10

days after the acute stress session. Knowing the importance of the locus coeruleus (LC)-BLA projections for fear extinction and that these projections are mostly norepinephrergic, we also investigated the effect of pre-conditioning intra-BLA norepinephrine signaling in the stress-induced fear extinction deficit. **Methods:** Injections of the FluoroGold (FG) retrograde tracer into the BLA or IL and intra-BLA administration of the non-selective-adrenergic antagonist propranolol were performed male Wistar rats. After the recovery period, rats underwent the 2h acute restraint stress session. Ten days later, animals were submitted to Pavlovian's contextual fear conditioning followed by a 6-day (10 min per session) extinction paradigm. A cohort of animals received an intra-BLA infusion of propranolol or saline 20 min before the conditioning training. Animals were transcardially perfused. Expression of FosB, cFos and FG was assessed by immunofluorescence. Freezing behavior was assessed via offline videos. All the experiments were conducted under the standards of the Ethics Committee for Animal Use of the Institute of Biomedical Sciences/University of São Paulo (CEUA-ICB 85/2016). **Results:** Acute stress promoted a delayed fear extinction deficit (CON=76.15; STR=145; n=9 (CON), 10 (STR) P=0.0450), and decreased the FosB expression in IL (CON=83.33; STR=55.18; n=5 (CON), 4 (STR) P = 0.0484) after fear extinction. We verified an increase in FosB expression in BLA neurons (CON=44.34; STR=80,48; n=5; P=0.0151), especially in BLA?IL neurons (CON=7.77; STR=16.08; n=5; P=0.0208). We observed a proportionally larger expression of FosB in IL?BLA projections in stressed animals (63.57% x 49.79%; n=2). In the PL?BLA projections, fewer FosB cells were observed in comparison with IL, both in controls and stressed animals (11.58% x 11.92%, respectively; n=2). Finally, we demonstrated that pre-conditioning BLA infusion of propranolol reverted the acute restraint stress effect in fear extinction deficit (F1, 33=38.22, P < 0.0001; treatment F1, 33=26.72, P<0.0001; stress-treatment interaction F1, 33=23.44, P< 0.0001; SAL=89.86%; SAL-STR=152.2%; PROP=88.01%; PROP-STR=95.99%; n=8 (SAL), 10 (SAL-STR), 8 (PROP), 11 (PROP-STR)). **Conclusion:** We demonstrated a possible mechanism for the acute stress effect over the fear extinction memory, involving IL activity during fear extinction and BLA noradrenergic signaling during conditioning. **Acknowledgments:** Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP 2020/06914-8, 2019/00908-9, and 2018/19599-3) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq 120633/2019-1 and 422523/2016-0). This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001. **License number of ethics committee:** All the experiments were conducted under the standards of the Ethics Committee for Animal Use of the Institute of Biomedical Sciences/University of São Paulo (CEUA-ICB 85/2016).

03.002 Toll-like receptor 4 (TLR4) knockout mice do not develop depressive-like behavior after repeated restraint stress but have worse anxiety behavior. Cunha LC¹, Lisboa SFS². ¹Ribeirão Preto Medical School; ²School of Pharmaceutical Sciences of Ribeirão Preto, Univ of São Paulo, USP

Introduction: Neuroimmunological factors are implicated in neuropsychiatric disorders and could be involved in the severity of symptoms and treatment response. Prolonged stress exposure can promote neuroinflammation and impair neural plasticity, especially in limbic areas, by affecting the release of neurotransmitters and immune mediators¹. Microglia is one of the cellular components that can regulate these responses. In rodents, repeated homotypical stress (RHS) activates microglia and increases expression of the pattern recognition receptor toll-like receptor type 4 (TLR4) in the brain¹. TLR4 is mostly expressed in microglia and its activation induces translocation of NF- κ B to the nucleus and the expression of pro-inflammatory cytokines and reactive oxygen species and RNS². Moreover, RHS also induces depressive- and anxiety-like behavior^{3,4}. TLR4 is implicated in the behavioral response to stress in animal models, but the data are still inconclusive. Therefore, we intend to investigate further the behavioral response to RHS and how the TLR4 pathway modulates that response. **Methods:** 8 weeks old male C57Bl/6 (WT) and TLR4 knockout mice were submitted to restraint stress (RS) for 10 days, 2 hours per day (9: 00-11: 00 a.m.). One day after the end of stress, animals were submitted to the Open Field Test followed by the Elevated Plus Maze (EPM). The Novelty Suppressed Feeding test (NSF) was performed the following day, after 24h-food deprivation. On the last day, mice were subjected to the Splash Test followed by the Forced Swim Test (FST). After FST all mice were euthanized, and brain tissues (hippocampus and prefrontal cortex) were collected for later analysis. **Results:** 10-day RS caused anxiety- and depressive-like behaviors. TLR4KO mice did not develop depressive-like behavior in the FST compared to WT mice (F_{2,11}=6.1 p=0.02, Tukey p=0.03), but they presented an anxiogenic effect in the NSF (F_{2,11}=9.2, p=0.005) compared to WT Naive (p=0.02, Tukey) and WT stressed (p=0.006, Tukey), and in the EPM (increase in enclosed arms entries; F_{2,12}=20.5, p=0.0001; %open arm entries F_{2,12}=2.9, p=0,09 tendency) compared to WT naive (p<0.0001, Tukey) and WT stressed (p<0.05, Tukey). **Conclusion:** Repeated RS-induced behavioral alterations are in part mediated by TLR4 since TLR4KO mice did not develop stress-induced depressive-like behavior. However, the worsening of anxiety-like behavior observed in TLR4KO mice after stress indicates that this receptor has a complex role in behavior. Further studies are needed to address

if pharmacological modulation of TLR4 promotes similar responses and if TLR4 located in microglia mediates the observed responses. This study was supported by Programa institucional de bolsas de iniciação científica (PIBIC) and National Council for Scientific and Technological Development (CNPq, Projeto Universal 420818/2018-9). 1 Walker, F. R., et al. *Curr. Drug Targets*, 14, 1262 (2013). 2 García Bueno, B., et al. *Neurosci. Biobehav. Rev.*, 64, 134 (2016). 3 Nie, X., et al. *Neuron*, 99, 464 (2018) 4 Femenia, T., et al. *Brain, behav, and immun.*, 69, 273 (2018). **License number of ethics committee:** All the procedures were approved by the local ethical committee (School of Pharmaceutical Sciences of Ribeirão Preto- FCFRP/USP; CEUA number 19.1.731 .60.9)

03.003 Investigation of the involvement of cannabinoid receptors in locomotor responses induced by methylphenidate and cocaine in male and female mice. Neves LS¹, Gobira PH¹, Joca, SRL^{1 2}, Lisboa, SF¹. ¹FCFRP-USP, Dpt of Biomolecular Sciences, Brazil ²Aarhus Univ, Dpt of Biomedicine, Aarhus, Denmark

Introduction: Methylphenidate, a psychostimulant widely used in treating attention deficit hyperactivity disorder, modulates mesocorticolimbic neurocircuitry through blockade of dopamine and noradrenaline reuptake transporters, increasing the levels of these neurotransmitters in the synaptic cleft (Demiral et. al, 2019). Studies have demonstrated the involvement of the endocannabinoid system (ECS) in the effects of other psychostimulants, such as cocaine and amphetamine. ECS is composed by CB1 and CB2 receptors, their endogenous ligands and enzymes involved in the synthesis and degradation of these ligands and plays an essential role in several biological processes (Di Marzo, 2009; Moreira et. al, 2008). Although cannabinoid receptors modulate behavioral and molecular responses induced by cocaine and amphetamine, their involvement in methylphenidate effects remain poorly understood (Maldonado et. al, 2006; Gobira et. al, 2019). Therefore, we aimed at evaluating the role of cannabinoid receptors in behavioral and molecular responses induced by methylphenidate. **Methods:** Male and female C57BL/6J mice, 7- 9 weeks old, received intraperitoneal injections of vehicle or the CB1 antagonist, AM 251 (3 and 10 mg/Kg/10 ml), and were immediately exposed for the first time to the open field test, for 20 minutes. After the first exposure, the animals were returned to their home cages and 15 minutes before a second exposure to the OFT, mice received methylphenidate (10 mg/Kg/10 ml), cocaine (20 mg/Kg/10 ml), or the respective vehicle. Then, all mice were exposed again to the OFT for 20 minutes. The distance travelled was analyzed using AnyMaze software. **Results:** We observed that AM 251 did not interfere with methylphenidate-induced hyperlocomotion in both male and female mice. However, AM 251 (10 mg/kg/10 mL) inhibited cocaine-induced hyperlocomotion in male mice. **Conclusions:** Our results suggest that CB1 receptors mediate cocaine-induced hyperlocomotion, but appear not to be involved in methylphenidate induced hyperlocomotion. Further investigation is necessary to evaluate the involvement of other cannabinoid receptors, such as CB2, GPR55 and TRPV1, in the psychostimulants' effects, and to conclude if the ECS participate in the behavioral responses induced by methylphenidate. **Financial support:** This work was financed by FAPESP (process number 2017/24304-0). The Institution's Animal Ethics Committee approved housing conditions and experimental procedures (process number: 19.1.1156.60.8/ 2019). **References:** Demiral, S. B., D. Tomasi, C. E. Wiers, P. Manza, E. Shokri-Kojori, Y. Studentsova, G. J. Wang, and N. D. Volkow. 2019, *Neuropsychopharmacology*, 44: 1389-97. Di Marzo, V. 2009. *Pharmacol Res*, 60: 77-84. Gobira, P. H., A. C. Oliveira, J. S. Gomes, V. T. da Silveira, L. Asth, J. R. Bastos, E. M. Batista, A. C. Issy, B. N. Okine, A. C. de Oliveira, F. M. Ribeiro, E. A. Del Bel, D. C. Aguiar, D. P. Finn, and F. A. Moreira. 2019. *Br J Pharmacol*, 176: 1541-51. Maldonado, R., O. Valverde, F. Berrendero. 2006. *Trends Neurosci*, 29: 225-32. Moreira, F. A., and B. Lutz. 2008. *Addict Biol*, 13: 196-212. **License number of ethics committee:** The Institution's Animal Ethics Committee approved housing conditions and experimental procedures (process number: 19.1.1156.60.8/ 2019)

03.004 Brain development during adolescence and the impact on psychiatric disorders and drug abuse: a scoping review. Lamarca LD¹, Poian LR², Chiavegatto S^{2,3} ¹Sch. Pharm. Sci. – Univ. of São Paulo, São Paulo, Brazil ²Pharmacol Biomed. Sci. Inst. – Univ. of São Paulo, São Paulo, Brazil ³Psychiatry – Sch. of Med – Univ. of São Paulo, São Paulo, Brazil

Adolescence is a period of transformations in which new experiences are fundamental for the transition between childhood and adulthood. During this period, there is a drastic change in behavior, characterized by lack of impulse control and increased emotional response. Although this behavioral change can be essential for human development, it is also related to problems such as alcohol and drug abuse, as well as psychiatric disorders. Our objective is: a. to review scientific articles on human brain development that permeate the reckless behavior of adolescents; b. to understand the motivations underlying those behaviors; c. to disseminate the information found, to assist in preventive actions to the problems faced by adolescents. **Methods:** A scoping review was carried out through the synthesis of articles published on the theme "Adolescent brain development", in the Medline database (PubMed) for the last 15 years. The

filters "reviews" and "study in humans" were selected. Finally, a characterization was made in relation to the year of publication, country of origin and impact factor of the published journal. **Results:** The analysis and synthesis of the 46 selected articles (out of 1,913) demonstrated that the period of adolescence is marked by a process of expressive changes in brain maturation. These occur asynchronously on different regions: primarily in areas of lower complexity (e.g. limbic subcortical systems) and later in more complex areas (e.g. cortical systems). Thus, in the adolescent brain, the limbic reward systems are already fully developed, while the cortical regulatory systems, still immature, are insufficient to perform adequate control, impairing the ability of adolescents to exert control over emotions, modify impulses and postpone gratification. Consequently, adolescence is characterized by an increase in risk behaviors and search for immediate rewards (e.g., drug abuse). Also, during this period, there is an increase in the incidence of psychiatric disorders, since the subcortical regions, which are less regulated during the maturation process (e.g., nucleus accumbens, amygdala), are the same described as altered in the pathology of the most common mental disorders (e.g., anxiety and depression). **Conclusion:** During adolescence, the active process of cerebral maturation characterizes a late plasticity, but the disparity between reward and control systems makes them more likely to suffer from various risks. Therefore, understanding the motivations behind the actions of this group is fundamental to ensure that environmental influences reach adolescents in a positive way, when developing strategies in public policies. **Acknowledgments:** FAPESP #2017/06100-8 **License number of ethics committee:** N/A

03.005 **What is the caffeine intake profile of the students from PUC Minas?** Almeida, LG^{1,2}; Cardoso, MCBS¹; Fonseca, MLA¹; Ribeiro, AF³ ¹Undergraduate in Biology, PUC-MG; ²Scholarship from the Tutorial Education Program, PET-Biology PUC-MG; ³PUC-MG

Introduction: Caffeine is considered the most commonly used psychoactive drug in the world and is present in a number of dietary sources and drugs, including coffee, tea, candy bars, soft drinks and over-the-counter cold medicine. Caffeine is a natural alkaloid (1,3,7-trimethylxanthine, chemical formula: C₈H₁₀N₄O₂) and it acts on the central nervous system as a stimulant. The excessive use of caffeine can trigger symptoms related to withdrawal or intoxication states. Therefore, this research was carried out to better understand the caffeine intake profile of the students from Pontifical Catholic University of Minas Gerais (PUC Minas). **Methods:** A descriptive, observational, and transverse study was made using an online form to collect sociodemographic information and daily pattern of caffeine use (High: ≥ 250 mg; Moderate: $100\text{mg} \leq x < 250\text{mg}$; Low: $< 100\text{mg}$) of undergraduate students of the Institute of Biological Sciences and Health (ICBS) from PUC Minas, campus Coração Eucarístico. The data collection was carried out between September and November 2019. All procedures were approved by the Ethics Committee in Human research (Protocol Number 16884719.9.0000.5137), PUC Minas. **Results:** Most part of the students were between 18 and 26 years old (ntotal= 174). They were upper-middle social class (A-B; n= 111, 63.8%), lived with their parents (n= 131, 77%) and were divided into four-people groups (n= 89, 51.1%). Caffeine intake was higher at night (almost 106 mg) than at any other time. Students exhibited more prevalent moderate to high caffeine consumption ($\chi^2= 44.18$, $p= 0.000$; n= 118, 67.8%). In more detail, the men increased their consumption during physical activities ($\chi^2= 4.50$, $p= 0.03$) while women in academic ($\chi^2= 86.92$, $p= 0.000$) or recreational activities ($\chi^2= 26.74$, $p= 0.000$). Almost half of them had a possible abstinence symptom or dental problem due to caffeine intake (n= 74, 42.5%; and n= 53, 30.5%, respectively). Also, intoxication symptoms were more frequent in the group with moderate (≥ 3 symptoms; n= 15, 30%) or high caffeine consumption (≥ 3 symptoms; n= 16, 23.5%). Similarly, abstinence symptoms (≥ 3) were higher in the group with moderate (n= 11, 22%; $\chi^2= 10.96$, $p= 0.001$) or high caffeine consumption (n= 20, 29%; $\chi^2= 16.97$, $p= 0.000$). **Conclusion:** Caffeine abuse was observed in the students. **Financial Support:** The fellowships were granted from PUC Minas – FIP-2019-22509-1S, and Fundo Nacional de Educação – FNDE. **Acknowledgements:** We thank the PUC Minas and FNDE for the fellowships awarded. We also thank the course coordinators and employees of the PUC Minas for their assistance. **License number of ethics committee:** All procedures were approved by the Ethics Committee in Human research (Protocol Number 16884719.9.0000.5137), Pontifical Catholic University of Minas Gerais, PUC Minas.

03.006 **Antagonism of TRPV1 receptors did not facilitate the impaired fear extinction in iNOS KO mice.** Ferreira BF¹, Silveira JRCA¹, Guimarães FS¹, Resstel LBM¹, Lisboa SF². ¹USP, Dpt of Pharmacology, Medical School of Ribeirão Preto, Brazil; ²USP, Dpt of BioMolecular Sciences, School of Pharmaceutical Sciences of Ribeirão Preto, Brazil

Introduction: We previously showed that iNOS knockout mice (iNOS KO) present increased contextual fear conditioning (CFC) expression and impaired fear extinction, suggesting these mice could be a suitable genetic model to investigate disorders with impaired extinction, such as PTSD. The behavioral changes

are associated with increased basal NOS activity in the medial prefrontal cortex (MPFC), but not hippocampus (HPC), and endocannabinoid system (eCB) dysregulation in both brain regions. An nNOS inhibitor or an inhibitor of the fatty acid amide hydrolase (FAAH) enzyme, which degrades the eCB anandamide-AEA, attenuates the behavioral changes observed in iNOS KO mice. AEA effects can be mediated by CB1 or TRPV1 receptors located in different brain regions, modulating the CFC expression through opposite mechanisms involving the NMDA/NO pathway. Therefore, the aim of this study was to investigate whether the behavioral changes observed in these animals would be attenuated by the antagonism of TRPV1 receptors. **Methods:** Male C57BL/6J and iNOS KO mice (8–12 weeks old) received three electrical footshocks (0.75mA, 2s each) in the conditioning box (UGO BASILE ANY-maze Fear Conditioning System). After 24h, the animals received injections of the TRPV1 antagonist, SB366791 (SB; 0.1, 0.3, 1.0 and 3.0 mg/kg; i.p.) or vehicle (10ml/kg) 30min before the re-exposure to the conditioning chamber (extinction acquisition session; 20min). After an additional 24h, the ability to evoke the extinction memory was evaluated in a 5-min session. Freezing behavior was automatically detected and quantified by the Anymaze® software (Stoelting). Data were analysed by repeated measures ANOVA, or one-way ANOVA. Experimental procedures were approved by the local Animal Research Ethical Committee (protocol 20.1.554.60.1). **Results:** Both WT and iNOS KO animals showed a similar increase in freezing behavior during the conditioning session, indicating there was no interference with memory acquisition. However, the iNOS KO mice presented impaired extinction of fear conditioning memory when compared to the WT mice. Treatment with SB neither changed behavior in WT mice nor attenuated the behavior of iNOS KO animals. **Conclusion:** In the present study we replicated our previous work showing that iNOS KO mice present impaired fear extinction in the CFC model. The absence of effect with SB suggests that the sole blockage of TRPV1 receptors is not sufficient to facilitate fear extinction, which could require additional CB1 receptors activation. Studies are under evaluation to clarify the involvement of these receptors in extinction deficits observed in iNOS KO mice. Financial support: FAPESP (2017/19731-6); Loreal, For Women in Science; CNPq/PIBIC fellowship. **License number of ethics committee:** Protocol 20.1.554.60.1

03.007 **S-Ketamine effects on female mice tested in the Marble Burying Test.** Ayub JGM, Tosta CL, Beijamini V. UFES Vitória, PPG Pharmaceutical Sciences, Health Sciences Centre, Brazil

Introduction: Obsessive-compulsive disorder (OCD) pharmacological treatments are not totally effective. Therefore, pre-clinical studies investigate potential new drugs to treat OCD. Our laboratory showed that a single dose of S-ketamine (S-KET) 10mg/kg, an NMDA antagonist, induces anticomulsive-like effects and anticipates the anticomulsive-like effect of fluoxetine (FLU) in male mice [1,2]. This study aimed to investigate the effects of S-KET administration, as monotherapy or as an augmentation to FLU effect, in female mice tested in the marble burying test (MBT). Also, investigate the influence of estrous cycle in S-KET anticomulsive-like effect. **Methods:** Adult female Swiss mice (25-30g) were used. Experiment 1 evaluated the effect of S-KET (3, 10, or 30 mg/kg) in the MBT and open field test (OFT) 45 min after administration. Experiment 2 investigated the response to FLU administered repeatedly (2.5 or 5 mg/kg, once a day, i.p.) in the MBT at days 1, 7, 14, and 21 of treatment. In experiment 3, we analyzed the association of sub-effective doses of FLU (2.5 mg/kg), administered once a day, and S-KET (10 mg/kg), administered once a week (4 injections). The animals were tested 45 min after last drug injection in MBT on days 1, 7, 14 e 21 of treatment. Lastly (experiment 4), we analyzed the estrous cycle and tested the animals in the MBT in one of the 3 phases (Proestrus - P, estrous - E, metestrus/diestrus - M/D) after acute administration of FLU (10 mg/kg) or S-KET (3 or 10 mg/kg). Our protocol was accepted by the Committee for the Ethical Use of Animals in Scientific Research (CEUA-UFES; nº 16/2018). **Results:** A single dose of S-KET 30 mg/kg, but not 3 or 10 mg/kg, decreased the number of buried marbles in the MBT ($F_{(3,43)} = 11.466$; $p < 0.001$; Tukey $p < 0.001$) without changing locomotor activity ($F_{(3,43)} = 2.16$; $p = 0.106$). FLU 5 mg/kg, but not FLU 2.5 mg/kg, reduced the number of marbles buried in the MBT in all sessions (1, 7, 14 and 21 days) compared with the control group (two-way ANOVA; $F_{(2,36)} = 4.938$; $p = 0.013$; Tukey $p < 0.05$ at each session) with no reduction of locomotor activity ($F_{(3,108)} = 0.937$; $p = 0.472$). The association of S-KET 10 mg/kg and FLU 2.5 mg/kg decreased the number of buried marbles since day 1 (two-way ANOVA; $F_{(1,48)} = 7.650$, $p = 0.008$) when compared with all the other groups (Tukey $p < 0.05$) in all sessions. S-KET 10 mg/kg once a week augmented the total distance traveled when compared with the control group in days 14 and 21 ($F_{(2,846, 144)} = 5.981$, $p = 0.01$, Tukey $p < 0.05$). We have preliminary results showing a reduction of marbles buried by S-KET 10 mg/kg administration in M/D estrous phase. **Conclusion:** Our results suggest that a single dose of S-KET induces anticomulsive-like effects and augments the effect of FLU in female mice. In addition, the estrous cycle seems to influence the S-KET anticomulsive response. More studies are necessary to understand the influence of sex hormones on the anticomulsive effect of S-KET. **Financial Support:** Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Universidade Federal do Espírito Santo (UFES). **References:** [1] TOSTA, C. L.,

Neuroph., 144, 233, 2019. [2] FRACALOSSO, M. P. DOS S., UFES, 2017. **License number of ethics committee:** Committee for the Ethical Use of Animals in Scientific Research (CEUA-UFES; nº 16/2018).

03.008 Behavioral and biochemical effects of acute triazophos intoxication in young and adult rats Vidigal, APP, Dos Santos, JG, Rodrigues, JVF, Minassa, VS, Batista, TJ, Sampaio, KN, Beijamini, V ¹UFES Vitória, PPG Pharmaceutical Sciences, Health Sciences Centre, Brazil

Introduction: Organophosphate compounds (OP) are acetylcholinesterase (AChE) inhibitors widely used in agriculture for pest control, especially in developing countries. Exposure to OP may increase the risk of developing depression, anxiety, and cognitive disorders. Recently, we showed that triazophos (TZP), an OP, impairs aversive memory extinction [1]. The behavioral consequences of acute TZP intoxication in tests related to depression and anxiety have not yet been investigated. OP tends to affect young animals to a greater extent than adults [2]. Therefore, we aimed to investigate whether acute exposure to TZP would induce differential anxiety and depressive-related behavioral changes in young and adult rats.

Methods: Male young (28 days) and adult (8 weeks) Wistar rats were acutely intoxicated (i.p.) with sub-lethal doses of a commercial formulation of TZP (3.75, 7.5, 15, and 30 mg/kg) or treated with saline (control group). The highest dose was 15 mg/kg for young rats, and 30 mg/kg for adult rats based on the previously defined maximum tolerated dose of this OP [2]. The animals were tested 24 hours after the intoxication in the open field (OF) and in the elevated plus-maze (EPM) to evaluate the locomotor activity and anxious behaviors. We also assessed cerebral AChE activity in the hippocampus and cortex of these animals. An independent cohort of animals was submitted to the forced swimming test (FST) 24 h and 8 days after intoxication to assess persistent behavioral effects. Our protocol was accepted by the Committee for the Ethical Use of Animals in Scientific Research (CEUA-UFES; nº 29/2017). **Results:** In adult rats, TZP 7.5 mg/kg increased the percentage of entries, while TZP 15 mg/kg decreased the percentage of time spent in the open arms of EPM, suggesting an anxiolytic and anxiogenic-like effect, respectively. In young animals, TZP 3.75 mg/kg decreased entries into the open arms. No motor impairment was observed for young and adult rats in the OF. TZP decreased the percentage of entries in the center at the highest dose in young and adult rats. At the highest doses, TFP inhibited the hippocampal and cortical AChE in young and adult rats. All doses of TZP increased the frequency of immobility and reduced the frequency of swimming in the FST 24 h after intoxication, suggesting a depressive-like effect in both young and adult animals. In young rats, TZP 15 mg/kg also decrease climbing frequency. The depressive-like effect of TZP persisted for 8 days in young animals treated with TZP 7.5 and 15 mg/kg and in adult animals treated with TZP 7.5 and 30 mg/kg. **Conclusion:** To date, our results demonstrate that young and adult animals show different patterns of behavioral changes even with a single exposure, suggesting that acute intoxication is sufficient to cause short and long damage to the mental health of exposed individuals. **Financial Support:** Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Fundação de Amparo à Pesquisa e Inovação do Espírito Santo (FAPES) and Universidade Federal do Espírito Santo (UFES). **References:** [1] Rodrigues JVF, Neurotoxicol Teratol. 82, 106929, 2020. [2] Singh M, Environ Toxicol Pharmacol. 19, 471, 2005 **License number of ethics committee:** Committee for the Ethical Use of Animals in Scientific Research (CEUA-UFES; nº 29/2017)

03.009 Behavioral effects induced by the cannabidiol analogs HU-556 and HU-502. Colodete, DAE¹; Silva, NR¹; Fogaça, MF²; Pedrazzi, JF²; Del Bel, EA³; Mechoulam, R⁴; Gomes, FV¹; Guimarães, FS¹ ¹FMRP-USP, Dpt of Pharmacology, Brazil ²FMRP USP, Dpt of Neurosciences and Behavioral Sciences, Brazil ³FORP-USP, Dpt of Physiology, Brazil ⁴Hebrew Univ, Jerusalem, Israel

Cannabidiol (CBD) is a phytocannabinoid that lacks the psychotomimetic properties of Δ^9 -tetrahydrocannabinol (THC), the main active component of Cannabis sativa. CBD has several potential therapeutic properties, including anxiolytic, antidepressant, and antipsychotic. However, CBD has low oral bioavailability, which can limit its clinical use. In the present study, we evaluated the effects induced by the CBD derivatives HU-556 and HU-502, with the perspective of finding that these compounds have similar but more potent CBD properties. Different doses of HU-556 (0.01-1 mg/kg i.p.) and HU-502 (1-3 mg/kg i.p.) were tested in male Swiss mice submitted to the elevated plus-maze (EPM), forced swimming test (FST), and amphetamine-induced prepulse inhibition (PPI) disruption and hyperlocomotion (CEUA #099/2019). Previous findings from our group indicate that CBD was effective in all these tests at a dose range of 15-60 mg/kg in mice. We also investigated if higher doses of HU-556 (3 and 10 mg/kg) and HU-502 (10 mg/kg) produced the cannabinoid tetrad (hypolocomotion, catalepsy, hypothermia, and analgesia), which are behavioral changes indicative of CB1 agonist activity. HU-556 (0.1 and 1 mg/kg) produced anxiolytic- and antidepressant-like effects in the EPM and FST, and attenuated amphetamine-induced PPI disruption. HU-502 (1 and 3 mg/kg) attenuated amphetamine-induced PPI impairment and hyperlocomotion. At higher doses, HU-556, but not HU-502, caused the cannabinoid tetrad. Taken together,

these findings suggest that, similar to CBD, HU-556 induces anxiolytic, antidepressant, and antipsychotic-like effects. HU-502, on the other hand, has antipsychotic properties. These effects were found at a dose range devoid of cannabinoid tetrad effects. **Keywords:** cannabidiol; analogs; depression; anxiety; schizophrenia; anxiolytic; antidepressant; antipsychotic; CB1; tetrad. **Financial Support:** Cnpq; Capes; FAPESP. **License number of ethics committee:** CEUA #099/2019

03.010 Involvement of cannabinoid type 2 receptors in aversive memory processing in mice evaluated in the contextual fear conditioning paradigm. Werworn LFM¹, Coelho AA², Galani, LC¹, Lisboa SFS^{1,2}. ¹FCFRP-USP Ribeirão Preto, Dpt of BioMolecular Sciences, Brazil; ²FMRP-USP Ribeirão Preto, Dpt of Pharmacology, Brazil

Introduction: Post-traumatic stress disorder (PTSD) is a debilitating psychiatric disorder that can be developed after trauma. In this disorder, there is an increased response to sensory cues related to trauma and alterations on fear memory processing mechanisms, particularly deficit in the fear extinction, which could explain the persistence of fear memories and responses¹. Conditioned fear paradigms in rodents are widely used to understand mechanisms involved in processing fear conditioning. Considering these mechanisms are altered in PTSD patients, they can help understand how fear mechanisms could be dysregulated in PTSD². The cannabinoid type 1 receptors (CB1) are implicated in the processing of aversive memories. CB1 receptors modulate contextual fear conditioning (CFC) extinction, but not acquisition or consolidation of fear-conditioned memory³. Regarding CB2 receptors, fewer studies evaluate their involvement in fear response, and the existing data is controversial^{4,5}. Therefore, considering the involvement of CB2 receptors in the extinction of CFC memory has not been investigated, we aimed at investigating if pharmacological activation or inhibition of CB2 receptors modulates CFC extinction in mice.

Methods: Male C57BL/6 mice (8 weeks old) were submitted to the CFC paradigm. Briefly, in the conditioning procedure (day 1), all mice received three random foot-shocks (0,75 mA, 2s) during a 7-min session. On day 2, vehicle, JWH133, a CB2 agonist (0.5, 1.0, 2.0, 4.0 mg/kg/10 mL/kg, i.p. injection), or AM630, a CB2 antagonist (0.5, 1.0, 2.0, 4.0 mg/Kg/10 mL/kg, i.p. injection), was administered 30 min before re-exposure to the same conditioning chamber for 20 min, no foot-shocks presented, for the purpose of evaluating the fear expression and acquisition of fear extinction memory. Evocation of memory extinction was evaluated 24 h later (day 3), during the last re-exposure to the chamber without foot-shocks for 10 min. **Results:** JWH133 did not affect conditioning memory evocation on Day 2, but it did attenuate (2.0 mg/kg) freezing behavior during extinction acquisition session ($p < 0.05$), indicating facilitation of fear memory extinction acquisition. No effect was observed in extinction memory evocation on day 3 ($p > 0.05$). Additional experiments are still under investigation to determine the impact of the CB2 antagonist in the CFC. **Conclusion:** Our data demonstrating that CB2 receptors activation attenuates aversive memory by facilitating fear memory extinction in the CFC suggest that CB2 receptors could be involved in aversive memory processing. **Financial Support:** FAPESP (2017/19731-6); Loreal, For Women in Science; FAPESP fellowship (2019/19226-5). **References:** 1 MORRISON, F.G. et al. *Depress Anxiety*, 31, 279, 2014 2 MAHAN, A.L. et al. *Trends Neurosci*, 35, 24, 2013 3 MARSICANO, G. et al. *Nature*, 418, 530, 2002 4 LI, Y. KIM, J. *Neural Plast*, 2016, 2015 5 LI, Y. KIM, J. *Hippocampus*, 26, 275, 2016 **License number of ethics committee:** Animal Use Ethical Committee of Pharmaceutical Sciences School of Ribeirao Preto registration: 19.1.926.60.4.

03.011 The role of anandamide in the anxiety-like behavior in female rats. Salemm BW, Raymundi AM, Sohn JMB, Stern CAJ. UFPR Curitiba, Dpt of Pharmacology, Brazil.

Introduction: Anxiety disorders have a higher prevalence in women, nonetheless pre-clinical studies in this area continue to be performed predominantly in male subjects. The influence of the endocannabinoid (eCB) system in controlling anxiety-like behaviors is well established, as well as the sexual dimorphism in the eCB system and in stress responses. However, it is still not clear whether eCBs, such as anandamide (AEA), mediate anxiety responses similarly in both sexes. In this sense, a previous study from our group showed that female rats are more sensitive to the effects of delta-9 tetrahydrocannabinol on anxiety than males. Furthermore, it is known that inhibition of fatty acid amide hydrolase (FAAH) by URB597 (URB) increases AEA levels and promotes anxiolytic-like responses in male rats. Thus, the aim of the present study was to evaluate the role of AEA in the anxiety-like behavior in female rats. **Methods:** Naturally cycling female Wistar rats were treated with vehicle or URB (FAAH inhibitor; 0.1 or 0.3 mg/kg), 30 min before the elevated plus maze (EPM) test. Open-arms and enclosed-arms time and entries were evaluated and the results were expressed as percentage of open-arms time (%OAT), open-arms entries (%OAE) and number of enclosed-arms entries (EAE). The estrous cycle was evaluated immediately after the test. One-way ANOVA followed by Newman-keuls post-hoc was used. All procedures were approved by the local ethics committee #1272. **Results:** When analyzed independently from the estrous cycle, the treatment

with URB did not change the %OAT [$F(2,69) = 1.1102, p = 0.33338$], %OAE [$F(2,69) = 0.69497, p = 0.50255$] or EAE [$F(2,69) = 0.50967, p = 0.60294$] when compared to controls. However, specifically in the estrus phase, the treatment with URB 0.3 significantly reduced the %OAT [$F(2,21) = 5.5028, p = 0.01198$] and the %OAE [$F(2,21) = 4.2006, p = 0.02921$], suggesting an anxiogenic-like effect. No effect was observed in the EAE [$F(2,21) = 0.3789, p = 0.68915$], suggesting no locomotor impairment from this dose of URB. At the tested doses, no effect was observed in the pro-estrus or diestrus. Importantly, there was no basal difference among the cycle phases. **Conclusion:** The results showed that enhancing AEA signaling specifically during the estrus phase promotes an anxiogenic-like effect, suggesting that the role of AEA in anxiety in females varies along the estrous cycle. The present results opposes the observed in males, where the same doses used here cause anxiolytic-like effects, suggesting that AEA's role in the anxiety-like behavior may differ depending on the sex. **Financial Support:** CAPES and CNPQ. **License number of ethics committee:** CEUA #1272

03.012 **Studies using the forced swim test: an interim meta-analysis.** Martins, T^{1,2}, Lino de Oliveira, C^{1,2}. ¹UFSC Florianópolis, PPG in Pharmacology, Brazil; ²UFSC Florianópolis, Dpt of Physiological Sciences, Brazil
Introduction: The forced swim test in laboratory rodents (FST) is predictive of the effects of antidepressants. The number of publications using FST has been growing since its standardization by Porsolt in 1977, thus, raising the questions about this massive literature: What experimental features do those studies have? What is their global result? Moreover, what is their quality? **Methods:** To answer these questions systematically and transparently, a systematic review and meta-analysis were conducted according to a pre-established protocol (PROSPERO, ID=CRD42020200604). Briefly, a search of term variations for rodents, forced swimming test and antidepressants was performed in the EMBASE, Pubmed, Web of Science and SCOPUS databases from the publication date 1977 to 2017. The 14719 references were exported to EndNote X9 for duplicate deletion ($n = 9582$) and selection using inclusion/exclusion criteria. Two reviewers performed the selection, and a third reconcile disagreements. Those that did not meet the selection criteria (see protocol) were excluded ($n = 2673$). A total of 2853 primary studies of any language that had the outcome of the duration of immobility of animals treated with antidepressants (not co-treatment) were included. Here, an interim study was carried out to obtain an overview of this immense library. Using the function "randbetween" in the applicative Excel, 32 publications with 74 studies using 1081 animals were randomly selected. Data extraction to evaluate external validity (experimental qualities), internal validity (risk of bias, RoB-Syrcler tool) and random-effects model meta-analysis was performed by a single reviewer. **Results:** Most of the studies used rat (58.67 %), Wistar strain (34.67 %, n strains = 7), and males (53.33 %). The predominant class of antidepressants ($n = 6$) was selective serotonin reuptake inhibitors (46.67 %), and the most common drug ($n = 15$) fluoxetine (28 %) followed by imipramine (18.67%). The most applied protocol was the 15 minutes pre-test with a 5 minute test 24h later (38.67 %, n protocols = 10). The combined effect size was large and significant with high heterogeneity (Hedges $g = 2.63, IC 95\% = 2.04-3.22, I^2 = 92.06\%, p < 0.0001, k = 74, power >99\%$) in favor to the antidepressant treatment compared to control, decreasing immobility in the forced swim test. Egger's regression test revealed a relationship between sample sizes and effect size ($z = 15.95, p < 0.0001$), suggesting asymmetry on funnel plot by small-studies effect. Trim-and-fill estimated one missing study in favor to control (adjusted Hedges' $g = 2.60, IC 95\% = 2.01-3.20, I^2 92.08\%, p < 0.0001, k = 75$). The likelihood ratio test from the weight-function model was significant ($X^2 = 12.94, p = 0.0003$), implying a possible publication bias. The risk of biases (selection, performance, detection, attrition, report) was unclear for most studies (upper 60%), low for 35%, and high for 3%. **Conclusion:** These results indicate a low external and internal validity in the studies using FST. Further studies are required to understand the heterogeneity within the literature using FST and summarize the knowledge to generate new research paths based on this literature. **Financial support:** CAPES. **License number of ethics committee:** N/A

03.013 **Functional inactivation of basolateral amygdala does not alter context-induced reinstatement of alcohol-seeking in rats.** Tavares GEB, Bianchi PC, Yokoyama TS, Palombo P, Cruz FC. Unifesp, São Paulo, Pharmacology Dpt, Brazil

Introduction: Relapse is a major problem in treating alcohol addiction. Environmental cues in which drugs are used can induce drug-seeking (SEO; SINHA, 2014; SINHA; LI, 2007; LUDWIG; STARK, 1974). We used a preclinical model of relapse in which a conditioned responding can be renewed by re-exposure to the conditioning context (A) following extinction in a different context (B), called ABA renewal (BOSSERT et al., 2013) The basolateral amygdala seems to encode learning association memories (KIM et al., 2018; SOTRES-BAYON; QUIRK, 2010). Here, we assessed the participation of the basolateral amygdala (BLA) in context-induced reinstatement of alcohol-seeking in rats. **Methods:** Male Long-Evans rats were trained to self-administer 10% alcohol in Context A, subsequently rats *underwent stereotaxic surgery* for bilateral

cannulas *implantation* in the BLA. After a recovery period, lever pressing was extinguished in a non-drug-associated context (Context B) in the presence of discrete cues. On the test day, we injected muscimol and baclofen (GABA_A and GABA_B receptor agonists) or vehicle into the basolateral amygdala 15 min before the rats being re-exposed to alcohol or extinction-associated context under extinction conditions. Next, three more extinction sessions were performed, and the rats were test again in contra balanced way. To rule-out the motor deficits caused by the effect of injecting muscimol + baclofen into the BLA, we re-trained the same rats to lever press for 0.2% saccharin solution for three sessions. Then, we determined the effect of muscimol + baclofen or vehicle injections into the BLA on a high-rate operant response for saccharin. All statistical analyses were performed by using Prisma 8.0.2. The data were analyzed by analysis of variance (ANOVA); Bonferroni test was used for *post hoc* analyses when the ANOVA indicated significant main or interaction effects ($p < 0.05$). **Results:** We did Reversible inactivation of the BLA with muscimol and baclofen did not impair the context-induced reinstatement of alcohol-seeking (active lever presses: 5.8 ± 3.5 , context B-BM; 7.3 ± 4.0 , context B-VEH; 11.2 ± 9.0 , context A-VEH and 14.6 ± 11.2 , context A-BM; $n=9-12$ per group; $p > 0.05$). Inactivation of the BLA with muscimol + baclofen did not affect the high-rate saccharin response (active lever presses: 119.3 ± 47.9 VEH and 108.8 ± 78.5 BM), indicating that GABA receptor agonists did not cause motor deficits. **Conclusion:** Our results suggest that BLA did not mediate context-induced reinstatement of alcohol-seeking. **Financial Support:** FAPESP (n^o 2018/15505-4; 2019/17799-8). Ethical committee: CEUA N^o 8253041219 **References:** BOSSERT, J. M. et al. The reinstatement model of drug relapse: recent neurobiological findings, emerging research topics, and translational research. *Psychopharmacology*, v. 229, n. 3, p. 453-476, 2013. KIM, E.J. et al. Dynamic coding of predatory information between the prelimbic cortex and lateral amygdala in foraging rats. *Science advances*, v. 4, n. 4, p.eaar7328, 2018. LUDWIG, A. M.; STARK, L. H. Alcohol craving. Subjective and situational aspects. *Quarterly journal of studies on alcohol*, v. 35, n. 3, p. 899-905, 1974. SEO, D; SINHA, R. The neurobiology of alcohol craving and relapse. In: **Handbook of clinical neurology**. Elsevier, p. 355-368, 2014. SINHA, R; LI, C. S. R. Imaging stress-and cue-induced drug and alcohol craving: association with relapse and clinical implications. *Drug and alcohol review*, v. 26, n. 1, p. 25-31, 2007. SOTRES-BAYON, F; QUIRK, G. J. Prefrontal control of fear: more than just extinction. *Current opinion in neurobiology*, v. 20, n. 2, p. 231-235, 2010. **License number of ethics committee:** CEUA N^o 8253041219

03.014 TRPV1 antagonism associated with FAAH inhibition attenuated the impaired fear extinction recall induced by social isolation in mice. Coelho AA^{1,2}, Werworn LFM¹, Lisboa SFS¹. ¹FCFRP-USP Ribeirão Preto, Dpt of BioMolecular Sciences, Brazil; ²FMRP-USP, Dpt of Pharmacology, Brazil

Introduction: Stress exposure and cannabinoid CB₁ receptor (CB1) blockade cause deficits in the conditioned fear extinction. In contrast, increasing the endocannabinoid anandamide levels with FAAH inhibitors and CB1 activation facilitates conditioned fear extinction. Anandamide also activates TRPV1 receptors, resulting in opposite effects to CB1 in defensive responses, indicating a balance between CB1/TRPV1 receptors in those responses. Social isolation might contribute to the development of psychopathologies, such as PTSD. Thus, in mice, social isolation stress could resemble PTSD features, such as alterations in conditioned fear processing. Therefore, the administration of a dual blocker of the FAAH enzyme and TRPV1 receptors, arachidonoyl serotonin (AA-5HT), before the extinction session could facilitate stress-induced impairment in contextual fear conditioning by activation of CB1 signaling. **Methods:** Male C57/Bl6 mice were stressed by social isolation during 7 days or kept in groups (4 or 5/cage) and then were submitted to an inescapable footshock conditioning session (3 electrical footshock, 0.75 mA, 2s/each; Ugo Basile FC System, Anymaze). 24h later, mice were placed in the same box to evaluate freezing behavior during fear extinction acquisition (20 min - Day 2). After an additional 24h, the extinction recall was evaluated for 10 minutes in the same box (Day 3). Extinction recall was tested comparing the last 5 minutes of Day 2 with the first 5 minutes of Day 3. 30 minutes before re-exposure to the box in Day 2, independent groups of socially isolated mice received AA-5HT (0.1, 0.3, 1.0, or 3.0 mg/kg; intraperitoneally) or vehicle. Data were analyzed by repeated measures ANOVA, one-way ANOVA or Student's paired t test. All procedures were approved by the Local Animal Ethical Committee approval (process 19.1.671.60.6.). **Results:** There was no effect of stress on conditioned fear memory acquisition or its recall on Day 2 ($p > 0.05$). Stress exposure also did not interfere with fear extinction acquisition ($p > 0.05$). However, socially isolated, vehicle-treated mice showed impaired fear extinction recall ($p < 0.05$), whereas grouped, vehicle-treated animals showed normal fear extinction recall ($p > 0.05$). AA-5HT administration attenuated stress effect in fear extinction recall in all tested doses, since all treated groups showed fear extinction recall on Day 3. **Conclusion:** The simultaneous blocking of FAAH and TRPV1 receptors could be helpful to attenuate impaired fear extinction induced by social isolation stress. Therefore, these mechanisms can help us to understand psychiatric conditions in which impaired fear extinction is observed, such as PTSD, providing potential pharmacological targets for treatment. **Financial**

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03.015 Mitochondrial inhibition during neurodevelopment leads to schizophrenia-like-phenotype, an outcome related to a decreased mitochondrial biogenesis. Santos AS¹, Garcia TV², Simões JGT², Henrique E², Ramos AC³, Rosenstock TR^{1,4}. ¹Dpt of Pharmacology, Inst of Biomedical Science, Univ of São Paulo, São Paulo, Brazil; ²Dpt of Physiological Science, Santa Casa de São Paulo School of Medical Science, São Paulo, Brazil; ³Dpt of Bioscience, Federal Univ of São Paulo, Santos, Brazil; ⁴Inst of Cancer and Genomic Sciences, Inst of Biomedical Research, College of Medical and Dental Sciences, Univ of Birmingham, United Kingdom

The neurodevelopment is a continuous process that depends on several cellular mechanisms, including mitochondrial function. In the past years, mitochondrial dysfunctions have also been linked with neuropsychiatric disorders, such as Schizophrenia (SZ). To investigate the relationship between mitochondrial dysfunction during neurodevelopment and neuropsychiatric-like-phenotype, we treated male Wistar rats puppies (P) with Rotenone (Rot; 0,1 mg/kg), an inhibitor of mitochondrial respiratory complex I, from day 5 to day 11 (P5-P11). At P60, we performed behavioral tests to evaluate the main SZ-like-phenotypes, named locomotion (LOC), social interaction (SI) and memory through the aversive condition memory; ACM). In parallel, we investigated the mitochondrial biogenesis pathway through the expression of *Pgc1alpha*, *Nrf1*, *Tfam* and *Tom20* genes, and mitochondrial DNA (mtDNA) copy number. In order to verify whether Rot could induce SZ-like behaviour, we challenged the animals injected with Rot with Haloperidol (Hal; 0.05mg/kg), an antipsychotic, in addition to Methylphenidate (MPD; 2.5mg/kg) and Amphetamine (AMP; 0.5mg/kg), psychostimulants. We performed Two-Way ANOVA followed by the *post hoc* Tukey (n=10) for *In Vivo* experiments, and student's *t* test (n=4) for gene analysis. All results were computed as mean±SEM in relation to control group (100% for behavioral analysis, and 1 for gene expression). We found that Rot-treated animals at P60 presented hyperlocomotion (163.1%±6.1; p=0.01), decreased SI (67.73%±4.512; p=0.01) and deficits in ACM (33.28%±11.22; p=0.05). We perceived that the behavioral abnormalities were exacerbated after AMP administration (LOC: 192.9%±17.36, p=0.001; SI: 75.94±5.24, p=0.01). Importantly, the behavioral deficits were fully reverted after Hal treatment (LOC: 94.37%±17.58, p=0.001; SI: 98.45%±3.266, p=0.05; ACM: 108.5%±25.29, p=0.05) but not by MPD (LOC: 161.4%±8.095; p=0.998; SI: 109.3%±7.496; p=0.001; ACM: 95.19%±20.81; p=0.2696). About mitochondrial function, we observed in the Rot-treated animals a decrease in the expression of genes related to mitochondrial biogenesis pathway (*Pgc1alpha*: 0.872±0.21, p=0.56; *Nrf1*: 0.478±0.12, p=0.001; *Tfam*: 0.453±0.103, p=0.0003; *Tom20*: 0.013±0.176, p=0.013), in addition to a diminishment in the mtDNA copy number (0.45±0.21, p=0.05). Our results suggest that Rot treatment during neurodevelopment induce SZ-like-phenotype in adult rats, an outcome that can be related to a decrease in mitochondrial biogenesis and in the organelle number. The study was approved by the Animal Research Ethical Committee (013/16; 006/18) and it was supported by FAPESP (2015/02041-1). **License number of ethics committee:** The study was approved by the Animal Research Ethical Committee (013/16; 006/18)

03.016 Antidepressant treatment and behavior responses in *Drosophila melanogaster*: a systematic review. Eckert FB^{1,4}, Triches FF^{1,4}, Costa JEM^{1,4}, Marino-Neto J², De Toni DC³, Lino de Oliveira C⁴. ¹UFSC Florianópolis, PPG Pharmacology, Brazil; ²UFSC Florianópolis, IEB, Brazil; ³UFSC Florianópolis, Dpt of Cellular Biology, Embryology and Genetics, Brazil; ⁴UFSC Florianópolis, Dpt of Physiological Sciences, Brazil

Flies may be model organisms in neuroscience, including psychopharmacology. Here, the aim was to investigate how the field of antidepressant (AD) research used flies to investigate the effects of AD on the behaviors of flies. A systematic review (SR) was planned to answer the following question: how do flies perform in behavioral tests after treatment with AD drugs compared to control? The SR plan included creating research questions using the PICO tool, search strategy, selection criteria, and planning of data extraction and analysis. Publications returned of the searches in Embase, Pubmed, Scopus, Web of Science, using a combination of terms related to the population (*Drosophila melanogaster* or flies), intervention (antidepressants, ADs) and primary outcome (behavior), were exported to the EndNote X7. Two independent reviewers and a conciliator screened 169 articles and applied the RoB-Syrcl tool to evaluate the 20 included in the SR. The risk of bias was judged unclear in 70% of the articles. Included articles provided 148 behavioral studies (k) in larvae and adult flies. Sex was unspecified in all studies with larvae. Studies in adults used males (k=40), females (k=27), mixture of sexes (k=6) or unspecified (k=3). All studies used the oral via to treatment with ADs. In larvae, time of treatments ranged from 30 min (k=1), longer than 1 h up to 5 days (k=61) or unspecified period (k=10), while in adults were acute (10 min to 6 h, k=18), intermediate (14 h to 24 h, k=25) or chronic (48 h to 10 days, k=33). In larvae, studies used fluoxetine (k=9), amphetamine (k=36 studies), methamphetamine (k=17), desipramine (k=8) and imipramine (k=2). In

adults, studies used fluoxetine (k=39), amphetamine (k=9 studies), methamphetamine (k=18), desipramine (k=6), amitriptyline and imipramine (k=2 each). The behavioral outcomes in studies that used larvae were locomotion (k=66), feeding (k=2) and time to pupation (k=4). In adults, outcomes were locomotion (k=21), immobility (k=17), mating or courtship (k=21), sleep (k=8), AMPH preference (k=4), sucrose preference (k=3), light preference, aggression, climbing, male fertility (k=1 each). Qualitative results indicate that, except for via of administration, there is low consensus within the design of studies on the effects of ADs in flies. Except for locomotion, most of the outcomes in the individual studies came from few publications (3 or less), nesting the design of putative meta-analyses. Independent of the development phase of flies, the more prevalent intervention investigated was fluoxetine, while locomotion was the most frequent outcome, which may be further appraised in the meta-analyses. The contribution of sex (adults), time of treatment, and type of behavioral test to the heterogeneity in the meta-analyses should be investigated. Moreover, present results indicate a scarcity of knowledge about the effects of ADs in flies tested in behavioral assays traditionally used to screen ADs, such as forced swimming test and sucrose preference. **License number of ethics committee:** N/A

03.017 **Effect of folic acid on anxiety-like behavior modulation of rats.** Bonancea AM¹, Estrada VB¹, Silva KGN¹, Miguel MVO¹, Pelosi GG¹. ¹UEL Londrina, Dpt of Physiological Sciences, Brazil

Introduction: Anxiety disorders have a high prevalence throughout life interfering with performance of the individual in various activities, as well as being associated with elevated costs to public health. Folic acid (FA) is a vitamin B complex that acts as cofactor of several biological processes and demonstrates an apparent modulation in some psychiatric disorders. However, there is few studies showing the effect of FA on anxiety modulation. Then, the goal of this work was to evaluate the effect of FA administration in rats submitted to the elevated plus maze (EPM) and open field (OF) tests and its modulation on anxiety-like behavior caused by previous acute restraint stress. **Methods:** Wistar rats (65 days age) received AF (2.5, 5, 10 or 50 mg/kg, i.p.) or saline (0.9% NaCl, i.p.) 48h, 24h and 5h before EPM and OF tests. At the second moment, FA was administrated in a single dose 1h before the behavioral tests. For acute restraint stress, rats were introduced into a restraint tube during 2h and 24 later were submitted to EPM and OF tests. In order to evaluate the effect of FA on anxiety caused by previous acute restraint stress, animals received FA (2.5, 5, 10 and 50 mg / kg, i.p.) 48h, 24h and 5h before behavioral tests. Blood samples for plasma corticosterone dosage were performed. Data were analyzed using the non-parametric Kruskal-Wallis test followed by Dunn's or Mann's posttest. All data are expressed as Median and a significance level of 5%. **Results:** FA administration caused an anxiogenic effect observed on OF test, (KW= 28.1; p= 0.0001) associated with a reduction of locomotor activity (KW= 13.6; p= 0.001). Acute restraint stress caused an anxiogenic effect on EPM described as a decrease on percentage of entry and time spent into the open arms and (p= 0.01) However, no change was observed with FA treatment in both anxiety animal models. Finally, no change in corticosterone concentration was observed 24 h after acute stress, (KW= 1.2; p= 0.5). **Conclusions:** The present work suggests a FA modulation of anxiety behavior and locomotor activity; however, further studies are needed to understand the mechanisms involved on that modulation. **License number of ethics committee:** CEUA protocol nº19030.2015.69

03.018 **Long-term consequences of maternal separation stress on ethanol intake in male and female mice.** Bertagna NB¹, Favoretto CA¹, Rodolpho BT¹, Loss CM¹, Palombo P¹, Yokoyama TS¹, Righi T¹, Anesio A¹, Miguel TT², Cruz FC¹. ¹Unifesp-EPM Dpt of Pharmacology, São Paulo, Brazil; ²UFU Uberlândia, Dpt of Pharmacology, Brazil

Introduction: Early life stress exposure is a significant environmental risk factor for developing psychopathologies in adulthood, such as alcohol use disorders (AUDs). Maternal separation stress is a predictive animal model to evaluate the effects of early stress exposure on alcohol consumption. Extended amygdala, constituted by Nucleus Accumbens (NAc) shell, Central Amygdala (CeA) and Bed Nucleus of Stria Terminalis (BNST), is a complex neural circuitry has been implicated in the modulation of stress responses and AUDs. We aimed to investigate the influence of maternal separation stress on alcohol intake of male and female adult mice, and the involvement of extended amygdala in this interaction. **Methods:** From postnatal day (PND) 1 to 14, C57BL/6J pups were separated from the dam (maternal separation, MS) daily for 180 min or were left undisturbed in their home cage (control). On PND 45, they were assigned to operant oral alcohol self-administration protocol. All mice were first exposed to 20% ethanol in tap water, in the home cage for 3 weeks (involuntary consumption). Next, they were trained in an operant oral alcohol self-administration under fixed-ratio schedule (FR), in which mice were reinforced for nose poking by delivery of oral 10% alcohol solution during daily 60-min sessions. Following the acquisition phase, "breakpoints" were determined. Later, mice were allowed 4 h access to the reinforcing solution with no dosage limitation (binge). Next, increasing concentrations of quinine (0.005g/L, 0.01g/L,

0.025g/L, and 0.05g/L) were added to ethanol to assess the drinking response to ethanol adulteration. Twenty-four hours later, the expression of Fos protein were analyzed in NAc Core and Shell, CeA and Basolateral amygdala (BLA) and BNST using the software ImageJ. **Results:** In involuntary consumption, female control and MS male group reduced their consumption over the weeks, while MS female group increased their consumption in the third week. In the operant ethanol self-administration protocol, male and female mice from MS and control groups did not show any differences in the reinforcement number and responses to active nose poke in FR schedule. In the breakpoint phase no differences were found among the groups, but considering stress factor, MS mice were more motivated to seek alcohol than the control mice. In the binge protocol, no differences in reinforcements and responses to active nose poke parameters were found. Further, in male mice, MS stress reduced ethanol aversion such that higher concentrations of quinine were necessary to decrease ethanol intake as compared to control mice. We did not find any difference among the groups in the number of Fos positive cells. in the NAc Core and Shell, BNST, CeA and BLA. **Conclusion:** Maternal separation stress may influence alcohol intake in adulthood dependent on sex and reinforcement schedule. **Financial Support:** CAPES, FAPESP 2018/15505-4. **License number of ethics committee:** CEUA/UNIFESP: nº 5360240918

03.019 **Impact of the schizophrenia associated nuclear distribution element genes on nematode *Caenorhabditis elegans* monoamines levels and behavior: effects of typical and atypical antipsychotics.** Nani JV¹, Campeiro JD¹, Monte GG¹, Mori MA², Hayashi MAF¹ ¹Dpt of Pharmacology, Escola Paulista de Medicina (EPM), Univ Federal de São Paulo (Unifesp), Brazil ²Dpt of Biochemistry and Tissue Biology, Inst of Biology, Univ Estadual de Campinas (UNICAMP), Campinas, Brazil

Introduction: Nuclear distribution element genes are involved in several neurodevelopmental processes, including neuronal proliferation, differentiation and migration, and therefore in brain formation, which are processes associated with the susceptibility to develop schizophrenia. In fact, the implication of human nuclear distribution genes, namely nudC and NDE1 (Nuclear Distribution Element 1)/NDEL1 (Nuclear Distribution Element-Like 1) in psychiatric disorders, which classically are known to present imbalance in neurotransmitter signaling, was confirmed by several ways. The monoaminergic system is composed by highly conserved neurotransmitters and pathways which promotes the adaptability of several species to different environment. In the nematode *Caenorhabditis elegans*, the biogenic amines are the main responsible for the modulation of several behaviors, as well as it occurs in mammals. Although NDEL1 null knockout (KO) leads to early embryonic lethality in mice, *C. elegans* KO for the orthologs of nuclear distribution element genes (nud) are viable, allowing us to evaluate the consequences of nud genes suppression in the monoamines levels and animal behavior in these worms. **Methods:** The behavior and monoamine levels were evaluated in wild-type (WT) (N2) and KO for the nuclear migration genes nud-1 (RB773) and nud-2 (RB1022) *C. elegans* at baseline (drug-naïve) and after the treatment with typical or atypical antipsychotics, which are main drugs used in the treatment of psychiatry disorders, such as schizophrenia and bipolar disorder. **Results:** The comparative analysis of worm behavioral response (e.g. egg laying, body bend and pharynx pumping) allowed to observe significant deficits for nud genes KO worms compared with WT animals at baseline, with a more evident effects observed for clozapine compared with haloperidol in nud KO worms. Additionally, the monoamines dopamine, serotonin and octopamine levels were significantly lower in nud KO worms compared with WT *C. elegans* at baseline, while the treatment with typical or atypical antipsychotics determined significant effects on monoamine levels only in WT worms. **Conclusion:** Taken together, the present results highlight the crucial role of nud genes for the pharmacological effects of typical or atypical antipsychotics on the animal behavior responses, as well as in monoamines homeostasis and levels, reinforcing the importance of nud genes family at the cornerstone of pathways involved in pathways associated with mental disorders, such as the dopaminergic and serotonergic pathways. In addition, the present work strengthens the validity and reliability of using nematode as an experimental animal model to explore the convergence between conserved neurochemical pathways and genes that are known to be essential for the neurodevelopmental processes and for the susceptibility to psychiatric disorders as schizophrenia. **Financial Support:** by FAPESP (2019/09207-3; 2019/13112-8; 2020/01107-7), CNPq and CAPES – Finance Code 001. **License number of ethics committee:** This study was approved by the Ethical Committee of the Federal University of São Paulo (UNIFESP/EPM), under CEUA N° 9635080315.

03.020 **Behavioral effects of the combined treatment with cannabidiol and antidepressants in stressed mice.** Scarante FF¹, Araújo MR¹, Campos AC¹. ¹Dpt of Pharmacology, Ribeirão Preto Medical School, Univ of São Paulo, Brazil

Antidepressants comprise a class of drugs indicated as first-line pharmacological treatment for the therapeutic of several psychiatric disorders. However, a high percentage of patients are poorly responsive

to antidepressant monotherapy. Combination/augmentation strategies are commonly employed for enhancing the action of antidepressants in psychiatric disorders¹. In this sense, cannabidiol (CBD) is a phytocannabinoid that displays potential therapeutic effects in animal models of anxiety and depression and arises as a potential candidate as an add-on therapy for the treatment of psychiatric disorders. Previous data from our lab showed that the combination of escitalopram with a low dose of CBD accelerated the anxiolytic-like effect of the antidepressant². In this study, we aimed to investigate (1) the potential antidepressant-like effect of the combination of escitalopram with a low dose of CBD in stressed mice and (2) the potential anxiolytic-like effect of the combination of imipramine with a low dose of CBD in stressed mice. The stress model employed was a 10-day protocol of chronic unpredictable stress (CUS) in C57BL/6 male mice (10-12 weeks old). A group of mice were submitted to CUS and during the last 7 days of the stress protocol received treatment with vehicle or escitalopram (10mg/kg) in combination with vehicle or CBD (7.5mg/kg). In the 11th day, they were submitted to the splash test (ST) for the evaluation of depressive-like behaviors. In the ST, after spraying a solution of 10% sucrose in the dorsal region of the mice, we quantified the latency for the first grooming episode, the total grooming time, the grooming time in the dorsal and in the facial region and the time of passive self-cleaning behavior. We did not observe any effects of stress or treatment in all the parameters evaluated in the ST, even though the stressed mice treated with the combination of escitalopram and CBD seemed to spend more time cleaning the dorsal region in comparison to the facial region, indicating that the treatment might be increasing the seeking for the sucrose solution sprayed in this region. Another group of mice were submitted to CUS and during the last 7 days of the stress protocol received treatment with imipramine (15mg/kg) in combination with vehicle or CBD (7.5mg/kg). In the 11th day, they were submitted to the novelty suppressed feeding test (NSF) for the assessment of anxiolytic-like responses. Treatment with imipramine alone or with the combination of imipramine and CBD decreased the latency for animals to feed in the NSF, indicating an anxiolytic-like effect. In conclusion, in stressed mice the combination with a low dose of CBD did not enhance the antidepressant-like effect of escitalopram in the ST nor did it improve the anxiolytic-like effect of imipramine in the NST. CEUA number: 47/2019. Financial support: CNPq and FAPESP. ¹Connolly KR; Thase ME. *Drugs*, 71(1), p.43, 2011. DOI: 10.2165/11587620-000000000-00000 ²Scarante FF. *bioRxiv*, 2021. DOI: 10.1101/2021.04.23.441143 **License number of ethics committee:** CEUA number: 47/2019.

03.021 CB2 receptors' spontaneous activity is relevant for the adverse effects of chronic unpredictable stress in mice treated with antidepressant. Araújo MR²; Aguiar RP¹; Füsse EJ³, Scarante FF²; Oliveira RMMW¹; Guimarães FS², Campos AC² ¹Dpt of Pharmacology and Therapeutics, State Univ of Maringá, Maringá, Brazil ²Dpt of Pharmacology - Ribeirão Preto Medical School, Univ of São Paulo- Ribeirão Preto, Brazil ³Mental Health Graduate Program, Ribeirão Preto Medical School, Univ of São Paulo Ribeirão Preto, Brazil

Introduction: The incomplete knowledge of the mechanisms involved in the regulation of emotional states and coping stress represents the primary limiting factor of the efficacy of antidepressants (ADs), as well as the development of new drugs to the treatment of mood disorders, such as anxiety and depression. Several lines of evidence suggest that the monoaminergic theory of depression cannot fully explain the behavioral and neuroplastic changes observed after ADs chronic treatment (1-4). Hence, other molecular targets, such as the CB2 receptors, have been associated with the chronic effects of these drugs, specially in stressed mice (5, 6). In the present study, we hypothesized that the behavioral and neuroplastic effects observed after repeated treatment with the AD escitalopram (Esc) in chronically stressed mice depend on CB2 receptor signalling. **Methods:** Male mice submitted to chronic unpredictable stress (CUS) paradigm for 21 days were treated with Esc (10mg/kg) once a day in the presence or not of AM630 (0.01, 0.03 and 0.30mg/Kg), a CB2 receptor antagonist/inverse agonist. At the end of the last stress episode and drug treatment, mice were submitted to the Novel Suppressed Feeding test to evaluate anxiety-like behavior and to the Tail-suspension test to evaluate depressive-like behavior. Animals were then euthanized and brains were removed to Doublecortin (DCX) immunohistochemistry assay. **Results:** Our results demonstrated that chronic blockade of the CB2 receptor by AM630 attenuated the neuroplastic and the antidepressant- but not the anxiolytic-like effects of Esc. AM630 alone or in combination with Esc decreased the expression of DCX+ cell in both the subgranular and granular layers of the dentate gyrus (DG), indicating a general reduction of DCX+ neuroblasts and a decrease in their migration through the DG layers. **Conclusion:** We suggest that the antidepressant-like behavior and the pro-neurogenic effect, but not the anxiolytic like behavior, promoted by Esc in stressed mice are, at least in part, dependent of CB2 activation. Financial support: CAPES, FAPESP, CNPq and Institute L'Oréal. **REFERENCES:** (1) Blier P, Ward NM. Is there a role for 5-HT_{1A} agonists in the treatment of depression? *Biol Psychiatry*. 2003;53(3): 193-203.; (2) Blier P, de Montigny C. Current advances and trends in the treatment of depression. *Trends*

Pharmacol Sci. 1994;15(7): 220-6.; (3) Santarelli L, Saxe M, Gross C, Surget A, Battaglia F, Dulawa S, et al. Requirement of hippocampal neurogenesis for the behavioral effects of antidepressants. *Science*. 2003;301(5634): 805-9; (4) Hill N, Ho WS, Sinopoli KJ, Viau V, Hillard CJ, Gorzalka BB. Involvement of the endocannabinoid system in the ability of long-term tricyclic antidepressant treatment to suppress stress-induced activation of the hypothalamic-pituitary-adrenal axis. *Neuropsychopharmacology*. 2006;31(12): 2591-9. (5) García-Gutiérrez MS, Pérez-Ortiz JM, Gutiérrez-Adán A, Manzanares J. Depression-resistant endophenotype in mice overexpressing cannabinoid CB(2) receptors. *Br J Pharmacol*. 2010;160(7): 1773-84; (6) Ortega-Alvaro A, Aracil-Fernández A, García-Gutiérrez MS, Navarrete F, Manzanares J. Deletion of CB2 cannabinoid receptor induces schizophrenia-related behaviors in mice. *Neuropsychopharmacology*. 2011;36(7): 1489-504. **License number of ethics committee:** Animal Research Ethical Committee: CEUA/FMRP 032/2015-1, 01/2019.

03.022 Zebrafish exposure to valproic acid as a translational platform for Autism Spectrum Disorders research and drug discovery. Costa KCM¹, Brigante TAV¹, Fernandes GG¹, Ferreira RR¹, Scomparin DS¹, Scarante FF¹, Pires-dos-Santos I¹, Oliveira DP², Campos AC¹. ¹FMRP-USP, Dpt of Pharmacology, Ribeirão Preto, Brazil; ²FCFRP-USP, Dpt of Clinical, Toxicological and Bromatological Analysis, Ribeirão Preto, Brazil **Introduction:** Autism Spectrum Disorders (ASD) is a neuropsychiatric condition with no effective treatment and is characterized by impaired social communication and behavioral domains. The Food and Drug Administration (FDA) recognizes only risperidone (RISP) and aripiprazole in treating ASD-aggressiveness symptoms, but with a potential risk for side effects. Previous studies have shown that exposure to valproic acid (VPA) during the embryonic period is associated with a higher incidence of ASD. Rodent models of ASD involve an increased number of animals, high costs, and time-consuming experiments. In this context, zebrafish models have emerged as a potential model for translational research of neuropsychiatric disorders. Zebrafish keeps significant genetic and physiologic similarities with mammals, including brain structure and functions. Besides, zebrafish grow in a cost-effective manner producing generation in a short time, increasing efficiency, and reproducibility between experiments. Our aim in the present study was to develop a quick and effective zebrafish model to study the mechanisms of autism and to generate a robust tool for screening new drugs for ASD treatment. **Methods:** All protocols were approved by CEUA-FCFRP (nº 19.1.243.60.4). Zebrafish embryos were exposed to different VPA concentrations (5, 25, 125, 625, or 1250 µM at 4-48 hours post-fertilization- hpf) and treated with RISP (1 or 3µM at 48-96 hpf) for the model validation. Mortality rate, the presence of malformations, spontaneous movements, motor activity, social interaction, and aggressiveness were assessed during the early embryo-larval stage (1 to 7 days post-fertilization-dpf). **Results:** First, we found that with only 24 hpf, embryos treated with 125µM VPA already showed an increase in spontaneous movements compared to the other groups (p<0.05; n=61-63/treatment), while embryos exposed to 1250µM AVP showed a significant reduction in spontaneous movements compared to the other groups (p<0.001; n=61-63/treatment). At 5dpf, the exposure to 625µM and 1250µM VPA concentrations in the early stages of development induced a higher mortality rate and developmental impairments (p<0.001; n=61-63/treatment). For larval behavior analysis at 7dpf, the 125µM VPA early exposure still leads to a hyperlocomotion in the open field (p<0.05; n=21-24/treatment), with no difference for social interaction profile (p>0.05; n=22-23/treatment), and also leads to an increase in the aggressiveness profile in the mirror attack test (p<0.05; n=22-24/treatment). Interestingly, we showed that the 1µM RISP treatment only rescued the non-aggressive pattern of VPA-exposed animals (p<0.05; n=21-23/treatment), but without returning to the baseline values of the control group (p<0.05; n=21-23/treatment). **Conclusion:** In conclusion, our fast zebrafish model of early embryonic exposure to VPA showed robust behavioral changes compatible with the broad phenotypic spectrum of autism, with RISP treatment being able to reduce only the aggressive behavior, in line with clinical data. These results reveal the potential of our model as a tool for ASD-drug screening. Financial support: CAPES, FAPESP. **License number of ethics committee:** CEUA-FCFRP (nº 19.1.243.60.4)

03.023 Glucocorticoid and noradrenaline signaling in the stress-induced dendritic remodeling in the amygdala and anxiety-like behavior in rats. Novaes LS¹, Bueno-de Camargo LM¹, Almeida, AS¹, dos Santos, NB¹, Goosens KA², Munhoz CD¹. ¹Dpt of Pharmacology, São Paulo. ²Dpt of Psychiatry, Icahn School of Medicine at Mount Sinai, New York

Introduction: Anxiety, a state-related to anticipatory fear, can be adaptive in the face of environmental threats or stressors. However, anxiety behaviors can also become persistent and manifest as anxiety- and stress-related disorders, such as generalized anxiety or post-traumatic stress disorder (PTSD). In rodents, systemic administration of glucocorticoids (GCs) or short-term restraint stress induces anxiety-like behaviors and dendritic branching within the basolateral complex of the amygdala (BLA) ten days later. Additionally, increased arousal-related memory retention mediated by elevated GCs requires concomitant noradrenaline

(NE) signaling, both acting in the BLA. It is unknown whether GCs and NE play a role in the delayed effects of acute stress on BLA dendritic plasticity and animal behavior. Given the tremendous burden of affective disorders, it is imperative to advance our understanding of stress neurobiology and translate this into improved treatments **Methods:** Male Wistar rats were used as subjects of this study. Animals were restraint stressed for 2 hours and the behavioral experiments were carried out 10 days after. For anxiety-like behavior assessment, we carried out the elevated plus maze and light-dark box tests. Intra-BLA expression of the dominant-negative form of GR (dnGR) and cannulae implantation for infusion of RU-486 (10ng in 5% DMSO), dexamethasone (30 ng in 0.5% ethanol), propranolol (2.5 µg in saline), or yohimbine (2.5 µg in saline), or their corresponding vehicle controls, were performed by stereotaxic surgeries. Intra-BLA drugs were infused 30 min before the stress session. Metyrapone (i.p.; 150 mg in saline) was administered 1.5 hours before restraint stress. BLA dendritic spine density was performed using the Rapid Golgi Kit (FD NeuroTechnologies, Inc.) or by detection of GFP expression. All the experiments were conducted under the standards of the Ethics Committee for Animal Use of the Institute of Biomedical Sciences/University of São Paulo (CEUA-ICB 85/2016). **Results:** Inhibiting corticosterone (CORT) elevation during 2-hour restraint stress prevents stress-induced increases in both delayed anxiety-like behaviors and BLA dendritic spine density in rats (two-way ANOVA: stress-Met interaction $F_{1, 39} = 9.282$, $P = 0.0041$). Also, we show that the behavioral (stress-dnGR interaction $F_{1, 39} = 8.047$, $P = 0.0072$) and morphological (two-way ANOVA: stress-dnGR interaction $F_{1, 69} = 7.85$, $P = 0.0066$) delayed effects of acute stress are critically dependent on glucocorticoid receptor (GR) genomic actions in the BLA. Unlike CORT, we found that pharmacological enhancement of NE signaling in the BLA was, in the absence of stress, not sufficient to drive delayed anxiety-related behavior (student's t-test: $P = 0.9058$); however, the delayed anxiety-like behavior observed after acute stress does require NE signaling in the BLA at the time it is expressed (student's t-test: $P = 0.0441$). **Conclusion:** These results suggest a model in which acute stressors induce morphological changes in the BLA via GR genomic activity; this requires NE signaling in the BLA to express stress-induced behavioral changes. **Financial Support:** FAPESP and CNPq **License number of ethics committee:** CEUA-ICB 85/2016

03.024 **Tolerance to the antidepressant-like effect of ketamine after sub chronic administration: the role of oxygen and nitrogen reactive species in the brain mice.** Contó MB¹, Camarini R¹. ¹USP São Paulo, Dpt of Pharmacology, Brazil

Introduction: The anesthetic ketamine has been recently approved for antidepressant treatment. This drug has important advantages over the traditional antidepressants: it has a rapid onset and long-lasting effect. In addition, ketamine produced therapeutic effect in patients that were refractory to the conventional pharmacological treatments (Monteggia, *Curr. Opin. Neurobiol.* 30, 139, 2015). However, there is some few evidence, both in humans and animals, demonstrating that chronic administration of antidepressant doses of ketamine can elicits tolerance to its antidepressant effect (Popik, *Psychopharmacol.* 198, 421, 2008; Bonnet, *J.Psychoactive Drugs*, 847, 276, 2015). Oxygen and nitrogen reactive species in the brain have been related to the pathophysiology of depression (Scapagnini, *CNS Drugs*, 26, 477, 2012). Thus, it is possible that ROS and NOS enhancement may underlie the tolerance to the antidepressant-like effect of ketamine. Therefore, the aim of this study was to investigate if the antidepressant tolerance induced by sub chronic treatment with ketamine would be associated to a higher lipidic and protein peroxidation in the brain. **Methods:** Naïve adult male Swiss mice, 80 days old, were housed in standard polycarbonate boxes, 4 mice/cage, food and water ad libitum. The experiment comprises two groups: 1) Group Ketamine (50 mg/kg) 1x, which received one intraperitoneal (i.p.) injection of ketamine; and 2) Group Ketamine (50 mg/kg) 4x, which received 4 i.p. injections of ketamine (4 intercalated administrations along 8 days, 1 administration every 2 days). Twenty-four hours after the single or the 4th injection, animals were sacrificed. We established this protocol, considering our results showing that the animals presented their maximal antidepressant effect 24 hrs after a single injection, while after the 4th ketamine administration, the antidepressant-like effect was no longer observed (tolerance phenomenon). The brains (without olfactory bulbs, cerebellum, and brainstem) were used to quantify the oxidative/nitrosative stress biomarkers by spectrophotometry. This work was approved by our institution's Ethics Committee on Animal Research (Protocol 132/2016). **Results:** The student's t test for independent samples (two-tailed) did not find significant differences between the groups Ketamine (50 mg/kg) 1x and Ketamine (50 mg/kg) 4x in the brain levels of carbonyls ($p = 0.1089$), malondialdehyde ($p = 0.7931$), sulfhydryls ($p = 0.5066$) and catalase activity ($p = 0.2916$). However, it was observed a decrease in the nitric oxide (NO) metabolite (nitrite) in the group Ketamine (50 mg/kg) 4x compared to the group Ketamine (50 mg/kg) 1x ($p = 0.0410$). **Conclusion:** We conclude that the tolerance to ketamine's antidepressant-like effect, developed along its sub chronic treatment, apparently, is not related to an oxidative/nitrosative stress in the brain. However, lower NO brain levels may play a role in the antidepressant tolerance developed by ketamine sub-chronic

administration. **Acknowledgements:** We thank CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior) for the financial support **License number of ethics committee:** Protocol 132/2016

03.025 The Impact of social isolation in alcohol use and mental health during the COVID-19 pandemic in Brazil Freese, L¹; Nin, MS^{1,2}; Almeida, FB¹; Heidrich, N¹; Constant, HMRM¹; Izolan, LR³; Bortolon, CB⁴; Gomez, R³; Barros, HMT¹. ¹UFCSA, PPG-Ciências da Saúde, Neuropsychopharmacology Lab, RS, Brazil; ²FURG, Dpto. de Farmacologia, Brazil; ³UFRGS, PPG-Farmacologia e Terapêutica, Brazil; ⁴Unifesp, PPG-Psiquiatria e Psicologia Médica, Brazil

Introduction: The world is currently going through a serious health crisis, the COVID-19 pandemic. The stress caused by social isolation and fear about contamination is affecting people, increasing the risk of mental disorders. Evidence shows that the prevalence (%) of symptoms of stress (81.9 to 8.1), post-traumatic stress disorder (53.8 to 7), anxiety (50.9 to 6.33) and depression (48.3 to 14.6) seem to much high during compared to before the pandemic through many countries¹. Moreover, the impact of the feelings of loneliness were accompanied by an alcohol intake increase during that period (58%)². Studies show that the social isolation led to elevated alcohol and drug use, and might also enhance the risk of a more severe infection^{3,4}. **Objective:** to assess whether the social isolation promoted by the COVID-19 pandemic affected the self-reported alcohol consumption and depression, anxiety and stress levels in Brazil. **Methods:** preliminary cross-sectional survey observed the general population in Brazil (>18 years old), and recruited people during the first wave of the pandemic, using social media (Facebook®, Instagram®, WhatsApp®) and institutional websites. The online questionnaire was built and filled by respondents on REDCap®; (UFCSA Ethics Committee #34840620.6.0000.5345). The first page had the informed consent, followed by 56 questions, which evaluate social, demographic, and drug use profile through the Alcohol, Smoking and Substance Involvement Screening Test (ASSIST), and mental health state through the abbreviated Depression, Anxiety and Stress Scale (DASS-21). The Wilson score interval was used for the CI95%, and the comparison of drug use according to social isolation was performed by the Chi-square test. Also, the Pearson Correlation test was used to verify the association between DASS-21 and ASSIST scores. **Results:** 2288 questionnaires were completed: 1533 (67%) were female, 741 (32.4%) male and 14 (0.6%) opted not to reply. 1716 (75%) self-reported white race, 1494 (65.2%) were single, divorced or widowed and the mean age was 31.83 (SD ± 10.56). Individuals who declared themselves more strictly isolated perceived reduced alcohol consumption, while the less isolated ones noticed an increase in the alcohol use (P<0.05). For the DASS form, correlation was higher between stress and anxiety (r=0.78, P<0.001), followed by stress and depression (r=0.76; P<0.001). **Conclusions:** The most restrictive isolated ones seem to significantly inhibit lower risk of alcohol use. Also there is an influence of stress for symptoms of depression and anxiety, meaning that the greater the symptoms of stress, the greater the other parameters. Finally, we can observe that the isolation affected the alcohol consumption and the stress caused by the pandemic seems to be affecting the depression and anxiety levels in the population studied. **Financial Support:** - UFCSA, CNPQ, CAPES 1. Abramson, A. Amer. Psych. Assoc. 52(2), 22; 2021. 2. Xiong, J. et al. J Affec Disord, 277,55; 2020. 3. Dar, K.A. et al. Asian J. Psych., 29, 129; 2019. 4. Banerjee, D. Asian J. Psych., 50, 102014; 2020. **License number of ethics committee:** 34840620.6.0000.5345

03.026 Association between hypnotics/sedatives use and social isolation during the COVID-19 pandemic in Brazil. Nin MS¹, Freese L², Almeida FB², Heidrich N², Constant HMRM², Izolan LR³, Bortolon CB⁴, Gomez R³, Barros HMT². ¹FURG, Dpto. de Farmacologia, Brazil; ²UFCSA, Dpto. Farmacociências, Neuropsychopharmacology Lab, Brazil; ³UFRGS, PPG-Farmacologia e Terapêutica, Brazil; ⁴Unifesp, PPG-Psiquiatria e Psicologia Médica, Brazil

Introduction: The COVID-19 pandemic is increasing hospitalization due to severe respiratory infection around the world, surpassing 4 million deaths in July of 2021. The stress caused by fear of getting infected or contaminating others might also increase the risk of drug abuse¹. Evidence shows that the prevalence of stress symptoms, PTSD, anxiety and depression are higher during the pandemic throughout many countries². Also, isolation due to the pandemic led to elevated drug use and might also enhance the risk of a more severe infection^{3,4}. The present work aimed to assess whether the social isolation promoted by the COVID-19 pandemic affected the self-reported consumption of hypnotic/sedative drugs among the general population in Brazil. **Methods:** This preliminary cross-sectional survey observed the general population in Brazil (>18 years old), and recruited people during the first wave of the pandemic (sept-oct/2020), using social media (Facebook®, Instagram®, WhatsApp®) and institutional websites. The online questionnaire was built and filled by respondents on REDCap® (Ethical Committee-UFCSA: 34840620.6.0000.5345). The first page of the questionnaire had the informed consent, followed by 56 questions, which evaluate social, demographic, and drug use profile through the Alcohol, Smoking and

Substance Involvement Screening Test (ASSIST), and mental health state through the abbreviated Depression, Anxiety and Stress Scale (DASS-21). The statistic test used were: Wilson score interval ($CI_{95\%}$), Chi-square test, and Pearson Correlation test. **Results:** A total of 568 questionnaires of hypnotic/sedative users were completed (23.2% of all questionnaires filled). Concerning the question did your hypnotic/sedative drug use change during the pandemic?, 26,2% ($IC_{95\%}$: 22.8%-30.0%) responded that it had decreased; 49.6% ($IC_{95\%}$: 45.6%-53.8%) maintained the same use pattern; and 24.1% ($IC_{95\%}$: 20.8%-27.8%) increased it. When the association between isolation and drug use was analyzed, there was no statistical association ($P=0.216$). Finally, the association between the ASSIT score for hypnotic/sedative use and DASS-21 score for depression was low ($r=0.305$; $P<0.001$), as well as for anxiety ($r=0.374$; $P<0.001$) and for stress ($r=0.366$; $P<0.001$). **Conclusion:** Approximately the same proportion of the sample increased and decreased the hypnotic/sedative use profile during the pandemic, and half of them continued using the same amount. Although many data point to an increase of several stress-evoked disorders and drug use, there was a weak association between stress/anxiety/depression scores and hypnotic/sedative use. Also, the isolation perception did not impact drug use change. Nevertheless, since the main target for the study was to detect alcohol use changes, the sample for the hypnotic/sedative use might not have been numerous enough to detect those changes. **Financial Support:** - UFCSPA, CNPQ, CAPES 1. Abramson, A. Amer. Psych. Assoc. 52(2), 22; 2021. 2. Xiong, J. et al. J Affec Disord, 277,55; 2020. 3. Dar, K.A. et al. Asian J. Psych., 29, 129; 2019. 4. Banerjee, D. Asian J. Psych., 50, 102014; 2020. **License number of ethics committee:** UFCSPA: 34840620.6.0000.5345

04. Inflammation and Immunopharmacology

04.001 Investigation of the anti-inflammatory mechanism of action of stigmasterol

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Introduction: Stigmasterol is a phytosterol present in many medicinal plants. It has been studied for many pharmacological properties, including the anti-inflammatory activity. It is known that stigmasterol downregulates the expression of inflammatory mediators through the inhibition of p65NFkB-IkB complex phosphorylation. However, its mechanism of anti-inflammatory action has not been completely elucidated yet. This research aimed to investigate the activity of stigmasterol on mice models of inflammation and nociception as well as its mechanism of action. **Methods:** Male Swiss mice (35-40 g) were used. Three doses of stigmasterol (10, 30 and 100 mg/kg, orally - p.o.) were tested to standardize its lowest effective dose. The antinociceptive activity was investigated by acetic acid-induced writhing test, formalin and hot plate tests. The anti-inflammatory activity was assessed by carrageenan-induced peritonitis and paw edema induced by arachidonic acid. The mechanism of action was investigated by molecular docking and by pretreating mice with RU-486 (glucocorticoid receptor -GR- antagonist) in the acetic acid-induced writhing test. The motor coordination and the locomotor activity were assessed by the open field and rota-rod tests, respectively. Indomethacin (10 mg/kg, p.o.), dexamethasone (10 mg/kg, p.o.) and morphine (30 mg/kg, p.o.) were the positive controls; saline + 1% polysorbate 80 (vehicle) was the negative control. The results were evaluated by one-way or two-way (repeated measures) ANOVA post hoc Student-Newman-Keuls (Ethics Committee approval: 007/17; 006/19). **Results:** Stigmasterol significantly ($p < 0.05$) reduced the number of abdominal writhes (10 - 100 mg/kg) and nociception time (s) in the first ($p < 0.05$) and second ($p < 0.01$) phases of the formalin test (10 mg/kg) comparing to the vehicle-treated group. No effect was observed in the latency time of the hot plate test. It also reduced leukocytes infiltration (cells/mm³) in the peritoneal cavity ($p < 0.05$) and the paw edema 30 min after the injection of arachidonic acid ($p < 0.05$) when compared to the vehicle-treated group. The binding affinity of stigmasterol and GR was -6.1 kcal/mol, and hydrophobic interactions and hydrogen bonds stable the GR-stigmasterol docking complex. Furthermore, RU-486 prevented its effect ($p < 0.05$). Stigmasterol-treated mice presented a number of crossings significantly lower ($p < 0.01$) than the animals treated with vehicle in the open field test. Nevertheless, the results of rota-rod test show that stigmasterol has no effects on mice motor coordination. **Conclusion:** Stigmasterol present anti-inflammatory activity mediated by the activation of GR and inhibition of arachidonic acid pathway. Theoretical evidence support the hypothesis that stigmasterol is a potential agonist of the GR. It also has antinociceptive effect (chemical nociception) and does not affect the motor coordination in mice, but acts as a sedative. Therefore, stigmasterol presents a potential therapeutic application as an anti-inflammatory drug. **Acknowledgements:** This work was supported by the Universidade Comunitária da Região de Chapecó and Programa de Bolsas Universitárias de Santa Catarina - Uniedu [Art. 171 CE]. **License number of ethics committee:** 007/17; 006/19

04.002 **Effects of α -Klotho protein in TNF- α signaling through TNFR1 in mice.** Araujo de Souza G, Scavone C, Kinoshita PF. USP São Paulo, Dpt of Pharmacology, Brazil I.

