

14. Pharmacology: Other

14.001 Zebrafish feeding of Gong-Cheng-You-Cha (a herb-based health food) delivers antifatigue effects. Zhao Y, Hao E, Qin L, Xie J, Huang J, Du Z, Hou X, Deng J Guangxi University of Chinese Medicine

Introduction: Gong-Cheng-You-Cha (GCYC) is a traditional herb-based health food of the Yao nationality in Guangxi Province, China. This study used swimming channel against controlled current and High-Throughput Fish Surveillance System to establish a zebrafish model for antifatigue research; meanwhile examined fatigue-relevant biochemical indices to systematically evaluate the antifatigue effects of GCYC, and to evaluate its health value. This study provides theoretical basis and support for the antifatigue effects of GCYC. **Methods:** Adult zebrafish were randomly divided into blank control group, positive control group, experimental groups with low-, medium- and high-dose of GCYC, and treated for 5 days. Exhaustive swimming and 6-minute swimming test were conducted in swimming channel against controlled current, and the exhaustive swimming time and zebrafish sport recovery ability were measured. **Results:** Compared with the blank control group, the exhaustive swimming time of low, medium and high dose groups of GCYC was prolonged, and the differences were statistically significant. Furthermore, the exercise ability of zebrafish in the GCYC medium and high dose groups recovered better in 6 min swimming test. Lactate dehydrogenase, catalase and coenzyme Q10 were significantly increased in the low-dose and the medium-dose groups. The levels of reactive oxygen species, hypoxic inducible factor-1, myoglobin and cortisol were significantly reduced in the high-dose group. **Conclusion:** We established a zebrafish antifatigue model and demonstrated that the Chinese health food GCYC has antifatigue effects in zebrafish, validating the health and commercial value of GCYC. This zebrafish-based antifatigue system can be further applied in drug discovery. **Acknowledgments:** We thank Guangxi key laboratory of efficacy study on Chinese materia medica for helping to make the trial possible. We thank Xiaoyan Wen for his assistance in writing this article. We also thank Cong Li and Weijie Xu for their assistance in conducting these experiments. **Funding:** This work is supported by the Guangxi Key Laboratory Construction Project (16-380-29); Construction of Guangxi Collaborative Innovation Center for Research on Functional Ingredients of Agricultural Residues CICAR 2017-Z1; Guangxi Science and Technology Base and Talent Project AD17129010). The animal use protocol listed above has been reviewed and approved by Guangxi University of Chinese Medicine Institutional Review Board.

14.002 Periprostatic adipose tissue from obese mice reduced the contraction induced by alpha-1 adrenoceptor agonist in isolated prostate smooth muscle from obese and lean mice. Passos GR, Oliveira MG, Bertolotto GM, Rocha NR, Antunes E, Mónica FZM Unicamp

Introduction: Benign prostate Hyperplasia is a common disease associated with aging and obesity. Obesity promotes an increase of adipose tissue, which is an important source of growth factors, hormones, pro-inflammatory, contractile and anti-contractile substances. Previous study has shown that periprostatic adipose tissue (PPAT) from obese mice presented higher area and increased expression of gp91phox (NOX2) and TNF-alpha (Alexandre et al., 2018). Because obese mice present prostate hypercontractility (Calmasini et al., 2018), this study is aimed to assess the effect of PPAT from obese mice in isolated prostate smooth muscle reactivity. **Methods:** Six-week old male C57BL/6 mice were fed for 10-weeks with high fat diet (obese group). Control mice (16-week old) were those that received normal chow (lean group). Bioassay was performed to analyze the interference of PPAT from obese mice on the prostate smooth muscle reactivity. Briefly, PPAT were isolated, weighted (~40 mg), kept in Krebs solution (30 min at 37°C) and the supernatant collected (1 mL). Prostate strips from lean and obese mice were incubated with and without PPATsupernatant for 30 min. Concentration-response curves to phenylephrine (PE) was carried out. In another set of experiments, the soluble guanylate cyclase inhibitor, ODQ (10µM) was incubated with PPAT for 30 minutes and then added in prostate strips from obese and lean mice. Data represent mean ± SEM. Unpaired t-test or one-way ANOVA were carried out. All experimental protocols were approved by the Animal Ethical Committee of UNICAMP (CEUA/UNICAMP 4836-1/2018). **Results:** When PPAT supernatant from obese mice was added in prostate strips from both lean and obese mice, a substantial reduction by 44 % (1.4 ± 0.30 mN, N=6) and 58 % (1.4 ± 0.28 mN, N=6) ($P < 0.05$), respectively was observed in PE-induced contraction in comparison with strips without PPAT supernatant (lean: 2.5 ± 0.9 mN vs obese: 3.4 ± 0.53 mN). In the presence of PPAT+ ODQ a tendency of increase (2.4 ± 0.4 mN, N=6, $P > 0.05$) in PE-induced contraction was observed in prostate from obese mice when comparing to strips incubated only with PPAT (1.4 ± 0.28 mN, N=6). On the other hand, PPAT + ODQ did not produce any effect in PE-induced contraction in prostate from lean mice. ODQ alone did not interfere significantly in the pharmacological parameters induced by PE in prostate smooth muscle from lean and obese mice. **Conclusion:** Based on our results, we conclude that PPAT from obese mice releases anti-contractile substances, which may be nitric oxide. **Disclosures:** none **Ethical approval:** **Financial support:** São Paulo Research Foundation 2018/05956

14.003 In vitro evaluation of the antileishmania and immunomodulatory activities of the monoterpenes limonene and carvacrol. Carvalho RCV¹, Santos IL¹, Alves MMM¹, Sousa VC¹, Cruz LPL¹, Santos LP¹, Carneiro SMP¹, Carvalho FAA² ¹UFPI, ²UFMG

Leishmaniasis are parasitic diseases of high incidence in development countries and hard to treat, besides the disease doesn't awaken the interest of pharmaceutical companies, existing drugs for their treatment have high level of toxin. It's estimated that 1,3 million new cases annually in 98 countries with 350 million people at risk of contracting the infection (SILVA et al., 2016). This way, the search for new antileishmania compounds, effective and less toxic has encouraged the isolation of substances from plant species popular use (OLIVEIRA et al., 2017). In this context, limonene and carvacrol (monoterpenes) present in plant, essential oil that have proven antiparasitic activity, may, in combination therapy, provide an alternative for the treatment of these diseases, focusing on dose reduction, in their respective individual components and in the duration of therapy. (MONZOTE et al., 2015). The aim of this study was to assess the antileishmanial activity, cytotoxic and immunomodulating association of monoterpenes: limonene-carvacrol (Lim-Car). For this purpose a mixture of monoterpenes Lim-Car were prepared in the proportions of: 1: 0; 1: 1; 1: 4; 2: 3; 3: 2; 4: 1 and 0: 1, respectively. Afterwards, the activity assays were performed on *Leishmania major* promastigotes, MTT cytotoxicity and immunomodulation assays, such as evaluation of lysosomal activity and phagocytic capacity, as well as the induction of nitric oxide synthesis in murine macrophages (RAW 264.7). The association of monoterpenes showed potential antileishmanial activity, with mean inhibitory concentration (IC₅₀) of 16,0; 14,2; 13,5; 19,0; 15,2; 15,4 and 5,8 µg.mL⁻¹, respectively. Compared with macrophages, the average cytotoxic concentration (CC₅₀) was 158,9; 118,9; 112,2; 73,6; 154,2; 176,0 and 94,1 µg.mL⁻¹, respectively. The Lim-Car association showed selectivity index ranging from 3,87 to 16,23, for the proportions 2: 3 and 0: 1, respectively. The monoterpene mixture wasn't able to induce macrophage activation. However, the 4:1 Lim-Car association is promising for the indexes presented. Complementary studies to know your mechanism of action and efficacy in the treatment of the experimental model *in vivo*. **Acknowledgments:** FAPEPI, Laboratory of antileishmanial activity, UFPI.

Monzote, L.; Pastora, J.; García AM.; Steinbauer, S.; Setzerc, W.N.; Sculld, R.; Gilleb, L. Combination of ascaridole, carvacrol, and caryophyllene oxide against *Leishmania*. *Acta Tropica*, v.145, p. 31–38, 2015. Oliveira, L.G.C.; Brito, L.M.; Alves, M.M.M.; Amorim, L.V.; Sobrinho-Júnior, E.P.; Carvalho, C.E.; RODRIGUES F.K.A.; Arcanjo, D.D.; Citó, A.M.G.L.; Carvalho, F.A.A. In vitro effects of the Neolignan 2,3-dihydrobenzofuran against *Leishmania amazonensis*. *Basic Clin Pharm Toxicol*, v. 1, n.1 p. 1-7, 2017. Silva, J.F.; Figueiredo, K.A.; Medeiros, M.G.F. Natural products for leishmaniasis treatment: exploratory technology. *Rev Cubana de Farmacia*, vol.50, n.2, 2016.

14.004 Validation of an *in vitro* cell culture bioassay for the bioactivity assessment of monoclonal antibody denosumab. Perobelli RF, Xavier B, Silva FS, Cardoso Júnior CDA, Cavalheiro TN, Walter ME, Dalmora SL UFSM

Introduction: Denosumab (DmAb) is a monoclonal antibody produced by recombinant engineering techniques, consisting of 2 heavy chains and 2 light chains, yielding a molecular mass of 147 kDa. Clinically, DmAb is approved for the prevention and treatment of diseases of bone loss and cancer-induced bone destruction. The aim of this study was to optimize and validate an *in vitro* bioassay for the potency assessment of DmAb in pharmaceutical products, and correlate the results with those of the high-performance liquid chromatography (HPLC) methods. **Methods:** The inhibition of osteoclast formation by DmAb was assessed in the RAW 264.7 cell line of macrophages (ATCC® TIB-71™), which serve as osteoclast precursors. The cells were seeded in 96-well microplates at a density of 6×10^3 cells/mL and were incubated with macrophage colony-stimulating factor (M-CSF) and human receptor activator of nuclear factor- κ B ligand (hRANKL) peptide (30 ng/mL) for 4 days to stimulate osteoclastogenesis. Then, the cultures were dosed upon seeding with five concentration ranges starting with 10 μ g/mL of DmAb, in triplicate, as a parallel line assay, and incubated again for 2 days. Then, 20 μ L of alamarBlue™ was added per well and the plates were incubated for an additional 4 h. All cultures were incubated at 37°C in an atmosphere of 5% (v/v) CO₂ and 95% air throughout this study. The bioassay responses were quantified in absorbance mode with a microplate reader. The Xgeva® pharmaceutical products (70.59 mg/mL) were used as the representative DmAb biological reference substance (BS-DmAb) and the bioassay was validated by using pharmaceutical products of Prolia® (60 mg/mL). **Results:** The robustness was determined by analyzing a sample under a variety of conditions of the bioassay parameters, such as: fetal bovine serum concentration, cell concentration, time of exposure to DmAb and incubation time with alamarBlue™ reagent. The results demonstrated that the potencies were within the acceptable relative standard deviation (RSD \leq 15%) with non-significant differences ($p > 0.05$), as calculated by analysis of variance (ANOVA). Then, the bioassay was considered robust under the conditions tested. The accuracy was evaluated by recovering known amounts of the BS-DmAb added to the sample solution. The absolute means obtained with a mean value of 102.33%, with bias lower than 3.60%, confirmed that the bioassay was accurate within the desired ranges. Moreover, bioassay validation demonstrated acceptable results for specificity, linearity and precision. The bioassay was applied for the analysis of DmAb in pharmaceutical products giving potencies between 88.80–112.60%, related to the potencies claimed by the manufacturers. **Conclusion:** The bioassay was validated and applied for the potency assessment of DmAb in pharmaceutical products, contributing to ensure batch-to-batch consistency and to support future biosimilarity studies of the biomolecule. **References:** HANLEY, D.A. et al. Int J Clin Pract. 66, 1139, 2012. PEROBELLI, R.F. et al. Int. J. Biol. Macromol. 119, 96, 2018. WANG, X. et al. Protein Cell. 9, 74, 2018. **Acknowledgments:** CAPES and FATEC for the financial support.