Introduction: α -Klotho is a protein expressed primarily in the kidney and brain, receiving attention by its capability of delaying aging and cognitive enhancement when overexpressed in mice. In chronic kidney diseases models, cognitive deficits and neuroinflammation are observed. In this context, our group has shown that there is a negative correlation between high TNF- α levels and lower expression of α -Klotho in the frontal cortex. This suggests that the administration and/or reestablish of α -Klotho concentrations could revert the inflammatory state. Although some protective actions of α -Klotho in the CNS were described, its role in neuroinflammation is poorly understood. TNF- α has an important role in neuroinflammation, but also in synapse modulation. This cytokine has two different receptors: TNFR1 is known as the receptor of the classical TNF- α activation and the TNFR2 has a more protective effect. The project aims to investigate the role of α -Klotho in TNF- α signaling through TNFR1 in wild-type (WT) and TNFR1 knockout mice (TNFR1 KO). **Methods:** 1. Animals: All procedures were approved by the Ethical Committee for Animal Research of Institute of the Biomedical Sciences under registration number 37 on page 15 of book 3. 2. Genotyping: a fragment of the animal's tail was used to extract DNA used to perform PCR assays to identify WT and TNFR1 KO mice. 3. Klotho treatment: Two to four-month mice were separated into 4 groups (n=5). The WT animals received an intraperitoneal (ip) injection of saline (WT sal), or an ip injection of recombinant klotho (rmKL) (10ug/kg) (WT kl). The same was performed in the TNFR1 KO mice that corresponds to the TNFR1 sal and TNFR1 kl groups, respectively. 4. Protein extraction: Four hours after treatment, animals were euthanized, and the cortex was removed. Cytosolic and nuclear proteins were extracted. 5. Western Blotting (WB): The primary antibodies used were: anti-GluN1, anti-p-GluN1, anti-GluN2B (subunits of N-Methyl-D-Aspartate receptor (NMDA), anti-AKT, anti-p-AKT, anti-p-FoxO3a, TNFR2 and anti-NFKB p65. 6. Statistical analysis: It was performed a two-way ANOVA, followed by Tukey's test. P values were considered significant for $p < 0.05$. **Results:** The TNFR1 expression nor the α -Klotho treatment change the protein expression of GluN1, AKT, p-AKT, p-FoxO3a, p65, TNFR2. Although there were no significant differences in p-GluN1 between the groups so far, it could have a trend of increase in phosphorylation in WT kl group compared to TNFR1 kl. The GluN2B is expressed different between groups: WT sal vs WT kl $p = 0,0103$; WT kl vs TNFR1 kl $p = 0,0025$. **Conclusion:** rmKL did not alter AKT, p-Foxo3a, p65 and TNFR2 expression independently of TNFR1 expression. The trend in an increase of GluN1 phosphorylation WT kl groups compared to TNFR1 kl suggests that kl effects can be dependable partially on TNFR1. There was also an effect in GluN2B expression, suggesting that the effects of α -Klotho are, in some matters, also dependent on TNFR1 signaling. Thus, it seems that there is a relationship between α -Klotho and TNFR1 concerning glutamate receptors modulation. **Acknowledgments:** This research is supported by a student fellow grant #2019/05970-4 and a project grand #16/07427-8, São Paulo Research Foundation (FAPESP). **License number of ethics committee:** Ethical Committee for Animal Research of Institute of the Biomedical Sciences under registration number 37 on page 15 of book 3

04.003 **The acute inflammatory response is attenuated in elastase-2 knockout mice.** Dantas P¹, Mestriner F¹, Silva-Jr E², Becari C¹. ¹USP Ribeirão Preto, Dpt of Surgery and Anatomy, Brazil ²UFRN Natal, Dpt of Biophysics and Pharmacology, Brazil

Introduction: Several studies have shown that Elastase-2 (ELA-2), an angiotensin II converting enzyme, plays an important role in regulating the cardiac autonomic system, myocardial infarction, and abdominal aortic aneurysm. However, the contribution of ELA-2 on inflammatory response is not fully understood. Therefore, we sought to investigate the role of ELA-2 on the acute inflammatory response induced by the administration of carrageenan or bradykinin into the mouse paw. **Methods:** We used ELA-2 knockout (ELA-2 KO; CELA-2aTm1Bdr) and BALB/c mice (control group), male and female, weighing 20-28g, from the Center for Special Mice Breeding at FMRP-USP. The acute inflammatory response was measured by the edema induced by the administration of carrageenan (1%) or bradykinin (BK; 3 nmol) into the subplantar region of the right paw of control mice (n = 18) and ELA-2 KO (n = 18). The left paw of both mice strains was injected with Saline (20 μ L) and used as edema control. The statistical significance considered was $P < 0.05$. All experimental procedures were previously approved by the local Animal Ethics Committee (n.131/2019). **Results:** The ELA-2 KO mice group showed reduced paw edema induced by carrageenan compared to the control group at the time of 30 min ($p = 0.0487$). The paw edema induced by BK was diminished in the ELA-2 KO group after 30 min ($p = 0.0047$) and 60 minutes ($p = 0.0001$) compared to the control group. Saline-induced paw edema was similar between ELA-2 KO mice and controls. **Conclusion:** Our data suggest that ELA-2 KO mice showed an altered inflammatory response compared to control animals, suggesting that ELA-2 could be in the acute inflammatory response. **Funding:**

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04.004 Influence of COX-1 and COX-2 pathways on hemorrhage and tissue ischemia events during skeletal muscle degeneration and regeneration induced by a snake venom. Correia MR¹, Han SW², Escalante T³, Moreira V¹. ¹Unifesp, Dpt of Pharmacology, Brazil; ²Unifesp. Dpt of Biophysics, Brazil; ³Universidad de Costa Rica, Inst Clodomiro Picado, SJCR

Microcirculation restoration is essential for skeletal muscle regeneration because it provides oxygen and nutrients, in addition to removal of metabolites and cellular debris. Despite the knowledge of pro-inflammatory mediator regulation in angiogenesis, the influence of arachidonic acid-derived mediators, as prostaglandins, generated by cyclooxygenase (COX)-1 and -2 pathways, is still poorly investigated. Experimental models using bothropic snake venoms are ideal for broad characterization of muscle regeneration, because they contain phospholipases A₂ and snake venom metalloproteinases (SVMPs) that promote inflammation and myotoxicity. Moreover, SMVPs affect the vascular structure due to matrix extracellular disruption, increasing the impairment of tissue regeneration course. The study aim was to investigate the regulatory role of prostaglandins from COX-1 and -2 pathways in hemorrhage, muscle ischemia and matrix metalloproteinases (MMP)-9, -10 and -13 production, during skeletal muscle degeneration and regeneration induced by *Bothrops asper* venom (BaV). Distinct groups of male Swiss mice (30g) received BaV (2.5 mg/kg/50µL) in gastrocnemius muscle or saline solution (0,9%) in contralateral muscle (control). After 30min, 2 and 6 days (d) i.m. injection, animals received lumiracoxib (LUM-selective COX-2 inhibitor/20mg/kg) or indomethacin (IND-non-selective COX inhibitor/5 mg/kg) or vehicle (Tween1%-TW) by oral administration. After 24h, 7 and 21d i.m. injection, mice were anesthetized with ketamine and xylazine (100 and 10 mg/kg) for flowmetry of both limbs by Laser Doppler (Moor Instruments). Next, animals were sacrificed by isoflurane saturated environment, and both gastrocnemius muscles were collected and processed to hemoglobin or MMPs-9, 10 and 13 quantifications, using Drabkin solution or enzyme immunoassay, respectively. In degenerative phase (24h), mice treated with BaV/IND showed significant decrease (p<0,05) of blood flow (39±4%) when compared to BaV/TW (58±3%). In regenerative phase (21d) animals pretreated with BaV/IND and BaV/LUM showed both significant increase (p<0,05) in limb blood flow (116±7 and 110±4%, respectively) in comparison to BaV/TW mice (94±6%). After 24h of i.m. treatment, BaV/IND mice showed significant presence of tissue hemoglobin (22±2 mg/g), when compared to BaV/TW animals (17±1 mg/g). In regeneration initial phase (7d), animals BaV/IND (424±42, 733±70 and 3247±150 ng/mL/mg, respectively) or BaV/LUM (427±10, 676±109 and 2919±256 ng/mL/mg, respectively) showed significant decrease (p<0.05) in MMP-9, -10 and -13 concentrations when compared to BaV/TW animals (587±46, 1172±98 and 4596±427 ng/mL/mg, respectively). After 21d when treatment with COX inhibitors had been stopped, animals that previously received BaV/LUM showed increase (p<0.05) of MMP-9 and -10 (596±84 and 1696±292 ng/mL/mg, respectively) in comparison to BaV/TW (483±30 and 708±46 ng/mL/mg, respectively). Groups BaV/LUM or BaV/IND showed an increase in MMP-13 (4874±495 and 4014±288 ng/mL/mg, respectively), compared to BaV/TW group (2932±28 ng/mL/mg). Previous data demonstrate that mediators from COX-1 pathway are involved in mechanisms that preserve the vascular disruption, which could contribute to reduction of skeletal muscle hemorrhage and ischemia, induced by BaV. In the tissue regeneration phase and absence of COX inhibitors effect, our data suggest that presence of prostaglandins in the tissue are involved in upregulation of MMPs, specially MMP-13, which should contribute to the increase in angiogenesis, and decreased tissue ischemia. **Financial Support:** FAPESP and Capes **License number of ethics committee:** (CEUA 9047230920)

04.005 Anti-inflammatory effect of the lipid-transferring protein, MCLTP1, isolated from noni seeds *Morinda citrifolia* L. (Rubiaceae) in mice burns model. Rabelo LMA¹, Kurita BM¹, Macedo FS¹, Rangel GFP¹, Souza TFG¹, Duarte RS¹, Costa AS², Oliveira HD², Alencar NMN¹. ¹UFC, Dpt of Physiology and Pharmacology, Biochemical Pharmacology Lab, Drug Research and Development Nucleus, Brazil; ²UFC, Dpt of Biochemistry, Medicinal Chemistry Lab, Drug Research and Development Nucleus, Brazil

Burns are defined as injuries to any organic tissue caused by thermal trauma and their complexity varies according to the extension and depth of the affected site. Currently, this pathology represents 38% of the main injuries seen in the Unified Health System (SUS) in Brazil and stands out due to the high mortality rate and degree of disability it can cause to those committed. Even with several therapeutic strategies available, the costs of treating burns are still quite high. Therefore, it is interesting to consider more cost-effective and effective therapeutic options. The Brazilian biodiversity profile suggests numerous plants with therapeutic potential to be considered as bioactive. In addition to their widespread popular use, they are low-cost and easily accessible options. *Morinda citrifolia* (noni) is a plant that has anti-inflammatory and antibiotic effects. Studies have associated the type 1 lipid transfer protein isolated from

Morinda citrifolia (McLTP1) with these effects in models of liver and enteric injury. So, this work investigated the effect of McLTP1 in mouse burn models. For this, a dermatological cream containing McLTP1 at concentrations of 0.25% and 0.5% was made to treat female Swiss mice (25±30 g) in a superficial burn model. All procedures were approved by the ethics committee according to the acceptance protocol: 02170619-0 and funded by CAPES and CNPq. The injury was induced by direct contact with a square stainless steel hot plate (1.5 cm²). The animals were treated topically with 0.9% NaCl saline solution (Sham; N=8), or with the dermatological creams: positive control with 1% silver sulfadiazine (Sulfa; N=8), cream containing McLTP1 a 0.25% and 0.5% (N=16). Euthanasia was made on the 3rd experimental day. Measurements of the activity of the enzyme myeloperoxidase, the levels of inflammatory cytokines such as TNF- α , IL-1 β and IL-6 and anti-inflammatory cytokines such as IL-10 and vascular mediators such as VEGF were made with samples from the back skin. Differences were considered significant when the comparison between means resulted in a p-value <0.05. It was observed that 0.5% McLTP1 prevented the increase in myeloperoxidase enzyme activity compared to the Sham group (p<0.05), suggesting its potential to reduce neutrophil recruitment to the injury site. This protein, at the same concentration, also had an anti-inflammatory effect by reducing the levels of TNF- α , IL-1 β and IL-6 (p<0.05) and increasing the levels of IL-10 when compared to Sham. 0.5% McLTP1 also decreased VEGF levels compared to the Sham group (p<0.05), which demonstrates its ability to decrease vasodilation and vascular permeability during acute inflammation. In short, McLTP1 modulated the initial acute inflammatory process in this experimental model and could, in the future, be used in treatment protocols for this type of injury. Its healing potential is being further investigated, so that it can be considered an acceptable alternative for the treatment of superficial burns. **Keywords:** Lipid transfer protein, *Morinda citrifolia*, burn, healing. **License number of ethics committee:** 02170619-0

04.006 Role of hyperglycemia and inflammation in bone marrow derived macrophages in Type 1 Diabetes. Sousa ESA, Queiroz LAD, Martins JO. Lab of Immunoendocrinology, Dpt of Clinical and Toxicological Analyses, School of Pharmaceutical Sciences of Univ São Paulo (FCF-USP), São Paulo, Brazil

Introduction: Hyperglycemia causes damage to the immune system, providing diabetic individuals with greater susceptibility to infections when compared to non-diabetic individuals. The high susceptibility to infections is, at least in part, due to an inadequate immune response. Thus, the aim of this study was to evaluate the influence of hypoglycemic in the autophagy process and lipopolysaccharide (LPS)-induced response in bone marrow derived macrophages (BMDM) from diabetic and non-diabetic animals. **Methods:** T1DM was induced in mice by alloxan (60 mg/kg, i.p) [CEUA/FCF/USP n°570/2018]. BMDM from diabetic and non-diabetic C57BL/6 mice were used. The macrophages were maintained in culture medium with normal concentration (5.5 mM) and high glucose concentration (25 mM) and were stimulated or not with LPS at the concentration of 100 ng/mL and Nigericine (20 μ M), at times of 30 minutes, 2, 4, 6, and 24 hours. The cytokines were dosed by enzyme-linked immunosorbent assay (ELISA) at all times. **Results:** It was possible to verify alterations in the secretion of pro-inflammatory cytokines IL-6, IL-1 β and TNF- α , where BMDMs showed an increase in the secretion of these cytokines when stimulated with LPS+Nigericin. BMDM from diabetic mice showed a decreased after 2 hours in TNF- α secretion in normoglycemic and hyperglycemic medium with LPS+Nigericin stimulation, when compared to the control group. On the other hand, at the times of 30 minutes, 4, 6 and 24 hours, BMDM of diabetic mice showed an increased in IL-1 β secretion in normoglycemic and hyperglycemic medium with LPS+Nigericin stimulation when compared to healthy animals. **Conclusions:** Alterations in pro-inflammatory secretion may directly interfere in the inflammatory response of diabetic individuals, causing an imbalance in the immune response of these patients to infections, making them susceptible to develop more severe pictures of the disease. Therefore, we can suggest that hyperglycemia plays an important role in the inflammatory response of BMDM in diabetic mice, causing an imbalance in cellular homeostasis. **License number of ethics committee:** CEUA/FCF/USP n°570/2018

04.007 Gamma-Terpinene improves pruritus and atopic dermatitis-like lesions in animal models. Bandeira SRM¹; Lima CMB¹, Reis-Filho AC¹; Oliveira LSA¹; Gonçalves RLG¹; Rezende DC¹; Nunes DB¹; Pinheiro-Neto FR¹; Trindade GNC¹; Barros RO², Ramos RM², MEDEIROS MGF¹, Almeida FRC¹, Oliveira FA¹ ¹Center for Research on Medicinal Plants, Federal Univ of Piauí, Teresina, Brazil ²Research Lab in Information Systems, Information Dpt, Environment, Health and Food Production, Federal Inst of Education, Science and Technology of Piauí, Teresina, Brazil

Itch is a common symptom of dermatological diseases (PARISER et al., 2020). Atopic dermatitis (AD) is a chronic inflammatory dermatosis characterized by lesions and intense desire to scratch (KU et al., 2017; KANG, et al., 2021). Gamma-terpinene (GT) is a monoterpene found in essential oils of various plant species and it has important biological activities (RAMALHO et al., 2015). In this work, we evaluated the

effect of GT on pruritus and AD models in mice (ECAE/UFPI 517/2018) and its interaction with the histamine receptor (H1) and X1 receptor coupled to the G protein related to human Mas (hMrgprX1) by molecular docking. Scratching behavior was induced by subcutaneous injection of compound 48/80 (C48/80) in the dorsal region of the head after treatment (v.o) with saline, GT (25, 50, 100 or 200 mg/kg) or cyproheptadine (10 mg/kg) (KURASHI et al., 1995). Dinitrochlorobenzene (DNCB) was used on the dorsal skin mice to induce AD-like cutaneous lesions and ear edema (CHAN et al., 2013). The levels of IgE, LDH and TNF- α , IL-1 β , IL-4 and IL-6 were measured. The results were calculated in relation to the saline group and the values were expressed as mean \pm S.E.M, considering $p < 0.05$. GT (100 and 200 mg/kg) reduced (30.0 \pm 2.70 and 27.16 \pm 8.04, respectively) the scratching induced by C48/80. Molecular docking suggests interaction between GT and H1 and hMrgprX1 receptors, with free binding energy of -6.47 and -5.8 respectively. In the DNCB-induced AD, GT (100 and 200 mg/kg) remarkably alleviated the AD-like skin lesions (3.37 \pm 0.62 and 4.12 \pm 0.48, respectively). Histological examination showed that GT (100 and 200 mg/kg) reduced skin thickening, spongiosis, cell infiltration, cell disarray and fibrosis; GT reduced ear edema (44.62 \pm 7.89 and 44.0 \pm 8.22) and IgE levels (45.19 \pm 2.10 and 48.76 \pm 3.46) at doses of 100 and 200 mg/kg, and LDH levels (750.75 \pm 26.57) only at the 200 mg/kg dose. Additionally, GT (100 and 200 mg/kg) reduced the levels of TNF- α (208.4 \pm 37.35 and 287.4 \pm 59.08), IL-4 (139.5 \pm 56.42 and 172.0 \pm 41.76) and IL-6 (85.11 \pm 33.03 and 90.78 \pm 16.25), respectively. Nevertheless, only at the dose of 100 mg/kg there was a reduction in IL-1 β levels (236.1 \pm 55.88). In conclusion, GT attenuated C48/80-induced pruritus and inflammation in DNCB-AD model, and it represents a potential therapeutic option to control the pruritus and inflammatory symptoms of AD. This work was supported by the UFPI and Research Support Foundation of the State of Piauí. **References:** PARISER, D. M. J. Am. Acad. Dermatol. v. 82, p. 1314, 2020. KANG, J. Biomed. Pharmacother., v. 137, 2021. KU, J. M. BMC Complement. Altern. Med., v. 17, p. 98, 2017. RAMALHO, T. R. Planta Med., v. 81, p. 1248, 2015. KURASHI, Y. Eur. J. Pharmacol., v. 275, p. 229, 1995. CHAN, C. C. J. Dermatol. Sci., v. 72, p. 149, 2013. **License number of ethics committee:** Ethics Committee for Animal Experimentation (ECAE)/UFPI: 517/2018

04.008 **Investigation of the effect of fluoxetine on sickness behavior in zebrafish (*Danio rerio*).** Petry F¹, Ultramari AR¹, Da Costa CAM¹, Kuhn KZ¹, Garbinato CLL¹, Aguiar GPS¹, Kreutz LC², Oliveira JV³, Siebel AM¹, Muller LG¹. ¹Unochapecó Chapecó, Brazil; ²UPF Passo Fundo, Brazil; ³UFSC Florianópolis, Brazil

Introduction: Inflammation is a physiological response of the body and a possible triggering factor for other diseases, including depression, which is currently considered the most disabling illness and a public health problem. Its neurobiology is based mainly on the monoaminergic theory. However, other hypotheses have been studied, including the involvement of the activation of immunoinflammatory pathways (increased production of proinflammatory cytokines). Thus, several theories support depression as a psychoneuroimmunological phenomenon, since the increase in proinflammatory cytokines in depression would result in symptoms related to it, such as the sickness behavior. However, there are no data regarding this hypothesis using zebrafish (*Danio rerio*) as the animal model. Thus, this research aimed to investigate the effects of immune response activation and fluoxetine on the development of sickness behavior in zebrafish, by the novel tank, social preference and novel object tests. **Methods:** Female and male zebrafish were used in the study (180 days of life; 0.3-0.5 g). The inflammatory response was induced by intraperitoneal (ip) inoculation of 10 μ l of *Aeromonas hydrophila* inactivated with formalin. The experimental groups were: sham (n=30, received 10 μ l sterile PBS ip), immunostimulated (n=30, received 10 μ l sterile PBS ip and 30 min after, 10 μ l *A. hydrophila* ip) and fluoxetine 10 mg/kg (n=30, received fluoxetine 10 μ l ip and 30 min after, 10 μ l *A. hydrophila* ip). After 24 h, the animals were evaluated in the novel tank test, social preference and novel object tests. After the behavioral tests, the fish were cryoanesthetized and euthanized by decapitation. The brains were dissected and will be used for *ex vivo* assays. Results were analyzed using one-way ANOVA post-hoc Tukey's test or Kruskal-Wallis post-hoc Dunn's test (non-parametric data) (significance for $p < 0.05$). **Results:** There was a significant difference in behavioral parameters between animals in the sham, immunostimulated and fluoxetine groups. In the novel tank test, there was a reduction in the total distance traveled (m), displacement speed (m/s) and crossings in males and females. The administration of fluoxetine prevented these alterations and increased the time (s) and distance traveled (m) at the top of the apparatus. In the social preference test, only immunostimulated females showed a significant reduction in the time (s) spent in the segment containing the conspecifics, but fluoxetine pretreatment prevented these effects. Finally, in the novel object test, only females showed a significant reduction in the time (s) of exploration of the novel object, and fluoxetine pretreatment decreased the latency (s) for entry into the novel object zone. **Conclusion:** The sickness behavior was successfully induced by *A. hydrophila* inoculation. Nevertheless, females were more susceptible to reduced social interaction and exploration caused by the inflammatory process. In addition,

fluoxetine pretreatment prevented the sickness behavior induced by the immune challenge. Financial support: FAPESC. CEUA Protocol Number: Unochapecó 009/2020 License number of ethics committee: Unochapecó 009/2020

04.009 **Investigation of the neurogenic component in the irritative effect of dithranol in mice.** Silva AMD, Ferreira J UFSC Florianópolis, Dpt of Pharmacology, Brazil

Introduction: Dithranol is one of the most effective topical drugs for the treatment of plaque-type psoriasis. However, its clinical use is limited by irritating adverse reactions at skin, such as edema, erythema and pruritus, whose mechanisms are not understood (LOWE et al. Arch. Dermatol., v. 117, p.698. 1981). The stimulation of TRPV1 positive peripheral terminals of sensory fibers may cause neurogenic inflammation, related to adverse effect of some drugs. Since the neurogenic component of dithranol irritation is unknown, the purpose of the present study was to verify whether the adverse effects of dithranol could be related to the stimulation of TRPV1 positive fibers. **Methods:** Dithranol cream was applied topically to the right ear of male C57BL/6 mice. Dose-response curve was performed to define the minimal irritative dose of dithranol (0.01%, 0.1% and 0.5% - $6\mu\text{g}/\text{ear}$). In control animals, only Lanette cream was applied. Edema (difference in ear thickness after treatment compared to baseline - Δ/mm), erythema (scores) and pruritus (scratching after von Frey filament 0.02 g application) were investigated from 2 hours to 6 days after dithranol application (VAN DER FITS et al. J. Immunol. Res., v. 182, p. 5836. 2009; HUANG et al. 565, Nature, p.86 2019). To investigate whether the desensitization of TRPV1 positive fibers reduced the irritant effects, animals were topically pretreated repeatedly (3 times, every other day) with cream containing 1% capsaicin, and posteriorly treated with dithranol. Data are expressed as mean \pm S.E.M (n=7-10), performed 2-3 independent experimental blocks, analyzed by one or two-way ANOVA, followed by Dunnett's Post hoc test. **Results:** Topical treatment with 0.5% dithranol caused edema and erythema, but not pruritus, starting at 6 am, peaking at 1 day and lasting up to 6 days after treatment. At the peak of irritation (1 day), the minimum dose of dithranol that caused edema was 0.5% (increase in ear thickness of 0.029 ± 0.015 , 0.032 ± 0.011 , 0.063 ± 0.017 and 0.164 ± 0.026 mm for vehicle, 0.01, 0.1 and 0.5% dithranol). At the same time, the minimum dose of dithranol that induced erythema was 0.1% (scores of 0.444 ± 0.176 , 0.556 ± 0.176 , 1.100 ± 0.100 and 1.300 ± 0.153 for vehicle, 0.01, 0.1 and 0.5 % dithranol). After the first treatment, topical application of capsaicin (1%, an agonist of TRPV1 receptor) produced a marked edematogenic response 1 hour after the first application (0.112 ± 0.021 mm) when compared to vehicle (0.026 ± 0.008 mm). Demonstrating the defunctionalization of TRPV1 positive fibers at the applied skin, capsaicin was unable to cause edema after the second and third application (0.012 ± 0.010 and 0.017 ± 0.012 mm), respectively. However, the edema or the erythema caused by dithranol (0.5%) was not altered by the pre-treatment with capsaicin (at 1 day, the ear thickness was 0.106 ± 0.042 and 0.117 ± 0.018 and the erythema was 0.833 ± 0.167 and 1.143 ± 0.143 in vehicle or capsaicin pre-treated ears). **Conclusion:** The present study shows that topical application of dithranol in mouse ears causes acute inflammatory responses with formation of edema and erythema, which are independent of the activation of TRPV1 positive fibers. These results suggest that skin irritation caused by dithranol does not have a neurogenic component. **Acknowledgments:** Financial support by CNPq, CAPES and INCT-INOVAMED (MCTI/CPES/CNPq/FAPESC). The project was approved by the CEUA - UFSC (6048210720). **License number of ethics committee:** 6048210720

04.010 **Renina is involved in increasing osteopontin expression in the jaws of diabetic mice with periodontitis.** Ribeiro BS^{1,2}, Balera VGB^{1,2}, Frasnelli SCT¹, Soares VL³, Santos CF⁴, Oliveira SHP¹ ¹Dept. Basic Science; São Paulo State Univ, School of Dentistry, Araçatuba, Brazil ²Multicenter PPG in Physiological Sciences, SBFis ³Dpt of Biological Science, Bauru School of Dentistry, Univ of São Paulo, Brazil ⁴Dpt of Stomatology, Bauru School of Dentistry, Univ of São Paulo, São Paulo, Brazil

Introduction: Periodontal disease (PD) is an inflammatory disease that affects the teeth supporting tissue, and systemic comorbidities, such as diabetes mellitus, may enhance its severity, leading to an exacerbated inflammation and increased alveolar bone loss. The renin-angiotensin system (RAS) is associated with the bone tissue dynamics by the action of angiotensin II, which acts indirectly on bone cells through vascular flow of the bone marrow or by the release of inflammation mediators that increase vascularization endothelial that stimulates osteoclastogenesis. In this work we used Aliskiren, a renin inhibitor, to better study the effect of this system on bone metabolism. **Aims:** To evaluate the role of the RAS on the gene expression of bone metabolism markers in the mandible of diabetic mice with PD. **Methods:** Adult male BALB/c mice were used. Diabetes mellitus was induced by streptozotocin (single intraperitoneal injection; 200mg/kg), and after 7 days mice were considered diabetics when presenting blood glucose > 250 mg/dL. PD was induced by the insertion of a bilateral silk thread ligature, in the first lower molars, maintained for 15 days, and Aliskiren (renin inhibitor) was administered once a day, for 16 days (gavage;

50 mg/kg), beginning the day before PD induction. Mandibles were harvested for gene expression analysis (real-time RT-PCR) of bone formation (Runx2, Osx, Catnb, Alp, Col1a1, Opn, Ocn, Bsp, Bmp2), remodeling markers (Opg, Rankl, Rank), and resorption markers (Trap, Ctsk, Mmp2 and -9, Oscar, Vtn, Itga5 and Itgb5). **Results:** Diabetes mellitus caused an increase expression of the transcription factors (Runx2, Osx, and Ctnnb), compared to non-diabetic mice, which were reduced with the induction of PD. The data obtained in relation to bone formation factors showed that diabetes increased the expression of Alp, Col1a1, Ocn and Bmp2 compared to the normal group, and PD induced a decrease in this expression, while in Normal animals (NPD) it led to an increase of the expression of Cola1a and Bsp. In the Opg, Rankl and Rank axis, diabetes led to a higher expression of Rankl and Rank, and a decrease in Opg, thus stimulating bone resorption. Treatment with Aliskiren was able to decrease Opg and Rankl expression only in normal animals (Alisk+PD). Regarding resorption factors, we observed that PD led to an increase in the production of Trap, Mmp9, Cstk, Oscar in both normal and diabetic animals. Treatment with Aliskiren promoted a decrease in the expression of Trap, Mmp2 and Itgb5, only in treated Normal animals (Nalisk). **Conclusion:** Diabetes mellitus provides a favorable environment for changes in bone metabolism and periodontal disease induction enhances these results, however aliskiren does not have a significant positive effect on these data. **Acknowledgments:** FAPESP (grant no. 2015/03965-2, and scholarship no. 2020/03068-9). **License number of ethics committee:** 000974-2016

04.011 **Role of TLR-4 in a model of COPD exacerbation induced by H1N1 virus infection** Almeida MD¹, Ferrero MR¹, Garcia CC², Martins MA¹. ¹Lab of Inflammation, Oswaldo Cruz Inst, Fiocruz, Rio de Janeiro, Brazil; ²Lab of respiratory virus and Measles, Oswaldo Cruz Inst, Fiocruz, Rio de Janeiro, Brazil

Introduction: Chronic Obstructive Pulmonary Disease (COPD) is mainly associated with smoking and is characterized by pulmonary airflow obstruction, seriously affecting patient's life quality. COPD patient's can experience acute exacerbations (AECOPD) of symptoms, frequently triggered by airway infections. Which decreases the sensitivity to available medications, becoming the main cause of hospitalization. Influenza virus infection is among the most common causes of AECOPD. Damage-associated molecular patterns (DAMPs) play a critical role in the pathophysiology of COPD, through binding to PRRs such as TLR4. During periods of AECOPD, an increase in TLR4 expression is observed in neutrophils from patients, which could contribute to the exacerbated recruitment of these cells into the airways. In this context, we developed a short-term EADPOC model, to assess the effect of treatment with the TLR4 antagonist (Tak-242) on pulmonary inflammatory exacerbation. **Methods:** Thirty-five female C57Bl/6 mice were equally distributed into 5 groups (n=7) as follows: (1) room air (AA), (2) cigarette smoke (CS), (3) H1N1, (4) - **Results:** We found increased Penh values in CSH1N1 animals when compared to the AA, Cs and H1N1 control groups. Leukocyte counts in the airways showed that the AA group and the CS group presented comparable leukocyte counts, viral infection induced a significant augmentation of both macrophages and neutrophils, whereas CSH1N1 group showed increased numbers of macrophages and exacerbated numbers of neutrophils compared to the H1N1 group. Both, lung function deterioration and exacerbated leukocyte counts in the airways were responsive to treatment with TAK-242. By analyzing the presence of the messenger RNA encoding TLR4, compared to control animals (AA), we observed that neither the CS group nor the infection with H1N1 significantly modulate TLR-4 expression, whereas, the combination of both stimuli (CSH1N1) significantly increased TLR-4 expression. **Conclusion:** Our results show that the CS-H1N1 combined insult led to an exacerbation of pulmonary inflammatory changes in C57Bl / 6 mice, which was responsive to the treatment with TAK-242. These findings suggest that the increased expression of TLR-4 can play a pivotal role in acute exacerbation of H1N1-induced lung inflammation in mice exposed to cigarette smoke. Financing source: CAPES, CNPq and FAPERJ. **License number of ethics committee:** CEUA - L030 / 15.

04.012 **Variation in the chemical composition and anti-inflammatory activity of the essential oil of *Schinus terebinthifolius* Raddi leaves in different Brazilian states.** Marangoni JM¹, dos Santos SM², Oliveira-Júnior PC¹, Vieira AR², da Silva ME², Narcizo LL¹, Cardoso CAL³, Formagio ASN^{1,2} ¹Faculty of Biological and Environmental Sciences, Federal Univ of Grande Dourados, Brazil ²Faculty of Health Sciences, Federal Univ of Grande Dourados, Brazil ³State Univ of Mato Grosso do Sul, Univ City of Dourados, Brazil

Introduction: *Schinus terebinthifolius* Raddi. (Anacardiaceae) is native to South America and can be found in different states of Brazil, popularly known as pink pepper and being widely used in traditional medicine to treat uterine inflammation, arthritis, and urinary tract infections¹. The objective of this work was to verify the chemical composition of the essential oil of *S. terebinthifolius* leaves (EOST), collected in the Brazilian states of Bahia (OEST-BA), Espírito Santo (OEST-ES), Paraná (OEST-PR), Rio Grande do Sul (OEST-RS), Mato Grosso do Sul (OEST-MS) and São Paulo (OEST-SP), and evaluated for anti-inflammatory and analgesic activity in mice. **Methods:** The OEST was extracted from the leaves by hydodistillation and

quantified by GC/MS². Male Swiss mice were divided into (6 animals/group) and treated orally with OEST-BA, OEST-ES, OEST-PR, OEST-RS, OEST-MS and OEST-SP, single dose (30mg/kg) and vehicle. In the positive control group, was used the steroidal anti-inflammatory dexamethasone (DEXA, 1.0mg/kg, subcutaneously). The animals received 50 µL of carrageenan solution in the right paw (Cg - 300µg). Edema was the difference in thickness of both legs by means of a digital micrometer (DIGIMESS 110-284) after 2 and 4 hours³. **Results:** The essential oil from *S. terebinthifolius* (EOST) presents a predominance of monoterpenes and sesquiterpenes, being identified in OEST-BA, OEST-RS and OEST-MS twenty substances; OEST-ES and OEST-PR twenty-one and OEST-SP sixteen substances. The major substances found in each sample were OEST-BA (α-Pinene, 22.34%), OEST-ES (Limonene, 17.01%), OEST-PR (Limonene, 14.01%), OEST-RS (Limonene 19.45%), OEST-MS (α-Pinene, 14.90%) and OEST-SP (α-Phellandrene, 20.13%). The Cg-induced paw edema in a single dose of EOST (30 mg/kg) inhibited the formation of Cg-induced paw edema in comparison to the control group, the percentage of inhibition was 39.99% (EOST-PR), 37.00% (EOST-BA), 34.90% (EOST-MS), 32.05% (EOST-ES and EOST-RS) and 27.52% (EOST- SP) at 2h, but this reduction does not continue after 4 h of observation, with inhibitions of 26.33% (EOST-BA and EOST-ES), 23.70% (EOST-PR and EOST-RS) and 21,05% (EOST-MS), EOST-SP did not have the same effect as the others, which may be correlated with the chemical composition which demonstrated a different constituent profile and the positive control (DEXA) showed an inhibition of 73.33% and 78.94% after 2h and 4h. **Conclusion:** The essential oil of the leaves showed potential anti-edematogenic activity in mice, proving to the traditional use of this plant for the treatment of inflammatory processes. **Financial Support:** Capes, Fundect and UFGD **References:** 1. Tlili, N., Ind. Crop. Prod., v. 122, p. 559, 2018. 2.Adams, R.P., Identification of essential oil components by gas chromatography/mass spectrometry, p.804, 2007. 3. Henriques, M.G., Br. J. Pharmacol., v. 99, p. 164, 1990. **License number of ethics committee:** CEUA/ UFGD protocol n° 33/2019

04.013 Role of RAS in glucose metabolism and autophagy in metabolically active tissues from type 1 diabetic and obese mice. Guimarães JPT^{1,2,3}, Menikdiwela KR², Ramalho T³, Queiroz LAD¹, Kalupahana NS^{2,4}, Jancar S³, Ramalingam L², Moustaid-Moussa N², Martins JO¹. ¹Lab of Immunoendocrinology, Dpt of Clinical and Toxicological Analyses. School of Pharmaceutical Sciences of Univ of São Paulo (FCF-USP), São Paulo, Brazil; ²Lab of Nutrigenomics, Inflammation and Obesity Research, Dpt of Nutritional Sciences, Texas Tech Univ (TTU), Lubbock, Texas, USA; ³Lab of Immunopharmacology, Dpt of Immunology, Inst of Biomedical Sciences, Univ of São Paulo (ICB-USP), São Paulo, Brazil; ⁴Dpt of Physiology, Univ of Peradeniya, Peradeniya, Sri Lanka

Introduction: Impaired metabolic functions underlie the pathophysiology of diabetes and obesity. Although these diseases have different mechanisms, they may share common physiological pathways. Renin-angiotensin system (RAS) is one of the physiological systems that can be related to the pathophysiology of both diseases. The over activation of RAS in metabolically active tissues exert pro-inflammatory effects via angiotensin II (Ang II), often linked to dysfunction in cellular processes such as autophagy, which is associated with obesity and diabetes. **Methods:** We have used C57bl/6 mice to induce Type 1 diabetic (T1D) and to overexpress angiotensinogen (Agt-Tg) to induce obesity. T1D was chemically induced with streptozotocin (STZ), while obesity was induced with high fat diet (HF), and to inhibit RAS we treated the diabetic and obese mice with captopril. After the treatments, plasma and mice tissues were uptake for further analysis. We evaluated (1) expression of RAS, autophagy, and insulin receptor gene markers by real-time PCR, (2) protein expression of RAS components, autophagy, and insulin receptor via Western blotting, (3) production of adipokines in plasma samples by bead-based multiplex assay. **Results:** We observed that T1D mice have lower plasma leptin and resistin levels and higher non-esterified fatty acids (NEFA), with similar results from T1D mice treated with angiotensin converting enzyme inhibitor captopril. Regarding gene expression, we found enhanced Agt, At1, Insr, Irs1 and Beclin1 in muscle and liver from T1D mice treated with captopril. In muscle, high fat (HF) fed Agt-transgenic (Tg) mice demonstrated increased Irs1, however both HF Agt-Tg and HF Agt-tg treated with captopril mice, showed decreased Agt, At1, Insr, Irs1, Ampk, Beclin1, Agt12 and Lc3 in the liver. **Conclusion:** Our results suggest that captopril treatment stimulates components of RAS, insulin signaling and autophagy process in both muscle and liver, indicating a possible role of captopril in insulin sensitivity and autophagy activation. **Animal Research Ethical Committee:** (CEUA no. 08/2014 - book 03 - ICB/USP) **Financial Support:** CNPq (310993/2020-2); FAPESP (2018/50004-6; 2018/23266-0; 2019/09983-3; 2020/03175-0) **License number of ethics committee:** CEUA no. 08/2014 - book 03 - ICB/USP

04.014 Investigating mechanisms behind angiotensin, leukotrienes and insulin signaling in type 1 diabetes. Guimarães JPT², Queiroz LAD¹, Jancar S², Moustaid-Moussa N³, Martins JO¹ ¹Lab of Immunoendocrinology, Dpt of Clinical and Toxicological Analyses. School of Pharmaceutical Sciences of Univ of São Paulo (FCF-

USP), São Paulo, Brazil ²Lab of Immunopharmacology, Dpt of Immunology, Inst of Biomedical Sciences, Univ of São Paulo (ICB-USP), São Paulo, Brazil ³Lab of Nutrigenomics, Inflammation and Obesity Research, Dpt of Nutritional Sciences, Texas Tech Univ (TTU), Lubbock, Texas, USA

Diabetes mellitus (DM) is a chronic metabolic disease that may be associated with other physiological disorders, such hypertension (HTN). Some studies have shown common pathways linking diabetes to insulin resistance and to the renin-angiotensin system(RAS), a leading regulator of blood pressure. Angiotensinogen (Agt) is the main precursor of RAS. Activation of Agt in adipose tissue exerts pro-inflammatory effects, often linked to dysfunctions in cellular processes, such as autophagy. In diabetes, the pro-inflammatory profile in adipose tissue leads to the production of adipokines and cytokines that promote glucose intolerance and insulin resistance. Several studies have shown that the lipid mediator Leukotriene (LT)B4 plays a central role in the establishment of insulin resistance and in the development of low-grade inflammation in DM. In macrophages, activation of the LTB4 receptor potentiates the pro inflammatory phenotype. In the adipose tissue of T2D mice, monocyte recruitment, macrophage polarization into M1 profile, pro inflammatory cytokine secretion and insulin resistance have been shown to be linked to LTB4 production. In the liver and muscle, LTB4 also induces inflammation and insulin resistance. Therefore, we investigated the involvement of RAS and LTs in the muscle and liver of mice with Type 1 diabetic (T1D), in the autophagy and insulin pathways. We used 129sve mice and 129sve mice knockout for leukotrienes (5LO^{-/-}). T1D was chemically induced with streptozotocin (STZ) and to inhibit RAS, we treated the mice with captopril. Results: We observed that plasma levels of non-esterified fatty acids (NEFA) from 5LO^{-/-} T1D mice are higher, but the treatment of captopril in these mice decreased NEFA levels. We also observed decreased plasma levels of resistin and leptin, despite the treatment with captopril or the presence of LTs. After insulin tolerance test, T1D 5LO^{-/-} mice showed enhanced insulin sensitivity compared to T1D Wt mice, and this effect was more pronounced in 5LO^{-/-} T1D mice treated with captopril. In muscle of T1D mice, treatment with captopril increased the expression of *Agt*, *At1*, *Insr*, *Irs1*, *Ampk*, *Beclin1*, *Atg5*, *Atg7*, *Atg12*, *Atg14* and *LC3*. In liver of T1D Wt and 5LO^{-/-} mice, the treatment with captopril increased the expression of *Agt*, *At1*, *Insr*, *Irs1* and *Ampk*. Were also observed that in the liver and muscle of these mice, the expression of autophagy markers was independent of LTs presence. **Conclusion:** Analyzing the set of results, we conclude that LTs contributes to the development of insulin resistance in T1D. In addition, treatment with captopril recovered the gene expression of the main markers of the insulin signaling pathway, as well as those related to the functioning of the autophagy pathway in muscle and liver, indicating a possible role of captopril in insulin sensitivity and activation of autophagy in these diseases. **License number of ethics committee:** (CEUA no. 08/2014 - book 03 - ICB/USP)

04.015 **Phenotypic and functional characterization of T lymphocytes of alloxan and streptozotocin induced diabetes.** Queiroz LAD¹, Guimarães JPT¹, Assis JB², Souza ESA¹, Milhomem AC³, Sunahara KKS⁴, Sá-Nunes A², Martins JO¹. ¹USP-FCF São Paulo, ²Dpt of Clinical and Toxicological Analyses, Brazil; USP-ICB São Paulo, Dpt of Immunology, Brazil; ³UFG-IPTSP Goiania, Dpt of Microbiology, Immunology, Brazil; USP-FM São Paulo, ⁴Dpt of Sciences-Experimental Physiopathology, Brazil

Introduction: Alloxan (ALX) and streptozotocin (STZ) are the most common diabetogenic agents used to induce type 1 diabetes (T1D) in animal models, with several studies reporting their toxic effects on the immune response. This study aims to evaluate the differences in immune parameters caused by ALX and STZ, with special attention to T cell phenotype, biology, and function in lymphoid and non-lymphoid tissues. To clarify these effects, this study aims to evaluate the differences in immune parameters caused by ALX and STZ, with special attention to T cell phenotype and function in lymphoid and non-lymphoid tissues. **Methods:** T1D was induced in C57BL/6J mice by ALX (a single dose of 60 mg/kg, i.v) or STZ (five daily doses of 65 mg/kg, i.p) [CEUA/FCF/USP n° 388] and the animals were followed up for 180 days and evaluated the following parameters: (a) total and differential count of bone marrow cells and peripheral blood; (b) characterization of the lymphocyte composition in the thymus, spleen and pancreas, with surface markers (CD11b, CD3, CD4, CD8, CD19 and CD25); (c) determination of cytokines tumor necrosis factor (TNF)- α , interferon (IFN)- γ , interleukin (IL)-1 β , IL-2, IL-4, IL-6, IL-10, IL12p70 and IL-17 in the spleen and pancreas homogenate; (d) morphological analysis of the thymus, spleen and pancreas; (e) activation of the adaptive response and production of immunoglobulins (IgG) 1 and IgG2a in animals immunized and challenged with ovalbumin; (f) activation of spleen T lymphocytes stimulated with concanavalin (ConA). **Results:** Both ALX and STZ induced a decrease in the total number of circulating leukocytes and lymphocytes, with an increase in granulocytes when compared to control mice (CT). Mice treated with STZ exhibited an increase in neutrophils and a reduction in the lymphocyte percentage in the bone marrow. In addition, while the STZ group showed a decrease in total CD3⁺, CD4⁺CD8⁺ and CD4⁺CD8⁺ T lymphocytes in the thymus, and CD19⁺ B lymphocytes in the pancreas and spleen, the ALX group showed an increase in CD4⁺CD8⁺ and CD19⁺ lymphocytes only in the thymus. Basal levels of splenic

interleukin (IL)-1 β and pancreatic IL-6 in the STZ group were decreased. Both diabetic groups showed atrophy of the thymic medulla and degeneration of pancreatic islets of Langerhans composed of inflammatory infiltration and hyperemia with vascular dilation. ALX-induced diabetic mice showed a further decrease in reticuloendothelial cells, enhanced lymphocyte and thymocyte cell death and increased number of . Reduced *in vitro* activation of splenic lymphocytes was found in the STZ group. Furthermore, mice immunized with ovalbumin (OVA) showed a more intense antigen-specific paw edema response in the STZ group, while production of anti-OVA IgG1 antibodies in both the ALX and STZ groups was similar. **Conclusions:** These findings suggest that the effects of the diabetogenic agents ALX and STZ influenced, at different times, lymphoid organ development and the biology of their cell populations. **Financial Support:** Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP – grant # 2020/03175-0); Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq – grant # 163410/2018-6 and 310993/2020-2). **License number of ethics committee:** CEUA/FCF/USP n^o 388

04.016 **Methylglyoxal aggravates lipopolysaccharide-induced mouse lung inflammation.** Medeiros ML, Oliveira AL, Oliveira MG, Mônica FZ, Antunes, E. Dept de Farmacologia, Faculdade de Ciências Médicas, Univ de Campinas, Campinas, Brasil

Introduction and Aim: Methylglyoxal (MGO) is a highly reactive dicarbonyl species implicated in diabetic-associated diseases (1). Evidence shows that MGO modifies free amino groups yielding advanced glycation end products (AGEs), which in turn interacts with its transmembrane receptor RAGE, initiating the tissue injury. High levels of MGO are found in plasma and urine of pre-diabetic, diabetic and obese individuals (2). The anti-hyperglycemic drug metformin decreases the plasma levels of MGO, which may be due to its ability to scavenge MGO from circulation (3). Acute lung injury (ALI) symptoms and prognosis are worsened in obesity (4). Here, we hypothesized that elevated MGO levels aggravates experimental ALI, and that metformin acts to reduce the airways inflammation. Therefore, this study evaluated the lung inflammation in lipopolysaccharide (LPS)-exposed mice treated with MGO in the absence and presence of metformin. **Methods:** C57Bl/6 male mice were divided into the following groups: Control, MGO and MGO plus metformin with 4 -7 animals per group . Animals from the MGO group received 0.5% MGO in the drinking water for 12 weeks, while animals in MGO plus Metformin group received the 12-week MGO treatment plus metformin (300 mg/kg, gavage, given in the last two weeks of MGO treatment). Next, mice were intranasally instilled with saline (50 μ l) or LPS (30 μ g) to induce ALI. After 6 h, bronchoalveolar lavage fluid (BALF) and lung tissues were collected to quantify the airway infiltration and levels of cytokines and reactive-oxygen species (ROS), as well as the mRNA expressions of RAGE, NADPH oxidase isoforms NOX-1 and NOX-2, and COX-2 enzyme. **Results:** Serum MGO concentration achieved after 12-week intake was 6.7-times higher than untreated mice ($P < 0.05$). LPS exposure markedly increased the neutrophil infiltration in BALF and lung tissue, which was accompanied by higher levels of IFN- γ , TNF- α and IL-1 β in BALF compared with untreated group ($P < 0.05$). In BALF and lung tissue, MGO promoted significant increases of neutrophil infiltration and mRNA expressions of RAGE, TNF- α and IL-1 β , whereas COX-2 expression remained unchanged. Methylglyoxal treatment also significantly increased the NOX-1 and NOX-2 expressions. MGO elevated by 45% ($P < 0.05$) the ROS levels in the lung tissues of LPS-exposed mice. Treatment with metformin fully normalized the exacerbation by MGO of LPS-induced neutrophil infiltration in BALF and lung tissues. Metformin also significantly reduced the mRNA expressions of RAGE and TNF- α in lung tissues of MGO-exposed mice. **Conclusion:** MGO intake potentiates the LPS-induced mouse lung inflammation, increases RAGE expression in favor of excessive ROS generation. Metformin significantly attenuated the MGO actions in LPS group. Scavengers of MGO may be a good adjuvant therapy to reduce ALI in patients with cardiometabolic diseases. **References:** 1. Schalkwijk C.G, et al. *Physiol. Rev.* (100): 407-461; 2020 2. Hanssen N.M.J, et al. *Diabetes Care*, (41): 1689–1695; 2018 3. Kinsky O.R, et al. *Chem Res Toxicol*, (15): 227-34; 2016 4. Bellani G, et al. *JAMA*, (315): 788-800; 2016 **Financial Support:** CAPES (88882.435314/2019-01) **License number of ethics committee:** All procedures with animals were approved by the Ethics Committee on Animal Use CEUA / UNICAMP (CEUA; No. 5200-1 / 2019) where 4-7 animals were used per group

04.017 **Antioxidant effect of mitochondrial H₂S donor (AP39) in topical treatment for burn injury.** Cerqueira ARA¹, Teixeira SA¹, Coavoy-Sanchez SA¹, Oliveira JP¹, Wood ME², Whiteman M³, Muscará MN¹, Lopes LB¹, Costa SKP¹. ¹USP São Paulo, Inst of Biomedical Sciences, Dpt of Pharmacology, Brazil; Univ of Exeter Exeter, ²Dpt of Biosciences, ³College of Medicine and Health, UK

Introduction: The cutting-edge therapeutic potential of hydrogen sulfide (H₂S) donor molecules has been shown in several inflammatory conditions, including skin diseases (for review, see Coavoy-Sánchez *et al.*, 2020). Drugs associated with nanostructured delivery systems are known to improve bioactive compounds applied to the skin surface area, thus enhancing their efficacy (Dolgachev *et al.*, 2021). This study seeks

to develop and evaluate a topic nanostructured delivery system for the mitochondrial H₂S donor, AP39, in 2nd degree burn injury in mouse dorsal skin. **Methods:** Cytotoxicity and wound healing assessments were performed on 3T3 fibroblast cells exposed to AP39 (0.2, 2 or 20 µM; n=3) during 24h. Three nanostructured delivery systems were developed to accommodate AP39. Dorsal skin injury was induced in anaesthetised (isoflurane 5% in O₂) C57BL/6 female mice (12 weeks old; n=6) by direct contact with a 5 mm diameter hot surface (80°C during 30 s), which induced a 2nd degree thermal burn wound. The nanoemulsion alone or containing AP39 (200 nmol g⁻¹) was applied topically twice daily for 8 days, and the animals were killed after. Skin samples were used to evaluate cell infiltration (as myeloperoxidase - MPO activity), antioxidant and oxidative stress markers. **Results:** AP39 was successfully incorporated into three delivery systems: i) alginate nanoparticle, ii) nanoemulsion or iii) beads, but the nanoemulsion exhibited higher stability and skin penetration when associated with AP39. In comparison with vehicle, AP39 at concentrations equal or higher than 2 µM reduced 3T3 cells viability, but at 0.2 µM, AP39 improved fibroblast migration and wound occlusion. Thermal skin burn increased MPO activity and carbonylated proteins, and reduced antioxidant enzyme activities (catalase, glutathione peroxidase and glutathione s-transferase) and protein expression of nuclear factor erythroid-derived 2-like 2 (Nrf2). All these parameters, except for the increased MPO activity (n=6), were reversed by the topical daily treatment with the nanoemulsion containing AP39. **Conclusion:** These findings provide new evidence that AP39 stimulates fibroblasts migration *in vitro*, which associated with the antioxidant activity, may lead to the observed beneficial effects of this compound on thermal wound healing *in vivo*. **References:** Coavoy-Sánchez *et al.* (2020) Br J Pharmacol. 177(4): 857-865. Dolgachev *et al.* (2021) J Burn Care Res. 18: 118. **Acknowledgments:** This study was financed in part by the CNPq (870212/1997-4), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001, FAPESP (2016/06146-5) for financial support. **License number of ethics committee:** The present work was performed under the Ethical Committee of Animal Experimentation number 9319020818

04.018 **Effects of tyrosine kinase inhibitor in experimental cerebral malaria.** Moraes BPT^{1,2}, Rodrigues SO^{1,2}, Soares GMV¹, Abreu VHP¹, Maron-Gutierrez T², Batista CN², Bozza PT², Castro Faria Neto HC², Silva AR², Albuquerque CFG¹. ¹Lab de Imunofarmacologia, Univ Federal do Estado do Rio de Janeiro, Rio de Janeiro, Brasil; ²Lab de Imunofarmacologia, Fundação Osvaldo Cruz, Rio de Janeiro, Brasil.

Introduction: Cerebral malaria is caused by Plasmodium falciparum infection and is responsible for mostly all malaria deaths. P. falciparum developed increasing drug resistance, requiring alternative drugs combinations (WHO 2020). P. falciparum secretes several different kinases into its host red blood cell (Lin *et al.* 2017) and use others that plays key signaling roles in motility, secretion and development in the blood stages of infection (Gaji *et al.* 2014). Dasatinib is a potent tyrosine kinases inhibitor, specially BCR-ABL and Src family kinases that is able to cross the blood brain barrier (BBB) and regulates inflammatory pathways as TLR4/AKT and TLR4/ERK pathways (Ryu *et al.* 2019). The aim of this study is to evaluate the effects of dasatinib on an experimental cerebral malaria model. **Methods:** C57Bl/6 mice were infected with *Plasmodium berghei* ANKA and treated orally with dasatinib from day 2 to 7 post-infection. The animals were divided into four groups with 7-8 animals each: DMSO, the control group, chloroquine (25 mg/kg), and dasatinib (1mg/kg and 10 mg/kg). Five- and seven-days post-infection clinical score and parasitemia were evaluated. Survival rate were evaluated up to day 14. Parasitemia was measured by blood smear staining analysis. On day seven, animals were euthanized, and samples as kidney, liver and brain were collected after perfusion. Cytokines were measures by ELISA and BBB permeability by Evans Blue dye assay. Statistical analysis was assessed using Prism one-way ANOVA. **Results:** Dasatinib 10 mg/kg treatment increased survival rate up to day fifteen and reduced clinical score and parasitemia on day seven. Dasatinib at 10 mg/kg dose prevented hepatosplenomegaly and thrombocytopenia found in groups who did not receive antimalarial drug. Dasatinib at 1 mg/kg did not alter any of above indicators. Dasatinib promotes immunomodulation as seen in total leukocytes count and cytokines production. MCP-1 and TNF-α cytokines levels expression in brain tissue were inhibited while IL-10 levels were increased with dasatinib 1 mg/kg and 10 mg/kg. Dasatinib 10 mg/kg prevented the increase in BBB permeability in cerebral malaria animals. **Conclusion:** Dasatinib mitigates the evolution phases of parasite and modulates inflammation ending up in increase survival rate and better systemic disease indicators, such as organ damage and BBB breakdown. The results suggest a potential use of dasatinib, a Src family tyrosine kinase inhibitor, in cerebral malaria. **Financial Support:** information: FAPERJ, UNIRIO, CAPES, European Community Seventh Framework Programme. **References:** Gaji, R. Y., L. Checkley, M. L. Reese, M. T. Ferdig, and G. Arrizabalaga. 2014. 'Expression of the essential Kinase PfCDPK1 from Plasmodium falciparum in Toxoplasma gondii facilitates the discovery of novel antimalarial drugs', *Antimicrob Agents Chemother*, 58: 2598-607. Lin, B. C., D. R. Harris, L. M. D. Kirkman, A. M. Perez, Y. Qian, J. T. Schermerhorn, M. Y. Hong, D. S. Winston, L. Xu, and G. S. Brandt. 2017. 'FIKK Kinase, a Ser/Thr Kinase Important to Malaria Parasites, Is

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04.019 Role of TLR4 regulation and activation in the hypothalamus-pituitary-adrenal axis hyperactivity in diabetic animals. Magalhães NS¹, Chaves AS¹, Torres RC², Gonçalves-de-Albuquerque CF³, Castro-Faria-Neto HC⁴, Martins PMRS¹, Martins MA¹, Carvalho VF¹ ¹Lab of Inflammation, Oswaldo Cruz Inst, Oswaldo Cruz Foundation, Rio de Janeiro, Brazil; ²Carlos Chagas Filho Biophysics Inst – Federal Univ of Rio de Janeiro, Rio de Janeiro, Brazil; ³Federal Univ of the State of Rio de Janeiro, Rio de Janeiro, Brazil; ⁴Lab Immunopharmacology, Oswaldo Cruz Inst, Oswaldo Cruz Foundation, Rio de Janeiro, Brazil

Introduction: Previously, we showed that increased HPA axis activity was related to the downregulation of the peroxisome proliferation activated receptor (PPAR) γ in the pituitary and adrenal glands of diabetic rats. On the other hand, the antagonistic functional relationship between PPAR γ and Toll-like receptor 4 (TLR4) is established. This work aimed to evaluate the role of TLR4 in HPA axis hyperactivity observed in diabetic animals. **Methods:** The animals were obtained from Oswaldo Cruz Foundation breeding colony and used in accordance with the guidelines of the Ethic Committee on Use of Laboratory Animals of the Oswaldo Cruz Foundation, License L-027/2016. Diabetes was induced by intravenous injection of alloxan in fasted animals. The TLR4 antagonist (TAK-242) and antibiotic cocktail (Metronidazole, Ampicillin, and Neomycin) were administered daily for 14 consecutive days, starting 7 days after diabetes induction.

Results: Our data showed that TLR4 expression increased in the pituitary and adrenal of diabetic rats compared to non-diabetic rats. Besides that, we observed the increased expression of its endogenous activators HSP70 and HMGB1 in the pituitary and adrenal. Furthermore, we observed that both oleic and stearic acids, assessed by HPLC, were increased in the plasma of diabetic rats compared to non-diabetic rats. To understand the importance of TLR4 in HPA axis hyperactivity under diabetic conditions, we used TLR4 signaling mutant mice (C3H. HeJ) or diabetic mice treated with TAK-242. Both C3H.HeJ diabetic mice and TAK-242 (81.7 ± 15.0 ; 75.1 ± 24.9 ; 627.7 ± 111.1 ; 245.7 ± 37.5 corticosterone levels of non-diabetic, non-diabetic plus TAK, diabetic and Diabetic plus TAK, respectively; mean \pm SEM; $n=7$; $p<0.05$) treated diabetic mice showed no increased plasma levels of corticosterone compared to untreated diabetic animals. Diabetic mice treated with TAK-242 showed reversal on the under-expression of GR in the pituitary and overexpression of MC2R in the adrenal of diabetic animals. Moreover, we stimulated diabetic rats with lipopolysaccharide (LPS) and analyzed corticosterone levels after different times. Although unstimulated diabetic rats showed hypercortidism, LPS induced increase glucocorticoid levels 1 hour after stimulation with LPS. However, LPS did not increase plasma glucocorticoid levels in non-diabetic animals at this time. Diabetic mice were treated with an antibiotic cocktail containing NEO, AMP, and METRO to eliminate gram-negative, gram-positive, and anaerobic bacteria, respectively. Diabetic animals treated with the antibiotic cocktail did not show an increase in the plasma corticosterone levels. We also observed that diabetic mice showed increased permeability of the intestinal barrier and an imbalance in the ratio of inflammatory cytokines in the intestine, IL17/IL22, and IL17/IL10, in diabetic animals. **Conclusion:** Our findings revealed that TLR4 appears to be a target to HPA axis hyperactivity observed in diabetic animals. **Financial support:** CNPq, INCT-NIM, FAPERJ and FIOCRUZ. **License number of ethics committee:** Ethic Committee on Use of Laboratory Animals of the Oswaldo Cruz Foundation, License L027/2016

04.020 PI3K- γ inhibition attenuates the irinotecan-associated intestinal mucositis in mice. Cajado AG¹, Rangel GFP¹, Nobre LMS¹, Quintela LCS², Paguada ALP³, Ferreira LMM⁴, Alves APNN², Wong DVT², Lima-Júnior RCP^{1,2,3,4}. ¹UFC Ceará, Dpt of Physiology and Pharmacology, Brazil; ²UFC Ceará, Dpt of Pathology, Brazil; ³UFC Ceará, Dpt of Pharmaceutical Sciences, Brazil; ⁴UFC Ceará, Dpt of Medicine, Brazil

Mucositis is a common toxicity of irinotecan-based colorectal cancer treatment. Its manifestation delays subsequent cycles of chemotherapy, dose reduction, or suspension. The associated inflammatory response can be accompanied by sepsis and increase the risk of patient death. The phosphoinositide 3-kinase- γ (PI3K γ) is expressed in leukocytes and plays an essential role in activating innate immune cells. PI3K γ inhibition is a promising therapeutic strategy for controlling inflammatory disorders. We then analyzed the role of PI3K γ in the model of intestinal mucositis induced by irinotecan. Male C57BL/6 mice (20-25g, $n=6$ /group) received an i.p. injection of vehicle, irinotecan (120 mg/Kg, one injection per day for four days) alone or combined with AS-605240 (a PI3K γ inhibitor, 10 mg/kg, *p.o.*). On day seven, post first dose of irinotecan, we determined the diarrhea severity and body mass variation. After animal euthanasia, we harvested the small intestine for measuring the morphometry (intestinal length in cm and the

villus/crypt ratio) and inflammatory parameters, such as the myeloperoxidase (MPO) activity, cytokine levels (IL1- β and IL-6) by ELISA, and the *Tlr9* gene expression by qRT-PCR. One-way ANOVA followed by the Bonferroni test was used to determine the statistical differences between the groups ($P < 0.05$). Ethics committee approval: CEUA N°5132240718. Irinotecan-associated body mass loss (12.25% vs. vehicle group) was unaffected ($P > 0.05$) by AS-605240 (10.09%). Conversely, PI3Ky inhibition partially attenuated the intestinal injury, as detected by the milder diarrhea scores [1 (1-2)] and improved villus/crypt ratio versus the irinotecan group (diarrhea: 2[1-3], $P < 0.05$). Additionally, PI3Ky inhibition significantly reduced myeloperoxidase activity, the levels of IL-1 β and IL-6, and *Tlr9* expression compared with the irinotecan group ($P < 0.05$). These findings suggest the deleterious role of PI3Ky in mediating the inflammatory response during irinotecan-related intestinal mucositis. Financial support: CNPq, Capes, and Funcap. **License number of ethics committee:** CEUA N°5132240718

04.021 Effect of physical exercise on a preclinical model of nonalcoholic fatty liver disease. Rodrigues KL¹, Silva VV¹, Pereira ENGS¹, Silveiras RR¹, Araujo BP¹, Flores EE¹, Ramos, IP, ²Daliry A¹. ¹Lab of Cardiovascular Investigation, Oswaldo Cruz Inst, Fiocruz, Rio de Janeiro, Brazil; ²National Center of Structural Biology and Bio-imaging, Federal Univ of Rio de Janeiro, Rio de Janeiro, Brazil

Introduction: Non-alcoholic fatty liver disease (NAFLD), considered the hepatic manifestation of metabolic syndrome (Met), affects between 20 and 30% of the adult population worldwide. NAFLD can progress to more severe conditions with the presence of inflammation and fibrosis (steatohepatitis), cirrhosis or hepatocarcinoma, but the exact mechanism that triggers this progression is not fully understood. Currently, the role of advanced glycation end products, or AGEs (Advanced Glycation End Products), in the etiology of NAFLD is discussed. AGEs are proteins modified by the action of reducing sugars, in a non-enzymatic way, which are formed throughout life, but increased in situations of oxidative stress and hyperglycemia, and which can damage tissues. The only non-pharmacological intervention capable of preventing the development and progression of hepatic steatosis is the change in eating habits and physical training. However, the exact mechanism involved in this improvement is not fully understood. Thus, the objective was to evaluate the effects of physical training on complications and progression of steatosis. **Methods:** C57BL/6 mice were fed a high fat and carbohydrate diet (HCHF) or a normocaloric diet (CTL) for 36 weeks. During the last 12 weeks, a subgroup of HCHF and CTL animals were submitted to aerobic physical training (CTL EX and HCHF EX), while other were kept sedentary (CTL SED and HCHF SED). At the end of the protocol, metabolic parameters, glycemic homeostasis, biochemical markers, liver oxidative damage, AGEs levels, steatosis severity, and liver and adipose tissue microcirculatory parameters were evaluated in all groups. Experimental procedures were approved by the Oswaldo Cruz Foundation Animal Welfare Committee (license L-012/2018 A1). **Results:** The HCHF EX group showed a decrease in body weight, blood pressure, and improvement in glucose metabolism, a smaller increase in total cholesterol and CTL-like levels in liver weight. The catalase enzyme activity had a greater increase in the HCHF EX group than in the HCHF SED group. Physical training prevented the increase in serum AGE levels and negatively modulated AGE levels in the liver. Physical training was also able to reverse the presence of steatosis and changes in liver microcirculation and adipose tissue. **Conclusion:** We conclude that physical training can be a potential non-pharmacological treatment for microcirculatory dysfunction and metabolic complications associated with NAFLD. Supported by FAPERJ, CNPq e FIOCRUZ. **License number of ethics committee:** L-012/2018 A1

04.022 Effect of Mo-CBP4, a purified chitin-binding protein from *Moringa oleifera* seeds, in irinotecan-induced intestinal mucositis in mice. Ferreira KQ¹, Carmo LD¹, Rangel GFP¹, Nunes MO¹, Duarte RS¹, Rabelo LMA¹, Lopes TDP², Sousa DOB², Alencar NMN¹. ¹Drug Research and Development Center, Dpt of Physiology and Pharmacology, Federal Univ of Ceará, Fortaleza, Brazil; ²Dpt of Biochemistry and Molecular Biology, Federal Univ of Ceará, Fortaleza, Brazil

Intestinal mucositis (IM) is a side effect that can affect patients who are being treated for colorectal cancer (CRC). About 85% of patients undergoing chemotherapy develop IM in varying degrees and the available treatment is only palliative (1). *Moringa oleifera* Lamarck is a species native from Northeast India, which is largely used due to its nutritional and therapeutic properties. Our research group has demonstrated that Mo-CBP4 (11.78 kDa) potent antinociceptive and anti-inflammatory activity both administered orally and intraperitoneally (2). The objective of this study was to study the effect of Mo-CBP4 on the intestinal mucositis model induced by irinotecan (CPT-11). For induction of IM male swiss mice (25-30 g) were divided into 3 groups: group 1 received saline (0.9%, i.p.) once daily for four days; Group 2 received irinotecan (75 mg/kg, i.p.) once daily for four days; Groups 3 treated for 7 days with Mo-CBP4 at dose 10 mg/kg e.v. respectively, 30 min before CPT-11 which was administered for 4 days. During the seven days the weight loss, presence of diarrhea by scores and the survival were evaluated.

On the seventh day, blood was collected for leukocyte count and then euthanasia for duodenum collection and evaluation of the following parameters: small intestine length, intestinal contractility, histopathological and morphometric changes, MPO, GSH, MDA, NO and cytokines (IL-1 β , IL-6, KC, and TGF- β). Statistical analysis used ANOVA/Bonferroni or Kruskal Wallis/Dunns test and $p < 0.05$ was considered significant. This study was approved by the UFC - CEPA Animal Research Ethics Committee (7796300120). CPT-11 caused loss of body mass, diarrhea, increased mortality, induced leucopenia, decreased intestinal length, increased intestinal contractility, caused villi flattening, loss of crypt architecture, presence of vacuolization, inflammatory cell infiltrate, and mucous and muscular layer. An increase in the levels of MPO, IL-1 β , IL-6, KC and TGF- β was observed in relation to inflammatory parameters. An increase in MDA and a decrease in GSH levels were also observed. Corroborating the results found them previously by our group (3). Comparatively, treatment with Mo-CBP4 10 mg/kg reduced diarrhea, increased survival and intestinal damage, attenuating the histopathological changes caused by CPT-11. McLTP1 was also able to decrease the levels of MPO (35%), NO (48%), IL-1 β (52%), IL-6 (98%), KC (88%) and TGF- β (62%), and decrease MDA levels and increase GSH. Therefore, Mo-CBP4 demonstrates important anti-inflammatory and antioxidant activities that make it a promising therapeutic option to prevent and attenuate the severity of intestinal mucositis during the chemotherapy treatment with CPT-11. Financial support: Capes. **References:** 1. Andreyev J. *Lancet Oncol.* 447: 60, 2014

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04.023 **Captopril reduces hypothalamus-pituitary-adrenal axis hyperactivation in diabetic mice.** Chaves AS¹, Magalhães NS¹, Cardoso CF¹, Martins PMRS¹, Martins MA¹, Carvalho VF¹. ¹Oswaldo Cruz Foundation, Oswaldo Cruz Inst, Lab of Inflammation, Rio de Janeiro, Brazil

Introduction: Diabetes Mellitus (DM) is a chronic metabolic disease accompanied by reduced insulin production and increased levels of glucocorticoid hormones (GC). Chronically, increased circulating glucocorticoids levels are related to the development of some diabetes comorbidities, including increased of peripheral insulin resistance, impaired wound healing, and neuropathy. Our hypothesis is that the hypothalamus-pituitary-adrenal (HPA) axis hyperactivity in diabetes is associated with the hyperstimulation of the renin-angiotensin system (RAS). Since diabetic individuals present an increase in the levels of Angiotensin II (Ang II) and this peptide induces adrenal steroidogenesis. This work aimed to investigate the role of the RAS on HPA axis in hyperactivity in diabetic animals. **Methods:** The animals were obtained from Oswaldo Cruz Foundation breeding colony and used in accordance with the guidelines of the Ethic Committee on Use of Laboratory Animals of the Oswaldo Cruz Foundation, License L-027/2016. Male Swiss Webster mice were induced to diabetes by intravenous injection of alloxan and treated with ACE inhibitor, Captopril (10 mg/kg/day) for 14 consecutive days, beginning 7 days after the diabetes induction. All analyses were performed 24 h after the last day of captopril treatment, including plasma Ang II and corticosterone quantification by ELISA, expression of MC2R, 11 β -HSD1 and evaluation of corticotropic cell numbers by immunohistochemistry, and expression of StAR, POMC, GR, and MR by western blot. **Results:** Our results show that treatment with captopril reduced plasma Ang II (7.168 \pm 4.75; 5.785 \pm 2.271; 16.503 \pm 5.567; 8.779 \pm 3.312 of non-diabetic plus saline, non-diabetic treated with captopril, diabetic plus saline and diabetic treated with captopril, respectively; mean \pm SEM; n=7; $p < 0.05$) and corticosterone levels (346.11 \pm 133.68; 345.19 \pm 155.86; 2281 \pm 868.77; 659 \pm 267.46 of non-diabetic plus saline, non-diabetic treated with captopril, diabetic plus saline and diabetic treated with captopril, respectively; mean \pm SEM; n=5; $p < 0.05$) in diabetic mice compared to untreated diabetic mice. We also observed that the captopril reduced the expression of 11 β -HSD1, StAR, and MC2R in the adrenal gland of diabetic mice compared to untreated diabetic mice. In the pituitary gland, we observed that captopril drops the ACTH expression by reducing the total number of positive adrenocorticotrophic cells and their precursor, POMC in diabetic mice compared to untreated diabetic mice. In addition, the treatment with captopril was not able to restore GR and MR expression in the pituitary gland of diabetic mice compared to untreated diabetic mice. **Conclusion:** In conclusion, our findings show that the inhibition of Ang II formation by captopril restores the HPA axis activity in diabetic mice. **License number of ethics committee:** Ethic Committee on Use of Laboratory Animals of the Oswaldo Cruz Foundation, License L-027/2016.

04.024 **Experimental Alzheimer's disease: Kinin-B2 receptor signaling in immune response and neuroinflammation in mice.** Viero FT¹, Mello CF¹, Pillat MM², Ulrich H³. ¹Pharmacology, Federal Univ of Santa Maria, Santa Maria, Brazil; ²Microbiology and Parasitology, Federal Univ of Santa Maria, Santa Maria, Brazil; Dep. of Biochemistry, Inst of Chemistry, Univ of São Paulo, Brazil

Introduction: Neuroinflammation is an inherent process in the pathogenesis of Alzheimer's disease (AD) that may act as initial deleterious trigger. In this context, recent data show that the kinin system is

stimulated by the pathologic amyloid- β ($A\beta$) peptide in AD patients. Kinin-mediated actions are primarily mediated by bradykinin (BK) and its kinin-B2 receptor (B2R), and the role of BK/B2R in AD is not completely understood. Thus, the aims of this study were to determine effects of B2R blockage in different AD models: the transgenic triple mouse as a model of the familial AD without B2R expression (APP^{swe}/PS1dE9/B2R^{-/-}) or injection of oligomeric $A\beta$ -peptide mouse model. **Methods:** Wild type mice received $A\beta$ 1–42 oligomers intracerebroventricular (i.c.v.) injection and some mice were treated with B2R selective antagonist, HOE 140, 50 pmol/site (Ethics process 5250180515). Animals were genotyped before the studies using specific primers. The blood-brain barrier (BBB) permeability was assessed by the fluorescein sodium method (0.1mg/mL), which was injected intraperitoneally. Reactive oxygen species (ROS) assays were performed by cell staining with in DCFH-DA, and the granulocyte death was detected as 7AAD⁺ cells using flow cytometry. **Results:** Our work is the first to show that $A\beta$ oligomers increased BBB permeability and gene expression and pharmacological blockade of B2R provided BBB protection in AD mice. Moreover, HOE-140 prevented the increase of ROS in hippocampus of $A\beta$ -treated mice. Interestingly, besides the neuroinflammation observed by an increased number of Iba⁺ microglia cells, the peripheral immune system of AD mice also presented some alterations, such as high levels of IL-6, which were modulated by BK/B2R signaling. Both HOE-140 and B2R^{-/-} knockout prevented long memory loss in different AD models, observed in new object recognition task experiments. Moreover, 100nM, 500nM and 1 μ M $A\beta$ oligomers progressively reduced neurosphere diameters *in vitro*, and 1 μ M HOE-140 prevented this effect. **Conclusion:** Taken together, these data suggest that the B2R presents a key role in neuroinflammation observed in AD, from disruption of the BBB and peripheral immune system alterations to memory loss. **Acknowledgements:** This work was supported by grants and fellowships from FAPESP and CNPq Brazilian funding agencies. **License number of ethics committee:** 5250180515

04.025 The reduction of inflammation and oxidative stress by a novel laticifer protein sub-fraction II of *Calotropis procera* decreases mucositis induced by irinotecan. Rangel GFP¹, Carmo LD¹, Rabelo LMA¹, Duarte RS¹, Costa ADC¹, Macedo FS¹, CAJADO AG¹, Souza TFG¹, Ramos MV², Alencar NMN¹. ¹UFC Fortaleza, Dpt de Fisiologia e Farmacologia, Brasil; ²UFC Fortaleza, Dpt de Bioquímica e Biologia Molecular, Brasil **Introduction:** *Calotropis procera* is a laticiferous plant (Apocynaceae) found in tropical regions all over the world. The ultrastructural characteristics of laticifers and anti-inflammatory potential in several models, make analysis for peptidases from latex very attractive for biological. This work aimed to evaluate the effects of a novel laticifer protein (LP) sub-fraction II (PII) of *Calotropis procera* presenting an iodoacetamide-inhibited cysteine proteinase (IAA) activity (LP-PII-IAA), on the development of intestinal mucositis following irinotecan administration in mice. **Methods:** Swiss mice were treated daily with saline or LP-PII-IAA (5mg/kg, i.v.) 24 h prior to CTP-11 (75 mg/kg/4 days, i.p) and for additional 6 days. Seven days after the first dose of irinotecan, the intestine was removed for histological evaluation, measurement of myeloperoxidase (MPO), glutathione (GSH), malondialdehyde (MDA), proinflammatory cytokines and chemokine (IL-1, IL-6, and KC levels - a murine homolog of human IL-8 chemokine) by elisa; analysis of cyclooxygenase 2 (COX-2) and nuclear factor kappa B (NF- κ B) expression by immunohistochemistry. The study was approved by the Comissão de Ética no Uso de Animais/Universidade Federal do Ceará 55/2017. Statistical analysis ANOVA / Bonferroni test was used. $p < 0.05$ was accepted. **Results:** Animals receiving co-administration of LP-PII-IAA presented a significant histological preservation. Clinical homeostasis was accompanied by a reduction in MPO activity and ameliorated levels of IL-1 β , IL-6 and KC, associated with an increase in IL-10 muscle ($p < 0.05$). COX-2 and NF- κ B expressions were reduced and oxidative markers (GSH, MDA) were normalized in animals that received LP-PII-IAA ($p < 0.05$). **Conclusion:** We suggest that peptidases from the latex of *Calotropis procera* were instrumental for the suppression of the adverse clinical and physiological effects of irinotecan. **Financial Support:** This research project was supported by the Fundação Cearense de Apoio ao Desenvolvimento Científico e Tecnológico (FUNCAP), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) (Award Number: PR2-0101-00054.01.00/15 PRONEX/FUNCAP/CNPq - Edital 02/2015). **License number of ethics committee:** This research was approved by the Animal Research Ethics Committee (CEPA) of the Universidade Federal do Ceará (protocol number 55/2017).