14.005 Development of an *in vitro* cell culture bioassay for the potency assessment of ramucirumab. Silva FS, Perobelli RF, Xavier B, Cardoso Júnior CDA, Nascimento BF, Mohr A, Diefenbach ICF, Dalmora SL UFSM

Introduction: Ramucirumab (RAM) is a human IgG1 monoclonal antibody that binds directly to vascular endothelial growth factor receptor 2 (VEGFR-2) developed to treat advanced solid malignancies. The structure consists of 2 heavy chains with 446 amino acids each and 2 light chains with 214 amino acids each, with a molecular mass of approximately 147 kDa. The aim of this study was to develop an *in vitro* A-549 cells proliferation assay for the potency assessment of RAM in biopharmaceutical formulations. **Methods:** The A-549 cell line of human alveolar adenocarcinoma (ATCC® No. CCL-185™), was maintained in culture medium DMEM supplemented with 10% (v/v) fetal bovine serum and 1% penicillin/streptomycin in 75cm² flasks. The cells were seeded in 96-well cell culture plates at a density of 1.0×10^4 cells/mL, and dosed upon seeding with five concentrations of RAM between 1.0 – 90.0 µg/mL, in triplicate. The plates were incubated at 37°C, 5% v/v CO₂, for 72 h. Then, 20 µL of alamarBlue™ was added per well, and the plates were incubated for a further 4 h. The response was calculated as the difference between the absorbances measured at 570 and 600 nm. **Results:** The A-549 cell culture assay was developed by using samples of RAM (10 mg/mL). The experimental conditions of the *in vitro* bioassay were investigated by changing the cell concentrations within 0.6×10^4 to 3.0×10^4 cells/mL, and the 1×10^4 cells/mL showed an improved discrimination related to the cells' control. Then, the incubation time with alamarBlue™ was tested between 3 to 6 h, selecting 4 h because of the higher reproducibility of the absorbances. Moreover, times of exposure of the cells with the sample were tested at 24, 48, 72 and 96 h, to determine the cell response, and was optimized as 72 h. The stability of the cell line was evaluated at three different stages (passages 5, 20 and 40), which showed that the responsiveness of the cells did not differ significantly. The method was applied for the analysis of RAM in biopharmaceutical formulations giving potencies between 92.30% and 114.10%, related to the potencies claimed by the manufacturers. **Conclusion:** The A-549 cells culture assay represent an advance toward the establishment of *in vitro* approaches. The assay was developed for the potency assessment of RAM in biopharmaceutical formulations, contributing to establish alternative which improve the quality control assuring the therapeutic efficacy. **References:** JANOUSEK, J. et al. Anticancer Res, v. 39, p. 735, 2019. MAIONE, P. et al. Curr Med Chem, v. 24, p. 3, 2017. FLEETWOOD, F. et al. Cell. Mol. Life Sci, v. 73, p. 1671, 2016. **Acknowledgments:** CAPES, CNPq e FATEC/UFSM.

14.006 Evaluation of probable interactions between food/nutrient and drugs used in treatment of non-communicable chronic diseases in seniors living in an asylum in Macaé (RJ). Campos WVA, Carmo PLC, Vianna KS, Ferreira CCD UFRJ

Introduction: The non-communicable chronic diseases are considered a global health issue. Some of them are diabetes, cardiovascular diseases, chronic pulmonary diseases and neuropsychiatric disorders. The nutrients contained on daily diet can interact with the drugs used in treatment of those diseases, so, the food can change the pharmacokinetics or pharmacodynamics of a drug (MOHN et al., 2018). Then, it is important to understand the possible interactions to avoid them and, consequently, make the treatment more effective and not allow the development of any complication to the clinical management. Thus, the objective of this study was to analyze the percentage of possible food-drug interactions in an asylum in Macaé (RJ). **Methods:** A retrospective analytical study was made with the medical records and diet of 62 seniors over 60 years old living in “Casa do Idoso” in the city of Macaé, of both the genders, with non-communicable chronic diseases during the period of time between September 10th and October 14th, 2018. The study was approved by the Human Research Ethical Committee of UFRJ-Campus Macaé (number 92088418.1.0000.5699). **Results:** Potential and consistent interactions with nutrients were found in 21 of the 71 drugs used by the seniors. The percentage of possibility of interaction by therapeutic class, as well as the individual percentages of interaction found for the class/drugs were: Inhibitors of the Renin Angiotensin Aldosterone System (21%): Captopril (100%); Vasodilators (3%): Nifedipine (67%); Beta-Blockers (11%): Atenolol (83%), Propranolol (100%) and Metoprolol (100%); Diuretics (3%): Hydrochlorothiazide (15%); Cardiotonics (2%): Digoxin (100%); Antiplatelet Agents (42%): Acetylsalicylic Acid (100%); Oral hypoglycemic agents (18%): Metformin (83%); Benzodiazepines (50%): Diazepam (66,7%), Clonazepam (10,3%); Anticonvulsivants (25%): Carbamazepine (60%); Antiparkinsonians (33,3%): Levodopa (60%); Selective Serotonin Reuptake Inhibitors (25%): Fluoxetine (100%); Non-steroidal anti-inflammatories: Acetaminophen (92,9%); Biphosphonates (50%): Alendronate Sodium (90,9%); β_2 -adrenergic agonists (33,3%): Formoterol (100%), Salbutamol (100%); Synthetic Hormones (100%): Levothyroxine (100%); Antiacids (100%): Calcium Carbonate (100%), Omeprazole (100%). **Conclusions:** There was a relevant percentage of possible food-drug interactions because of the time of meal and administration of the drug, besides the kind of food, which can harm the health of the seniors. It is suggested that a bigger attention, by the health professionals, to the drug administration and the offer of meal would reduce the non-desirable interactions and optimize the efficiency of the drugs used in the medical management. **References:** MOHN, E.S; et al. *Pharmaceutics*, 10(1), 36, 2018.

14.007 Use of delta-9-Tetrahydrocannabinol (DELTA-9-THC) and cannabidiol in the treatment of neurological disorders: A systematic review. Lopes VB¹, Silva VIAP², Araújo HFP², Mendonça TPS¹, André ACGM¹, Santos FMR¹ ¹Unit, ²UFPE

There thousands of years *Cannabis*, but known as marijuana by Brazilians, has been used as a medical source for the treatment of diseases. There are reports of its use for intestinal constipation and as analgesic in China around 2700 BC and in India 1000 BC for the control of diseases such as anxiety and even depression. In the 1960, israeli scientist Raphael Mechoulan discovered two chemical structures isolated from *Cannabis sativa*, which called attention to their effect on the human body, the higher-amount Delta-9-tetrahydrocannabinol (delta-9-THC), which has the psychotropic and psychoactive effect and Canabidiol (CBD), which is in smaller quantity and has no psychoactive effect? In this work, an integrative literature review was used to summarize previous researches and to obtain general conclusions to analyze the productions about the theme approached between the years 2014 and 2019, obtained in the database *Pubmed* and *Scielo* for articles that related the action of delta-9-THC and that of CBD for the treatment of neurological diseases. In the treatment of severe epilepsies, the use of CBD is beneficial because it reduces the seizures of these patients who no longer respond to conventional drugs on the market, and because they do not cause any side effects in these patients, studies have also revealed the antipsychotic effect, since this component, the CBD, has the ability to improve the symptoms of schizophrenic patients and with depression. The delta-9-THC for being a psychoactive substance, in patients suffering from insomnia, shows a considerable increpasse in deep sele, being administered at the correct dose, 10-30 mg / kg, since one of the side effects of delta-9-THC is the hypnotic effect. In neurodegenerative diseases such as Parkinson's and Alzheimer's, studies in guinea pigs show that the uses of synthetic cannabinoids have a neuroprotective effect, inhibiting the excessive synthesis of glutamate, consequently decreasing excitotoxicity and neuronal damage, improving the symptoms of these diseases, such as loss of progressive memory and tremor in the members. Considering the beneficial effects of the rational use of extracts of *Cannabis sativa*, it is evident the necessity of its study to improve the quality of life of patients who use it in a therapeutic way, so as to ameliorate its side effects and symptoms of the diseases that are carriers. It is important to note that although it is a controversial issue because it involves political, religious and social issues, it is clear that its use as a medicinal plant shows how *Cannabis sativa* has a very great pharmacological potential for the improvement of those patients suffering from pathologies related to the central nervous system.

Descriptors: Cannabinoids, Neurological Disorders, Cannabidiol, Delta-9-THC.

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14.008 Evaluation of the cytotoxic activity of nanostructured sodium alendronate.

Machado FS, Iles B, Dourado FF, Araújo GS, Souza JMT, Barros AB, Medeiros JVR, Silva DA, Araújo AJ, Marinho Filho JDB UFPI

Introduction: Recent studies have shown that bisphosphonates have direct antitumor effects in vivo as well as therapeutic antireabsorptive properties. These inhibit proliferation and induce apoptosis of several cancer cell lines. Among bisphosphonates, alendronate sodium has been used in studies in the treatment of cancer, mainly breast and prostate. However, it is described as a drug toxic to gastric mucosa, acting locally in the stomach, causing gastric discomfort such as abdominal pain, diarrhea and ulcerations involving both the stomach and the esophagus, as well as other side effects. Taking into consideration the clinical problems related to the use of sodium alendronate, the search for new pharmaceutical formulations that reduce these side effects and/or potentiate their therapeutic effects is of great importance. Thus, the present work seeks to develop and study the effect of polymeric nanoparticles with sodium alendronate on the antitumor activity of cancer cells. **Methods:** The nanoparticles were synthesized by nanoemulsion at room temperature, under constant shaking at 1000rpm for 30 minutes, 0.1% protonic anhydride cashew gum (GCAD) and 15 mg sodium alendronate, diluted in 2% DMSO, organic phase. For the aqueous phase, 10 mg of red angico gum (GAng) was used as a surfactant diluted in distilled water. The nanoparticles were characterized by UV-Vis, DLS, Zeta and FTIR spectroscopy. The incorporation potential was analyzed by UV-Vis, using a calibration curve. For the cytotoxicity test, the MTT assay with alendronate (ALD) and its nanoformulation (NALD) in serial dilution with a higher concentration of 50 μM were performed in non-tumor MRC-5 (human fibroblast) and HCT-116 tumor cells (colorectal cancer) and MDA-MB-231 (breast cancer) with treatment for 72h. **Results:** It was observed with this study that the formulated nanoparticles obtained average diameter of 71.60nm. Zeta potential values of -29.1mV, indicative of good colloidal stability, were verified. The incorporation assays demonstrated that the nanoparticles containing 15mg of ALD incorporated a total of 98.5%. In the FTIR bands were observed in the regions of 807 cm^{-1} , 747 cm^{-1} and 1200 cm^{-1} , characteristics for the ALD and for the polysaccharides used. The spectrum obtained for the nano demonstrated the union of these bands, indicating interaction between the components in the formulation. The MTT assay showed cytotoxic activity in all strains tested, with IC₅₀ of 15.59 μM (ALD) and 28.40 μM (NALD) for MRC-5, 2.2 μM (ALD) and 1.56 μM (NALD) for HCT-116 and 4.78 μM (ALD) and 2.95 μM (NALD) for MDA-MB-231. **Conclusion:** Thus demonstrating that the nanoparticle was adequately formulated, indicating that nanoformulation maintained the effect of alendronate. **Support:** CAPES, CNPq, FAPEPI e INCTBioNat.

14.009 Phytochemical prospection and antibacterial evaluation of the ethanolic extract of *Croton tricolor* Klotzsch ex Baill. Neri TS¹, Silva DC¹, Costa JG², Santos AF³, Maior LPS¹, Rocha TJM⁴, Fonseca SA¹ ¹Cesmac, ²UECE, ³UFAL, ⁴UPE