04.026 Low birth weight induced by maternal malnutrition prejudices phagocytic activity of alveolar macrophages and increases susceptibility to infections. Azevedo GA¹, Gil NL¹, Balbino AM¹, Silva MM¹, Landgraf MA², Landgraf RG¹. ¹Dpt of Pharmaceuticals Sciences, Univ Federal de São Paulo-Diadema, Diadema, Brazil; ²Univ Paulista-Rangel, Santos, Brazil

Introduction: Maternal food restriction during pregnancy induces morphological and metabolic fetal adaptations and predisposes to metabolic diseases including diabetes and hypertension, in offspring. Metabolic disturbances can also be induced by the imbalance of pro and anti-inflammatory factors, as

observed in sepsis. In previous studies, our group demonstrated that food restriction during pregnancy resulted in low birth weight, hypocellularity in bone marrow and peripheral blood, reduction in leukocyte migration, and a defective inflammatory response in Wistar rats at 12 weeks. The aim of this study was to evaluate the influence of maternal malnutrition during the pregnancy on the development of sepsis, in adult offspring. **Methods:** Timed mating was carried out in age-matched (12- to 16-wk-old) female and male Wistar rats. Day 1 of the pregnancy was defined as the day on which spermatozoa were detected in the vaginal smear. After confirmation that mating had occurred, female rats were randomly divided into 2 groups: nourished - diet and water *ad libitum*; malnourished - 50% food restriction and water *ad libitum*. At 12 weeks of age, male offspring rats from both groups were submitted to cecal ligation and puncture (CLP) or submitted to sham operation and six hours after, the animals were euthanized and the inflammatory parameters were evaluated. It was evaluated temperature and glycaemia levels, in blood, and creatinine and urea, in urine. The cytokines IL-1 β , IL-10, IL-6, MIP-2, TNF- were evaluated in serum and lung tissue. To assess phagocytic activity of alveolar macrophages, these cells were incubated with *Saccharomyces cerevisiae*, in a 4: 1 ratio. **Results:** Malnutrition during gestation caused a drop in fetal birth weight (LBW) compared with offspring from control group (normal body weight at birth-NBW). Similar hypothermia and hyperglycemia were observed in both groups submitted to sepsis protocol; in addition, increase in creatinine and urea levels, in urine, was also observed in these groups. Although serum IL-6, IL-1 β and IL-10 levels were enhanced by sepsis protocol in both groups, the levels of these cytokines were higher in LBW than in NBW group. Sepsis protocol did not induce increase in serum TNF- α and MIP-2 levels in NBW group, different from that observed in LBW group. The count of colony forming units (CFU) in the peritoneal fluid (in groups submitted to sepsis) was significantly higher in LBW when compared to NBW group. LBW group showed defective phagocytic activity because the number of yeasts phagocytosed by alveolar macrophages is lower than NBW group. **Conclusion:** low birth weight induced by maternal malnutrition during pregnancy prejudices phagocytic activity of alveolar macrophages and could compromise immune response and increases susceptibility to infections. **Financial Support:** FAPESP (2019/05242-9, 2020/16020-4), CNPq (306631/2018-0) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001 **License number of ethics committee:** CEUA n^o 2849110517

04.027 Dissecting the mechanisms involved in differential responses and outcomes of identical mice to sepsis. Cebinelli GCM, Nascimento DCB, Damasceno LEA, Tavares AC, Donate PB, Cunha TM, Farias JC, Cunha FDQ. Center for Research in Inflammatory Diseases (CRID) - Dpt of Pharmacology, Ribeirão Preto Medical School Univ of São Paulo São Paulo, Brazil

Introduction: Sepsis is defined as a life-threatening organ dysfunction caused by a dysregulated host response to infection. There is estimate an annual occurrence of 31.5 million cases of sepsis causing potentially 5.3 million deaths. New therapies, such as immunotherapies, have been tested in sepsis, but with no success yet. One possible explanation is that the therapeutic targets used for the development of these immunotherapies were not adequate, suggesting that sepsis pathophysiology is not fully understood. Thus, studies using advanced methods may contribute to the identification of new therapeutic targets in sepsis. By inducing cecal ligation and puncture sepsis model (CLP) in C57/Bl6 mice genetically equal and having the same grown-up environment, these animals have different response to sepsis in which half of the mice survive and the other half die from sepsis. That way, our study aims to identify new therapeutic targets by analyzing the profile of the different leukocyte subtypes comparing surviving and non-surviving septic animals **Methods:** We induced sepsis in C57/Bl6 male mice using Cecal ligation and puncture (CLP), the gold standard model of sepsis. Moderate and severe intensities of CLP sepsis were performed using a 21- or 18-gauge needle, respectively. Of note, induction of severe CLP was followed by antibiotic treatment (ertapenem sodic). In both cases, CLP promoted mortality of 50%. Animal Research Ethical Committee - CEUA FMRP/USP - number: 151/2019. **Results:** After 6 hours of CLP induction, surviving and non-surviving mice displayed the same serum levels of cytokines (IL-6, IL-10, CXCL1, CXCL2, and CCL2). However, at 12h, 24h, or 48h, animals that survived from sepsis presented reduced levels of these cytokines, while in non-surviving mice these levels remained higher. Non-surviving mice also showed an increase of plasma concentration of liver, kidney, and heart lesion biomarkers and bacteremia. Likewise, non-surviving mice showed increased concentrations of chemokines and cytokines in the lungs, kidneys, heart, liver, and also in the primary infection focus (peritoneal cavity) in comparison with surviving animals at 24h after CLP. By evaluating the activation of neutrophils, which are key cells promoting infection control in the CLP model, we observed that both surviving and non-surviving animals displayed decreased CXCR2 expression and an increased CD11b expression at 6h on blood neutrophils. However, after 24h and 48h of CLP induction, once neutrophils of surviving mice reestablish CXCR2 and CD11b expression levels similar to the control mice, the neutrophils of non-surviving mice remained with

an internalized CXCR2 and high CD11b expression. **Conclusion:** We observed that identical mice responded to the experimental sepsis differently. Notably, non-surviving animals had a persistently higher level of inflammatory cytokines in plasma and organs, and persistent activation of neutrophils. Currently, we are evaluating what conditions might be responsible for these different outcomes in identical mice. Besides, the differential gene expression of leukocytes from surviving and non-surviving septic mice is being determined by using single-cell RNA sequencing.

Keywords: sepsis; new therapeutic targets; sepsis outcome; leukocytes alterations. **Financial Support:** FAEPA, FAPESP, CNPq and CAPES. **License number of ethics committee:** Animal Research Ethical Committee – CEUA FMRP/USP – number: 151/2019

04.028 Treatment with the flavonoid quercetin accelerates resolution of lung changes caused by silica particles in mice. Guimarães FV¹; Ferreira TPT¹; Arantes ACS¹; Jannini-Sá YAP¹; Silva CD¹; Moraes JA²; Carvalho VF¹; Martins MA¹; Silva PMR¹; ¹Lab de Inflamação, Inst Oswaldo Cruz/Fiocruz; ²Labio Redox, UFRJ; RJ - Brazil

Introduction: Silicosis is a chronic, potentially fatal and irreversible lung disease caused by inhalation of silica particles, characterized by fibrosis and granuloma formation. To date, no effective therapy is available for treatment of silicosis. Quercetin is a flavonoid present in several plants including fruits, vegetables and some grains, known by its antioxidant and anti-inflammatory activities. This study was undertaken in order to investigate mechanism involved in the suppressive effect of quercetin on experimental silicosis in mice. **Methods:** Male Swiss-Webster mice were anesthetized with halothane, and then instilled with crystalline silica (10 mg; particle size 0.5 ? 10 ?m) by intranasal via. Treatment with quercetin (2.5 - 10 mg/kg, p.o.) was performed daily, from day 21 to 27. The antioxidant N-acetylcysteine (NAC) was used for comparison. The analyses were performed 1 day after the last drug administration. Parameters included *In Vivo* and *in vitro* systems, addressing oxidative stress as well as inflammatory and fibrotic markers. All experimental procedures were performed according to the guidelines of the Committee on Use of Laboratory Animals of the Oswaldo Cruz Foundation (LW57-14/L001-19). **Results:** Mice challenged with silica particles when treated therapeutically with quercetin showed reversion of decreased lung function, collagen deposition and granuloma formation. Quercetin also restored basal levels of antioxidant enzymes such as catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx). Increased levels of NADPH oxidase 4 (NOX-4) in silicotic lungs was suppressed by the flavonoid. Additionally, quercetin also inhibited the release of microvesicles from macrophages. By means of *in vitro* assays, we noted that quercetin directly suppressed mice alveolar macrophages (lineage AMJ2C11), blocking their activation and reducing cell death. Treatment with N-acetylcysteine (NAC) inhibited some alterations associated with the oxidative stress, but did not affect lung fibrosis. **Conclusion:** Altogether, our findings show that therapeutic treatment with quercetin reversed important pathological features of silica particle inhalation, suggesting that it seems to be a promising compound for future application in chronic lung diseases such as silicosis. **Financial Support:** FIOCRUZ, CNPq, FAPERJ, CAPES (Brazil) **License number of ethics committee:** LW54/14 and L-001/2019

04.029 Therapeutic treatment with gold nanoparticles suppresses lung fibrosis in silica-challenged mice. Ribeiro NBS¹, Janinni-Sá YAP¹, Arantes ACS¹, Chaves AS¹, Pelajo-Machado M², Silva-Aguiar RP³, Peruchetti DB³, Caruso-Neves C³, Santos-Oliveira R⁴, Martins MA¹, Silva PMR¹ ¹Lab of Inflammation; ²Lab of Pathology, Oswaldo Cruz Inst; ³Lab of Biochemistry and Cellular Signalling, UFRJ; ⁴Inst of Nuclear Engineering, RJ, Brazil

Introduction: Silicosis is a pneumoconiosis caused by inhalation of silica particles, characterized by marked inflammation and collagen deposition with granuloma formation. Gold nanoparticles (AuNPs) are known as an important anti-inflammatory tool in experimental models of chronic diseases. This study was undertaken to investigate the effect of aerosolized AuNPs as well their distribution in silica-challenge mice. **Methodology:** Anesthetized male Swiss-Webster mice were intranasally instilled with silica particles (10 mg) or vehicle (saline 0.9%). AuNPs (1.5 µg/mL) were aerosolized every two days, from day 21 to 27 post-silica, and the analyzes performed 1 day after the last treatment. For some assays, samples of 197AuNPs were irradiated in an Argonaut reactor, and several organs (lungs, heart, brain, stomach, pancreas, intestine, bladder, kidney, lung, liver, spleen, mediastinal lymph node) were immediately recovered, weighted and the activity determined by gamma spectrometry. Other parameters analyzed included: i) Body weight; ii) Lung function (invasive plethysmography); iii) Histology (H&E); iv) Blood and plasma samples were used for cellularity and biochemical determination of liver and kidney markers. All experimental procedures were approved by Ethical Committee of the Oswaldo Cruz Foundation (CEUA L-001/19). **Results:** We showed that therapeutic aerosolization of silicotic mice with AuNPs modified fibrogenesis component, disrupting granuloma structure and opening alveolar space, phenomenon

correlated with improvement of lung function. By transmission electron microscopy, osmiophilic black particles were detected in the silicotic tissue, indicating the presence of AuNPs in the lungs. No changes in body weight and blood parameters (red and white cells, hemoglobin, hematocrit and platelets) were detected after AuNPs. Likewise, markers of liver (alanine aminotransferase (ALT) and aspartate aminotransferase (AST)) and of kidney toxicity (creatinine and blood ureic nitrogen (BUN)) were not altered by AuNPs. Negligible amounts of AuNPs were detected in the other tissues. Trace amounts were detected in the bladder and kidneys, and increased levels were detected in the liver. No difference of AuNP biodistribution was noted when silicotic mice were compared to controls. Mice given intravenous AuNPs (2 μg) exhibited similar pattern of tissue distribution, with a marked increase in the liver. **Conclusion:** Our data show that therapeutic aerosolization of AuNPs ameliorated lung function and reduced granulomatous response caused by silica particles in mice. They also indicate that AuNPs seem to be preferentially eliminated by the liver, with less excretion through the renal system via the kidneys. Importantly, AuNP-treatment did not cause measurable liver and kidney toxicity, though histological analysis requires further investigation. Altogether our findings support the idea that AuNPs seem to be a promising approach to fibrotic diseases such as silicosis. **Financial Support:** FIOCRUZ, FAPERJ, CNPq (Brazil). **License number of ethics committee:** Ethical Committee of the Oswaldo Cruz Foundation (CEUA L-001/19)

04.030 **STING is an intrinsic checkpoint inhibitor that restrains the TH17 Cell Pathogenic Program.** Damasceno LEA¹, Cebinelli GCM¹, Oliveira SC², Cunha TM¹, Cunha FQ¹, Alves-Filho JC¹. ¹Center for Research on Inflammatory Diseases, Dpt of Pharmacology, Ribeirão Preto Medical School, Univ of São Paulo, Ribeirão Preto, Brazil; ²Inst of Biological Sciences, Dpt of Biochemistry and Immunology, Federal Univ of Minas Gerais, Belo Horizonte, Brazil

Introduction: external and intrinsic factors regulate the transcriptional profile of T_H17 cells, thereby affecting their pathogenic potential and revealing their context-dependent plasticity. The Stimulator of Interferon Genes (STING) is a nucleic acid sensor that triggers innate immune response but remains largely unexplored T cells. Here we described an intrinsic role of STING signaling in limiting the T_H17 cell pathogenic program. **Methods:** naive CD4⁺CD62L^{high}CD44^{low} T cells were sort-purified from mice lymph nodes and spleen. Thereafter, cells were TCR-activated with plate-bound anti-CD3e and anti-CD28 in skewing conditions for non-pathogenic T_H17 (cT_H17; rhTGF β , rmlL-6) or pathogenic T_H17 (pT_H17; rmlL-6, rm-IL-1b, rmlL-23). When indicated, aryl hydrocarbon receptor (AhR) antagonist (CH223191), STING inhibitor (C-176) and/or STING ligands (DMXAA, c-di-AMP or c-di-GMP) were used. T_H17 cells were collected at the indicated timepoints for WB, RT-qPCR and/or flow cytometric analysis. **Results:** we demonstrated that cT_H17 cells showed higher expression of STING than those cells generated under pathogenic conditions. STING activation induced T_H17 transdifferentiation into an IL-10-producing cell, besides decreasing IL-17A and *Il23r* expression in a type-I IFN-independent manner. Mechanistically, STING-induced IL-10 production was dependent on AhR signaling, while the decrease in IL-17A expression was associated with a reduced Ror γ t transcriptional activity through IRF3-Ror γ t nuclear interaction. **Conclusion:** our findings reveal an undescribed regulatory function of STING in T_H17 cell plasticity, proposing it as a valuable target to limit T_H17 cell pathogenicity. **Financial Support:** FAPESP and CNPq **License number of ethics committee:** Animal Research Ethical Committee: CEUA-FMRP 095/2019

04.031 **Lipopolysaccharide (LPS)-induced inflammation on proximal and distal epididymis differentially affects sperm motility parameters.** De Andrade AD, da Silva AA, Kushima H, Silva EJR. Unesp, Dpt of Biophysics and Pharmacology, Botucatu, Brazil

Introduction: Epididymitis, inflammation of the epididymis, is one of the most prevalent diseases of the male urogenital tract and a relevant factor of male infertility. Its main etiological factor involves retrograde urethral bacterial ascent. We and others have previously shown that the proximal (initial segment) and distal (cauda epididymidis) epididymal regions mount different inflammatory responses to lipopolysaccharide (LPS) from *E. coli*, which may influence the reproductive disease outcomes. Here, we evaluated whether LPS-induced inflammation of the initial segment and cauda epididymidis differentially affect sperm motility parameters. **Methods:** Male C57BL/6 mice (90 days, n=5/group) were anesthetized with ketamine/xylazine (60/20 mg/kg, i.p.), and then epididymitis was induced by sterile saline (control) or ultrapure LPS from *E. coli* (50 μg) injections as follows: 1) interstitial injection into the initial segment; and 2) retrograde intravasal injection into the vas deferens towards the cauda epididymidis. Mice were euthanized 1 or 7 days after treatment; spermatozoa were isolated from their cauda epididymides in HTF medium supplemented with 0.75% BSA (w/v) and processed for computer-assisted sperm analysis (CASA). Sperm tracks were classified as motile, progressive, hyperactivated, and static. We evaluated the following sperm kinematics parameters: average path velocity (VAP; $\mu\text{m/s}$), straight-line velocity (VSL; $\mu\text{m/s}$), curvilinear velocity (VCL; $\mu\text{m/s}$), straightness (STR; %), linearity (LIN; %), and amplitude of lateral head

displacement (ALH; μm). Data were analyzed by Student's t-test or Mann-Whitney test, for data parametric or non-parametric, respectively; $p < 0.05$ was considered significant. **Results:** Interstitial LPS injection into the initial segment did not affect sperm motility after 1 and 7 days. Regarding kinematics parameters, we observed that interstitial LPS injection decreased VSL and VCL after 1 and 7 days, respectively, whereas VAP was decreased at both time points. Conversely, intravasal LPS injection decreased both total and progressive motility after 7 days. In addition, intravasal LPS injection decreased VAP, VSL, and STR after 1 and 7 days, and VCL and ALH after 7 days only. **Conclusion:** LPS-induced acute inflammation of the cauda epididymidis induces a more severe impairment on sperm motility parameters compared to the initial segment. Our results suggest that region-specific inflammatory responses of the epididymis contribute to epididymitis reproductive outcomes. Financial support: FAPESP (2015/08227-0, 2017/20102-3) and CAPES. Ethics approval: 1029-IBB/CEUA. **License number of ethics committee:** 1029-IBB/CEUA

04.032 Glucagon prevents airway hyperreactivity, inflammation and remodeling induced by ovalbumin in a murine model of asthma. Insuela DBR¹, Azevedo CT¹, Coutinho DS¹, Magalhães NS¹, Ferrero MR¹, Ferreira TPT¹, Cascabulho CM², Pons AH², Martins PMRS¹, Martins MA¹, Carvalho VF¹ ¹Lab of Inflammation, Oswaldo Cruz Inst, Oswaldo Cruz Foundation (Fiocruz), Rio de Janeiro, Brazil; ²Lab of Innovations in Therapies, Education and Bioproducts, Oswaldo Cruz Inst, Oswaldo Cruz Foundation (Fiocruz), Rio de Janeiro, Brazil **Introduction:** Although great attention is given to the effect of glucagon (GLU) on glucose metabolism, endogenous levels of this hormone are altered in patients with some inflammatory diseases such as asthma. Asthmatic patients present a reduction in the circulating levels of GLU. In contrast, GLU administration induces bronchodilation in asthmatics. In this study, we evaluated the effect of GLU on allergen-induced airway hyperreactivity (AHR), inflammation and remodeling in a murine model of asthma. **Methods:** The animals were obtained from Oswaldo Cruz Foundation breeding colony and used in accordance with the guidelines of the Ethic Committee on Use of Laboratory Animals of the Oswaldo Cruz Foundation, License L-027/2016. Male A/J mice were treated with glucagon (100 $\mu\text{g}/\text{Kg}$, i.n.) once a day, 1 h before ovalbumin (OVA)-challenge, for 2 consecutive days. In some experiments, the mice were pretreated with COX inhibitor (indomethacin; 10 mg/Kg , i.p.) 30 min before GLU. All analyses were performed 24 h after the final challenge, including: AHR to methacholine through an invasive barometric plethysmography; lung inflammation and remodeling through bronchoalveolar lavage (BAL) and histological analyses. The effects of GLU (3 μM) on proliferation and activation of TCD4⁺ cells from lymph nodes *in vitro* were evaluated by BrdU incorporation using ELISA, and quantification of cytokines using CBA kit, respectively. **Results:** GLU inhibited OVA-induced AHR, eosinophil and T lymphocytes accumulation in lung and BAL ($4 \times 10^2 \pm 0.002$; $48 \times 10^2 \pm 0.005$; $12 \times 10^2 \pm 0.003$ TCD4⁺ cells in BAL of saline, OVA and OVA plus glucagon, respectively; mean \pm SEM; $n = 5$; $p < 0.05$), and subepithelial fibrosis. In parallel, GLU prevented production of IL-4, IL-5, IL-13, TNF- α , eotaxin-1, and eotaxin-2, but not MDC/CCL22 and TARC/CCL17 in the lungs induced by OVA challenge. Indomethacin abrogated the protective effect of GLU on OVA-induced AHR to methacholine and collagen deposition in the lungs. In addition, GLU increased cAMP intracellular levels in TCD4⁺ cells *in vitro*, and inhibited the proliferative response and IL-2, IL-4, IL-10, and TNF- α production by these lymphocytes stimulated with anti-CD3 plus anti-CD28 *in vitro*. **Conclusion:** GLU prevents AHR, lung inflammation and remodeling in a murine model of acute asthma. These protective effects seem to be related with reduction of eosinophils and T lymphocytes recruitment to the lungs and by inhibition of TCD4⁺ cell proliferation and activation by GLU (Insuela DBR et al., Sci Rep. 9: 1. 2019). **Financial Support:** CNPq, INCT-NIM, FAPERJ and FIOCRUZ. **License number of ethics committee:** The animals were obtained from Oswaldo Cruz Foundation breeding colony and used in accordance with the guidelines of the Ethic Committee on Use of Laboratory Animals of the Oswaldo Cruz Foundation, License L#027/2016.

04.033 Low birth weight induced by maternal food restriction decreases lipogenesis activity, in mesenteric adipocytes. Andreoti S^{1,2}, Reis GB², Komino ACM², Silva FF², Gil NL¹, Azevedo GA¹, Ramos APA¹, Balbino AM¹, Sertié RAL², Lima FB², Landgraf RG¹, Landgraf MA³. ¹Dpt of Pharmaceuticals Sciences, Univ Federal de São Paulo - campus Diadema, Diadema, Brazil; ²Dpt of Physiology, Univ de São Paulo, São Paulo, Brazil; ³Univ Paulista - campus Rangel, Santos, Brazil

Introduction: studies have shown that intrauterine malnutrition (ITM) promotes low birth weight (LBW) and when associated with accelerated growth in the first weeks of age may promotes obesity into adulthood. The aim of this study was to verify, in rats, if ITM (50% food restriction) modifies metabolic (lipogenesis and lipolysis) and inflammatory processes in the mesenteric adipose tissue (meAT) of offspring with 12 weeks old, before the development of obesity **Methods:** we evaluated the functional capacity of meTA fat cells to perform lipogenesis (¹⁴C-labeled glucose incorporation into lipids) and lipolysis (primary adipocyte incubation with or without isoproterenol) and also the expression of genes linked to these processes (by

Real-Time RT-PCR); the analysis of pro inflammatory markers in the meTA of these animals was also performed using Multiplex assay. **Results:** malnutrition during gestation caused a drop in fetal birth weight (LBW) compared with offspring from control group (normal body weight at birth-NBW). At 12 weeks old the LBW group presented body weight and naso-anal length equivalent to the NBW. However, the meTA of LBW animals was reduced and, since the mesenteric adipocytes volume was equal, we were able to conclude a decrease in the total amount of adipose cells in BPN group. Circulating insulin and glucose levels were the same between the groups; however, increase in circulating triacylglycerol (TG) levels and decrease in both basal and insulin-stimulated lipogenic capacity were observed in LBW (55% and 64% respectively) compared to NBW. The gene expression related to lipogenesis (LPL, G6PDH, ME1, ACLY, ACACA, FABP4, FAT / CD36, DGAT1, DGAT2, AGPAT1, AGPAT2) is also suppressed, reinforcing the finding. At the same time, the elevation observed in the pro-inflammatory cytokines IL1, IL6 and TNF α , in both tissue extract and adipocytes in culture, may explain the suppression of the lipogenic process and insulin resistance found in this tissue. The ability of LBW adipocytes to perform lipolysis was also decreased. In addition, we observed the gene expression of the anti-lipolytic ADRA2A increased and the pro-lipolytic genes (ADRB2, AQP7, PLIN, ATGL, HSL and MGL) repressed, corroborating with the data from the functional assay. **Conclusion:** these data suggest that intrauterine food restriction might, in adulthood, increase production of inflammatory cytokines and compromise lipogenic pathway, which could lead to an important impairment in energy storage and an increase in ectopic fat deposition. **Financial Support:** FAPESP (2019/05242-9, 2020/16020-4), CNPq (306631/2018-0) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001 **License number of ethics committee:** CEUA nº 9816040716

04.034 **Nebulized gold nanoparticles down-regulate inflammation and lung remodeling in a murine model of steroid-resistant asthma** Cotias, AC¹, Serra, MF¹, Pimentel, AS¹, Lanzetti, M², Hickmann, J³, Arante, ACS¹; Silva, PMR¹; Cordeiro, RSB¹, Barreto, E⁴, Martins, MA¹ ¹Lab of Inflammation, Oswaldo Cruz Inst, Fiocruz, Brazil; ²Lab of Cell Biology, Federal Univ of Alagoas, Maceió, AL, Brazil; ³Inst de Física, Univ Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil; ⁴Univ Federal do Rio de Janeiro (UFRJ), Inst de Ciências Biomédicas, Rio de Janeiro, Brazil

Introduction: The reduction of glucocorticoid (GC) anti-inflammatory efficacy is an important barrier to treat severe asthma patients. Gold nanoparticles (AuNPs) are widely studied for the treatment of several inflammatory diseases, due to their anti-inflammatory and antioxidant properties. Recently, our group demonstrated that intranasal instillation with AuNPs can prevent central features of the pathophysiology of asthma in distinct murine models of GC-sensitive asthma. In the present study, we investigated the effectiveness of aerosolization of gold nanoparticles in a murine model of asthma, which has been proved to be resistant to GC treatment. **Methods:** A/J mice were sensitized on days 0 and 7 by a suspension of Al(OH)₃ and ovalbumin (OVA) given subcutaneously, and challenged intranasally, once a week, for 9 consecutive weeks, starting on the second-week post-sensitization. Three weeks after the onset of OVA challenge, the mice were submitted to daily nebulizations of AuNPs (0.4 μ g/mL and 4 μ g/mL) or budesonide (7,5 mg/mL) 1 h before challenge. Airway hyper-reactivity (AHR), leukocyte infiltration, adverse airway remodeling, cytokine generation and oxidative stress were evaluated 24 h after the last challenge. Western blotting was used to investigate Nitrotyrosine, Nrf2, PI3K?, AKT and HDAC2 expression in the lung tissue. The Committee on Use of Laboratory Animals of the Oswaldo Cruz Institute (license L-030/2015; Rio de Janeiro, Brazil) approved all protocols and experimental procedures involving animals. **Results:** We found that mice challenged with OVA had airway hyperreactivity, eosinophil and neutrophils infiltrates in the lung, increased peribronchial fibrosis, mucus production and cytokine generation compared to mice challenged with saline. All of these changes were inhibited in mice treated with AuNPs, but not by budesonide. In GC-resistant asthma model, there was a substantial increase in oxidative imbalance, which seemed to contribute to reduction of antioxidant defenses (Catalase, SOD and Nrf2) leading to oxidative damage (TBARS and Nitrotyrosine), increased PI3K?/AKT expression and reduction of HDAC2 expression. All these changes were clearly sensitive to AuNPs, but not budesonide. **Conclusion:** Our findings show that AuNPs, can improve AHR, lung inflammation, tissue remodeling and oxidative stress in a murine model of GC-insensitive asthma. This effect seems to be at least in part accounted for by down-regulation of PI3K?/AKT expression, which leads to restoration of HDAC2 expression. Taken together, these results suggest that AuNPs should be further investigated as a therapeutic alternative for the management of difficult-to-treat asthma and other severe respiratory diseases. **Financial Support:** CNPq, FAPERJ and CAPES. **Keywords:** Severe asthma, resistance, glucocorticoid, gold nanoparticle. **License number of ethics committee:** The Committee on Use of Laboratory Animals of the Oswaldo Cruz Institute (license L-030/2015; Rio de Janeiro, Brazil) approved all protocols and experimental procedures involving animals.

04.035 **Role of toll-like receptor (TLR) 3 in the fibrosis triggered by silica particles in the lungs of mice.** Jannini-Sá, YAP¹, Ferreira TPT¹, Ribeiro, NBS¹, Guimarães, FV¹, Souza LM¹, Alves-Filho, JC², Hogaboam, CM³, Martins, MA¹, Silva PMR¹. ¹Lab of Inflammation - Oswaldo Cruz Inst, Brazil, ²Lab of Inflammation and Pain FMRP-USP, Brazil, ³Cedars-Sinai Medical Center Medicine Dpt Los Angeles, CA, USA

Introduction: Silicosis is a chronic, irreversible occupational disease, characterized by intense inflammatory and fibrotic response, with marked formation of granulomas. Cell death is a process that contributes to the establishment of silicosis, through the release of ligands for “Toll like” receptors, with emphasis on TLR3. Canonically, the TLR3 is expressed in endosomes and can recognize viral dsRNA promoting anti-viral response, but recently this receptor was related to the recognition of self-RNA released from necrotic cells. It is also known the lack of functional TLR3 may be related to bad prognosis in other lung fibrotic diseases. This study was undertaken to investigate the role of TLR3 in the lung fibrosis triggered by silica particles in mice and its role in lung fibroblast activation. **Methods:** C57BL/6 mice (TLR3+/+) and knockouts (TLR3-/-) were instilled with silica particles (13 mg). Mice instilled with saline 0.9% were used as controls. The analyses were performed 7 (early phase) and 28 (late phase) days post-challenge and parameters included: lung function and airways hyper-reactivity (AHR) to methacholine (invasive plethysmography) and morphology/morphometry of lungs by classical hematoxylin and eosin staining of tissue slides. Cytokines were quantified by ELISA. The reactivity of primary lung fibroblasts (human and murine) was evaluated *in vitro* against Poly(I: C) (10 µg/mL) challenge by means of proliferation (BrdU incorporation), collagen and α-SMA expression (ELISA) and invasion. All experimental procedures with Swiss-webster, knockout mice and murine lung fibroblasts were approved by the Animal Ethics Committee of the Oswaldo Cruz Foundation (CEUA L-057/14 and L-001/19). All the procedures with human primary lung fibroblasts were approved by Institutional Review Boards at Cedars-Sinai Medical Center (Los Angeles - California - EUA).

Results: Primary human lung fibroblasts challenged with the TLR3 ligand Poly(I: C) showed lower levels of collagen production, α-SMA expression and invasion as compared to the controls. Increased levels of MCP-1 chemokine were detected in the supernatant of murine fibroblasts after activation with Poly(I: C). In another set of experiments, TLR3+/+ silicotic mice exhibited a marked inflammatory response, with fibrosis and granuloma formation, at 7 and 28-days post-silica. Also, they showed normal levels of airway resistance and dynamic elastance increased in the silicotic mice as compared to controls. In contrast, TLR3-/- mice showed worsen of lung function, at 7 days, and restoration to basal condition, at 28 days-post silica. Collagen deposition and granuloma fibrosis, time-dependently, TLR3-/- showed no alteration and reduced levels as compared to TLR3+/+ at day 7 and 28 post-silica, respectively. Lower levels of the profibrotic cytokines MCP-1 and IL-13 were detected in the lungs of TLR3-/- mice, at 28 days.

Conclusion: Our data show the down-regulatory effect of TLR3 on lung fibrogenesis though promoting inflammatory response. The knockout animals revealed that TLR3 may play a dual role, controlling the fibrotic response, at early stages, while maintaining the granuloma and fibrotic response at late stages of murine silicosis. **Financial Support:** FIOCRUZ, CAPES, CNPq, FAPERJ

Keywords: silicosis; tlr3; lung fibroblasts; fibrosis **License number of ethics committee:** All experimental procedures with Swiss-Webster, TLR3 knockout mice and primary murine lung fibroblasts were approved by the Animal Ethics Committee of the Oswaldo Cruz Foundation (CEUA L-057/14 and L-001/19). All the procedures with human primary lung fibroblasts were approved by Institutional Review Boards at Cedars-Sinai Medical Center.

04.036 **Effect of H1N1 viral infection on lung fibrosis induced by silica particles in mice.** Ferreira TPT¹, Arantes, ACS¹, Jannini-Sá YAP¹, Hogaboam CM², Martins MA¹, Silva PMR¹ ¹Lab of Inflammation, Oswaldo Cruz Inst-Fiocruz, RJ, Brazil, ²Cedars Sinai Medical Center, LA, USA

Introduction: Silicosis is an occupational lung disease caused by inhalation of low levels of crystalline silica over many years. It is characterized by extensive lung damage and progressive inflammatory and fibrotic response with irreversible loss of lung function. The therapeutic arsenal aims to relieve symptoms and improve quality of life but no cure is available. Silicotic patients are continuously exposed to agents present in the air that may trigger concurrent inflammatory response when inhaled. Influenza A and B, agents of flu, promote an inflammatory response with intense leukocyte recruitment and activation of various cell types. Usually, flu is resolved by the immune system, but airway infection is a threat for patients with previous chronic lung inflammatory diseases. Influenza is the most common agent responsible for exacerbation, a condition characterized by worsening of the symptoms, accelerating the progression of some lung diseases and the main cause of hospitalization. The exacerbation in patients with silicosis is not well described and documented, and in this study, we used a previous established exacerbation model by influenza virus in silicotic mice to understand this phenomenon. **Methods:** Male Swiss-Webster mice were intranasally (i.n.) instilled with silica particles (10 mg/50 µL). After 21 days, viral infection was set by instillation of H1N1 A/PR/8/34 strain. Seven days after H1N1 provocation (28 days post-silica),

the following analyses were performed: i) weight loss; ii) lung function (resistance and elastance) and airways hyperreactivity (AHR) to methacholine (invasive plethysmography – DSI System), iii) leucocyte infiltrate in bronchoalveolar lavage fluid (BALF), iv) lung morphology, and v) cytokine/ chemokine generation (ELISA). All experimental procedures were approved by the Committee on Use of Laboratory Animals of the Oswaldo Cruz Foundation. **Results:** The weight of silicotic mice infected with H1N1 (Sil+H1N1) was marked decreased when compared to saline, silica and H1N1 groups. Moreover, silica-induced alteration of lung function (increased resistance and elastance) and airway hyperreactivity (AHR), as compared to H1N1 group. Interestingly, Sil+H1N1 group exacerbated this response but does not the AHR. We showed that leukocyte infiltration in BALF increased in all the groups when compared to control group, including increase in the number of mononuclear cells and neutrophils. The silica, H1N1, and Sil+H1N1 group increased the production of cytokines (IL-1b and TNF-a) and chemokines (KC, MIP-1a, MIP-2 and MCP-1), but only MIP-1a and MCP-1 were increased in Sil+H1N1 group compared to the respective control groups. Histological analyzes revealed that H1N1 mice showed an intense inflammatory infiltrate in the lungs compared to saline group. Similarly, an intense fibrotic response and granuloma formation was noted in silicotic as in Sil+H1N1 group. **Conclusion:** Our preliminary results show that infection with H1N1 in silicotic mice may promote an exacerbation response as observed in other chronic lung diseases. However, additional studies are needed to better understand the mechanism of this response in silicosis. **Financial Support:** FIOCRUZ, CNPq, FAPERJ. **License number of ethics committee:** License LW-057/14 and L0-01/19

04.037 *Lantana canescens* (Kunth) inhibits inflammatory responses in murine models Lencina JS¹; Moslaves ISB¹; Muller JAI¹; Alves FM³ Silva DB²; Toffoli-Kadri MC¹ ¹Lab of Pharmacology and Inflammation, FAFAN-Federal Univ of Mato Grosso do Sul, Campo Grande, Brazil ²Lab of Natural Products and Mass Spectrometry, FAFAN-Federal Univ of Mato Grosso do Sul, Campo Grande, Mato Grosso do Sul, Brazil ³Lab of Botany, INBIO/Federal Univ of Mato Grosso do Sul, Campo Grande, Brazil

Introduction: *Lantana canescens* is popularly known in Brazil as *cidreira* or *chumbinho-branco*, it is found in Pantanal biome and its flowers and leaves are used in traditional medicine to inflammation. Information about this species is limited to the activity of isolated essential oils. Studies with different extracts, composition, and biological properties are still scarce. This study was to evaluate the anti-inflammatory activity of the hydroethanolic extract of *L. canescens* aerial parts (HELc). **Methods:** The HELc was provided by LAPNEM/UFMS. Protocols approved by UFMS Ethics Committee in Animal Use (1.039/2019). Male *Swiss* mice weighing 18-25 g were used in the *In Vivo* assays. Anti-inflammatory activity through paw edema, mast cell degranulation and peritonitis, were evaluated using the doses of 3, 30 e 300 mg/Kg. In assays for anti-inflammatory activity, mice were pretreated orally (p.o.) with water (10 mL/Kg, control) or indomethacin (5 mg/kg, standard anti-inflammatory). Results were expressed as mean \pm S.E.M., ANOVA and Bonferroni test ($p < 0.05$) **Results:** In the carrageenan-induced paw edema model, the peak of edema was at 240 minutes (0.098 ± 0.016 mL) and at that time the edema was inhibited by indomethacin in 74.0 % and by HELc (3, 30 and 300 mg/Kg) by 44.0 %, 54.0 % and 72.0 %, respectively. For the compound 48/80 increased mast cell degranulation by 64.4% when compared to the control group (15.5%), and the indomethacin reduced by 67.8 %. HELc in the same previous doses reduced by 64.9 %, 45.4 % and 40.8 %, respectively, with no difference between them. For leukocyte influx, the predominant cells in the inflammatory focus were polymorphonuclear (3085.0 ± 548.2 cells/mm³) reduced by indomethacin in 74.4% and by HELc in 54.2 %, 56.3 % and 62.3 % at doses of 3, 30 and 300 mg/kg, respectively. HELc did not alter the influx of mononuclear leukocytes. Statistical analysis showed no difference between the doses assessed ($p > 0.05$). **Conclusion:** These results confirm the anti-inflammatory effects of HELc, validating the use of this plant in folk medicine as an infusion. **Keywords:** medicinal plants, *cidreira*, *chumbinho-branco*, inflammation **Funding:** CNPq and CAPES **References:** Galvão-Nascimento et al., 2010. *Toxicon*. v. 55, p. 343. Martell et al., 2019. *Arch. Heal. Investig*. v. 8, p. 79. Souza et al., 1985. *Agents Actions* v. 17, p. 97. Winter et al., 1962. v. 111, p. 544. **License number of ethics committee:** 1.039/2019

05. Pain and Nociception Pharmacology

05.001 Role of TRPA1 expressed in bone tissue and the antinociceptive effect of the TRPA1 antagonist repeated administration in a breast cancer pain model. Mazzochi N¹, Almeida AS², Pereira GC², Brum ES³, Silva CR⁴, Antoniazzi CTD², Ardisson-Araújo D³, Oliveira SM³, Trevisan G². ¹Medical Graduate Student, Health Sciences Center, Federal Univ of Santa Maria, Santa Maria, Brazil ²Pharmacology Postgraduate Program, Health Sciences Center, Federal Univ of Santa Maria, Santa Maria, Brazil ³Biological Science: Toxicological Biochemistry Postgraduate Program, Biological Sciences, Federal Univ of Santa Maria, Santa Maria, Brazil ⁴Genetics and Biochemistry Postgraduate Program, Biotechnology Inst, Federal Univ of Uberlândia, Uberlândia, Brazil

Introduction: Advanced breast cancer is commonly associated with chronic pain due to bone metastasis, thus opioids are used to treat the pain¹. However, these drugs can cause adverse effects, such as tolerance, requiring an increase in the dose and, consequently, an increase in adverse effects.² Transient receptor potential ankyrin 1 (TRPA1) is involved in cancer pain; moreover, endogenous TRPA1 agonists are associated with cancer pain development.³ The aim of the study was to investigate the antinociceptive effect of a TRPA1 antagonist after its repeated administration and the production of TRPA1 endogenous agonists and TRPA1 immunoreactivity in the bone of mice submitted to a breast cancer pain model. Furthermore, it was used a sequence read archive (SRA) strategy to observe the TRPA1 presence in mice bone cell lines. **Methods:** BALB/c mice were submitted to tumor cell inoculation (4T1 strains) in the fourth mammary gland. Treatment with HC-030031 (a TRPA1 antagonist) was done after tumor inoculation. On the 11th day after tumor cell inoculation, treatment with the antagonist TRPA1 (HC-030031, 100 mg/kg intragastric, i.g.) started for the next ten consecutive days, once a day. The 100 mg/kg dose was used previously and induced an antinociceptive effect; this was the dose selected to be used in that study. Nociceptive tests of mechanical pressure and cold sensitivity were done. TRPA1 expression and biochemical oxidative stress tests were performed in the femur of mice. The SRA strategy was used to detect the presence of TRPA1 in mice bone cell line. **Results:** The repeated administration of TRPA1 antagonist for 10 days, HC030031 (100 mg/kg, i.g.), showed an antiallodynic effect in a model of breast carcinoma in mice. The maximum inhibition (Imax) for the mechanical threshold was $80 \pm 18\%$ and for allodynia to cold was $65 \pm 10\%$, respectively, in relation to the first assessment, which was carried out 24 hours after the 9 injected doses. The Imax for the mechanical threshold was $60 \pm 9\%$ and for cold allodynia it was $69 \pm 5\%$, referring to the second assessment (1 h after the last administration of HC-030031 within 20 days after tumor inoculation. Furthermore, there was an increase in the levels of hydrogen peroxide, NADPH oxidase and superoxide dismutase activities, but the expression of TRPA1 in bone tissue was not altered. SRA did not show the residual transcription of TRPA1 in osteoblast and osteoclast cell lines, as well as in mouse cranial tissue and in mouse osteoclast precursors. **Conclusion:** The study showed that the TRPA1 receptor is a potential target for the development of new analgesics for the treatment of bone cancer pain. **References:** 1. P. Mantyh, Pain, v. 154, p. S54, 2013. 2. A. Satija, Indian J. Med. Res., v. 139, p. 216, 2014. 3. A.S. de Almeida, Pharmacol. Res. V. 152, p. 104576, 2020. **Financial support:** National Council for Scientific and Technological Development (CNPq) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES). **License number of ethics committee:** 7536250417

05.002 Evaluation of antinociceptive and anti-inflammatory activities of substituted synthetic chalcones LC24, LC31 and LC41 in vivo. Melo EDN¹, Botinhão MC¹, Reis JVR¹, Souza ROMA², Leal ICR², Raimundo JM¹, Bonavita AGC¹, Muzitano MF¹, Carmo PL¹. ¹UFRJ Macaé, Brasil; ²UFRJ Rio de Janeiro, Brasil

Introduction: There is a need to develop drugs for the treatment of acute pain and many works describe chalcones as a source of new bioactive products. The objective of this work was to evaluate the *In Vivo* antinociceptive and anti-inflammatory effects of the substituted synthetic chalcones LC24 (4'-methoxychalcone), LC31 (2'-chlorochalcone) and LC41 (4-nitro-2'-chlorochalcone), whose *in vitro* anti-inflammatory effects have already been described (Ventura T.L.B. et al., Molecules, 20, 8072, 2015). **Methods:** Male Swiss mice, pretreated intraperitoneally with the DMSO vehicle or with the positive controls (morphine or indomethacin) or with the chalcones, were used. The formalin test was performed, being injected intraplantar (20 μ L, 2,5%), and then the time spent by the animal licking the paw in the neurogenic (0-5 min) and inflammatory (15-30 min) phases was timed. The second test was the hot plate test, in which the animals were placed on the hot plate at 54 °C prior to injection and 30, 60, 90 and 120 min after injection of the samples, and the time the animal remained on the plate was measured without licking the paws (time maximum of stay = 30 s). And finally, the modified hot plate test, in which 50 μ L of saline and carrageenan (500 μ g/paw) intraplantar injected were into the right and left hind paws, respectively. Afterwards, the animals were placed on the hot plate at 52 °C and the difference in latency times between the paws was calculated. Experiments were approved by Ethics Committee on the Use of Animals of the Federal University of Rio de Janeiro-Macaé, protocol MAC044. **Results:** 1) Formalin test: The chalcones were evaluated at the same doses (10, 30 and 60 mg/kg). In the neurogenic phase, all of them had an antinociceptive effect. LC24 (10, 30 and 60 mg/kg) reduced paw lick time from 60.3 ± 4.9 s (DMSO) to 38.9 ± 4.1 , 28.1 ± 3.4 , and 35.0 ± 5.5 s ($P < 0.05$) respectively; LC31 (30 mg/kg), for 34.8 ± 5.4 s ($P < 0.05$); and LC41 (60 mg/kg), for 36.5 ± 5.6 s ($P < 0.05$). In the inflammatory phase, LC24 (60 mg/kg) had an antinociceptive effect, reducing paw licking time from 361.6 ± 17.8 s (DMSO) to 271.2 ± 13.1 s ($P < 0.05$); and LC41 (10; 30 and 60 mg/kg) to 280.2 ± 23.6 ; 232.4 ± 24.8 and 285.1 ± 23.5 s, respectively ($P < 0.05$). 2) Hot Plate Test: None of the chalcones tested had significant results in this test. 3) Modified hot plate test: LC24 (60 mg/kg) and LC41 (10, 30 and 60 mg/kg) showed antinociceptive and anti-inflammatory effects in all evaluated times. In 15 minutes, for example, the LC24 (60 mg/kg)

reduced the latency variation between the paws from 4.7 ± 0.7 s (DMSO) to 1.5 ± 1.0 ($P < 0.05$); and LC41 (10, 30 and 60 mg/kg), for 0.0 ± 0.0 ; 0.0 ± 0.0 and 0.3 ± 0.2 s ($P < 0.05$), respectively. Conclusions: LC24, LC31 and LC41 had antinociceptive effects *in vivo*, by central mechanisms when pain was triggered by chemical stimulus, but not by thermal stimulus. Furthermore, the LC24 and LC41 chalcones also had an antinociceptive effect related to anti-inflammatory mechanisms. Financial Support: PIBIC/UFRJ. **License number of ethics committee:** Experiments were approved by Ethics Committee on the Use of Animals of the Federal University of Rio de Janeiro-Macaé, protocol MAC044.

05.003 Evaluation of *Vitex polygama* extract and fractions effects on vincristine-induced peripheral neuropathic pain in mice. Ramos IFO, Melo EDN, Santos IS, Carmo PL, Muzitano MF, Bonavita AGC. UFRJ - Campus Macaé, Bioactive Products Pharmacology Lab, Brazil

Introduction: Neuropathic pain is a health problem since most usual pharmacological treatments are inefficient or promote several side effects to patients (Vranken, 2015). The study of natural products is promising to the development of new drugs including the antinociceptive drugs. In the present work we evaluated the effects of *Vitex polygama* Cham. (Lamiaceae) plant extract (VPE) and its fractions on a model of vincristine-induced neuropathic pain in mice. **Methods:** Neuropathic pain was induced by intraperitoneal injection (i.p.) of vincristine (0,1 mg/kg) for 10 days and thermal hyperalgesia and mechanical allodynia was attested by the Hot plate test ($52 \pm 0,5$ °C) and Von frey filaments test, respectively. Five weeks old Swiss mice (30-40g) with food and water *ad libitum* were used in the experiments. VPE (or fractions) were injected (5-30 mg/kg i.p.) 1h before the nociception evaluation. Saline (0,9% NaCl) or Morphine (10mg/kg) were used as negative and positive controls, respectively. All experiments conducted were approved by the UFRJ Animal Care and Use Committee under protocol #MAC051. **Results:** Vincristine-induced pain as observed by the decrease of mice hind paw withdraw (latency of 5.03 ± 0.25 s) when compared with saline treated group (latency of 11.95 ± 1.11 s) on the hot plate test. When animals were treated with VPE was observed an antinociceptive effect in all doses used. For purposes of comparison treatment with 10 mg/kg of VPE produced a latency of 10.6 ± 1.3 s compared with the 8.75 ± 1.11 s of morphine, 1 hour after treatments. The VPE fractions were also capable to reduce thermal hyperalgesia, especially the butanolic and ethyl acetate fractions, with hind paw latency of 9.0 ± 0.77 s and 8.2 ± 0.92 s, respectively (both at 10 mg/kg and 1 hour after treatment). VPE was able to affect the hind paw withdrawal after stimulation with the Von frey filaments when compared with saline-treated group. **Conclusion:** We concluded that *Vitex polygama* Cham. plant extract and its fractions promoted antinociceptive effects in animals with neuropathic pain suggesting that the plant extract could be source for new antinociceptive drugs to the treatment this condition. Financial support: FAPERJ; CNPq. References: Vranken, JH. Current Approaches to the Management of Peripheral Neuropathic Pain. J Pain Palliat Care Pharmacother. 2015 Sep;29(3): 307-10. **License number of ethics committee:** UFRJ Animal Care and Use Committee under protocol #MAC051

05.004 Nonclinical investigation of the analgesic efficacy of gabapentin infiltration in a mice surgical pain model. Martins F¹, Ferreira M², Schran R², Ferreira J². ¹Undergraduate student in Pharmacy, Federal Univ of Santa Catarina, Florianópolis, Brazil ²PPG in Pharmacology, Federal Univ of Santa Catarina, Florianópolis, Brazil

Introduction: Postoperative pain is a common type of pain in clinical practice (POGATZKI-ZAHN, Pain Rep., v. 2, p. 588, 2017). The drug gabapentin, used orally, reduce both pain and opioid consumption postoperatively, but it causes several systemic adverse effects (KUMAR, Cur. Op. Anaesthesiol., v. 32, p. 629, 2019). An alternative to avoid systemic adverse effects of drugs is the use of analgesia by analgesic/anesthetic drug infiltration at the incision site. However, the analgesic efficacy of gabapentin infiltration in postoperative pain is unknown. Thus, the aim of the study was to carry out a nonclinical investigation of analgesic efficacy through gabapentin infiltration in a mice postoperative pain model. **Methods:** female C57BL/6-UFSC mice (N= 8-10, 20-25 g) were submitted to the plantar incision model, (Cowie & Stucky, BioProtoc, v.9, p.1, 2019). Gabapentin (3.0%), bupivacaine (0.5%, used as positive control) or vehicle were administered locally immediately before incision (10 µl) and before suturing (5 µl). We detected paw withdrawal thresholds (PWTs) and affective motivational behaviors (AMBs) after von Frey filaments application near the incision. PWTs were determined by using the up-and-down method and AMBs were detected by scoring the behaviors (0-normal paw, 1-sideways paw, 2-raised paw and 3-licking, shaking or biting the paw (CORDER, Nat Med., v. 23, p. 164, 2017). The paw thickness (an indicative of edema) was assessed with a caliper. The experimental measures were carried out before and from 15 min to 24 hours after surgery. **Results:** Animals infiltrated with vehicle had a reduction of PWTs (mechanical hyperalgesia) and an increase of AMBs postoperatively (thresholds of 0.665 ± 0.012 or 0.029 ± 0.013 g and AMBs of 2.143 ± 0.670 or 12 ± 2 scores, before or 1 hour after surgery). The infiltration with bupivacaine

was able to fully (100% of inhibition at 15 min after surgery), but transitorily (this effect was vanished from 1 to 24 hours), reduce postoperative hyperalgesia and AMBs. The gabapentin treatment was partially capable of prevent the hyperalgesia (thresholds of 0.234 ± 0.065 and 0.029 ± 0.013 g, for gabapentin and vehicle groups, 1 hour after surgery), but its efficacy was prolonged (from 1 to 4 hour after surgery). Gabapentin treatment also prevented AMBs 2 hours after surgery (mean of scores 12 ± 1 and 8 ± 1 for vehicle and gabapentin groups, respectively). Finally, gabapentin, but not bupivacaine, infiltration increased the postoperative edema observed 2 hours after surgery (0.4 ± 0.081 , 0.3 ± 0.084 and 0.8 ± 0.092 mm of paw thickness for vehicle, bupivacaine and gabapentin groups, respectively). **Conclusion:** Our results demonstrate that gabapentin infiltration produced an effective and long-lasting antinociceptive effect in a postoperative pain non-clinical model. However, the increase of the postoperative edema may be a limitation to its infiltrative use and its safety is under further investigation. **Acknowledgments:** This study was approved by the Ethics Committee on the Use of Animals (CEUA) number: 1704260819. Financial support for this project was provided by Instituto Nacional de Ciência e Tecnologia-Inovação em Medicamentos (INCT-INOVAMED) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq). **License number of ethics committee:** This study was approved by the Ethics Committee on the Use of Animals (CEUA) number: 1704260819.

05.005 Participation of the transient potential receptor ankyrin 1 (TRPA1) in facial mechanical allodynia induced by chronic administration of corticosterone. Silva NAR¹, Dalenogare DP¹, Pereira GC¹, Bochi GV¹, Trevisan G¹.¹UFSM Santa Maria, Dpt of Physiology and Pharmacology, Brazil

Introduction: Migraine is a neurovascular disease that is difficult to treat, as its mechanisms and causes have not yet been fully elucidated. One of the most relevant factors that lead to the development of migraine attacks is stress. Thus, it is necessary to search for new pharmacological targets for its treatment, such as transient ankyrin 1 potential receptors (TRPA1), which are expressed in sensory neurons and are involved in the transduction of pain stimuli. Therefore, we aim to evaluate the participation of TRPA1 in facial nociception induced by stress caused by chronic administration of corticosterone. **Methods:** Adult male Swiss mice obtained from the UFSM vivarium were used (Animal Research Ethical Committee – CEUA: 6412121218). Animals received subcutaneous corticosterone for 21 days to induced facial mechanical allodynia, a pain response observed in migraine, while control mice received vehicle. After the 21st day, the animals were divided into groups (n=8) where they received treatment with the TRPA1 receptor antagonist (HC-030031 and A967079), the antimigraine compound (sumatriptan) and the antioxidant compound (apocynin). Animals were evaluated before the start of corticosterone administration (basal), post-administration at day 21 and post-treatments again at day 21 for nociception parameters, those including facial mechanical allodynia, facial nociception score and measurement of locomotion. After nociception tests, the animals were euthanized and samples from the trigeminal ganglion and brainstem were harvest. Within these samples, oxidative stress parameters such as hydrogen peroxide and the antioxidant enzyme NADPH, evaluation of TRPA1 receptor expression levels and evaluation of CGRP levels were assessed. **Results:** Animals induced with chronic administration of corticosterone showed a decrease in the mechanical threshold in comparison to control animals from day 14th to 21st. The treatment with sumatriptan, an antimigraine compound drug already used in the clinic, mice showed an increased threshold after 1 hour. The CGRP antagonist, olcegepant, also showed an antinociceptive effect 1 hour after treatment. The same occurred to both the TRPA1 receptor antagonists (HC-030031 and A967079), which showed antinociceptive effect after 1 hour of treatment. H₂O₂ levels and NADPH oxidative activity were increased in comparison to control groups in trigeminal ganglion and brainstem samples. When treated with an antioxidative agent, apocynin, animals showed an increased threshold after 1 hour. **Conclusion:** We were able to conclude that chronic stress reached through continuous administration of corticosterone was capable to cause facial nociception and that the oxidative stress could be involved in the activation of TRPA1 receptor. **Financial Support:** CNPq, Capes. **Acknowledgments:** Conselho Nacional de Desenvolvimento Científico e Tecnológico-CNPq, and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior-CAPEs. We thank CNPq and CAPES for their fellowship support. **License number of ethics committee:** Animal Research Ethical Committee – CEUA: 6412121218

05.006 Mechanism of action of the antinociceptive and anti-inflammatory activities of a synthetic substituted 4-dimethylaminochalcone in acute pain models. dos Santos IS, Melo EDN, Reis JVR, Muzitano MF, Bonavita AGC, do Carmo PL, Raimundo JM. UFRJ-Macaé, Bioactive Products Pharmacology Lab, Brazil **Introduction:** Chalcones and their derivatives are substances of medicinal interest since they show a broad spectrum of biological activities¹. The objective of this study was to evaluate the antinociceptive and anti-inflammatory activities, as well as the mechanism of action, of a synthetic substituted 4-dimethylaminochalcone (LC4) in acute pain models. **Methods:** All protocols were approved by the Ethics

Committee on the Use of Animals of UFRJ-Campus Macaé (license MAC044). The experiments were performed on male Swiss mice (18-30 g, n= 7-13). DMSO (30 μ L), LC4 (3, 10 and 30 mg/kg) or positive controls (morphine or indomethacin) were administered intraperitoneally (i.p.) 30 min before the tests. 1) Formalin test: animals received 20 μ l of 2.5% formalin in the right hind paw. The time spent licking the paw was measured during the neurogenic (0-5 min) and inflammatory phases (15-30 min). 2) Hot plate test: animals were placed on the hot plate (54°C) before and 30, 60, 90 and 120 min after the treatment. Latency to paw lifting or licking response was recorded. 3) Modified hot plate test: 50 μ L of saline and carrageenan (500 μ g/paw) were injected via intraplantar into the right and left hind paws, respectively, and the animals were placed on the hot plate (51°C). The withdrawal latency for each paw was recorded at 15, 60, 180 and 360 min after injection, and the difference between paws latency was calculated. 4) Mechanism of action: in formalin and hot plate tests, naloxone (1 mg/kg), atropine (2 mg/kg) or L-NAME (30 mg/kg) were administered i.p. 15 min before the administration of LC4 (10 mg/kg). **Results:** 1) Formalin test: LC4, 10 and 30 mg/kg, reduced paw licking time in the neurogenic phase from 59.4 ± 5.3 s (DMSO) to 28.2 ± 4.8 and 23.1 ± 2.8 s ($P < 0.05$), respectively. In the inflammatory phase, 3, 10 and 30 mg/kg of LC4 reduced paw licking time from 361.6 ± 1.7 s (DMSO) to 200.6 ± 16.7 , 226.4 ± 12.3 and 238.5 ± 16.0 s ($P < 0.05$), respectively. The effect of LC4 (10 mg/kg) was reversed by atropine in both phases of the formalin test (1st phase: 51.2 ± 2.4 s; 2nd phase: 320.3 ± 22.3 s; $P < 0.05$). L-NAME pre-treatment significantly inhibited the effect of LC4 only in the first phase, while naloxone reversed LC4 effect only in the second phase of the test. 2) Hot plate: LC4 (10 and 30mg/kg) significantly increased the time on the hot plate at all evaluation times. The antinociceptive effect of LC4 (10 mg/kg) was significantly inhibited by the pre-treatment with L-NAME, atropine and naloxone. L-NAME pre-treatment reduced latency from 15.6 ± 2.0 s to 9.0 ± 0.5 s at 120 min ($P < 0.05$). 3) Modified hot plate: LC4, at 15 and 360 min, reduced the latency variation between the paws from 4.1 ± 0.8 s (DMSO) to 0.6 ± 0.3 s ($P < 0.05$) and from 6.6 ± 1.3 s (DMSO) to 1.1 ± 0.7 s ($P < 0.05$), respectively. **Conclusion:** LC4 shows antinociceptive activity through peripheral anti-inflammatory and central mechanisms, which seem to involve the inhibition of inflammatory factors and the activation of muscarinic and opioid receptors and the production of nitric oxide. Financial support: FAPERJ. **Reference:** ¹Ferreira MKA et al. Rev Virtual Quim 10: 1455, 2008. **License number of ethics committee:** UFRJ Animal Care and Use Committee under protocol #MAC044

05.007 **Aldehyde dehydrogenase-2 activation alleviates neuropathic pain by controlling neuroinflammation in the spinal cord.** Alcantara QA¹, Neto BS¹, Mochly-Rosen D², Cury Y¹, Zambelli VO¹. ¹Lab of Pain and Signaling, Butantan Inst, Brazil; ²Chemistry and Systems Biology, Stanford Univ, USA

Introduction: Aldehyde dehydrogenase-2 (ALDH-2) is a mitochondrial enzyme responsible for detoxifying aldehydes generated from the lipid peroxidation. We previously showed that Alda-1, which is a small molecule that activates ALDH-2, has a potent antinociceptive effect in an acute model of inflammatory hypernociception and a chronic neuropathic pain model induced by the chronic constriction injury of the sciatic nerve (CCI) in mice. The analgesic effect was followed by a decrease in reactive aldehydes, such as 4-hydroxynonenal (4-HNE) at the injured site. Since the key mechanism involved in the neuropathic pain onset and chronification is the activation of glial cells, and that reactive aldehydes are involved in pain, we hypothesize that 4-HNE in the spinal cord may contribute to CCI-induced neuroinflammation. Therefore, we aimed to investigate the role of ALDH2 on the CCI-induced neuroinflammation, evaluating the aldehydic load and glial cells activation in the spinal cord. **Methods:** The nociceptive threshold was assessed by von Frey filaments, before surgery (baseline), 7, and 14 days afterwards. The modulation of ALDH2 function was achieved by either its activator, Alda-1 (16 mg/Kg/day), delivered by an osmotic pump of continuous release, or transgenic mice with an inactivating mutation found commonly in asiatic populations that impairs ~95% in ALDH2 activity (ALDH2*2). The 4-HNE protein adducts and the activation of glial cells (microglia and astrocyte) in the dorsal horn of the spinal cord (DHSC;L3-L5) was assessed by using immunofluorescence techniques. **Results:** Our data show that the CCI decreases the nociceptive threshold when compared to the baseline on the 7th (89 ± 2.7 and $91 \pm 3.4\%$), and 14th (91 ± 1.8 and $83 \pm 8.5\%$) days after surgery, both in wildtype (WT) and in ALDH2*2 animals, respectively. Alda-1 decreases CCI-induced hypernociception in WT (to 18.8 ± 7 and $39.2 \pm 8\%$), and ALDH2*2 (to 4.5 ± 14 and $38.4 \pm 4,7\%$) in both periods, respectively. Also, we detected an increase in immunostaining for 4-HNE adducts (2.5- and 1.7-fold), IBA-1 (microglia) (2.4- and 2.7-fold) and GFAP (astrocyte) (4- and 1.2-fold) in WT animals on both days after surgery, respectively. Alda-1 reduces the 4-HNE adducts and prevents the activation of glial cells to control levels in WT mice. Interestingly, ALDH2*2 mice display greater 4-HNE adducts and GFAP staining, but decreased microglial activation. Interestingly, Alda-1 reduces the 4-HNE levels without interfering with glial activation. **Conclusion:** Thus, the data suggest that ALDH2 plays an important role in

hypernociception by increasing the aldehyde levels and activating glial cells in the DHSC in a model of neuropathy. Alda-1 has an antinociceptive effect by preventing the 4-HNE adducts formation and reducing the neuroinflammation in WT animals. Interestingly, the genetic impairment in ALDH2 activity changes the dynamics of glial activation upon nerve injury. Finally, Alda-1 is a promising molecule for neuropathic pain treatment. Acknowledgments: This work was supported by CNPq 119077/2019-1; 139215/2018-2 and FAPESP 2017/16071-5; 2020/04998-0. We thank CENTD and DDC at Butantan Institute for Confocal Microscopy availability. **License number of ethics committee:** Experiments approved by the Butantan Institute Ethical Committee (7044040219).

05.008 Isopulegol presents antinociceptive activity in a cancer pain model: *In Vivo* study and *in silico* predictions. ¹Dias WA, ¹Pimentel VD, ¹Sales SCS, ¹Acha BT, ²Silva JN, ²Paulo Ferreira PMP, ¹Almeida FRC ¹Lab of Pain Pharmacology, Medicinal Plants Research Center; ²Lab of Experimental Cancerology, Dpt of Biophysics and Physiology, Federal Univ of Piauí, Teresina, Brazil

Introduction: Cancer pain is not a diagnosis, and it is not a syndrome, in fact, it is a pain resulting from the sum of or combination of several causes. Due to the particularities of this neuropathy, it is treated with various pharmacological agents. Among them are non-steroidal anti-inflammatory drugs (NSAIDs), opioids, serotonin-norepinephrine reuptake inhibitors (SNRIs), tricyclic antidepressants (TADs) and others. In this scenario, the inclusion of new pharmaceutical agents becomes increasingly necessary. Among these, monoterpenes are secondary metabolites of essential oils, such as Isopulegol (ISO), that has a great prominence since it is reported to present important biological activities such as anti-inflammatory, antinociceptive and antioxidant properties. In view of above, the aim of this study was to investigate a possible antinociceptive effect of ISO in animal model of cancer pain, as well as *in silico* predictions of the pharmacokinetic and toxicological properties of ISO. **Methods:** Female Swiss mice weighing between 25 and 35 g were used and the experiments were previously approved by the Ethics Committee on Animal Use (CEUA / UFPI No. 148/16). For induction of hypernociception, 2.5 million sarcoma cells were inoculated into the right paw of mice, and mechanical allodynia was assessed using von Frey filaments in acute treatment during a 12-hour evaluation period. Furthermore, the animals were divided into 6 groups: sham (normal animals), vehicle (NaCl 0.9% with 2% Tween 80), positive control (pregabalin 10 mg/kg po), and 3 doses of ISO (12.5, 25 and 50 mg/kg po). The relative mass of the animals' organs was evaluated to investigate possible toxicity. *In silico* evaluation was performed using ACD/ChemSketch software version 14.0 and the PreADMET online server. **Results:** Reduction in hypernociception was measured by comparing between group means in the Von Frey test from the first until the twelfth hour of evaluation. The best result achieved was at the 4th hour of evaluation (sham = 7.63 ± 0.43 ; vehicle = 1.09 ± 0.8 ; ISO 12.5mg/kg= $3.75 \pm 1.04^*$, ISO 25mg/kg= $4.75 \pm 1.49^*$; ISO 50mg/kg= $5.75 \pm 0.71^*$ and pregabalin= $4.50 \pm 1.41^*$) (*p <0.05). ISO improved the animal locomotion in the open field test when compared to the negative and positive controls, but it was not able to reduce paw licking time. Regarding pharmacokinetics, we noticed that ISO is able to cross the blood-brain barrier, corroborating the opioid effect already demonstrated in previous studies by our research group. In addition, it inhibits the following P450cytochrome (CYP) enzymes, CYP2C19, CYP2C9, CYP3A4, and is a substrate of CYP3A4. The human intestinal absorption of ISO is 100%, and it also shows no carcinogenicity. **Conclusion:** ISO has a potential antinociceptive effect in cancer hypernociception model, presumably by opioid action and antioxidant action as demonstrated by other studies. Additionally, due to the numerous pharmacokinetics advantages presented by ISO in this study such as 100% intestinal absorption, not metabolized in the liver, besides demonstrating neither cardiotoxicity nor carcinogenicity activities. Thus, this substance appears to be a safe well-tolerated drug that can be administered orally without inconvenience. **Financial Support:** UFPI, CAPES. **License number of ethics committee:** CEUA-UFPI No. 148/2016

05.009 The neuroinflammatory process and TRPA1 role in two multiple sclerosis mouse models. Dalenogare PD¹, Trevisan G¹, Araújo DSM², Landini L², Titiz M², De Logu F², Nassini R², Geppetti P². ¹UFMS Santa Maria, PPG Pharmacology, Brazil; ²UNIFI Firenze, Dpt Health Sciences, Italy

Introduction: Transient Receptor Potential Ankyrin 1 (TRPA1) channel is expressed in neuronal and non-neuronal cell types (FERNANDES et al., 2012; STORY et al., 2003), and it was recently described its expression in astrocytes (SHIGETOMI ET AL., 2011) and oligodendrocytes (HAMILTON et al., 2016). Furthermore, TRPA1 channel plays a main role in neurodegenerative diseases, such as Alzheimer's disease and multiple sclerosis (MS) (LEE et al., 2016; SÁGHY et al., 2016). Previously, in a multiple sclerosis mouse model induced by cuprizone demonstrated the activation of TRPA1 expressed by astrocytes might induce the release of pro-inflammatory mediators which contribute to the progression of oligodendrocyte apoptosis (KRISZTA et al., 2019). The TRPA1 deletion prevented the microglia activation, modulating the ionized calcium binding adaptor molecule 1 (IBA-1), after hyperalgesia model induced by bradykinin B1 receptor agonist, revealing an important role of this channel in the nociception pathways (MEOTTI et al.,

2017). Nevertheless, the relation between TRPA1 expression and regulation of neuroinflammatory markers in the MS rodent models by experimental autoimmune encephalomyelitis (EAE) was not demonstrated. Previously, it was demonstrated the enhance of IBA-1 and glial fibrillary acidic protein (GFAP), microglial and astrocyte markers, in an EAE model of induction in rats. In addition, a model of relapsing remitting EAE in mice it was demonstrated the increased of the same neuroinflammatory markers. Our group newly published that the pharmacological blockade of TRPA1 was capable to attenuate the mechanical and cold allodynia in two EAE models in mice (DALENOGARE et al., 2020; RITTER et al., 2020). Thus, our main purpose here was evaluated if the TRPA1 deletion could modulate the nociception behaviors and neuroinflammatory process in two different clinical course models of MS. **Methods:** C57BL/6J female, littermate wild-type (*Trpa1^{+/+}*) and TRPA1-deficient (*Trpa1^{-/-}*) (KWAN et al., 2006) (female, 20-30 g, 4-6 weeks) were induced to the progressive (PMS) (RITTER et al., 2020) or relapsing-remitting (RR) (DALENOGARE et al., 2020) multiple sclerosis models by experimental autoimmune encephalomyelitis (EAE). To induce two EAE models, it was used the subcutaneous injection of myelin oligodendrocyte glycoprotein (MOG₃₅₋₅₅) antigen (200 µg) and two different adjuvants: Complete Freund Adjuvant (CFA) for PMS model or Quil A (saponin mixture of *Quillaja* sp. bark) for RR model. All the induced and control animals received two intraperitoneal doses of Pertussis toxin, 300 ng to PMS-EAE or 250ng to RR-EAE, one at induction day and the other 48 hours later. The control/ non-induced animals received only the adjuvant injection and Pertussis toxin. The clinical signs of PMS and RRMS-EAE models were evaluated using a clinical score scale to measure neurological impairment and weighted animals for eventual significative weight loss. To test the locomotor activity, we used Rotarod® apparatus and the open field test. The nociception behaviors were measure by von Frey test (mechanical allodynia) and acetone test (cold allodynia) (DALENOGARE et al., 2020; RITTER et al., 2020). The neuroinflammatory markers, ionized calcium binding adaptor molecule 1 (IBA-1) and glial fibrillary acidic protein (GFAP) antibodies, were assessed in the spinal cord (L4-L6) samples by immunohistochemistry assay. The experimental procedures were approved by the Italian Ministry of Health (protocol #1194/2015-PR) and followed the Animal Research Reporting *In Vivo* Experiments (ARRIVE) guidelines. **Results:** As previously published, PMS-EAE induced *Trpa1^{+/+}* female mice demonstrated alteration in the clinical score, without motor impairment, and mechanical and cold allodynia development from 7 to 14 days post-induction (p.i). Likewise, *Trpa1^{-/-}* female mice developed the PMS-EAE scores, without motor impairment, however, did not present alterations in the nociception behavior tests. After RR-EAE induction, without motor impairment *Trpa1^{+/+}* female mice presented the first clinical score, without motor impairment, at 14 days p.i. and the maintenance of the relapsing-remitting course until 35 days p.i. Otherwise, RR-EAE induced *Trpa1^{-/-}* female mice developed the clinical score, without motor impairment, but did not present significative alteration in the cold and mechanical allodynia, when compared to induced wild-type group. We observed an increase of neuroinflammatory (IBA-1 and GFAP) markers in the PMS- and RR-EAE *Trpa1^{+/+}* mice. Remarkably, the TRPA1 deletion was able to attenuate the neuroinflammatory process in both EAE models. **Conclusion:** Here, as unprecedented results, we confirm our previous results that demonstrated the TRPA1 involvement in nociception behaviors using now a TRPA1 deletion approach and the important role in the neuroinflammatory regulation of this receptor in MS mouse models. **References:** DALENOGARE, D. P. et al. TRPA1 activation mediates nociception behaviors in a mouse model of relapsing-remitting experimental autoimmune encephalomyelitis. *Experimental Neurology*, v. 328, 1 jun. 2020. SHIGETOMI, E. et al. 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05.010 **The CB2 receptor agonist beta-caryophyllene attenuates oxaliplatin-induced peripheral neuropathy in a tumor-bearing mice model.** Agnes JP¹, Neves RN¹, Gonçalves RM¹, Delgobo M¹, Silva RC², Ferreira AR², Senna EL², Zanotto-Filho A¹ ¹UFSC Florianópolis, PPG Pharmacology, Dpt of Pharmacology, Brazil; ²UFSC Florianópolis, PPG Pharmacy, Dpt of Pharmaceutical Sciences, Brazil

Introduction: Chemotherapy-induced peripheral neuropathy (CIPN) is a morbidity that affects patients receiving chemotherapeutic agents such as platinum-based anticancer drugs. The symptoms include tingling in hands and feet, cold sensitivity and, in severe cases, pain. These symptoms, besides impairing the quality of life of cancer patients, can also lead to changes in the treatment protocol by either reducing doses or increasing intervals between chemotherapy cycles, facilitating the emergence of chemoresistant clones and hampering the success of the treatment. To date, there are no effective treatments to relieve the CIPN symptoms. Given that cannabinoid receptors type 2 (CB2) are predominantly expressed in immune cells, and that the activation of these receptors reduces the release of inflammatory mediators and consequently the neuroinflammation, we have hypothesized that activation of the endocannabinoid system through CB2 receptors may be an alternative to reduce CIPN. In this study, we evaluate the effects of the CB2 agonist beta-caryophyllene (BCP) administered as a nanoemulsion or a free oil on CIPN and tumor growth kinetics. **Methods:** Female Swiss mice were used in experiments; experimental protocols were approved by CEUA-UFSC nº 3722260417. BCP nanoemulsions (BCP-NE) displaying droplet size of around 200 nm were prepared by a spontaneous emulsification method. For induction of CIPN, oxaliplatin (OXA) was i.p administered at a dose of 5 mg/kg, every 48 hours, for 14 days. Both free oil and BCP-NE were administered daily through oral gavage, at doses ranging from 5 to 250 mg/kg. The effect of BCP (100 mg/kg) was also evaluated after the establishment of the CIPN phenotype. The mechanical nociception was evaluated by using the Von Frey filament test "up and down" method, and the thermal nociception was evaluated by the cold plate test. The effect of the free oil on tumor growth was also evaluated after the implant of Ehrlich cells (3×10^6 cells/mouse) into the mammary fat pad of mice to induce a solid tumor. **Results:** Oral administration of BCP at doses of 50 and 100 mg/kg was efficient in attenuating nociceptive responses to mechanical and thermal/cold stimuli in mice treated with OXA. However, the effect observed for BCP at the dose of 250 mg/kg was not higher than those obtained for the doses of 50 e 100 mg/kg, while the smallest effect was verified when the dose of 10 mg/kg was tested. In addition, BCP was also effective in attenuating both mechanical and cold nociception in mice with established neuropathy. The administration of the BCP-NE produced a more significant effect on mechanical and thermal nociception when compared to the free oil, at low dose (10 mg/kg), probably due to the increase of the BCP bioavailability upon nanoemulsification. Regarding the tumor volume, no differences were observed between the groups treated with BCP and OXA, when compared to animals treated only with OXA. **Conclusions:** This study demonstrated that BCP is effective in reducing the mechanical and thermal nociception when concomitantly administered with OXA and after neuropathy induction. The BCP-NE showed a more consistent antinociceptive effect when administered at the lower dose. Regarding tumor growing, BCP seems did not affect the antitumor activity of OXA, at least in the tumor model used herein. **Acknowledgments:** We thank the CNPq and CAPES for supporting this work and also our multiuser laboratory (LAMEB-UFSC) for technical support. **License number of ethics committee:** CEUA-UFSC n.º 3722260417

05.011 **Nociceptive effect of TLR2 on a mice model of postoperative pain.** Schran RG, Ferreira M, da Silva AM, Martins F, Ferreira J. Dpt of Pharmacology, Federal Univ of Santa Catarina, Florianópolis, Brazil

Introduction: Postoperative pain affects millions of people each year in worldwide, being more prevalent in women. However, safe and efficacious analgesics to treat postoperative pain remains quite limited. The toll-like receptor 2 (TLR2) has already been implicated in chronic and inflammatory pain, but not postoperative pain. Thus, we used TLR2 gene deletion or antagonism in mice to elucidate the role of TLR2 in postoperative pain. **Methods:** Female or male C57BL/6-UFSC wild-type (WT) or TLR2 knockout (KO) mice (N= 8-10, 20-25 g) were submitted to the plantar incision (skin+muscle) model (Cowie & Stucky, BioProtoc, v.9, p.1, 2019). We detected paw withdrawal thresholds (von Frey filaments) and thickness (caliper) before and from 15 minutes to 28 days after skin+muscle. A separated groups of WT animals were treated with vehicle or the TLR2 selective antagonist vanillin (0.045%) locally immediately before incision (10 µl) and before suturing (5 µl). **Results:** The surgery produced an intense hyperalgesia from 1 hour to 2 days after operation in both sexes (thresholds fell from baseline values of about 1 to 0.008 g 1 day after operation in male or female wild-type mice). This hyperalgesia was slowly subsiding after day

3 and lasted up 14 days in males and 21 days in females. Surgery also induced a marked edema from 4 hour to 2 days after operation in both sexes (paw thickness increase of 0.026 ± 0.041 and 0.027 ± 0.043 mm 1 days after operation in male or female mice, respectively). This edema was quickly subsiding after day 3 and lasted up 4 days in males and females. The TLR2 gene deletion in female mice did not alter the intensity of hyperalgesia (thresholds of 0.047 ± 0.025 and 0.020 ± 0.009 g 1 hour after operation in female wild-type or knockout mice, respectively), but it was subsiding more quickly from the 7th day. However, the TLR2 gene deletion in male mice reduced both the intensity (thresholds of 0.017 ± 0.009 and 0.097 ± 0.019 g 1 hour after operation in male wild-type or knockout mice, respectively) and the duration of postoperative hyperalgesia, subsiding from the 4th day. The TLR2 gene deletion in mice also presented a reduced intensity, but not duration, of edema compared to wild-type animals, being this reduction more effective in female mice (47 ± 9 vs. $34\pm 4\%$ of inhibition in female vs. male mice). The infiltration of the TLR2 antagonist was able to reduce the intensity of hyperalgesia in male (thresholds of 0.023 ± 0.008 and 0.107 ± 0.036 g), but not in female wild-type mice (0.091 ± 0.048 and 0.126 ± 0.082 mm in vehicle and antagonist-treated mice, 15 min after operation). On the other hand, TLR2 antagonist treatment did not alter the postoperative edema in male mice but increased such edema in female mice (increase in thickness of 0.325 ± 0.084 and 0.650 ± 0.110 mm in vehicle and antagonist-treated female mice, 2 hours after operation). **Conclusion:** TLR2 is important for the development of postoperative pain in a sexually dimorphic way, being a potential target for its treatment. **Acknowledgments:** This study was approved by the Ethics Committee on the Use of Animals number: 3505290719. Financial support for this project was provided by Instituto Nacional de Ciência e Tecnologia-Inovação em Medicamentos (INCT-INOVAMED) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq). **License number of ethics committee:** 3505290719

05.012 **Benefits of hydrogen sulfide-releasing non-steroidal anti-inflammatory drug, ATB-352, on postoperative pain and gastric safety: role of endocannabinoid system** Dallazen JL¹, Santos LG¹, Teixeira SA¹, Muscará MN¹, Wallace JL^{2,3}, Costa SKP¹; ¹Dpt of Pharmacology, Inst of Biomedical Sciences, Univ of São Paulo; ²Antibe Therapeutics, Inc., Toronto, Canada; ³Dpt of Physiology and Pharmacology, Univ of Calgary

Introduction: It is estimated that 80% of patients undergoing surgery report postoperative pain, a condition with suboptimal treatment choices, and often associated with low quality of life and high morbidity rate. Besides opioids and non-steroidal anti-inflammatory drugs (NSAIDs) exerting potent analgesic effects, opioids possess the risk of addiction whilst NSAIDs induces gastrointestinal (GI) mucosa damage, thus limiting their use. This study was undertaken to investigate the analgesic and GI irritative effects of a new H₂S-releasing ketoprofen derivative (ATB-352) in a murine model of postoperative pain (plantar incision surgery, PIS) and involved mechanisms. **Methods:** All experiments were conducted with male BALB/c mice (~25 g, CEUA-ICB/USP: 2055050819). Previously (basal values, B) and 24 h after to PIS, the mechanical (von Frey filaments) and heat hyperalgesia (hot plate, 52 ± 0.1 °C), were measured. Subsequently, the animals were orally (p.o.) treated with vehicle (V: 0.5% carboxymethylcellulose, CMC, 10 mL/Kg), ketoprofen (KETO: 3, 10, and 30 mg/Kg) or ATB-352 (ATB-352: 4.6, 15, and 46 mg/Kg, equimolar doses). In a separate set, a group of mice were intraperitoneally pretreated with the CB1 receptor antagonist (AM-251: 3 mg/Kg, i.p.), 30 min before KETO or ATB. The nociceptive behaviors were assessed for 5 h. After this, blood samples were collected by intracardiac puncture for measurement of plasma levels of anandamide (AEA) via ELISA kit, and the stomachs were removed to measure gastric ulcer area and wall mucus levels. **Results:** After PIS procedure, mice developed mechanical allodynia and thermal hyperalgesia to heat. The sham (non-operated) group did not exhibit changes in either mechanical or thermal parameters. Only the 30 mg/Kg of KETO treatment reverted the mechanical hyperalgesia in 53.5%. ATB-352 treatment at 15 and 46 mg/Kg revert in 30.8% and 58.8% the mechanical hyperalgesia, respectively, compared to the vehicle group. The heat latency was reversed by KETO and ATB in 78.9% and 80.9%, respectively, only with the highest tested dose. CB1 receptor antagonism by AM-251 abolished the analgesic effect of ATB-352 in mechanical and heat hyperalgesia, but did not affect KETO effects. KETO-induced gastric lesions at 30 mg/kg, and the CB1 receptor antagonism exacerbated the ulcers in 660.2% and 126.4% in animals treated with 10 and 30 mg/Kg of KETO, respectively. ATB-352 treatment did not induce gastric lesions, even when administered after CB1 receptor antagonist. Gastric mucus levels increased in 67.6% with 46 mg/Kg ATB-352 treatment, which was reversed by CB1 receptor antagonism, compared to the vehicle group. While 10 mg/kg KETO reduced AEA levels in 33.9%, 15 mg/Kg ATB increased it in 27.7%, comparing to the sham group. AM251 pretreatment did not alter this parameter. **Conclusion:** The H₂S-releasing NSAID (ATB-352) shows enhanced analgesic properties and gastric safety as compared to its parent drug ketoprofen. The endocannabinoid system seems to be the mediated mechanism involved in both analgesia and GI protection, indicating a promising treatment for postoperative

pain. **Financial Support:** FAPESP, CAPES (Finance Code 001) and CNPq (142343/2020-0; 312514/2019-0). **License number of ethics committee:** CEUA-ICB/USP: 2055050819

05.014 **Succinate/sucnr1 signaling in non-Nav1.8⁺ nociceptors drive paclitaxel-induced neuropathic pain.** Gomes FIF, Kusuda R, Silva CEA, Guimarães RM, Silva NR, Carmo BR, Mendes AS, Paiva IM, Oliveira AER, Lopes AHP, Alves-Filho JCF, Cunha FQ, Cunha TM Dpt of Pharmacology, Ribeirão Preto Medical School, Univ of São Paulo

Introduction: Paclitaxel is an anti-cancer agent, but the ensuing neuropathic pain is a major side-effect. The pathogenesis of paclitaxel-induced neuropathic pain involves bioenergetic imbalances in primary sensory neurons, affecting oxidative phosphorylation in mitochondria, disrupting energy-producing pathways. Succinate levels can rise upon mitochondrial perturbations, leading to activation of its receptor (Sucnr1). Here, we aimed to study the pronociceptive role of Sucnr1 in the physiopathology of paclitaxel-induced peripheral neuropathic pain. **Methods:** Ethics committee approval CEUA-FMRP/USP 153/2019. Bioinformatics re-analyses of Seq-RNA datasets (Accession number GEO: GSE131230) from mouse primary sensory neurons. Sucnr1 expression was assessed by immunofluorescence. Behavioral responses of wild-type (WT), full knockout for Sucnr1 (Sucnr1^{-/-}), and mice lacking TRPV1⁺ or Nav1.8⁺ nociceptors, and conditional knockout (Nav1.8^{CRE/0}-Sucnr1^{flox/flox}) animals were verified. Succinate levels were determined in DRG samples of WT mice after paclitaxel treatment (8 mg/kg, i.p.) by 1H-NMR (600Hz) analyses. WT and Sucnr1^{-/-} mice received paclitaxel (8 mg/kg, i.p.) or saline solution (i.p.) and behavioral nociceptive responses were analyzed. Data were analyzed by ANOVA or Student's t-test, significance level was set at 0.05. **Results:** Bioinformatics analyses showed the expression of Sucnr1 more confined to A-delta low threshold mechanoreceptors in comparison to the other subsets analyzed (p<0.01). Double-labelling using immunofluorescence assays confirmed the expression of Sucnr1 mostly in large-diameter neurons, NF200 positive cells (p<0.05). Succinate injection induced mechanical and cold allodynia (p<0.05) in a dose-dependent manner (p<0.05). Depletion of TRPV1⁺ and Nav1.8⁺ nociceptors did not alter the pronociceptive effect of succinate (1 nmol/paw) (p>0.05), whilst Sucnr1 gene expression was preserved after neuronal depletions of TRPV1⁺ and Nav1.8⁺ nociceptors in comparison with non-depleted animals (p>0.05). Behavioral responses of conditional knockout and littermate control mice did not differ upon succinate injection (1 nmol/paw) (p>0.05). Paclitaxel treatment increased the levels of succinate in DRG samples overtime, peaking on day 10 compared to vehicle-treated animals (p<0.05). Finally, Sucnr1^{-/-} mice treated with paclitaxel showed attenuated nociceptive responses compared to neuropathic WT animals (p<0.05). **Conclusion:** Succinate/Sucnr1 pathway elicited nociception independent of Nav1.8⁺ neurons and contributed to the development of neuropathic pain induced by paclitaxel. **Financial Support:** FAPESP 13/08216-2, FAPESP 2019/14285-3. **License number of ethics committee:** CEUA-FMRP/USP 153/2019.

05.015 **Aldehyde dehydrogenase-2 is a critical enzyme involved in 4-hydroxy-2-nonenal-induced pain: role of protein kinase Cε.** Martins BB¹, Zambelli VO^{1 2}, ¹Lab. Especial de Dor e Sinalização, Butantan Inst, Brazil, ²Dep. de Anestesia, Univ de Stanford, EUA

Introduction: Aldehyde dehydrogenase-2 (ALDH2) is a mitochondrial enzyme responsible for metabolizing toxic aldehydes including 4-hydroxynonenal (4-HNE), a sub-product of oxidative stress-induced lipid peroxidation (1). We have previously shown that 4-HNE is a key mediator of inflammatory pain. Of interest, ALDH2 activation by a small molecule called Alda-1 induces analgesia by decreasing 4-HNE levels in the inflamed tissue (2). ALDH2 enzyme is a substrate for protein kinase Cε (PKCε), a protein highly expressed in nociceptors. On activation, PKCε translocates to multiple subcellular sites, including plasmatic membrane, where it contributes to pain by phosphorylating ion channels. However, PKCε also induces cytoprotection when imported to mitochondria and activates ALDH2 and reduced 4-HNE levels. Therefore, our hypothesis is that PKCε has a dual effect in nociception. Our aim was to investigate whether PKCε and ALDH2 substrates participate in 4-HNE induced nociception. **Methods:** To answer this question, we used loss and gain of function strategies. For the loss of function, we used transgenic mice having the common inactivating ALDH2 mutation present in Asians, ALDH2*2, PKCε knockout mice (PKCεKO) or the PKCε inhibitor (εV1-2, 2µg/paw). The gain of function was achieved with ALDH2 activator (Alda-1, 10mg/kg, s.c.) or PKCε agonist (ψεRACK, 1µg/paw). The mechanical nociceptive threshold was assessed by von Frey filaments before (baseline) and after 4-HNE injection (60 nmol/paw). Cold allodynia was accessed by acetone test. The procedures were approved by the Animal Care Committee, Butantan Institute. **Results:** 4-HNE reduces the mechanical (78%) and cold (60%) nociceptive threshold (hypernociception) with a peak at 30 min, persisting for 8h in wild-type mice. ψεRACK induces hypernociception with a peak in 30 min (80%). The local injection of εV1-2 partially reverts 4-HNE-induced nociception (67%). However, the genetic ablation of PKCε (PKCεKO mice), does not modify the 4-HNE-induced hypernociception. Interestingly, 4-HNE induces a long-lasting hypernociception, that lasts 24h in ALDH2*2 mice with impaired ALDH2 activity.

The activation of ALDH2 with Alda-1 blocks 4-HNE-induced hypernociception but does not interfere with Ψ RACK-induced hypernociception. Taken together, our data suggest that the PKC ϵ activation may contribute to both nociception and antinociception with the downstream mitochondrial ALDH2 activation as a key pathway for pain control. The development of new strategies to better understand the mechanisms involved in 4-HNE signaling may open new perspectives for pain management. Conclusions: Taken together, our data suggest that the PKC ϵ activation may contribute to both nociception and antinociception with the downstream mitochondrial ALDH2 activation as a key pathway for pain control. The development of new strategies to better understand the mechanisms involved in 4-HNE signaling may open new perspectives for pain management. FAPESP 2017/16071-5, CAPES. CEAUIB Protocol 4239090318 1.Che-Hong Chen, Science, 12;321(5895): 1493-5, 2008 2.Vanessa O Zambelli, Sci Transl Med., 6(251), 2014
License number of ethics committee: CEAUIB Protocol 4239090318

06. Cardiovascular and Renal Pharmacology

06.001 Hypertensive aged rats showed altered vasomotion in testis

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Introduction. Hypertension (HA) is a multifactorial chronic disease that affects one in four adults in Brazil and men are often more affected than woman. Several organs are compromised by hypertension, including heart, brain, eyes, and kidneys. Recently, our group showed hypertension affects testis and sperm quality, specially increasing the testicular hypoxia due alteration in the local microcirculation which brought to light the testis as a new target organ of HA. However, this study and others studied the effect of hypertension on testis just using young adult animal models. It is important to point out that a parallel decline in testis function and alterations in the microcirculation occurs with aging. In fact, humans have been experiencing a profound reduction in the sperm concentration (50% in 50 years) and higher frequency of abnormalities in the male reproductive system in the last decades. Since changes in the male reproductive system can be considered today as a marker of the men's global health, we aim to verify the effect of hypertension in the microcirculation of aged rats. **Methods:** Sixty to sixty six week-old spontaneously hypertensive rats (SHR) and normotensive rats Wistar were used in this study (CEUA protocol #8026181120). Indirect blood pressure (BP) was measured by tail plethysmography in Wistar rats and SHR treated with losartan (15 mg/kg/day) or prazosin (1 mg/kg/day) by gavage and in the drinking water, respectively, for 15 days. Tap water with no drug was given to a group of SHR that served as control. Fluorescence intravital microscopy was used to quantify the leukocyte adhesion, vasomotricity and reactive oxygen species (ROS) presence in the testicular microcirculation. **Results:** BP was reduced the same percentage (20%) in SHR treated with losartan or prazosin for 15 days compared to the SHR control group ($P < 0.05$). Leukocyte adhesion and ROS generation was not different in the testicular microcirculation of SHR and Wistar rats ($P > 0.05$). On the other hand, SHR showed increased number of spontaneous blood flow stops than Wistar ($P < 0.05$), which was not observed in SHR previously treated with losartan ($P > 0.05$). **Conclusion:** Hypertension increases the number of blood flow stops in the testis what can probably enhance tissue ischemia and impairs the sperm function. Funding support: FAPESP (#2020/12616-0) License number of ethics committee: CEUA protocol #8026181120

06.002 Characterization of caffeine effect on cardiovascular changes induced by adenosine. Albino LB, Oliveira JG, Fernandes D. UFSC Florianópolis, Dpt of Pharmacology, Brazil.

Introduction: Adenosine is an endogenous nucleoside with a short half-life that regulating many physiological functions. The adenosine action is exhibit through four G-protein coupled receptors: A₁, A_{2A}, A_{2B}, and A₃. The A_{2A} activation causes vasodilation, while A₁ induces vasoconstriction of renal afferent arterioles. During metabolic stress, adenosine is extracellularly released by endothelial cells and myocytes and is involved in hemodynamic abnormalities as sepsis. Caffeine is a non-selective adenosine receptor antagonist with a higher affinity to A₁ and A_{2A} receptors. Thus, caffeine can be an important pharmacological tool for understanding adenosine receptor's role in cardiovascular changes during an inflammatory response. Therefore, this study aims to determine a proper dose and response duration of caffeine to antagonize adenosine-induced cardiovascular changes. **Methods:** Wistar male rats were anesthetized and prepared for heart rate, blood pressure, and renal blood flow measurement. Two consecutive dose-response curves to adenosine (1, 3, 10, 30, 100, and 300 nmol/kg, i.v.) were obtained before or 60 min after caffeine administration (10, 30, or 100 mg/kg, s.c.). In another set of experiments, the rats were randomized to receive caffeine (30 mg/kg, s.c.) or vehicle (saline, 1 ml/kg). Four or eight hours later, the animals were prepared to measure the cardiovascular and renal parameters, and a dose-response to adenosine was performed (University Institutional Ethics Committee (Protocol number 9770210519). **Results:** The adenosine reduced blood pressure and renal blood flow when administered in

animals. The lower caffeine dose (10 mg/kg) reduced the adenosine-induced hypotension (control -19.3 ± 7.9 ; caffeine -10.2 ± 4.5 , 300 nmol/kg, $p < 0.05$), but did not change adenosine-induced reduction in renal blood flow. The higher doses of caffeine (30 and 100 mg/kg) reduced the hypotension (control -18.6 ± 11.9 ; caffeine 30 mg/kg -2.3 ± 3 , 300 nmol/kg, $p < 0.05$) and the reduction in the renal blood flow (control -58.7 ± 25.5 ; caffeine -30.2 ± 21.7 , 300 nmol/kg, $p < 0.05$) induced by adenosine. The caffeine effect (30 mg/kg) on adenosine response was observed by 4 hours on blood pressure (control -24.25 ± 9.39 ; caffeine -14.30 ± 5.73 , 300 nmol/kg, $p < 0.05$) and renal blood flow (control -84.15 ± 44.53 ; caffeine -28.16 ± 21.33 , 300 nmol/kg, $p < 0.05$) and was not observed 8 hours later the caffeine administration. **Conclusion:** Although the lower dose of caffeine reduced the adenosine-induced hypotension it was not able to affect the adenosine response in the renal blood flow. However, the higher doses antagonized adenosine effects in both systems. The effect of caffeine on adenosine response was transitory (4 h), agreeing with caffeine short half-life. In view of this information, it is now possible to plan experiments to access the effect of caffeine on inflammatory processes, which are associated with increased extracellular adenosine levels. **Financial Support:** This work was supported by FAPESC, CAPES and CNPq. **License number of ethics committee:** University Institutional Ethics Committee (Protocol number 9770210519)

06.003 **Wedelolactone as a possible treatment for cardiotoxicity induced by *Bothrops jararacussu* venom.** Albernaz LCS¹, Pinto HMC¹, Romanelli MA², Lara LS², Melo PA², Gonzalez SR¹. ¹UFRJ, Campus Macaé, Brazil; ²UFRJ, Inst of Biomedical Sciences, PPG Biological Sciences (Pharmacology and Medicinal Chemistry), Brazil

Introduction: In Brazil, *Bothrops* snakes are responsible for approximately 90% of reported snakebites with high prevalence in Rio de Janeiro [1]. Surviving patients burden the health system by the need for Intensive Care Unit (ICU) admission, hemodialysis and possible progression to chronic kidney disease [2]. *Bothrops* venom is an important myonecrotic agent with cytolytic effect, characterized by prominent local tissue damage. [3-5]. Some studies showed its venom infusion in isolated hearts produced hypotension, reduction in myocardial contraction force and bradycardia with a block in electric conduction in the heart [6-7]. Wedelolactone (WED), the major component of *Eclipta prostrata* plant, is a potent candidate for kidney and cardiac damage recovery post-poisoning due to its antioxidant and anti-inflammatory actions. [8-10]. **Methods:** Male Wistar rats weighing 100-120g, were randomly divided into 8 groups (n = 5/group; CEUA 128/2018): (1) Control (Ctrl), receiving NaCl 0,9% solution; (2) *Bothrops jararacussu* (Bj): received 3.5 mg/kg of venom intramuscularly (IM); (3, 4 and 5) Bj + WED received doses of 2.0; 5.0 and 10 mg/kg of WED treatment IM 2 hours after intoxication and their controls (6,7, and 8) Ctrl + WED. After treatments, the animals were allocated in metabolic cages for 24 hours (with food and water ad libitum) and then euthanized for heart, blood and urine collection, and for measuring heart weight and water intake. After the hearts were weighed and isolated, microsections were taken and stained with hematoxylin & eosin for light microscopic analysis. **Results:** There was no statistical difference in the correlation between heart weight and body weight among all groups. However, the study follow-up will allow the verification of these preliminary data. Heart tissue analysis from Bj group detected damaged tissue and swollen cardiomyocytes with separation of bundles of myofibrils. Thereby, it was induced a disorganization of the cardiac fibers. **Conclusion:** The observations suggests that WED 2.0 mg/kg was able to partially preserve cardiac muscle tissue, protecting against the cytotoxic effect of the venom compounds. Further studies and results obtained will define if WED is potentially useful and safe drug in the prevention of acute cardiotoxicity induced by *Bothrops* venom. Financial support: FAPERJ. References: 1- GUTIÉRREZ, J. M. et al., Nat Rev Dis Primers, v. 03, 01p., 2017. 2- SPRIGNOLLI, L. R. et al. Nephron Clin Pract, v. 119, 131p., 2011. 3- QUEIROZ, L.S. et al., Toxicon, v. 22, 339p., 1984. 4- SOARES, A.M., et al., Biochem. Mol. Biol. Int., v. 43, 1091p., 1997. 5- GUTIÉRREZ, J. M. et al., Toxicon, v.54, 976p., 2009. 6-EVANGELISTA, I.L. et al., Toxicon, v. 55, 1061p., 2010. 7- SIFUENTES D.N. et al., Toxicon, v. 51, 28 p., 2008. 8- MELO, P.A. et al. Toxicon, v. 32 (5), 595p., 1994. 9- ZHU, MAO-MAO et al., Biomed. Pharmacother., v. 117, 01p., 2019. 10- KARTHIKUMAR, P. et al., Sci. Res. Essays, v. 24, 101p., 2007. **License number of ethics committee:** CEUA 128/2018

06.004 **Wedelolactone as a possible treatment for acute kidney injury induced by *Bothrops jararacussu* venom.** Pinto HMC¹, Albernaz LCS¹, Santos MARF², Souza PDN², Lara LS², Melo PA², Gonzalez SR¹. ¹Univ Federal do Rio de Janeiro – Campus Macaé, Brazil ²PPG em Ciências Biológicas (Farmacologia e Química Medicinal), Inst de Ciências Biomédicas, Univ Federal do Rio de Janeiro, Brazil

Introduction: Acute kidney injury (AKI) is defined as the sudden loss of kidney function within hours or days and is one of the main complications resulting from snakebites. [1,2] The *Bothrops* species is responsible for almost 90% of snakebites in South America, representing a serious public health problem due to its morbidity and mortality. [2,3] Surviving patients burden the health system due to the need for

hemodialysis and possible progression to chronic kidney disease. [4] Wedelolactone (WED) is the main metabolite derived from the *Eclipta prostrata* plant. Also, it can neutralize the myotoxic, proteolytic and hemorrhagic activities of *Bothrops jararacussu* (Bj) venom. [5] The main goal was to investigate whether WED protects kidney function from Bj-induced kidney injury. **Methods:** Male Wistar rats weighing 100-120 g, were randomly divided into 8 groups (n = 5/group); CEUA 128/18: AKI was induced by the administration of the venom, forming the Bj (1) received 3.5 mg/kg of the venom intramuscularly (IM) in the left paw and treated groups (2, 3 and 4) Bj + WED 2, 5 and 10 group with different doses of WED 2, 5 and 10 mg/kg IM in the right paw 2 hours after the venom inoculation, respectively. In parallel, the respective control groups were made. (5) Control (Ctrl), which received NaCl 0.9 % solution IM in the left paw and (6, 7 and 8) Ctrl + WED 2, 5 and 10, which received NaCl 0.9 % solution in the rat right paw plus WED 2, 5 and 10 mg/kg in the rat left paw 2 hours later, respectively. After treatments, the animals were allocated in metabolic cages for 24 hours (with food and water ad libitum) and then euthanized for kidneys, blood and urine sample collection and for measuring kidneys weight, water intake and the extent of damage to muscle tissue. **Results:** There was no difference in water intake or in kidney weight between groups. However, analyzing urinary volume (UV), the Bj group present an increased UV (64%) when compared to control group (Control 11.25±0.66 to Bj 18.4±2.41). WED at the dose of 5 mg/kg returned the UV to the Ctrl values (decreased in 42%) (Bj 18.4±2.41 to Bj + Wed 5 10.6±1.06). Histological analysis detected the dose of 2 mg/kg of WED did not show significant improvement in kidney damage while in the dose of 5 mg/kg, a partial improvement was observed (when compared to Bj group): the glomeruli had their size normalized, the capsular space and medullar tubular lumen, both increased in the Bj group, obtained a significant improvement, presenting a smaller Bowman's capsule space and smaller medullary tubular lumen, similar to control group; the treatment with 10 mg/kg of WED showed a worsening in the tissue aspect compared to control or to other groups (possible toxic dose). **Conclusion:** These preliminary data suggests that WED promoted nephroprotection pharmacological for Bj envenomation. Financial support: FAPERJ License number of ethics committee: CEUA 128/2018

06.005 **Acute toxicity and molecular docking of ruthenium complexes FOR811A and FOR811B.** Oliveira JPH¹, Nogueira PMM¹, Alves RS¹, Santos PN², Rocha DG², Nogueira-Júnior FA², Lopes LGF³, Gouveia-Júnior FS³, DE Sousa EHS³, Braz HLB⁴, Olivier DS⁵, Monteiro SMN⁶, Monteiro HSA⁶, Silveira JAM⁶, Jorge RJB⁶. ¹UFC Fortaleza, Dpt de Farmácia, Brasil; ²UFC Fortaleza, PPG Farmacologia, Brasil; ³UFC Fortaleza, Dpt de Química Orgânica e Inorgânica, Brasil; ⁴UFC Fortaleza, PPG Ciências Morfofuncionais, Brasil; ⁵UFT Palmas, Campus Araguaína, Brasil; ⁶UFC Fortaleza, Dpt de Fisiologia e Farmacologia, Brasil

Introduction: Coordination complexes based on ruthenium presents lower toxicity than platinum-based complexes, furthermore, previous studies have showed that these compounds have an ability to release nitric oxide (NO), that it's known to have a protective effect against acute kidney injury. Therefore, these compounds have shown promising characteristics for clinical use. Gentamicin is a aminoglycoside that about 5% of the dose accumulates in epithelial cells of the renal cortex, after glomerular filtration, through the megalin and calreticulin pathways, causing nephrotoxic effects. So, the aim of this study was to evaluate the acute toxicity and molecular docking of the ruthenium complexes FOR811A and FOR811B. **Methods:** For acute toxicity, it was performed the Up-and-Down protocol, from the Organization for Economic Cooperation and Development (OECD) Alternative Methods Guideline 425, using female Swiss mice. This protocol provides an estimative of the median lethal dose (LD50) and an evaluation of clinical signs suggestive of toxicity. Using the software Acute Oral Toxicity 425 (AOT425), the following doses were obtained for use in the protocol: 10 mg/kg; 28 mg/kg; 70 mg/kg and 175 mg/kg). Auto dock 4.2 was used to perform molecular docking with these metallocompounds, targeting important pathways for gentamicin induced kidney injury (Megalina and Calreticulin). The experimental model involving animals was approved by the Animal Research Ethics Committee of the Center for Drug Research and Development (NPDM) of the Federal University of Ceará (UFC) (nº 13010819-0). **Results:** Molecular docking, taking as a reference measure for high interaction the energy value of -6 kcal/mol, showed a good affinity between both compounds and Calreticulin (FOR811A: -7.27 kcal/mol; FOR811B: -8.09 kcal/mol). However, considering the megalin pathway, only FOR811B showed good interaction, with a value of -7.0 kcal/mol, while the energy value of FOR811A was -5.35 kcal/mol. Analyzing the results of the Up-and-Down protocol, was estimated a LD50 of 115.5 mg/kg for FOR811A and 45.72 mg/kg for FOR811B. Furthermore, concerning the physiological and behavioral observations, FOR811A caused vasodilation and lethargy at 70 and 175 mg/kg doses and the last one was lethal. In addition, the FOR811B caused pruritus, lethargy and vasodilation and 70 mg/kg was lethal. **Conclusions:** We can conclude that the ruthenium complex FOR811B showed higher lethality in relation to FOR811A, as well as greater interactions with the targets in docking, being a candidate for gentamicin induced kidney injury treatment. Thus, demonstrating the great importance of studies of the toxic effects of possible future drugs such as ruthenium complexes

presented in this abstract. Financial support: This study received financial support from the National Council for Scientific and Technological Development (CNPQ). Acknowledgments: to the Laboratory of Toxinology located in the NPDM. **License number of ethics committee:** The experimental model involving animals was approved by the Animal Research Ethics Committee of the Drug Research and Development Center (NPDM) of the Federal University of Ceará (UFC) by protocol number: 13010819-0.

06.006 Antihypertensive action of new N-acylhydrazonic derivatives. Rocha BS^{1,2}, Da Silva JS^{1,2}, Pedreira JGB¹, Barreiro EJ¹, Zapata-Sudo G^{1,2}. ¹ICB-UFRJ, Rio de Janeiro, Brazil; ²UFRJ, PPG in Medicine - Cardiology, ICES, Rio de Janeiro, Brazil

Introduction: Systemic arterial hypertension is a multifactorial condition and considered a risk factor for cardiac, encephalic, renal dysfunction and metabolic complication^{1,2,3}. The search for new strategies for the prevention and treatment of cardiovascular diseases like systemic arterial hypertension, led to the design and synthesis of new N-acylhydrazones, to identify agents with vasodilator activity and positive cardioinotropic effect. New molecules containing selenium (-Se) were designed in order to improve the interaction with the adenosine receptor. Furthermore, selenium is an essential microelement with antioxidant properties⁴, which could reduce the oxidative stress characteristic of hypertension. **Methods:** Protocols were approved by Animal Care and Use Committee at Universidade Federal do Rio de Janeiro n. 017/19. Vascular reactivity was evaluated using isometric tension recording of pre-contracted thoracic aorta from male Wistar rats (240-290 g) after exposure to increasing concentrations of each derivate (0.1 – 100 µM). To investigate the antihypertensive effect, systolic (SBP), diastolic (DBP) and mean (MBP) blood pressure and heart rate (HR) were determined after intravenous administration of 30 µmol/kg of the selected compounds LASSBio-2062 and LASSBio-2063 in spontaneously hypertensive rats (SHR) (12-14 weeks old). **Results:** Vasodilation was induced by different analogues, LASSBio-2062 (n= 7), LASSBio-2063 (n= 5), LASSBio-2076 (n= 7), LASSBio-2084 (n= 3), LASSBio-430 (n= 5), and LASSBio-2092 (n= 3) with a mean effective concentration of 14.7 ± 5.2; 14.6 ± 2.9; 8.8 ± 3.3; 59.4 ± 31.7; 5.9 ± 3.6; and 27.9 ± 9.2 µM, respectively. LASSBio-2062 and LASSBio-2063 (30 µmol/kg) reduced MBP in SHR from 131.8 ± 17.5 to 87.7 ± 16.7 mmHg and from 117.7 ± 3.9 to 80.5 ± 12.3 mmHg, respectively. **Conclusion:** The new N-acylhydrazones showed antihypertensive effect possibly through their prominent vasodilator action. **References:** 1- Radovanovic, C.A.T. Rev Latino-Am Enfermagem, v. 22, p. 547, 2014.; 2- VI Diretriz Brasileira de Hipertensão Arterial. Arq Bras Cardiol, v. 107, p. 1, 2016.; 3- VI Diretrizes Brasileiras de Hipertensão. Arq Bras Cardiol, v. 95, p. 1, 2010.; 4- Gierus, Martin. Cienc. Rural, v. 37, p. 1212, 2007. Financial support: CNPq, FAPERJ, CAPES, INCT-Inofar. **License number of ethics committee:** Animal Care and Use Committee at Universidade Federal do Rio de Janeiro n. 017/19.

06.007 Evaluation of the cardiovascular effects of new acilhydrazone derivatives in the acute phase of myocardial infarction. Carlos-Nascimento B¹, da Silva JS¹, Beltrame F^{1,2}, Montagnoli TL¹, Rocha BS¹, Maia RC¹, Barreiro EJ¹, Zapata-Sudo G.^{1,2} ¹ICB-UFRJ, Rio de Janeiro, Brazil ²ICES-UFRJ, Rio de Janeiro, Brazil

Introduction: The intense inflammatory response that occurs in the acute phase of myocardial infarction (MI) causes cardiac remodeling that leads to heart failure (HF). Purpose (hypothesis): Activation of adenosine receptor by LASSBio-1860 (heteroaryl N-acylidrazone derivative) could reduce cardiac remodeling preventing HF. **Methods:** The protocols were approved by the Ethics Committee for the Use of Animals at Federal University of Rio de Janeiro (nº 103/17). MI was induced by the ligation of the anterior descending coronary artery in male Wistar rats. Experimental groups were divided into: false-operated (Sham) treated with vehicle, infarcted treated with either vehicle and infarcted treated with LASSBio-1860 (70 µmol/kg). After 7 days of oral administration (gavage), hemodynamic parameters were obtained and hearts were prepared for histological evaluation of fibrosis and cellular infiltration. Immunohistochemistry and western blot analysis were used to determine the involvement of TNF-α p-ERK1/2, t-ERK1/2, p38 and c-fos in inflammatory and cardiac remodeling processes. **Results:** MI reduced the ejection fraction from 91.5 ± 0.5% to 45.2 ± 1.3% when compared to the sham group (p < 0.05), which improved the infarcted group treated with LASSBio-1860 to 60.1 ± 10.3%. The filling pressure (E/e?) was increased by MI from 18.6 ± 1.5 to 31.3 ± 4.4 (p<0.01) indicating diastolic dysfunction. Oral administration of LASSBio-1860 (70 µmol/kg) prevented diastolic dysfunction because E/e? reduced to 15.8 ± 5.5. An increase in collagen content and TNF-α expression as well as nuclear p38 labeling was detected in the heart from MI group, which was normalized after LASSBio-1860 treatment. Furthermore, p-ERK1/2 increased from 0.75 ± 0.01 to 0.89 ± 0.01 (p<0.5) in the AMI group, while treatment with LASSBio-1860 reduced to 0.79 ± 0.01 (p<0.05). In contrast, there was no significant change in t-ERK1/2 expression. c-fos expression was also increased to 0.73 ± 0.05 by MI while sham animals had 0.39 ± 0.04 and it was observed a reduction to 0.55 ± 0.01 in the treated infarcted group. **Conclusions:** Agonist of adenosine receptor LASSBio-1860, prevented diastolic dysfunction in AMI possibly consequent to the

improvement of the inflammatory response and cardiac remodeling. **Financial support:** FAPERJ, CNPQ, INCT-INOVAR, CAPES **License number of ethics committee:** nº 103/17

06.008 Cilostazol exerts positive effects on cardiovascular system of Wistar rats exposed to doxorubicin. Autran LJ¹, Brazão SC¹, Lima GF¹, Mendes ABA², Lopes RO¹, Motta NAV¹, Brito FCF¹. ¹ Lab de Farmacologia Experimental - LAFE, Univ Federal Fluminense. ²Grupo de Pesquisa, Inovação e Desenvolvimento em Endocrinologia Experimental, Univ Federal do Rio de Janeiro, RJ

Introduction: Doxorubicin (DOX) is an antineoplastic drug used in treatment of cancer in human and veterinary medicine (Hader, *Am J Physiol Heart Circ Physiol*, 317: H705, 2019; Marquardt, *J Am Vet Med A*, 254: 236, 2019). Despite its wide use, DOX has the potential to cause toxic effects, such as cardiovascular toxicity. Research on cardiotoxicity prevention methods is important to improve survival of cancer patients in treatment with this drug. Cilostazol (CILO), a phosphodiesterase III inhibitor, presents anti-inflammatory, antioxidant and cardioprotective activities (Paronis, *J Cardiovasc Pharmacol Ther*, 25: 273, 2020; Chattipakorn, *J Geriatr Cardiol*, 11: 151, 2014). This work studied the cardioprotective effect of CILO in an animal model of DOX-induced cardiotoxicity. **Methods:** The animal protocols were approved by the Ethics Committee for Animal Use (CEUA/UFF 5633100719). Adult male Wistar rats (400-500g) were divided into four groups: control (C), control treated with CILO (C+CIL), group exposed to DOX only (DOX), and group treated with CILO and DOX (DOX+CIL). CILO (30mg/kg) or vehicle were administered daily for 5 weeks. From the 2nd week until the end of the protocol, saline solution (0.9%) was injected weekly intravenously in groups C and C+CIL and DOX (2.5 mg/kg) in DOX and DOX+CIL groups. Electrocardiography and echocardiography analysis were performed. In 36th day, animals were anesthetized with ketamine (100 mg/kg) and xylazine (10mg/kg), blood was collected by cardiac puncture and thoracic aorta was dissected for vascular reactivity study. Statistical analysis was performed using GraphPad Prism 9.02 (San Diego, CA). Data were analyzed using One Way (ANOVA) and Bonferroni post-test ($p < 0.05$). **Results:** DOX caused a reduction in the erythrocyte count (C: 8.47 ± 0.20 million/mm³; DOX: 5.95 ± 0.51 million/mm³), hemoglobin dosage (C: 14.9 ± 0.21 g/dl; DOX: 10 ± 0.89 g/dl) and hematocrit (C: $46.52 \pm 1.16\%$; DOX: $31.58 \pm 1.07\%$). Cilostazol was able to significantly increase the hemoglobin dosage (DOX: 10 ± 0.89 g/dl; DOX+CIL: 12.58 ± 0.41 g/dl) and hematocrit (DOX: $31.58 \pm 1.07\%$; DOX+CIL: $41.35 \pm 1.43\%$) in the DOX+CIL group. In the electrocardiographic results, the DOX+CIL group had a significantly shorter QTc interval when compared to the DOX group on the 27th day (DOX: 105.5 ± 7.12 ms; DOX+CIL: 92 ± 1.83 ms) and 35th day of protocol (DOX: 105.4 ± 3.74 ms; DOX+CIL: 92 ± 2.05 ms). On echocardiographic evaluation, a reduction in left ventricular systolic shortening fraction (C: $55.33 \pm 0.95\%$; DOX: $44.0 \pm 2.14\%$) and ejection fraction was observed in DOX group (C: $89.83 \pm 0.64\%$; DOX: $80 \pm 2.02\%$). DOX+CIL group (FE: $84 \pm 1.05\%$; FS: $49.20 \pm 1.24\%$) showed no significant difference in relation to the DOX group or in relation to group C in both parameters. In vascular reactivity, DOX group showed a reduction in the maximum relaxation induced by acetylcholine. Cilostazol was able to prevent reduction in maximum relaxation in DOX+CIL group (DOX: $87.94 \pm 0.90\%$; DOX+CIL: $106.57 \pm 6.6\%$). **Conclusion:** Cilostazol had positive effects on cardiovascular and hematological parameters in an animal model of doxorubicin-induced cardiotoxicity. **License number of ethics committee:** CEUA/UFF 5633100719

06.009 Adjuvant-induced arthritis causes contractile dysfunction in rat aorta by a mechanism related to Nitric Oxide. Araújo TS¹, Spadella MA², Tirapelli CR³, Pinheiro JCD⁴, Chies AB¹. ¹FAMEMA Marília, Dpt of Pharmacology, Brazil; ²FAMEMA Marília, Dpt of Human Embriology, Brazil; ³USP Ribeirão Preto, Dpt of Cardiovascular Pharmacology, Brazil; ⁴FAMEMA Marília, Dpt of Pharmacology, Brazil

Introduction: Arthritis has important cardiovascular repercussions. Phenylephrine (PHE)-induced vasoconstriction in rat aortas is impaired in the early phase of the adjuvant-induced arthritis (AIA), around the 15th post-induction(1,2). Therefore, the present study aimed to investigate the mechanisms underlying AIA-induced contractile dysfunction. **Methods:** Male Wistar rats were submitted to AIA by intradermal administration of 100µL of "Mycobacterium tuberculosis" (3.8mg/mL), in the right hind paw. Control animals (false-immunized) received only the vehicle. All animals were killed 15 or 36 days after AIA induction. The thoracic aorta was cut into rings (3mm) and then transferred to organ baths. Concentration-response curves for PHE were determined in endothelium-intact or denuded rings in the absence or presence of NG-nitro-L-arginine methyl ester [(L-NAME), 10⁻⁴ M, non-selective nitric oxide synthase (NOS) inhibitor], 1400W [10⁻⁶ M, selective inducible NOS (iNOS)]; indomethacin [10⁻⁵ M, non-selective cyclooxygenase (COX) inhibitor] or 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one [(ODQ), 10⁻⁶ M, selective inhibitor of guanylyl cyclase enzyme]. PHE-induced contraction was recorded by isometric force transducers and expressed as grams (g). Log of EC50 (pEC50), obtained from the concentration-response curves, as well as maximal response (Rmax) were compared among groups (n=7-14) by two-way ANOVA/Bonferroni. The aortic concentration of nitrate/nitrite (NOx) was determined by Griess reaction and compared by

Student “t” test. Differences were statistically significant when $p < 0,05$. All procedures were carried out with the approval of the Ethics Committee on Animal Use of Marília Medical School (protocol nº 2935/2020). **Results:** There was a significant reduction of PHE-induced contraction 15 days after AIA induction (R_{max} : from 2.26 ± 0.15 g to 1.33 ± 0.20 g; pEC_{50} : from 7.00 ± 0.20 to 6.22 ± 0.18), but not 36 days after AIA induction. This AIA-induced decrease in the contractile response of PHE was not observed in endothelium-denuded rings or in endothelium-intact rings incubated with L-NAME, 1400W or ODQ. Indomethacin also prevented the hypocontractility to PHE induced by AIA. However, in this case, instead of preventing AIA-induced reduction of PHE responses, indomethacin reduced responses of control animals (R_{max} : from 2.26 ± 0.15 g to 1.81 ± 0.20 g). AIA also increased the aortic concentration (g/g tissue) of NOx (from 3.54 ± 0.46 to 5.72 ± 0.80). **Conclusion:** In aorta taken from rats submitted to AIA, nitric oxide (NO) produced by endothelial iNOS, stimulates guanylate cyclase in smooth muscle cells, thereby impairing PHE-induced contraction. Financial Support: CAPES (scholarship 88887.480973/2020-00). **References:** 1. Haruna Y, et al., *Arthritis Rheum*, 54, 1847, 2006. 2. Ulker S, et al., *Pharmacology*, 60, 136, 2000. **License number of ethics committee:** Ethics Committee on Animal Use of Marília Medical School, protocol nº 2935/2020

06.010 Preventive effects of kefir in the behavioral and renal changes induced by chronic unpredictable stress in mice. Silva AO¹, Ribeiro JM¹, Amorim GE¹, Pereira-Junior AA¹, Ângelo ML¹, Torres LHL¹, Paula FBA², Leitão SGR³, Dias MVS⁴, Ceron CS⁵. ¹Unifal Alfenas, Dpt of Food and Drugs; ²Unifal Alfenas, Dpt of clinical and toxicological analysis; ³Unifal Alfenas, Dpt of Physiological Sciences; ⁴Unifal Alfenas, Nature science institute; UFOP Ouro Preto, Dpt of biological Sciences

The stress directly affects the quality of life of the world population, favoring the emergence of diseases related to the central nervous system and damage to other systems, such as the renal system (MARIN et al., 2007; HERMAN et al., 2016). Kefir is a probiotic that can be beneficial to the body, acting on changes caused by stress due to its antioxidant and anti-inflammatory effect (PIMENTA et al., 2018), and its high concentration of tryptophan (NOORI et al., 2014). Chronical unpredictable stress was induced in Swiss mice during 21 days (LEPSCH et al., 2005). Mice were treated with kefir (SK) (0,3ml/100g) or regular milk (SM) (0,3ml/100g) for 10 days before the stress induction and during the stress protocol. Behavioral alterations, renal function (urea and creatinine), renal Superoxide Dismutase (SOD) and Catalase (CAT) activities and the levels of 3-nitrotyrosine and metalloproteinase-se (MMP-2) were evaluated. Control mice received kefir (CK) or regular milk (CM). The constitution of kefir was previously evaluated (BERGMANN et al., 2010). In the elevated cross maze test, Kefir had an anxiolytic-like effect ($p < 0.05$, CM: 51.35 ± 2.166 ; CK: 50.07 ± 3.2206 ; SM 41.02 ± 2.896 ; SK 56.92 ± 3.294). No change in plasma urea levels was observed ($p > 0.05$; CM: 150.60 ± 5.829 ; CK: 130.70 ± 5.309 ; SM: $139,50 \pm 9.016$; SK: 147.50 ± 8.698). Kefir treatment decreased creatinine plasma levels in both control and stress groups ($p < 0.05$; CM: 0.22 ± 0.036 ; CK: 0.02 ± 0.003 ; SM: 0.30 ± 0.041 ; SK: 0.03 ± 0.005), suggesting a protective effect in the renal tissue. Stress decreased SOD and CAT activities and increased MMP-2 and 3-nitrotyrosine levels, suggesting the induction of an early kidney damage, which was not detected on urea and creatinine tests. Kefir treatment increased SOD and CAT activities in the stress group ($p < 0,05$; SOD: CM 1.73 ± 0.205 ; CK 3.19 ± 0.460 ; SM 2.73 ± 0.190 ; SK 4.19 ± 0.420 / CAT: CM 0.04 ± 0.007 ; CK 0.05 ± 0.009 , SM 0.03 ± 0.003 ; SK 0.08 ± 0.011), and decreased MMP-2 levels ($p < 0.05$; CM 115.6 ± 3.385 , CK 121.2 ± 1.908 , SM 131.1 ± 1.398 , SK 118.5 ± 1.555), but not the nitrotyrosine levels ($p > 0.05$; CM 122 ± 1.531 , CK 120 ± 1.623 , SM 127.8 ± 1.493 , SK 122.9 ± 1.138) induced by stress. These results suggest that Kefir has anxiolytic effect, improves the activity of the antioxidant kidney system and the early kidney tissue damage oxidative caused by chronic unpredictable stress in mice. Approval by the Ethics Committee, Registration No. 61/2018. **Financial Support:** FAPEMIG **References:** HERMAN, J. P. et al. *ComprPhysiol*, v. 6, p. 603, 2016. HU, C. et al. *Plos One*, v.12, 2017. LEPSCH, L. B. et al. *Addiction Biology*, V. 10, p. 251, 2005. MARIN, M. T. et al. *Physiology & Behavior*, v. 90, p. 29, 2007. NOORI, N. et al. *Adv. Biomed Res.*, v. 3, 2014. PIMENTA, F. S. et al. *Cell Physiol Biochem*, v. 48, p. 1901, 2018.

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06.011 Nox5 contributes to vascular hyperreactivity associated with pre-eclampsia. Barbosa NC¹, Machado MR², Alves JV², Oliveira Neto JT², Silva JF², Cavalli RC³, Passaglia RCAT², Costa RM². ¹UFJ, Jataí Academic Unit of Health Sciences, Federal Univ of Jataí, Brazil ²FMRP USP Ribeirão Preto, Dpt of Pharmacology, Ribeirão Preto Medical School, Univ of São Paulo, Brazil ³FMRP USP Ribeirão Preto, Dpt of Gynecology and Obstetrics, Ribeirão Preto Medical School, Univ of São Paulo, Brazil

Introduction: Nox5 is a major pro-oxidant enzymes found in humans. Its activity depends on several stimuli, for example, TNF- α cytokine. In this context, there is a direct link between oxidative stress and inflammation. Inflammatory and oxidative processes in hypertensive disorders such as pre-eclampsia (PE)

damage the vascular endothelium, causing disturbances in vascular homeostasis. **Methods:** Peripheral venous blood and umbilical cord arteries were collected from normotensive (NT) pregnant and from PE pregnant. ELISA was used to determine cytokine levels. Endothelial cells (EA.hy926) were stimulated with serum from normotensive pregnant and with PE (20% v/v for 1 hour), in the presence or absence of infliximab (1 μ M for 30 minutes), nifedipine (1 μ M for 30 minutes) and ML090 (0.01 μ M for 30 minutes). Calcium (Ca^{2+}) influx was measured by FLIPR probe. Reactive oxygen species (ROS) generation was evaluated by lucigenin and amplex red. Nitric oxide (NO) was evaluated by Griess assay. Reduction of Nox5 expression in endothelial cells was done using Nox5 SiRNA. Umbilical artery rings from normotensive pregnant were incubated with serum from normotensive pregnant and with PE, concentration-effect curves for serotonin were performed. **Results:** In the serum of pregnant with PE, there is an increase in the TNF- α and IL-6 levels (in pg/mL, NT: 5.6 \pm 0.3 vs. PE: 19.1 \pm 0.5 and NT: 3.2 \pm 0.3 vs. PE: 16.1 \pm 0.7, respectively). In endothelial cells, stimulation with serum from pregnant with PE and with recombinant TNF- α increased Ca^{2+} influx (in relative fluorescence units, NT: 532 \pm 110 vs. PE: 4568 \pm 134 and Vehicle: 273 \pm 56 vs. TNF: 2846 \pm 417, respectively). Pretreatment with infliximab caused partial inhibition (2626 \pm 127) and pretreatment with nifedipine, abolished this increase (207 \pm 66). In addition, serum from pregnant with PE promoted an increase in the O_2^- (in relative chemiluminescence units, NT: 92 \pm 19 vs. PE: 751 \pm 26) and H_2O_2 (in relative fluorescence units, NT: 8.6 \pm 0.4 vs. PE: 22.5 \pm 0.9) generation and pretreatment with infliximab partially inhibited this increase (478 \pm 23). Pretreatment with nifedipine as well as with ML090 abolished this increase (109 \pm 16 and 132 \pm 18, respectively). Basal NO production in PE serum-stimulated cells was lower (in nmol, NT: 23.5 \pm 0.7 vs. PE: 7.0 \pm 0.5) and pretreatment with infliximab partially prevented this decrease (15.6 \pm 0.6). Pretreatment with nifedipine as well as ML090 abolished this decrease (21.7 \pm 0.5 and 22.0 \pm 0.9, respectively). Stimulation with serum from pregnant with PE increased Nox5 gene expression in endothelial cells (in $2^{-\Delta\text{Ct}}$, NT: 1.7 \pm 0.01 vs. PE: 5.8 \pm 0.1) pretreatment with infliximab abolished this increase (2.4 \pm 0.1). Furthermore, silencing of the Nox5 gene reduced the protein expression of the enzyme. Nox5 gene silencing was able to prevent the increase in ROS generation and the reduction of NO levels. There was a higher gene and protein expression of Nox5 in the umbilical arteries of pregnant with PE (in chemiluminescence intensity, NT: 0.10 \pm 0.02 vs. PE: 0.15 \pm 0.01). There was an increase in O_2^- (in relative chemiluminescence units, NT: 742 \pm 121 vs. PE: 2752 \pm 201) and H_2O_2 (in relative fluorescence units, NT: 16.3 \pm 0.9 vs. PE: 45.2 \pm 0.8) generation and reduction in NO levels (in nmol, NT: 26.2 \pm 0.4 vs. PE: 14.2 \pm 0.8) when compared to the umbilical arteries from normotensive pregnant. The serotonin-induced contractile response was greater in umbilical arteries stimulated with serum from pregnant with PE when compared to umbilical arteries stimulated with serum from normotensive pregnant [in E_{max} (g), NT: 2.7 \pm 0.03 vs. PE: 3.3 \pm 0.01]. **Conclusion:** TNF- α promotes an increase in intracellular Ca^{2+} , increased expression and activation of the Nox5 enzyme, and the ROS generation in endothelial cells and umbilical arteries. Such effects reduce NO bioavailability and may justify umbilical artery dysfunction under PE conditions. **Financial support:** CAPES, CNPq. The study was approved by Research Ethics Committee of the Federal University of Goias, under approval n $^\circ$ 3.780.533. **License number of ethics committee:** The study was approved by Research Ethics Committee of the Federal University of Goias, under approval n $^\circ$ 3.780.533.

06.012 Chronic ethanol consumption induces oxidative stress and NLRP3 activation in thoracic aorta via mineralocorticoid receptor activation. Dourado TMH, Assis VO, Awata WMC, Tirapelli CR Lab. of Pharmacology, EERP-USP, Ribeirão Preto, Brazil

Introduction: Several experimental studies have shown that chronic ethanol consumption increases blood pressure through activation of the renin-angiotensin-aldosterone system (RAAS) leading to an increase in vascular contractility and oxidative stress. Aldosterone acts on mineralocorticoid receptor (MR) to promote vascular inflammation and oxidative stress leading to vascular dysfunction. It is well known that aldosterone can induce vascular damage through activation of NOD-LRR-and pyrin domain-containing protein 3 [NLRP3 inflammasome]. This study aimed to investigate whether aldosterone would modulate NLRP3 activation and oxidative stress in the vasculature of rats chronically treated with ethanol. **Methods:** Male Wistar Hannover rats (250-300g) were distributed in 4 groups: Control: animals received water *ad libitum* for 5 weeks and daily gavage (DG) of vehicle (CV) or an MR antagonist [potassium canrenoate (PC), 30mg/kg/day, (CP)]; Ethanol: animals were treated with ethanol 20% (v/v) for 5 weeks and DG of vehicle (EV) or PC (EP). At the end of the treatment, blood and the thoracic aorta were collected for biochemical analysis. Concentration-response curves for phenylephrine were obtained in aortic rings with (E $^+$) or without (E $^-$) endothelium, in the absence or after incubation (30min) with one of the following drugs: tiron (10 μ mol/L, superoxide scavenger) and MCC950 (1 μ mol/L, NLRP3 antagonist). All procedures were approved by Ethics Committee on Animal Use (CEUA#20.1.402.22.4). Results were analyzed using two-way ANOVA. **Results:** Ethanol increased serum levels of IL-1 β (pg/ml) (CV=96 \pm 4.1; CP=90 \pm 3.5; EV=122 \pm 6.8*; EP=100 \pm 3.7; n=5-

8) and maximal response to phenylephrine (mN) in E⁺ aortic rigs (CV=10.8±0.4; CP=11.5±0.5; EV=15.8±0.5*; EP=9.9±0.6; n=5-8) and E⁻ aortic rings (CV=16.1±0.7; CP=15.1±0.2; EV=21.1±0.9*; EP=15.3±1.3; n=5-8). Treatment with potassium canrenoate prevented these responses. Furthermore, incubation with tiron, reversed ethanol-induced hyper-reactivity of aortic E⁺ rings (11.6±1.2; n=6) and E⁻ rings (14.5±0.6; n=6), indicating the participation of superoxide in such response. Incubation with MCC950 reversed the increase in the maximal response induced by ethanol in aortic E⁺ rings (12.4±0.9; n=5) but not in E⁻ rings (19.5±0.8; n=6), indicating a role for NLRP3 in such response. **Conclusion:** MR modulates the vascular hyper-contraction induced by ethanol, which is associated to NLRP3 activation and reactive oxygen species generation. Financial support: CAPES. **License number of ethics committee:** CEUA#20.1.402.22.4

06.013 Phenotypical and pharmacological differences evoked by *in vitro* aging of LLC-PK1 proximal tubule renal cells. Barros GMO¹, Silva AAC², Quintas, LEM³, Inst of Biomedical Sciences, Federal Univ of Rio de Janeiro, Brazil

Introduction: Cell aging comprises the loss of repair and adaptation capacity, resulting in phenotypic transformation that can culminate in cell death and modified drug responses. The kidneys are one of the organs most severely affected which can lead to renal failure. We have shown that bufalin, a cardiotonic steroid that selectively binds to Na/K-ATPase (NKA), induces epithelial-mesenchymal transition (EMT) in LLC-PK1. Our objective was to evaluate phenotypical and bufalin-induced response in LLC-PK1 cells of low (P<40) and high (P>80) *in vitro*. **Methods:** LLC-PK1 cells (porcine proximal renal tubule) cultured in DMEM with 5% FBS were serum-deprived and treated with 20 nM bufalin for 48 h and were evaluated by phase-contrast microscopy. In 12-well plates (5,000 cells/well) Tripan blue-free cell counting was evaluated for 24-72 h in Neubauer chamber. Cell viability was assessed using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) test (1,000 cell/well) for 24-72 h. The cell migration assay with the wound healing method was evaluated during 24-72 h. NKA activity as well as the protein expression NKA α 1 isoform was evaluated according to Fiske and Subbarow method and Western blot, respectively. Western blot was also performed for phospho- and total ERK1/2. The comparison between groups was performed by Student's t test or two-way ANOVA, followed by Sidak posttest ($p < 0.05$ was considered statistically significant). The data are expressed as mean \pm SEM. **Results:** Although P<40 and P>80 cells are morphologically comparable by phase-contrast microscopy without bufalin treatment, 20 nM bufalin induced a deep change in cell shape only in P>80, consistent with EMT. Cell number after 96 h was 70% higher for P<80 ($p < 0.05$, n=6). MTT test showed 50% higher absorbance for P>80 cells compared to P<40 after 72h ($p < 0.05$, n=5). P>80 cells also presented a migration rate 2.6 and 2.2-fold greater than P<40 after 24 and 48 h, respectively ($p < 0.05$, n=10). NKA activity (in micromol Pi/mg/h: 7.4 ± 1.2 P<40 vs 6.7 ± 1.3 P>80; n=11) as well as the protein expression of the α 1 isoform was similar between both groups. The expression of the active form (phospho) of the MAP kinases ERK1/2 was 2.2-fold higher in P>80 ($p < 0.05$, n=4). **Conclusion:** Our data show that P<40 and P>80 LLC-PK1 cells present different phenotypes promoted by successive passages *in vitro*. Increased proliferative capacity, viability and motility of P>80 cells may be related to MAP kinase activity. In contrast to P<40, P>80 cells were sensitive to EMT-like phenomenon induced by bufalin, but no alteration in NKA activity and expression was detected. Our results indicate that *in vitro* aging has an important role in cellular phenotype and the pharmacological response to cardiotonic steroids. Financial support: PIBIC/UFRJ, CAPES, FAPERJ e CNPq. **License number of ethics committee:** N/A

06.014 Blood pressure effects in male and female rats due to antidepressant use: A systematic review and pilot meta-analysis. Santos TM, Linder AE. UFSC - PPG Pharmacology - Florianópolis, Brazil

Antidepressants (AD), due to their different mechanisms of action, can modulate the availability of neurotransmitters, regulate receptors and enzymes, and lead to remodeling of physiological responses, including blood pressure control. Furthermore, despite the differences between male and female hormonal mechanisms, responses to treatment with ADs may also diverge, leading to the development of distinct pressor effects. The question arises: Does the use of antidepressants induce different blood pressure effects in male and female rats? A systematic review and meta-analysis were planned to answer the question, which includes creating a search strategy, inclusion/exclusion criteria, and planning data extraction and analysis. Initially, searches were performed on variations of terms related to population (rats), intervention (antidepressant), and outcome (blood pressure) in Medline (via Pubmed), SCOPUS, and EMBASE databases. All publications (n=2863) returned from searches were exported to EndNote X9 for duplicate exclusion (n=299) and selection. To get an overview of the extensive library, a pilot study was carried out. For this, we used the "randbetween" function of the Excel application to select 29 publications randomly; among them, those that did not meet the inclusion and exclusion criteria were excluded (n=23), and a total of 6 publications in any language that described the pressor effects of animals treated with

ADs, were included. A single reviewer performed the selection, qualitative data extraction, and meta-analysis. **Results:** In preliminary results, most studies used Wistar rats (83,33%), males (83,33%), the intraperitoneal route for AD administration (50%), and the cannulation of the artery method for blood pressure measurement (50%). The decreasing prevalence for each class of ADs found was: selective serotonin reuptake inhibitors (50%), tricyclic antidepressants (16,66%), monoamine oxidase inhibitors (16,66%), and serotonin and norepinephrine reuptake inhibitors (16,66%). A total of 8 studies (91 subjects) were included in the meta-analysis. The standardized mean differences observed ranged from -0,46 to 2,75, with most estimates being positive (75%), in favor of a blood pressure effect of ADs treatments. Based on the random-effects model (Hedges'g=0,9273 95% CI: 0,18 ?1,67), the mean result differed significantly from zero (p=0,0148) and appeared to be heterogeneous (I²=60,18). Trim-and-fill (value=0,00) and Egger regression test (p=0,061) did not indicate any asymmetry of the funnel plot. These results are indicative of the blood pressure effects resulting from the use of ADs. The contribution of sex to heterogeneity should be investigated in the meta-analysis through subgroup analysis. In addition, further studies should be carried out to identify the influence of sex and ADs in modulating blood pressure parameters to improve understanding of the possible mechanisms associated with these responses. **Financial Support:** Capes. **License number of ethics committee:** N/A

06.015 **Serum from COVID-19 patients decreases endothelial cell antioxidant defense via downregulation of the Nrf2 transcriptional factor.** Rodrigues D¹, Machado MR¹, Costa RM^{1,2}, Tostes RC¹, ¹Dpt of Pharmacology, Ribeirão Preto Medical School, Univ of São Paulo, Brazil, ²Academic Unit of Health Sciences, Federal Univ of Jatai, Brazil

Background: Coronavirus disease 2019 (COVID-19), caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), very often leads to severe respiratory failure, long-term hospitalization and death. COVID-19 is considered an endothelial disease since endothelial cell activation by SARS-CoV-2 or by the associated cytokine storm shifts the endothelium phenotype to a pro-inflammatory profile, linked to increased levels of cytokines, adhesion molecules and reactive oxygen species (ROS). Nrf2 transcriptional factor is the major transcriptional factor related to cellular antioxidant defense. A few studies have proposed that Nrf2 activation could be targeted in COVID-19 therapeutics. However, little is known about the impact of COVID-19 on Nrf2 activity and regulation in endothelial cells. **Hypothesis:** Serum from COVID-19 patients decreases endothelial antioxidant defense through downregulation of the Nrf2 transcriptional factor activity. **Methods:** All experiments were performed on Human Umbilical Vein Endothelial Cells (HUVECs). ELISA was performed to assess proinflammatory cytokines in the serum of healthy and COVID-19 subjects; ROS generation was evaluated by dihydroethidium (DHE) fluorescence and OxyBlot detection kit. Nrf2 activity and protein expression of its negative regulators were assessed by translocation assay and western blot analysis, respectively. **Results:** Serum from COVID-19 patients exhibited increased levels of IL-6, but not of TNF- α or IL-1 β . COVID-19 serum-treated HUVECs showed increased ROS generation with a peak after 12 and 24 hours (h) of exposure. This was accompanied by increased protein carbonylation and reduced nitric oxide bioavailability (at 60 min and 24 h, respectively). Nrf2 activity was reduced in HUVECs after treatment with serum from COVID-19 patients. In HUVECs treatment with COVID-19 serum increased expression of Bach-1, a negative regulator of Nrf2 signaling pathway. However, expression of Keap-1 (another negative regulator of Nrf2) was decreased after treatment with serum from COVID-19 patients. **Conclusions:** These data indicate that COVID-19 serum from patients decreases endothelial cell antioxidant defense via downregulation of Nrf2 activity. **Financial Support:** FAPESP (CRID), CAPES and CNPq **License number of ethics committee:** The Brazilian National Committee for Ethics in Research (CONEP) approved all procedures performed in the study (CONEP CAAE: 30248420.9.0000.5440 and 30816620.0.0000.5440)

06.016 **Evaluation of endothelial function in vascular reactivity and antioxidant systems in mice with chronic kidney disease.** Garlet TC¹, Moecke DMP¹, Martins GHC², Probst JJ², Hahmeyer MLS³, Dafre AL², da Silva-Santos JE³, Hizume-Kunzler DC¹. ¹UDESC Florianópolis, Dpt de Fisioterapia, Brazil; ²UFSC Florianópolis, Dpt de Neurociências, Brazil; ³UFSC Florianópolis, Dpt de Farmacologia, Brazil

Introduction: Physiological disorders of chronic kidney disease (CKD) are closely related to the development of cardiovascular injuries, as well as imbalance in the redox system and significant endothelial dysfunction. Therefore, the aim of this study was to evaluate the endothelial function of thoracic aorta in an ex-vivo vascular reactivity (VR) assay and the activity of antioxidant systems in an experimental model of CKD. **Methods:** 30 Male Swiss mice, 4 to 6 weeks old, were divided in Control (CTL, n=13) and CKD groups (CKD, n=17; in order to induce CKD, mice were submitted to a powdered diet containing adenine (SIGMA®) 0.2% w/w [i.e., 0.2g adenine/100g of powdered feed for 4 weeks]). 24 hours after last day of experimental protocol, animals were anesthetized, euthanized and thoracic aorta was extracted and divided in rings

with and without endothelium (E+ and E-, respectively) for VR evaluation. Rings were sequentially exposed to 120 mmol/L potassium chloride (KCl), 1 micromolar phenylephrine (Phe) and cumulative concentrations (1 to 30 micromolar) of Phe, acetylcholine (ACh) and sodium nitroprusside (SNP). We also collected blood and kidney tissue to evaluate nitrate/nitrite (NOx) levels, catalase (CAT) and glutathione peroxidase (GPx) activities. **Results:** The adenine-induced CKD model showed significant improvement of urea and creatinine serum levels ($p=0.001$ and $p<0.0001$, respectively), as well as reduced creatinine clearance values ($p=0.0002$), ratifying the CKD. The studied kidney disease model showed high serum urea and creatinine levels ($p=0.001$ and $p<0.0001$, respectively), as well as reduced creatinine clearance values in CKD group animals ($p=0.0002$), showing to be a viable model of CKD induction. E+ rings exposed to KCl and Phe showed a significant increase in mean contraction in the CKD group ($p=0.0003$ and $p=0.0249$, respectively), which was not observed in E- rings ($p>0.05$). Under cumulative concentrations of Phe, E+ rings showed a significant increase in the maximum contractile responses in CKD mice when compared to CTL group ($p=0.0001$), but no significant difference was observed in E- rings ($p>0.05$). Regarding to the relaxation process, there was no significant difference between groups, for either ACh or SNP analysis ($p>0.05$). The antioxidant enzymatic analysis showed a significant decrease in the level of CAT in the kidney tissue of CKD animals ($p=0.0001$), but this difference was not observed in the GPx analysis ($p>0.05$). Parallel, we observed that serum levels of NOx were not changed by CKD, but in kidney tissue there was a significant decrease of NOx levels ($p=0.002$). **Conclusion:** These results suggest that adenine-induced CKD in mice result in preponderant endothelium-dependent VR impairment in the aortic rings, in addition to decreased antioxidant levels in kidney tissue. **Financial Support:** This research was supported by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES, Grant 88882.447369/2019-01) and Fundação de Amparo à Pesquisa e Inovação do Estado de Santa Catarina (FAPESC, Grant PAP-2019TR715). **License number of ethics committee:** This study was approved by UDESC Animal Ethics Committee (protocol number: CEUA 2306201018).

06.017 Salt-inducible Kinase (SIK): A pharmacological target of salt-sensitive hypertension. Gomes DS¹, Visniauskas B², Prieto MC², Lara LS¹ ¹UFRJ, Inst de Ciências Biomédicas, Programa de Pesquisa em Farmacologia e Inflamação, Brazil ²Tulane Univ School of Medicine, Dpt of Physiology and Renal Hypertension Center of Excellence, USA

Introduction: Salt-inducible kinase (SIK) is a serine/threonine kinase involved in intracellular sensing network. However, its role in the development of salt-sensitive hypertension remains uncertain. We used a mouse model of salt-sensitive hypertension to test the hypothesis that SIK inhibition decreases systolic blood pressure (SBP) and renal injury. **Methods:** Male C57BL/6J mice (20-25g) were randomly fed either normal salt (NS; 0.5% NaCl) or high salt (HS; 4% NaCl) diet and daily treated or not with a SIK inhibitor (iSIK, YKL-05-099, 20 mg/Kg, via IP; $n=6$ /group; CEUA/UFRJ: 013/19). In these mice: 1) SBP was measured by telemetry for 15 days; 2) Urine metabolic collection for 24 h was performed on day 14; and 3) Blood and kidney samples were collected after euthanasia on day 15. **Results:** HS diet increased SBP during both day and night times (Day: from 114 ± 1 to 136 ± 1 mmHg; Night: from 127 ± 3 to 141 ± 4 mmHg; $p<0.0001$). Treatment with iSIK prevented increases in SBP (Day: 112 ± 1 and Night: 123 ± 2 mmHg). iSIK did not change SBP nor renal Na^+ handling in NS diet. In HS mice, iSIK prevented the augmentation of urine osmolality (HS: 1692 ± 14 vs. HS+iSIK: 1104 ± 34 mOsm/Kg H_2O ; $p=0.0019$) but did not change increases in urine volume and Na^+ excretion neither decreases in urine creatinine. Moreover, HS mice displayed glomerular segmentation, tubular interstitial disruption, infiltration of inflammatory cells and collagen accumulation. These responses were attenuated by iSIK. Increased kidney SIK activity in HS mice were sensitive to iSIK (HS: 222.8 ± 45 vs. HS+iSIK: 19.10 ± 15 pmol.mg⁻¹.min⁻¹; $p=0.0026$). The augmentation of (Na^++K^+)ATPase activity in HS mice was exacerbated during iSIK (HS: 517 ± 112 and HS+iSIK: 763 ± 18 mmol.mg⁻¹.min⁻¹ $p<0.0001$). Equally important, SIK inhibition in HS mice: 1) Decreased plasma MCP-1 levels (HS: 79 ± 7 vs. HS+iSIK: 51 ± 2 pg/dL; $p=0.0019$); 2) Prevented HS-dependent macrophages renal infiltration; 3) Decreased kidney ROS; and 4) Decreased kidney TGF- β protein expression (HS: 1.9 ± 0.01 vs. HS+iSIK: 1.4 ± 0.3 au. $p=0.0007$). **Conclusion:** Thus, the data indicate that SIK inhibition attenuates salt-sensitive hypertension and prevents kidney injury, without altering Na^+ handling in response to HS. In perspective, SIK might be a pharmacological target for salt-sensitive hypertension treatment. **License number of ethics committee:** 013/19 CEUA/UFRJ

06.018 Endothelium dysfunction in gestational hypertension in rats induced by the reduced uteroplacental perfusion pressure model. Santos-Silva, ML; Souza-Paula, E and Dias-Júnior, CA Dpt of Pharmacology, São Paulo State Univ, Botucatu, Brazil

Introduction: During the gestational period, several cardiovascular adaptations occur to provide a healthy pregnancy. Preeclampsia (PE) is a disease that arises during pregnancy and is characterized by elevated

maternal blood pressure (140x90mmHg) accompanied by 24-hour proteinuria after the 20th week of pregnancy. Its pathogenesis is still unclear; however, the placenta can play a key role. With pregnancy, many changes take place in the cardiovascular system to accommodate the increased blood volume. The vasodilation process is mediated by the formation of nitric oxide (NO) which reduces peripheral vascular resistance so that pregnancy can occur in a healthy way. Studies have shown that deficiencies in the production of this mediator lead to hypertensive disorders. The experimental model of gestational hypertension and PE induced by RUPP (reduced uteroplacental perfusion pressure) has shown great promise for manifesting the main features found in human PE, including hypertension, endothelial dysfunction and increased vascular reactivity. **Methods:** On the 14th day of gestation, the rat was anesthetized with isoflurane (1.5-2%) and, subsequently, a median incision was made for implantation of a 0.203mm silver clip in the lower abdominal aorta (above the iliac bifurcation) and another two 0.100mm (each) silver clips were implanted in the right and left branches of each ovarian artery. Norm-Preg Group: Pregnant rats that received 0.9% saline solution by gavage. HTN-Preg group: pregnant rats submitted to the RUPP model on the 14th day of pregnancy. Thoracic arteries were dissected in 3 to 4 mm segments. The rings placed in Krebs-Henseleit nutrient solution and suspended between two wires, one close to the support and the other connected to an isometric force transducer. The nutrient solution was adjusted to pH 7.4 and maintained at 37 °C in the presence of a mixture of carbogen (95% O₂ and 5% CO₂). Changes in aortic tension were recorded using isometric force transducers. **Results:** We compared the relaxation responses to acetylcholine between strips of aortic vessels from pregnant and hypertensive RUPP rats and found that endothelium-dependent vasodilation was significantly attenuated in hypertensive RUPP rats (66.86±9.47 vs. 41.32±16.80g). Increases in the contraction of the α -adrenergic receptor agonist phenylephrine have been observed in RUPP group (1.12±0.25 vs. 0.63±0.18g). **Conclusion:** Thus, RUPP-induced hypertension in pregnant rats has many of the characteristics of preeclampsia in women, objective to simulate the main conditions of preeclampsia, being the way to advance studies of new treatments and solutions for investigating the pathophysiology of this condition that affects thousands of women. **License number of ethics committee:** IBB/UNESP (Protocol# 6707090320)

06.019 **H1-Receptors May Mediate the Vasodilator Activity of a Natural Substance** 06.019 **H1-Receptors may mediate the vasodilator activity of a natural substance extracted from piper rivinoides.** Barenco TS¹, Souza PDN¹, Marques AM², Ramalho TC³, Nascimento JHM¹, Ponte CG⁴. ¹UFRJ, IBCCF, Brazil; ²Fiocruz, Dpt of Chemistry of Natural Products, Brazil; ³UFLA, Dpt of Chemistry, Brazil; ⁴IFRJ, NCBA, Brazil

Introduction: The genus Piper is the most representative in Piperaceae family, with approximately 2000 species distributed in tropical and subtropical regions (Quijano-Abril et al.2006). Phytochemical studies have led to the isolation of a large number of physiologically active substances with pharmacological value, such as the neolignan conocarpan, found in leaves and roots, especially from Piper rivinoides (Parmar et al. 1997; Sartorelli et al. 2001; Moreira et al. 2016). Conocarpan has some important biological characteristics, however, non is known about its effects in cardiovascular system. **Methods:** Male Wistar rats weighing 200-300g were used (CEUA/CCS/UFRJ protocol No. 087/15). The effect of conocarpan in vascular reactivity, as well as in presence of different inhibitors/blockers were evaluated in rings from the 2nd branch of mesenteric artery, using a Mulvany's miograph. The rings were kept in Krebs-Henseleit solution at 37 °C, aerated with carbogen mixture and contracted with phenylephrine. Molecular docking procedures were performed by Molegro Virtual Docker (Thomsen and Christensen. 2006), with the overall energy of the complex calculated as a sum of van der Waals, electrostatic, hydrogen-bonding and torsion stress terms. **Results:** In vascular reactivity, conocarpane presented vasodilator activity in tension generated by the rings of mesenteric arterioles with intact endothelium (IC₅₀ = 1.08 μ M); this effect was significantly reduced in rings without endothelium (IC₅₀ = 11.9 μ M). In presence of different inhibitors/blockers, we observed that the vasodilator effect of conocarpan was inhibited by loratadine (H1 receptor antagonist). In docking analysis, within the receptor interaction site, the structures of conocarpan and histaprodifene, dimethylistaprodifene and vilazodone were docked; doxepin was re-docked. All of them had a great affinity/interaction in the enzyme active cavity, with vilazodone showing the major (-219.6 kcal mol⁻¹) and conocarpan the minor (-150.4 kcal mol⁻¹). The same occurs with the stability of the structures; conocarpan had one intermolecular interaction (Ser 111), and this interaction also happens in dimethylistaprodifen. **Conclusion:** We conclude that conocarpan has a vasodilatory effect on resistance arteries, with great influence of endothelium. This mechanism may be related to histamine receptors antagonism, once loratadine was able to inhibit this effect and the docking analysis showed the affinity between conocarpan and H1-receptors active sites. However, some studies are still needed to confirm this antagonistic effect. **Financial Support:** CAPES, CNPq, IFRJ **References:** QUIJANO-ABRIL, M. A. Areas of endemism and distribution patterns for Neotropical Piper species (Piperaceae). J Biogeog. 33: 1266. 2006. PARMAR, V. S. Phytochemistry of the genus Piper. Phytochemistry. 46(4): 597. 1997. SARTORELLI, P. Enantioselective

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06.020 Type 1 Collagen (COL-1) proteolysis by Matrix Metalloproteinase (MMP)-2 may contribute to FAK activation and increased vascular smooth muscle cells proliferation in aorta of acute hypertensive rats.

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Increased activity of MMP-2 contributes to accentuated vascular smooth muscle cells (VSMC) proliferation in early hypertension. MMP-2 also proteolyzes many proteins of the extracellular matrix of VSMC, including type 1 collagen (COL-1). The cleaved products from COL-1 may induce focal adhesion kinases (FAK) that trigger the proliferation signal in VSMCs. We hypothesized that increased activity of MMP-2 proteolyzes COL-1 in aortas of hypertensive rats, which activates integrin receptors and FAK phosphorylation, thus leading to increased VSMC proliferation and hypertrophic remodeling. Male Sprague-Dawley rats were submitted to renovascular hypertension by two kidney-one clip (2K-1C) model and treated with doxycycline (Doxy, 30mg/kg/day) by gavage from the third to seventh day post-surgery. Control rats were submitted to sham surgery (CEUA-USP 165/2019). We also worked with a protocol of ten weeks of hypertension to consider whether doxycycline would ameliorate the established VSMC proliferation and hypertrophy. Rats were treated with doxycycline (Doxy, 30mg/kg/day) from the second until ten weeks of hypertension (CEUA-USP 119/2017). Systolic blood pressure (SBP; tail-cuff plethysmography) was daily (acute) or weekly (chronic) measured, and aortas were processed for gelatin and *in situ* zymography, MMP-2, pFAK/FAK, integrin and COL-1 levels by Western blot, morphological analysis by hematoxylin and eosin (H&E) and picrosirius red stain, immunofluorescence to Ki-67. Statistical analysis was done by two-way ANOVA followed by Tukey post-test with $p < 0.05$. The 2K-1C rats developed high SBP at the first week (150 mmHg \pm 3.9 vs. Sham $p < 0.001$), which was higher at ten weeks (219 mmHg \pm 6, $p < 0.0001$). The expression and the activity of MMP-2 were increased in the aortas of 2K-1C rats at first and tenth weeks of hypertension ($p < 0.05$ vs. Sham vehicle and doxy). Doxycycline reduced these parameters in the acute situation ($p < 0.01$ vs. 2K-1C vehicle). Decreased levels of COL-1 as well as increased in its potential degradation products were observed in the aortas of 2K-1C rats at the first week ($p < 0.05$ vs. Sham vehicle and doxy). Treatment with doxycycline prevented this effect in the hypertensive rats ($p < 0.01$). The 2K-1C rats showed an increase in FAK activation ($p < 0.001$ vs. Sham vehicle and doxy) and in VSMCs proliferation ($p < 0.05$ vs Sham vehicle and doxy) at first week, although the levels of integrin were not changed. Doxycycline reduced FAK activation ($p < 0.01$) and the Ki-67 marker ($p < 0.05$) in the hypertensive rats. Hypertrophic vascular remodeling occurred for both acute and chronic hypertensive situation ($p < 0.05$ vs Sham vehicle), although we see more collagen deposition by picrosirius staining in the aortas of chronic hypertensive animals. Doxycycline did not reduce this fibrotic situation in chronic hypertension. In conclusion, increased expression and activity of MMP-2 may be associated with COL-1 cleavage, which activates FAK and induces VSMCs proliferation and hypertrophic remodeling in the early phase of hypertension. Late inhibition of MMP-2 would may be not enough to prevent fibrotic remodeling in hypertension. **Keywords:** MMP-2; COL-1; FAK; remodeling; aorta; hypertension **Financial Support:** CAPES/ FAPESP/ CNPQ/ FAEPA **References:** Belo VA; et al; *J Vasc Res*. 2015;52(4): 221-231. Belo VA; et al; *Biochem. Pharmacol*. 2016,, vol. 118, pp. 50-58, Newby, A. C.;et al;*Cardiovasc. Res*.2006, vol. 69, no. 3, pp. 559-561 **License number of ethics committee:** (CEUA-USP 165/2019)/(CEUA-USP 119/2017)

06.021 Analysis of the action of H2S from the perivascular adipose tissue in different vascular vessels of hypertensive pregnant rats. Paula ES, Santos-Silva MLSS, Bozoni FT, Dias Junior CAC São Paulo State Univ, Botucatu, Brazil

Introduction: Preeclampsia is a pregnancy related hypertensive disorders characterized by increased blood pressure, often proteinuria and other clinical symptoms that affect 8-12% pregnant women after the 20th gestational week (ACOG, 2013). Perivascular adipose tissue (PVAT), may participate in the control of vascular tone through the release of vasoactive substances, including hydrogen sulfide (H₂S) (Beltowski,J; *Can J Physiol Pharmacol*, v93, p. 889, 2015). H₂S is a gaseous molecule, enzymatically produced by cystathionine gamma lyase (CSE) that has been shown to exert vascular tonus regulation, predominantly vasodilation (Aydinoglu, F; *Nitric Oxide*, v 70, p.51, 2017). We assess the difference between brown PVAT and beige PVAT in tone regulation from the thoracic and abdominal aortae. **Methods:** Female Wistar rats (220-300g) were mated and then allocated in individual cages. Animals were divided into four different groups, as follow: Normal Pregnant (Preg),Pregnant+desoxycorticosterone acetate (DOCA) (Preg+DOCA-

salt), virgin (virgin) and virgin+ desoxycorticosterone acetate (DOCA) (Virgin+DOCA-salt). Animals from Preg+DOCA-salt and Virgin+DOCA-salt groups received desoxycorticosterone acetate (DOCA) 12.5 mg on gestational day one or correspondent day, followed by weekly injections of DOCA 6.25mg and water was replaced by 0.9% saline solution. Systolic blood pressure (SBP) was measured by tail cuff plethysmography before DOCA-salt treatment and on days 14th and 19th. On pregnancy day 21, animals were killed under isoflurane overdose. Thoracic and abdominal aortae were removed and divided into four rings as follow: +PVAT + endothelium (E), +PVAT -E, -PVAT +E, -PVAT -E. Preparations were pre-contracted with phenylephrine (PHE) (10^{-6} M) followed by ACh (10^{-4} M) to test endothelial integrity. After tissue equilibration, aortic rings were challenged with pre-contracted with PHE (10^{-6} M) and challenged with L-cysteine (L-Cys - 10^{-2} M) for 5, 10 or 15 minutes. **Results:** Injections of DOCA increased Systolic blood pressure during pregnancy but not in virgin animals. No differences were found in thoracic aortae on the groups preg, virgin and virgin+DOCA-salt and Abdominal aortae on the groups preg, preg-DOCA-salt and virgin+DOCA-salt on the times 5, 10 or 15 minutes. In contrast, we observed anti-contractile effect of PVAT in thoracic aortae of 15 minutes in preg+DOCA-salt group and in abdominal aortae on the group virgin in 5 minutes. The placental and fetus parameters (fetal weight, placental weight and litter size) are statistically differences in the preg+DOCA-salt group in compared in the preg group. The antiangiogenic factor sFLT-1 is high and de factor angiogenic PLGF it's decreasing in the group preg+DOCA-salt compared on the other groups. **Conclusions:** The H₂S derivate of PVAT brown or beige exerts effects on vascular modulation in the thoracic aortae and abdominal, this effect is directly proportional to the time supplemented with the enzyme cystathionine gamma-lyase (CSE) with the substrate L-Cysteine. **Financial Support:** FAPESP and CAPES **License number of ethics committee:** CEUA 1083-2018

06.022 Platelet activity from Antiphospholipid Syndrome (APS) patients is enhanced: possible role of the ADP signaling pathway. Leonardi G¹, Lescano CH¹, dos Santos APR², Jacinto BC², Mazetto BM², Orsi FA³, Mónica FZ¹. ¹Dpt of Pharmacology, Faculty of Medical Sciences, Univ of Campinas, Campinas, Brazil; ²Faculty of Medical Sciences, Univ of Campinas, Campinas, Brazil; ³Lab of Haemostasis, Hematology and Hemotherapy Center, Univ of Campinas, Campinas, Brazil

Introduction: Several studies have evaluated the direct effect of antiphospholipid antibodies in isolated platelets from healthy volunteers, but the literature is scarce about platelet activity obtained from patients with APS. **Aims:** To evaluate platelet aggregation obtained from patients with primary APS with thrombosis (t-PAPS) or healthy volunteers with no history of diabetes, hypertension or dyslipidemia. **Methods:** Twenty-four patients with t-PAPS (66.6% females, mean age: 38 years) and 48 healthy volunteers (58.5% females, mean age: 33 years) were included. All protocols were approved by the Human Ethics Committee of the University of Campinas (CAAE nº 59362216.7.0000.5404). Firstly, platelet-rich plasma (PRP) was obtained and stimulated with adenosine diphosphate (ADP, 3 or 10 μ M), collagen (1 μ g/ml) or arachidonic acid (AA, 300 μ M). Next, PRP was pre-incubated with platelets inhibitors, as nitric oxide donor, sodium nitroprusside (SNP, 3 or 10 μ M) or the stable analogue of prostacyclin, iloprost, 3 or 10 nM) and then stimulated with ADP 30 μ M. Washed platelet (WP) was obtained by centrifuging PRP at 800 g for 12 minutes, followed by resuspension of the platelet pellet with Krebs Ringer's solution (3.0×10^8 platelets/ml). For western blot, WP just at basal level was used and electrophoresis was performed on 8% agarose gel. **Financial support:** CAPES and CNPq. **Results:** ADP-induced platelet aggregation was significantly higher in t-PAPS group than in controls (3 μ M: $70\% \pm 26.4\%$ vs $55.5\% \pm 23.3\%$, $P=0.02$) and (10 μ M: $82\% \pm 21.3\%$ vs $70\% \pm 13.4\%$, $P=0.02$). No difference in AA- ($49.7\% \pm 37.3\%$ vs $49\% \pm 29.4\%$, $P=0.95$) or collagen- ($72\% \pm 20.9\%$ vs $68.2\% \pm 18.6\%$, $P=0.51$) -induced aggregation was seen between groups. The aggregation inhibition induced by SNP (3 μ M: $26.4\% \pm 40.2\%$ vs $50\% \pm 26.1\%$, $P=0.001$) and (10 μ M: $15.93\% \pm 11.23\%$ vs $35\% \pm 25.87\%$, $P=0.01$) or iloprost 3 nM ($59.5\% \pm 39.8\%$ vs $80.7\% \pm 22.9\%$, $P=0.01$) was less prominent in platelets from t-PAPS than in healthy volunteers. As the P₂Y₁₂ receptor is essential in the ADP-induced platelet aggregation, we performed the protein quantification of this receptor via western blot. We observed an increased expression of the P₂Y₁₂ receptor in platelet membrane of patients with t-PAPS (Control: 0.53 ± 0.24 vs t-PAPS: 0.81 ± 0.23). All data represent the mean values \pm SD. **Conclusions:** Our results showed that ADP-induced aggregation was increased and the inhibition induced by endothelial mediators was reduced in platelets from t-PAPS patients when compared to controls. Furthermore, the increased expression of the P₂Y₁₂ receptor on the platelets of t-PAPS patients may contribute to greater platelet aggregation against ADP. Our findings suggest that platelets activity is increased in t-PAPS and point towards a possible role of the ADP signaling pathway in the thrombotic event seen in these patients. **License number of ethics committee:** CAAE nº 59362216.7.0000.5404

06.023 **Chronic ethanol consumption induces loss of the anticontractile effect of perivascular adipose tissue: role for Angiotensin II.** ^{1,2}Awata, WMC; ^{1,2}Sousa, AH; ²Tirapelli CR ¹FMRP-USP – PPG em Farmacologia, ²EERP-USP Lab de Farmacologia

Introduction: Renin-angiotensin system plays a role in ethanol-induced hypertension and vascular dysfunction. Hypertension-induced vascular inflammation is initiated in perivascular adipose tissue (PVAT) and angiotensin II (ANGII) is an important mediator of this response. However, there are no studies describing the impact of chronic ethanol consumption on the modulatory action exerted by PVAT on vascular tone. **Objectives:** To investigate the consequences of ethanol consumption on the anti-contractile effect of PVAT. **Methods:** Male Wistar Hannover rats (260-280 g) were randomized in 4 groups: 1)Control; 2)Ethanol(20%, vol./vol.); 3)Losartan(10 mg/kg/day;p.o. gavage); 4)Ethanol(20%, vol./vol.)+Losartan(10 mg/kg/day;p.o. gavage). Concentration-response curves for phenylephrine and serotonin were obtained in thoracic aortas with or without PVAT [PVAT(+) and PVAT(-)] with [Endo(+)] and without endothelium [Endo(-)] after three weeks of treatment. Periaortic PVAT was isolated for biochemical assays. Results are shown as mean±SEM (two-way ANOVA/Bonferroni) [CEUA 19.1.937.22.3]. **Results:** Ethanol increased phenylephrine-induced contraction in arteries Endo(+) and Endo(-) in the absence of PVAT. The consumption of ethanol impaired the anti-contractile effect of PVAT in an endothelium-dependent manner [PVAT(+)/Endo(-): Control: 11.3±0.4mN, n=9; Ethanol: 15.7±1.7mN*, n=12; PVAT(+)/Endo(+): Control: 8.1±0.5mN, n=15; Ethanol: 14.6±0.1mN*, n=11]; [PVAT(-)/Endo(-): Control: 15.9±0.5mN, n=8; Ethanol: 20.9±1.3mN*, n=9; PVAT(-)/Endo(+): Control: 11.9±0.4mN, n=8; Ethanol: 16.3±1.1mN*, n=15]. Treatment with losartan prevented this effect [PVAT(+)/Endo(+): Ethanol+losartan: 9.8±0.5mN, n=17]. Ethanol did not affect serotonin-induced contraction in arteries PVAT(-). However, ethanol impaired the anti-contractile effect of PVAT in an endothelium-dependent manner [PVAT(+)/Endo(+): Control: 7.4±0.5mN, n=13; Ethanol: 12.3±0, 7mN, n=10]. We noticed that carboxy-Ptio and L-NAME did not change the contraction induced by phenylephrine in aortas from the ethanol group. Furthermore, it was seen that the loss of the anti-contractile effect of PVAT was reversed only in after incubation with tiron[(PVAT(+)/Endo(+): Ethanol: 14.6±0.1mN, n=11; Ethanol+tiron: 8.6±0.8mN, n=7)]. However, there were no changes in the levels of O₂⁻ (URL/mg protein) in the PVAT after treatment with ethanol. Catalase did not affect phenylephrine-induced contraction in aortas from the ethanol group [PVAT(+)/Endo(+): 13.8±1.5mN, n=7]. Decreased levels of H₂O₂ (nmol/mg protein) were detected in periaortic PVAT from ethanol-treated rats and losartan prevented this effect [(Control: 2.2±0.1, n=4; Ethanol: 1.5±0.1*, n=5; Losartan: 2.1±0.1 n=5; Ethanol+losartan: 2.5±0.2, n=6). RO1138452 (antagonist of PGI₂ receptor) did not change the vascular response induced by ethanol [PVAT(+)/Endo(+): Etanol: 14.6±0.1mN, n=11; Ethanol+RO1138452: 15.9 ± 1.4 mN, n=7; **Conclusion:** Ethanol induced loss of the anti-contractile of PVAT in an endothelium-dependent manner and ANGII played a role in such response. The mechanism associated with this effect involves reduction in NO and H₂O₂ bioavailability in PVAT. **Financial Support:** FAPESP, CNPq **License number of ethics committee:** CEUA 19.1.937.22.3

06.024 **BMP9 / ALK1 / BR-SMAD signaling pathway regulates cardiac remodeling of the offspring of dexamethasone-treated mothers.** Sodr  FSS¹, Pereira GA¹, Amaral AG¹, Murata GM¹, Castelo-Branco RC¹, Campos CV², Almeida LS², Teixeira CJ¹, Couto GK¹, Rossoni LV¹, Anh  GF², Bordin S¹. ¹Dpt of Physiology and Biophysics, Inst of Biomedical Sciences, Univ of S o Paulo, S o Paulo, Brazil ²Dpt of Pharmacology, Faculty of Medical Sciences, State Univ of Campinas, Brazil

Background: Low birth weight (LBW) is associated with an increased risk of cardiovascular disease in adulthood. Although epidemiological studies have shown that LBW caused by prenatal overexposure to glucocorticoid increases the incidence of heart disease, the mechanisms underlying this disorder remain largely unknown. **Aims:** To investigate molecular alterations in the left ventricle (LV) of LBW rats, focusing on the TGF-  superfamily signaling. **Methods:** Body weight and Blood Pressure (BP) were monitored in male Wistar rats born to mothers treated with dexamethasone (0.2 mg / kg / day in the drinking water) during the last week of pregnancy (DEX), and in untreated age-paired offspring (CTL). At 120 days of life rats were euthanized, and LV was processed for qPCR and Western blot. **Results:** DEX were lighter than CTL (21%, p=0.0001). BP increased (128 ± 1.3 to 135 ± 1.2 mmHg, p=0.0063). There was a reduction in gene expression (19%, p=0.0494) and protein content (16%, p=0.0172) of BMP9 in the DEX liver, resulting in a lower amount of circulating BMP9 (32%, p=0.0023). The expression of its receptor Alk1 and its downstream signaling proteins Smad1 and Smad5 reduced 16% and 27% respectively (p=0.0343, 0.0022); phosphorylation of BR-Smad (Smad1/5/9) and expression of its classical target gene Id1 (Inhibitor of DNA Binding 1) were reduced 47% (p=0.0187). In parallel, there was an increase in the protein content of mTOR (255%, p=0.0002), phosphorylation of S6 Kinase (70%, p=0.0005) and Col1a2 (Cola Collagen, type I, alpha 2) expression (208%, p=0.0001) in LV of DEX rats. **Conclusion:** Rats exposed to glucocorticoid excess *in utero* show, in adult life, a reduction in BMP9 synthesis and signaling in LV. As BMP9 has been

previously described as an endogenous inhibitor of cardiac fibrosis due to LV pressure overload, it is likely that the BMP9/Alk1/BR-Smad signaling pathway is involved in the maladaptive remodeling in the heart of the offspring born to DEX-treated mothers. **Ethical approval:** CEUA/USP (8500250619/2019) **Funding support:** FAPESP (Grants 2013/07607-8, 2019/03196-0, 2020/09717-9), CAPES and CNPq **License number of ethics committee:** CEUA/USP (8500250619/2019)