Introduction: The Caatinga constitutes a rich Biome exclusively Brazilian, with great diversity of species and high incidence of endemism. Among the existing species stands out *Croton tricolor* Klotzsch ex Baill (Euphorbiaceae) which is a native shrub from the Brazilian northeast. Where it is popularly called "silvery quince" or "sacatinga". Possessing a high medicinal value being widely used as: antiparasitic, antibacterial, analgesic, diuretic, antimalarial and with antioxidant action and larvicidal activity against *Aedes aegypti*. This work aimed to evaluate the phytochemical and antibacterial prospection of *Croton tricolor* ethanolic extract. **Material and methods:** Phytochemical prospecting tests, Müller-Hinton agar tests in three strains *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 25922 were performed. Minimal Inhibitory Concentration (MIC) using the microdilution protocol in Müller- Hinton at serial dilutions of 1% to 0.06%. **Results and discussion:** In the phytochemical analysis the crude ethanolic extract of *C. tricolor* showed interest in phenols such as flavones, flavonols, alkaloids, steroids, tannins and xanthenes. The antibacterial evaluation showed a positive result against *S. aureus* strain and negative strains of *P. aeruginosa*s and *E. coli*. And in the analysis of CIM showed that there was no growth in *S. aureus* bacteria, proving the efficacy of the extract against this bacterium. **Conclusion:** Taking into account the high resistance of these microorganisms, it is important to emphasize the relevance of studies with medicinal plants as a therapeutic alternative. **Thanks:** I thank God and the University Center Cesmac and all its teaching and administrative staff who provided the window that today I can see a higher horizon, in which I gained confidence in the merit and ethics present here.

14.010 Establishment of a diet-induced animal model of non-alcoholic fatty liver disease for application in pre-clinical studies. Araújo BP, Pereira ENGS, Martins CSM, Silveira RR, Flores EEI, Rodrigues KL, Daliry A Fiocruz

Non-alcoholic fatty liver disease (NAFLD) is the hepatic manifestation of Metabolic Syndrome (MetS), affecting about 30% of the world's adult population. It comprises a spectrum of clinical and histological events ranging from simple fatty liver to steatohepatitis (NASH) and cirrhosis. In addition, a group of patients with end-stage liver disease may develop hepatocellular carcinoma and finally death. However, there is no generally accepted effective treatment for NAFLD/NASH and the pathophysiology of the disease is still poorly understood. Therefore, the development of animal models suitable for the evaluation of the pathophysiology and pharmacological alternatives for NAFLD in order to reduce the impact on the quality of life of the population and the economy of the countries is urgent and necessary. For this purpose, male C57BL/6 mice were fed with a hyperlipid and hypercarbohydrate diet (HLHC, n=10) composed of 60% kcal of fat associated with the addition of 55% fructose and 45% of sucrose in drinking water, offered ad libitum, during 30 weeks. The control group were fed with a standard chow diet with 10% kcal of fat and water ad libitum (CTL, n=10). The weight of the animals and water and food consumption were monitored weekly. At the end of the experimental protocol, systolic arterial blood pressure and fasting blood glucose levels were evaluated. Rolling and adhesion of leukocytes and tissue perfusion in hepatic microcirculation were examined using in vivo microscopic and laser speckle contrast imaging (LSCI), respectively. Abdominal and epididymal adipose tissue content, kidneys and liver were dissected and weighed, and serum and hepatic tissue were collected for analyses. Regarding the cardiometabolic parameters, it was observed that fasting blood glucose and epididymal fat content were increased in animals fed with the HLHC diet. No difference in water and food intakes was observed. The hepatic basal blood flow was decreased in the animals with the HLHC diet, and the rolling and adhesion of leukocyte in the hepatic microcirculation were markedly increased in the HLHC group when compared with the controls. Thus, until now, the evaluation of the results has shown that the diet-induced experimental model has important characteristics consistent with NAFLD, including the increase in the fasting blood glucose, increased in the fat content and liver microcirculatory alterations, suggesting that this is a suitable model for preclinical studies. **Financial Support:** CNPQ, FAPERJ e PAPES / FIOCRUZ. Todos os procedimentos experimentais foram conduzidos de acordo com os princípios internacionalmente aceitos para o Cuidado e Uso de Animais de Laboratório (Licença L-012/2018 A1).

14.011 Toxicogenetic evaluation of hydroxyureia associated with ascorbic acid in allium cepa model. Rêgo NTDS, Aguiar RPS, Melo APM, Marinho Filho JDB UFPI

Introduction: Hydroxyurea (HU) is a molecule known for more than 100 years. It is used in the treatment of myeloproliferative diseases, neoplasias, HIV and also used in single daily doses for the treatment of Sickle Cell Anemia. However, studies have shown the production of free radicals in sickle cell anemia. **Objective:** This study aimed to evaluate the toxic, cytotoxic and mutagenic potential of the hydroxyurea, associated with ascorbic acid via the *Allium cepa* assay. **Methods:** Three groups were used with Isolated Hydroxyurea (75 mM HU/mL, 150 mM HU/mL, HU 300 mM/mL), three with the association with ascorbic acid (2 µM/mL) and two groups as positive and negative controls (negative control - 0.9% saline solution, positive control - 0.6 µg/mL copper sulphate). The toxicity was evaluated by observing root growth length, the cytotoxicity by mitotic index evaluation and mutagenicity by micronucleus formation. The results obeyed a parametric distribution and were analyzed by analysis of variance (two-way ANOVA), followed by the Turkey test (post hoc test). **Results:** *In vitro* tests demonstrated the toxic, cytotoxic and mutagenic capacity of hydroxyurea with a good decrease in these effects when ascorbic acid was used ($p < 0.05$). **Conclusion:** Although the hydroxyurea therapy induces changes in the genetic material, the results obtained in the present study showed that these effects may be diminished by the association of this drug with ascorbic acid, suggesting that this damage may happen due to oxidative stress. **Acknowledgments:** CAPES, CNPq, INCTBioNat.

14.012 Relaxing effect of purified cashew gum (*Anacardium occidentale* L.) on rodent uterus *in vitro* and *in vivo*. Souza ORB, Savia SFLD, Pereira LCA, Santos DF, Oliveira MM, Reis Filho ACF, Silva AM, Silva DA, Nunes LCC, Santos RFS, Meneses Oliveira RC UFPI

Introduction: Primary dysmenorrhoea is defined as cyclic pain of uterine origin, without pelvic pathology; affects up to 81% of menstruating women in their reproductive years, producing a significant negative impact on their quality of life. Pharmacological treatments include the oral contraceptive pill (OCP) and/or non-steroidal anti-inflammatory drugs (NSAIDs); however, these medications have demonstrated numerous side effects. Because of this, the use of medicinal plants as an alternative for the treatment of dysmenorrhea is increasing among women (PELLOW, J. et al. 2018). This study proposed the relaxing effect of purified cashew gum (PCG) on rodent uterine contractility. **Methods:** To investigate the relaxing effect in female rat isolated uterus (*in vitro*) virgin Wistar rats (*Rattus norvegicus*) weight (180-230g) were used. All animals were pretreated with estradiol prior to the experiments. The studies were performed in uterus pre-contracted with oxytocin (10^{-2} IU/mL) or KCl (60mM) at different concentrations of PCG (10^{-3} – 10^3 µg/mL). The effect of PCG on a concentration-response curve to oxytocin was investigated. Comparison of the effect of purified Cajueiro Gum (GCP) and Ibuprofen on tonic contractions induced by oxytocin. The mechanism of action was studied through the participation of potassium channels in the presence of TEA⁺ 1 mM and 5 mM. For the *in vivo* study, virgin female mice (Swiss) weight (25-35g) were used. The oxytocin-induced contortion test was used as a model for in-vivo dysmenorrhea. **Results:** PCG relaxed the uterus when it was precontracted with 10^{-2} IU / mL oxytocin significantly and concentration-dependent ($EC_{50} = 2.53 \pm 0.54$ µg / mL) with maximum efficacy of $97.8 \pm 0.8\%$. The displacement of the curve to the right is observed, in a non-parallel manner with reduction of E_{max} in relation to the concentration, which characterizes a non-competitive antagonism and confirms that the PCG does not act at receptor levels. In the presence of the blocker (TEA⁺) the value of E_{max} was only $49.05 \pm 4.33\%$ for 5mM and $58.87\% \pm 3\%$ for 1mM, which made it impossible to calculate the EC_{50} . Thus, suggesting the participation of K⁺ channels. Oral PCG significantly reduced the number of oxytocin-induced contortions compared to the negative control group, maximum inhibition percentage of $78.86 \pm 8.97\%$ at the dose of 0.3 mg / kg. **Conclusion:** GCP decreases oxytocin-induced contraction of uterine smooth muscle *in vitro* and *in vivo*. The tocolytic mechanism appears to be associated with the activation of K⁺ channels. **References:** PELLOW, J; et al. Medicinal plants for primary dysmenorrhoea: A systematic review. *Complementary Therapies in Medicine*. (2018) 13–26p. DIAS, Sávia Francisca Lopes. Relaxing effect of purified cashew gum (*Anacardium occidentale* L.) on rodent uterus *in vitro* and *in vivo*. 2019. 105f. Tese (doutorado em Biotecnologia) - Universidade Federal do Piauí, Teresina, 2019.

14.013 Nanogels for Topical Drug Delivery: Development and Influence of Composition on Cutaneous Permeability. Mojeiko G, Lopes L ICB-USP

Introduction: In spite of the high incidence of breast cancer worldwide, there are few strategies for its chemoprevention, and they have limited adherence mainly due to their serious adverse effects. In this study, topical nanogels for self-administration of a combination of celecoxib and endoxifen directly on the breast skin were developed to optimize deep skin penetration and localization of these drugs in the breast tissue. Nanogels were structured as nanoemulsions stabilized by lamellar phases. In addition to nanogel development, its ability to disrupt cutaneous barrier and increase its permeability was assessed as a function of composition. **Methods:** Nanogels were developed using water, phosphatidylcholine as surfactant, and sunflower oil mixed with Shea butter as oil phase. As penetration enhancers, the effect of caprylic and oleic acid (2-5% w/w) was compared. The relationship between composition, type of phase formed, nanoemulsion droplet size and incorporation of the drugs was evaluated using polarized light microscopy and light scattering. The influence of the penetration enhancer type and concentration on the nanogelability to disrupt the skin barrier function was evaluated by assessing transepidermal water loss (TEWL), while their effect on the cutaneous bioavailability of the drugs was studied using Franz cells and porcine ear skin as tissue model. **Results:** The content of water and the ratio between surfactant and oil phase (S: O) largely influenced formation of nanostructures. S:O ratios above 2: 8 (m/m) and aqueous phase content at 60-80% produced nanoemulsions stabilized by lamellar phase (the desired nanogel). Four formulations were selected, composed of S: O at 5: 5, 80% of water, and 2 or 5% of either oleic or caprylic acid as penetration enhancer. The droplet size was not influenced by the penetration enhancer. Compared to water (control), all nanogels increased TEWL by 3.1-4.2-fold. Even though no significant differences among the nanogels was observed, caprylic acid at 5% promoted the most pronounced enhancement compared to control ($p < 0.01$), indicating a stronger ability to improve cutaneous permeability. **Conclusion:** Desired nanogels were obtained with S: O at 5: 5, 80% of water, and either oleic or caprylic acid at 2 or 5% as penetration enhancer. The droplet size was within the nanometer range. Even though all nanogels disrupted the skin barrier, compared to control, addition of caprylic acid at 5% promoted the most pronounced enhancement in cutaneous permeability. **Acknowledgements:** This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES, Finance Code 001) and FAPESP (2013/16617-7, 2019/00196-9). The study was considered exempt from IACUC approval.

14.014 Pleiotropic effects of Simvastatin in an experimental model of non-alcoholic fatty liver disease. Pereira ENGS, Martins CSM, Araújo BPD, Silveiras RR, Flores EEI, Rodrigues KL, Daliry A Fiocruz