06.025 **Cardiac dysfunction in sepsis: the involvement of intercalated discs.** Hahmeyer MLS, Assreuy J, da Silva-Santos JE. UFSC Florianópolis, Dpt of Pharmacology, Brazil

Introduction: Sepsis is a serious condition, defined as a life-threatening syndrome, in which organ dysfunction occurs due to the host dysregulated immune response to an infection. Cardiovascular dysfunction is the main dysfunction resulting from sepsis and nitric oxide (NO) has a relevant role in it. Intercalated discs, structures that connect the myocytes, play a fundamental role in cardiac mechanic and electrical coupling. The present study investigated the involvement of intercalated discs and nitric oxide (NO) in cardiac dysfunction in sepsis. **Methods:** The cecum ligation and perforation (CLP) model was used to induce sepsis in female rats (Protocol 2291220319 CEUA/UFSC). Hearts were collected 6, 12 and 24 hours after surgery. Cardiac tissue was collected for immunostaining assay for the protein N-cadherin, an important structural element of the intercalated discs and for the investigation of protein S-nitrosylation levels. **Results:** Immunostaining for N-cadherin was already seen 6 hours after sepsis, and reached a maximum 12 hours after CLP, showing a tendency to return to normal levels 24 hours after surgery. Protein S-nitrosylation marked increased in septic hearts, reaching a peak 12 after CLP. To directly assess the role of NO in the disruption of N-cadherin labeling, normal (non-septic) animals were treated with a NO donor, S-nitroso-N-acetylpenicillamine (SNAP) and the heart was collected 6 hours later. Treatment with SNAP disrupted the immunostaining for N-cadherin by dilating the structure of the intercalated discs. Other group of normal animals was ere treated with the denitrosylating agent, 5,5-dithio-bis- (2-nitrobenzoic acid; DTNB) 3 hours after SNAP administration. The treatment with DTNB partially reversed the effect of SNAP. **Conclusion:** Our results suggest that the role of N-cadherin in the mechanisms involving the pathophysiology of sepsis seems to be early and more correlated with morbidity than with the mortality of this syndrome. The findings that sepsis disrupts the immunostaining of N-cadherin and that this effect is reproduced in animals treated with a NO donor indicates that the disruption of intercalated discs is an important element for cardiac dysfunction and that NO has a relevant role in this disruption. More important however, the improvement in the disc structure induced by DTNB clearly shows that protein S-nitrosylation may provide an interesting target for possible new approaches to sepsis. **Keywords:** Sepsis. N-Cadherin. Nitric oxide. Heart. Intercalated disc. **Financial Support:** CAPES **License number of ethics committee:** Protocol 2291220319 CEUA/UFSC

06.026 **TNF-alpha inhibition reverses endothelial dysfunction and renovascular hypertension-induced ROS formation.** Vitorino TR^{1,2}, Mantovani B¹, Bonácio GF¹, Batista RIM³, Tanus-Santos JE³, Rizzi E¹ ¹Biotechnology Unit, Univ of Riberão Preto, São Paulo, Brazil ²Dpt of Pharmacology, School of Medical Sciences, Univ of Campinas, São Paulo, Brazil ³Dpt of Pharmacology, Ribeirão Preto Medical School, Univ of São Paulo, São Paulo, Brazil

Introduction: TNF-alpha seems to be important for the reactive oxygen species (ROS) formation induced by angiotensin II (Ang II), leading to vascular alterations, such as nitric oxide (NO) bioavailability reduction. TNF-alpha affects NO production, but the relationship between this cytokine and NO is not fully defined, particularly when Ang II levels are increased. Thus, the present study hypothesizes that chronic treatment with Etanercept (ETN; selective TNF-alpha inhibitor) decreases ROS formation, resulting in greater NO bioavailability in 2-kidneys and 1-clip (2K1C) hypertensive animals. **Methodology:** Male Wistar (180-200g) Sham and 2K1C rats were treated with ETN (1 mg/kg) or vehicle three times/week for 4 weeks. Systolic blood pressure (SBP) was evaluated weekly. Endothelial dysfunction was performed using concentration-effect curves of acetylcholine (ACh) and sodium nitroprusside (SNP) in aortic rings pre-contracted with phenylephrine. Dihydroethidium (DHE) assay was used to evaluate ROS in aortas. Plasma nitrite concentrations were evaluated using chemiluminescence. The results were analyzed with two-way ANOVA and Tukey test. Statistically different values were considered when $p < 0.05$. Ethics Committee Number 15/2016. **Results:** SBP increased progressively in 2K1C animals, reaching 187 ± 4 mmHg ($p < 0.05$ vs. Sham) in the week 4. ETN treatment did not decrease SBP in 2K1C rats (181 ± 9 mmHg). ACh-induced relaxation was reduced in 2K1C untreated rats ($71 \pm 3\%$) compared to Sham ($95 \pm 8\%$; $p < 0.05$) while ETN treatment restored endothelial dysfunction in hypertensive animals ($p < 0.05$). No differences were observed in SNP-induced relaxation curves in among all groups. ROS production was increased 2K1C rats ($194 \pm 21\%$ vs Sham), and ETN treatment produced antioxidant effects ($91 \pm 24\%$). There were no statistical differences in plasmatic nitrite concentrations. **Conclusion:** TNF-alpha inhibition did not affect SBP, but reversed

endothelial dysfunction and ROS formation in hypertensive rats. Thus, ETN could improve hypertension-induced target organs damage. **Financial Support:** FAPESP and UNAERP. **License number of ethics committee:** Ethics Committee Number 15/2016

06.027 Proteolytic action of Matrix Metalloproteinase (MMP)-2 on sarcoplasmic reticulum calcium ATPase (SERCA) and the morphofunctional vascular alterations of hypertension. Mello MMB1; Pernomian L1; Parente JM1; Neves VGO1; Silva PHL1; Castro MM1 1 Dpt of Pharmacology, Ribeirão Preto Medical School, Univ of São Paulo, Brazil

Introduction: Hypertension-induced chronic vascular remodeling may result from increased activity of matrix metalloproteinase (MMP)-2 that contributes to vascular smooth muscle cells (VSMCs) migration, proliferation and extracellular matrix proteolysis. MMP-2 also proteolyzes many intracellular proteins, such as calponin-1, thus contributing to switch the phenotype of VSMCs and promote remodeling and dysfunction. MMP-2 also proteolyzes the sarcoplasmic reticulum calcium ATPase (SERCA) in ischemic-reperfused rat hearts, thus impairing the cardiac contractile function. In hypertension, the protein levels and the activity of SERCA are reduced in the arteries. The hypothesis is that hypertension-induced increased activity of MMP-2 contributes to reduce SERCA level and activity, thus resulting in vascular remodeling and dysfunction. **Method:** Male Sprague-Dawley rats were submitted to renovascular hypertension by two kidney-one clip (2K-1C) model and treated with doxycycline (Doxy, 30mg/kg/day) by gavage from the third to seventh day post-surgery. Control rats were submitted to sham surgery. Systolic blood pressure (SBP; tail-cuff plethysmography) was daily measured and after seven days, aortas were processed for gelatin and *in situ* zymography, SERCA protein levels by Western blot, calcium immunofluorescence using Rhod-2AM, morphological analysis by hematoxylin and eosin (H&E) stain, immunofluorescence to Ki-67 and vascular reactivity to phenylephrine. Statistical analysis was done by two-way ANOVA followed by Tukey post-test. The Ethics Committee for Animal Research of the Ribeirão Preto Medical School approved all protocols (122/2019). **Results:** SBP was increased in 2K-1C rats and Doxy did not reduce it (n=5-9; p<0.05). Meanwhile, increased MMP-2 activity in 2K-1C rats (vs. controls; p<0.05, n=5-8) was reduced by Doxy (n=5-8; p<0.05). SERCA proteolysis was increased in 2K-1C aorta (n=4-8; p<0.05 vs. controls) and Doxy prevented it (0.49±0.07 2K-1C+Doxy vs 1.42±0.42 2K-1C). Cytosolic calcium concentrations trended to increase in 2K-1C aorta (n=3; p=0.08) and Doxy decreased it (n=3; p<0.05). Calcium accumulation results in VSMC proliferation and vascular remodeling. H&E showed that cross sectional area (CSA) was increased in the hypertensive rats vs controls (n=3-4; p<0.05). Doxy decreased the increased cell proliferation by immunofluorescence to Ki-67 (n=5; p<0.05). Additionally, 2K-1C rats presented impaired vascular contraction in response to phenylephrine and Doxy seems to prevent this effect. **Conclusions:** SERCA is decreased by MMP-2 activity in the aortas of hypertensive rats and this effect may contribute to the morphofunctional vascular alterations in hypertension. Furthermore, MMP-2 inhibition by doxycycline reduced hypertension-induced SERCA proteolysis and the resulting vascular changes, thus preventing morphofunctional vascular damage. **Financial support:** CAPES, CNPq and FAPESP. **License number of ethics committee:** 122/2019

06.028 NLRP3 inflammasome mediates testosterone-induced cardiac dysfunction. ¹Dpt of Pharmacology, Ribeirão Preto Medical School, Univ of São Paulo, Ribeirão Preto, Brazil ²Special Academic Unit of Health Sciences, Federal Univ of Goiás, Jataí, Brazil ³Dpt of Physiology, Ribeirão Preto Medical School, Univ of São Paulo, Ribeirão Preto, Brazil

Introduction: Testosterone (Testo) modulates vascular tone and cardiac performance. Both supraphysiological and subphysiological testosterone levels are associated with increased cardiovascular risk. Athletes who use Testo at supraphysiological doses exhibit increased blood pressure, higher inflammatory markers levels, vascular dysfunction, and cardiac hypertrophy. NLRP3 inflammasome activation as part of the innate immune system response contributes to proinflammatory cytokines production, which leads to cardiac hypertrophy as one of its effects activation. However, whether NLRP3 participates in Testo-induced cardiac dysfunction still unclear. **Hypothesis:** We hypothesized that supraphysiological levels of Testo promote NLRP3 inflammasome activation in cardiac macrophages, leads cardiac dysfunction. **Methods:** Male, 12 week-old C57Bl/6J (WT) and NLRP3 knockout (NLRP3^{-/-}) mice were used. Mice were treated with testosterone propionate [Testo-P (10 mg/kg)] or vehicle for 30 days. Cardiac function was evaluated using echocardiography. After *In Vivo* experiments, western blot and ELISA assays were performed to evaluate NLRP3 inflammasome components. In addition, bone marrow-derived macrophages (BMDMs) were isolated, primed with lipopolysaccharide (LPS [500 ng/mL 4 h]) and stimulated with Testo [10⁻⁷ M], for 4, 6, 12 and 24 h. **Results:** Echocardiography outcomes showed severe cardiac dysfunction in WT mice treated with Testo-P, characterized by a reduction of the ejection fraction, shortening fraction, cardiac output and systolic volume. In addition, there was an increase in

interventricular septum, left ventricle posterior wall and decrease left ventricle internal diameter. All these effects were prevented in NLRP3^{-/-} mice. Furthermore, WT mice treated with Testo-P showed an increase in the cardiac expression of NLRP3 receptor [WT_Vehicle: 100.0 ± 0.00 (AU) vs. WT_Testo-P: 217.9 ± 36.28 (AU)], Caspase-1 [WT_Vehicle: 100.0 ± 0.00 (AU) vs. WT_Testo-P: 532.3 ± 89.28 (AU)] and, IL-1β cardiac levels was increased in WT mice treated with Testo-P [WT_Vehicle: 15.26 ± 7.88 (pg/mg) vs. WT_Testo-P: 51.97 ± 36.43 (pg/mg)]. These effects were prevented in NLRP3^{-/-} [NLRP3^{-/-}_Vehicle: 19.88 ± 2.60 (pg/mg) vs. NLRP3^{-/-}_Testo-P: 20.22 ± 10.94 (pg/mg)]. In addition, Testo-P treated WT mice showed increased macrophage infiltrate in the left ventricle, however this effect was not seen in the WT vehicle mice. *In vitro* experiments, bone marrow-derived macrophages primed with LPS showed increased NLRP3 receptor expression [Control: 100.00 ± 0.00 (AU) vs. Testo: 370.0 ± 72.47 (AU)] and IL-1β levels [Control: 0.001 ± 0.00 (pg/mL) vs. Test: 259.0 ± 17.08 (pg/mL)] 12 and 24 h after Testo stimulation. **Conclusion:** These data indicate that NLRP3 inflammasome activation induce cardiac dysfunction associated supraphysiological levels of testosterone. **Financial support:** FAPESP – 2019/20692-0, CAPES, CNPq. **License number of ethics committee:** This study was approved by the Ethics Committee on Animal Experimentation of the Ribeirao Preto Medical School (020/2021).

06.029 **Resistin contributes to PVAT dysfunction in a rheumatoid arthritis experimental model.** Fedoce AG, Veras PF, Silva JF, Cunha FQ, Tostes RC. USP-FMRP

Introduction: Patients with rheumatoid arthritis (RA) experience 50% more risk of mortality attributed to cardiovascular disease (CVD) independently of traditional risk factors. Perivascular adipose tissue (PVAT) dysfunction and the release of proinflammatory adipokines such as resistin contribute to CVD. Besides, circulating and synovial resistin concentrations are increased in RA patients. The present study tested the hypothesis that resistin contributes to PVAT dysfunction in a RA experimental model. **Methods:** Antigen-induced arthritis (AIA) was induced in male, 12 weeks-old C57BL/6 mice. AIA immunized mice received i.a. injection of mBSA (10 µg/10 µl of PBS) or PBS (10 µl) in C57BL/6 control group (CT). Disease activity was determined by lymph nodes flow cytometry and mediolateral knee joint diameter measurement. Thoracic aortae, with or without PVAT, were isolated after three weeks of AIA onset for functional, cellular and molecular assays. Data are represented as mean and standard error, using student's T test (p<0.05) for statistical analysis. **Results:** Inguinal lymph nodes of AIA showed an increase of CD4⁺/IL-17 cells compared to control (AIA: 10.4 ± 1.06 vs. CT 1.8 ± 1.06,%, n=6) and mediolateral knee diameter was increased in AIA compared to control mice (AIA 4.38 ± 0.06 vs. CT 3.50 ± 0.04, mN, n=6). PVAT from AIA mice was dysfunctional, with decreased phenylephrine (Pe) maximum responses (Emax) and no changes in Pe logEC50 compared to CT [Emax (mN): CT -PVAT 10.6 ± 0.3 vs. CT +PVAT 8.7 ± 0.2; AIA -PVAT 6.8 ± 0.3 vs. CT +PVAT 7.0 ± 0.2, n=6-8). The delta of Pe responses in aortas with and without the PVAT was also lower in AIA mice compared to control [Delta Pe (mN): CT 3.3 ± 0.3 vs. AIA 0.15 ± 0.1, n=6-8). Resistin concentrations were increased in the PVAT, plasma and knee of AIA mice vs. control [(pg/ml) Serum: AIA 899.2 ± 11 vs. CT 837.9 ± 18; PVAT: AIA 217.0 ± 24 vs. CT 121 ± 18; Knee: AIA 28.3 ± 1.7 vs. CT 19.5 ± 1.1, n=4-8). Moreover, the mRNA gene expression of monocyte chemoattractant protein-1 (CCL2); resistin-like molecule alpha (Retnla); Mannose Receptor C-Type 1(mrc1) and interleukin-1beta (IL-1b) was increased in PVAT from AIA mice compared to control (CCL2: AIA 2.6 ± 0.3 vs. CT 0.82 ± 0.10; Retnla: AIA 2.0 ± 0.1 vs. CT 1.0 ± 0.2; Mrc1: AIA 1.4 ± 0.2 vs. CT 0.75 ± 0.2; IL-1b: AIA 2.0 ± 0.4 vs. CT 1.0 ± 0.1, 2^(-ΔΔct)n=4-8). **Conclusion:** AIA-induced PVAT dysfunction, i.e. loss of its anti-contractile effect, is linked to increased resistin and inflammatory cytokines and vascular inflammation. **Financial support:** FAPESP 2013/08216-2 and 2019/24921-4 and CAPES.CEUA #15/2020. All experimental procedures were approved by the Ethics Committee on Animal Research of the Ribeirao Preto Medical School, University of Sao Paulo (Protocol: 15/2020). **License number of ethics committee:** 15/2020

06.030 **PDE3 inhibition in an experimental model of sepsis.** Oliveira JG¹, Sordi R¹, Amarantes ELA¹, Oliveira MRP², Fernandes D¹ ¹UFSC Florianópolis, Dpt of Pharmacology, Brazil; ²UEPG Ponta Grossa, Dpt of Structural Biology, Molecular, and Genetic, Brazil

Introduction: Sepsis is a life-threatening organ dysfunction caused by a dysregulated immune response to infection, impairing the cardiovascular system, platelet aggregation, neutrophil migration, endothelial stabilization, and lastly, multiorgan dysfunction establishment. Reduction in cyclic nucleotides (cAMP and cGMP) production has been associated with hemodynamical collapse in sepsis. Therefore, blocking cyclic nucleotide hydrolysis catalyzed by phosphodiesterase (PDEs) would improve organ perfusion in sepsis. Furthermore, the PDE3 family isoforms are able to hydrolyze both cAMP and cGMP, and cilostazol (CLZ), a PDE3 inhibitor approved for the supportive treatment of chronic peripheral vascular diseases, would represent a therapeutic strategy in sepsis. Thus, we investigate PDE3 inhibition by CLZ on cardiovascular and inflammatory parameters in an experimental sepsis model. **Methods:** Sepsis was performed by cecal

ligation and puncture (CLP) procedure in male Wistar rats. Cilostazol (CLZ 15 mg/kg, og) or vehicle was administered six h later. Twenty-four h after the CLP procedure, pressure, heart rate, renal blood flow, isolated aorta vascular reactivity were obtained. After that, blood samples were collected to cyclic nucleotides (cAMP and cGMP), nitrite/nitrate (NO_x), aminotransferases AST and ALT, lactate, and hematological analyzes. Lastly, tissues were collected for myeloperoxidase activity (MPO), Evans blue (EB) leakage, histopathology, and Western blotting analysis. In the second experimental protocol, blood pressure and heart rate were measured at different times over 24 h. Lastly, the percent survival during sepsis was analyzed until five days after the CLP procedure. University Institutional Ethics Committee (Protocol number 1667100417). **Results:** CLP procedure caused hypotension, hyporesponsiveness to vasoconstrictors, renal blood flow reduction, systemic inflammation, multiorgan dysfunction development, and mortality over time (CLP 20% survival in five days; control 100%). Moreover, CLP reduced the plasmatic cGMP levels 12 h later the surgery (CLP 4.5 ±0.4, control 11.7 ±1, p<0.001), no difference was observed in the cAMP levels. Interestingly, CLZ increased cAMP levels 12 h later the surgery only in the sepsis group (CLP 12.7 ±1.6, CLP CLZ 25.1 ±4.6, p<0.05). In addition, CLZ improved renal blood flow (CLP 251.9 ±42; CLP CLZ 394.2 ±25.9, p<0.05), responsiveness to vasoconstrictors *in vivo*, and organ bath, and the hyporeactivity an essential marker in septic shock and poor prognosis. Furthermore, the CLZ reduced plasma biomarkers of poor blood perfusion as lactate level (CLP 50.9 ±7.9; CLP CLZ 27.1 ±4.7, p<0.05), and systemic inflammation as lung MPO (CLP 4.2 ±1.6; CLP CLZ 0.5 ±0.1, p<0.05). CLZ also reduced lung histopathologic score and lung EB leakage, but the differences were not statistically different. Ultimately, both CLP and CLZ did not change PDE3A expression in the heart and thoracic aorta. **Conclusion:** CLZ, when appropriately administered in sepsis, improved tissue blood perfusion and reduced organ injury, thus avoiding the septic shock development. Together with other treatments, CLZ can represent a useful supplementary tool in sepsis management, preventing organ damage and mortality. **Financial Support:** This work was supported by FAPESC, CAPES, and CNPq. **License number of ethics committee:** Protocol number 1667100417

06.031 **Evaluation of the role of gastroenteric xanthine oxidoreductase in the hypotensive effect of sodium nitrite.** Medeiros CFA, Neves EMN, Santana IV, Lopes JMS, Nogueira RC, Tanus-Santos JE Lab of Cardiovascular Pharmacology, Univ of São Paulo, Ribeirão Preto, Brazil

Introduction: The antihypertensive effects of sodium nitrite have been confirmed in several studies. However, the biochemical mechanisms responsible for these beneficial effects are still not completely understood. Enzymatic pathways are identified as capable of promoting the reduction of nitrite to NO. For example, during hypoxia and low pH, Xanthine Oxidoreductase (XOR) converts nitrite to NO which can and may be involved in the antihypertensive effect exerted by nitrite. **Aim:** the aim of the present study was to evaluate and compare the effect of the administration of sodium nitrite in different portions of the gastrointestinal tract, as well as to investigate whether these effects would be dependent on the distribution of XOR in these tissues. **Methods:** For this, Wistar rats were initially pretreated with a vehicle or allopurinol, and underwent a surgical procedure to assess mean arterial pressure and XOR participation. Hypertension was induced by the administration of 60mg/kg of NO synthases inhibitor (L-NAME). Then, a dose-response curve was performed with the doses of 1, 5 and 15mg/kg of sodium nitrite or corresponding volume of vehicle, to evaluate the effects of nitrite in the three portions of the gastrointestinal tract (gastric and enteric proximal). At the end of the protocol, plasma samples were collected for biochemical analysis. **Results and Conclusions:** The results obtained in this work show that sodium nitrite surprisingly produced an antihypertensive effect much more pronounced in the proximal enteric route than in the gastric routes. Possibly, this more pronounced hypotensive effect is due to the higher levels of nitrite and plasma S-nitrosothiols concentrations as compared to the other groups. Pretreatment with allopurinol was able to abolish the hypotensive effect of nitrite in the gastric route. However, in the proximal enteric route, allopurinol was not able to significantly reduce the effect of nitrite. These results suggest that XOR, although more expressed in the proximal small intestine, is not relevant to the effects of nitrite. The results after pre-treatment with allopurinol, on the other hand, suggest that XOR is fundamental for the effects of nitrite in the stomach. When evaluating the activity of XOR in the two tissues, it was found that there is a much higher activity of this enzyme in the proximal portion of the small intestine, compared to the stomach; and that in the presence of allopurinol, this activity was reduced by approximately half in all evaluated tissues. We conclude that there is a differentiated participation between the upper digestive organs in relation to the effects of nitrite. **Keywords:** Nitrite, xanthine oxidoreductase, nitrosothiols, allopurinol. **License number of ethics committee:** This research was approved by the Ethics Committee on Animal Experiments, Faculty of Medicine of Ribeirão Preto, University of São Paulo, under protocol number 0213/2019.

06.032 **NONO2P, a new nitric oxide donor, induce vasorelaxation in superior mesenteric artery from rats.** Moraes RA^{1,2}, Araújo FA^{1,2}, Jesus RLC¹ Silva LB¹, Meira CS², Capinan Filho JWS², Soares, MBP², Sá DS³, Silva CDS³, Silva DF^{1,2} ¹Lab of Cardiovascular Physiology and Pharmacology, Bioregulation Dpt, Federal Univ of Bahia (UFBA), Salvador, Bahia, Brazil ²Gonçalo Moniz Inst, Fiocruz, Salvador, BA, Brazil ³Federal Inst of Bahia, IFBA, Salvador, BA, Brazil

Cardiovascular diseases are the leading cause of death globally. Additionally, damage to the endothelium impairs the production and/or bioavailability of nitric oxide (NO), which can show a role in hypertension, myocardial infarction and atherosclerosis. NO is involved in several roles as the maintenance of vascular and heart homeostasis. NO donors are potent vasodilators, but currently available NO donors may have toxicity and/or vascular tolerance. In this way, NONO2P, nitric oxide donor, was synthesized, and there are still no reports in the literature on the cardiovascular activity. The aim of this study was to investigate the vascular activities of the NONO2P in the superior mesenteric artery, with propose of describing the mechanisms of action involved in the observed responses. **Methods:** Male Wistar rats (200-300g) were euthanized and the superior mesenteric artery was isolated for recordings of isometric tension in an organ bath. It was approved by the Ethics Committee on Animal Use from the Federal University of Bahia (CEUA/UFBA nº 4169290420). **Results:** In human umbilical vein endothelial cells (HUVEC), NONO2P was non-toxic at the concentrations tested (6.25, 12.5, 25 or 50 μ M). Cumulative administration of NONO2P (10^{-13} to 3×10^{-6} M) in pre-contracted mesenteric artery rings with phenylephrine, 1μ M, induced endothelium-independent vasorelaxation. Similar results were obtained after pre-treatment of the rings with L-NAME (100μ M) (nitric oxide synthase inhibitor). However, the presence of the specific soluble guanylyl cyclase inhibitor, ODQ (10μ M), abolished the vasorelaxant effect induced by NONO2P. Additionally, NONO2P induced vasorelaxation in rings exposed to a depolarizing-tyrode solution containing 60 mM KCl or 20 mM KCl, which was significantly attenuated compared to the control, suggesting the participation of K^+ channels. In this way, the vasorelaxant response induced by NONO2P was significantly attenuated by ATP-sensitive potassium (K_{ATP}) channels blocker, glibenclamide (10μ M), inward rectifier potassium (K_{ir}) channels blocker, $BaCl_2$ (30μ M), voltage-gated potassium (K_v) channels blocker, 4-AP (1mM) and large conductance Ca^{2+} -activated K^+ (BK_{Ca}) channels blocker, TEA (1mM). Besides, the free radical ($NO\cdot$) scavenger (carboxy-PTIO; 100μ M and hydroxocobalamin; 30μ M) and nitroxyl anions (NO^-) scavenger (L-cysteine; 3mM) decreased relaxations promoted by NONO2P. **Conclusion:** This is the first evidence of the vasorelaxant effect induced by the novel NO donor, NONO2P. Interestingly, NONO2P can release different types of NO, free radical species of nitric oxide ($NO\cdot$) or nitroxyl anions (NO^-) species. The endothelium-independent vasorelaxant effect induced by NONO2P involves soluble guanylyl cyclase and K^+ channels activation, becoming a promising molecule with vascular activity as a new possible therapeutic alternative for the treatment of cardiovascular diseases. **Financial Support:** CAPES, FAPESB and CNPq **License number of ethics committee:** CEUA/UFBA nº 4169290420

06.033 **Bariatric surgery impairs the nitrate-nitrite-NO pathway and prevents the beneficial cardiovascular effects of nitrate-rich beetroot extract supplementation.** Sanches-Lopes JM, Barros AC, Tanus-Santos JE Dpt of Pharmacology, Ribeirão Preto Medical School, Univ of São Paulo, Ribeirão Preto, Brazil.

Introduction: Supplementation with nitrate-rich vegetables such as beetroot, offer cardioprotective properties. The nitrate-nitrite-NO pathway is the key to these health benefits. For this pathway, dietary nitrate is reduced to nitrite and the acid gastric pH is essential for the reduction the nitrite to NO and nitrosylated species, consequently the cardiovascular effects. In bariatric surgery, Roux-en-Y gastric bypass, anastomosis is made between a smaller portion of the stomach and a jejunal strap. Resulting in a reduction in stomach volume and high on gastric pH. Objectives: To evaluate the effects of sodium nitrate present in beetroot extract on blood pressure, vascular function and redox biology in health patients and whether bariatric surgery impairs these effects, as a result of surgery-induced alterations in the nitrate-nitrite-NO pathway. **Methods:** Women between 18 and 60 years with controlled blood pressure, and who underwent Roux-en-Y gastric bypass surgery or not (controls). These patients were divided into a control group and a bariatric group and ingested beetroot extract supplemented with sodium nitrate (0.1 mmol/kg) for 14 days. Before and after the treatment, the pulse wave velocity (PWV) and endothelial function using EndoPat were measured. Blood pressure was monitored for 24 hours using an ambulatory blood pressure monitoring and plasma were collected for the biochemical measurements of nitrate, nitrite, nitrosylated species concentrations and for evaluate oxidative stress and antioxidant assay. **Results:** 15 volunteers from each group were evaluated. After the treatment, the control group presented a decreased daytime systolic blood pressure ($p < 0.05$), reduced daytime heart rate ($p < 0.05$), reduced mean arterial pressure ($p < 0.05$) and improve endothelial function ($p < 0.05$). In addition, the treatment in control group increase nitrite and nitrosothiols concentrations ($p < 0.05$), improved the redox status by reduced thiobarbituric acid reactive substances (TBARS) and hydrogen peroxide concentration and improve total antioxidant capacity

(TAC) by antioxidant assay ($p > 0.05$). Nighttime blood pressure, nighttime heart rate and PWV remained unchanged ($p > 0.05$). In the bariatric surgery group, there were no changes in systolic, diastolic and mean blood pressure, heart rate, endothelial function and PWV ($p > 0.05$) after treatment. In relation to biochemical measures, interestingly, the treatment in this group increase only nitrite concentration ($p > 0.05$), decrease nitrosothiols formation ($p < 0.05$) and did not change the other biochemical measures. Conclusions: Sodium nitrate treatment reduces blood pressure, improves endothelial function and improves the redox status of women with normal stomach by increasing nitrite and nitrosothiols formation. They also support the hypothesis that bariatric surgery impairs the beneficial effects of sodium nitrite on cardiovascular health, due to anatomical changes that impair the nitrate-nitrite-NO pathway and especially impairs the formation of nitrosothiols. **Financial Support:** CNPq and FAPESP. **License number of ethics committee:** This study was approved by the Research Ethics Committee (CEP) of Ribeirao Preto Medical School, University of Sao Paulo, CAAE: 88796918.2.0000.5440.

06.034 Acute chikungunya infection induces vascular dysfunction related to changes in ROS/NF- κ B/iNOS/NO pathways. ¹Dpt of Pharmacology, Ribeirão Preto Medical School, Univ of São Paulo, Ribeirão Preto, Brazil ²Dpt of Cellular and Molecular Biology and Pathogenic Bioagents, Ribeirão Preto Medical School, Univ of São Paulo, Ribeirão Preto, Brazil ³Special Academic Unit of Health Sciences – Federal Univ of Goiás, Jatai, Brazil

Introduction: Chikungunya virus (CHIKV) is a reemerging arbovirus, whose infection causes febrile illness associated with severe joint pain, myalgia and cardiovascular impairment. There is evidence that Chikungunya is associated with symptoms of bradycardia and hypotension, in addition to other cardiovascular effects, including the generation of reactive oxygen species (ROSs) and production of pro and anti-inflammatory cytokines. We hypothesized that CHIKV infection compromises arterial function by direct actions on vascular cells and by mechanisms involving oxidative stress. **Methods:** Male C57BL/6J mice, six weeks old, were used. The mice were divided into two experimental groups: 1) infected with CHIKV and 2) Mock-vehicle. The mice were infused with CHIKV (1.0×10^6 PFU - *Plaque-Forming Unit*) or culture medium via the intracaudal route. Vascular function was assessed in thoracic aortic rings using a myograph to record isometric tension. Endothelial cells (EA.hy926) were infected with CHIKV (1.0 MOI - *Multiplicity Of Infection*). The production of ROS was evaluated by chemiluminescence with lucigenin and the expression of proteins by western blot. Significantly statistical differences were considered when $p < 0.05$. **Results:** Infected mice showed less vasoconstrictor response to FEN 48 hours (h) after infection (R_{max} CHIKV = 126.8 ± 4.34 *; R_{max} Mock = 178.8 ± 5.04), which was restored by the NOS inhibitor (L-NAME 300 300 mM) (R_{max} CHIKV = 219.7 ± 10.66 ; R_{max} Mock = 214.4 ± 6.81) and removal of the endothelium. There was no difference in the vasodilator response to ACh (R_{max} CHIKV = 88.22 ± 2.85 ; R_{max} Mock = 85.37 ± 1.78). Aortas of mice infected with CHIKV showed increased ROS generation [Lucigenin (RLU) - Basal: 88.6 ± 4.7 ; CHIKV: 163.7 ± 17.0]. CHIKV infection increased the protein expression of iNOS and phospho-NF- κ B p65, but decreased the expression of NF- κ B p65. In endothelial cells, CHIKV induced the production of ROS and increased the expression of the endothelial protein ICAM-1, related to endothelial activation processes. **Conclusion:** These data suggest that the increase in ROS production and iNOS expression, triggered by the direct action of CHIKV in vascular cells, alters NO signaling, promoting activation of endothelial cells and inflammatory processes, with consequent vascular dysfunction. Therefore, this study provides new evidence for the involvement of vascular dysfunction linked to alterations in ROS/NF- κ B/iNOS/NO pathways in the pathogenesis of Chikungunya. **Financial support:** CAPES, CNPq. The Ethics Committee on Animal Experimentation of the Ribeirao Preto Medical School (171/2019) approved this study. **License number of ethics committee:** The Ethics Committee on Animal Experimentation of the Ribeirao Preto Medical School (171/2019) approved this study.

06.035 Temporal changes of renal and hepatic protein SUMOylation in hemorrhagic shock. Oliveira FRMB, Soares ES, Ramos HP, Cimarosti HI, Sordi R. UFSC, Dpt of Pharmacology, PPG Pharmacology

Introduction: Hemorrhagic shock (HS) is a condition commonly associated to multiple organ dysfunction (MOD) and death. Excessive bleeding followed by reperfusion cause a type of damage known as ischemia-reperfusion injury (Eltzschig et al, Nat. Med. 17, 1391, 2011). The precise mechanisms of MOD are not completely elucidated. It is well known that severe cell stress may lead to increased small ubiquitin-like modifier (SUMO) levels, and several SUMO targets may be involved in organ protection (Yang et al, Open Biol. 7, 170167, 2017). Mammals usually express three SUMO paralogues (SUMO1–3). SUMO1 participates mainly in normal cellular processes whereas SUMO2/3 is primarily involved with pathological responses (Yeh, J. Biol. Chem. 284, 8223, 2009). The hypothesis of our study is that SUMOylation increases after HS to protect organs against injury. In this study we evaluated temporal changes of SUMO levels in the liver and kidney at 3 time points after HS. **Methods:** Male Wistar rats were given tramadol, then,

anesthetized with ketamine and xylazine, and submitted to the HS protocol. After cannulating the left femoral artery and vein, blood was withdrawn at a rate of 1 mL/min until blood pressure reached 40 ± 2 mmHg. The animals were kept under this condition for 90 min and, then, reperfused with the previously shed blood. Two, 6 or 24 h after reperfusion, blood, kidney, and liver were harvested. MOD markers in plasma, cardiovascular parameters, and blood cell counts were assessed. SUMO1, SUMO2/3, and conjugating enzyme Ubc9 levels were determined by dot blotting. **Results:** Serum lactate levels were elevated in animals that underwent HS when compared with sham group. The elevation was observed immediately after the surgery and remained high until 2 h after the reperfusion. Urea and creatinine levels increased 2 and 6 h after the surgery; however, unlike hepatic aminotransferases (AST and ALT) that were elevated throughout the time, these kidney damage markers values were similar to sham 24 h after the insult. Animals were hypotensive 2 and 6 h after the surgery, but not at 24 h. Granulocytosis and lymphocytosis were observed 6 and 24 h, respectively. Analysis of hepatic tissue showed no elevation in SUMO1 or SUMO2/3; still, Ubc9 was elevated 6 h after the HS when compared to the 2 and 24 h groups. Contrastingly, in renal tissue, protein SUMOylation and Ubc9 levels were more elevated than the sham group. **Conclusions:** The HS surgery caused systemic organ failure that lasted, at least, for 24 h. Although most organ damage markers increased over time, we observed a reduction in urea and creatinine levels at later time points. This fact coincided with a higher protein SUMOylation in kidneys, which could explain, at least in part, the improvement in renal function over time. Financial support: CAPES and CNPq. **License number of ethics committee:** Research approval by UFSC Ethics Committee on Animal Use: Protocol 7396250219.

06.036 Post-occlusive reactive hyperemia in the skeletal muscle of rats at model of adjuvant-induced arthritis. Santos WP, Souza-Silva E, Dornelles FN, Tonussi CR. UFSC Florianopolis, PPG Pharmacology, Brazil

Introduction: Rheumatoid arthritis (RA) is a chronic inflammatory disease that affects skeletal muscle tissue. Although RA is speculated to be associated with an increased risk of developing cardiovascular disease (CVD), little is known about how endothelial dysfunction (ED) can affect blood perfusion in the skeletal muscle around affected joints. Peripheral microvascular function can be assessed by post-occlusive reactive hyperemia (PORH), which is the measure of reperfusion after a brief period of occlusion-induced ischemia (flow-mediated vasodilation). This study was designed to detect whether PORH is impaired in skeletal muscle in an animal model of rheumatoid arthritis such as adjuvant-induced arthritis (AIA). Furthermore, we explore the hypothesis whether PORH in skeletal muscle might be influenced differently after adjuvant-induced intra-articular stimulation. **Materials and Methods:** Female Wistar rats (200-250 g) were administered with vehicle or immunized with an injection of complete Freund's adjuvant (CFA; 0.5 mg/mL; Mycobacterium tuberculosis) in a total volume of 50 μ L at the base of the tail. After 21 days, the control group received a vehicle solution, while the test group received CFA (5.0 mg/mL) in the right knee (50 μ L; i.a.). Five days later, the animals were anesthetized with ketamine (100 mg / kg, im) and xylazine (20 mg / kg, im), and the cuff positioned around the animals' thigh was inflated to 300 mmHg simultaneously in both legs or in the individual leg. Blood flow was assessed by laser Doppler in the tibialis anterior muscle of both legs, before and immediately after 5 min of blood flow occlusion. PORH was characterized by four parameters: peak, hyperemia peak time, half-recovery time and total hyperemia. **Results:** Cuff deflation resulted in a rapid increase in local blood flow in both legs in all experimental groups, characterizing PORH after 5 min of cuff occlusion. In animals immunized with CFA and control, we found no difference in blood perfusion and in the profile of PORH in the skeletal muscle between the legs. Blood perfusion was reduced in the right leg in CFA animals compared to control and no difference was observed in the left leg. Individual occlusion reduced the profile of PORH (peak and total hyperemia) in the right leg in CFA animals compared to control. In contrast, simultaneous occlusion reduced the peak in the left leg but not in the right leg. **Conclusion:** We demonstrate that blood perfusion and PORH profile in the tibialis anterior muscle of rats are reduced in adjuvant-induced arthritis and changes in the detected blood flow profile associated with microcirculation can be used to explore the adaptive behavior of small arterioles to meet physiological demands of muscle blood perfusion. Financial support: CAPES, CNPq. **License number of ethics committee:** 1914250220

06.037 Acute kidney injury in Wistar rats experimentally envenomated with *Bothrops jararacussu* venom. Romanelli MA¹, Soeiro PA¹, da Silva RC¹, Taveira-da-Silva R², Melo PA¹, Lara LS¹ ¹PPG em Farmacologia e Química Medicinal, Inst de Ciências Biomédicas, Univ Federal do Rio de Janeiro, Brazil ²Inst de Biofísica Carlos Chagas Filho, Univ Federal do Rio de Janeiro, Rio de Janeiro, Brazil

Introduction: The *Bothrops* genus is one of the most clinically relevant venomous snakes' genus in Latin America, next to *Crotalus*, *Lachesis* and *Micrurus* genus (Chippaux, 1998). However, in Brazil, the

snakebites occur mainly with Bothrops, as *Bothrops jararacussu* (Bj) causing severe local (as necrosis) and systemic reactions, as Acute Kidney Injury (AKI) (Pinho, 2001). Nowadays, the only available treatment is the serotherapy, but there is no consensus on whether serotherapy prevents the progress of AKI and there is no available pharmacotherapy to prevent AKI progression, so preclinical study models are needed. We aimed to elaborate an AKI model induced by the administration of Bj venom for preclinical studies. **Methods:** Male Wistar rats (CEUA 128/18) were randomly divided into 3 different groups: (1) Bj-IV: intravenous administration of 0.4 mg/kg Bj; (2) Bj-IP: intraperitoneal administration of 2.0 mg/kg Bj; (3) Bj-IM: intramuscular administration of 3.5 mg/kg Bj. For each corresponding control group, a 0.9% saline solution was administered. Kidneys, blood and urine samples were collected 24 or 72 h after administration of the Bj venom for renal function analysis. To analyze the renal tubular injury, a blind evaluation was made, scoring kidney tissue as follows: score 1 (between 0 to 25% of damaged renal tissue); score 2 (25% to 50%), score 3 (50% to 75%) and score 4 (more than 75%). **Results:** The IV- and IP-Bj groups presented a moderate tubular injury (score 3) and a time-dependent kidney dysfunction. Both IV- and IP administrations routes showed increased proteinuria in the first 24 hours (35% and 110%, respectively, compared to control group) that returned to control values 72 hours after poisoning. Besides that, IV- and IP-Bj groups presented increased PCr in the first 24 hours (38% and 100%, respectively) that also returned to control values after 72 hours after poisoning. In the Bj-IM group, renal tubular injury was aggravated (score 4) with collagen deposition and renal dysfunction was observed in the first 24 h: hyperfiltration, proteinuria, albuminuria and decreased fractional sodium excretion (FENa). Over time, the glomerular lesion was intensified, with a decrease in glomerular filtration rate (GFR; 67%), blood urea-nitrogen (BUN; 68%) and urine volume decrease (71%). Proteinuria and tubular function returned to control levels after 72 h. We attributed the pronounced kidney injury and reduced filtration function in the Bj-IM to the muscle damage provoked by the IM administration. It should be emphasized that myoglobin deposits in renal tissue are closely related to acute tubular necrosis, a very common diagnosis in cases of AKI resulting from bothropic accidents (Santos et al., 2009). **Conclusion:** We concluded that the Bj-IM is administration route to establish a preclinical model of AKI with the monitoring of the progression of renal function in the periods of 24 and 72 h. **Financial Support:** FAPERJ, CAPES/CNPq **References:** CHIPPAUX, J.P., 1998. Bull. World Health Organ. 76, 515-524. PINHO, F.M.O. et al., Ren Failure, v. 23(2), 269p., 2009. SANTOS, M.F.L., et al., 2009J. Bras. Nefro. 31, 132-138, 2009. **License number of ethics committee:** CEUA 128/2018

06.038 **SARS-CoV-2-induced endothelial cell damage by mitochondrial DNA release and TLR9 activation.** Costa TJ¹, Potje SR¹, Fraga-Silva² TFC, Silva-Neto JA¹, Barros PB¹, Rodrigues D¹, Machado MR¹, Martins RB³, Santos-Eichler RA⁴, Bonato VLD², Arruda E³, Bomfim GF⁵, Tostes RC¹. ¹Dpt of Pharmacology, Ribeirão Preto Medical School, Univ of São Paulo, Brazil ²Dpt of Biochemistry and Immunology, Ribeirão Preto Medical School, Univ of São Paulo, Brazil ³Virology Research Center, Ribeirão Preto Medical School, Univ of São Paulo, Brazil ⁴Dpt of Pharmacology, Inst of Biomedical Science, Univ of São Paulo, Brazil ⁵Inst of Biological and Health Sciences, Federal Univ of Mato Grosso, Brazil

Mitochondria and Toll-like receptor (TLR) activation play a central role in the host response to viral infection and immunity have been suggested as potential targets in SARS-CoV-2 infection. However, the involvement of mitochondria and TLR9-activation in SARS-Cov-2-induced endothelial dysfunction and potential contribution to cardiovascular complications in COVID-19 has not been demonstrated. To determine whether infection of endothelial cells by SARS-CoV-2 affects mitochondrial function and induces mitochondrial DNA (mtDNA) release. We also questioned whether TLR9 signaling mediates the inflammatory responses induced by SARS-CoV-2 in these cells. Human umbilical vein endothelial cells (HUVECs) were infected by SARS-CoV-2 and immunofluorescence was used to confirm the infection. Mitochondria function was analyzed by specific sondes and real-time polymerase chain reaction (RT-PCR) confirmed the mtDNA levels. Inflammatory parameters were measured by ELISA, protein expression by western blot and calcium (Ca²⁺) by FLUOR-4. Vascular reactivity was evaluated using an isometric myography. SARS-CoV-2 infection HUVECs promoted mitochondrial dysfunction, mtDNA release, activation of TLR9, and release of cytokines. SARS-CoV-2 also decreased nitric oxide synthase (eNOS) levels and inhibited cell Ca²⁺ responses in endothelial cells. TLR9 blockade reduced SARS-CoV-2-induced IL-6 release and prevented decreased eNOS protein levels. mtDNA increased vascular reactivity to endothelin-1 (ET-1) in arteries from wild type, but not TLR9 knockout mice. These effects were recapitulated in samples from COVID-19 patients. Serum from COVID-19 patients exhibited high levels of mtDNA compared to sex- and age-matched healthy subjects and patients with comorbidities. Critical, but not mild-to-severe, COVID-19 patients presented high levels of ET-1. SARS-CoV-2 infection impairs mitochondrial function and activates TLR9 signaling. TLR9 triggers inflammatory responses that contribute to endothelial cell dysfunction, potentially contributing to the severity of symptoms in COVID-19. Targeting mitochondrial metabolic pathways may help to define

novel therapeutic strategies for COVID-19. Financial support: FAPESP (17/25116-2; 13/08216-2), CNPq and CAPES. **License number of ethics committee:** (CONEP CAAE: 30248420.9.0000.5440 and 30816620.0.0000.5440); CEUA 144/2020

06.039 **Effect of Paroxetine for the treatment of sepsis.** Galant LS¹, Borges V¹, Rodrigues FC¹, Kanashiro A¹, Monteiro V¹, Cebinelli GM¹, Duarte DA², Costa-Neto C², Pupo AS³, Cunha FQ¹ ¹Faculdade de Medicina de Ribeirão Preto, Univ de São Paulo – USP – Dpt. de Farmacologia Ribeirão Preto (SP) - Brasil. ²Faculdade de Medicina de Ribeirão Preto, Univ de São Paulo, Dpt de Bioquímica, Ribeirão Preto, Brasil. ³Univ Estadual Paulista, Botucatu – Dept de Farmacologia, Inst de Biociências SP- Brasil

Introduction: Recent studies demonstrate that paroxetine has the property of inhibiting GRK2 (Thal DM, *ACS Chem Biol*, 2012). In sepsis, GRK2 activation is involved in the internalization of alpha1 adrenoceptors in vasculature cells and CXCR2 receptors expressed in neutrophils. Therefore, we investigated the effect of paroxetine on vasoplegia and neutrophil migration deficiency at the focus of infection in a severe sepsis model. **Methodology:** Wild mice of the C57Bl/6 lineage were used between 8 -10 weeks. The procedures were approved by the ethics committee registered with CEUA-FMRP protocol number 241/2019. In the experiments with the 18G needle-induced severe sepsis model, the animals received treatment with the antimicrobial agent ertapenem (30 mg/kg 12/12h via intraperitoneal) and paroxetine (5 mg/kg 12/12h) starting at the sixth hour after ligation and perforation of the cecum (CLP) for seven days in the survival analyses. To assess blood pressure and heart rate, telemetry transmitters were implanted in the animals. The quantification of neutrophils in the peritoneal lavage was evaluated, as well as local and systemic bacteremia. Pro-inflammatory cytokine levels were also determined. The assessment of internalization of CXCR2 receptors in circulating neutrophils was determined by flow cytometry. Furthermore, in an *in vitro* experiment, HEK-293 cells were transfected with specific plasmids and evaluated for CXCR2 internalization after stimulation with MIP2 through the BRET assay. **Results:** Our results show that paroxetine is able to reduce the internalization of CXCR2 receptors in circulating neutrophils after sepsis. These data were confirmed by BRET analysis. This result supports the improvement in the migration of neutrophils to the infectious focus, reducing bacteremia at the site of infection and increasing the survival of septic animals. Regarding cardiovascular parameters, paroxetine was able to increase blood pressure and heart rate in animals with severe sepsis. Furthermore, treatment with paroxetine decreased inflammatory mediators such as CXCL2 and CXCL1. **Conclusion:** Paroxetine was effective in improving the survival of septic animals. This event was accompanied by improvement in cardiac function and migration of neutrophils to the site of infection. These effects resulted in a decrease in local bacteremia and inflammatory mediators. The mechanism of action of paroxetine in a septic condition is possibly due to the inhibition of GRK2 and, consequently, avoiding the internalization of CXCR2 receptors in neutrophils and alpha-1 adrenoceptors in the vasculature. This project is funded by FAPESP, CNPq and Capes. **License number of ethics committee:** CEUA-FMRP protocol number 241/2019

07. Endocrine, Reproductive and Urinary Pharmacology

07.001 Experimental model of insulin resistance in Swiss mice female and male

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Introduction: The establishment of an animal model of insulin resistance is important to understand the pathological process of insulin resistance and to develop therapeutic drugs. The aim of this study was to characterize the biochemical changes in a model of insulin resistance in mice with characteristics of pathology in humans. **Methods** Swiss mice male and female (4 weeks) were fed on standard rodent chow plus high fat emulsion (10 mL/kg) once *per day* by intragastric (i.g) (after fasting 4 h from 10 h a.m) for 8 weeks. After 6 h of fasting, control and insulin resistant mice were submitted to an insulin tolerance test – ITT – (1IU/kg insulin). Mice were injected with insulin and blood samples were collected from the tail at 0, 5, 10, 15, 20, 25 and 30 min for serum glucose determination. After determination of insulin resistance, glycemia, creatinine, aspartate transaminase (AST), alanine transaminase (ALT) cholesterol, serum and hepatic triglycerides, were measured. Slices of the gastrocnemius muscle and adipose tissue were preincubated (30 min) and incubated (60 min) at 37°C in Krebs Ringer-bicarbonate (KRb) buffer with ¹⁴C-DG (0.1 µCi/mL), O₂/CO₂ (95%: 5%, v/v) until pH 7.4, to analyze glucose uptake. Glycogen content was measured in liver and muscle and the activity of disaccharidases was evaluated in intestine (Protocol CEUA-UFSC PP0045). **Results:** Insulin resistance was achieved with high-fat emulsion. Blood glucose, cholesterol, plasma and liver triglycerides, total liver weight and serum creatinine were higher in groups

that received the emulsion. The activity of intestinal, sucrase and maltase disaccharidases were increased. The muscle and liver glycogen content as well as ^{14}C -DG uptake in muscle and adipose tissue were reduced in groups that received the emulsion. **Conclusion:** So, according to the biochemical parameters stated over here and the data collected, suggests an animal model of insulin resistance for the studies on diabetes late complications similar to the human frame reported for insulin resistance. **Financial Support:** CNPq/Grant # 472071/2013-0, CAPES, Fundação de Amparo à Pesquisa e Inovação do Estado de Santa Catarina (FAPESC) and Programa de Pós-graduação em Bioquímica/UFSC. **License number of ethics committee:** PP0045

07.002 **Hypoglycemic effect of *Lippia origanoides* Kunth hydroalcoholic extract in an experimental model of alloxane-induced diabetes.** Pereira YLG, Diniz LA, Miranda VC, Jóia-Mello V, Hamoy M Federal Univ of Pará, Inst of Biological Sciences, Brazil

Introduction: *Lippia origanoides* Kunth is an aromatic shrub present in North and South America, especially in the Amazon region of Brazil. The extracts of this plant have an increasing economic and medicinal value due to their antimicrobial, antiprotozoal bioactivity against *Leishmania chagasi* and *Trypanosoma cruzi*, antiviral against yellow fever virus *in vitro* and antigenotoxic against bleomycin^{2,3}. In addition, there are studies on the anti-inflammatory, analgesic, antipyretic, gastroprotective and healing potential of plants of the same genus^{4,5,6}. Ethnopharmacological reports also point to its use in diabetes. Thus, it is necessary to develop experimental studies on new forms of clinical use of the species *L. origanoides*.

Methodology: The extract was obtained by maceration in a solvent extracting EtOH/H₂O (1: 1;v/v). A total of 152 female and male Wistar rats, 90 days old (250-300g), kept in a 12/12h light/dark cycle, with food and water ad libitum, in the vivarium of the Laboratory of Pharmacology and Toxicology of Natural Products were used. of the ICB-UFPA. Diabetes was induced by intraperitoneal administration of 120 mg/kg of alloxane (Sigma-Aldrich Brazil) after a 12-hour fast. Diabetic rats (serum glucose > 250 mg/dL) were listed in the following experiments: Evaluation of the Acute Hypoglycemic Effect (up to 120min) and Chronic in 21 days using 150 mg/kg of extract (v.o); and Dose-Response Curve at doses 250, 150 and 75 mg/kg (po) of extract (groups I, II and III, respectively), dose of 5mg/kg (po) of glibenclamide (group IV) and vehicle (negative control). Blood samples were collected at 0, 7, 15, 21 and 28 days. At the end of each experimental period, the animals were euthanized by deep anesthesia (ketamine and xylazine). **Results:** As for the acute hypoglycemic activity, a time-dependent decrease in fasting blood glucose was noted in animals with alloxan diabetes (with a dose of 150 mg/kg), obtaining a reduction of 37.83%, 39.07% and 55.96% at 30, 60 and 120 min respectively (Anova, Turkey p<0.05). In continued treatment under the same dose, blood glucose was close to the normoglycemic range on the 15th day. In the dose-response curve, all doses reduced the glycemia of diabetic animals (Anova Turkey p<0.05). The animals treated with 150 mg/kg of extract showed results similar to those using Glibenclamide 5 mg/kg and normoglycemic control on the 21st day of treatment, while the 250 mg/kg group obtained this result on the 15th day. The negative control with untreated alloxan hyperglycemia showed increasing mortality up to 100% on the 17th day of the experiment. The hypoglycemic activity of the extract for the content of flavonoids such as Naringenin and Apegenin. **Conclusion:** *L. origanoides* has broad pharmacological action in the body and, through this study, its hypoglycemic activity was experimentally proven, with an effect similar to drugs used in the clinic, such as glibenclamide, and with a dose (150 mg/dL) effective to reduce blood glucose levels normoglycemic. **Research Support:** CNPq, CAPES and UFPA. **References:** 1Mar JM. Ind Crop e Prod, v.111, p.292, 2018; 2Silva SS. Res Soc Dev, v.9, p.1, 2020; 3Sarrazin SLF. Molecules, v.20, p.1860, 2015; 4Olivero-Verbel, J. Braz J Microbiol, v.45, p.759, 2014; 5Forestieri, A.M. Phytother Res, v.10, p.100, 1996; 6Khalil, H. E. Pharmacogn Mag, v.3, p.258, 2007. **License number of ethics committee:** 3807311019 CEUA-UFPA

07.003 **Food supplementation with *Spirulina platensis* prevents strength training-induced oxidative stress on rat uterus.** Lacerda-Júnior FF², Ferreira PB², Diniz AFA², Silva, MCC², Silva AS³, Silva, BA^{1,2}. ¹DCF-CCS-UFPB, ²PPgPNSB-CCS-UFPB, ³PAPGEF-UFPB

Introduction: Strength training promotes excessive increase in reactive oxygen species (ROS) due to oxygen consumption. ROS lead to oxidative stress reactions such as lipid peroxidation and activation of protein kinase C, increase uterine contraction. In this context, *Spirulina platensis* (SP), an alga with antioxidant potential (Mazo et al. Vopr. Pitan, 73: 45, 2004), decreased oxidative stress in aorta (Brito et al., Oxid. Med, 17: 4, 2017) and rat ileum (Ferreira, Dissertation, UFPB, 2017). Thus, we evaluated the participation of NADPH oxidase, superoxide dismutase (SOD), catalase, malondialdehyde (MDA) and antioxidant capacity (CAT) in the effects of SP on uterine reactivity. **Methods:** Virgin Wistar rats (150-250 g) were divided into a control (CG), trained (TG) and trained supplemented (TG100) orally with SP dissolved in saline solution (NaCl 0.9%) at the dose of 100 mg/kg. The female rats were submitted for eight weeks to a progressive

training and supplemented with SP, 24 hours before euthanasia, was administered diethylstilbestrol (1 mg / kg, s.c.) for estrus induction. After, the uterus was removed for analysis. The results were expressed as mean and standard error of the mean and analyzed by one-way ANOVA followed by Tukey's post-test. **Results:** In the oxytocin curve at presence of apocynin a NADPH inhibitor, on TG the efficacy and contractile potency of oxytocin were reduced ($E_{max} = 212.6 \pm 8.1\%$; $pEC_{50} = 3.3 \pm 0.1$), when compared to absence of inhibitor ($E_{max} = 222.0 \pm 7.1\%$; $pEC_{50} = 2.1 \pm 0.1$). Moreover, the efficacy and contractile potency of oxytocin increased in the presence of apocynin ($E_{max} = 188.0 \pm 11.5\%$; $pEC_{50} = 2.8 \pm 0.4$) in the TG100 compared to the absence of the inhibitor ($E_{max} = 169.0 \pm 7.7\%$; $pEC_{50} = 3.5 \pm 0.1$). In the presence of tempol a sod inhibitor, the curve with oxytocin, in TG, decreased the contractile efficacy and potency ($E_{max} = 203.0 \pm 12.6\%$; $pEC_{50} = 3.0 \pm 0.2$), when compared in the absence ($E_{max} = 222.0 \pm 7.1\%$; $pEC_{50} = 2.1 \pm 0.1$). TG100, on the other hand, decreased the contractile efficacy of oxytocin in the presence of tempol ($E_{max} = 169.1 \pm 7.7\%$) compared to its absence ($E_{max} = 134.6 \pm 4.8\%$), but did not change the potency ($pEC_{50} = 3.3 \pm 0.1$ and $pEC_{50} = 3.5 \pm 0.1$) respectively. In the relaxation with isoprenaline at presence of catalase in the TG, observed increase in the relaxing efficacy and potency of this agonist ($E_{max} = 100\%$; $pEC_{50} = 12.3 \pm 0.2$) when compared to curve in the absence ($E_{max} = 89.6 \pm 3.6\%$; $pEC_{50} = 9.8 \pm 0.3$). In TG100 there was no change in the relaxing efficacy of isoprenaline in the presence of catalase ($E_{max} = 100\%$), but increased potency ($pEC_{50} = 14.5 \pm 0.3$) comparing in the absence ($pEC_{50} = 12.3 \pm 0.2$; $E_{max} = 100\%$). When evaluating the MDA concentration in the uterus, an increase was observed in the TG (3.9 ± 0.1) and decrease in TG100 (1.6 ± 0.1). Besides that, CAT increased in TG ($97.4 \pm 1.3\%$) and this increase was accentuated in TG100 ($130.8 \pm 4.0\%$). **Conclusions:** This study demonstrates an antioxidant effect of SP in preventing changes in uterine reactivity, possibly decreasing superoxide anion and lipid peroxidation and increasing H_2O_2 and CAT, evidencing a role of SP in uterine disorders related to oxidative stress such as inflammatory processes and uterine hypercontractility. **Support:** CNPq, PPgPNSB. **Research approval:** CEUA/UFPB 0211/14. **License number of ethics committee:** Research approval: CEUA/UFPB 0211/14.

07.004 Analysis of phenotypic and metabolic changes in neurolysin knockout animals in a diet-induced obesity model. Caprioli B, Gewehr MCF, Eichler RAS, Ferro ES Dept de Farmacologia, Inst de Ciências Biomédicas, Univ de São Paulo

Introduction: Neurolysin (Nln) is an oligopeptidase present in mammals that degrades several functional peptides, both inside and outside cells. Nln knockout mice ($Nln^{-/-}$) present a slight but significant decrease in body weight, as they are more tolerant to glucose, more sensitive to insulin and produce more glucose in the liver using pyruvate as a precursor. **Objectives:** Our present aim is to compare the metabolic effects of diet-induced obesity in $Nln^{-/-}$ and wild type C57BLc mice. **Methods:** Animals four weeks old started to receive, during eight weeks, either standard diet (SD) or high fat diet (HFD) supplemented with condensed milk. The caloric content of SD is 3.8 kcal/g (carbohydrate, 70%; protein, 20%; fat, 10%; Nuvilab CR1, Nuvital Nutrientes S.A., Brazil). HFD caloric content is 5.3 kcal/g (carbohydrate, 27.44%; protein, 13.55%; fat, 59%; 1% of vitamin AIN-93; Rhoster) and was supplemented with condensed milk of 3.25 kcal/g (68% carbohydrate; 9% protein; 23% fat). Body weight was determined weekly, while fasting glucose and insulin levels were determined together with insulin-tolerance test (ITT) and glucose tolerance test (GTT) after the eight weeks of diet. **Results:** Initial data suggest that $Nln^{-/-}$ fed with SD gained $1,1 \pm 0,1$ (SEM; $n = 5$) g of body weight after six weeks of experiments. $Nln^{-/-}$ fed with HFD gained $8,5 \pm 1,0$ (SEM; $n = 5$) g of body weight after six weeks of experiments. Basal glucose levels were not altered in $Nln^{-/-}$ animals fed either SD or HFD. **Conclusion:** Our initial data suggest that $Nln^{-/-}$ fed with HFD gain significant more body weight along six weeks than $Nln^{-/-}$ fed with SD, whereas the fasting glucose levels were not altered in animals fed either Sd or HFD. Future investigation will allow to determine whether Nln plays a role in diet-induced obesity metabolic regulation. **Financial support:** CAPES, CNPq (302809/2016-3) and Fapesp (2016/04000-3). **License number of ethics committee:** 4112010621

07.005 Cinnamaldehyde action on muscular glucose uptake and insulin resistance. Roriz RNS¹, Sulis PM², Padilla DPR², Padilla DPR², Alencar NMN¹, Silva FRMB², Frederico MJS¹. ¹UFC Fortaleza, Dpt de Fisiologia e Farmacologia, Brasil; ²UFSC Florianópolis, Dpt de Bioquímica, Brasil

Introduction: Cinnamaldehyde (CINNA) have beneficial effects on glycemia and insulin resistance (ALLEN et al., 2013; HOSNI et al., 2017), however its mechanism of action has not yet been revealed. CINNA mechanism of action on muscular glucose uptake, insulin resistance and lipid metabolism were evaluated. **Methods:** To induce insulin resistance male Wistar rats (180–200 g) received dexamethasone (0.1 mg / kg, sc) for 5 consecutive days and were divided into 4 groups; 1-control saline, 2-insulin resistant (IR) 3-IR + CINNA (20 mg / kg i.p.); and 4-CINNA. After, insulin tolerance test (ITT), liver glycogen content and serum lipid profile were performed. Rat soleus muscle were incubated for 60 min in HEPES-KRb with $^{45}Ca^{2+}$

and [U-¹⁴C]-2-deoxy-D-glucose (¹⁴C-DG) at 37 ° C, pH 7.4 with O₂: CO₂ (95: 5 v/v) to verify CINNA (100 μM) mechanism of action on calcium influx and glucose uptake. Electrophoresis analysis from skeletal muscle with anti-GLUT-4, anti-p38 and anti-phospho-p38 antibody were evaluated. Finally, lipid tolerance test (LTT) was performed (Protocol CEUA/UFSC/PP00398/749). Data were expressed as mean ± S.E.M. ANOVA followed by Bonferroni post-test. *p* ≤ 0.05. **Results:** Treatment with CINNA 20 mg / kg for 5 days was able to reverse insulin resistance and increased the hepatic glycogen content. This treatment increased glucose uptake in muscle and fat tissue *in vivo*, decreased serum triglycerides, total and LDL cholesterol of insulin-resistant animals. Furthermore, CINNA treatment increased serum HDL cholesterol particles, GLUT4 content and p38 phosphorylation. Glucose uptake stimulation activates PI3K, PKC and p38 MAPK pathways; and depends on type L calcium channels and intracellular calcium, *in vitro*. This CINNA mechanism also involves the integrity of microtubules and actin filaments. In LTT, CINNA decreased serum triglycerides. **Conclusion:** Glucose uptake stimulated by CINNA activates PI3K, PKC and p38 MAPK pathways. CINNA treatment has beneficial effects on glucose and lipid metabolism in insulin resistant animals. Financial Support: CNPq, CAPES-PPGBQA/UFSC. ALLEN, R. W. et al. Ann Fam Med, v. 11, p. 452, 2013. HOSNI, A. A. et al. Biomedicine & Pharmacotherapy, v. 88, p. 52, abr. 2017. **License number of ethics committee:** Protocol CEUA/UFSC/PP00398/749

07.006 New insights into the bladder dysfunction caused by long-term methylglyoxal intake: reversal by metformin. Oliveira AL, Medeiros ML, Oliveira MG, Mónica FZ, Antunes E Dept de Farmacologia, Faculdade de Ciências Médicas, Univ de Campinas, Campinas, Brasil

Introduction: Methylglyoxal (MGO) is a reactive carbonyl species found at high levels in circulating blood and urine of diabetic and obese patients (1). MGO reacts with endogenous molecules, leading to the formation of advanced glycation end products (AGEs). Glyoxalase1 (Glo1) enzyme converts MGO to its end-product D-lactate, thus efficiently preventing MGO accumulation (2). The anti-hyperglycemic drug metformin is able to scavenge MGO, thereby reducing the AGEs formation (3). Diabetic bladder dysfunction (DBD) is a highly prevalent urological complication (4). A previous study showed that four-week MGO intake mimics DBD phenotype in mice (5). Here, we aimed to investigate the effects of 12-week intake of MGO on bladder dysfunction, looking at serum levels of AGEs and glucose, Glo1 mRNA expression and activity in bladder tissues, as well as the *In Vivo* voiding behavior and *in vitro* contractile responses. The protective effects of metformin in all of these parameters have also been investigated. **Methods:** The study design was experimental with animals and approved by the Animal Use Ethics Committee, protocol number 5573-1/2020. Male C57/BL6 mice received 0.5% MGO in drinking water for 12 weeks, and metformin (300 mg/kg, daily gavage) was given in the last two weeks. The bladder functions were evaluated *In Vivo* by performing voiding behavior assays, cystometry, *in vitro* bladder contractions and histological analysis. **Results:** MGO intake markedly elevated the levels of MGO and fluorescent AGEs in serum while glucose levels remained unaffected. MGO reduced the mRNA expression and activity of Glo1 in bladder tissues and increased the urothelium thickness and collagen content. Void spot assays in conscious mice revealed an increased void volume in MGO group. In MGO group, the cystometric assays revealed increases of basal pressure, non-voiding contractions frequency, bladder capacity, inter-micturition pressure and residual volume, accompanied by reduced voiding efficiency. *In vitro* bladder contractions to carbachol, α , γ -methylene ATP and electrical-field stimulation were significantly greater in MGO group. Metformin normalized the levels of MGO, AGEs, Glo1 expression and activity, urothelium thickness and collagen content. The *In Vivo* MGO-induced voiding dysfunction and *in vitro* bladder contractions were all restored by metformin treatment. **Conclusion:** Our findings show that metformin suppresses the enhanced serum levels MGO, then improving the voiding dysfunction promoted by MGO exposure. The beneficial effects of metformin in diabetic patients may reflect in part its ability to inactivate MGO and restore the glyoxalase system. **References:** 1. de la Cruz-Ares, S., et al., Nutrients 12, 238. 2020 2. Rabbani, N., et al., Glycoconj J. 38, 331. 2021. 3. Kinsky, O.R. et al., Chem. Res. Toxicol. 29, 227, 2016. 4. Daneshgari, F., et al., J. Urol. 182, S18-26. 2009. 5. de Oliveira, M.G., et al., Front. Physiol. 11: 290. 2020. **Financial Support:** CAPES (88882.435315/2019-01) **License number of ethics committee:** All animal procedures were approved by the Ethical Committee on Animals Use CEUA/UNICAMP, protocol number 5573-1/2020

07.007 Are the anti-inflammatory and metabolic effects of mometasone furoate dependent on glucocorticoid receptor activation? Zimath PL^{1,2}, Almeida M², Rafacho A^{1,2}. ¹UFSC Florianópolis, PPG Pharmacology, Brazil; ²UFSC Florianópolis, Lab. of Investigation of Chronic Disease, Brazil

Introduction: Exogenous glucocorticoids (GCs) including dexamethasone (DEX) are widely used based on their immunosuppressive and anti-inflammatory actions. Despite their therapeutic effects, GCs in excess result in metabolic disturbances such as glucose intolerance, insulin resistance, and dyslipidemias (Bijsmans et al., 2015). Thus, new GC ligands with the potential therapeutic application and minor adverse effects

are welcome. Mometasone furoate (MF) is a GC used for the topic and inhaled purposes, and based on *in vitro* evidence, it potentially exhibits lower side effects because it seems to act mainly through the Farnesoid X receptor (FXR) instead of the glucocorticoid receptor (GR) (Pasięka et al., 2016). Thus, we aimed to evaluate whether the anti-inflammatory and/or metabolic impact of systemic MF treatment is dependent on GR. **Methods:** To evaluate the anti-inflammatory effect of GCs, we performed the carrageenan (Cg)-induced peritonitis test (500 µg/cavity, intraperitoneally (ip)) on male Wistar rats (3 months old) treated with MF (0.1, 0.3, 1.0 or 3.0 mg/kg body mass (bm) by oral gavage (og) or 1.0 mg/kg bm ip, diluted in corn oil) or DEX (1.0 mg/kg bm og or ip, diluted in saline). To analyze metabolic parameters related to glucose and lipid metabolism, rats were treated daily with MF (0.1 or 1.0 mg/kg bm both through ip or og) or DEX (1 mg/kg bm both through ip or og) for 7 consecutive days. In another set of metabolic experiments, rats were treated daily with MIFE before the GCs treatment to evaluate the involvement of GR on these metabolic effects. The study was approved by the Institutional Ethical Committee (CEUA Nº 5012250518). **Results:** MF and DEX administered by systemic routes presented an anti-inflammatory effect in a GR-dependent manner. As expected, rats treated with DEX, irrespective of the route of administration, exhibited lower body mass and food intake and became glucose-intolerant, insulin-resistant and dyslipidemic. Almost all parameters altered by DEX treatment were reproduced in rats receiving MF through the ip route. Only minor alterations (i.e., reduction in the insulin sensitivity) were observed in rats receiving MF through the og route. MIFE pre-treatment prevented the most metabolic outcomes, except triacylglycerol liver content, which were induced by the ip administration of MF. **Conclusions:** MF maintains anti-inflammatory activity when administered through systemic routes and exhibits a minor impact on metabolism when orally delivered. In addition, MF does not act exclusively on GR, suggesting anti-inflammatory and metabolic adverse effects may occur through another pathway, possibly FXR. Support: CAPES, CNPq. MF was kindly donated by Aché Pharmaceuticals **References:** Bijmans A. et al. *Sci. Rep.*, 5, 14086, 2015. Pasięka A. et al. *Metabolites*, 6, 24, 2016. **License number of ethics committee:** CEUA Nº 5012250518

07.008 Targeting the new H₂S-releasing ketoprofen derivative for visceral pain and cystitis: A potential therapeutic approach. Santos LG¹, Teixeira SA¹, DE Oliveira MG², Dallazen JL¹, Oliveira JP¹, Mónica FT², Wallace J^{3,4}, Muscará MN¹, Antunes E², Costa SKP¹. ¹USP, Dpt of Pharmacology, Brazil; ²UNICAMP, Dpt of Pharmacology, Brazil; ³Antibe Therapeutics, Inc., Toronto, Canada; ⁴Univ of Calgary, Dpt of Physiology and Pharmacology, Canada

Introduction: Interstitial cystitis/bladder pain syndrome (IC/BPS) is an inflammatory and debilitating visceral pain disease that affects the lower urinary tract. The etiology is uncertain and the treatment is difficult. Evidence suggests that the endogenous mediator hydrogen sulfide (H₂S) plays an important role in the urinary tract function¹. Interestingly, we showed that the new H₂S-releasing nonsteroidal anti-inflammatory ketoprofen compound ATB-352 reduced postoperative pain². This study was carried out to investigate the beneficial role of ATB-352 on mice model of cyclophosphamide (CYP)-IC/BPS. **Method:** Cystitis model was established in male C57Bl/6 mice (8-10 weeks old; n=6) by intraperitoneal injection of cyclophosphamide (CYP; 300 mg kg⁻¹) or vehicle (saline; 10 ml kg⁻¹) for control group. Behavioural tests were performed as well as isolated bladder in an organ bath apparatus tested to investigate bladder nociceptive pain and function, respectively. At 21h of cystitis induction, mice were orally treated with ATB-352 (4.6, 15 or 46 mg kg⁻¹) or vehicle (carboxymethylcellulose; 0.5% CMC) and visceral (abdominal) referred mechanical allodynia was measured at 21, 22, 23 and 24 h, via the von Frey test. At 24h, bladder was isolated for either setting in an organ bath apparatus to measure spontaneous or carbachol (0.001–30 µM; n=6)-mediated contractile responses or used for protein expression of H₂S synthesizing enzymes: cystathionine gamma-lyase (CSE); cystathionine beta-synthase (CBS); 3-mercaptopyruvate sulfurtransferase (3-MST) and H₂S production. Data were analyzed by one-way ANOVA plus Tukey's test and were considered statistically significant when p<0.05. **Results:** ATB-352 (4.6, 15 or 46 mg kg⁻¹; n=6) in a dose-dependent fashion reduced (P<0.05) CYP-induced abdominal mechanical allodynia by 38, 58 and 66 %, respectively compared to IC/BPS untreated group (100%). The treatment of IC/BPS mice with H₂S-releasing anti-inflammatory compound ATB-352 (4.6, 15 or 46 mg kg⁻¹) also prevented carbachol- or KCl-mediated reduced contractile in isolated bladder. Both the CBS and CSE protein expression were decreased in IC/BPS bladder, but 3-MST expression was increased. Neither protein expression was affected by treatment with ATB-352 (15 mg kg⁻¹) nor significant changes of H₂S production were observed between IC/BPS and control groups. **Conclusion:** ATB-352 alleviates bladder pain/referred allodynia and improved bladder contractile function elicited by CYP-induced IC/BPS, indicating a potential therapeutic benefit for it. **References:** [1] Masaki *et al.* (2020) *Nitric Oxide* 1(104-105): 44-50. [2] Costa *et al.* (2020) *Antioxid Redox Signal* 33(14): 1003-1009. **Acknowledgments:** Fapesp (2017/15175-1), CAPES (Finance Code 001; 88887.621476/2021-00) and CNPq

(312514/2019-0). **License number of ethics committee:** ETHICS COMMITTEE ON ANIMAL USE (CEUA-ICB/USP, Protocol no. 2055050819)

07.009 **Are muscarinic receptors and EGF involved in sperm activity?** Gontijo LS¹, Moreira TJ¹, Corrêa-Ramos TL¹, Ribas JAS¹, Porto CS², Maróstica, E¹. ¹Lab of Experimental Pharmacology, UFF-Niterói, RJ; ²Experimental Endocrinology, Unifesp-SP, Brazil

Introduction: The expression of muscarinic receptor subtypes (mAChRs) was shown in efferent ducts and epididymis (M₁-M₃). AChE positive nerve fibers with free endings to the lumen were detected in the epididymis cauda (Avellar et al, J Mol Neurosci 40: 127,2010), suggesting the release of ACh into the epididymal fluid and its interaction with mAChRs on the spermatozoa (sptz). EGF receptors (EGFR) is also express in mature sperm of mammals and they can be transactivated by GPCR (Etkovitz et al, *Dev Biol*, 334: 447,2009). Thus, the aim of this study was characterized mAChRs in rat sptz and their possible correlation with EGFR transactivation in sperm function. **Methods:** (CEUA/UFF: 1026/18) Male Wistar rats (120-days old) were anesthetized with ketamine/xylazine (80/10mg.Kg⁻¹,i.p.) and the epididymis were removed and dissected. The spermatozoa (sptz) were obtained from caput and/or cauda of epididymis for sperm evaluation and western blotting (WB) assays or separated in Percoll gradient for immunofluorescence (IF) assays. IF studies were performed with polyclonal goat anti-mAChR primary antibody (M₁-M₅) and rabbit anti-goat IgG, conjugated with Alexa Fluor 594. Blocking peptide was used for the negative control. For *In Vivo* sperm evaluation, the rats were treated (i.v.) and sptz were isolated from epididymis cauda. For *in vitro* evaluation, sptz were incubated with 10⁻⁵M carbachol (CA) or 10⁻⁴M bethanechol (BE) in the absence and presence of 10⁻⁵M atropine (AT) and progressive motility, vigor, membrane integrity and functionality were evaluated. WB assays were performed with primary mouse anti-EGFR and anti-pEGFR antibodies and secondary anti-mouse IgG conjugated with peroxidase. Values were expressed as mean±SEM; ANOVA,Newman-Keuls, P<0.05. **Results:** Immature sperm showed the presence of M₁ subtype in the head and flagellum, M₂ in a punctate pattern that outlines the head region, and M₃ in the acrosome, centriole and flagellum. Mature sperm showed M₁ subtype only in the flagellum, M₂ mainly in the head and M₃ concentrated in a punctiform pattern only in the centriole region. In the sperm evaluation, no statistical differences were found among different treatments, *In Vivo* or *in vitro*, when compared to the control group, in any parameter analyzed. WB assays for EGFR showed the expression of these receptors in both immature and mature gametes, with greater expression in the latter. Our preliminary results also showed that in immature sptz, treatment with BE, increased the p-EGFR(Tyr845) expression, effect abolished in the presence of AT. On the other hand, mAChR activation by BE seems to decrease the expression of p-EGFR (Tyr1068, extracellular via) in these sptz. In mature sperm, the results were not conclusive. **Conclusion:** Our studies showed the presence of mAChR subtypes M₁, M₂ and M₃ in rat sperm, but not M₄ and M₅. The mAChR subtypes are redistributed in the gamete during its transit through the epididymis, suggesting their involvement in the maturation. These mAChRs are not directly involved with gamete membrane motility or functionality but preliminary data from WB assays suggest that these receptors can transactivate EGFR intracellular pathways and inhibit EGFR activation via extracellular pathway, at least in immature sperm. **Financial Support:** CNPq, CAPES, PROPI/UFF. **License number of ethics committee:** CEUA/UFF: 1026/18

07.010 **Intrauterine and lactational exposure of male rats to supraphysiological doses of manganese: short-term reproductive and development toxicity.** Silva APG¹, Correia MH², da Silva LN¹, Santiago MSA¹, Perobelli JE¹. ¹Dpt de Ciências do Mar, PPG Interdisciplinar em Ciências da Saúde ¹⁻²Unifesp, Brasil

Introduction: Manganese (Mn) is an essential micronutrient for the development of the animal organism, but in supraphysiological levels it can be harmful. A previous study showed that adult male rats exposed to 5 or 15 mg of Mn for 30 days present changes in their reproductive system indicating toxicity of Mn on Sertoli cells, whose development begins in intrauterine life and ends in the lactation period. Despite the relevance of the fetal/perinatal period for reproductive toxicology studies with Mn, there are still no data with animals exposed to Mn during this period of life regarding their reproductive development. In this context, the present study aims to assess the risks of exposure to supraphysiological levels of Mn during intrauterine and neonatal life on the reproductive parameters of male rats, focusing on the function of Sertoli cells in 15-day-old animals, when Sertoli cell proliferation ceases. **Methods:** To achieve these objectives, a first experimental stage was to perform the "Acute Toxicity Test", to define the effective doses of Mn to be used in the present study, following the recommendations of the OECD(guideline 423, GUIDELINE FOR TESTING OF CHEMICALS-Acute Oral). The LD50 for manganese chloride under these experimental conditions was 900mg/kg. From this, the experimental doses were defined, being "high dose" (1/10 of the LD50=90mg/kg) and "low dose" (1/100 of the LD50=9mg/kg). Subsequently, the 2^o experimental stage was developed, in which pregnant rats on the gestational day 13 (GD 13) (when the

population of sertoli cells in fetuses begins), were allocated into 3 experimental groups that received the treatment from DG13 to the 15th day of lactation, orally by gavage: G1-Control: received only the vehicle, i.e. distilled water (n=6); G2: Received MnCl₂ at a dose of 9mg/Kg (n=8); G3: Received MnCl₂ at a dose of 90mg/Kg (n=9). On postnatal 15, one or two male puppies of each litter (G1, n=8; G2, n=9; G3, n=8) were euthanized to assess reproductive parameters in the late infantile period. **Results:** The histopathological evaluation of the testes showed an increase in the occurrence of vacuoles and acidophilic cells in G3, indicative of increased apoptosis rates. This result was associated with an increase in the intensity of immunostaining for 8OHdG exclusively in the germ cells of the groups treated with Mn, which demonstrates oxidative damage to the genetic material of these cells. Immunostaining analysis for connexin 43, indicative of Sertoli cell integrity, revealed a decrease in staining intensity in the groups exposed to Mn. There were no histopathological changes in the epididymal tissue. **Conclusion:** So far, we can conclude that exposure to high doses of Mn during the embryonic and lactational period can cause immediate damage to the testis, that includes possible oxidative damage to the genetic material of germ cells and Sertoli cells, culminating in increased cell death rates and observation of vacuoles in the seminiferous epithelium, without apparent epididymal alterations. Long-term effects are under investigation to better understand the impact of manganese on male reproductive development and function. **Financial Support::** FAPESP/ Ethics Committee approval: 6104311018 **License number of ethics committee:** 6104311018

07.011 *Spirulina platensis* reverses damage to erectile function *in vivo* and oxidative stress in obese rats fed a hypercaloric diet. Diniz AFA¹, Souza ILL³, Ferreira ES¹, Ferreira PB¹, Barros BC¹, Lacerda-Júnior FF¹, Silva MCC¹, Silva BA^{1,2} ¹UFPB-PPgPNSB, ²DCF-CCS-UFPB, ³UERR-DCM

Introduction: The incidence of obesity has increased rapidly in recent years. Several pathophysiological complications have been associated with abnormal and excessive accumulation of body fat, representing a potential risk to human health. Among obesity-induced complications, erectile dysfunction (ED) is the most common disorder that affects about 40% of men (Kwaifa, Biomolecules, v. 10, p. 291, 2020.). Recently, it was shown that dietary supplementation with *Spirulina platensis*, an alga rich in proteins and antioxidants, restores damage to erectile function in rats fed a hypercaloric diet (Diniz, Oxidative Medicine and Cellular Longevity, v.2020, p. 1, 2020). Thus, we aimed to evaluate whether *S. platensis* supplementation also reverses the damage to erectile function *in vivo*, as well as the oxidative stress parameters, triggered by the consumption of a hypercaloric diet. **Methods:** Wistar rats (8 weeks of age) were divided into rats that received standard diet (SD), hypercaloric diet (HCD) or hypercaloric diet + orally supplementation with *S. platensis* powder at 25, 50 or 100 mg/kg (HCD25, HCD50 and HCD100, respectively). Animals received different diets for 16 weeks. The erectile function *In Vivo* was monitored and the parameters of total antioxidant capacity (CAT) and malonaldehyde (MDA) were analyzed. Results were expressed as mean and standard deviation of the mean and analyzed by one-way ANOVA followed by the Tukey post-test (n=5). **Results:** In the HCD group (0.3 ± 0.2) it was observed that the number of penile erections was lower than in the SD group (2.0 ± 0.4). Interestingly, when rats were supplemented with *S. platensis* at a dose of 50 mg/kg (HCD + SP50 1.8 ± 0.5) they showed an increase in the number of penile erections when compared to the HCD group (0.3 ± 0.2). The latency to obtain penile erection in the HCD group (26.7 ± 2.2 min) was higher than in the SD group (8.0 ± 1.0 min). In rats fed the hypercaloric diet and supplemented with seaweed, it was observed that this parameter was reduced and consequently reversed by supplementation at doses of 25 (12.8 ± 2.6 min), 50 (15.3 ± 3.3 min) and 100 mg/kg of the alga (13.4 ± 1.0 min), when compared to the HCD group. Regarding MDA levels, the HCD group (0.9 ± 0.40 µmol/L) showed an increase in concentration compared to the SD group (0.5 ± 0.05 µmol/L). However, the supplementation of rats with *S. platensis* at doses of 25 (0.5 ± 0.1 µmol/L), 50 (0.3 ± 0.02 µmol/L) and 100 mg/kg (0.4 ± 0.02 µmol/L) reduced MDA levels both in relation to the HCD group and also to the SD group. The total antioxidant capacity of the corpus cavernosum was increased in rats fed a hypercaloric diet and supplemented with *S. platensis* at a dose of 50 mg/kg (91.0 ± 0.8%) compared to the HCD group. **Conclusion:** Food supplementation with *Spirulina platensis* reverses the damage caused to erectile function *In Vivo* and on oxidative stress in the corpus cavernosum of Wistar rats fed a hypercaloric diet. Financial support: CNPq, CAPES, PPgPNSB/UFPB. Research approval: Ethical Committee on Animal Use/UFPB (0201/14). **License number of ethics committee:** Research approval: Ethical Committee on Animal Use/UFPB (0201/14).