Liver abnormal fat accumulation is a feature of non-alcoholic fatty liver disease (NAFLD) and strongly associated with the metabolic syndrome (MS). The NAFLD is a public health, affecting about 30% of the world's adult population. The search for safe therapeutic alternatives that minimize damage resulting from NAFLD is urgent and necessary. Current guidelines for clinical practice support the use of statins in the treatment of dyslipidemia in patients with NAFLD; however, the pleiotropic effects of statins in the management of NAFLD-derived complications are not well elucidated. Therefore, this study aims to investigate the protective effect of statins in hepatic microcirculation using an animal model of NAFLD. The experimental model of NAFLD was induced in 20 male C57BL/6 mice by 13 weeks of feeding with a high-fat high-carbohydrate (HFHC) diet containing 60% fat (% kcal) associated with 55% fructose and 45% sucrose in the drink water. For the non-NAFLD control (CTL, n=20), standard rat diet and water was administered. The animals were subdivided into: untreated animals, which received vehicle between 7-13 weeks (CTL+VEI, n= 10 and HFHC+VEI, n= 10) and treated animals that received simvastatin (SV) (20 mg/kg/day) between 7-13 weeks (CTL+SV, n= 10 and HFHC+SV, n= 10). Rolling/adhesion of leukocytes and tissue perfusion in hepatic microcirculation were examined using *in vivo* microscopic and laser speckle contrast imaging (LSCI), respectively. Oxidative parameters were analyzed by TBARS, and catalase (CAT) and superoxide dismutase (SOD) enzyme activities. The participation of advanced glycation end-products (AGE) was evaluated by quantification of fluorescent AGEs in liver samples by spectroscopy. Animals with NAFLD presented overweight and increased fasting blood glucose, serum and hepatic triglyceride, serum cholesterol and fat deposition. Treatment with SV was able to prevent the hyperglycemia and the increase in serum triglycerides in NAFLD animals and protected against the increase of hepatic triglyceride observed in the HFHC group. Liver histology confirmed the presence of severe steatosis and prominent ballooning in the HFHC-fed animals, which was markedly reduced by SV. Concerning oxidative stress parameters, NAFLD group had decreased CAT enzyme activity and increased SOD enzyme activity and lipid peroxidation, however, SV treatment was able to protect against these alterations. Concomitantly, the increase in liver AGE content and hepatic microcirculation alterations (increased leukocyte recruitment and decreased perfusion) observed in HFHC-fed mice was prevented by SV, suggesting that microcirculation represents an important target for the pleiotropic action of SV. Therefore, the microvascular and metabolic effects of SV, independent of cholesterol lowering, may contribute greatly to the drug repurposing of statins and its therapeutic indications for NAFLD. Este trabalho foi financiado por CNPQ, FAPERJ e PAPES / FIOCRUZ. Todos os procedimentos experimentais foram conduzidos de acordo com os princípios internacionalmente aceitos para o Cuidado e Uso de Animais de Laboratório (Licença L-012/2018 A1).

14.015 Validation of alternative method for the content/potency assessment of Botulinum Toxin Type A. Xavier B, Silva FS, Perobelli RF, Cardoso Júnior CDA, Cossetin LF, Escobar AF, Dalmora SL UFSM

Introduction: Botulinum neurotoxin type A (BoNTA) is a polypeptide that contains 1296 amino acids and has a molecular mass of 150 kDa. Therapeutic applications have increased for the treatment of migraine, pain, strabismus and blepharospasm, and as cosmetic. The LD₅₀ bioassay is the gold standard method to estimate the potency of BoNTA. Besides, this assay requires a large number of animals, and is time-consuming and expensive. Thus, substantial effort has been spent to find alternatives, following the principles of the refinement, reduction, and replacement (3 Rs). In this context, the aim of this study was to optimize and validate an alternative *in vitro* bioassay to evaluate the content/potency of BoNTA in biopharmaceutical products. **Methods:** The *in vitro* bioassay was performed based on the inhibitory effects of BoNTA on the proliferation of the cell line T-47D(ATCC HTB-133). The cells were seeded at a density of 3×10^5 cells mL⁻¹, and the assay was performed with doses of BoNTA, between 3 and 81 U mL⁻¹, in triplicate. The Biological Reference Substance of Botulinum Neurotoxin Type-A (BRS-BoNTA) was used as the standard, and the control was RPMI 1640 medium. The responses were assessed using 10 µL of alamarBlue™. The absorbances were read at 570 and 600 nm, and the CombiStats™ software, licensed by the European Directorate for the Quality of Medicines & HealthCare (EDQM) of the Council of Europe (Strasbourg, France), was used to calculate the biological activity using the parallel-line statistical method. **Results:** The bioassay was validated by using samples of BoNTA (100 U/mL and 200 U/mL). The specificity was established with BoNTA formulations spiked independently with known amounts of interferon beta, insulin glargine, and interleukin-11 with showed non-significant differences ($p > 0.05$) related to the un-spiked sample. The accuracy was 101.11% with bias lower than 3.30%. The intraday, interdays and between-analysts precision was evaluated, giving an RSD lower than 15%. Moreover, method validation demonstrated acceptable results for linearity and robustness. The method was applied for the analysis of BoNTA in biopharmaceutical formulations giving potencies between 85.30% and 113.20%, related to the potencies claimed by the manufacturers. **Conclusion:** The results demonstrated the capability of the *in vitro* bioassay for the potency assessment of BoNTA. Besides, the bioassay represents an advance toward the establishment of *in vitro* approaches, in the context of the Three Rs, improving the quality control and assuring the efficacy and safety of the biotherapeutics. **References:** Fonfria, E. et al. *Toxins*, 10, 1, 2018. Pirazzini, M. et al. *Pharmacol. Rev.*, 69, 200, 2017. Xavier, B. et al. *Toxins*, 11, 1, 2019. **Acknowledgments:** CNPq and FATEC for the financial support.

14.016 Antioxidant evaluation of hydroxyurea associated with ascorbic acid in model of *Saccharomyces cerevisiae*. Marinho Filho JDB, Rêgo NTDS, Aguiar RPS, Melo APM UFPI

Introduction: Hydroxyurea (HU) is a molecule known during more than 100 years old and it is used in a range of activities: antifungal, psoriasis treatment, antimicrobial and most recently it was recognized for use in myeloproliferative diseases. Even that it approved for use, its effects can be challenged against its ability to produce oxidative stress by reactive oxygen species generation. **Objective:** The aim of this study was to evaluate the antioxidant/oxidative potential of the association of Ascorbic Acid with Hydroxyurea in *Saccharomyces cerevisiae* strains. **Methodology:** Three groups were used with Isolated Hydroxyurea (HU 75 mM, 150 mM HU and HU 300 mM) and another three groups with the association with ascorbic acid (2 μ M) and two groups for the negative control (0.9% Saline Solution) and positive (10mM Hydrogen Peroxide) respectively. The *Saccharomyces cerevisiae* assay was applied for the determination of antioxidant/oxidant activity by inhibiting yeast growth of pro-efficient and mutated strains (Cu-Zn superoxide dismutase (cytosolic), Mn superoxide dismutase (mitochondrial), cytosolic and mitochondrial superoxide dismutase, Catalase Cytosolic) inoculated in YEL medium). The results obeyed a parametric distribution and were analyzed by analysis of variance (two way ANOVA), followed by the Turkey test (post hoc test). **Results:** In the present study, hydroxyurea presented modulatory effects against oxidative damage induced by hydrogen peroxide in proficient and mutated strains in antioxidant defenses. This suggests that in situations of oxidative stress, hydroxyurea may induce antioxidant effects. **Conclusion:** The test with *Saccharomyces cerevisiae* strain demonstrated that hydroxyurea decreased the damage caused by H₂O₂ and signaling an antioxidant activity, however in association with ascorbic acid it acts synergistically and oxidantly. **Acknowledgments:** CAPES, LCCDELTA, UFPI, LAPGENIC, CNPq.