07.012 **NADPH oxidase but not uncoupled eNOS is the source of ros in murine experimental cystitis.** Oliveira MG¹, Mónica FZ¹, Passos GR¹, Victorio JA², Davel AP², Silva FH³, Antunes E¹ ¹Dpt of Translation Medicine (Pharmacology Area), Faculty of Medical Sciences, Univ of Campinas. ²Dpt of Structural and Functional Biology, Inst of Biology, Univ of Campinas. ³Hematology and Hemotherapy Center, Univ of Campinas

Interstitial Cystitis/Bladder Pain Syndrome (IC/BPS) is a multifactorial inflammatory disease characterized by suprapubic pain, urgency and excessive urinary frequency, which profoundly impairs patient's quality of life (BJU Int 2018, 122(5): 729-743). Among mediators implicated in cystitis, the overproduction of reactive oxygen species (ROS) seems to play a key role (Am J Physiol Renal Physiol 2016, 311(1): F85-93); however, no studies have elucidated the source(s) of ROS and therapies targeting selective ROS generators are demanded. NADPH oxidases isoforms (NOX) generate ROS that are critical in regulating a variety of cellular functions in many physiopathological conditions. Moreover, endothelial nitric oxide synthase (eNOS) activity may become "uncoupled" in inflamed tissues, driving to ROS rather than nitric oxide (NO) production, contributing to tissue damage (Antioxid Redox Signal 2015, 23: 1171-1185). This study aimed to investigate the contribution of NOX and eNOS in ROS generation and voiding dysfunction of cyclophosphamide (CYP)-induced mouse cystitis. Ten-week-old female C57BL/6 (wild-type) and eNOS^{-/-} knockout mice were treated with a single injection of CYP (300 mg/kg, ip). Time course from 0 to 24 h (considering 0 h as control) were carried out to evaluate the voiding behavior and to collect bladder tissue. RT-PCR for eNOS, NOX2 and NOX4, as well as western blotting for eNOS expression and dimerization were determined in bladder lysates. Superoxide dismutase (SOD) activity and levels of NO and superoxide anion (O₂⁻) were determined by histochemistry in frozen bladder sections using DAF and DHE, respectively. Additionally, DHE was carried out in the presence/absence of L-NAME (NOS inhibitor; 1 mM), GSK2795039 (selective NOX2 inhibitor; 25 μ M) and GKT 137831 (NOX1/4 inhibitor; 50 μ M) in bladder sections. Data represents mean \pm SEM, unpaired t-test. All animal procedures were previously approved by CEUA/UNICAMP 4882-1. CYP exposure caused progressive increases over time in bladder weight (0.9 \pm 0.02 and 1.9 \pm 0.22 mg, for 0 and 24 h, respectively) and voiding frequency (3.7 \pm 0.5 and 54.6 \pm 4.0 number/min for 0 and 24 h, respectively) consistent with bladder overactivity. The mRNA expressions of eNOS (1.3 \pm 0.1 and 1.5 \pm 0.1 a.u for 0 and 24 h, respectively) and NOX2 (1.0 \pm 0.1 and 1.6 \pm 0.16 a.u for 0 and 24 h, respectively) were significant increased in bladder tissues, but significant reductions of NOX4 (1.05 \pm 0.1 and 0.14 \pm 0.1 a.u for 0 and 24 h, respectively) were observed from 3 to 24 h after CYP (p<0.05 vs 0h). The eNOS protein expression was significantly increased from 3 to 12 h after CYP (p<0.05), but no significant differences were observed in the heterodimer/monomer ratio for all times evaluated. Additionally, NO and O₂⁻ productions increased from 6 to 24h after CYP (p<0.05), whereas SOD decreased from 6 to 24 h (p<0.05). CYP injection in eNOS^{-/-} mice did not further increased O₂⁻ levels in comparison to WT animals, thus excluding eNOS as the O₂⁻ major source. On the other hand, the NOX2 and NOX4 inhibitors fully prevented the increased O₂⁻ levels, suggesting these NADPH oxidases act as potential O₂⁻ sources. In conclusion, our data indicate that NOX2 and NOX1/4, rather than eNOS, act as major source of the excessive ROS in CYP-induced mouse cystitis. Thus, NOX selective inhibition may be promising targets for future therapies for IC/BPS. Financial support: FAPESP 2018/09765-3; 2017/15175-1. License number of ethics committee: CEUA/UNICAMP 4882-1

08. Respiratory and Gastrointestinal Pharmacology

08.001 Intestinal health assessment of patients diagnosed with celiac disease. Marques CRS¹, Couto PEA¹, Costa LATJ², Santos, AT³; Aragao, KS¹ ¹Centro Universitário Estácio de Ceará ²Univ de Fortaleza ³Univ Federal do Ceará

Introduction: Celiac disease (CD) is an autoimmune enteropathy characterized by gluten intolerance. It is considered an underreported public health problem that causes serious damage to the health of the celiac, which can cause villous atrophy, inflammation and damage to the intestinal lining, directly interfering with the absorption of essential nutrients to the body. The main clinical evidence of this pathology is chronic diarrhea, tiredness, bloating, flatulence, vomiting, weight loss, muscle weakness and loose stools. The only treatment available to date for CD is a permanent and definitive gluten-free diet. Therefore, the objective of the present study was to evaluate intestinal health through social, economic and food analysis, as well as such as its association with the level of adherence to the gluten-free diet in patients diagnosed with Celiac Disease. **Methods:** The present study was cross-sectional, quantitative and descriptive, using the Google Forms platform, through an electronic address in the period from October 2020 to March 2021. A sample calculator was used for health research, with a margin of error of 5% and 95% confidence level and 10% of the population, in a total of 57 patients. Descriptive analyzes were performed, with categorical variables expressed as simple frequency and percentages, and numerical as mean and standard deviation. Statistical analyzes were performed using Pearson's chi-square and Fisher's exact tests, with p <0.05 being considered significant. **Ethical Committee:** The present study was approved by Human Research Ethical Committee (3.581.929). **Results:** The frequencies of gastrointestinal symptoms were assessed in the last 30 days and in the 48 hours preceding the completion of this questionnaire. In the last 48 hours, the association was statistically significant between the percentage of adherence to

the gluten-free diet and the symptoms of bloating / bloating and stomach / intestinal pain, in which individuals with a percentage of adherence to bed equal to or less than 75% had higher prevalence of these symptoms ($p < 0.05$) when compared to individuals with 100% adherence to diet. It was found that the total of 61.4% of patients had adequate intestinal health according to the Bristol scale. This result corroborated with the nutritional follow-up rate of these patients (59.6%). Another important factor was the difficulty in accessing gluten-free foods reported by 80.7% of patients. **Conclusion:** These results could be reversed with nutritional monitoring and strategies to improve the intestinal health of these patients. **Financial support:** authors' own support. **Keywords:** Celiac. Gluten. Intestine. Microbiota. Immunity. **Acknowledgments:** We would like to acknowledge the Ceara Association of Celiacs to support on their site. **References:** 1. MUNIZ, et al., *Arq. Gastroenterologia*, v.53, n.4, p 267-272, Dezembro de 2016. 2. GALGLIARDIA, et al., *Rebuilding the Gut Microbiota Ecosystem*. *Int Environ Res Public Health*. 2018;15(8). pii: E 1679. **License number of ethics committee:** 3.581.929

08.002 Histamine H4R antagonist (LINS01007) reduces development of colon inflammation, in DSS-induced colitis in mice. Lippi BK¹, Balbino AM¹, Fernandes GAB¹, Landgraf MA², Fernandes JPS¹, Landgraf RG¹. ¹Dpt of Pharmaceutical Sciences, Univ Federal de São Paulo, Campus Diadema, São Paulo, Brazil; ²Univ Paulista, Campus Rangel, Santos, São Paulo, Brazil

Introduction: Ulcerative colitis is a chronic inflammatory bowel disease affecting any aspect of the colon, and its incidence is increasing worldwide. The most common manifestations of intestinal inflammation are abdominal pain, bloody diarrhea, weight loss, reduced appetite, anorexia, and fever. In this study, we evaluated the involvement of the histamine H4 receptor in a mice model of ulcerative colitis using the new compounds from the 5-Substituted 1-[(2,3-dihydro-1-benzofuran-2-yl)methyl]piperazines (LINS01 series), specifically the LINS01007 molecule. **Methods:** Experimental acute colitis was induced in male BALB/c mice by administering 3% DSS in the drinking water for six days. Histamine antagonist H4R LINS01007 (5 mg/kg) were administered i.p. during the 6 days of colitis induction. To assess the development of the disease, the weight of the animals, as well as the consumption of water and feed were monitored throughout the treatment. The presence of fecal blood was also evaluated. Seven days after the start of the protocol, the animals were euthanized and the colon removed for histological analysis. **Results:** It was not observed changes in water consumption, in experimental group. Both feed intake and animal weight were significantly reduced 4 days after starting the experimental protocol, and these findings were completely reversed with LINS01007 treatment. Presence of blood in the stool was detected after the sixth day of treatment and LINS01007 administration did not reduced this parameter. Histological analysis showed edema and presence of granulocytes in the colon of experimental group, and these findings were reduced in animals treated with LINS01007. **Conclusion:** In a DSS-induced colitis model, blockade of H4R by LINS01007 resulted in a reduction of disease development, in addition to diminish edema and granulocyte infiltration in the colon of experimental group. **Financial Support:** FAPESP (2019/05242-9, 2020/16020-4, 2020/16258-0), CNPq (306631/2018-0) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001 **License number of ethics committee:** CEUA Nº 7601230317

08.003 Mechanism of action of tracheal hyper-reactivity in a model of asthma exacerbated by obesity. Martins AMO¹, Ferreira SRD², Araújo MPA¹, Figueiredo IAD², Pessoa RF², Cavalcante FA^{2,3}, Vasconcelos LHC^{2,3} ¹UFPB, ²UFPB - PPgPNSB, ³UFPB - DFP P

Introduction: Obesity is known to be a major risk factor and disease modifier of asthma in adults (ASTRUP, *Lancet*, v. 374, p. 1606, 2009). To better understand the physiopathology of those diseases associated, various animal models have been used to emulate like condition in humans. Dietary changes to induce obesity and exposure to allergen ovalbumin (OVA) is one of such models (HUR, *Pulm. Pharmacol. Ther.*, v. 67, 2021). Thus, the aim of this study was characterized the mechanism of action involved in the increased tracheal contractility caused by associated diseases. **Methods:** Male Wistar rats ($n = 5$) that were divided into: control (CG), asthmatic (AG), obese (OG) and obese asthmatic (OAG) groups. For inducing obesity, the animals of OG and OAG received high glycemic level index (HGLI) diet for 16 weeks (Adapted of LUZ, *Biosci rep*, v. 38, p. 1, 2018). For asthma induction, of the last 22 days of the 16 weeks, the animals were submitted to sensitization (i.p., on days 1-3) and challenges with OVA (on days 6, 9, 12, 15, 18, 21). On 22nd they were euthanized, had the trachea removed, cut in rings and suspended in organ bath and isometric contractions were evaluated (Adapted by GALVÃO, *J. Inflamm. Res*, v. 66, p. 1117, 2017). All experimental protocols were approved by Ethical Committee on Animal Use of UFPB (1162100918). All results were expressed as mean \pm standard error of the mean (S.E.M.) and statistically analyzed by analysis of variance followed by Tukey's post-test using GraphPad Prism® software. **Results:** In the evaluation of the nitric oxide (NO) pathway, it was observed that, in the presence of L-NAME, only

in the OG there was a reduction in contractile efficacy of carbachol (CCh) ($E_{\max} = 80.5 \pm 3.2$), with no change on its potency, compared to CG, AG and OAG ($E_{\max} = 94.3 \pm 2.0$; 97.9 ± 2.1 and $89.6 \pm 5.9\%$ to CG, GO, GA and GOA, respectively). To investigate a cyclooxygenase and oxidative stress pathway, the organs that were pre-incubated with indometacin, a cyclooxygenase inhibitor, or apocynin, an NADPH oxidase inhibitor, and only AG showed decrease in the contractile efficacy, with no change on its potency when compared with the other groups ($E_{\max} = 94.5 \pm 3.2$; 85.9 ± 3.2 ; 74.2 ± 3.3 ; $87.2 \pm 1.9\%$, respectively) and apocynin ($E_{\max} = 93.3 \pm 5.9$; 99.8 ± 0.2 ; 73.0 ± 5.6 ; 99.3 ± 3.8 , respectively). In the presence of the tempol, a mimetic of superoxide dismutase, SOD, there was no change in the contractile response in any group. The same result was found to zileutona, a lipoxygenase inhibitor. For ROCK pathway investigation, was observed that only the OAG had decreased inhibitory effect caused by Y-27632, a blocker of ROCK, showing increase in the contractile efficacy ($E_{\max} = 17.9 \pm 3.6$; 23.5 ± 2.9 ; 18.3 ± 4.8 ; $30.9 \pm 4.7\%$), but without changing potency. **Conclusions:** The mechanism involved on obesity is probably the increase on the NO production. On the other hand, the asthma seems to be modulated by increase of the synthesis of contractile prostanoids, and main mechanism involved in obesity-induced asthma severity seems to be the upregulation of the ROCK pathway. **Financial Support:** PIBIC, CNPq, PPgPNSB/CCS/UFPB. **License number of ethics committee:** 1162100918

08.004 **Carvacrol attenuates cigarette smoke-induced acute lung injury.** Holanda-Pereira, AK¹, Santos, CA¹, Pereira-Gonçalves, A², Oliveira-Melo, P³, Felix, RGS¹, Moura, MJN¹, Borges, CS¹, Silva, GM¹, Lima, CC², Kennedy-Feitosa, E¹ ¹Univ Federal Rural do Semi-Árido, Mossoró, Brazil; ²Inst Superior de Ciências Biomédicas, Univ Estadual do Ceará, Fortaleza, Brazil; ³Inst do Coração, Univ de São Paulo (USP), São Paulo, Brazil

Introduction: Cigarette smoke is able to induce acute lung injury (ALI). This condition is characterized by tissue damage and increase of inflammatory markers. Carvacrol is a monoterpene present in the essential oil of some plants and has been used to treat respiratory disease because of anti-inflammatory and antioxidant activity. **Aim:** Investigate the pharmacological activity of carvacrol (CAR) in the acute lung injury induced by cigarette smoke in mice. **Methods:** CEUA (23091.007638/2018-95). Mice C57BL/6, male, (25-28g) were divided into two groups: control and cigarette smoke (CS). The CS group were exposed to 12 cigarettes per day for 5 days. The control group was exposed to sham smoking. The CS group was treated with CAR (1, 3 or 10 mg/mL) or vehicle by inhalation (15 min/daily) for 5 days. After 5 days bronchoalveolar lavage (BAL), trachea and lungs were collected for inflammatory profile, functional and morphological analysis. Significant difference when $p < 0.05$. **Results:** The number of inflammatory cells increased in the BAL of the CS group when compared to control one (0.02 ± 0.01 vs 0.34 ± 0.03 ; $p < 0.001$). The treatment with CAR 1, 3 and 10 mg/mL reduced this number (0.23 ± 0.02 , $p = 0.02$; 0.18 ± 0.01 , $p = 0.002$ and 0.010 ± 0.01 , $p = 0.001$, respectively). The treatment with CAR 10 mg/mL reduced the airway hyperresponsiveness in tracheal segments in the electro and pharmacomechanical coupling ($p < 0.05$). The pulmonary parenchyma analysis showed an increase of the epithelium damage ($76,3 \pm 2,26$ vs $112 \pm 3,62$ control group; $p < 0.001$), lung inflammation (3.5 ± 0.17 vs 1.9 ± 0.21 control group; $p < 0.001$) and bronchoconstriction index (4.66 ± 0.2 vs 2.99 ± 0.18 control group; $p < 0,05$). CAR recovery epithelium damage with 1 mg/mL ($91,9 \pm 2,12$ $p < 0.01$) and 10 mg/mL ($102,3 \pm 2,51$ $p < 0.0001$). The lung inflammation was reduced by all the doses (1 mg/mL $2,25 \pm 0,09$; 3 mg/mL $2,40 \pm 0,11$ e 10 mg/mL $2,59 \pm 0,08$; $p < 0.001$). The bronchoconstriction index was reduced in 1 mg/mL ($3,79 \pm 0,17$; $p < 0.05$) and 10 mg/mL ($3,85 \pm 0,16$; $p < 0.05$). Alveolar septal volume density (VvSept) and mean linear intercept (Lm) did not show any difference. **Conclusion:** Carvacrol has anti-inflammatory and bronchodilator activity without change in the lung parenchyma in the lung injury induced by cigarette smoke. **License number of ethics committee:** 23091.007638/2018-95

08.005 **Anti-inflammatory activity of eugenol in acute lung inflammation induced by cigarette smoke in mice.** Barbosa MCO¹, Araújo BVS¹, Holanda-Pereira AK¹, Santos CCA¹, Oliveira-Melo P², Silva AGG¹, Borges CS¹, Silva GM¹, Kennedy-Feitosa E¹ ¹Univ Federal Rural do Semi-Árido, Mossoró, Brazil; ²Inst do Coração, Univ de São Paulo, São Paulo, Brazil **Introduction:** Cigarette smoke can induce the lung acute injury with inflammatory cells and lung parenchyma damage. Eugenol (EUG) is a component of clove oil, with anti-inflammatory and antioxidant activities. **Aim:** To evaluate the pharmacological activity of the EUG in cigarette smoke-induced acute lung injury. **Methods:** (CEUA: 23091.007638/2018-95). C57BL/6 mice, male were exposed to 12 cigarettes per day for 5 days (CS group). The control group was exposed to sham smoking. The CS group was treated with EUG (100 mg/mL) or vehicle by inhalation (15 min/daily) for 5 days. The anti-inflammatory markers and lung morphology were evaluated. **Results:** The leucocytes number from mice BAL increased 3x on the CS group compared to control and the treatment with EUG (100 mg/mL) reduced 46% when compared to the CS group ($p < 0.05$). The number of macrophages (142%)

and neutrophils (3x) increased in the CS group. EUG (100mg/mL) reduced in 60% ($p < 0.001$) and 63% ($p < 0.001$), respectively. The lung inflammation score showed the increase of 100% of inflammation in the CS group ($p < 0.001$). The treatment with EUG 100 mg/mL reduced this score by 30% ($p < 0.01$). The bronchoconstriction index was increased in the CS group (65%; $p < 0.01$) and reduced in 29% ($p < 0.05$) by treatment with EUG 100 mg/mL. **Conclusion:** Eugenol has an anti-inflammatory effect to attenuates the inflammatory response induced by cigarette smoke. **License number of ethics committee:** 23091.007638/2018-95

08.006 Consumption of *Spirulina platensis* prevents deleterious effects on the contractile reactivity of the ileum of Wistar rats fed a hypercaloric diet. Francelino DMC¹, Diniz AFA², Souza PPS¹, Claudino BFO¹, Duvirgens MV¹, Lacerda-Júnior FF², Barros BC², Ferreira PB², Silva BA^{1,2} ¹DCF-CCS-UFPB, ²PPgPNSB-CCS-UFPB

Introduction: The consumption of hypercalorics diets is associated with the development of obesity in humans and animals, which is characterized by a chronic inflammatory condition, representing the main axis in the genesis of other diseases, such as those that affect the intestinal smooth muscle, such as diarrhea and constipation (SHIN et al., BMJ Open Gastroenterology, v. 6, 2019). Recently, *Spirulina platensis* (SP), a blue-green alga, has been shown to have anti-inflammatory and antioxidant effects, in addition to preventing intestinal changes in Wistar rats fed a high-calorie diet (CARVALHO et al., ENIC, 2018). Thus, the objective is to evaluate the mechanism of action by which SP prevented changes in contractile reactivity in rat ileum. **Methods:** Wistar rats (170-190 g) were randomly divided into 4 experimental groups, (1): rats fed standard diet (SD), (2): fed SD and supplemented with Spirulina at a dose of 25 mg/kg (SD+SP25); (3): fed with a hypercaloric diet (HCD), (4): fed with HCD and supplemented simultaneously with 25 mg/kg of SP (HCD + SP25). All diets and supplementation were offered for a period of 8 weeks, after which the animals were euthanized and the ileum removed to carry out the experiments. The results were expressed as mean \pm standard error of the mean, being statistically analyzed by the *t* test or one-way ANOVA followed by Tukey's post-test ($p < 0.05$, $n=5$). **Results:** In the HCD group, the cumulative concentration-response curves to carbachol (CCh) showed a decrease in contractile efficacy ($E_{max} = 31.4 \pm 6.4\%$) when compared to the SD group ($E_{max}=100\%$), with no change in relative potency. in the presence of atropine (10^{-6} and 10^{-5} M), a non-selective antagonist of muscarinic receptors, a shift of the curve to the right was observed, with a reduction in potency and maintenance of E_{max} respectively ($pEC_{50} = 3.5 \pm 0.2$ and 2.3 ± 0.01). However, the HCD + SP25 group ($E_{max} = 63.87 \pm 1.0 \%$) showed increased efficacy and contractile potency when compared to the HCD group ($E_{max} = 31.4 \pm 6.4$ and $pEC_{50} = 6.6 \pm 0.01$). **Conclusion:** Given the results obtained and previous studies, it is concluded that food supplementation with SP prevents the deleterious effects promoted by the consumption of a high-calorie diet on the contractile reactivity of the ileum, possibly to the downregulation of muscarinic receptors. Support: CAPES, CNPq, PPgPNSB/UFPB. **License number of ethics committee:** Research approval: CEUA/UFPB - 6061090318.

08.007 Food supplementation with *Spirulina platensis* prevents obesity and deleterious effects on ileal histomorphometry induced by hypercaloric diet. Claudino BFO¹, Diniz AFA², Duvirgens MV¹, Souza PPS¹, Francelino DMC¹, Ferreira PB², Lacerda-Júnior FF², Alves AF³, Silva BA^{2,4} ¹CCS-UFPB, ²PPgPNSB-CCS-UFPB, ³DFP-CCS-UFPB, ⁴DCF-CCS-UFPB

Introduction: Classified as a chronic and inflammatory disease, obesity is responsible for causing several damages to human and animal health, including gastrointestinal disorders, some types of cancer and metabolic syndrome (WHO, 2020). Thus, it is extremely important to search for new therapeutic alternatives, especially of natural origin, which have preventive effects in combating these disorders. In this context, *Spirulina platensis* (SP), an alga with antioxidant and anti-obesity effects, emerges as a potential alternative for the prevention of diseases associated with the gastrointestinal tract induced by the accumulation of adipose tissue ZEWEIL et al., 2016; FINAMORE et al., 2017). Thus, the objective of this work is to evaluate the effects of hypercaloric diet on parameters of experimental obesity and ileal histomorphometry of Wistar rats, as well as to elucidate the possible preventive effect of SP supplementation on such alterations. **Methods:** Wistar rats (170-190 g) were divided into 3 experimental groups, fed standard diet (SD), hypercaloric diet (DHC) and/or fed hypercaloric diet and supplemented simultaneously with 25 mg/kg of SP (DHC+ SP25). SP in the form of lyophilized powder was dissolved in saline (NaCl 0.9%) administered by gavage for 8 weeks. The results were expressed as mean \pm standard error of the mean and analyzed by one-way ANOVA (Tukey's post-test) ($p < 0.05$, $n=5$). **Results:** The hypercaloric diet increased all murinometric and ileal histomorphometric parameters analyzed in this study, confirming the induction of obesity in animals. Interestingly, supplementation with SP at a dose of 25 mg/kg was responsible for preventing most changes in the obesity parameters, such as final body mass, naso-anal length, abdominal circumference, thoracic circumference, adiposity index and masses of adipose tissue deposits

(retroperitoneal, inguinal and epididymal) showing the anti-obesity effect of the alga. In addition, the alga also prevented the deleterious effects of the diet on histomorphometric parameters such as villi length, villi width and muscle layer. Such effects are justified and are related to the nutritional and antioxidant composition of SP. Conclusions: Given what was evidenced in this research, it can be concluded that SP emerges as an important and potential promising source in the prevention of obesity and its comorbidities, especially those associated with the intestinal tract, becoming a future drug candidate with preventive potential-therapeutic. Research approval: CEUA/UFPB (6061090318). Financial support: CNPq, CAPES, PROEX. Acknowledgments: CAPES, CNPq, PROEX, UFPB. **License number of ethics committee:** CEUA/UFPB: 6061090318

08.008 A comparative approach to evaluate *in vitro* contractile and relaxing responses of the gastrointestinal smooth muscle of mice. Oliveira SM, da Silva-Santos JE. UFSC Florianópolis, PPG Pharmacology, Brazil

Introduction: In the gastrointestinal tract (GIT), the contractile and relaxing properties are regulated by various sympathetic, parasympathetic, enteric, and hormonal mediators. Nevertheless, the magnitude of effects of each mediator on the muscle tone of GIT remains unclear. Therefore, we hypothesized that the responses to these mediators show differences dependent on the portion of the GIT. **Methods:** Male Swiss mice (3-4 months) were anesthetized (ketamine/xylazine, 100/20 mg/kg, i.p.) and euthanized to remove their GIT. The tissues were washed in physiological saline solution (PSS) and placed in Petry dishes to clean and isolate strips of the gastric body and segments (~ 2 cm length) of jejunum, ileum, and colon. The preparations were mounted in organ baths containing PSS (37 °C), under a basal tone of 0.5 g, and continuously bubbled with an aerator. The tissues were allowed to rest for 30 min before the addition of any drug. Then, we evaluated the spontaneous motility (frequency and force generated by the slow waves) and the contractile responses to a modified PSS containing 120 mM KCl. After washing and a new stabilization period, different preparations were subjected to: i) cumulative (gastric body) or non-cumulative concentrations of acetylcholine (ACh; 10-300 mM), or; ii) cumulative concentration-response curves (1-300 mM) of dopamine (DA), norepinephrine (NOR), or isoprenaline (ISO), constructed under ACh (30 mM)-stimulated preparations. The results (n =6/group) were presented as the mean ± standard error of the mean of contraction in grams or as the percentage of relaxation. The data were analyzed with one- or two-way ANOVA followed by Tukey's test. This study was authorized by our institutional Ethics Committee on Animal Use (5366250520). **Results:** Compared with intestinal preparations, the gastric body strips presented reduced spontaneous and KCl- and ACh-stimulated contractility. However, the gastric preparations were more sensitive than the ileum for DA-, NOR-, and ISO-induced relaxation. For instance, the maximal relaxation induced by DA was 72.6 ± 9.7 and 48.3 ± 5.2 in the stomach and ileum preparations, respectively. Among intestinal preparations, the colon was the one with the lowest motility and spontaneous tone. However, the maximal contractile responses to ACh in the colon were ~60% and 160% higher than in the ileum and jejunum, respectively. Additionally, the maximal relaxation induced by DA was higher in the colon ($110.2 \pm 4.0\%$) than in the ileum (48.3 ± 5.2) or jejunum ($76.9 \pm 5.4\%$). Moreover, despite the similar maximal effects, the EC₅₀ for NOR and ISO were lower in the colon. For instance, the EC₅₀ (mean and 95% confidence intervals) obtained for ISO were 1 (0.7-1.4), 7 (5-10), and 3 (2-5) nM in the colon, ileum, and jejunum, respectively. **Conclusion:** All portions of the GIT showed differences in responses to the agonists used. Interestingly, contractile responses mediated by muscarinic acetylcholine receptors increased from the jejunum to the colon, and we found the opposite order of sensitivity for ISO-induced relaxation. Understanding this differential profile of responses is essential in the search for pharmacological targets. **Acknowledgments:** CAPES and CNPq for fellowship and financial support. **License number of ethics committee:** CEUA/UFSC nº 5366250520

08.009 Acid exposure impairs the duodenal mucosal integrity - A translational study. Sousa MKA¹, Saraiva LGM², Borges IC³, Costa Filho HB¹, Sales TMAL², Freire PRP¹, Ribeiro TA⁴, Souza MC⁴, Lederhos QR⁴, Araújo GAC³, Paula SM², Souza MHL². ¹Dpt of Pharmacology, Federal Univ of Ceará. ²Dpt of Medical Sciences, Federal Univ of Ceará. ³Federal Univ of Ceará. ⁴Estácio Univ Center of Ceará

Introduction: Dyspeptic symptoms, such as postprandial fullness, early satiety, epigastric pain, and burning, affects up to 16% of otherwise healthy individuals in the general population. Functional dyspepsia is a the most common digestive disorder related to the gastroduodenal region, which still has no obvious organic cause. The pathophysiology of functional dyspepsia remains incompletely understood. Recently, studies using duodenal biopsies from dyspeptic patients have shown that there is impairment in the duodenal barrier function in addition to the presence of underlying inflammatory mechanisms. However, the role of acid exposure in impairment the duodenal functional barrier is still poorly understood. In this sense, the functional assessment of acid exposure in the duodenal mucosa can provide evidence for a

better understanding of the pathophysiology of dyspeptic symptoms. **Objective:** Assess the integrity of the functional barrier of the duodenal mucosa of healthy individuals and healthy mice to acid (HCL) exposure. **Methods:** A group of 8 individuals without complaints of dyspeptic symptoms or chronic-inflammatory disease were selected by Roma III symptom questionnaire validated for dyspepsia. Patients underwent upper digestive endoscopy at the endoscopy service of Walter Cantídio University Hospital, Federal University of Ceará, and during the procedure, duodenal biopsies were collected (Ethic committee No. 3009133). Duodenum of male Swiss mice (n=8) were also dissected (Ethic committee No. 14444050421). Both human and mouse duodenal biopsies were mounted in an Üssing chamber to record the transepithelial electrical resistance (TEER) and assess the integrity of the functional barrier. During the experiments, Krebs solution pH 7.4, considered control, and Krebs solutions pH 2, pH 1.75, pH 1.5, pH 1.25 and pH 1 were used to evaluate the effect of acid exposure, under the same experimental conditions. **Results:** Duodenal biopsies from healthy individuals had lower mean basal TEER when compared to mouse duodenum TEER (human duodenum: 25.87 ± 2.23 vs. animal duodenum: $44.74 \pm 2.29 \Omega/\text{cm}^2$, $p < 0.05$). After exposure to acidic saline solutions, there was a significant drop in the TEER of duodenal biopsies when adding a pH 1 solution when compared to a pH 7.4 solution (pH 7.4: 5.5 ± 4.1 vs. pH 2: 7.3 ± 2.1 vs. pH 1.75: 2.3 ± 14.5 vs. pH 1.5: 28.2 ± 14.6 vs. pH 1.25: 7.3 ± 7.7 vs. pH 1: $78.5 \pm 7.7\%$, $p < 0.05$). When the duodenum of healthy animals was evaluated, a percentage drop in transepithelial resistance was observed at pH 1.5, pH 1.25 and pH 1 when compared to pH 7.4 (pH 7.4: 6.5 ± 2.2 vs. pH 2: $23.7 \pm 8, 6$ vs. pH 1.75: 36.6 ± 10.0 vs. pH 1.5: 46.1 ± 6.5 vs. pH 1.25: 59.8 ± 8.2 vs. pH 1: $75.8 \pm 3.7 \%$, $p < 0.05$). **Conclusion:** Acid exposure impairs the integrity of the both duodenal mucosa of human and mouse. Furthermore, the duodenal mucosa of mouse, despite having higher basal TEER, was weaker to acid exposure compared to human. **Financial Support:** CAPES, FUNCAP and CNPQ. **License number of ethics committee:** Duodenal biopsies - Ethic committee No. 3009133 / Duodenum of male Swiss mice Ethic committee No. 14444050421.

08.010 **Diet-Induced Nonalcoholic Steatohepatitis (Nash) animal model for preclinical pathogenesis and therapy research.** Araujo BP¹, Pereira ENGDS¹, Martins CSM¹, Silvaes RR¹, Rodrigues KL¹, Flores EEI¹ and Daliry A¹. ¹Lab of Cardiovascular Investigation, Oswaldo Cruz Inst, Oswaldo Cruz Foundation, Rio de Janeiro, Brazil

Introduction: Non-alcoholic steatohepatitis (NASH) is one of the diseases in the spectrum of non-alcoholic fatty liver disease (NAFLD). NASH is characterised by hepatocellular injury with lobular inflammation and presence or absence of fibrosis. NASH is a serious disease associated with the development of cirrhosis and the need for liver transplantation. Despite the significant prevalence and adverse consequences of NAFLD, there are no approved drugs to treat this condition. Furthermore, there are few animal models that fully replicate the spectrum of NAFLD phenotypes in human disease and are suitable for pathogenesis studies and predicting the efficacy of therapies in humans. **Methods:** C57BL/6 control animals (CTL) received a standard diet and water ad libitum, whereas the diet-induced NASH model was induced by a hyperlipid (high fat) and hypercarbohydrate (HFHC) diet consisting of 55% kcal fat or by the same diet plus 2% cholesterol (HFHC+COL) added during the last 12 weeks of the experimental protocol. Both diets involved the addition of 250 g/L fructose in the drinking water and the induction lasted 43 weeks. All procedures were approved by the Oswaldo Cruz Foundation Animal Welfare Committee (license L-012/2018 A1). At the end of the experimental protocol, weight and fasting blood glucose (FBG) were evaluated. All fat deposits, kidneys, heart and liver were dissected and weighed. Liver histopathology was performed. **Results:** Water and food consumption was higher in CTL compared to HFHC or HFHC+COL. The weights of body, liver, heart and all deposits of adipose tissue were increased in the HFHC and HFHC+COL compared to CTL. HFHC had impaired glucose metabolism when compared to CTL, which was not observed in HFHC+COL animal group. HFHC and HFHC+COL had an increase in serum cholesterol and hepatic triglyceride when compared to CTL. HFHC+COL had LDL, HDL, ALT, AST and hepatic cholesterol levels significantly increased compared to CTL and HFHC. Serum triglycerides levels were not altered among the analyzed groups. Histological analysis showed that CTL had no steatosis or had a mild microvesicular steatosis, while HFHC had moderate and severe steatosis and HFHC+COL had severe steatosis. HFHC and HFHC+COL showed a significant increase in global hepatic steatosis and inflammatory infiltrates compared to CTL, with significant increased values for HFHC+COL group. HFHC and HFHC+COL had non-alcoholic fatty liver disease activity score (NAS) of 5 and 8, respectively, being classified as NASH. **Conclusion:** We conclude that HFHC and HFHC+COL showed pathophysiological changes consistent with NASH and could be a suitable model for the study of pharmacological and non-pharmacological therapies for the NAFLD spectrum. This study was supported by CNPq, FAPERJ AND FIOCRUZ. **License number of ethics committee:** L-012/2018 A1

08.011 **Relaxation of airway smooth muscle induced by classical phosphodiesterase (PDE) inhibitors involves inhibition of ecto-PDE.** Satori NA¹, Pacini ESA¹, Jackson EK², Godinho RO¹. ¹Division of Cellular Pharmacology, Dpt of Pharmacology, Escola Paulista de Medicina, Univ Federal de São Paulo (EPM/Unifesp), São Paulo, Brazil ²Dpt of Pharmacology and Chemical Biology, Univ of Pittsburgh School of Medicine, Pittsburgh, PA, USA

Introduction: β_2 -adrenoreceptor agonists and phosphodiesterase (PDE) inhibitors are effective bronchodilators drugs used for asthma treatment, due to their ability to increase intracellular 3',5'-cyclic AMP (cAMP) levels and induce airway smooth muscle (ASM) relaxation. Interestingly, we have shown that increases in intracellular cAMP levels in ASM is followed by cAMP efflux, leading to an increase in extracellular cAMP levels and constriction of ASM (Pacini et al., J. Pharmacol. Exp. Ther. 366; 75, 2018). Assuming that in many tissues extracellular cAMP is converted to adenosine by ectoenzymes (ecto-PDEs and ecto-nucleotidases), we evaluated whether classical inhibitors of intracellular PDEs 3-isobutyl-1-methylxanthine (IBMX) and aminophylline could also inhibit ecto-PDEs, and as consequence affect the contracting effects of extracellular cAMP in the ASM. **Methods:** Isolated tracheal segments from adult male rats (Wistar, 3–4-month-old; 250-350 g) were pre-contracted with carbachol in order to measure the relaxing effects of these PDE inhibitors using an isometric force transducer. In another set of experiments, isolated tracheal segments were incubated for 60 min with exogenous cAMP (30 μ M) in the presence of IBMX (non-selective PDE inhibitor), and the concentrations of extracellular 5'-AMP, adenosine and inosine were measured using ultraperformance liquid chromatography–tandem mass spectrometry (UPLC-MS/MS). All values were expressed as mean \pm S.E.M. **Results:** The PDE inhibitors induced a concentration-dependent relaxation of tracheal segments (pEC_{50} : IBMX = 5.5 ± 0.1 ; aminophylline = 4.0 ± 0.1 ; n= 5-6). Pretreatment of tracheas with 20 μ M CGS-15943, a nonselective adenosine receptor antagonist, did not change the relaxation curve of PDE inhibitors but shifted to the left the relaxation curve of the β_2 -adrenoreceptor agonist salbutamol (pEC_{50} = 6.9 ± 0.1 versus pEC_{50} = 7.4 ± 0.1). Preincubation of tracheal segments with 1 mM IBMX reduced the extracellular conversion of cAMP to 5'-AMP by 42% (17.15 ± 1.30 versus 9.92 ± 1.42 ng/mL), to adenosine by 42% and to inosine by 57% (n=6, p < 0.05). **Conclusion:** These results indicate that inhibitors of intracellular PDEs could also be acting as ecto-PDE inhibitors, thus preventing extracellular degradation of cAMP to the contracting metabolite adenosine. **License number of ethics committee:** CEUA #1021240519 and #9987150714

08.012 **The treatment with virgin coconut oil (*Cocos nucifera* L.) improves the murinometric parameters and tracheal reactivity of obese-asthmatic Wistar rats**

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Introduction: Virgin coconut oil (VCO) obtained from the pulp of fresh mature coconut (*Cocos nucifera* L.) has already shown pharmacological activities such as improvement of obesity parameters in both animal models (ZICKER, J Nutr Biochem, v. 63, p. 117, 2019), human (LIAU, ISRN Pharm., v. 2011, p. 1, 2011), in addition to reversing tracheal hyperresponsiveness in asthmatic guinea pigs (VASCONCELOS, LHC, Oxid Med Cell Longev, v. 2020, p. 1, 2020). Thus, the aim of this study was to evaluate whether treatment with VCO would reverse the changes caused by asthma/obesity association in Wistar rats, in both murinometric parameters and tracheal hyperresponsiveness. **Methods:** Wistar rats (n = 5-6) were randomly divided into control group (CG), fed with a standard diet and not sensitized; obese asthmatic group (OAG), fed with a high glycemic index diet (HGLI) and sensitized with ovalbumin (OVA); and obese asthmatic group supplemented with 1000 or 2000 mg/kg/day of VCO p.o (OAVCOG1000 or 2000) during the last 30 days of disease induction. All experimental protocols were approved by the Ethical Committee on Animal Use of UFPB (9133040520/ID-001135). All results were expressed as mean \pm standard error of the mean (S.E.M.) and statistically analyzed by Student's *t*-test or one-way ANOVA, followed by Tukey's post-test using GraphPad Prism[®] software version 5.01. **Results:** Treatments with VCO (1000 and 2000 mg/kg) prevented the increase in blood glucose caused by ingestion of HGLI after 16 weeks (95.3 ± 3.3 mg/dL and 104.2 ± 6.2 mg/dL, respectively) compared to CG (92.0 ± 5.1 mg/dL). Otherwise, the treatment with VCO did not modify final body mass of animals, the average weekly food consumption, nasoanal length, body mass, Lee index and chest circumference after 16 weeks of feeding with HGLI compared to the CG. Moreover, treatment with VCO 2000 mg/kg decreased waist circumference (20.1 ± 0.4 cm) compared to OAG (21.4 ± 0.1 cm); however, did not completely reverse compared to CG (18.8 ± 0.3 cm). Visceral adipose tissue deposits, mainly epididymal and retroperitoneal fat, did not decrease with VCO 1000 and 2000 mg/kg. Differently, treatment with VCO 2000 mg/kg reversed the inguinal fat accumulation (6.6 ± 0.6 g) caused by HGLI (9.5 ± 1.5 g) compared to CG (3.1 ± 0.2 g). Interestingly, treatment with VCO 1000 and 2000 mg/kg decreased the adiposity index (5.9 ± 0.3 and $5.8 \pm 0.1\%$ respectively), compared to OAG ($7.6 \pm 0, 5\%$), but it did not completely prevent since had not difference of CG (3.9

$\pm 0.2\%$). Furthermore, treatment with VCO 1000 mg/kg did not prevented tracheal hyperresponsiveness to carbachol (CCh) ($E_{\max} = 157.4 \pm 7.3\%$; $EC_{50} = 9.8 \pm 0.7 \times 10^{-7}$ compared to OAG ($E_{\max} = 150.1 \pm 6.0\%$; $EC_{50} = 2.3 \pm 1.2 \times 10^{-6}$ M) and to CG ($E_{\max} = 100\%$; $EC_{50} = 9.7 \pm 0.8 \times 10^{-7}$ M), while treatment with VCO 2000 mg/kg completely prevented tracheal hyperresponsiveness to CCh ($E_{\max} = 86.1 \pm 4.5\%$; $EC_{50} = 7.9 \pm 0.9 \times 10^{-7}$ M) compared to CG ($E_{\max} = 100\%$; $EC_{50} = 9.7 \pm 0.8 \times 10^{-7}$ M). **Conclusions:** Treatment with VCO 1000 and 2000 mg/kg/day was able to prevent the effects of obesity induced by HGLI, such as fasting glucose, waist circumference and adiposity index. The most effective dose was 2000 mg/kg/day, that improved contractile reactivity, in a perspective of VCO as a promising candidate for treating obesity/asthma association. **Financial Support:** CNPq, PIBIC, PROPESQ/UFPB, PPgPNSB/CCS/UFPB. **License number of ethics committee:** Ethical Committee on Animal Use of UFPB (9133040520/ID-001135).

08.013 Role of the Pepsin in the Pulmonary Inflammatory Dysfunction induced by gastroesophageal reflux in mice. Sales TMAL¹, Sousa MKA², Costa-Filho HB², Gadelha KKL², Dias-Júnior GJ², Paula SM¹, Ribeiro TA³, Lederhos QR³, Magalhães PJC², Soares PMG², Sifrim D⁴, Souza MHL¹. ¹UFC, Fortaleza, Dpt of Medicine, PPG Medical Sciences, Brazil; ²UFC, Fortaleza, Dpt of physiology and pharmacology, PPG pharmacology, Brazil; ³Estacio Univ Center of Ceará, Fortaleza, Brazil; ⁴Queen Mary Univ of London, London, United Kingdom

Introduction: The relationship between gastroesophageal reflux and airways disorders has long been postulated. Pepsin represents a potential biomarker for reflux disease when detected in airways, however a direct role for pepsin in lung dysfunction has not been clearly established. In the present study, we aimed to evaluate the effect of pepsin inhibitor on the pulmonary dysfunction induced by experimental gastroesophageal reflux. **Methods:** The surgical model consists of a pyloric substenosis and complete ligation of the gastric fundus in the Swiss mice (30-35 g). First, a time course was performed to define the day with the highest pulmonary damage. The sham (false operated) group was the control. After the 7, 21 or 28 days of the surgery, the animals were sacrificed and the lungs were removed for measurement of MPO activity, GSH or MDA concentrations. In addition, lung function was assessed by spirometry. In another experimental group, after surgery the animal were treated daily with pepstatin (0.3 mg / kg, gavage, a pepsin inhibitor) or saline solution (control). At the end of 28 days, the animals were sacrificed, and the same parameters were evaluated. **Results:** After 28 days post-surgery, there were highest ($p < 0.05$) pulmonary inflammation (MPO= 16.3 ± 3.0 U/mg), pro- oxidant state ($p < 0.05$) (GSH = 126.1 ± 3.9 pg/mg, MDA= 35.0 ± 10.2 pg/mg) and pulmonary dysfunction ($p < 0.05$) (expiratory flow= 0.012 ± 0.0007 ml/sec; current/min= 123.0 ± 0.9 ml/min) compared with the sham (MPO= 2.2 ± 0.3 U/mg, GSH = 180.3 ± 31.3 pg/mg, MDA = 12.3 ± 0.9 pg/mg, expiratory flow= 0.020 ± 0.0006 ml/s; current/min= 134.5 ± 2.9 ml/min). Pepsin inhibitor (pepstatin) reversed the inflammation ($p < 0.05$) (MPO= 6.8 ± 0.5 U/mg), pro- oxidant state ($p < 0.05$) (GSH= 237.3 ± 23.5 pg/mg MDA= 17.2 ± 1.4 pg/mg) and pulmonary dysfunction ($p < 0.05$) (expiratory flow= 0.016 ± 0.0005 ml/sec, current/min= 131.3 ± 1.5 ml/min) associated with the surgical reflux model. **Conclusion:** The experimental reflux in mice induced pulmonary inflammation and pro-oxidant state associated with lung dysfunction. The inhibition of pepsin activity was able to protect the lung against the reflux induced these alterations. **Financial support:** CNPq, CAPES **License number of ethics committee:** Animal Ethics Committee: 2346010321. Federal University of Ceará

08.014 Impair in mucosal integrity associated with colitis correlates with microscopic inflammatory damage: a translational study. Costa-Filho HB¹, Lopes AKM², Sales TMAL², Paula SM¹, Sousa MKA¹, Dias-Júnior GJ¹, Souza MC³, Silva LMG³, Araújo GAC², Soares PMG⁴, Barbosa ALR⁵, Souza MHL¹. ¹UFC Fortaleza, Dpt of Physiology and Pharmacology, Brazil; ²UFC Fortaleza, Dpt of Medicine, Brazil; ³Estácio Univ Center of Ceará, Fortaleza, Brazil; ⁴UFC Fortaleza, Dpt of Morphology, Brazil; ⁵UFPI Parnaíba, Dpt of Physiotherapy, Brazil

Background: The pathogenesis of inflammatory bowel disease (IBD) is a multifactorial process. In IBD, the defective mucosal barrier, with increased intestinal permeability, promotes intestinal inflammation. Experimental models of IBD have provided significant contributions not only to the pathogenic mechanism, but also to the development of new therapeutic strategies for IBD. Translational studies in IBD are not common.

Aim: To evaluate the correlation between colonic mucosal integrity and microscopic inflammatory damage both in the trinitrobenzenesulfonic acid (TNBS)-induced experimental colitis in rats and in ulcerative colitis patients. **Methods:** Colitis was induced in Wistar rats by intracolonic administration of 20 mg TNBS in 50% ethanol. The control group (Sham) received only saline solution. Human biopsies were obtained by colonoscopy from patients with active ulcerative colitis (UC) or from patients with normal colonoscopy (Control). From these samples, the transepithelial electrical resistance (TEER) was analyzed using the Ussing Chamber and the histopathological scores using the Riley criteria in humans and Appleyard and

Wallace in rats. The Ethics Committees of the Federal University of Ceará approved this study (128/2017 and 3245,468). **Results:** There was a reduction in TEER in the Colitis group (Sham: 35.6 ± 0.47 vs. Colitis: $31.9 \pm 0.6 \Omega/\text{cm}^2$, $p < 0.05$), in addition, an increase in histopathological scores was also observed, mainly regarding the loss of mucosal architecture and formation of crypt abscess. In human biopsies, a reduction in TEER was also observed in the ulcerative colitis group (Control: 43.2 ± 5.2 vs. UC: $22.6 \pm 2.1 \Omega / \text{cm}^2$, $p < 0.05$), in addition to higher histopathological scores, mainly in relation to the epithelium, surface integrity and irregularity in the architecture of the crypts. Based on these data, the main finding of the study was a negative correlation between TEER and histopathological damage, both in rats (Spearman's $r = -0.74$; $p < 0.05$) and in humans (Spearman's $r = -0.73$; $p < 0.05$). **Conclusion:** During colitis, both experimental and in patients, mucosal integrity was negatively correlated with microscopic inflammatory damage, demonstrating an important association between morphological and functional aspects in the pathogenesis of IBD. These results may be relevant to new goals for the IBD treatment. **Financial Support:** FUNCAP and CNPq. **License number of ethics committee:** Rats (128/2017); Humans (3245,468).

08.015 **Experimental gastroprotective potential of the dry hydroalcoholic extract from flowers of *Tagetes erecta* L., a useful medicinal plant in gastrointestinal diseases.** Silva TFQ¹, Meurer MC¹, Felisbino F¹, Muller FB¹, Somensi LB^{1,2}, Cury BJ¹, Jerônimo DT¹, Venzon L¹, França TC¹, Mariott M¹, Santos AC¹, Boeing T¹, Cruz AB¹, Souza P¹, Silva LM¹ ¹PPG in Pharmaceutical Sciences, Chemical Pharmaceutical Research Nucleus, Univ of Vale do Itajaí, Itajaí, Brazil ²PPG in Development and Society, Alto Vale do Rio do Peixe Univ, Caçador, Brazil

Introduction: *Tagetes erecta* L. (Asteraceae), known as Aztec marigold, is used in folk medicine to treat gastrointestinal diseases, but there is no study regarding its antiulcerogenic potential and therefore the anti-gastric ulcer effect of the dry extract of *T. erecta* flower (DETe) was studied in this work. **Methods:** The models of acute ulcer induced by acidified ethanol or indomethacin were reproduced in mice pretreated with DETe (3 - 300 mg/kg) and morphological and biochemical parameters were evaluated in the gastric mucosa. The anti-ulcer activity of DETe against acidified ethanol was also verified in mice pretreated with NEM, L-NAME, indomethacin, or yohimbine. The antisecretory effect of the extract was verified in rats and the anti-*Helicobacter pylori* activity was determined *in vitro*. **Results:** Oral administration of DETe only at the dose of 300 mg/kg reduced the ulcer area induced by ethanol and indomethacin by 49 and 93%, respectively, compared to the vehicle group. In parallel, the administration of DETe at 300 mg/kg increased GSH levels, and GST activity, and reduced LOOH levels, and SOD, and MPO activity, compared to the vehicle group. In addition, pre-treatment with L-NAME, NEM, yohimbine abolished the gastroprotective effect of the extract. The administration of DETe (300 mg/kg, id) did not change the volume, pH, acidity, or peptic activity in pylorus ligated rats, and DETe up to 2500 $\mu\text{g}/\text{L}$ had no antimicrobial effect against *H. pylori*. **Conclusion:** Therefore, confirming the popular use, the extract has an antiulcerogenic potential mediated by the reduction of oxidative stress and the involvement of nitric oxide, non-protein sulfhydryl groups, α_2 adrenergic receptors, and prostaglandins. This work was approved by CEUA under the number 055/18 and financial support: CAPES/CNPQ. **License number of ethics committee:** This work was approved by CEUA under the number 055/18

08.016 **Evaluation of the geraniol gastric healing mode in rodents.** Venzon L¹, Meurer MC¹, França TCS¹, Longo B¹, Mariott M¹, Somensi LB², Mariano LNB¹, Boeing T¹, Cazarin CA¹, Pereira LN¹, da Silva LM¹. ¹Univali Itajaí, Pharmaceutical Sciences Graduate Program, Brazil; ²Uniarp Caçador, PPG in Development and Society, Brazil

Introduction: Gastric ulcers are necrotizing lesions that affect the entire surface of the gastric mucosa. Current treatment consists of the use of proton pump inhibitors (PPIs) from the parietal cells of the stomach, such as omeprazole. However, prolonged use of these drugs is associated with adverse effects and inefficient gastric healing, which can promote the occurrence of lesions. Thus, the search for new therapeutic alternatives for the auxiliary treatment of gastric ulcers is increasing and necessary. This study evaluates the mode of action through which the monoterpene geraniol, administered orally or inhaled, accelerates the process of gastric healing. **Methods:** The gastric healing effect of geraniol was evaluated in the experimental model of chronic gastric ulcer induced by 80% acetic acid in female rats. The effects of geraniol on oxidative stress and inflammation during gastric healing were then evaluated. In parallel, histological and histochemical analysis of the ulcer site was performed to measure the outcome of geraniol on healing quality and mucin levels. In another experiment using male mice, the effect of geraniol in reducing the severity of recurrence of chronic gastric ulcer induced by 10% acetic acid with subsequent administration of Interleukin-1 β (IL-1 β) was evaluated. And also myeloperoxidase (MPO) activity, and the levels of some cytokines. Other tests were performed to assess the activity of geraniol in gastric emptying, intestinal transit, and evacuation index. Finally, behavioral tests were conducted to verify the influence of

treatment with the monoterpene on the locomotor action of ulcerated animals with 80% acetic acid, to determine the influence of the anxiolytic effect in the results obtained. All procedures were approved by the Committee on Ethics in Animal Use (CEUA) of the University of Vale do Itajaí (UNIVALI) with protocol numbers 036/16 and 053/17. **Results:** In summary, the data revealed that geraniol has healing potential when administered orally (30 mg/kg) and by inhalation (30 mg/mL), twice daily for seven days, both routes being mediated by increased mucin levels and favoring antioxidant defenses, including maintenance of reduced glutathione (GSH) levels and an increase in catalase (CAT) and glutathione s-transferase (GST) activity, as well as a reduction in MPO levels (indicating a reduction in neutrophilic migration at the ulcer site). Thus, regardless of the route of administration, or compound, it minimized the occurrence of ulcers through anti-inflammatory mechanisms, measured by the decrease in tumor necrosis factor (TNF) and IL-6 levels, and also by the maintenance of mucin levels. However, administration of geraniol (3, 10, and 30 mg/kg, vo) did not change gastric emptying, neither did it demonstrate any antidiarrheal property. On the other hand, it decreased intestinal transit at reduced doses (3 and 10 mg/kg, vo). The results of the behavioral effects also indicate that inhaling the compound may also exert an anxiolytic effect. **Conclusion:** This study clarifies some of the mechanisms that act both in gastric healing and in preventing the reappearance of the lesion from treatment with geraniol. In addition, they have also observed some other factors that may be associated with the treatment with the compound, which demonstrated that it, despite being an oil, does not have side effects to the gastrointestinal tract, and its calming effect, as previously described by other authors, may have some involvement in the healing process of gastric ulcers, since the anxiety and stress are risk factors for the emergence of these situations. However, more studies are needed to elucidate this hypothesis. **Acknowledgments:** This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001. We also thank the support received from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Universidade do Vale do Itajaí (UNIVALI). **License number of ethics committee:** CEUA 036/16 and 053/17

08.017 Experimental model of obesity-induced exacerbated asthma: an analysis of *in vivo* and *in vitro* dysfunctions on airways of Wistar rats. Ferreira SRD¹, Pessoa RF¹, Figueiredo IAD¹, Martins AMO², Araújo MPA², Alves JLB³, Alves AF⁴, Vasconcelos LHC⁴, Cavalcante, FA^{1,4}. ¹UFPB PPgPNSB, João Pessoa, Brazil; ²UFPB PIBIC/CNPq, João Pessoa, Brazil; ³UFPB Dpt of Nutrition and PPgCN, João Pessoa, Brazil; ⁴UFPB - Dpt of Physiology and Pathology, Brazil

Introduction: The obesity-induced exacerbated asthma phenotype is characterized by aggravation of symptoms, lack of asthma control and different responses to usual treatment (PETERS, J. Allergy Clin. Immunol, v. 141, p.1169, 2018), however underlying mechanisms of obesity influence on asthma severity are still uncertain, being necessary more studies in this field. Obesity model by high glycemic index (HGLI) diet previously standardized induced murinometric alterations (FERREIRA, Annals SBFTE, 2019). Thus, this study aimed to standardize an obesity-induced exacerbated asthma model by HGLI diet and ovalbumin (OVA) in Wistar rats. **Methods:** Wistar male rats (n = 5-6) were randomly divided into groups: control (CG), obese (OG), asthmatic (AG) and obese asthmatic (OAG). For inducing obesity, the animals were fed with a HGLI (Adapted of LUZ, Biosci Rep, v. 38, p. 1, 2018) for 16 weeks and to induce asthma they were sensitized and challenged with ovalbumin (OVA) (Adapted of GALVÃO, J. Inflamm Res, v. 18, p. 48, 2017). *In Vivo* and *in vitro* evaluations were performed. All experimental protocols were approved by Ethical Committee on Animal Use of UFPB (1162100918). All results were expressed as mean ± standard error of the mean (S.E.M.) and statistically analyzed by analysis of variance one-way followed by Tukey's post-test using GraphPad Prism[®] 5.01 software. **Results:** after the 16 weeks of obesity and asthma induction there was an increase in final body mass of OAG compared to CG and AG, despite the average weekly food intake was not increased into groups. Fasting blood glucose was increased only in OAG after 16 weeks. Furthermore, abdominal circumferences, body mass index, retroperitoneal, epididymal, and inguinal adipose tissues and adiposity index were increased in OAG compared to CG and AG. Respiratory function was assessed using a spirometer during the course of asthma implantation (days 0, 11 and 21). There was no differences in the tidal volume between groups; however, on 11th day respiratory frequency was reduced in OAG (96.8 ± 10.9 resp/min), compared to CG (144.8 ± 7.8 resp/min). Likewise, on 21st day the tidal volume of OG (105.4 ± 1.1 mL/kg) and AG (103.9 ± 4.9 mL/kg) was reduced, compared to CG (143.8 ± 10.8 mL/kg). Moreover, AG and OAG had reduced 54 and 46%, respectively, its minute-volume (627.0 ± 133.8 and 646.0 ± 47.0 mL/kg/min, respectively) on 21st day, compared to CG (1162.0 ± 112.9 mL/kg/min). Contractile and relaxant reactivity was evaluated in the trachea. The cumulative concentration-response curves for carbachol (CCh) did not show any change in contractile potency between the groups; however, there was an increase in contractile efficacy in OG ($E_{max} = 133.2 \pm 10.4\%$), AG ($E_{max} = 126.1 \pm 2.4\%$) and OAG ($E_{max} = 152.6 \pm 5.5\%$) compared to CG ($E_{max} = 100\%$), being OAG showed greater contractile efficacy also compared to AG. Otherwise, aminophylline was equipotent and

maintained the same relaxant efficacy. Ultimately, histological sections of lungs stained with hematoxylin-eosin and Masson's trichrome showed an increased peribronchovascular inflammation area on OAG compared to diseases alone, as well as smooth muscle hypertrophy and remodeling area filled by extracellular matrix, compared to CG. **Conclusions:** These results allow concluding that obesity-induced exacerbated asthma model was successfully established, characterized by functional and morphologic respiratory changes. This methodology allows future molecular investigations and the search for new therapeutic alternatives for the treatment of this condition. **Financial Support:** CNPq, PIBIC/UFPB, PPgPNSB/CCS/UFPB. **License number of ethics committee:** 1162100918

08.018 Controlled release of JME-173 from nanocapsules improves lipopolysaccharide-induced lung inflammation in mice. Coutinho DS¹, Bernardi A¹, Guterres SS², Pohlmann AR³, Silva PMR¹, Martins MA¹
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Prior studies have shown that local anaesthetics, such as lidocaine and mexiletine, can be beneficial for asthma patients. However, the anaesthesia of the airways results in the blockade of protective neuronal bronchodilator reflexes, limiting their application as anti-asthmatic agents. Our group has planned and synthesized a structural mexiletine analogue, JME-173, marked by reduced anaesthetic effect and improved anti-inflammatory and spasmolytic activity as compared to the prototype. Nevertheless, JME-173 bioavailability following oral administration has been shown to be very poor. Nanotechnology enables the drug vectorization, which favors the increase in the bioavailability of the administered compound and could be an alternative for the low oral bioavailability of JME-173. The aim of this study was the development and physical-chemical characterization of nanocapsules containing JME-173 and the evaluation of its potential pharmacological effect on lipopolysaccharide (LPS)-induced mice lung inflammation. The nanocapsule formulations were prepared by interfacial deposition of preformed biodegradable polymers method. The analysis indicated that formulations of Eudragit® S-100 polymer containing JME-173 presented an average diameter of 120-180 nm, zeta potential of -39.9 mV and a low polydispersity index. The average content of JME-173 in formulation was 95%, with 97% encapsulation efficiency. Concerning the pharmacological evaluation of nanoformulations, male A / J mice were treated 4 h before LPS instillation, with free or nanoencapsulated JME-173 (8-25 mg/kg, orally), and 18 h after LPS exposure the analysis were performed (CEUA license number - LO30/15). Results showed that treatment with nanocapsules containing JME-173, but not with free JME-173 or with unloaded nanocapsules vehicle, reduced leukocyte influx in bronchoalveolar lavage, myeloperoxidase activity, and levels of KC, IL-1 β , MCP-1 and RANTES in the lung of mice challenged with LPS. Treatment with JME-173-loaded nanocapsules also abolished LPS-induced airway hyperreactivity, as attested by increased lung elastance response, whereas both unloaded nanocapsules and free JME-173 were clearly inactive. In conclusion, these results suggest that the biodegradable formulation of the Eudragit® S-100 polymer containing JME-173 can improve the anti-inflammatory effect of this compound following oral administration in mice. These are new insights into the putative applicability of non-anesthetic mexiletine derivatives in drug discovery for lung respiratory diseases. **License number of ethics committee:** (CEUA license number - LO30/15

09. Natural Products and Toxinology

09.001 Short and Long-term effects of *Salacia impressifolia* on management of hyperglycemia for glucose homeostasis. Furtado IP¹, Da Luz G², Altenhofen D², Ruani AP³, Pizzolatti MG³, Silva FRMB², Frederico MJS¹. ¹UFC Fortaleza, Dpt de Fisiologia e Farmacologia, Brasil; ²UFSC Florianópolis, Dpt de Bioquímica, Brasil; ³UFSC Florianópolis, Dpt de Química, Brasil

Introduction: The aim of the present study was to investigate the *In Vivo* and *in vitro* effect of bark of *Salacia impressifolia* on glucose tolerance test (GTT), insulin secretion, advanced glycation end-products (AGE) and intestinal disaccharidase activity. In addition, a phytochemical characterization of crude extract and fractions was investigated. **Methods:** Fasting male *Wistar* rats (48-50 days) (16h) were treated with CE (25, 50 and 100 CF (12.5, 25 and 50 mg/kg, i.p), and/or glipizide (control, 10 mg/kg, i.p) in GTT. After 30 min of treatment, they received a glucose load (4 g/kg, i.p) and blood was collected at time 0, 15, 30, 60 and 180 min for serum glucose and insulin measurements. The serum insulin was measured by enzyme-linked immunosorbent assay (ELISA). Chromatogram (HPLC) of the aqueous fraction from *S. impressifolia* barks were performed. The intestine homogenates were preincubated at 37°C for 5 min, in the absence (control) or in the presence of different fractions of *S. impressifolia* barks (treated). Sucrase (EC 3.2.1.48), and maltase (EC 3.2.1.20) activities were determined using a glucose diagnosis kit based on the glucose oxidase reagent. AGE measurements were formed in the *in vitro* system using BSA (10 mg/mL)

in phosphate buffered saline (PBS, pH 7.4) incubated with glucose (500 mM) or fructose (100 mM) at 37 °C for 0, 7, 14 and 28 days. AGE formation it was measured the characteristic fluorescence (Protocol CEUA-UFSC PP00398). **Results:** The triterpenes α -amyrin, β -amyrin, melaleucic acid and the flavan-3-ol (-)-epicatechin were isolated and characterized from *S. impressifolia* barks. HPLC analyses showed epicatechin and catechin in the WF. Oral administration of glipizide, CE, WF and CF significantly improved the glucose tolerance in hyperglycemic rats. Both fractions, WF (25 mg/Kg) and CF (12.5 mg/Kg), induced serum insulin secretion at 30 min after oral treatment. In addition, these fractions significantly inhibited the intestinal sucrase and maltase activities. The *in vitro* formation of advanced glycation end-products was significantly reduced by WF, EF, CF and/or CE in a long-term treatment. **Conclusion:** *S. impressifolia* barks has important antihyperglycemic and insulin secretagogue effect. The WF and CF, with the highest triterpenes content, showed an inhibitory *in vitro* effect on sucrase and maltase activities. Additionally, CE and fractions exhibited an inhibitory effect on AGE formation, resulting in short and long-term influence on the prevention of protein glycation. Taking these data in account, *S. impressifolia* presents short- and long-term biological effect contributing for glucose homeostasis, which may ameliorate significantly the diabetes mellitus status. **Financial Support:** CNPq, CAPES-PPGBQA/UFSC. **License number of ethics committee:** CEUA-UFSC PP00398

09.002 Amazonian guaraná and açai: conjugated extract improves the healing of *Eisenia fetida* submitted to tail-amputation. Bonotto NCA¹, Felin FD¹, Maia-Ribeiro EA², Barbisan F³, Felin CD⁴, Teixeira CF¹, Roggia I¹, Turra BO¹, da Cruz IBM¹.¹UFSC Santa Maria, Dpt of Morphology, Brazil;² FUnATI Manaus, Brazil; ³UFSC Santa Maria, Dpt of Patology, Brazil; ⁴UFN Santa Maria, Dpt of Medicina, Brazil

Introduction: Previous studies have suggested that guarana (*Paullinia cupana*) and açai (*Euterpe oleracea*) have antioxidant, anti-inflammatory, and proliferative properties, indicating their potential therapeutic action on wound healing. In order to test this hypothesis, we produced a conjugated guarana-açai (GA) extract, whose healing action was tested on earthworms (*Eisenia fetida*) submitted to tail amputation by surgical incision. **Methods:** A hot-water extract obtained from roasted guarana seeds and fresh açai seeds berries was chemically characterized by high-resolution mass spectrometry (ESI-ToF-MS). The antioxidant and genoprotective capacity of GA-extract was tested by DPPH and Picogreen DNA assays at concentrations of 0, 1, 3, 5, 10, and 30 μ g/mL. The concentration with the most remarkable healing potential was used in the other tests. The last three posterior segments of the clitellate-earthworm tail reared under standardized conditions were surgically amputated with a scalpel. Next, a topical PBS or GA-extract application (2 μ l) was performed on the surgical wound. After that, the rate of cell migration and tissue regeneration in the wound local was histologically evaluated in 1, 3, 6, 12, and 24 h after the procedure using Masson-Goldner staining. The expression of the SOX-4 gene that acts on epithelial-to-mesenchymal transition was determined by qt-PCR assay. Statistical tests: repeated-measured analysis of variance or Student t-test. **Results:** 16 bioactive molecules, including some no previously described substances. All concentrations tested showed antioxidant and genoprotective capacity. A concentration of 5 μ g/ml was used in the tail surgical procedure. The GA- extract accelerated the healing processes observed through macroscopic and histological analysis and increased the expression of the SOX-4 gene. **Conclusion:** The set of results indicates that the GA extract has a potential role in the healing of surgically produced wounds. **License number of ethics committee:** N/A

09.003 Methyl cinnamate suppress migration and induce cell cycle arrest at S phase in NIH 3T3 fibroblasts. Barros A, Ferreira E, Aquino F, Silva J, Carmo J, Barreto E UFAL, Lab of Cell Biology

Introduction: Methyl cinnamate (MC), a natural derivative of cinnamic acid, have been shown to have anti-inflammatory and antioxidant potential. However, the pharmacological effects of MC on fibroblasts are still poorly understood. In present study, we evaluated the pharmacological effect of MC in 3T3 fibroblast cells. **Methods:** 3T3 fibroblast cells were cultured in DMEM medium supplemented with 10% fetal bovine serum, 2 mM L-glutamine, and 0.02% penicillin/streptomycin, and maintained at 37°C with 5% CO₂ in a humidified atmosphere. Cells were exposed at 0.1, 1, 3, 10 and 30 μ M MC during 24 hours. Cell viability was evaluated using the MTT assay, while cell migration was measured using the scratch wound healing assay. Fibroblasts were cultured until confluent, and then a linear scratch wound was created and treated with MC. Two representative areas of the scratch in each culture were photographed and the scratch area was quantitated using image analysis software (ImageJ). Propidium iodide staining and flow cytometry analyses were used to evaluate cell-cycle distribution. Statistical significance between groups was determined by ANOVA followed by Tukey's test ($p < 0.05$). **Results:** We demonstrate that MC had no cytotoxic effect on fibroblasts at concentration ranges from 0.1 to 30 μ M at 24 h. According to the scratch assay, the treatment with MC at 10 μ M significantly inhibited (47%) the migratory capacity of fibroblasts compared to untreated cells. Cell cycle analysis showed that exposure to MC at 10 μ M did not affected the

proportion of cells in the G2/M phases, but decreased the proportion of cells in the G0/G1 and increased the proportion of cells in the S phase. **Conclusion:** Taken together, these results suggest that MC may be a potent regulator of fibroblast function by inhibited the cell migration and induced cell cycle arrest without any cytotoxic effects. **Financial Support:** CNPq **License number of ethics committee:** N/A

09.004 **Methyl cinnamate inhibits migration and inflammatory response in A549 human epithelial cells.** Ferreira E, Barros A, Nascimento L, Silva J, Carmo J, Barreto E. UFAL, Lab of Cell Biology

Introduction: Methyl cinnamate (MC) has aroused interest by its antioxidant and tyrosinase inhibitor activities. However, the bioactivity of methyl cinnamate on epithelial cell functions in both the cell migration and inflammatory response remain poorly understood. Thus, the present study sought to assess A549 human alveolar epithelial cells responses to MC treatment. **Methods:** A549 cells were cultured in DMEM medium supplemented with 10% fetal bovine serum, 2 mM L-glutamine, and 0.02% penicillin/streptomycin, and maintained at 37°C with 5% CO₂ in a humidified atmosphere. Cells were exposed at 0.1, 1, 3, 10 and 30 µM methyl cinnamate during 24 hours. Next, cell viability was determined using MTT assay, while cell migration was investigated using the scratch assay. Epithelial cells were cultured until confluent. Then a linear scratch wound was created and treated with MC. Two representative areas of the scratch in each culture were photographed and the scratch area was quantitated using image analysis software (ImageJ). The effects of MC on secretion IL-1b and IL-6 in TNF-α-stimulated A549 cells were determined by ELISA assays. Statistical significance between groups was determined by ANOVA followed by Tukey's test (p<0.05). **Results:** We noted through the MTT assay that all concentrations tested of MC had no cytotoxic effects. Compared to untreated cells, treatment with 10 and 30 µM MC significantly decreased A549 cells migration in 28% and 32%, respectively. The treatment with 10 and 30 µM MC in TNF-α-stimulated A549 cells inhibited the release of proinflammatory cytokines IL-1β (in 40% and 30%) and IL-6 (in 43% and 51%), which play important roles in triggering the inflammatory response. **Conclusion:** These results indicated that MC may be a potent regulator of cell migration and suppressor the pro-inflammatory abilities of A549 cells. **Financial Support:** CNPq **License number of ethics committee:** N/A

09.005 **Blockade of calcium channels in the vasorelaxant effect of (E)-N-(4-metoxifenetil)-3-(tiofen-2-il)acrilamid in Wistar rats.** Moura TMC¹, Silva ARLFC², Pessoa RF², Fernandes JM¹, Silva LAA³, Rodrigues LC⁴, Cavalcante FA^{2,5} ¹PIBIC-UFPB, João Pessoa, Brazil; ²PPgPNSB-UFPB João Pessoa, Brazil; ³PPgDITM-UFPB João Pessoa, Brazil, ⁴UFPB João Pessoa, Dpt of Biotechnology, Brazil, ⁵UFPB João Pessoa, Dpt of Physiology and Pathology, Brazil

Introduction: Chemical synthesis are structural modifications to generate a new compound that is less toxic or has greater biological activity (BARREIRO, Quim Nova, v. 29, n. 2, p. 326, 2006). Thus, there is an interest in investigate substances obtained by synthesis that act on smooth muscles. Smooth muscle lines the wall of several hollow organs, dysregulation in the process of contraction and relaxation can cause pathophysiological processes such as asthma, diarrhea, erectile dysfunction and hypertension (WATTERSON, Cell Signal, v. 17, n. 3, p. 289, 2005). In the context of organic synthesis, amides stand out, playing an important role in the preparation and composition of biological systems, being present in natural products and in various types of synthetic molecules, including several drugs on the market (KUNG, J Med Chem, v. 53, n. 1, p. 499, 2010). Activities of natural and synthetic amides have been reported on smooth muscle reactivity, including vasorelaxant activity in rat aorta (ARAÚJO-JÚNIOR, Emir J Food Agric, v. 23, n. 3, p. 265, 2011). Therefore, this study aimed to investigate spasmolytic mechanism of action of synthetic thiophenic amide (E)-N-(4-metoxifenetil)-3-(tiofen-2-il)acrilamid (MFTA) on rat aorta. **Methods:** The rat aorta was removed and suspended in organ baths under appropriate conditions for each experimental protocol and isotonic contractions were monitored. The results were statistically analyzed by the Student's *t*-test or one-way ANOVA (variance analysis) followed by Tukey's post-test when appropriate (n = 4 - 5). The values were expressed as the mean and standard error of the mean. All experimental protocols were approved by the Ethical Committee on Animal Use of UFPB (8073300419/ID 633). **Results:** MFTA relaxed in a concentration-dependent and equipotent manner the pre-contracted rat aorta with phenylephrine 3 x 10⁻⁷ M, both in the presence (EC₅₀ = 9.4 ± 1.6 x 10⁻⁵ M) and absence (EC₅₀ = 1.2 ± 0.3 x 10⁻⁴ M) of functional endothelium, suggesting that it's vasorelaxant effect appears to be due to a mechanism independent of endothelium-derived relaxing factors. Thereby, we decided to evaluate the participation of the K⁺ and Ca²⁺ channels in the vasorelaxant effect of MFTA. And it was observed that the amide inhibited the contractions induced by both high and moderate K⁺, being 2.8 times more potent when the contractions were induced by KCl 80 mM (EC₅₀ = 5.3 ± 1.2 x 10⁻⁵ M) than KCl 30 mM (EC₅₀ = 1.5 ± 0.3 x 10⁻⁴ M), indicating a possible participation of the voltage-dependent Ca²⁺ channels (Ca_v). This hypothesis was confirmed by the observation that, in the presence of the amide (3 x 10⁻⁶; 10⁻⁵; 3 x 10⁻⁵; 10⁻⁴ and 3 x 10⁻⁴ M), there was a shift to the right of the cumulative control curves to CaCl₂

in depolarizing medium ($E_{\max} = 100\%$), in a non-parallel manner and with reduction in E_{\max} to 88.0 ± 2.4 ; 74.0 ± 3.9 ; 60.2 ± 7.9 ; 35.6 ± 9.2 and $19.4 \pm 3.5\%$, respectively. Conclusions: These results suggest that the vasorelaxant effect of MFTA is due to inhibition of calcium influx through Ca_v , however, other protocols are needed to confirm this hypothesis. **Financial Support:** PIBIC/CNPq, PPgPNSB/CCS/UFPB. **License number of ethics committee:** (8073300419/ID 633)

09.006 Screening of oleanolic acid and its derivatives for monoamine oxidase inhibitory activity. Cabral IB¹, Martins JLR¹, Pedrino GR¹, Costa EA¹, Fajemiroye JO¹. ¹UFG, Dpt de Farmacologia, PPG Ciências Biológicas, Brasil

Introduction: Depression and anxiety disorders are psychiatric pathologies that can compromise the quality of life, generate high costs for health systems and society (Fajemiroye et al., *Psychopharmacol*, v. 28, p. 923, 2014). According to the monoamine hypothesis, the reduction or deficiency of neurotransmitters such as serotonin (5-HT), dopamine (DA), and/or norepinephrine (NE) plays a role in depression and anxiety (Liu et al., *Front Psychol*, v.9, 2018). A low concentration of these neurotransmitters could be associated with the degradation process by monoamine oxidase (MAO). The two isoforms of MAO; MAO-A and MAO-B preferentially metabolize 5-HT/NE and DA respectively. Thus, the inhibitors of MAO (MAOIs) could increase the concentration of monoamine levels in the synaptic cleft (Follmer et al., *Quím Nova*, v. 32, p. 306, 2013; Opielak. *Advanc in Medic and Biol*, v. 87, p. 195, 2015). In clinical practice, MAOIs are used in the treatment of depression and anxiety, and Parkinson's diseases (Larit et al., *Phytomed*, v.40, p. 27, 2018). However, the adverse effects which limit the applications of the available MAOIs provide an opportunity for the screening of new compounds (Fajemiroye et al., *Scient Reports*, v. 5, 2015; Perna et al., *Curr Psychiatry Rep*, v. 18, 2016). The pentacyclic triterpene oleanolic acid (OA) is an isolated of several medicinal plants with antioxidant, neuroprotective, antidepressant, and/or anxiolytic activities (Guo et al., *Clin Exp Pharmacol Physiol*, v. 47, p. 1263, 2020; Fajemiroye et al., *Psychopharmacol*, v. 28, p. 923, 2014). Previous studies demonstrated the blockade of the antidepressant or anxiolytic-like activity of this triterpene by pharmacological antagonists that suggest the involvement of the monoamines (Fajemiroye et al., *Scient Reports*, v. 5, 2015; Fajemiroye et al., *Psychopharmacol*, v. 28, p. 923, 2014). To provide a better understanding of the mechanisms of antidepressant or anxiolytic action, this study investigated the effects of OA and its derivatives on the activity of MAO-A and/or MAO-B. **Methods:** Recombinant human monoamine oxidase A and monoamine oxidase B were purchased from BD Biosciences (Bedford, MA, USA). Quinuramine, clorgyline, deprenyl, and DMSO were obtained from Sigma Chemical (St Louis, MO, USA). To investigate the inhibition of MAO-A and MAO-B activities by natural products and their derivatives, the quinuramine deamination assay was used. A fixed substrate concentration (80 μ M kynuramine) and concentrations of 10⁻⁹ to 10⁻⁴ M for OA and its derivatives (acrylate, methacrylate, methylfumarate, and ethylfumarate) or 10⁻¹² to 10⁻⁵ M for standards [selective inhibitor of MAO-A (Clorgyline) and MAO-B (Deprenyl)] were tested to determine 50% and 90% inhibition of enzyme catalytic activity (IC₅₀ and IC₉₀, respectively). After incubation of the preparation (enzyme-substrate and inhibitor), the inhibitory activity was calculated as a percentage of product formation compared to the corresponding control (enzyme-substrate incubation without the inhibitors). Reactions were performed in 0.1 M potassium phosphate buffer at pH 7.4. Incubation mixtures contained 5 μ g / ml MAO-A (50 μ l in buffer) and 12.5 μ g / ml MAO-B (50 μ l in buffer). The inhibitor was dissolved in DMSO. The total reaction volume was 200 μ l at the final concentration of 1.0% DMSO in the mixture. Reaction mixtures were pre-incubated for 10 min at 37 °C, followed by the addition of MAO-A or MAO-B. Reactions were incubated for 20 min at 37 °C and were immediately stopped by the addition of 75 μ l of 2N NaOH. The formation of 4-hydroxyquinoline was determined fluorometrically by a plate reader (SpectraMax M5, Molecular Devices, Sunnyvale, CA) with an emission and excitation wavelength of 380 nm and 320 nm, respectively, using the SoftMax Pro program. **Results:** The IC₅₀ of OA, methacrylate-OA and ethylfumarate-OA were greater than 100 μ M while acrylate-OA and methylfumarate-OA exhibited lower (48.85 and 78,09 μ M, respectively) for MAO-A inhibition. The IC₉₀ values for OA and its derivatives were all greater than 100 μ M. Except for methylfumarate-OA (13.83 μ M), other compounds showed higher (>100 μ M) IC₅₀ and IC₉₀ values for MAO-B. Deprenyl presented IC₅₀ and IC₉₀ values of 0.06 and 0.23, respectively, for MAO-B while clorgyline presented IC₅₀ and IC₉₀ values of 0.003 and 0.01, respectively, for MAO-A. **Conclusion:** Altogether, the MAO-A inhibitory activity of OA, methacrylate-OA and ethylfumarate-OA seems less potent than acrylate-OA, methylfumarate-OA, and clorgyline while methylfumarate-OA and deprenyl seem to be more potent against MAO-B as compared to OA and other derivatives. **Financial support:** FUNADESP, CNPq, Capes, FAPEG **License number of ethics committee:** N/A

09.007 Counteracting action of *Coutarea hexandra* (Rubiaceae) stem bark extract on the systemic toxicity induced by *Lachesis muta muta* (Viperidae: Crotalinae) venom in rats. Torres AGL¹, Moraes AM¹, Moraes-

Santos LS¹, Sales-silva MS¹, Santarém CL², Nogueira RM², Giuffrida R², Silva EO², Gerez JR³, Santos MG⁴, Silva-junior NJ⁵, Pilon GD⁶, Oshima-franco Y⁶, Floriano RS¹ ¹Lab of Toxinology and Cardiovascular Research, PPG in Health Sciences, Univ of Western São Paulo, Presidente Prudente, Brazil; ²PPG in Animal Science, Univ of Western São Paulo, Presidente Prudente, Brazil; ³Dpt of Histology, State Univ of Londrina, Londrina, Brazil; ⁴PPG in Environmental Sciences, Tocantins Federal Univ, Palmas, Brazil; ⁵PPG in Environmental Sciences and Health, School of Medical, Pharmaceutical and Biomedical Sciences, Pontifical Catholic Univ of Goiás, Goiânia, Brazil; ⁶PPG in Pharmaceutical Sciences, Univ of Sorocaba, Sorocaba, Brazil

Introduction: *Lachesis muta muta* is found in Amazon river basin and occasionally causes severe human envenomation, with the treatment being conditioned to polyvalent antivenoms. *Coutarea hexandra* is a native plant from South of Brazil and exhibits high antioxidant activity. In this work, we investigated the counteracting action of *C. hexandra* aqueous stem bark extract (*Ch-E*), associated with a commercial antivenom or as a stand-alone therapy, on the acute systemic envenomation induced by *L. m. muta* in rats. **Methods:** Male Wistar rats (300–350 g) were exposed to *L. m. muta* venom (1.5 mg/kg – i.m.) and then treated with a polyvalent antivenom (antivenom: venom ratio 1: 3 ‘v/w’ – i.p.), *Ch-E* (100 mg/kg – i.p.) or combining both of these agents. After 120 min envenomation, animals were anesthetized in order to collect blood samples through intracardiac puncture and, subsequently, euthanized for collecting tissue samples; the hematological-biochemical and histopathological analyses were performed through conventional **Methods: Results:** *Ch-E* administered alone or associated with antivenom effectively prevented the venom-induced increase of serum creatine kinase (CK) and alanine aminotransferase (ALT) release ($p < 0.05$ vs. venom group, $n = 6$); *Ch-E* reduced the release of the serum biomarker (CK-MB) for cardiotoxicity only in association with antivenom ($p < 0.05$ vs. venom group, $n = 6$). Venom caused pronounced inflammatory responses associated with neutrophilia, eosinophilia and monocytosis; *Ch-E* alone, similarly to antivenom, also contributed to reduce the venom-induced leukocytosis mainly due to its suppressive action on neutrophils ($p < 0.05$ vs. venom group, $n = 6$). *Ch-E* prevented the venom-induced increase of reticulocytes either alone or in the presence of antivenom ($p < 0.05$ vs. venom group, $n = 6$). *Ch-E* administered alone or associated with antivenom produced effective protection against venom-induced hepatic and renal morphological alterations ($p < 0.05$ vs. venom group, $n = 6$). **Conclusion:** *Ch-E* shows to be an important source of biomolecules with therapeutic potential to prevent efficiently a number of systemic disorders caused by *L. m. muta* venom in rats, e.g., myotoxicity, hepatotoxicity, inflammatory responses. **Financial Support:** This work was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP, process no. 2019/21147-6). **License number of ethics committee:** Approval by Animal Research Ethical Committee: The experimental procedures were approved by Committee for Ethics in Animal Use of the University of Western São Paulo (CEUA/UNOESTE, Protocol No. 6099).