14.017 Genotoxic, mutagenic and enzymatic evaluation of hydroxyurea associated with ascorbic acid. Nascimento CC, Aguiar RPS, Melo AP, Marinho Filho JDB UFPI

Introduction: - Hydroxyurea (HU) approved by the Food and Drug Administration (FDA) has been used in single daily doses since 1998 as a form of treatment for patients with sickle cell anemia, however despite the approval for the use, its effects can be questioned. Against their ability to produce reactive oxygen species, oxidative stress, and electron transfer. **Objective:** The objective of this work is to investigate the genotoxic, aneugenic/clastogenic and antioxidant potential of Hydroxyurea and its association with ascorbic acid in mice model. Methodology Animal Experimentation Ethics Committee of the Federal University of Piauí (CEEAA/UFPI) approved all experiments proposed under registration 401/17. 8 groups with 5 animals each were treated with HU 7.5 mg/kg, HU 15 mg/kg and HU 30 mg/kg and another 3 groups with the hydroxyurea in association with ascorbic acid (2 μ M/ml) and two groups for the control negative (Saline Solution at 0.9%) and positive (cyclophosphamide 50mg/kg) treated for 7 days. Genotoxic damage analysis was carried out using the comet test methodology; micronucleus test as an indicator of clastogenic and/or aneugenic action; and enzymatic analysis of the enzymes Catalase, Glutathione reductase and Malondialdehyde was carried out as oxidative evaluation. **Results:** Hydroxyurea alone and in combination with ascorbic acid induced genotoxicity, assessed by increased of damage index and damage frequency index in nucleated cells of peripheral blood, liver and bone marrow in all tested concentrations in relation to CN ($p < 0.05$); no clastogenic/aneugenic events were seen in both polychromatic erythrocytes of bone marrow; there was a significant ($p < 0.05$) decrease in catalase and glutathione dosage levels during treatment with hydroxyurea and an increase in malondialdehyde levels; Pearson's correlation showed a negative correlation for catalase and glutathione levels with damage index and damage frequency index in all tissues evaluated and a positive correlation with malondialdehyde and damage index and damage frequency index. **Conclusion:** Hydroxyurea therapy suggests an increase in oxidative stress, but this effect has been diminished by the action of ascorbic acid, suggesting better therapeutics in the use of both molecules. **Acknowledgments:** CAPES, LCCDELTA, UFDFPAR, LAPGENIC, CNPq.

14.019 Investigation of the presence of drugs as emerging pollutants in Bengal River (Nova Friburgo, RJ) and implications for health. Fujimaki CMO, Bernardo RRB, Santos BLR, Lima CKF, Miranda ALP UFRJ

The growing use of pharmaceuticals around the world gave rise a new class of environmental pollutants called emerging pollutants (PE). PE are potentially toxic substances found in the environment at low concentrations, whose long-term risks to various living organisms and to human health are not yet known. These drugs and their metabolites are introduced into aquatic environments through excreta or discharges and when exposed cause the same consequences for health as persistent organic pollutants (POPs) due to their continued entry into the environment. The objective of this work is to determine and quantify the presence of PE, such as ibuprofen and its metabolites, in the Bengal River (Nova Friburgo, RJ) and to evaluate the impact of this contamination on health through tissue impregnation tests, behavioral memory studies, motor activity and nociceptive development. The experimental groups, consisting of swiss female mice, $n = 4$, were divided into: Group 1 - control group -drinking water from the laboratory; Group 2 - intervention group - water from a source in the city of Nova Friburgo, RJ containing ibuprofen at nano concentration. All groups underwent intervention for a period of 60 days. On the sixtieth day of intervention, animals were anesthetized, euthanized and blood sample, liver, pancreas, brain, gonads and adipose tissue collected for further analyses (CEUA 098/17). Blood and tissues samples were submitted to high performance liquid chromatography coupled to the mass spectrometry (LC-MS) (LCQ FLEET apparatus, LCF10576, with electron spray ionizer (ESI), Ion Max-S, ION TRAP analyzer) for identification and quantification of ibuprofen. A C18 chromatographic column (50 x 2.1 mm; 100Å; SilaChrom) was used. The total running time was 40 minutes, Samples (10µL) were injected and eluted with different gradients of H₂O/ACN (acetonitrile), 95%/5% - from 0 to 25 min, 10%/90% - from 25 to 37 min, and 95%/5% from 37 to 40 min, T1 scanning from 100 to 420 Da, positive mode. Mass spectrometry analysis showed the presence of ibuprofen through the m/z 161 fragment in adipose, hepatic and gonadal tissues, where the content of the impregnation presents variability among the animals. Mass spectrum analyses of plasma of animal 1 of the intervention group showed the presence of ibuprofen, m/z 161 ion, but the detection method should be improved. In animals of the control group, the presence of ibuprofen was not observed by LC/MS-MS analyses. During the experiment weight, glycemia and mechanical allodynia were evaluated. Impregnation of adipose, hepatic and gonadal tissues was successfully achieved. Drugs that act as a non-steroidal anti-inflammatory drug, such as ibuprofen, may act as emerging pollutants and may behave as endocrine disrupting agents, corroborating work already described in the literature (1). **References:** (1) Fujimaki CMO; J EnvironSciEng. 443. Agradecimentos: CAPES. CNPq FAPERJ