09.008 **Antinociceptive activity of gamma terpinene in rats with chemotherapy-induced peripheral neuropathy: Possible involvement of opioid system and KATP channels.** Reis Filho AC, Acha BT, Pinheiro Neto FR, Bandeira SRM, Gomes LS, Lopes EM, Almeida, FRC. Medicinal Plants Research Center, Federal Univ of Piauí, Teresina, Brazil

Introduction. Among several pathologies, cancer stands out for the disordered growth of cells, which invade tissues and organs, with the potential to cause neuropathic pain. For the pharmacological treatment of cancer, there are several chemotherapy drugs, including microtubule stabilizers, such as paclitaxel, which has peripheral neuropathy as a serious adverse effect. The treatment of neuropathic pain consists of anti-inflammatory drugs, antidepressants, anticonvulsants, opioids, among others. However, recurrent use can generate adverse effects such as gastric problems, hepatotoxicity, insomnia and dependency. It has been approached that essential oils are the target of research as a new therapy for analgesia, since it is addressed in the literature several pharmacological activities of its constituents such as antinociceptive, antibacterial, antifungal, anticancer, antidiabetic, antiviral and antiprotozoal properties. The gamma-terpinene (gamma-TPN), monoterpene very common in aromatic plants has already described in the literature with anti-inflammatory and antinociceptive activity. The aim of this study was to investigate the possible antinociceptive effect of gamma-TPN in an animal model of neuropathic pain due to paclitaxel. **Methods:** Female Swiss mice (25-35 g, $n = 6$) were used under authorization from the Animal Ethics Committee (CEEA/UFPI Nº 148/2016). To induce neuropathic pain, paclitaxel (PACLI, 2 mg/kg, i.p.) was administered for 4 consecutive days. The animals were evaluated on different days for the parameters of mechanical hyperalgesia, using the von Frey filaments as well as thermal stress tests (acetone and hot plate). The groups used were sham (NaCl 0.9% without neuropathy), vehicle (NaCl 0.9% with Tween 80 at 2% p.o.), positive control (morphine 10 mg/kg, s.c.), and 2 doses of gamma-TPN (100 and 200 mg/kg p.o.). On the eighth day (4 days after the end of PACLI administration), the animals were submitted to mechanical and thermal evaluation at 0, 60, 120, 180 and 240 minutes. **Results:** The vehicle group showed an average reduction in the nociceptive threshold of 89 % (1 g) during the entire observation

period in relation to sham group (9 g), while the gamma-TPN (200) showed a reduction of 72 % (2,5 g) at 120 min and morphine of 44 % (5 g) at 60 min. In the acetone test gamma-TPN (200) decreased response time by 52 % (13 s) compared to control group (27 s), while the morphine reduction was 88 % (2 s) at 180 min. The evaluation in the hot plate presented an increase in response latency by 55 % (14 s) in the gamma-TPN group (200) and 100 % (18 s) in the morphine group at 120 min. Gamma-TPN antinociceptive effect was inhibited in the presence of naloxone (2 mg/kg, i.p.) and glibenclamide (3 mg/kg, i.p.). **Conclusion:** These preliminary results suggest that oral gamma-TPN produces an antinociceptive effect in the chemotherapy-induced neuropathic pain model through the involvement of the opioid system via K⁺ATP channels. UFPI, CAPES and Research Support Foundation of the State of Piauí supported this work. **License number of ethics committee:** CEUA-UFPI No. 148/2016

09.009 Evaluation of the anti-inflammatory activity of the ethanolic extract from the roots of *Eriosema campestre* on RAW 264.7 cells stimulated by bacterial lipopolysaccharide Ottoni MHF¹, Barra A², Fernandes-Braga W³, Santos MG¹, Pereira WF¹, Klein A², Melo GEBA¹. ¹UFVJM Diamantina, Integrated Center of Postgraduate and Research in Health, Brazil; ²UFMG Belo Horizonte, Inst of Biological Sciences, Dpt of Pharmacology, Brazil; ³UFMG Belo Horizonte, Inst of Biological Sciences, Dpt of Biochemistry and Immunology, Brazil

The medicinal plant *Eriosema campestre* is commonly used by the population of Vale do Jequitinhonha, in Minas Gerais (Brazil) as an anti-inflammatory agent. Popularly known as *Pustemeira*, its ethanol extract from roots has already shown antiproliferative effect on human lymphocytes stimulated with phytohemagglutinin, however, there are no studies evaluating its action on innate immunity mechanisms associated with the inflammatory response. Thus, the aim of this study was to evaluate the pharmacological activity of the ethanol extract from roots of *E. campestre* (ECEXT) on RAW 264.7 cells (murine macrophages) stimulated by bacterial lipopolysaccharide (LPS). For this purpose, RAW 264.7 cells were treated with ECEXT at 10, 20 or 40 µg/ml (E10, E20 and E40 cultures, respectively) or with dimethylsulfoxide at 0.4% (DMSO culture), the solvent used in the solubilization of the plant extract. There was also a cell culture that did not receive any treatment or LPS stimulation (Negative Control). Cells remained with treatments 1 hour before addition of LPS at 100ng/ml. One of the cultures received only LPS, without any other treatment (LPS culture). Cells were then incubated for 24 hours at 37°C and 5% CO₂. After that, the cultures supernatants were evaluated for the presence of nitric oxide (NO) by the Griess method, in addition to the production of cytokines IL-6 and TNF and the chemokine MCP-1, by ELISA assay. An assay was also performed to evaluate the effect of ECEXT on the phagocytic activity of RAW 264.7 cells in the presence of zymosan-FITC particles. Analyses were performed by confocal microscopy. Data are represented as mean ± standard deviation and were analyzed by one-way ANOVA test with Tukey's post-hoc. Statistical difference was considered when p<0.05. It was observed that cultures E20 and E40 had lower NO production than LPS cultures (LPS: 7.61 ± 1.62µM vs E20: 2.77 ± 1.08 µM; LPS vs. E40: 2.00 ± 0.98 µM, p<0.05 in both). It was also verified that cells from E40 cultures showed lower production of IL-6, when compared to LPS cultures (LPS: 122.5 ± 11.9 pg/ml vs. E40: 69.9 ± 7.2 pg/ml, p<0.05), which also occurred for MCP-1 production (LPS: 1879 ± 125 pg/ml vs. E40: 804 ± 86 pg/ml, p<0.05), including in E10 and E20 cultures (LPS: 1879 ± 125 pg/ml vs. E10: 1345 ± 186 pg/ml; E20: 1286 ± 75, p<0.05 in both). However, there was no effect of ECEXT on TNF production. ECEXT also did not decrease the percentage of phagocytic cells nor their phagocytic efficiency, when compared to LPS cultures. The results so far obtained suggest an anti-inflammatory action of ECEXT mediated especially by the decrease in the production of IL-6, MCP-1 and NO, not affecting the phagocytic activity of macrophages. **Financial Support:** CAPES and UFVJM. **License number of ethics committee:** N/A

09.010 Chemical characterization and gastroprotective effect of *Lonchocarpus sericeus* on ethanol-induced gastric ulcers in mice. FREIRE GP¹, Almeida-Filho LCP², Nunes PIG¹, Silva AVL³, Lima RP³, Ribeiro PRV⁴, Brito ES⁴, Carvalho AFFU², Santos FA³. ¹UFC Fortaleza, PPG Medical Sciences, Brazil; ²UFC Fortaleza, PPG Biochemistry; ³UFC Fortaleza, PPG Pharmacology, Brazil; ⁴Embrapa Tropical Agroindustry, Brazil

Introduction: Gastric ulcer is a very prevalent gastrointestinal disorder, affecting more than 10% of the world's population. Etiological factors of gastric ulcer include alcohol abuse, smoking, stress, drug overuse, and *H. pylori* infection. The disease is often associated with loss of quality of life and high expenses for treatment of its complications. Currently, there is a need to seek new drugs that are more effective, less toxic and less expensive for treatment of gastric lesions. Based on this, we studied the potential of the hexane extract of *L. sericeus* seeds (LsHE) by performing its chemical characterization and investigating its gastroprotective activity and possible antioxidant effect. **Methods:** The chemical composition of LsHE was determined through gas chromatography. Its gastroprotective potential was investigated using the ethanol-induced gastric ulcer model, where male Swiss mice (25-30g, n=8) were treated orally with vehicle

(saline), LsHE (0.8, 1.6 or 3.2 mg/kg) or N-acetylcysteine (NAC) (200 mg/kg). Sixty minutes later, the mice received absolute ethanol (99.5%, 10mL/kg, p.o.) to induce gastric ulcer, and 30 minutes later they were euthanized. The stomachs were excised and the lesion area was quantified using the ImageJ software. The stomach tissues were used for the preparation of 10% homogenates for catalase (CAT), malondialdehyde (MDA), reduced glutathione (GSH) and superoxide dismutase (SOD) analyses. Results were expressed as the mean \pm standard error of the mean (SEM). Statistical analysis was performed using ANOVA, followed by Tukey's multiple comparison test. Values of $p < 0.05$ were considered statistically significant. **Results:** Chemical analysis of LsHE showed a high level of confidence between the experimental retention index and the US National Institute of Standards and Technology (NIST) Mass Spectral Library, identifying the presence of eight components. The major component was methyl oleate (67.03%), followed by methyl hexadecanoate (11.17%) and methyl docosanoate (10.74%). When compared to the vehicle group, LsHE in oral doses of 0.8, 1.6 and 3.2 mg/kg decreased the severity of ethanol-induced gastric lesions by 36.8, 61.8 and 64.2%, respectively. The NAC control, using the same comparison, was able to reduce gastric ulcers by 60.6%. LsHE at a dose of 1.6 mg/kg was also able to increase CAT levels by 52.37%, GSH by 38.36% and SOD by 60.83%, in comparison with the values of these markers found in the vehicle group. NAC increased CAT, GSH and SOD levels by 60.58, 55.17 and 80.0%, respectively, when performing the same comparison. When compared to the values found in the vehicle group, at the same dose, 1.6 mg/kg of LsHE was able to reduce the levels of MDA and MPO by 46.02 and 74.75%, respectively. The NAC control reduced MDA by 57.8% and MPO by 74.66%, when compared to the vehicle group. **Conclusion:** These results provide initial evidence of the gastroprotective effect of LsHE, which may be related to its antioxidant activity. Financial support: CNPq; CAPES; FUNCAP. **License number of ethics committee:** This work was approved under CEUA no, 1933011019.

09.011 **Linalool antinociceptive activity evaluation in acid-induced nociceptive-like behavior in fish.** Rodrigues P, Barbosa LB, Ferrari FT, Bianchini AE, Baldisserotto B, Heinzmann BBM Post-Graduation Program in Pharmacology, Federal Univ of Santa Maria, Santa Maria, RS, Brazil Pharmacy School, Federal Univ of Santa Maria, Santa Maria, RS, Brazil

Introduction: Natural products used as anesthetics and sedative drugs together with analgesics effect can be a more complete drug for fish welfare (HUNTINGFORD et al. J. Fish Biol. 68, 332-72, 2006). Linalool presents pharmacological potential as an anesthetic or sedative drug in fish farming (HELDWEIN et al. Veterinary anaesthesia and analgesia.41: 621-9, 2014). The pharmacokinetics of S-linalool in silver catfish plasma was investigated and the results supported its application in fish farming due to its rapid absorption, distribution, and elimination (BIANCHINI et al. Aquaculture. 506, 302-7, 2019) . Thus, we hypothesized the antinociceptive effect of linalool in an acid-induced nociceptive-like behavior method. **Methods:** The antinociceptive effect was evaluated on acid-induced nociceptive-like methodology using silver catfish (*Rhamdia quelen*) (Process number: 5591250219) as an animal model (RODRIGUES et al. Physiol Behav. 112648, 2019). Fish behavior was videotaped for 20 min and analyzed by ANY-maze[®] software. The locomotor activity assay of intramuscularly linalool (i.m. 5-50 mg/kg) was used in order to choose the dose to be employed to assess antinociceptive activity. Afterwards, the linalool was injected before acid for the antinociceptive assay and naloxone (5 mg/kg, i.m.) was used to block the opioid receptor. **Results:** Linalool (5 mg/kg i.m.) decreased the number of line crossings, average speed, mobile time and distance traveled. On the other hand, 10 mg/kg linalool decrease the immobile time, while it increases the number of line crossings, average speed, absolute turn angle, and the distance traveled in the top and middle of the tank arena which may suggest an anxiolytic-like activity. The heat map also shows the increased fish distance traveled after 10 mg/kg linalool treatment and decrease after 5 mg/kg linalool treatment. Furthermore, 50 mg/kg linalool did not alter fish locomotion behavior when compared to the saline group. The injection of acid resulted in a decrease in all metrics of swimming activity and immobile time compared to vehicle controls. Linalool (50 mg/kg) prevented the acid-induced nociceptive-like behavior, and naloxone-blocked linalool effect suggesting an opioid-involvement. agent. **Conclusion:** In conclusion, these results may suggest the use of linalool as a natural approach to increase fish welfare in farming activities, and opioid-mediated antinociceptive effect against acid-induced nociceptive-like behaviour. Moreover, linalool-induced anxiolytic-like behaviour is another advantage for its use as a veterinary drug. Therefore, linalool (50 mg/kg) is suitable as an antinociceptive agent and our results suggest the use of linalool as an antinociceptive. **License number of ethics committee:** Process number: 5591250219

09.012 **Treatment with pyridoxamine protects kidney endothelial dysfunction caused by diet-induced metabolic syndrome.** Silveiras, RR¹, Pereira ENGDS¹, Flores, EEI¹, Rodrigues, KL¹, Silva, AR², Gonçalves-de-Albuquerque, CF² ³ and Daliry, A¹. ¹Lab of Cardiovascular Investigation, Oswaldo Cruz Inst, Oswaldo Cruz

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Introduction: Metabolic syndrome (MS) has become a major public health problem worldwide and is considered a risk factor for the development of diabetes and cardiovascular diseases. MS refers to the co-occurrence of several known cardiovascular risk factors, including insulin resistance, obesity, atherogenic dyslipidemia and hypertension. However, the pathophysiology of renal endothelial dysfunction associated to MS and other related diseases, including diabetes, are not fully understood. This study aimed to investigate changes in renal function and the AGE-RAGEaxis in the kidney of rats with MS. Additionally; we evaluated the protective effect of pyridoxamine (PM), a vitamin B6 analog with anti-AGE effects, in the context of diet-related renal endothelial dysfunction. **Methods:** The MS animal model was induced in Wistar rats for 28 weeks of feeding with HFD. Rats were treated daily with PM (60mg/kg/day) between weeks 20 and 28 and all analyzes were performed at the end of the protocol. Tissue perfusion in the renal microcirculation was assessed by laser speckle contrast imaging (LSCI). The oxidative stress parameters were analyzed by thiobarbituric acid reactive species (TBARS) and catalase enzyme activity, while inflammatory markers, TNF- α and IL-1 β , by ELISA. eNOs, IL-6, vascular cell adhesion molecule (VCAM), catalase and receptor for AGE (RAGE) gene expression were studied by RT-PCR. All procedures were approved by the Oswaldo Cruz Foundation Animal Welfare Committee (License L-019/2016). **Results:** Wistar rats fed a HFD showed changes in renal function, with decreased urinary volume and increased serum creatinine and uric acid compared to control animals (CTL). Compared to CTL, renal tissue of MS animals showed decreased catalase gene expression and catalase enzyme activity and increased IL-1 β . Regarding the microcirculation, the HFD group showed renal endothelial dysfunction in response to the vasodilator acetylcholine (Ach), but without significant differences in basal microvascular blood flow compared to CTL animals. PM significantly improved renal Ach-induced vasorelaxation in HFD-fed rats. The expression of the eNOS, VCAM and RAGE genes, the AGE content and the TBARS were not altered in the kidney of rats with MS compared to controls. **Conclusion:** Our results suggested that HFD-induced kidney microvascular dysfunction is an early manifestation of MS and is associated to defects in the antioxidant machinery and activation of inflammation. In addition, PM is a promising agent in the management of MS-related renal endothelial dysfunction, probably due to its metabolic and antioxidant effects. **License number of ethics committee:** License L-019/2016

09.013 **Antagonism of *Apis mellifera* venom activities by *Eclipta prostrata* extract and wedelolactone.** Nogueira-Souza PD, Rocha-Junior JRS, Pinheiro AN, Cesar MO, Strauch MA, Monteiro-Machado M, Patrão-Neto FC, Melo PA CCS-ICB- UFRJ Lab de Farmacologia das Toxinas

Introduction: Africanized bee (*Apis mellifera*) mass attacks are a common cause of accidents in rural and urban areas. Human envenoming can lead to some clinical conditions due to the complex composition of bee venom that contains enzymes, peptides, biogenic amines, and toxic substances. The *Eclipta prostrata* (EP) plant popularly known in Brazil as erva-botão is commonly used in Chinese traditional medicine and for victims of snakebite accidents [1]. One of the EP component is wedelolactone (WED), known to anti-inflammatory [2], and antiophidic activities [3]. This study aims to evaluate the ability of the EP hydroalcoholic extract and the substance WED to inhibit the activity of *A. mellifera* venom (BV) in different protocols. **Methods:** We investigated the antagonism of EP and WED in different experimental models *in vitro*, such as phospholipase A₂ (PLA₂) activity in which the BV (1 μ g/mL) was preincubated with EP or WED (3–100 μ g/mL or μ M) for 30 min at 37 °C. The BV hyaluronidase activity (10 μ g/ml) was observed in presence of 10–150 μ g/mL or μ M of WED and EP, respectively. In the myotoxicity study were used *Extensor digitorum longus* muscles (EDL) isolated from Swiss mice (25–30 g, protocol CEUA UFRJ n° DFBCICB072-04/16; CEUA IVB n° 001/20, to evaluate the myotoxic activity of the BV alone (10 - 25 μ g/mL) or in the presence of EP or WED (1–10 μ g/mL or μ M) by the releasing rate, creatinocinase (CK), in U/g/h. This study also evaluates the effects of WED and EP on *In Vivo* protocols, such as myotoxicity, myeloperoxidase (MPO) and edematogenic activity, vascular permeability alteration and lethality of BV. All of these tests were done in two protocols, which the venom (1 mg/kg) was administered after being incubated to EP extract (50 mg/kg) or WED (30 mg/kg) for 15 min at 37 °C or EP (250 mg/kg) was *per os* administered 30 and 60 min before the BV injection, and WED (30 mg/kg) was administered ip. 30 min before the venom. **Results:** The BV enzymatic activities were inhibited by EP extract and almost 80 to 100% by WED. The CK release induced by BV *in vitro*, 90 min after EDL muscle perfusion was reduced by both EP and WED at the highest concentration from 13,29 \pm 0,85 to 3,11 \pm 0,64 U/g/h and 11,77 \pm 1,42 to 1,18 \pm 0,34 U/g/h, respectively. Inhibition of myotoxicity *in vitro* by WED proved to be dependent on the concentration. At *In Vivo* experiments the BV injection increased the CK activity in

plasma and only the EP extract incubated with the BV was able to prevent the increase of the plasma CK activity. The MPO activity in the EDL muscles stimulated by BV injection was abolished by EP extract when preincubated with the BV and reduced by almost 40% by pretreatment with WED. The BV preincubation with EP extract or WED reduced the BV edema by approximately 40% and 20%, respectively, but only the EP group showed a decrease in vascular permeability. The BV lethality was lower in the preincubated EP group, but both pretreatments delayed this effect of the BV. **Conclusion:** The EP extract and WED showed ability to reduce toxic activities of BV *in vitro* and *In Vivo* and further studies should be done to better understand this effect. **Financial Support:** CAPES, FAPERJ and CNPq. **References:** [1] MORS, W. B., *et al. Toxicol.*, v. 27, p. 1003, 1989. [2] WEI, W., *et al. Biomedicine and Pharmacotherapy*, v. 94, p27, 2017. [3] MELO, P.A., *et al. Toxicol.*, v. 37, p. 199, 1999. **License number of ethics committee:** CEUA UFRJ n° DFBICB072-04/16; CEUA IVB n° 001/20

09.014 **Evaluation of the effect of *Hesperozygis ringens* (Benth.) Epling extract on the total antioxidant capacity of silver catfish infected by *Aeromonas hydrophila*.** Rosa IA¹, Bressan CA¹, Ferrari FT², Pavanato MA¹, Baldisserotto B¹, Heinzmann BM^{1,2}. ¹UFSM Santa Maria, Dpt of Physiology and Pharmacology, Brazil; ²UFSM, Santa Maria, Dpt of Industrial Pharmacy, Brazil

Introduction: In the current aquaculture scenario, outbreaks of bacterial infections represent one of the biggest challenges in this setor (Bandeira Junior and Baldisserotto, 2020). The hexane extract of leaves of *Hesperozygis ringens* (Benth.) Epling (HEHR) (Lamiaceae), known as “espanta-pulga”, provided an increase in the survival rate of silver catfish (*Rhamdia quelen*) experimentally infected by the bacterium *Aeromonas hydrophila*, one of the main pathogens in fish farming (Rosa et al., 2019). For this reason, the effect of HEHR on the total antioxidant capacity (TAC) in the muscle of silver catfish experimentally infected by *A. hydrophila* was evaluated. **Methods:** Silver catfish juveniles (8.24±0.28g; 11.25±0.25cm) were kept in the Fish Physiology Laboratory under appropriate acclimatization conditions (protocol approved by the Animal Use Ethics Committee – UFSM 074/2014). Subsequently, the animals were divided into eight experimental groups, four of which received saline injection (n=24) and another four, bacterial suspension of *A. hydrophila* MF 372510 (n=24). After 5 hours, each group (n=6) was submitted to a treatment: control, HEHR 15 mg/L, HEHR 30 mg/L and florfenicol (FLOR) 4 mg/L. After the 7-day experimental period, the fish were euthanized by medullary section and muscle tissue samples were collected. To determine the TAC in the silver catfish muscle, a solution containing 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) in buffer phosphate pH 7.0 was incubated for 50 minutes at 45°C. The resulting solution was cooled and held at 4°C until use. The assay was performed by adding sample aliquots to plates containing the ABTS-AAPH solution at 22°C. Absorbance was recorded at 734 nm for 1 minute. The TAC assay was calibrated with an ascorbic acid standard and the results (mean ± standard error) were expressed in μmol mg protein⁻¹ (Cao and Prior, 1998). For statistical analysis, two-way ANOVA followed by Tukey post-hoc was used and the minimum significance level assumed was 95% (p<0.05) (Statistica® 7.0). **Results:** The animals infected and treated with FLOR (50.5 ± 11.27) and HEHR15 (40.59 ± 4.93) had significantly lower TAC levels compared to their groups per se (FLOR: 143.72 ± 16.57; HEHR15: 173.59 ± 25.42). Only the infection by *A. hydrophila* did not reduce the TAC of the animals, however, when infected and exposed to the FLOR and HEHR treatments, they showed a reduction in the TAC, which demonstrates a possible pro-oxidant effect. The other treatments did not differ statistically from each other. **Conclusions:** HEHR30 was not characterized as a stressor in the presence of *A. hydrophila* infection and, therefore, it is the target of new studies to assess safety and efficacy in silver catfish infected by *A. hydrophila*. **References:** Bandeira Junior, G. J. Appl. Microbiol. 2020. Cao, G. Clin. Chem. 44. 1309. 1998. Rosa, I. A. J. Appl. Microbiol. 126. 1353. 2019. **Financial Support:** PIBITI-CNPq and CAPES. **Acknowledgments:** To CAPES and CNPq for their financial support. **License number of ethics committee:** Protocol approved by the Animal Use Ethics Committee – UFSM 074/2014

09.015 **Alpha, beta-amyrin improves glucose uptake through membrane GLUT4 expression of TNFα-induced insulin resistance in 3T3-L1 cells.** Lima RP¹, Oliveira FTB², Viana AFSC¹, Silva RAC², Nunes PIG², Silva AVL¹, Freire GP², Carvalho AA³, Chaves MH³, Santos FA¹ ¹UFC Fortaleza, PPG Pharmacology, Brazil; ²UFC Fortaleza, PPG Medical Sciences, Brazil; ³UPI Teresina, Dpt of Organic Chemistry, Brazil

Introduction: Insulin resistance (IR) is a state of deficient response of cells to insulin and plays a key role in the development of type 2 diabetes (T2DM) and obesity. Adipose tissue-derived TNF-α suppresses the expression of many proteins that are required for insulin-stimulated glucose uptake in adipocytes, such as the insulin receptor, IRS-1 and GLUT4. Due to the increasing prevalence of T2DM and obesity in recent decades and the search for new therapeutic options, many natural triterpenoids have been found to have promising anti-diabetic and anti-obesity properties. We investigated the effects of α, β-amyrin

(AMY), a triterpene isolated from *Protium heptaphyllum*, on TNF α -induced IR in 3T3-L1 adipose cells. **Methods:** 3T3-L1 pre-adipocytes were differentiated into mature adipocytes for 8 days. AMY was dissolved in DMSO (0.1% v/v) and added to the medium for final concentrations of 3.12–400 $\mu\text{g}/\text{mL}$ on day 8 and its effect on 3T3-L1 cells' viability was evaluated by the MTT assay. Vehicle group cells were treated with the same volume of 0.1% DMSO. To assess the effect of AMY on TNF α -induced IR, mature adipocytes were divided into the following groups: vehicle control, insulin-stimulated control, TNF α -induced IR (TNF α -IR), TNF α -IR treated with AMY (12.5, 25 and 50 $\mu\text{g}/\text{mL}$) and TNF α -IR treated with rosiglitazone (20 μM) for 24 h, followed by assessment of glucose uptake with 2-NBDG. Total and membrane proteins of 3T3-L1 cells were extracted to evaluate the translocation of GLUT4 by western blotting. The protein concentration of each sample was determined by the Lowry method. Results were expressed as mean \pm SEM of three independent experiments. For multiple comparison of parametric data, one-way ANOVA was used, followed by the Student Newman-Keuls test. P-values < 0.05 were considered statistically significant. **Results:** AMY (3.12–400 $\mu\text{g}/\text{mL}$) did not reduce 3T3-L1 cell viability in comparison with the vehicle group. In mature adipocytes, insulin increased glucose uptake by 39.3% compared to the vehicle group. TNF α reduced glucose uptake by 65.5% after insulin stimulation compared to the insulin-stimulated control group. In the TNF α -IR cells, AMY (12.5, 25 and 50 $\mu\text{g}/\text{mL}$) and rosiglitazone (20 μM) increased glucose uptake by 137.5%, 137.3%, 142.3 and 126.7%, respectively, in comparison with the TNF α -IR group. TNF α reduced membrane GLUT4 protein expression when stimulated with insulin (0.5874 \pm 0.021 arbitrary units) compared to the insulin-stimulated control group (1.341 \pm 0.099 arbitrary units). In the TNF α -IR cells, AMY 12.5, 25 and 50 $\mu\text{g}/\text{mL}$ and rosiglitazone 20 μM increased membrane GLUT4 protein expression (1.068 \pm 0.72, 1.147 \pm 0.071, 1.168 \pm 0.087, 1.103 \pm 0.067 arbitrary units, respectively) compared to the TNF α -IR group (0.458 \pm 0.048 arbitrary units). **Conclusion:** These results suggest that AMY improves glucose uptake in TNF α -induced insulin resistance in 3T3-L1 cells by increasing GLUT4 translocation to the plasma membrane. Support or financing information: CAPES; CNPq; FUNCAP **License number of ethics committee:** N/A

09.016 **Effect of alpha,beta-amyrin, a triterpenoid mixture from *Protium heptaphyllum* on insulin resistance in skeletal muscle of high fat diet-induced obese mice.** Nunes PIG¹, Oliveira FTB¹, Lima RP², Viana AFSC², Silva RAC¹, Freire GP¹, Silva AVL², Carvalho AA³, Chaves MH³, Santos FA². ¹UFC Fortaleza, PPG Medical Sciences, Brazil; ²UFC Fortaleza, PPG Pharmacology, Brazil; ³UFPI Teresina, Dpt of Organic Chemistry, Brazil

Introduction: Insulin resistance (IR) is a state in which the insulin responses of liver, muscle and adipose tissues are significantly reduced, and is strongly correlated with obesity and type 2 diabetes mellitus (T2DM). Skeletal muscle is the major site of postprandial peripheral glucose uptake, but in insulin-resistant states, insulin-stimulated glucose disposal is markedly impaired. In the search for new therapeutic options for the treatment of T2DM, triterpene compounds isolated from medicinal plants are being investigated for their promising potential to treat IR. We investigated the preventive effect of a mixture of alpha,beta-amyrin (AMY) triterpenes isolated from *Protium heptaphyllum* on the IR of skeletal muscles of high fat diet-induced obese mice. **Methods:** Male Swiss mice (n=10/group) were divided into five groups: Vehicle (2% Tween 80) + standard diet (SD); high fat diet (HFD); AMY 10 mg/kg + HFD; AMY 20 mg/kg + HFD; and fenofibrate (FEN) 50 mg/kg + HFD. Mice were treated for 15 weeks and at the end of the experiment the animals were weighed and the serum was collected to determine the blood glucose and lipid profiles. The gastrocnemius muscle was collected for the quantification of glycogen, oxidative stress markers (NO, malondialdehyde (MDA), reduced glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT)) and expression of GLUT4 membrane protein by western blotting. CEUA/UFC No. 5347120318. Results were expressed as mean \pm SEM and submitted to ANOVA and the Student-Newman-Keuls post-test, where $p < 0.05$ was statistically significant. **Results:** The HFD increased the animals' weight gain by 27.3% compared to the SD group. Blood glucose (178.8 \pm 9.3 mg/dL), total cholesterol (146.6 \pm 4.7 mg/dL) and triglycerides (98.7 \pm 6.0 mg/dL) increased in comparison with the HFD group to SD (93.0 \pm 6.8 mg/dL, 94.5 \pm 2.7 mg/dL and 73.6 \pm 1.6 mg/dL, respectively). AMY 10 and 20 mg/kg and FEN 50 mg/kg significantly reduced animal weight gain by 17.8%, 23.4% and 9.9%, blood glucose levels by 56.5%, 51.8% and 58.6%, total cholesterol by 16.9%, 22.6% and 36.2%, and triglycerides by 38.1%, 34.4% and 27.7%, respectively. All the treatments were able to reduce oxidative stress, bringing it to levels similar to the SD group. HFD reduced concentrations of muscle glycogen (0.3 \pm 0.03 mg/100 mg of tissue) and membrane GLUT4 expression (0.48 \pm 0.11 a.u.) when compared to SD (0.6 \pm 0.0 mg/100 mg of tissue and 1.00 \pm 0.04 a.u., respectively). AMY 10 and 20 mg/kg and FEN 50 mg/kg significantly increased tissue muscle glycogen concentrations by 78.8%, 78.8% and 42.4%, as well as membrane GLUT4 expression by 1.29, 1.12 and 1.04 times, respectively. **Conclusion:** The results demonstrate a protective effect of AMY on insulin

resistance in skeletal muscle of mice with high fat diet-induced obesity. Support or financing information: CAPES. CNPq. FUNCAP. License number of ethics committee: CEUA/UFC No. 5347120318.

09.017 **Thymic epithelial cells are sensible to the triterpene friedelin.** Porto FL¹, LINS MP^{1,2}, Barreto EO^{1,2}, Smaniotto S^{1,2}, Reis MDS^{1,2}. ¹Lab of Cell Biology, Inst of Biological and Health Sciences, Federal Univ of Alagoas, Maceió, Brazil; ²Brazilian National Inst of Science and Technology on Neuroimmunomodulation, Oswaldo Cruz Inst, Oswaldo Cruz Foundation, Rio de Janeiro, Brazil

The thymus is a primary lymphoid organ responsible for the maturation and differentiation of immunocompetent T lymphocytes. One of the main elements that constitute the thymic stroma are the thymic epithelial cells (TECs). They produce extracellular matrix molecules and soluble factors necessary for thymocyte differentiation and survival. TECs also present important antigens coupled to major histocompatibility molecules (MHC) during thymocyte positive and negative selection processes. Alterations in TEC function can lead to an impaired adaptive immune response, resulting in immunodeficiency or autoimmune disorders. Therefore, the investigation of new drugs with immunomodulatory capacity can contribute to the development of strategies to improve thymic functions. Friedelin (FD) is a natural triterpene with known activities on immune response, however, its effects on the thymus, particularly in TECs, has not been described so far. In this context, the study aimed to evaluate the effects of FD in TEC biology *in vitro*. For this, 2BH4 cells (murine TEC line) were treated with 0.1 and 1 μ M of FD for 24 h. After this period, TECs were submitted to cell viability assay by MTT, and immunofluorescence assay to observe laminin, fibronectin and CXCL12 production. Fresh thymocytes obtained from C57BL/6 mice were cocultured with TECs, in the presence or not of FD, for evaluation of cell-cell adhesion and survival. After coculture, thymocyte and TECs were harvested to analyse the expression of membrane surface receptors by flow cytometry. All experimental strategies were approved by the animal research ethical committee (CEUA/UFAL: protocol number 47/2016). As results, it was observed that the production of fibronectin and laminin were increased in FD-treated TECs, however, FD did not alter the expression of the chemokine CXCL12. Moreover, total thymocytes and CD4⁺CD8⁺ and CD4⁺CD8⁻ subsets had higher adhesion to TECs treated with FD. Also, an augment in the thymocyte survival in FD-treated coculture was observed, while the FD treatment upregulated MHC I and MHC II molecules in TECs surface after coculture with thymocytes. These findings show that FD has an important role in the physiology of murine TEC, indicating that it can be used as a candidate molecule to improve thymus function in immune-related disorders. The authors thank Julianderson Carmo for assistance in the friedelin preparation and dilution, also Juliane Pereira for handling in the cytometer. **Financial Support:** CNPq (N^o. 408677/2016-3 and N^o. 304408/2018-2) and FAPEAL (N^o. 60030 001260/2017). **License number of ethics committee:** CEUA/UFAL: protocol number 47/2016

10. Cancer Pharmacology

10.001 **Simulation of pharmacokinetic parameters of an antitumor prototype LOE420 with the aid of the Certara SimCyp® program.** Manso MP¹; Pereira JVM¹; Carvalho GGC¹; Silva-Neto JA¹; Sales SLA¹; Costa PMS¹; Guimarães CJ^{1,2}; Miranda-Furtado CL¹; Pessoa C¹. ¹UFC Fortaleza, Dpt of Physiology and Pharmacology. ²Amazonas State Foundation Center of Oncology Control

Introduction: Cancer ranks second among the diseases with the greatest cause of mortality in the world and searching for synthetic compounds that have *in vitro* antitumor potential, a derivative of combretastatin, LOE420, was developed. This compound showed previous results that determined the cytotoxic action. To follow up on the analysis of the potential of this molecule, a *In silico* simulation of their pharmacokinetic profile was performed using the Certara Simcyp® software version 19 (UK) and the evaluate human total plasma compound concentration-time profile for LOE420. **Methods:** The simulation inputs consisted of compound specific physicochemical properties such as logP, pKa and molecular weight. The human virtual population (n=100) was constructed with 10 trials (n=10/trial), 20-50 years old, with cancer disease, 50% female, fasted and infusion doses of 18, 36, 60 and 90 mg/m² (during 10 min). Was considered a pharmacokinetic analysis for 24 h evaluating the main pharmacokinetic parameters, such as maximum concentration (C_{max}), maximum time (T_{max}), area under the curve (AUC), clearance total (CL), blood/plasma concentration ratio (B/P), distribution volume (V_d), distribution volume at steady state (V_{ss}) and plasma half-life (t_{1/2}). **Results:** The values obtained for the C_{max} was 0.33 μ M for 18mg/m²; 0.67 μ M for 36 mg/m²; 1.11 μ M for 60 mg/m²; and 1.67 μ M for 90 mg/m² of the administered dose. The T_{max}, CL and B/P value for the administered doses was the same, being 0.2-hour, 25.72 L/h and 0.916, respectively. The V_{ss} was 4 L/Kg, for all administered doses, while the V_d had a slight variation of 81.5, for the lowest administered dose, and 79.6 for the highest administered dose. And t_{1/2} of 2.19h for the lowest administered dose and 2.14h for the highest administered dose. **Conclusion:** Considering the results, the C_{max} and AUC increases with the increase of the administered dose and

T_{Max} remained the same, demonstrating proportionality with the change in doses. The CL stands for drug clearance and indicates the blood flow that has been completely cleared of the drug per unit of time^{1,2}. The value for the administered doses was the same, indicating a zero-order kinetics, where a constant amount of drug is eliminated per unit of time and the rate of metabolism remains constant with the time, even at high drug concentrations. This can result in dangerously high plasma concentrations, which can cause toxic effects¹. Considering B/P, it is a parameter that indicates the blood / plasma concentration ratio², indicating that the highest concentration of the drug is in the plasma. Which may suggest that the compound could have high affinity with plasma proteins. The V_d and V_{ss} represent the volume of fluid needed to contain the total amount of drug absorbed into the body and the volume of distribution at steady state, respectively³. The V_{ss} remains the same for all administered doses, while the V_d was considered a high value⁴ while the t_{1/2} demonstrated a rapid elimination of LOE420 from the plasma in the human simulate module. These results guide the preclinical *In Vivo* experiments that will be performed in the future. **Acknowledgment:** FCECON, FUNCAP, CAPES **References:** 1 - Currie G M, J Nucl Med Technol, 46, 221, 2018 2 - Mamada H, Molecular Diversity, 2021. 3 - Rodgers T, Pharm. Resear., 24, 2007. 4 - Di L, Pharmacokinetics, 2016. **License number of ethics committee:** N/A

10.002 Simulation of tissue partition coefficients (K_p) of LOE420 antitumor prototype, with the aid of the Certara SimCyp® program. Pereira JVM¹; Manso MP¹; Carvalho GGC; Vieira-Neto JB¹; Sales SL¹; Costa PMS¹; Guimarães CJ²; Miranda-Furtado CL¹; Pessoa C¹ ¹UFC Fortaleza, Dpt of Physiology and Pharmacology, Brazil ²Amazonas State Foundation Center of Oncology Control, Manaus, Brazil

Introduction: Cancer ranks among the second disease with the greatest cause of word mortality. The searching for synthetic compounds that have *in vitro* antitumor potential may improve cancer treatment. Combretastatin is a class of natural phenols related to vascular disruption in tumors. A derivative of combretastatin, LOE420, was developed. This compound showed previous results that determined the cytotoxic action. **Methods:** To follow up on the analysis of the potential of this molecule an *in silico* analysis of their plasma partition coefficients (K_p) was performed using the Certara Simcyp® software version 19 (UK), in the human and animal module. The K_p is an important drug-specific input parameter in PBPK models and is used to quantify the distribution of drugs between tissues and plasma under steady-state conditions. The simulation inputs consisted of compound specific physicochemical properties such as logP, pKa and molecular weight. The human virtual population (n=100) was constructed with 10 trials (n=10/trial), 20-50 years old, with cancer disease, 50% female, fasted and infusion doses of 18, 36, 60 and 90 mg/m² (during 10 min). Was considered a pharmacokinetic analysis for 24 h evaluating the tissue partition coefficient (K_p). The animal virtual population was constructed with healthy rats, weighing 170 g, fasted infused (IV route; during 10 min) with 0.7, 1 and 4 mg/kg, and oral doses of 10 and 30 mg/kg. **Results:** In the human module, the tissues with the highest value were the liver, with 15.04; followed by the heart, with a K_p value of 9.06, gut with 8.65, spleen, with 8.40; muscle, with 7.79 and kidney, with 7.44. While the tissues with the lowest values, were bone, adipose, brain and lung with K_p of 2.52; 1.96; 1.79 and 1.62, respectively. Different from the values obtained in the human simulation module, in the animal simulation module the highest tissue plasma partition coefficient value was the kidney, with a value of 4.48, followed by adipose tissue 3.97 and lung, with 3.92. Whereas for tissues with less K_p was muscle, bone and liver, with values of 2.09, 1.36 and 1, respectively. **Conclusion:** The K_p in the human module stands out with the highest values in the liver, heart, gut and spleen. It is suggested that the justification for the increased value presented for the liver is due to the possibility of predominant hepatic metabolism. Whereas other values, it can be important data to predict possible toxic effects and targeting treatments for tissue whose antiproliferative activity of the tumor cell lines showed promising results, such as HCT-116; HCT-8 and PC-9. In the animal simulation module, the highest K_p value was observed for the kidney tissue, followed by adipose and lung. These data may suggest a predominance of renal excretion in rats. The values obtained in animals' models will be used to start *In Vivo* studies and the results of the simulations will be compared with the real ones, hoping that there will not be great variations. The financial support from CAPES, FUNCAP and CNPQ. **License number of ethics committee:** N/A

10.003 Evaluation of the antineoplastic activity of pentacyclic triterpene Friedelin in mice bearing Ehrlich's ascites carcinoma. Silva ELES¹, Santos DLF¹, Almeida JH¹, Souza TPM¹, Silva LMP¹, Barreto E¹, Ferro JNS¹. ¹Federal Univ of Alagoas (UFAL), s/n, 57072-970, Maceió, AL, Brazil

Introduction: Friedelin (FD) is a natural triterpene exhibiting many pharmacological activities, such as anti-inflammatory (FARIAS. Inflammation. v.2, p.764, 2011) and antioxidant (UTAMI. Pharm. Sci. v.2, p.245, 2013). Recently, it has been reported that FD has cytotoxic and apoptotic effect on cancer cells *in vitro* (SUBASH-BABU. Exp Toxicol Pathol. v.8, p.630, 2017), illustrating it as a promising potential candidate for the

development of anti-cancer drugs. **Aim:** Hence, we aimed to investigate the antitumoral activity of FD against Ehrlich ascites carcinoma tumor model (EAC). **Methods:** Female Swiss mice 10–14-week-old were injected by intraperitoneal route with EAC cells (5×10^6 ; i.p.) and distributed into groups ($n=5$ /group; duplicate experiment); named tumoral control (TM), 5-Fluorouracil (Sigma®-51-21-8, 200 $\mu\text{mol/Kg}$, 5-FU solubilized in PBS) and friedelin (Sigma®-559-74-0, FD50 or FD200 $\mu\text{mol/Kg}$; solubilized in 1% ethanol). Animals were treated (i.p.) with 5-FU or FD once a day on days 6 to 10 after tumor induction. On the days 1, 6 and 11 after tumor induction photographs and x-ray images were taken as well as the measurements of weight and abdominal volume. After euthanasia (thiopental 200 mg/Kg, i.v) cellularity from tumor fluid, intracellular reactive oxygen species (ROS) and the area of vessels present in the abdominal region were quantified. All experimental protocols were approved by the UFAL Animal Use Ethics Committee, CEUA n° 15/2019 and the results were significative when $p < 0.05$. **Results:** The treatment with FD was able to reduce all parameters of tumoral development evaluated, which were reflected in the macroscopic imaging, where a reduction in the abdominal distension was observed. Similarly, 5-FU treatment also reduced all parameters evaluated. The ascitic fluid from peritoneal cavity of the FD-treated animals demonstrated a significant reduction in total cell content (57% to FD50 and 39.8% to FD200). This reduction reflected a lower number of tumor cells after treatment with FD at 50 or 200 $\mu\text{mol/Kg}$ to 61.2% and 35.6%, respectively. The leukocyte cells counting were also reduced after treatment with FD at 50 or 200 $\mu\text{mol/Kg}$ to 60.5% and 63.4%, respectively. The leukocyte profile in ascitic fluid showed a neutrophilic and macrophilic infiltrate, and the treatment with FD50 and FD200 reduced, respectively, the counts of neutrophils in 62% and 68% and macrophages in 83% and 77%. The tumor cells recovered from peritoneal lavage fluid were distinguished based on size and granularity and had its intracellular ROS levels measured by DCFH-DA. Compared with the control group, cells from FD50 and FD200-treated animals showed a reduction in ROS levels at 31% and 43%, respectively. The area of vessels that coat the peritoneal walls of tumor-bearing mice were significantly reduced after treatment with FD50 and FD200 to 38% and 31%, respectively. Similarly, animals treated with 5-FU showed a reduction at 30.9% at the vessel's area. **Conclusion:** These findings indicate that friedelin is a potential natural product that has promising antineoplastic efficacy. **Financial support:** CNPq (Chamada MCTIC/CNPq N° 28/2018). **Keywords:** Cancer. Terpene. Angiogenesis. **License number of ethics committee:** CEUA 15/2019

10.004 **Antiproliferative effects of telocinobufagin on human colorectal adenocarcinoma.** Godoy TM¹, Godoy TM¹, Lopes BJ², Castelo-Branco MTL², Moraes JA³, Quintas LEM¹ ¹Lab of Pharmacology and Molecular Biochemistry, ICB - Federal Univ of Rio de Janeiro, Brazil ²Lab of Immunology, ICB - HUCFF - Federal Univ of Rio de Janeiro, Brazil ³LABIO-Redox, ICB - Federal Univ of Rio de Janeiro, Brazil

Introduction: Na/K-ATPase is a transmembrane protein present in cells, responsible for the transport of sodium and potassium ions using the energy from ATP hydrolysis. Ion pumping activity can be selectively inhibited by cardiotonic steroids (CTS), recognized as classic inotropic drugs such as digoxin. In recent decades, new therapeutic perspectives have been proposed for CTS, through the modulation of signaling pathways by protein-protein interaction. *In vitro* and *In Vivo* studies report the antiproliferative and antitumor activity of some CTS. However, telocinobufagin (TCB), a bufadienolide present in the parotoid secretion of *Rhinella* toads and a component of Chan'Su, a traditional Chinese medicine, is poorly studied experimentally. Here, we investigated its effect on human colorectal adenocarcinoma HCT8 and on normal human fibroblast HFF1 cell line. **Methods:** HCT8 were cultured in DMEM 10% FBS and HFF1 in DMEM 15% FBS + 1% glutamine and antibiotics. Cell Count. In 24-well plates, the cells were treated with 30, 100 and 300 nM TCB for 24, 48 and 72 h, were trypsinized, centrifuged, stained with Trypan blue and counted in the Neubauer chamber. [3H]Thymidine incorporation assay. The cells were grown in plates of 96 wells and treated with 10, 30, 100, 300 and 1000 nM TCB. After 24 h, 50 nCu of [3H] thymidine was added to each well for 6 h, followed by trypsinization and radioactivity count. MTT assay. The cells were grown in plates of 96 wells and treated with the same TCB concentrations for 48 h. MTT solution was added and absorbance was measured after 4 h incubation. Cell cycle. In 6-well plates, the cells were cultured and treated for 24 h with 30, 100 and 300 nM TCB. After the incubation period, the cells were resuspended in buffer containing RNase, Triton and propidium iodide and was evaluated by flow cytometer. NFkB activation. In 24-well plates, 24 h before treatment, the cells were transfected with NFkB luciferase reporter plasmid. Subsequently, they were treated with 10, 30 and 100 nM TCB for 24 h, in one of the groups, 20 min after treatment, 1 $\mu\text{g/ml}$ of LPS was added. Statistics. The comparison between groups was performed by the ANOVA test, followed by Dunnett's test and $p < 0.05$ was considered as statistically significant. **Results:** TCB significantly decreased the number of cells after 24 h (25%, 100 nM; 37%, 300 nM, $n=$, $p < 0.05$) and 48 h (33%, 100 nM; 46%, 300 nM, $n=$, $p < 0.05$) and with 72 h in 30, 100 and 300 nM (20%, 38% and 59%, respectively; $n=$, $p < 0.05$). The MTT assay showed a significant reduction in

viability after 48 h at 100, 300 and 1000 nM (54%, 71% and 78%, respectively; n=4, p<0.05) and the decline of [³H] thymidine incorporation (n=6) was observed after 24 h at 300 and 1000 nM (63% and 78%, respectively, n=6, p<0.05). A concentration-dependent reduction in the G2 phase, increase in G0/G1 phase and moderate increase in subG0 was observed (n=2), as well as the activation of NFκB by LPS, with a full inhibition at 100 nM (n=2). Interestingly, treatment with TCB at the same time and concentrations did not affect HFF1 in any experiment performed. **Conclusion:** TCB exhibits an antiproliferative effect in a concentration and time-dependent manner and is selective for tumor cells. Besides, the results indicate cell cycle arrest in G0/G1, increase of sub-G0 phase by 300 nM (suggestive of cell death by apoptosis or necrosis) and inhibition of NFκB-induced gene products. Further studies are underway to characterize the type of cell death and signaling pathways involved in this cellular response. **Financial Support:** FAPERJ, CNPq, CAPES **License number of ethics committee:** N/A

10.005 **CD39 inhibition enhance temozolomide effect in non-sensibile glioma cells.** Scheffel TB¹, Merino BM¹, Kist LW², Bogo MR², Rockenbach L¹, Morrone FB¹. ¹PUCRS, Porto Alegre, Lab de Farmacologia Aplicada, Escola de Ciências da Saúde e da Vida, Brazil ²PUCRS, Porto Alegre, Lab de Biologia Genômica e Molecular, Escola de Ciências da Saúde e da Vida, Brazil

Introduction: Glioblastoma multiforme is the most aggressive and lethal brain tumor. Despite the advance in therapeutic research, surgical resection followed by radiotherapy and temozolomide remains the standard treatment. However, therapeutic resistance is frequent, and the median survival rate is just around of 15 months. The tumor resistance has been related to immunomodulation by adenosine. The main enzyme involved in adenosine production is CD39, which acts hydrolyzing ATP released in the extracellular environment and has been the focus of studies as a therapeutic target in glioma therapy. Studies focused on improving the therapeutic efficacy of temozolomide and overcoming tumor resistance can directly impact patient survival; furthermore, the study of patient-derived tumors has been an excellent strategy in the search for results closer to reality. The aim of this study was to investigate the role of CD39 as a key regulator of temozolomide cytotoxicity in patient-derived glioma (LS12) and human glioma cells (M059J and U251). **Methods:** Glioma cells were cultured in DMEM (M059J and U251) or DMEM-F12 medium (LS12) supplemented with fetal bovine serum and maintained under ideal conditions. LS12 was obtained in accordance with ethical protocols (Ethical committee 429.849/2013). CD39 expression and ATP hydrolysis profile were performed by real time PCR and malaquite green, respectively. The temozolomide effect on cell viability was measured by MTS ([3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt) assay. **Results:** Data shown that CD39 is similarly expressed in all glioma cells, however the CD39-dependent ATP breakdown was higher in LS12 (14.94 ± 1.44 nmol of inorganic phosphate/min/mg of protein ± standard deviation) compared to cell lines (U251: 3.35 ± 0.45; M059J: 4.66 ± 0.69). CD39 activity was confirmed using POM-1, which was able to inhibit in 80% the hydrolysis of ATP in patient-derived glioma. The glioma cell viability was compromised by the CD39 inhibition, and POM-1 was able to induce the cell sensibility to temozolomide in all glioma cultures, reducing around of 40% the cell viability in relation to the control group in LS12. **Conclusion:** Glioblastoma treatment is a challenge and the discovery of new targets or improvements in existent therapies may contribute to increase patient survival. The modulation of CD39 activity shown to be a good strategy to improve temozolomide effect in patient-derived glioma cells. **Financial Support:** CAPES, CNPq, FAPERGS, PUCRS. **License number of ethics committee:** PUCRS 429.849/2013

10.006 **Co-encapsulation of 5-fluorouracil in multiple nanoemulsions containing short chain triglycerides improves drug cytotoxicity against colorectal cancer cells.** Fukumori C, Branco PC, Lopes LB. ICB-USP – Dpt of Pharmacology, Brazil

Colorectal cancer (CRC) is the third most prevalent cancer worldwide. The chemotherapeutic regimen uses 5-Fluorouracil (5FU), the drug of first choice for CRC treatment, but due its low oral bioavailability and short plasma half-life, it is administered intravenously at high dosage and frequency which, combined with poor selectivity for tumor cells, leads to serious systemic adverse effects and limits the patient quality of life. Considering that short chain fatty acids, available in the large intestine as a result of fiber fermentation by the intestinal microbiota, have beneficial effects on CRC, we proposed their co-encapsulation with 5FU in nanocarriers to boost 5-FU cytotoxicity and reduce the dose necessary for treatment. We employed a nanotechnology-based approach for oral administration of 5FU to increase its concentration at the colon through a multiple w/o/w nanoemulsion and evaluated its cytotoxicity in cell lines of CRC presenting various mutations (HCT-116 wt, HCT-116 p53 null and HT-29). The nanocarrier was composed of tricaprylin, monocaprylin, sorbitan monooleate, polysorbate 80, polyoxyethylene 10 oleyl ether and sodium alginate. They contained either tributyrin or tripropionin as triglycerides of short chain fatty acids (SCT). The nanoemulsion displayed droplets of 400 nm and polydispersity index of 0.2.

Consistent with multiple nanoemulsions, transmission electron microscopy analysis revealed spherical aggregates of less than 500 nm with smaller droplets in their interior. The nanoemulsion was stable when incubated for 4h at 37°C in buffers mimicking gastrointestinal fluids. Compared to its solution, incorporation of 5FU in nanoemulsions without SCT produced a shift of the viability curve with an increase in cytotoxicity and a reduction of 5FU IC₅₀ (4,6 µM to 2,7 µM, respectively) in HCT-116 wt ? a KRAS mutated cell line. Inclusion of the SCT in the nanoemulsions produced a more pronounced reduction of the IC₅₀ (1,8 µM and 1,5 µM for tributyrin and tripropionin, respectively). HCT-116 p53 null, which still has a deletion in TP53 gene, seemed less sensitive to 5FU as solution (IC₅₀ = 15,8 µM) and when incorporated in multiple nanoemulsion without SCT (IC₅₀ = 8,2 µM). A reduction on IC₅₀ values was observed when tributyrin was incorporated (IC₅₀ = 0,4 µM) but not tripropionin (IC₅₀ = 10,3 µM). For HT-29, a TP53 and BRAF mutated cell, we observed higher IC₅₀ values (IC₅₀ = 47,2 µM for 5FU solution), even when incorporated in the nanoemulsion (IC₅₀ = 4,9 µM), and neither tributyrin nor tripropionin inclusion further reduced IC₅₀ values. These results demonstrated that co-encapsulation of 5FU with SCT in multiple nanoemulsions may potentiate the cytotoxic effects of 5FU, with tributyrin effects being consistently observed in HCT-116 cells independently on the mutation status.

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10.007 Effects of soluble polysaccharide fraction in the cytochrome P450-inflammation-cancer triad. Stipp MC¹, Kulik JD², Galindo CM¹, Corso CR³, Adami ER¹, Nardin JM⁴, Ioshii S⁴, Winnischofer SMB², Sasaki GL², Cadena SMSC², Acco A¹ ¹Dpt of Pharmacology, Federal Univ of Paraná, Curitiba, PR, Brazil ²Dpt of Biochemistry and Molecular Biology, Federal Univ of Paraná, Curitiba, Brazil ³Pelé Pequeno Principe Research Inst ⁴Erasto Gaertner Hospital, Curitiba, Brazil

Introduction: Cytochrome P450 system (CYPs) is responsible for the biotransformation of many endogens and exogenous substances and can be regulated by several factors such as inflammatory process. Pro-inflammatory cytokines are present in tumor microenvironment, having an important role in carcinogenesis and in modulation of CYPs. This work has two goals: to evaluate the frequency of CYP1B1 in human breast tumor; and to test the influence of soluble fraction of polysaccharides (SFP) from cabernet franc red wine, a compound with antitumor effect, in regulation of CYPs and inflammatory parameters *In Vivo* and *in vitro*. **Methods:** Experiments were divided in: 1) human luminal A/B and triple negative breast cancer biopsies: tumor tissue of 166 breast cancer patients were used to detect CYP1B1 by immunohistochemistry; 2) SFP study: SFP and vincristine (positive control) were used isolated or in combination in human HepG2 cells (*in vitro*), and in ascitic or solid Walker-256 tumor-bearing rats (*in vivo*, 60 mg/kg v.o. SFP; 0.5 mg/kg i.p. vincristine). Inflammatory parameters, CYP levels and gene expression of *Cyp1a1*, *Cyp2e11* and *Cyp3a9* were measured in liver of animals. Gene expression of *CYP1A2* and *CYP2B6* was performed in HepG2 cells. HepG2 cell viability was followed by MTT assay, while Walker-256 tumor growth was evaluated by volume and weight. All the experimental protocols were approved by the Ethical Committee for Animal Use (CEUA) of UFPR (#1065) and by Certificate of Presentation of Ethical Appreciation (CAAE # 23709119.3.0000.0098). **Results:** CYP1B1 was overexpressed in breast cancer tissues, with 75% of patients presenting high levels of CYP1B1 protein. In rats, the total CYP levels were reduced in the vehicle group and in all groups treated with SFP and vincristine (isolated or combined), compared with naïve (no tumor). SFP inhibited the gene expression of *CYP1A2* in HepG2 cells and *Cyp1a1* in the liver of solid tumor-bearing rats. *Cyp2e1* was reduced by the SFP treatments, and *Cyp3A9* was reduced in basal group, which received SFP but did not have tumor. SFP modulated inflammatory parameters, with increased hepatic N-acetyl-beta-D-glucosaminidase (NAG) activity in rats with ascitic and solid tumor, and hepatic TNF-α levels of solid tumor-bearing rats. Additionally, SFP reduced the weight and volume of solid Walker-256 tumor compared with the control (vehicle), but did not present cytotoxic effect in ascitic tumor and HepG2 cells. **Conclusion:** Increase of inflammatory response could be responsible for the decrease of hepatic CYP levels and expression in Walker-256 solid-tumor bearing rats treated with SFP. These data indicate that SFP immune modulation effects result in CYP inhibition. Moreover, CYP1 family was affected by SFP *in vitro* and *in vivo*, thus its effect could be beneficial to breast cancer treatment, where CYP1B1 was increased in tumor tissue of patients. **Financial Support:** CAPES, CNPq, UFPR. **License number of ethics committee:** Ethical Committee for Animal Use (CEUA) of UFPR (#1065) and by Certificate of Presentation of Ethical Appreciation (CAAE # 23709119.3.0000.0098).

10.008 Thioredoxin reductase-1 levels are associated with NRF2 pathway activation and tumor recurrence in non-small cell lung cancer. Delgobo M¹, Gonçalves RM¹, Delazeri MA², Falchetti M¹, Zandoná A²,

Nascimento Das Neves R¹, Almeida Lima K¹, Fagundes AC¹, Isidro Fracasso J³, Baroni Macedo G³, Priori L³, Pens Gelain D⁴, Forcelli CM², Fonseca Moreira JC⁴, Zanotto-Filho A¹. ¹UFSC Florianópolis, Dpt of Pharmacology, Brazil; ²UPF, Passo Fundo, Dpto of Medicine, Brazil; ³HSVP, Passo Fundo, Brazil; ⁴UFRGS, Porto Alegre, Dpt of Biochemistry, Brazil

Introduction: Activating mutations in the KEAP1 / NRF2 pathway have been characterized and associated with chemoresistance and poor prognosis in a subset of non-small cell lung cancer (NSCLC) patients. To protect themselves from ROS-induced damage, cells have developed multifaceted antioxidant systems such as those involving glutathione (GSH) and the thioredoxin/thioredoxin-reductase-1 (TXNRD1/TXN) pair amid others. Due to their role in various cancer-related processes, the TXNRD1/TXN pair has emerged as a potential target for cancer therapy, especially in tumor types that are susceptible to oxidative stress. Drugs such as auranofin (AUR) have gained interest as an anticancer agent through TXNRD1 activity inhibition. We hypothesized that TXNRD1/TXN upregulation and the NRF2 pathway provide a survival advantage to lung tumors. **Methods:** We firstly evaluated the relationship between the expression of 64 oxidative stress-related genes with patient survival in 35 published lung cancer datasets. Based on this initial screening, the survival impact of the selected gene (TXNRD1) was then evaluated by immunohistochemistry (IHC) in an observational hospital-based cohort, which included 65 patients diagnosed with NSCLC at the Hospital São Vicente de Paulo (HSVP), Passo Fundo, RS, Brazil. Genetic features associated with TXNRD1 upregulation were also examined in NSCLC samples from The Cancer Genome Atlas (TCGA). Cell viability experiments (MTT assay) were carried out in the A549 lung cancer cell line. **Results:** Kaplan Meier survival screen revealed TXNRD1 as the strongest predictor of poor survival among the 64 oxidative stress-related genes evaluated. In our cohort, a high IHC score of TXNRD1 in tumors was associated with shorter disease-free survival (DFS), distant metastasis-free survival (DMFS), and overall survival (OS). Bioinformatics analysis revealed that *TXNRD1* expression correlates with NRF2 target gene signature score, and TXNRD1 overexpression overlaps with tumors harboring *KEAP1*, *NFE2L2*, and *CUL3* mutations, and *NFE2L2* amplification, but no other genetic changes typical of NSCLC. Functional cell assays revealed that high TXNRD1 in KEAP1 mutant lung cancer cells is not a major determinant of malignancy; NRF2 seems to be more relevant. KEAP1 mutant cells are resistant to TXNRD1 inhibitor AUR, which also affected neither cell migration and nor sensitivity to cisplatin. KEAP1 mutant cells compensate for TXNRD1 inhibition by upregulating NRF2 and dependence upon GSH; NRF2 knockdown and glutathione depletion sensitized cells to AUR and cisplatin. **Conclusion:** The herein presented results indicate that high TXNRD1 at diagnosis predicts shorter time to local and distal recurrences after tumor resection and platinum-based adjuvant chemotherapy, an effect possibly associated with upregulated NRF2 pathway, and not directly to a TXNRD1-dependent malignant phenotype. **Financial Support:** This study was funded by Programa de Pesquisa para o SUS [PPSUS; 17/2551-0001-408-1] to JCFM and AZF, and in part by the CAPES – Finance code 001. **Acknowledge:** CNPq, CAPES, and FAPESC research funding agencies; our core facility LAMEB/UFSC for providing equipment and technical support. **License number of ethics committee:** All aspects related to ethics in human research were approved by Institutional Ethics Committee (CEP-UFRGS and CEP SH-UFSC), reference number CAAE 83271317.1.0000.5347.

10.009 **Extracellular vesicles released by adipose tissue from obese subjects can induce epithelial-mesenchymal transition in breast cancer cells.** Ramos-Andrade I¹, De Jesus ME¹, Moraes JA^{1,2}, Renovato Martins M³, Barja-Fidalgo C¹ ¹UERJ Rio de Janeiro, Dpt of Cell Biology, Brazil; ²UFRJ Rio de Janeiro, Dpt of Basic and Clinical Pharmacology, Brazil; ³UFF Niterói, Dpt of Molecular and Cell Biology, Brazil

Introduction: Obesity is a multifactorial disease characterized by adipose tissue (AT) meta-inflammation, presenting association with several types of cancer, including breast cancer, which is the most common type among women. The AT of obese individuals, in addition to secreting pro-inflammatory adipokines, releases a greater number of extracellular vesicles (EVs), which are small membrane vesicles capable of to transfer several molecules to target cells. It has been described that EVs are involved in the tumor microenvironment modulation, as in the epithelial-mesenchymal transition (EMT), which is defined by the loss of epithelial characteristics and the acquisition of the mesenchymal phenotype, triggering the increase in tumor cells malignancy. The aim of this study was to analyze the profile of AT secretome (conditioned medium (CM) and EVs) from lean and obese subjects and to evaluate whether this obese AT secretome is able to induce EMT in two human breast adenocarcinoma cells lineages: MCF-7 (non-invasive) and MDA-MB-231 (invasive). **Methods:** Explants of subcutaneous and visceral AT were obtained from obese or lean patients (control group), who underwent to bariatric or plastic surgery, respectively. The adipokines profile released by AT were analyzed by Milliplex; Protein load in the EVs released by AT were analyzed by proteomics and Western blotting; Expression of EMT-related markers was assessed in breast tumor cells by Western blotting; Wound healing assay was performed to evaluate cell migration. **Results:** Our results have shown that, compared to lean AT, the CM from obese AT presented increased levels of pro-

inflammatory adipokines (TNF- α , IL-6, TGF- β 1, 2 and 3). Compared to lean AT, EVs released by obese AT presented higher content of proteins related to tumor progression, as S100A4, perlecan, galectin-1, and increased expression of TGF- β . The EVs released by obese AT induced a decrease in the epithelial marker E-cadherin and an increase in the mesenchymal markers α -SMA in MCF-7 cells, and vimentin in MDA-MB-231 cells, compared to EVs released by lean AT. Reinforcing the pro-EMT effect of the obese AT-derived EVs on MCF-7 cells, we also observe increasing cell migration when compared to the CM and EVs released by lean AT. **Conclusion:** Together, our data indicate that EVs-derived obese AT present proteins with pro-tumor properties that appear to be capable of inducing EMT in breast tumor cells increasing their migratory capacity and thereby increasing their malignancy. **Financial Support:** CAPES, CNPq, FAPERJ **License number of ethics committee:** CAAE 03769618.3.0000.5646

11. Clinical Pharmacology, Pharmacokinetics, Pharmacogenomics and Toxicology

11.001 **Evaluation of the treatment effectiveness of second-generation direct-action antivirals for Hepatitis C.** Silva-Neto MR¹, Ziolkowski MI¹, Santos RB¹, Mocellin LP¹, Haas SE¹ ¹Unipampa Uruguaiiana, Pharmacology Lab

Introduction: Hepatitis C virus (HCV) in the absence of treatment or spontaneous viral elimination may progress to the chronic form or cirrhosis over time, constituting a major challenge to public health. The World Health Organization (WHO) has assumed the eradication of viral hepatitis until 2030¹. In Brazil, the southern region having the second highest number of cases reported in the country. Treatment with second-generation direct-acting antivirals (DAA) showed better results with Sustained Virologic Response (SVR) rates around 90%, being incorporated as of 2015 by the Unified Health System, available to all virus carriers regardless of disease stage². This study aimed to determine the clinical and laboratory characteristics and the effectiveness of treatment with DAA. **Methods:** Medical records of patients treated at the Outpatient Clinic in Uruguaiiana/RS during a 2-year period. Statistical and multivariate analysis was performed using SPSS software. **Results:** 199 patients were analyzed. The average age was 55.08 \pm 9.91 years. 55.8% were male and 68.2% were white. The initial fibrosis grade F0-F1 was predominant (47.8%), as well as genotype 1 and its subtypes (63.3%). Post-treatment evaluation of laboratory parameters resulted in a significant decrease in AST, ALT, alkaline phosphatase, Gamma GT, total bilirubin, and glycated hemoglobin levels. There was a significant increase in total and LDL cholesterol. **Results:** In multivariable analysis, genotypes 1a and 3 were more likely to result in detectable SVR when compared to genotype 1b, and the variable cardiovascular comorbidity indicated a higher risk of undetectable SVR when compared to individuals without this comorbidity. **Conclusion:** In agreement with other studies in different countries and regions of Brazil, DAA showed a high rate of effectiveness. The adoption and expansion of access to these treatments collaborate for the country to meet the goal of the Plan for the elimination of Hepatitis C proposed by the WHO. **References:** ¹World Health Organization. Global health sector strategy on viral hepatitis 2016-2021. June 2016. ²BRASIL. Ministry of Health. Secretariat of Health Surveillance. Dpt of Surveillance Prevention and Control of STIs HIV/AIDS and Viral Hepatitis. Clinical Protocol and Therapeutic Guidelines for the Prevention of Vertical Transmission of HIV, Syphilis and Viral Hepatitis. Brasília: Ministry of Health, 2019. **Financial support:** FAPERGS **License number of ethics committee:** CAAE 92602618.3.0000.5323

11.002 **Predictions of complex drug-drug-disease interactions with carvedilol using physiologically based pharmacokinetics (PBPK).** Micheletto AL¹, Yamamoto PA², DE Moraes NV¹. ¹Unesp, School of Pharmaceutical Sciences, Dpt of Drugs and Medicines, Araraquara, Brazil ²USP, School of Pharmaceutical Sciences of Ribeirão Preto, PPG Toxicology, Ribeirão Preto, Brazil

Introduction: Complex drug-drug-disease interaction are rarely investigated in clinical trials. Physiologically based pharmacokinetic (PBPK) modelling and simulation (M&S) is a valuable approach to simulate these interactions in virtual populations. Carvedilol is a beta-adrenergic receptor antagonist commonly prescribed to treat arterial hypertension, angina, and congestive heart failure. It is mainly metabolized by CYP2D6, CYP1A2 and CYP2C9. **Methods:** A full PBPK model of carvedilol [1] was implemented on Simcyp[®] (v.20) to simulate the carvedilol x quinidine (CYP2D6 inhibitor) and carvedilol x fluvoxamine (CYP1A2 inhibitor) in adult and elderly virtual patients, in subjects with normal renal function, moderate [estimated glomerular filtration ratio (eGFR): 30-60 mL/min] or severe renal impairment (eGFR < 30 mL/min), obesity or morbidly obesity, and cirrhosis [Child-Pugh (CP) score A, B or C] after multiple doses of 25 mg of carvedilol per day. All simulations were carried out in 250 subjects each group, 20-50 years, with a proportion of 50% females. Elderly virtual patients aged 65-98 years. **Results:** The co-administration of multiple doses of 200 mg/day quinidine (Q), 36.65 mg/day fluvoxamine (F), or Q+F combined, and 25 mg/day carvedilol resulted

in 2.1, 1.3 and 2.9-fold increase in carvedilol exposure in healthy volunteers. Compared with healthy virtual subjects taking carvedilol only, concurrent moderate or severe renal impairment plus quinidine resulted in 2.2 and 2.2-fold increase in exposure. Compared with healthy virtual subjects taking carvedilol only, concurrent quinidine treatment in virtual patients with cirrhosis CP-A, CP-B or CP-C resulted in 2.1, 1.5 and 1.2-fold increase in exposure. The worst-case scenario was observed for obese or elderly patients taking quinidine, fluvoxamine and carvedilol which resulted in 3.0-fold increase on carvedilol exposure. **Conclusion:** The predictions suggest that complex drug-drug-disease interaction may significantly increase carvedilol exposure and toxicity. PBPK simulations are valuable approach for mechanistic and quantitative predictions of drug-drug-disease interactions. **Financial Support:** Sao Paulo Research Foundation (FAPESP), Process 2018/06569-9. **References:** [1] Rasool et al. Clin Pharmacokinetic, 54: 943, 2015. **License number of ethics committee:** N/A

11.003 Population pharmacokinetic modelling of tobramycin lung and epithelial lining fluid disposition due to biofilm-forming *Pseudomonas aeruginosa* infection. Dias BB¹, Carreño F², Helfer VH¹, Garzela PM¹, Barreto F³, Araújo BV¹, Dalla Costa T¹. ¹UFRGS PPG Pharmaceutical Sciences Porto Alegre, Brazil; ²Univ of North Carolina at Chapel Hill, US; ³Federal Lab of Animal and Plant Health and Inspection, Porto Alegre, Brazil

Introduction: Tobramycin (TOB) is an aminoglycoside widely used to treat biofilm-forming *P. aeruginosa* lung infection and investigating its availability at the infection site is important to assure efficacious outcomes^[1]. Population pharmacokinetic (popPK) modeling can be applied to rationalize antimicrobial use, improving treatments by allowing dose adjustments based on pharmacokinetic alterations due to infectious processes and biofilm formation^[2]. This work aimed to develop a popPK model describing TOB lung and epithelial lining fluid (ELF) pharmacokinetics in healthy and biofilm-forming *P. aeruginosa* infection. **Methods:** TOB plasma, lung^[3] and ELF^[4] concentrations were measured in healthy and *P. aeruginosa* PA14 acutely (7 d) infected Wistar rats (CEUA/UFRGS #32345). Free TOB lung and ELF samples were collected using microdialysis (CMA 20, 4 mm and 2 mm probes, mDialysis®) after TOB 10 mg/kg i.v. bolus dosing. The PopPK model was developed in NONMEM 7.4 (Icon®) using the first order conditional estimation method with interaction. **Results:** Plasma concentrations were best fitted to a two-compartment model with first-order elimination. Microdialysis lung data were incorporated into the model as a third compartment, linked to the central compartment, with an input (Q_{in}) and an output (Q_{out}) intercompartmental clearances. Typical estimate for clearance, central, peripheral and lung volumes of distribution, intercompartmental clearance to peripheral compartmental, Q_{in} and Q_{out} were, respectively: 0.0337 L/h, 0.0951 L, 0.0909 L, 0.00048 L, 0.0776 L/h, 0.00253 L/h and 0.00465 L/h (relatively standard error (RSE) <37%). Interindividual variabilities at CL and Q_{out} were estimated to be 48.9 and 88.4%, respectively (Shrinkage <18%). In order to account for the interaction between TOB and the extracellular components of the biofilm matrix^[5] a lung output clearance was included to the infected animals model, estimated as 0.00402 L/h. The lower TOB concentrations in the ELF for both healthy and infected groups was explained using a penetration factor ($P_{factor} = 0.179$; RSE 20%) derived from lung compartment. Post-hoc estimates of area under the concentration-time curve (AUC_{0-12}) showed 72% reduction in TOB exposure in the lungs of infected animals compared to healthy ones. **Conclusions:** The model was able to describe 72% reduction in infected lung concentrations, and demonstrate that TOB concentrations in ELF are around 18% of its lung concentrations, independent of the infection. Simulations can be used to determine adequate TOB doses to reach effective free lung and ELF concentrations to cure *P. aeruginosa* acute infection. **Acknowledgements:** Financial support and master scholarship from CNPq/Brazil. **References:** [1] DHANANI, J. et al. Int J Antimicrob Agents 36, 491, 2010. [2] VELDE, F de. et al. Pharmacol Res. 134, 280, 2018. [3] BERNARDI, P. M. Dissertação. UFRGS, 2016. [4] DIAS, B. B. Trabalho de Conclusão de Curso. UFRGS, 2019. [5] TSENG, B. S. et al., Environ Microbiol, 15(10), 2865, 2013. **License number of ethics committee:** CEUA/UFRGS #32345

11.004 Population pharmacokinetic model of Ceftaroline distribution to muscle and subcutaneous tissue of healthy subjects and cerebrospinal fluid of neurosurgical patients. Helfer VE¹, Zeitlinger M², Zavaski A³, Verlindo de Araújo B¹, Dalla Costa T¹. ¹UFRGS, PPG Pharmaceutical Sciences, Brazil; ²Medical Univ of Vienna, Dpt of Clinical Pharmacology, Austria; ³Hospital de Clínicas de Porto Alegre, Brazil

Introduction: Ceftaroline (CFT), has been considered for the treatment of central nervous system infections (CNS)^[1] owing to its activity against methicillin-resistant *Staphylococcus aureus* and penicillin-resistant *Streptococcus pneumoniae*^[2]. We aimed to develop a population pharmacokinetic model (popPK) to describe CFT muscular, subcutaneous, and cerebrospinal fluid (CSF) distribution in healthy and neurosurgical patients. **Methods:** Data from two studies were used^[1,2]. Briefly, 12 healthy subjects and 9 neurosurgical patients [38 yo (range 24-75 yo); BW 76 kg (range 49-107 kg)] received CFT-fosamil as

single and multiple doses [600 mg either q8h 2h IV infusion (n = 6) or q12h 1h IV infusion (n = 15)]. Total plasma, CSF and free peripheral tissue concentrations were available, totalizing 947 data points. Data were analyzed with a nonlinear mixed effects modeling approach (NONMEM®, version 7.4), with the first-order conditional estimation method with interaction (FOCEI). Age, creatinine clearance (CL_{cr}), BW, healthy status (HST), and glucose brain concentration (GLUC) were tested as covariates on the parameters of the final model. **Results:** Plasma concentrations of CFT were described by a two-compartment model, with an assumption of 100% conversion of CFT-fosamil into CFT and 20% plasma protein binding. Tissue concentration data were incorporated to the model as three compartments linked to the central compartment with bi-directional transport parameterized as CL_{in} and CL_{out}, and apparent volumes fixed as physiological interstitial values of 3.91, 2.29 and 0.15 L for muscle, subcutaneous, and CSF respectively^[3]. Typical estimates for CL, central volume of distribution (V_c), intercompartmental clearance, and peripheral volume of distribution, were, respectively, 12.8 L/h, 18 L, 4.6 L/h, and 8.1 L (RSE < 12%). CL_{in} was estimated to be 5.9 L/h, 3.3 L/h, and 1.4 mL/h and CL_{out} 11.4 L/h, 5.7 L/h, and 16.3 mL/h for muscle, subcutaneous, and CSF tissue, respectively (RSE < 40%). The IIV (CV%) applying an exponential model for CL, V_c, CL_{in,muscle}, and CL_{in,subcutaneous} was 18.4%, 16.3%, 29.9%, and 33.3%, respectively, whereas for CL_{in,CSF} and CL_{out,CSF}, was 57.6% (Shrinkage < 40%). HST and BW were identified as significant covariates for V_c and CL_{cr} for CL. GLUC was significantly correlated with CL_{in,CSF}. A decrease of GLUC leads to an increase in the penetration ratio of CFT to CSF, suggesting that, in cases of meningeal inflammation, the penetration would be much greater than the observed (9%). **Conclusion:** The popPK model was able to describe tissues concentrations in healthy and neurosurgical patients. The inverse correlation of GLUC and CL_{in,CSF} indicates that in cases of CNS infections, CFT could achieve efficacious concentrations. **Financial Support:** CAPES/Brazil. **References:** 1. Chauzy A et al. J Antimicrob Chemother 74, 675, 2019. 2. Matzneller P et al. Antimicrob Agents Chemother 60, 3617, 2016. 3. Johanson C E et al. Cerebrospinal Fluid Research 5, 1, 2008. **License number of ethics committee:** Ethics Committee of the Medical University of Vienna (reference 1930/2012) and Comité de Protection des Personnes Est-I (reference 2014/56)

11.005 Pharmacogenetic testing in psychiatry and cardiology: strategies for obtaining robust scientific data, and for building overview of systematic reviews

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Introduction: Pharmacogenetic testing (PGx) is a tool for precision medicine, guiding drug selection and preventing adverse events. An overview of systematic reviews of PGx tests for relevant drug-gene pairs in the therapeutic areas of psychiatry and cardiology was performed. **Methods:** A search for systematic reviews was carried out in the Cochrane Library, Embase and PubMed, without period or language limitations. The selection process was conducted by two authors independently. The overview of systematic reviews included in this study was conducted as recommended by the Cochrane Collaboration, and quality assessments were performed for each review by two authors, using A Measurement Tool to Assess Systematic Reviews (AMSTAR-2). **Results:** Thirty systematic reviews were included. The most studied drug categories were anticonvulsants, selective serotonin reuptake inhibitors, P2Y12 inhibitor and antithrombotic. These drugs were analyzed in association with the human leukocyte antigen (HLA-A, HLA-B), cytochrome P450 (CYP2C9, CYP2D6, CYP2C19), and vitamin K epoxide reductase complex subunit 1 (VKORC1) genes. Although we only included systematic reviews, it is noteworthy that the majority (n = 21, 70%) did not specify the type of primary study included. In addition, many had significant methodological issues, classified as critically low (n = 17, 57%) or low quality (n = 8, 27%). **Conclusion:** Few systematic reviews were conducted with high methodological rigor. There is an opportunity for improvement, both in the selection of primary studies and in their evaluation, so that the certainty of evidence can be assessed. Primary studies need to be more robust and have adequate design, in order to support decision-making on the implementation of pharmacogenetics in health services and systems. **Acknowledgments:** Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPQ (grant number: 141302/2020-8), Fundação de Amparo à Pesquisa do Estado de São Paulo – FAPESP (grant number: 2019/08338-7). **License number of ethics committee:** N/A

11.006 Transgenerational reproductive effects (F2) in male offspring mediated by F0 generation exposure to Benzo(A)Pyrene from juvenile period to peripuberty in rats. Jorge BC¹, Reis ACC¹, Stein J¹, Paschoalini BR¹, Nogueira JB¹, Moreira SS¹, Manoel BM¹, Arena AC^{1,2}. ¹Dpt of Structural and Functional Biology. PPG Pharmacology and Biotechnology, Inst of Biosciences of Botucatu, Univ. Estadual Paulista-Botucatu, Brazil ²Center of Toxicological Assistance, Inst of Biosciences of Botucatu, Univ. Estadual Paulista-Botucatu, São Paulo, Brazil

Introduction: Benzo(A)pyrene (BaP) is a chemical substance formed by the union of a molten benzene into a pyrene produced by the incomplete burning of organic compounds (US-EPA, 2017). Because it is ubiquitous, it can cause adverse events and behave as an endocrine disruptor at low doses (Jorge et al., 2021); also targeting the reproductive system. Puberty, a critical period of development, is vulnerable to the effects of substances, leading to effects throughout life or in next generations. Our objective was to evaluate the transgenerational (F2) reproductive repercussions in male rats offspring which the paternal (F0) generation was exposed to benzo(a)pyrene during the juvenile period and peripuberty. **Methods:** For this, juvenile male Wistar rats (23 days, n = 16 animals/group) were allocated in control group (corn oil + DMSO - vehicle) and BaP (vehicle + 0.1 µg/kg) for 31 consecutive days (gavage). Half of these animals were killed for immediate toxicological and testicular evaluation (n = 8 animals/group). In adult life, the other half was mated with untreated females to obtain male offspring (F1) and, later, the F2 generation, which was evaluated in relation to their initial development and reproductive parameters (n = 16 litters/group). All data obtained in this analysis were submitted to statistical tests. **Results:** There was a reduction in anogenital distance in post-natal days (PND) 1, 13 and 22, and body weight (PND 1), advanced preputial separation and decreased body weight in this day. In adulthood, there were no changes in male sexual behavior and fertility parameters. But there was a reduction in body weight, a reduction in type A sperm (progressive motile) and increase in type C sperm (immobile) and epididymal transit time. **Conclusion:** We can conclude that F2 generation showed altered reproductive parameters, probably due to changes in the germ cells of the generation directly exposed to benzo(a)pyrene. Ethics committee, nº 1148/2019. **Financial Support:** CNPq - 140826/2019-0 and FAPESP - 2019/03264-5 **References:** US-EPA. Toxicological Review of Benzo(a)pyrene. n. January, 2017. Jorge, et. al. Chemosphere, 263 (2021) 128016. **License number of ethics committee:** nº 1148/2019

12. Drug Discovery and Development

12.001 **Synthesis of galectin-3 inhibitors with applications in neurobehavioral disorders.** Leite FT, Campo VL. Centro Universitário Barão de Mauá, Brazil

Introduction: The dissemination of COVID-19 caused by SARS-CoV-2 virus, from the end of 2019, resulted in several deaths, requiring government actions to control the disease in the population, including sanitary blocks and social distance. Although the actions have varied in severity between and within countries, they have changed the daily lives of people around the world, bringing consequences in health, economy, politics and society [1]. Due to the many adverse effects of COVID-19, the rate of mental disorders is increasing fastly. Therefore, neurological disorders such as anxiety and depression are affecting thousands of people and calls public health concern. These neurological disorders are among the most common diseases experienced by young people and adults, affecting people in different ways and causing a wide variety of symptoms [2]. In neurobehavioral disorders, such as anxiety and depression, there is an increase in a specific lectin, known as galectin-3, which is associated with a prognosis in patients affected by these disorders. Thus, considering the influence of this lectin on such disorders, synthetic drugs able to inhibit this galectin may represent an important therapeutic strategy [3]. In this context, this work presents the synthesis of galectin-3 inhibitors represented by triazole galactosyl arylsulfonamides 1 and 2. **Methods:** The compounds 1 and 2 were synthesized by routes involving the preparation of azide and alkyne precursors, followed by cycloaddition reactions to afford triazole derivatives [4]. All obtained compounds were purified by column chromatography and characterized by NMR spectroscopy. **Results:** The synthesis of the triazole galactosyl arylsulfonamides 1 and 2 involved the previous preparation of the alkyne-sugar propynyl-βGal 3 and azide-aryl-sulfonamides 4 and 5. The sugar 3 was obtained in 4 sequential steps involving isopropylideneation, nucleophilic reaction with propargyl bromide, deprotection with TFA and acetylation reaction with pyridine and acetic anhydride, being obtained in 30% overall yield [5]. Subsequently, the synthesis of azide-aryl-sulfonamides 4 and 5 was carried out by reactions with sodium azide and urea, affording compounds 4 and 5 in corresponding yields of 90% and 45%. Once the alkyne and azide precursors were obtained, the next step was the cycloaddition reactions between the alkyne-sugar 3 and azide-aryl-sulfonamides 4 and 5, which were realized in a sealed microwave tube, in DMF, and in the presence of CuSO₄/ sodium ascorbate as a catalytic system. The reactions were carried out under microwave irradiation, at 100°C (150 W) for 15 minutes [4]. Under these conditions, the final triazole galactosyl arylsulfonamides 1 and 2 were obtained in 49% and 30% yields, respectively, after purification by column chromatography. **Conclusions:** The applied synthetic methodologies were effective to afford the novel galectin-3 inhibitors represented by triazole galactosyl arylsulfonamides 1 and 2. These compounds will be submitted to neurobehavioral tests utilizing depression and anxiety models, and may represent important tools for the investigation of new therapies against these disorders, which will be highly relevant, especially in this pandemic period. **Financial Support:** FAPESP (São Paulo Research

Foundation). **References:** [1] VELAVAN, T. P. *Tropical Medicine & International Health*, v. 25, p. 278, 2020. [2] ASHRAF, G. M. *Frontiers in Neuroscience*, v. 12, p. 1, 2018. [3] KAJITANI, K. *Psychopharmacology*, v. 234, p. 2919, 2017. [4] MARCHIORI, M. F. *Bioorganic & Medicinal Chemistry*, v. 25, p. 6049, 2017. [5] KARTHA, K. P. R. *Tetrahedron Letters*, v. 27, p. 3415, 1986. **License number of ethics committee:** N/A

12.002 **Cytotoxic effects of lipid nanoparticles for sustained release of fenretinide and *in vivo* localization in the mammary tissue.** Malagó ID, Salata GC, Lopes LB. USP São Paulo, Inst of Biomedical Sciences, Dpt of Pharmacology, Brazil

Introduction: Chemoprevention is an important strategy to reduce breast cancer incidence, but the options available cause severe adverse effects, leading to a decrease in patient adherence. Therefore, there is an urgent need for developing new chemopreventive strategies to this disease¹. In this study, nanostructured lipid carriers (NLCs) were developed for intramammary administration and sustained local release of fenretinide, a synthetic retinoid that has shown strong potential for breast cancer prevention².

Methods: The NLCs were composed of glyceryl dibehenate, trycaprilin, polysorbate 80, Span 80, perillyl alcohol (POH) and water. Fenretinide was incorporated at 1% and we investigated the *in vitro* cytotoxic effects of NLCs in T47D breast cancer cells through the MTT assay over 48 and 72 h. For the *In Vivo* localization study, NLCs stained with Alexa Fluor 647 were administered in the mammary tissue of female rats and monitored using the IVIS Spectrum imaging system. Histological analysis was conducted after staining with hematoxylin and eosin to assess local irritation and tissue alterations mediated by the NLCs administration. **Results:** The NLCs displayed size of $376,4 \pm 35,2$ nm (with polydispersity index <0.2) and zeta potential of $-31,2 \pm 1,9$ mV. The nanocarrier cytotoxicity increased with treatment time. After 72 h, the unloaded nanocarrier reduced cell viability below 50% only when used over 10 mg/mL ($IC_{50}=10.3$ mg/mL). As expected, co-encapsulation of fenretinide and POH increased the nanocarrier cytotoxicity and reduced IC_{50} to 0.5 mg/mL, which corresponded to 12.9 μ M of fenretinide. Compared to a drug solution ($IC_{50}=11.9$), encapsulation of fenretinide in this NLC did not alter its cytotoxicity, despite the slower release of the drug. The NLCs were capable of forming a depot in the mammary tissue of the animals that persisted over 30 days, indicating the nanocarrier ability to prolong the release of encapsulated compounds. Histological analysis showed no signs of infiltration of inflammatory cells or changes in the tissue architecture after NLC administration, suggesting its overall safety. **Conclusion:** Fenretinide encapsulation did not preclude its cytotoxic effects in breast cancer cells. The NLCs were able to form a depot and prolonged the release of Alexa Fluor for 30 days when administered in the breast tissue of rats without signs of irritation or other tissue alterations, suggesting their potential safety and applicability for prolonged release upon mammary tissue administration. **Funding:** FAPESP (grants #2018/13877-1 and #2020/04633-1). **References:** 1. Cazzaniga, M. et al. Breast cancer chemoprevention: old and new approaches. *J Biomed Biotechnol* 2012, 2012. 2. Cazzaniga, M. et al. Fenretinide (4-HPR): a preventive chance for women at genetic and familial risk? *J Biomed Biotechnol* 2012, 2012. **License number of ethics committee:** The *In Vivo* localization study protocol was approved by the Institute of Biomedical Sciences Animal Use Ethical Committee in accordance with project #4906211117.

12.003 **Analysis of the anti-inflammatory potential of novel of new phenylbenzohydrazides.** Souza, ACN¹ Paiva JPB¹, Carvalho PR¹, Branco LOP¹, Lima EC², Fernandes, PD¹ ¹Federal Univ of Rio de Janeiro, Inst of Biomedical Science, Lab of Pain and Inflammation. Rio de Janeiro, Brazil ²Federal Univ of Rio de Janeiro - Macaé, Dpt of Chemistry, Lab of Catalysis and Synthesis of Bioactive Substances. Macaé, Brazil

Introduction: Inflammation is a beneficial response of the body to an infectious agent or injury. However, if occurring in higher intensity it can cause damage to the host. Non-steroidal and steroidal anti-inflammatory drugs are the gold treatment. However, the amount and intensity of the adverse effect limit their use. In this context, the objective of this study is to evaluate the anti-inflammatory activity of new phenylbenzohydrazides designed through molecular simplification of the arylidenehydrazinyl-quinazolinones.

Methods: Female Swiss Webster mice (28-32g, n=6) were used in carrageenan-induced cell migration (SAP) model. Molecular simplification between isatoic anhydride (AISTC) and arylidenehydrazinyl-quinazolinones resulted in two new phenylbenzohydrazides, INL06 and INL07. The substances INL06, INL07, AISTC (10, 30, 100 μ mol/kg) or saline were oral administered to mice 1h before injection of carrageenan (0,5%, 1 mL) in SAP. After 24h mice were euthanized and exudate collected for further measurements. A possible mielo and haematological toxicity was evaluated by counting bone marrow and blood cells from mice treated with higher doses. Protein quantification was done in exudate collected from SAP. Results are presented as media \pm SD. Statistical analysis were performed by ANOVA followed by Bonferroni test ($*p<0.05$). **Results:** None of the substances caused any hematological changes or myelotoxicity. INL06 and INL07 significantly inhibited cell migration at 30 and 100 μ mol/kg doses (vehicle-treated group: $78.3 \pm 13.7 \times 10^6$ cells/mL versus dexamethasone: $37 \pm 7.4 \times 10^6$ cells/mL; INL06: 10 μ mol/kg =

63.0±20.1⁶cells/mL; 30 µmol/kg = 47.3±14.9*⁶x10⁶cells/mL; 100 µmol/kg: 49.3±11.7*⁶x10⁶cells/mL. INL07: 10 µmol/kg = 81.8±19.8⁶cells/mL; 30 µmol/kg = 53.8±21.4*⁶x10⁶cells/mL; 100 µmol/kg = 51.01±9.9*⁶x10⁶cells/mL). AISTC did reduce cell migration only at higher dose (10 µmol/kg = 46.5±23.5⁶cells/mL; 30 µmol/kg = 57.8±16.1x10⁶cells/mL; 100 µmol/kg = 24.8±14.0*⁶x10⁶cells/mL). It is interesting to note that all substances significantly reduced protein extravasation (vehicle-treated group: 654.1±155.9 µg/mL versus dexamethasone: 343.3±69.5*µg/mL; INL06: 10 µmol/kg = 319.5±170µg/mL; 30 µmol/kg = 253.4±89*µg/mL; 100 µmol/kg = 230.6±97.5*µg/mL; INL07: 10 µmol/kg = 192±71*µg/mL; 30 µmol/kg = 113.6±57.1*µg/mL; 100 µmol/kg = 206.9±38.9*µg/mL; AISTC: 10 µmol/kg = 417.2±90.1*µg/mL; 30 µmol/kg = 363.2±150.8*µg/mL; 100 µmol/kg = 313.9±129.6*µg/mL. **Conclusion:** The new phenylbenzohydrazides synthesized (INL06 and INL07) showed significant effect in the preclinical model used demonstrating better effect than the isatoic anhydride (AISTC). Data suggest that both substances are potential compounds to further studies in other models of inflammation. **Acknowledgments:** Alan Minho for technical assistance and Institute Vital Brazil for animal donation. **Financial Support:** CAPES, CNPq and FAPERJ **License number of ethics committee:** CEUA/UFRJ 35/19.

12.004 **LQFM 247, a synthetic derivative of anandamide, has antioxidant activity *in vitro*.** Jesus FSD¹, Farias ERA¹, Souza RRLS¹, Pereira RM¹, Campos HM¹, Port's NMS², Orellana AMM², Marques TR³, Scavone C², Menegatti R³, Ghedini PC¹, Leite JA¹. ¹Dpt of Pharmacology, Inst of Biological Sciences, Univ Federal de Goiás, Goiânia, Brazil ²Dpt of Pharmacology, Inst of Biomedical Sciences, Univ of São Paulo. ³Lab de Química Farmacêutica Medicinal, Faculdade de Farmácia

Introduction: Oxidative stress and neuroinflammation are important pathological factors involved in the development of neurodegenerative diseases such as Parkinson's and Alzheimer's diseases. As physiological signaling molecules, reactive oxygen species (ROS) are important in different biological processes. However, excessive amounts of ROS overload the antioxidant defense system, compromising cellular integrity and functions, which can lead to neuronal death. It has been shown that alterations in the endocannabinoid signaling pathway can be triggered during tissue damage by oxidative stress and inflammation, in addition to anandamide has shown an antioxidant and neuroprotective potential due to its CB1 receptor agonistic action. The compound LQFM 247 was synthesized from anandamide by a molecular hybridization strategy. **Aim:** In this context, the work aimed to evaluate the antioxidant capacity of the synthetic derivative of anandamide *in vitro*. **Methods:** Initially, the cytotoxicity of different concentrations of LQFM 247 (0.1; 1; 10 and 100 µg/ml) was evaluated in primary cultures of rat glial cells using the MTT assay. In addition, the whole brain of rats was used to determine the effect of different concentrations of LQFM247 on the activity of catalase (CAT) and superoxide dismutase (SOD) enzymes *in vitro*. All procedures were The research was approved by the Committee on Ethics in Animal Use (CEUA/USP-SP n°74/2017 and CEUA/UFG n° 014/21). **Results:** The results showed that LQFM247 at the different concentrations evaluated did not show a cytotoxic effect in the primary culture of glial cells. Furthermore, it was observed that concentrations of 0.1; 1; 10 and 100 µg/ml increased the *in vitro* activity of the CAT enzyme by 36%; 46%; 50% and 72% respectively, as well as the same concentrations were able to increase the SOD enzyme activity by 23%; 34%; 34% and 41%, respectively. **Conclusion:** Our preliminary results may suggest that LQFM247, new synthetic derivative of anandamide, had an antioxidant effect *in vitro*. **Financial Support:** CNPq. **License number of ethics committee:** All procedures were The research was approved by the Committee on Ethics in Animal Use (CEUA/USP-SP no74/2017 and CEUA/UFG n° 014/21).

12.005 **Effect of the mimetic peptide Ac9-22 derived from Annexin A1 on skeletal muscular function after myotoxicity induced by a bothropic venom.** Alecrim NN¹; Damico MV ¹; Icimoto MY²; Escalante T³; Moreira V¹ ¹EPM-Unifesp São Paulo, Dpt of Pharmacology, Brazil ²EPM-Unifesp São Paulo, Dpt of Biophysics, Brazil ³Universidad Costa Rica, Inst Clodomiro Picado, San José, Costa Rica

Accidents with bothropic snake venoms lead to local clinical signals, as myonecrosis and prominent inflammatory reaction, accompanied by systemic manifestations. Myonecrosis is caused by components of venom, as phospholipase myotoxins and metalloproteinases, which cause necrosis of muscle fibers and affect the vascular network surrounding the muscle injury. These tissue-damaging effects lead to an unsuccessful regeneration process and permanent sequelae due to fibrosis and loss of tissue function. Auxiliary drugs for the treatment of envenoming are a recurrent target of interest, as the bothropic antisera are effective in neutralizing the systemic but not the local effects caused by venom. Among the possibilities of new bioactive molecules, there are peptides derived from Annexin A1 (AnxA1), an endogenous protein secreted by several cells and exerts anti-inflammatory effects, maintenance of cytoskeleton and extracellular matrix integrity, in addition to stimulation of proliferation and cell differentiation. The aim was to analyze the regulatory role of a mimetic peptide of AnxA1 on muscle tissue contractile activity, during the regenerative phase, induced by a bothropic venom. Distinct groups

of male Swiss mice (CEUA 1996230920) (30g) received intramuscular (i.m) injection of Bothros asper venom (BaV) (50 μ g/50 μ L) in the right gastrocnemius muscle, and 5 μ L of saline solution (SS) in the left limb muscle. After 30 min, 24, 48 and 72h of i.m injection, groups were treated intraperitoneally with peptide Ac9-22 (1mg/kg/500 μ L) or SS (control). After 28 days, mice were euthanized and both muscles were collected and processed for analysis of contractile function. Gastrocnemius were submerged in Tyrod buffer and connected to isometric voltage transducer coupled to PowerLab® amplifier and analyzed for: i) isometric twitch contractions; ii) curve of contraction versus frequency, and iii) fatigue curve. Data showed that BaV/SS animal muscles presented increase of muscle contractile resistance ($p < 0.05$; $5 \leq n \leq 8$) in 3 (81.25 \pm 3.35; mean \pm SEM, in %), 4 (64.75 \pm 3.35), 5 (53.99 \pm 3.07), 6 (45.75 \pm 3.24), 7 (40.16 \pm 3.15), 8 (36.39 \pm 3.19) and 9 (33.41 \pm 3.02) min of the test, when compared to SS/SS (67.00 \pm 3.18; 48.44 \pm 2.65; 36.18 \pm 2.08; 31.36 \pm 2.20; 26.62 \pm 2.33; 23.23 \pm 2.07; 21.94 \pm 1.78; mean \pm SEM, in %). However, BaV/Ac9-22 muscle present significant less level of resistance ($p < 0.05$; $5 \leq n \leq 8$) in 5 min (41.96 \pm 5.32 mean \pm SEM, in %), in comparison to BaV/SS (53.99 \pm 3.07 mean \pm SEM, in %), and similar values of resistance to showed by SS/SS group. Regarding to other applied protocols, isometric twitch contractions and the force versus frequency, no statistical difference were observed among distinct groups ($5 \leq n \leq 8$). These preliminary results suggest that although administration of the mimetic peptide Ac9-22 does not influence muscle endurance, it may be responsible for regulating mechanisms that lead to the formation of new muscle fibers content, such as Type II fiber, which are fast fibers and compose physiological constitution of gastrocnemius muscle. Therefore, treatment with this ANX A1 mimetic peptide leads to the early functional composition of skeletal muscle after tissue damage induced by the BaV. Financial support: FAPESP and Capes. **License number of ethics committee:** CEUA 1996230920

12.006 Pre-clinical evaluation of the anti-inflammatory effects of novel capsaicin-curcumin hybrid molecules. Paiva JPB¹, Carvalho PR¹, Etienne R², Viegas Júnior CV², Fernandes PD¹ ¹Federal Univ of Rio de Janeiro, Inst of Biomedical Science, Lab of Pain and Inflammation, PPG in Pharmacology and Medicinal Chemistry. Rio de Janeiro, Brazil ²Federal Univ of Alfenas, Medicinal Chemistry Research Lab. Minas Gerais, Brazil

Introduction: Inflammation is a beneficial response of the body to a tissue injury, infection or invasion of microorganisms and proposes to maintain the homeostasis and integrity of the injured tissue. The inflammatory process is characterized by a series of cellular and vascular reactions that serve to repair tissue damage. However, when this response is exacerbated, it can have detrimental effects on the organism, thus leading to the onset of pathogenesis. Due to several side effects of non-steroidal anti-inflammatory drugs the continue search for new substances is still a goal for researchers. In this respect capsaicin and curcumin was firstly described with significant anti-inflammatory properties. A hybridization between capsaicin and curcumin resulted in PQM310 and PQM311. So, the aim of the present work was to evaluate the anti-inflammatory effects of both substances using a traditional method of inflammation.

Methods: Male Swiss Webster mice (28-32g, n=6) were used in model of carrageenan-induced cell migration into the subcutaneous air pouch (SAP). Mice were orally treated with PQM310 or PQM311 (1, 3 or 10 mg/kg) 1 hour before carrageenan (0.5%, 1 mL) or saline (NaCl 0.9%, 1 mL) injection into SAP. After 24 hours mice were euthanized, BAS was injected with 1 mL saline and exudate collected for leukocyte count, cytokines (IL1 β and TNF- α) measurements (. Results are presented as media \pm sd. Statistical analysis were performed by ANOVA followed by Bonferroni test ($*p < 0.05$). **Results:** PQM310 (1, 3 or 10 mg/kg) inhibited leukocyte migration in a dose-dependent manner: vehicle-treated group: 79.0 \pm 20.3 $\times 10^6$ cells/mL; PQM310: 1mg/kg: 39.6 \pm 4.3 $\times 10^6$ cells/mL; 3mg/kg: 32 \pm 11.9 $\times 10^6$ cells/mL; 10mg/kg: 19.3 \pm 7.2 $\times 10^6$ cells/mL. PQM311 also inhibited leukocyte migration into SAP at all doses: 1mg/kg: 37.9 \pm 6.5 $\times 10^6$ cells/mL; 3mg/kg: 41.9 \pm 13.7 $\times 10^6$ cells/mL; 10mg/kg: 30.7 \pm 13.2 $\times 10^6$ cells/mL. Both compounds significantly reduced the production of the cytokine IL-1 β . 93,7 \pm 22,9 pg/ml in saline-injected in SAP; 774.14 \pm 249.9 pg/mL in carrageenan-injected in SAP and orally treated with vehicle, and PQM310: 1mg/kg: 130.8 \pm 73.9*pg/mL; 3mg/kg: 119.1 \pm 49.8*pg/mL; 10mg/kg: 380.2 \pm 131.8*pg/mL and PQM311: 1mg/kg: 180.1 \pm 82.4*pg/mL; 3mg/kg: 244.6 \pm 36.1*pg/mL; 10mg/kg: 164.6 \pm 97.7*pg/mL. the quantification of TNF- α indicated that PQM310 almost completely abolished the production of this cytokine: control (saline-injected in SAP): 365 \pm 224 pg/mL *versus* 1,175 \pm 466 pg/mL in carrageenan-injected in SAP and orally treated with vehicle, and PQM310: 1mg/kg: 36.8 \pm 7.8*pg/mL; 3mg/kg: 32 \pm 11.9*pg/mL; 10mg/kg: 23.3 \pm 12.5*pg/mL. PQM311: 1mg/kg: 751.3 \pm 85.8*pg/mL; 3mg/kg: 631.3 \pm 187.6*pg/mL; 10mg/kg: 692 \pm 132.5*pg/mL. Inhibitory effect of PQM311 was also significant when compared with mice pretreated with dexametasone (464 \pm 261 pg/mL). **Conclusion:** Our data suggest that both substances present anti-inflammatory activity since both reduced cell migration and production of cytokine IL-1 β . Further assays are under development to characterize their mechanism of action. **Financial Support:** CAPES, CNPq, FAPERJ

and Institute Vital Brazil (donation of mice) **License number of ethics committee:** The protocol for use of animals was approved by CEUA/UFRJ: 35/19.

12.007 Targeting myeloperoxidase ameliorates edema in a gouty arthritis model: discovery of inhibitors with biological activity through virtual screening. Matos IDA¹, Dallazen JLD², Costa SKP², Meotti FCM¹ ¹Dpt of Biochemistry, Inst of Chemistry, Univ of São Paulo ²Dpt of Pharmacology, Inst of Biomedical Sciences, Univ of São Paulo

Introduction: Myeloperoxidase (MPO) is a key enzyme present in neutrophils and has an important role during inflammatory conditions. This enzyme converts hydrogen peroxide and chloride to hypochlorous acid (HOCl), a strong oxidizing agent. MPO activity is elevated in gout, although neutrophils are not involved in the resolution of this inflammatory condition, showing that MPO is a promising pharmacological target for the develop of new drugs to treat gouty arthritis. Thus, the aim of this study was to discover new MPO inhibitors with *In Vivo* activity. **Methods:** Molecular properties of 143 know MPO inhibitors was analyzed and an inhibitor-like rule elaborates. Application this rules in Zinc15 database recover 6546 compounds with good theoretical pharmacokinetic properties, that after a structure-based virtual screening selects 28 potential inhibitors. These compounds were validated by enzymatic and cell assays. Four compounds named IRL6, IRL7, IRL21, and IRL22 (Specs, Holland) were tested in a murine model of gouty arthritis (CEUA 100/2018). Male C57BL6 mice (7-week-old, ~25 g) were intraperitoneally pretreated with vehicle, mefenamic acid (MEF: 30 mg/kg), IRL6 (3, 10, and 30 mg/Kg), IRL7 (0.3, 3, and 30 mg/Kg), IRL21 (3, 10, and 30 mg/Kg), or IRL22 (3, 10, and 30 mg/Kg) 15 min before the intraplantar injection of monosodium urate crystals (MSU: 1.5 mg/30 μ L, i.pl.), or its vehicle (sterile PBS, 30 μ L, i.pl.). The paw edema was measurements using pletismometer (mL) for 6 hours after MSU injection. The results were evaluated in AUC of antiedematogenic effect (mL/h) obtained from each compound. **Results:** Enzymatic assays by measuring both, peroxidase and chlorinating MPO activity, indicated that 60% of the hits were able to inhibit MPO activities. The dose-response curve of the five best compounds shows IC₅₀ between 0.3 and 16 μ M and a reversible inhibition. Cell assays shows that the inhibitors also prevented HOCl production by neutrophil-like dHL-60 cells and by peripheral blood neutrophils human at the same range as known irreversible inhibitors. The intraplantar injection of MSU induced paw edema since 2 h after its administration, until 5 h of evaluation, comparing to vehicle injected group. The pretreatment with MEF reduced in 67.58% the paw edema, comparing to vehicle treated group (V: 0.29 \pm 0.03 mL/h). IRL6 pretreatment reduced paw edema in 59.65% and 61.72% with 10 and 30 mg/kg, respectively. IRL7 reduced in 61.37% and 66.89% the paw edema at 3 and 30 mg/Kg, respectively. IRL21 reduced in 51.37%, 60.34% and 88.96% the paw edema, with 3, 10, and 30 mg/Kg, respectively. IRL22 reduced the paw edema in 69.31% only with 30 mg/Kg. **Conclusion:** Our results demonstrate that the virtual screening leads us to the discovery of MPO inhibitors with systemic activity that produce antiedematogenic effects on mouse model of gouty arthritis. **License number of ethics committee:** 100/2018

12.008 Effects of naturally occurring and synthetic indole molecule in the open field and conditioned place preference paradigms. Heidrich N¹, Bif TF¹, Feddern CF^{2,3}, Silva IA da², Fiore RL², Fonseca AR^{2,3}, Almeida FB¹, Fernandes PR¹, Freese L¹, Barros HMT^{1,2} ¹UFCSPA Porto Alegre, PPG Health Sciences, Brazil; ²UFCSPA Porto Alegre, Behavioral Neurosciences Lab, Brazil; ³UFRGS Porto Alegre, Dpt of Pharmacology, Brazil

Substance-related disorder has always been an issue for public health. Cocaine is a strong stimulant with great capacity for causing addiction. However, current therapies, which include cognitive-behavioral approaches and some pharmacological antagonists, are specific for one type of drug, but do not work well for every kind of drug, or do not consider patients' individualities (Kampman, 2019). These could account for failure rates of therapies for substance dependent patients, which leads to a search for more effective and more specific therapies. Considering this, a naturally occurring indole molecule, ibogaine, and a synthetic indole molecule, SM7, have been tested in male and female rats, regarding its potential to affect locomotor activity and place preference behaviors in association with cocaine, and its cytotoxicity profile. Wistar albino male (n=58) and female (n=57) rats on postnatal day (PND) 21 were allocated in standard housing. At PND 50, a classical CPP was initiated. Pretesting and testing lasted 15 min, while conditioning sessions lasted 30 min. Treatment was administered on day PND 61 (Testing) and 62 during open field test (OF) around 5 hours before procedures: vehicle (VEH: DMSO 80% + saline 20%); ibogaine (IBO): 40 mg/kg; SM7: 10 mg/kg. Vaginal smear cytology was done daily to verify the female estrus cycle. Experiments were approved by UFCSPA's Ethics Committee on the Use of Animals (#233/18). Neither of the two molecules has shown effects on time spent in center or defecation, however, locomotion showed differences for males between IBO and the other groups (Means: IBO = 69.15; VEH = 151.7; SM7 = 135.8; p < 0.00), but no difference was shown between VEH and SM7. Rearing counts were greater for

VEH group compared to SM7 group, as well as VEH compared to IBO group (VEH = 27.5 vs SM7 = 22.5; $p = 0.025$. VEH vs IBO = 9.2; $p < 0.001$). There were differences also for cocaine exposed and cocaine-naïve males (COC = 22.4; COC-Naïve = 17.07; $p < 0.001$). For females, locomotion showed differences between IBO and the other groups (Means: VEH = 161.7; SM7 = 156.3; IBO = 107.461; $p < 0.001$), but not VEH vs. SM7. Rearings also showed similar differences (VEH = 27; SM7 = 23.4; IBO = 14.083; $p \leq 0.001$). None of the molecules showed to potentiate or reduce cocaine's reinforcing properties. The cytotoxicity profile has revealed no changes in viability for SM7 in higher concentrations when compared to the NC group (Medians: NC = 1,014; 200 mM = 0,727; 400 mM = 0,576; $p < 0,001$). These results indicate there is little evidence of a toxicity of SM7 to the central nervous system (CNS), although there seems to be an influence on locomotion and emotionality, considering rearing counts, indicating serotonergic synergy. IBO changed locomotion and rearings, as expected, but did not detain movement. More studies should be performed to evaluate if this molecule could influence other aspects of behavior *in vivo*. **Financial Support:** CAPES, CNPQ, UFCSPA. **License number of ethics committee:** UFCSPA's Ethics Committee on the Use of Animals (#233/18)

12.009 Positive screening for new antiepileptic drugs induced by kainic acid and maximal electroshock in Wistar pups. Feuerharmel F1, Silva RB2, Schneider PH2, Schneider JMFM1, Barros HMT1; ¹Federal Univ of Health Sciences of Porto Alegre, Porto Alegre, Brazil; ²Inst of Chemistry, Federal Univ of Rio Grande do Sul, Porto Alegre, Brazil

Introduction: Epilepsy is a chronic disorder and about 25% of affected children develop drug-resistant seizures. Uncontrolled seizures and chronic use of some antiepileptic drugs (AED) may be responsible for neuronal damage and developmental delay in children. Despite the discovery of new AED, there is still a need for development of AEDs not only to overcome drug-resistant epilepsy but also to provide neuroprotection. **Methods:** We performed a screening for antiepileptic effects of five new selenobenzimidazoles (BZiSe) compounds using the kainic acid (KA) induced seizures (N=118) and maximal electroshock seizures (MES) (N=124) in Wistar rat pups. The doses of 1, 2, 4 and 8mg/kg were evaluated for each BZiSe in comparison to negative control for both models. The compounds were injected intraperitoneally (ip) 30 minutes before the administration of KA ip or electrical stimulation. Latency for seizures was evaluated on the KA model with Kruskal-Wallis chi-squared test followed by Dunn's test with Benjamini-Hochberg correction as post-hoc analysis. The presence or not of severe seizures was evaluated on the MES model with Fisher's exact test followed by pairwise comparison using Benjamini-Hochberg correction for multiple comparisons. The study was approved by the UFCSPA Ethics Commission on Animal Experimentation (Protocol 188/16). **Results:** Three of the five BZiSe compounds showed activity against seizures by enhancing latency for KA minimal clonic seizures (Dose 4mg/kg of BZiSe a, $p=0.03$; dose 8mg/kg of BZiSe b, $p=0.04$; and dose 8mg/kg of BZiSe e, $p=0.03$) and one compound decreased the number of maximal electroshock severe seizures (BZiSe b, $p=0.03416$). **Conclusion:** The BZiSe compounds showed efficacy effect against seizures for both animal models revealing potential new AEDs. Since previous benzimidazoles have already shown anti-inflammatory properties and Selenium is enrolled in antioxidant activity, we believe that these molecules might provide not only antiepileptic effect, but also neuroprotection, that still needs to be evaluated. In addition, this study brings light into the preclinical research for antiepileptic drugs because animal models using neonatal pups are infrequently studied, which leaves the neonatal epilepsy seizures uncovered in preclinical research for antiepileptic drugs and their likely adverse effects. The authors declare no financial support. HMTB receives a CNPQ 1B researcher scholarship. **License number of ethics committee:** The study was approved by the UFCSPA Ethics Commission on Animal Experimentation (Protocol 188/16)

12.010 Fenretinide-loaded microemulsions reduce cell migration, spheroid growth and breast cancer development in a chemically induced carcinogenesis model. Salata GC, Malago ID, Costa SKP, Marçal-Pessoa AF, Lopes LB Dpt of Pharmacology, Inst of Biomedical Sciences, Univ of São Paulo

Despite breast cancer's high incidence, very few pharmacological strategies are available for prevention in the high-risk population and, due to serious adverse effects, the existing ones have low acceptability, highlighting the need for new well-accepted forms of prevention therapies. Fenretinide is a synthetic retinoid and promising candidate for chemoprevention mainly for its ability to accumulate preferentially in the breast tissue, regulating cellular processes such as cell growth and differentiation. However, its high lipophilicity (logP 6.31), low systemic bioavailability and systemic adverse effects hinder its clinical use. To overcome these limitations, we developed a bioresponsive microemulsion (ME) capable of transitioning into a liquid-crystalline gel upon intramammary administration for sustained and local release of fenretinide. After characterization, we evaluated the *in vitro* and *In Vivo* ME efficacy and assessed mammary tissue alterations. Phosphatidylcholine-based precursor MEs (droplet size of 173 nm) were

developed, and the system's ability to sustain *In Vivo* release and inhibit cellular migration and spheroid viability and growth using T47D cells were assessed. Formulation efficacy in reducing breast cancer tumors incidence and modify histological parameters was evaluated in a chemically induced breast cancer *In Vivo* model, in which tumors were induced by n-methyl-n-nitrosourea (50 mg/kg). The ME transformed into lamellar phases *In Vivo* after 3 h, and prolonged *In Vivo* Alexa Fluor 647 release for 30 days. Migration of T47D cells was inhibited by 4-fold upon treatment with the formulation at IC₁₅ (concentration necessary to reduce cell viability by 15%) compared to the unloaded formulation and untreated cells. Treatment for 5 days with the ME at IC₁₅ reduced tT47D spheroid size (1.4-fold) of T47D spheroids compared to untreated or unloaded ME treatment. Fenretinide solution did not display a similar effect most likely due to drug precipitation in the medium. Intramammary administration of ME reduced the incidence of breast tumors by 4.5-fold compared to untreated animals and an epithelial reorganization and decrease in the presence of vacuoles and pyknotic nuclei was observed in contrast to induced but untreated animals. Additionally, the ME normalized collagen III (which has been described to limit breast cancer development) levels in skin and enhanced its expression in the mammary tissue of treated animals compared to control (untreated). These results suggest the potential applicability of this ME as a platform for inhibition of breast cancer development in high-risk patients. **License number of ethics committee:** 4906211117

12.011 Anticholinesterase-antimuscarinics: study of dual agents aiming at application for Alzheimer's disease. Guimarães MJR¹, Neves GA¹, Romeiro LAS², Castro NG¹. ¹Univ Federal do Rio de Janeiro, Inst de Ciências Biomédicas, Brazil ²Univ de Brasília, Dept de Farmácia, Brazil

Alzheimer's disease (AD) is a progressive neurodegenerative disease that affects cognitive abilities mostly due to synaptic dysfunction and death of entorhinal, hippocampal and frontal cortical neurons. In the areas affected by AD there is a drop in the levels of acetylcholine, which contributes to impair cognition by reducing activation of nicotinic and muscarinic receptors. Aiming at restoring cholinergic neurotransmission, cognitive symptoms of AD are treated using anticholinesterase drugs, such as donepezil, galantamine and rivastigmine. However, the high cost and high incidence of side effects, mainly due to the activation of peripheral muscarinic receptors, drive the demand for new drugs. Two collaborative drug development projects yielded anacardic acid derivatives and phenylpiperidine donepezil analogues that were planned to have anticholinesterase activity associated with other beneficial activities in AD, such as anti-inflammatory and antioxidant. Among these novel substances we sought to discover an additional antimuscarinic activity, which might reduce peripheral adverse effects. Ninety nine substances were screened for their anticholinesterase effect and 28 were selected, showing IC₅₀ between 2.8 and 29.7 μM for acetylcholinesterase and being non-competitive inhibitors. The anacardic acid derivative LDT532 at 10 μM was active in a M3 receptor inhibition screening assay using Ca²⁺ fluorimetry in human colon epithelial cells (HT29). It also inhibited carbachol-induced bradycardia in isolated rat atrium, suggesting M2 antagonism. Thus, LDT532 has both anticholinesterase and antimuscarinic activities, inhibiting M2 and M3 receptors that are the main targets of peripheral adverse effects of acetylcholinesterase inhibitors. **License number of ethics committee:** CEUA: 087/2018

12.012 N-octadecanoyl-5-hydroxytryptamide reduces neuroinflammatory responses induced by Aβ₁₋₄₂ in microglial cells. Giorno TBS¹, Lima FA², Brand ALM², Oliveira CM², Rezende CM², Fernandes PD¹ ¹UFRJ, Inst of Biomedical Sciences, Brazil; ²UFRJ, Chemistry Inst, Brazil

Introduction: The C18 5-HT, a new *N*-alkanoyl-5-hydroxytryptamide is naturally found in the surface wax of coffee beans (Speer et al., Braz. J. Plant Physiol., 18(1): 201, 2006) and has antinociceptive effect (Giorno et al., Sci. Rep., 8: 10027, 2018). Some amides of the serotonin class demonstrated an anti-inflammatory effect by inhibiting the expression of caspases participants in inflammatory process (Meijerink et al., Br. J. Pharmacol. 169: 772, 2013). In this study, we investigated the *in vitro* anti-inflammatory and neuroprotective activity of C18 5-HT. **Methods:** Inflammation was mimicked by Aβ₁₋₄₂ treatment of N9 microglia cell line and then were treated with different concentrations of C18 5-HT (0,01, 0,03, 0,1, 0,3, 1, 3 e 3 μM) for 24 h or 48h to evaluated cell viability by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-difenltetrazolium). These Aβ₁₋₄₂-stimulated cells were also treated with C18 5-HT (0,1, 0,3, or 1 μM) to assess nitric oxide (NO) levels and cytokines (TNF-α, IL-1β, IL-6 and IL-10). **Results:** The results showed that the cell viability was significantly reduced after 24h and 48h incubation with 3μM of C18 5-HT. Aβ₁₋₄₂ caused fold increase in NO production (54.4 ± 4.9 μM). Pretreatment of Aβ₁₋₄₂-stimulated cells with C18 5-HT significantly suppressed NO production at 0,1, 0,3 and 1 μM concentrations (25.7±2.8*; 23.2±5.1*; 19.4±4.2*μM, respectively *versus* control group=54.4 ± 4.9 μM). C18 5-HT also decreased the levels of TNF- α (0,1μM=115.5±1.8*; 0,3 μM= 103.1± 1.6*; 1 μM= 71.4±1.2* *versus* control group = 130.6±1.5pg/mL and IL-1 β (0,1 μM= 16.9±1.9*; 0,3 μM=14.3±2.6*; 1 μM=11.1±2.7* *versus* control group=33.5±3.5pg/mL) in a dose-dependent manner. The compound also significantly decreased IL-6 levels in the 1 μM

concentration, promoting a reduction of 38.5 % when compared to control group (1 μM =20.8 \pm 4.4* *versus* control group=33.8 \pm 3.3 pg/mL) and increased the level of IL-10 at 0.3 μM (102.6 \pm 6.6*) and 1 μM (132.1 \pm 10.4*) concentration *versus* control group (75.2 \pm 5.6pg/mL). **Conclusion:** This study highlighted the inhibitive effect of C18 5-HT on inflammation induced by A β_{1-42} in cultured microglia by inhibiting pro-inflammatory markers such as NO and cytokines (TNF- α , IL-1 β and IL-6). **Acknowledgements:** Alan Minho for technical assistance. **Financial Support:** CAPES, CNPq and FAPERJ. **License number of ethics committee:** N/A

12.013 Preparation and characterization of capsules with a standardized dry extract from leaves of *M. ilicifolia* to be used in a clinical trial: Scale up from laboratory to industrial scale. Meirelles GC^{1,2}, Bianchi SE², Bassani VL², Siqueira IR^{1,3}. ¹UFRGS, Porto Alegre, Dpt of Pharmacology, Brazil; ²UFRGS, Porto Alegre, PPG Pharmaceutical Sciences, Brazil, ³UFRGS, Porto Alegre, PPG Pharmacology and Therapeutics, Brazil
Introduction: *Monteverdia ilicifolia* (Mart. ex Reissek) Biral (= *Maytenus ilicifolia* Mart. ex Reissek), popularly known as “espinheira-santa”, is a native South America shrub which its infusions have been extensively used in the folk medicine for gastroprotective properties. Frequently these properties are attributed to the presence of phenolic compounds (BAGGIO et al., 2007). Although *M. ilicifolia* products are available in the pharmaceutical market, there is a lack of standardization of the dry extracts. This study is part of a broader project designed to evaluate the efficacy of *M. ilicifolia* capsules in a clinical trial specifically in the management of dyspepsia related to Gastroesophageal Reflux Disease (GERD). Thus, the main goal of the present work was to develop and characterize capsules with standardized dry extract from leaves of *M. ilicifolia*. **Methods:** Leaves of *M. ilicifolia* were grounded and the powder with mean particle size of 500 μm was selected for extraction with boiled water (2% w/v) for 15 minutes. The content of the chemical markers total tannins and epicatechin in the corresponding aqueous extractive solution (ES) was evaluated according to the methodologies described in the plant monography (BRAZIL, 2019). The ES was concentrated (8-fold). A spray dried *M. ilicifolia* extract was prepared using a mixture of the excipients starch: colloidal silicon dioxide (92: 8) at an excipient: dry residue of the plant ratio of 3: 7. The dried extract presenting the best process yielded 53.5% and its *in vitro* dissolution profile was carried out in triplicate using the USP II dissolution test apparatus. The industrial scale has been performed by SUSTENTEC®. **Results:** The content of chemical markers in the raw material was 2.13-3.27 % and 0.683-1.338% to total tannins and epicatechin, respectively. These values are in accordance with the official plant monograph. The spray drying technique afforded a technological suitable powder with an epicatechin content of 23.70-29.12 $\mu\text{g}/\text{mg}$ (w/w). Hence, samples containing an equivalent to 9.48 mg of epicatechin were filled in hard gelatin capsules and their dissolution profile was performed. The results demonstrated that almost 100% of the epicatechin contained in the capsules was released in 2 hours. The process was successfully scaled-up from laboratory to an industrial scale. **Conclusion:** The outcomes from this study evidenced that the developed technology was able to produce suitable spray dried powder to be employed as gelatin capsules in a clinical trial. This technological development was transferred to a Pharmaceutical Industry for scale-up and producing capsules for clinical trials. **Financial support:** This research was supported by the Brazilian Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq/MS/SCTIE/Decit Nº 19/2018 – Fitoterápicos – 405888/2018-0). **Acknowledgments:** SUSTENTEC®, CNPq, MS. PERICO, L.L. et al. Medicinal and Aromatic Plants of South America, Springer, p. 323, 2018. BAGGIO, C.H. et al. J. Ethnopharmacol., v. 113, p. 433, 2007. BRASIL, Farmacopeia Brasileira, volume 2, 6ed.: Anvisa, 546 p., 2019. **License number of ethics committee:** N/A

12.014 Impact of *Achyrocline satureioides* on clinical outcomes in viral respiratory infections, such as COVID-19: preliminary results of an open randomized clinical trial. Bastos CIM¹, Cechinel LR¹, Dani C¹, Rasia FB¹, Neves AHS¹, Possa LR², Meirelles GC², Bassani VL³, Siqueira IR^{1,3}. ¹UFRGS, Porto Alegre, PPG em Farmacologia e Terapêutica, Brazil ²UFRGS, Porto Alegre, Dpt de Farmacologia, Brazil ³UFRGS, Porto Alegre, PPG em Ciências Farmacêuticas, Brazil
Introduction: The outbreak of the disease COVID-19 spread rapidly to several countries. *Achyrocline satureioides* (“macela” tea) has several ethnopharmacological and *in vitro* and *In Vivo* antiviral evidences to be considered an innovative strategy for support in the treatment of patients with viral respiratory infections, such as COVID-19. Objective: to study the impact of infusion of *A. satureioides* inflorescences on clinical outcomes and white blood cell counts in individuals with viral respiratory infections, comparing with control group, *Malus domestica* fruits infusions (apple tea). **Methods:** The project was approved by CEP/UFRGS [CAAE: 40787320.6.2001.5530]. Inclusion criteria: subjects with persistent and mild symptoms of viral respiratory infection between March and May of 2021 in Southern Brazil. The inflorescences of *A. satureioides* and dehydrated apple infusions were supplied by the Kambo de Ervas®. The reference flavonoids content at the plant was evaluated using the high liquid chromatographic analysis method. The

results obtained demonstrate that the used *A. satureioides* contains 1.52 mg% of achyrobichalcone; 1.83 mg% of quercetin; 0.69 mg% of luteolin and 7.74 mg% of 3-O-methylquercetin for every 100g of inflorescences, which were in accordance with the Brazilian Pharmacopoeia (VI Edition). Inclusion criteria: subjects with 18 years or older, who needed to test for COVID-19 by polymerase chain reaction (PCR), demonstrating some symptoms associated with COVID-19, such as fever and dyspnea. A survey of the results of the PCR test was carried out from the patient medical records, in order to differentiate between SARS-COV-2 +/- cases. The treatment lasted for 14 days using the tea twice a day. Whole blood was collected on the first and last day of treatment. Clinical outcomes as number of days with cough and dyspnea, need for hospitalization, and mortality were evaluated by questionnaires, medical records, as well as immunological examinations. **Results:** At this moment, 125 patients were allocated to the experimental groups, and 57.6% (72) completed the study. A total of 57 participants were included in the *A. satureioides* group (intervention) and 67 in the *M. domestica* infusion (control). The participant dropout rate was 40% (51 individuals), the rate of severe cases requiring hospitalization was 1.6% (2), and mortality rate was 0.8% (1), all of which were excluded from the study. The subjects that received *A. satureioides* showed a statistically significant reduction in the average days with cough and dyspnea, comparing to control group, according to two-way ANOVA ($P<0.05$). COVID-19 cases showed reduced leukocytes, lymphocytes, eosinophils and monocytes, and higher values of segmented neutrophils ($P<0.05$). **Conclusion:** The *A. satureioides* infusion significantly reduced the latency for symptom remission, such as dyspnea and cough in subjects with respiratory infection, accelerating their recovery, without any effect on white blood cell counts. **Financial support:** CAPES/ME/FCAPNS/ Notice 11—Development of prototypes of antiviral drugs and their formulations - 23038.003950/2020-16 **License number of ethics committee:** The project was approved by CEP/UFRGS [CAAE: 40787320.6.2001.5530].

13. Pharmacology Education and Technology

13.001 **A patent landscape of pregnancy treatments.** Pereira KV¹, Pacheco CO², Haas SE¹ ². ¹PPG in Pharmaceutical Sciences, UFSM, Santa Maria, Brazil ²PPG- Unipampa in Pharmaceutical Sciences, Uruguaiiana, Brazil

Introduction: Pregnancy is the phase in which profound changes occur, related to both the fetus and the mother, and it is during this period that the maternal organism undergoes anatomical and physiological changes. In some cases, obstetric complications such as miscarriage, fetal growth restriction, pre-eclampsia or preterm birth may occur in approximately 15% of human pregnancies. As pregnant women, they are not always sure about prenatal exposure to drugs to treat this type of complications due to the scarcity of studies related to pregnant women. Due to the great technological advance, the filing of patents is one of the promising areas for the discovery and development of new drugs that can be used during pregnancy. However, it is important to consider that there are some limitations when it comes to this target audience, due to the few clinical trials and possible risks to maternal-fetal health. **Methods:** A survey of patent deposits in specialized databases was carried out, using the keywords “pregnancy and drugs” for the search. The selection system was in accordance with a systematic review and meta-analysis guidelines (PRISMA). The research interval was established between the years 2005-2020. **Results:** The data obtained in the research show 225 studies, of which they were published, and excluded by pre-selected selection criteria, reaching a final value of 42 studies. Among the selected studies, it was possible to identify patents that indicate treatments derived from natural products and their associations with synthetic drugs, as well as new methods and technological innovations to treat and prevent pathologies during pregnancy. **Conclusion:** Based on the results found, patents with herbal medicines lead the findings, considering that many women are limited to the consumption of natural products due to the lack of clinical trials with drugs in pregnant women. Therefore, the need for more important investments and discoveries in obstetric research is highlighted, providing a balance between maternal well-being and the risk of adverse fetal outcome. **Acknowledgments:** The authors would like to thank the Federal University of Pampa, Campus Uruguaiiana, the Science Foundation of Rio Grande do Sul (FAPERGS), grants 16 / 2551-0000207-0 and 19 / 2551-0001970-0, National Development Council Scientific and Technological (CNPq) (universal scholarship) and by Capes for the Improvement of Higher Education Personnel - Brazil. **References:** Alegre-del Rey, E.J. et al. Riesgo de medicamentos en el embarazo: un problema de transferencia del conocimiento con repercusiones éticas. Cuad. Bioét. p. 199, 2019. Bannerman, S., The World Intellectual Property Organization and the sustainable development agenda. Futures. 122: p. 102586, 2020. Cockburn, I. and G. Long, The importance of patents to innovation: updated cross-industry comparisons with biopharmaceuticals. Expert opinion on therapeutic patents. 25, p. 739, 2015. Racicot, K., et al., Understanding the complexity of the immune system during pregnancy. American Journal of Reproductive Immunology. 72, p. 107, 2014. Pereira, K. V. et al. "The challenge of using nanotherapy

during pregnancy: Technological aspects and biomedical implications." *Placenta* no. 100: 75-80, 2020. Sarmiento, S. R. et al. "Pre-eclampsia in pregnancy: emphasis on nursing care." *Nursing Brazil* no. 19, p.3, 2020. Tasnif, Y, J. et al. "Pregnancy-related pharmacokinetic changes." *Clinical Pharmacology & Therapeutics* no. 100 (1): 53-62, 2016. **License number of ethics committee:** N/A

13.002 *As aventuras de Farmaquinhos: teaching through gamification.* Farias RHC, Meneses JRL, Bayerlein MJ, Lorga ACM, Antonini HK, Pereira MLL, Carlos CP, Rodrigues AC USP, Inst of Biomedical Sciences, Brazil

Introduction: Traditional teaching methods are being challenged, especially when considering the new technologies available, which led to changes on students' routine, and on the means required to arouse their interest. In that context, gamification is a form to include study in students' new habits, since approximately 97% of teenagers play video games nowadays (Erenli, Kai. *iJET*, v. 8, p. 15. 2013). By bringing learning into a known atmosphere, this method allows greater engagement and understanding, reaching beyond the limits of the physical classrooms (Kalogiannakis, M. *Educ. Sci.* v. 11, p. 22. 2021). In pharmacology teaching, those benefits are notable, providing a clearer view of the learning process and students immersion, through the association of information, images and sounds of the storytelling design, combined with the sense of reward every time the player completes an objective, and also the use of brief breakdowns to elucidate the subject. By virtue of that, *As aventuras de Farmaquinhos* seeks to be an active way of learning and recalling basic concepts on pharmacology, on a trip through the human body, demonstrating the course of a drug. As a result, the main goal of the game is to promote learning in an interactive, immersive, and fun fashion. **Methods:** The game was developed on Construct 3 platform and published on itch.io website. Graphics and design were made through Canva and Photoshop, and sound effects were obtained from Freesound. Notably, the game's soundtracks made reference to classical game themes. A small sample of students from the Pharmacology course (n=11) was selected to evaluate the game. Four aspects of the game on a scale from 0 to 10 were considered: gameplay; pharmacological content; difficulty and learning objectives. The results are presented as mean \pm standard deviation. **Results:** Ten different stages were created to present the drug's route over the body, combined with tutorials, questions and explanations. The initial four stages display the drug administration and its passage through each part of the GI tract. First-pass metabolism and drug bioavailability are also considered in stages 5 and 6, respectively. Next, stages 7 to 9 consider pharmacodynamics aspects of drugs provoking biological effects, to be later excreted. As regards to students' game evaluation, the mean score for gameplay was 8.3 ± 0.8 , the themes approached had a mean score of 9.6 ± 1.2 , difficulty had a mean score of 7.1 ± 1.4 , and the game helpfulness on learning had a mean score of 8.3 ± 1.3 . **Conclusion:** Highlighting the playful learning program, we focused on the benefits of recurrence and the role of the student as protagonist of their own learning (Lee, Joey. *AEQ*, v. 15, p. 1. 2011). The game disrupts the idea that knowledge is restricted to the classroom and promotes memorable associations between images and stories, which support apprenticeship. The emotional component is also benefited, as the game objectives are well-known, encouraging players to retry without fear of failure. **Acknowledgments:** We would like to thank our advisor, professor Alice Cristina Rodrigues. **License number of ethics committee:** N/A

14. Pharmacology: Other

14.001 Use of medications for the treatment of Covid-19 in Brazil: A cross-sectional online survey. Alves GMS, Botinhão MC, Bonavita AGC, Carmo PL, Gonzalez SR, Raimundo JM. UFRJ-Campus Macaé

Introduction: On March 11, the World Health Organization (WHO) declared COVID-19 a pandemic. The first case in Brazil occurred in the end of February 2020, and in June 2021 there were over 18 million confirmed cases (1). The Federal Council of Pharmacy showed that since the first case in Brazil, the sales of some drugs that were associated with the prevention or treatment of this disease have increased (2). The aim of this study was to assess the prevalence of the use of medications by people who had a positive diagnosis for COVID-19 in Brazil. **Methods:** The study was a cross-sectional survey conducted through self-administrated online questionnaires from September 2020 to March 2021. The online questionnaires were elaborated in the Google Forms software and were disclosed in our website (www.farmacologiainforma.com), e-mails and social networks. The inclusion criteria were general population aged 18 years and over living in Brazil. One questionnaire was for people who had already been diagnosed with COVID-19 and the other questionnaire was for people who did not have the disease. The questionnaires included sociodemographic and socio-economic characteristics, presence of comorbidities and use of drugs for the prevention or treatment of COVID-19. The participation in the study was voluntary and anonymous. This study was approved by the Human Research Ethics Committee of UFRJ-Campus Macaé (CAAE 35948820.2.0000.5699). **Results:** A total of 2024 participants attended the questionnaires, being 1806 individuals without a previous diagnosis for COVID-19 and 218 who had already

been diagnosed with COVID-19, which represents approximately 10% of the total. Of the 218 individuals, 19 needed to be hospitalized (8.7%). These results slightly differ from the data provided by the Ministry of Health, which show that 5.9% of the Brazilian population was infected by the SARS-CoV-2 and 12.7% of those infected needed to be hospitalized (3). This difference may be related to the fact that 74.3% of the survey participants live in Rio de Janeiro state. Most of the diagnoses were made in the private health service (61.5%), through the RT-PCR test (53.7%). Among the risk factors for COVID-19 severity, hypertension (16.5%), obesity (13.8%) and asthma (9.2%) were the most prevalent. The most used medications for the treatment of COVID-19 were azithromycin (83.0%), ivermectin (60.8%), dipyrone (53.6%), vitamin C (47.1%), vitamin D (38.6%), zinc (35.3%), prednisone (28.8%) and hydroxychloroquine (14.4%). **Conclusion:** The two most used drugs for COVID-19 treatment were azithromycin and ivermectin, which are part of the “COVID-19 kit”, although there is no scientific evidence that they are effective. The irrational use of these medicines may increase the incidence of adverse effects, drug interactions and drug resistance. Thus, there is a need for promoting the rational use of medicines during the COVID-19 pandemic. **References:** (1) <https://covid.saude.gov.br/>. Accessed June 15, 2021. (2) <https://www.cff.org.br/noticia.php?id=6197&titulo=Sale+de+rem%C3%A9dios+sem+efic%C3%A1cia+proven+again+st+Covid+dispara> Accessed June 16, 2021. (3) Ministry of Health. Special Epidemiological Bulletin - Coronavirus Disease COVID-19 56: 1, 2021. **License number of ethics committee:** Human Research Ethics Committee of UFRJ-Campus Macaé (CAAE 35948820.2.0000.5699).

14.002 Use of medicines for COVID-19 prevention in Brazil: a cross-sectional study. Botinhão MC, Alves GMS, Gonzalez SR, Bonavita AGC, Montani JR, Carmo PL. UFRJ-Macaé

Introduction: Coronavirus disease 2019 (COVID-19) was first reported in Wuhan (China) in December 2019 and rapidly spread around the world. COVID-19 prevention is still based on non-pharmacological interventions, since no medicine has yet been shown to be safe and effective for preventing this disease¹. However, self-medication and off-label prescribing of medicines have been observed during COVID-19 pandemic. Thus, the objective of this study was to analyze the profile of use of medicines for the prevention of COVID-19 in Brazil. **Methods:** The study was a cross-sectional survey conducted through self-administrated online questionnaires from September 2020 to March 2021. Questionnaires were sent via WhatsApp, e-mail, Instagram, Facebook, and the link was also published on the Farmacologia Informa website. The inclusion criteria were general population aged 18 years and over living in Brazil. One questionnaire was for people who had already been diagnosed with COVID-19 and the other questionnaire was for people who did not have the disease. The questionnaires included sociodemographic and socio-economic characteristics, presence of comorbidities and use of drugs for the prevention or treatment of COVID-19. The participation in the study was voluntary and anonymous. This study was approved by the Human Research Ethics Committee of UFRJ-Campus Macaé (CAAE 35948820.2.0000.5699). **Results:** Questionnaires were completed by 2.024 people, where 1.806 had not been diagnosed with COVID-19. Most participants were aged between 18-29 years (33.1%) and between 30-39 years (20.9%). Answers were obtained from all regions of the country, being 86.2% from the southeast, 5.9% from the northeast, 4.3% from the south, 2.3% from the midwest and 1.3% of the northern region. Of the 1.806 participants, 891 declared to have certain risk factors associated with COVID-19 diseases exacerbation. Regarding the use of medicines to prevent COVID-19, 396 participants reported the use of at least one medicine, where 38.6% were prescribed by doctors, 34.3% were indicated by a friend or family member, 7.1% by referral through a social network and 2.5% by referral through websites. The most used drugs were ivermectin (77.3%), vitamin C (48.0%), vitamin D (48.0%), zinc (34.6%) and azithromycin (11.1%). 3.3% of participants who used any medication to prevent the disease had adverse effects. An increase in the sales of these medicines during the COVID-19 pandemic were reported by the Brazilian Federal Council of Pharmacy², as well as the risks associated with the irrational use of ivermectin³. **Conclusion:** Self-medication and off-label prescription without scientific evidence were common practices for COVID-19 prevention in Brazil, which could be associated with risks such as the use of excessive drug dosage and adverse drug reactions. Our results highlight the importance of educational initiatives to promote the rational use of medicines. **References:** ¹<https://www.who.int/emergencies/diseases/novel-coronavirus-2019/advice-for-public/myth-busters>. Accessed 10 July 2021. ²<https://www.cff.org.br/impressao.php?noticia=6197>. Accessed 6 April 2021. ³<https://www.cff.org.br/noticia.php?id=6242>. **License number of ethics committee:** This study was approved by the Human Research Ethics Committee of UFRJ-Campus Macaé (CAAE 35948820.2.0000.5699).

14.003 Cytotoxicity of new indole molecules in rat glioma cells. Amorim, I, Heidrich N, Steinmetz A, Almeida FB, Freese L, Barros HMT ¹UFCSA, Neuropsychopharmacology Lab, Brazil; ²UFCSA, Genotoxicity Lab, RS, Brazil

Introduction: The field of neuropharmacology research is constantly seeking for new compounds and molecules to better fit efficacy and safety profiles to enhance therapeutic strategies. Indole molecules are one of the broadest types of molecules with great biological relevance, as serotonin (5-HT) is an indolamine. The use of indole compounds has been showing interesting results for the treatment of drug dependence, as 5-HT is involved in the pathophysiology of numerous mental and systemic diseases, such as addiction (Heidrich, 2020). For this purpose, discovery and development of new indole molecules can be very promising. One of the required preclinical tests for that intent are *in vitro* tests, which are able to give a safety profile in target tissue or cells, enabling reduction in the use of animal models and in the failure rates of candidate compounds (Tonholo et al., 2020). **Objective:** To determine the biocompatibility (cytotoxicological potential) of new indole molecules (SM12 and SM7) through the *in vitro* cell viability test to investigation of the addiction treatment potential *In Vivo* testing. **Methodology:** The neutral red assay was used, which was initially developed to assess cell survival/viability. The method is based on the ability of viable cells to take up and bind to the vital neutral red dye. This weakly cationic dye penetrates, by passive nonionic diffusion, through cell membranes and accumulates intracellularly in lysosomes (lysosomal pH < cytoplasmic pH). In this sense, neutral red dye binding is a highly sensitive indicator of cell viability. Rat glioma C6 Cells - American Type Culture Collection (ATCC, Rockville, Maryland, USA) cultures were used. Cells were seeded in culture medium and grown for 24 hours and then treated for another 24 hours with SM7 and SM12 at different concentrations. The neutral red assay was used at the following molecules concentrations: 1, 10, 50, 100, 200 and 400 mM; as Controls: negative (NC) and 10% DMSO. Absorbance was measured at 540 nm, NC as reference. (UFCSA's Ethics Committee on the Use of Animals (#233/18). **Results:** Cytotoxicity assays showed that the SM7 molecule does not show cytotoxicity at the different concentrations administered and the SM12 molecule only shows cytotoxicity at higher concentrations (200 and 400 mM). Comparisons with other studies indicate that different glioma cell lines may have low sensitivity to these molecules (Santos, 2019). These results indicate that these molecules have a low toxicity profile in central nervous system cells, which points to security in *In Vivo* studies. **Conclusions:** The *in vitro* neutral red assay in glioma cells, assessed the safety of new indole molecules for further *In Vivo* testing. **Financial Support:** - UFCSA, CNPQ, CAPES **References:** HEIDRICH, N. Master's Thesis — Porto Alegre: UFCSA, 2020. TONHOLO, D.R., et al. Chem.-Biol. Interact. 315: 108896, 2020. SANTOS, S. Master's Thesis - Porto Alegre: UFCSA, 2019. **License number of ethics committee:** (UFCSA's Ethics Committee on the Use of Animals (#233/18)

14.004 **Standardization and evaluation of changes induced by primary dysmenorrhea in Wistar rats.** ¹Souza PPS, Lacerda-Júnior FF ² Barros BC², Diniz AFA², Ferreira PB², Costa BA³ ¹DCF-CCS-UFPB; ²PPgPNSB-CCS-UFPB; ³DCF-UFPB

Introduction: Primary dysmenorrhea (DysP) is defined by severe pelvic pain during the menstrual period, without prior cause, affecting many women of childbearing age, and may become disabling, thus being considered a public health problem (AVIDON et al, HRU, v21. p762. 2015). Therefore, we aimed to implement and standardize a method for inducing DisP and evaluate the alterations in uterus of virgin Wistar rats, in our laboratory. **Methods:** Female rats were divided into four experimental groups: control group (CG), primary dysmenorrhea group (DysP), and groups standard treated with scopolamine + dipyrone (DysP + SD) or ibuprofen (DysP + I). For induction of DysP, the female rats received dietilestilbestrol subcutaneously for ten days at a dose of 2.5 mg/kg on the first and tenth day and from the second to the ninth day, at a dose of 1.0 mg/kg. For the DisP group, twenty-four hours after the last administration the rats received 1.0 IU/kg of oxytocin intraperitoneally and were placed in a glass box for observation for 30 minutes; the groups standard received doses of 1.2 mg/kg or 50 mg/kg of the scopolamine + dipyrone or ibuprofen, respectively, orally thirty minutes before receiving oxytocin. For *in vitro* evaluation, the rats were euthanized, the uterus was removed, and suspended in bath tubs for isolated organs under physiological conditions. The results were expressed as mean and standard error of the mean and analyzed by one-way ANOVA followed by the Tukey post-test ($p < 0.05$, $n=5$). **Results:** In DysP it was observed an increase in the number of contortion (119) compared to CG (2.6), and DysP + SD or DysP + I showed a decrease in the number of contortions (63.5 and 38.67). In the evaluation of uterine contractility, an increase in the power and contractile efficacy of oxytocin was observed ($pEC_{50} = 3.7 \pm 0.2$ and $E_{max} = 145.1 \pm 8.7$) when compared to CG ($pEC_{50} = 3.1 \pm 0.1$ and $E_{max}=100 \pm 0.3\%$). Similar results were observed in the cumulative curve at KCl, where an increase in power and efficacy to KCl was observed ($pEC_{50} = 1.8 \pm 0.1$ and $E_{max} = 153,2 \pm 15,56\%$) when compared to CG ($pEC_{50} = 0.9 \pm 0.13$; 100%). Similar results were observed in the cumulative curve at PGF_{27} , where an increase in power and efficacy to PGF_{27} was observed ($pEC_{50} = 7,07 \pm 0,20$ and $E_{max} = 170.5 \pm 12.0\%$) when compared to CG ($pEC_{50} = 6,15 \pm 0,08$; $100 \pm 0,08\%$). In the *in vitro* relaxing reactivity, it was demonstrated that there was a displacement of the cumulative curve of naphedipine to the right with decreased power and maximum

effect ($pEC_{50} = \pm 0.04$ and $E_{max} = 73.5 \pm 1.3$) when compared to CG ($pEC_{50} = 9.5 \pm 0.1$ e $E_{max} = 100 \pm 0.03\%$ respectively). Similar results were observed in the isoprenalin curve, which showed a decrease in power and maximum effect ($pEC_{50} = 11.0 \pm 0.23$ and $E_{max} = 79.4 \pm 2.7$) when compared to CG ($pEC_{50} = 15.1 \pm 0.2$ and $E_{max} = 100 \pm 0.03$). **Conclusion:** Thus, we conclude that the method implemented for the induction of DisP was effective, proven by behavioral changes as well as by the potentiation of contractile reactivity and difficulty of relaxation in Wistar rats, making it possible to investigate the mechanisms by which the disease settles and the evaluation of new therapeutic alternatives for the treatment of this disorder. We thank the UFPB and PPgPNSB/UFPB for the financial support. CEUA/UFPB (No. 1886010520). **License number of ethics committee:** CEUA/UFPB (No. 1886010520).

14.005 Transcriptional analysis of TRPA1, TRPV1, TRPV4, TRPM8 in human systems. Kudsi SQ¹, Piccoli BC², Araújo DA², Trevisan G¹. ¹UFSM Santa Maria, PPG Pharmacology, Brazil; ²UFSM Santa Maria, PPG Biochemistry Toxicology, Brazil

Introduction: Transient receptor potential (TRPs) are a large family of non-selective cationic channels that act as polymodal sensors in many tissues in mammalian organisms. These channels are unique for their wide diversity of activation mechanisms and their cationic selectivity. TRP channels are involved in several physiological processes, including chemical detection, nociception, and mediation of cytokine release. They also play an important role in regulating inflammation through sensory function and neuropeptide release. **Objective:** This article aims to summarize the distribution of *TRPA1*, *TRPV1*, *TRPV4*, and *TRPM8* receptors, to understand and demonstrate the main tissues where transcription levels of each receptor are found, as well as provide essential suggestions for the discovery of new therapeutic targets. **Methods:** Using the RNA-seq data set from The National Center for Biotechnology Information's (NCBI) Gene database, we determined and compared the transcriptional levels of the *TRPA1*, *TRPV1*, *TRPV4*, and *TRPM8* found in 95 human individuals representing 33 different tissues to determine the tissue-specificity of all protein-coding genes. **Results:** Therefore, we observed that there is a higher transcriptional level of *TRPV1* in the duodenum and ovary compared to *TRPA1*, *TRPV4*, and *TRPM8*. In renal tissue, we observed higher transcriptional levels of *TRPV4* compared to other three genes. Interestingly, we observed very high transcriptional levels of *TRPM8* in the prostate and liver. **Conclusion:** In this study, we demonstrated the distribution of *TRPA1*, *TRPV1*, *TRPV4* and *TRPM8* receptors in 33 tissues where the transcription levels of each receptor was discovered. This information about *TRPA1*, *TRPV1*, *TRPV4* and *TRPM8* transcriptional levels in human systems may provide essential suggestions to new studies in this area. **Acknowledgments:** Conselho Nacional de Desenvolvimento Científico e Tecnológico-CNPq, Coordenação de Aperfeiçoamento de Pessoal de Nível Superior-CAPEs, and Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul-FAPERGS. We thank CNPq and CAPEs for their fellowship support. **License number of ethics committee:** N/A

14.006 Chronic ethanol consumption causes oxidative stress in the thymus via mineralocorticoid receptor activation. Assis VO Dourado TMH, Tirapelli CR. EERP-USP Ribeirão Preto, Lab of Pharmacology, Brazil

Introduction: Ethanol consumption (EC) induces activation of the renin-angiotensin system (RAS) with increased circulating aldosterone levels and further increase in blood pressure and vascular oxidative stress¹. In the vasculature, aldosterone acts on mineralocorticoid receptors (MR) promoting increased oxidative stress under different conditions, but in animals treated with spironolactone, an MR antagonist, vascular damage and oxidative stress caused by aldosterone were decreased². Considering a relationship between cells of the immune system and the vascular actions of aldosterone³ and the fact that the molecular mechanisms underlying the effects of EC in the thymus are not yet defined, we hypothesized that EC could, via increased circulating aldosterone levels, affect the maturation process of T lymphocytes in the thymus. Here, we investigated a possible role for MR in the effects in the thymus induced by EC. **Methods:** Male Wistar Hannover rats (260g) were divided into 4 groups: Control: animals received water ad libitum for 5 weeks and daily gavage (DG) of vehicle (CV) or an MR antagonist, potassium canrenoate (PC-30mg/kg/day - CP); Ethanol: animals were treated with ethanol 20% (v/v) for 5 weeks and DG of vehicle (EV) or PC (EP). At the end of the treatment, blood and thymus were collected for biochemical analysis. The levels of superoxide anion (O₂⁻) were detected by chemiluminescence of lucigenin, while the levels of thiobarbituric acid reactive species (TBARS) were measured by colorimetric assay and the levels of hydrogen peroxide (H₂O₂) were measured by commercially kit AmplexRed®. Two-way ANOVA followed by Bonferroni was used to compare the **Results:** The protocols were approved by the Ethics Committee (CEUA#20.1.402.22.4). **Results:** EC reduced the thymus weight/body weight ratio and also increased O₂-generation (RLU/mg protein; n=4-8) (CV=258±63; CP=383±78; EV=746±107*; EP=342±84) and TBARS levels (mol/mg protein=5-8) (CV=1,7±0,2; CP=1,2±0,4; EV=2,7±0,6*; EP=0,9±0,6) in the thymus and treatment with potassium canrenoate prevented these responses. EC also increased serum TBARS levels (mol/mL=6-

8) (CV=21,9±4,4; CP=25,6±6,4; EV=40,8±11,6*; EP=24,5±7,7) and treatment with potassium canrenoate prevented these responses. **Conclusion:** EC increases oxidative stress in the thymus via MR activation. Financial support: CAPES ¹ Passaglia P, Ceron CS, Mecawi AS, Antunes-Rodrigues J, Coelho EB, Tirapelli CR. Angiotensin type 1 receptor mediates chronic ethanol consumption-induced hypertension and vascular oxidative stress. *Vascul Pharmacol* 74: 49-59, 2015. ² Virdis A, Neves MF, Amiri F, Viel E, Touyz RM, Schiffrin EL. Spironolactone improves angiotensin-induced vascular changes and oxidative stress. *Hypertension* 40(4): 504-10, 2002. ³ Kasal DA, Barhoumi T, Li MW, Yamamoto N, Zdanovich E, Rehman A, Neves MF, Laurant P, Paradis P, Schiffrin EL. T regulatory lymphocytes prevent aldosterone-induced vascular injury. *Hypertension* 59(2): 324-330, 2012. **License number of ethics committee:** CEUA#20.1.402.22.4

14.007 Influence of periprostatic adipose tissue from obese mice on prostate smooth muscle contraction and human epithelial cell viability. Passos GR, Oliveira MGD, Ghezzi AC, Antunes E, Mónica FZ. Dpt of Translation Medicine, Faculty of Medical Sciences, Univ of Campinas

Introduction: Lower urinary tract symptoms (LUTS) secondary to benign prostatic hyperplasia (BPH) are highly prevalent. Studies showed that obese men are more likely to develop LUTS-BPH than non-obese. Recently, the adipose tissue in direct contact with specific organs, e.g. periprostatic adipose tissue (PPAT), have gained attention due to their potential paracrine role, through the release of several factors involved in tissue growth and contraction. Therefore, this study aimed to evaluate the effect of PPAT on mouse prostate *in vitro* reactivity, as well as human normal prostate epithelial cell (PNT1A) proliferation in the presence/absence of lean and obese mouse PPAT. **Methods:** Male C57BL/6 mice were fed with standard (lean) or high-fat diet (obese) from six to 18-weeks old. To evaluate the role of PPAT on prostate reactivity a bioassay was performed. Briefly, PPAT was isolated from obese mice, kept in Krebs (37°C) for 30 min, and the supernatant was transferred to a miograph for additional 30 min. Concentration-response curve to phenylephrine (PE) was carried out in prostates from lean and obese mice. Additionally, PPAT was first incubated for 30 min with/without guanylyl cyclase inhibitor ODQ (10 µM), nitric oxide synthase (NOS) inhibitor L-NAME (1 µM), inducible NOS inhibitor 1400W (10 µM) or adenosine receptor (A2A) antagonist ZM241385 (1 µM). To evaluate cell proliferation, PNT1A were cultured in RPMI medium using 96-well plates (3x10³ cells/well). Isolated PPAT from lean or obese mice was incubated within the cells, in the presence/absence of 1400W (10 µM) for 24 to 72 h, and cell viability was determined by MTT assay. Data represent mean ± SEM. T-test or ANOVA were used accordingly. All protocols were approved from the ethic committee (CEUA/UNICAMP 4836-1/2018). **Results:** The addition of obese PPAT to the prostate strips produced a significant reduction in PE-induced maximal contractions (Emax) by about 44 % (1.4 ± 0.30 mN, N=6) and 58% (1.4 ± 0.28 mN, N=6) in lean and obese mice, respectively, compared with strips without PPAT supernatant (Emax 2.5 ± 0.9 mN and 3.4 ± 0.53 mN, for lean and obese, respectively). Incubation with ODQ (2.8 ± 0.5 mN, N=8), L-NAME (2.94 ± 0.4 mN, N=6), 1400W (4.13 ± 0.65 mN, N=5) or ZM241385 (3.78 ± 0.48 mN, N=4) prevented (p<0.05) the inhibitory effect induced by PPAT. The incubation of PNT1A cells with PPAT from obese mice significantly increased cell viability after 72 hours (192 ± 39 %, N=12, p<0.05), in comparison to control cells (without PPAT or with PPAT from lean mice), N=17). 1400W did not affect significantly PPAT-induced increase in PNT1A viability (171 ± 19 %, N=7). **Conclusion:** PPAT from obese mice releases NO and adenosine. Whether these mediators also interfere in cell viability, thus contributing to the development of prostate hyperplasia seen in obese mice, more studies are needed. (FAPESP 18/21880-2; 18/05956-9) **License number of ethics committee:** CEUA/UNICAMP 4836-1/2018

14.008 Investigation of pleiotropic effects of simvastatin in a non-alcoholic fatty liver disease model. Pereira ENGS, Martins CSM, Araujo BP, Rodrigues KL, Silveiras RR, Flores EEI and Daliry A. Lab of Cardiovascular Investigation, Oswaldo Cruz Inst, Oswaldo Cruz Foundation, Rio de Janeiro, Brazil

Introduction: Non-alcoholic fatty liver disease (NAFLD) is one of the most common chronic liver diseases, affecting about 30% of the adult population. NAFLD encompasses a wide spectrum of liver disease ranging from simple steatosis to non-alcoholic steatohepatitis (NASH), the inflammatory form of the disease that can progress to liver failure. Despite the significant incidence and adverse consequences of NAFLD, there are no approved drugs to treat this condition, justifying the need to search for safe therapeutic alternatives that minimize the cellular and vascular damage caused by NAFLD. Studies on the beneficial effects of statins on liver disease are still scarce, with research focusing on their lipid-lowering effects. However, recently, there has been a growing interest in their pleiotropic effects, which are independent of their lipid-lowering effects. On this basis, we aim to investigate the pathophysiology of liver dysfunction in NAFLD and the protective effects of simvastatin (SV) against metabolic and microcirculatory complications in NAFLD. **Methods:** NAFLD was established by a high-fat, high-carbohydrate diet (HFHC) for 13 weeks.

Oral treatment with SV was administered between weeks 6 and 13. Leukocyte recruitment was assessed by intravital microscopy and Laser Speckle Contrast Imaging flowmetry was used to assess microcirculatory perfusion. **Results:** NAFLD animals showed obesity, an increase in fasting blood glucose and impaired glucose metabolism, an increase in serum and liver triglycerides, serum LDL levels, and the enzymes ALT and AST, as well as fat deposition and a decrease in HDL. SV treatment was able to normalize glycemic metabolism, serum and liver triglycerides, serum LDL and HDL levels, and liver enzymes ALT and AST, as well as fat deposition. Liver histology confirmed the presence of severe steatosis and marked hepatocellular ballooning in HFHC-fed animals, which was significantly reduced by SV. Regarding oxidative stress parameters, the NAFLD group showed a decrease in the activity of catalase enzyme and an increase in the enzymatic activity of SOD and lipid peroxidation in liver tissue and an increase in the activity of catalase enzyme and lipid peroxidation in visceral adipose tissue, while the animals treated with SV did not show these changes. Concomitantly, there was an increase in iNOS expression, higher NO bioavailability, increased AGE content and RAGE protein expression in the liver in HFHC group, which was not observed after SV treatment. The hepatic and visceral adipose tissue microcirculatory dysfunction: (i) increased leukocyte recruitment (ii) increased stellate cells activation, (iii) decreased sinusoidal density, (iv) decreased perfusion, and (v) endothelial dysfunction, marked by the deficient microcirculation vasodilatory responses to acetylcholine, observed in mice fed a HFHC diet were not observed in SV treated animals. **Conclusion:** Therefore, the microvascular and metabolic effects of SV, independent of cholesterol lowering, may contribute to reposition statins for NAFLD treatment. Supported by CNPQ, FAPERJ and PAPES/FIOCRUZ. **License number of ethics committee:** Experimental procedures were approved by the Oswaldo Cruz Foundation Animal Welfare Committee (CEUA licence L-012/2018 A1).

14.009 **Standardization of rodent occlusion trauma model and effectiveness of ATB-352, a H₂S-releasing non-steroidal anti-inflammatory.** Oliveira, JP¹, Santos LGK¹, Teixeira, SA¹, Contin, I², Wallace, J³, Muscará, MN¹, Costa, SKP¹. ¹ICB-USP Dpt of Pharmacology, Brazil; ²Dpt of Prosthesis of Dentistry Faculty-USP, Brazil; ³Dpt of Physiology and Pharmacology, Univ of Calgary, Canada

associated with inflammatory pain response in the orofacial cavity due to neurons sensitization and lesions in masticatory muscle, gingiva and temporomandibular joint (TMJ)¹. Non-steroidal anti-inflammatory drugs NSAIDs (eg. ketoprofen) are used for mild-to-moderate acute inflammation, but their long-term use induces gastrointestinal (GI) damage². Interestingly, the H₂S-releasing ketoprofen derivative ATB-352 enhanced the anti-inflammatory and analgesic effects of ketoprofen without inducing GI damage³. This study aimed to standardize an occlusal trauma model in rats and to test the effectiveness of ATB-352 in the resulting inflammatory pain. **Methods:** Male Wistar rat was killed by isoflurane overdose followed by exsanguination. The mandibula was removed and molded with condensation silicone to obtain a tooth mold in a type IV plaster. Resin crowns of 1mm thickness were produced and placed on the right lower 1st to 3rd molars by using photopolymerizable micro-hybrid composite resin. At day 1, male Wistar rats were anaesthetized with isoflurane 5% in O₂ and the oral cavity opened with a mouth opener. The resin crown of 1 mm thickness was placed in the right lower molar for 7 days. Nociceptive and inflammatory parameters in the rat with (or without – sham) the tooth crown was carried out on days 1, 3 and 7. Establishing the optimum occlusal installation period, a set of rats were orally (p.o.) treated with ATB-352 (4.6, 15 or 46 mg/kg; -1h; n=4-5) or its vehicle (CMC 0,5%; n=4). At days 1, 3, 5 and 7 post crown interference, either orofacial mechanical hyperalgesia *In Vivo* via von Frey or inflammatory parameters such as myeloperoxidase activity (MPO) or 3-nitrotyrosine (3-NT), 4-hydroxynonenal (4-HNE) and glial fibrillary acid (GFAP) expressions were evaluated in masseter muscle, gingiva and trigeminal ganglia via SLOT or Western blot test. Data are expressed as mean ± SEM and p <0.05 are taken as significant. **Results:** In the rat gingiva with interference occlusal, MPO activity increased significantly from day 1 to 7 as compared to sham group. In the masseter muscle MPO increase was only observed at day 7. Increased expression of 3-NT, but not 4-HNE, occurred in the rat gingiva with occlusal interference at day 7. These changes were paralleled by reduced nociceptive threshold and increased GFAP expression in trigeminal ganglia. ATB-352, at doses of 4.6; 15 and 46 mg/kg, reduced nociceptive behavior and MPO activity in the gingiva and masseter muscle. **Conclusion:** The resin crown placed on the right molar induced orofacial inflammation and nociceptive behavior, which was more evident on day 7, and mimicked the human occlusion. ATB-352 treatment ameliorated inflammation and nociceptive behavior, thus suggesting this compound can be useful to treat inflammation by occlusal interferences. **References:** 1. Mediotrusive. J Prosthodont 2021 30(S1): 43-51. 2. J Pain Res. 2014; 7: 99–115. 3. Antioxid Redox Signal. 2020 33(14): 1003-1009. **Animal ethics committee:** CEUA-ICB/USP, Protocol n° 2055050819. **Acknowledgments:** CAPES, CNPq (n° 142342/2020-3; 312514/2019-0). **License number of ethics committee:** Animal ethics committee: CEUA-ICB/USP, Protocol n° 2055050819.

14.010 *Spirulina platensis* modulates the NO and COX pathways and prevents the increase in uterine contractility promoted by strength training

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Introduction: Physical exercise is a factor responsible for reducing risks related to chronic diseases, however, inadequate training can be harmful (ACSM, 10. ed., 2018; CHENG et al., v. 35, 2020). In this context, Ferreira (Tese, 2019), demonstrated that female rats submitted to progressive strength training show increased contractile reactivity and decreased potency to KCl, and dietary supplementation with *Spirulina platensis* (SP) (100 mg / kg) prevented such effects. Thus, it decided to investigate whether the electromechanical mechanism of action of exercise and SP supplementation on uterine contractile reactivity involves the nitric oxide (NO), cyclooxygenase (COX) pathways and the crosstalk between these pathways.

Methods: Female rats were divided into 3 groups: control (CG); trained (TG); trained and supplemented with SP100 mg/kg (TGSP100), orally. Strength training was based on a progressive fluid jump protocol, 24 hours before euthanasia received diethylstilbestrol (1 mg/kg, s.c.) for estrus induction. The results were expressed as mean and standard deviation of the mean and analyzed by one-way ANOVA followed by Tukey's post-test. **Results:** In the evaluation of uterine reactivity in TG, there was an increase in potency and a decrease in efficacy in the presence of L-NAME ($E_{max} = 122.1 \pm 9.1\%$; $pEC_{50} = 2.4 \pm 0.05$) when compared to the curve in its absence of the inhibitor ($E_{max} = 172.7 \pm 8.1\%$; $pEC_{50} = 1.0 \pm 0.03$), and when compared to the CG there was an impediment to the increase in efficacy and potency ($E_{max} = 100\%$; $pEC_{50} = 2, 0 \pm 0.07$). In TGSP100, there was an increase in contractile efficacy to KCl ($E_{max} = 203.0 \pm 27.8\%$), with no change in potency ($pEC_{50} = 2.1 \pm 0.2$) when compared to its absence ($pEC_{50} = 2.0 \pm 0.05$; $E_{max} = 119.7 \pm 9.1\%$) When evaluating GT, there was an increase in potency and a decrease in contractile efficacy, in the presence of indomethacin ($pEC_{50}=2.2\pm 0.06$, $E_{max}=93.4\pm 11.3\%$; $pEC_{50}=1.0\pm 0.03$, $E_{max}=172.7\pm 8.1\%$, respectively), the curve in the presence of the inhibitor prevented the increase in efficacy, but increased the potency when compared to the GC ($E_{max}=100\%$; $pEC_{50}=2.0\pm 0.07$). In GTSP100 there was an increase in the efficacy of KCl, without changing the contractile potency ($E_{max}=256.0\pm 22.9\%$; $pEC_{50}=2.0\pm 0.03$; $pEC_{50}=2.0 \pm 0.05$; $E_{max}=119.7\pm 9.1\%$, respectively). The simultaneous presence of L-NAME and indomethacin in the TG shifted the curve to the left, without changing the contractile efficacy of KCl when compared to the curve in the absence of these inhibitors ($pEC_{50} = 2.3 \pm 0.05$; $pEC_{50} = 1.0 \pm 0.03$, respectively). In the TG, it avoided the decrease in potency, but did not prevent the increase in efficacy when compared to the CG ($pEC_{50} = 2.0 \pm 0.07$; $E_{max} = 100\%$). In TGSP100, it increased the efficacy of KCl and shifted the curve to the left ($E_{max} = 227.8 \pm 14.0\%$; $pEC_{50} = 2.5 \pm 0.005$) when compared in the absence of these inhibitors ($E_{max} = 119.7 \pm 9, 1\%$; $pEC_{50} = 2.0 \pm 0.05$). **Conclusions:** It was observed that strength training increases uterine contractile efficacy by having NO reacting with free radicals, increased contractile prostanoids and inhibitory crosstalk of the NO and PLA2 pathways, in addition, these effects are prevented by SP, through the activation of NO pathway, COX inhibition and crosstalk activation. Thus demonstrating a promising role of algae in the face of disorders related to uterine hypercontractility such as abortion, dysmenorrhea, etc. **Support:** CAPES, PPgPNSB/UFPB. **Research approval:** CEUA/UFPB (5191200320). **License number of ethics committee:** CEUA/UFPB (5191200320).

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