08. Respiratory and Gastrointestinal Pharmacology

08.001 JME-209 I: A novel orally active mexiletine analogue exhibiting antispasmodic properties – mechanism of action and translation to an animal model of bronchoconstriction. Carvalho KIM[1], Oliveira MTP[1], Coutinho DS[1], Silva ET[2], Costa JCS[2], Faria RX[3], Silva PMR[1], Martins MA[1] [1]Fiocruz – Inflammation, [2]Farmanguinhos-Fiocruz, [3]Fiocruz – Cellular Communication

Introduction: Prior studies showed that some lidocaine analogues, screened for reduced local anesthetic (LA) activity, exhibited better antispasmodic, anti-inflammatory and safety profile as compared to the prototype. Since the low oral bioavailability is a limitation of these analogues, we hypothesized that the orally active LA mexiletine should be a better molecular template for the generation of novel dual anti-inflammatory and anti-spasmodic agents. **Aim**: The current study was undertaken in order to evaluate the antispasmodic profile of JME-209, a mexiletine analogue previously selected for the highly attenuated activity on sodium channels. **Methods**: Rat trachea rings were mounted in tissue baths filled with Krebs' solution, and the contractile response to distinct stimuli was measured in the presence or absence of JME-209. A complementary assay involving epithelium-denuded tracheas was also employed. Bronchoconstriction was measured in unrestrained mice using whole-body plethysmography. Penh measurements were performed 3 h after JME-209 (10–100 mg/kg) or vehicle (0.9% NaCl) administered orally. The effect of mexiletine (10-100 mg/kg) was assessed for comparison. All animal experiments occurred under the CEUA FIOCRUZ license number LW-23/10. **Results**: Exposure to JME-209 and mexiletine inhibited tracheal contraction induced by allergen (IC_{50s}= 237 µM and 436 µM, respectively), carbachol (IC_{50s}= 88 µM and 373 µM, respectively) or extracellular Ca^{2+} under high K+ depolarization (IC_{50s}= 28 µM and 381 µM, respectively). Notably, the relaxing effect of 100 µM JME-209 was abolished following epithelium removal while the spasmolytic action of 300 µM mexiletine remained unaltered. In *in vivo* settings, JME-209 (10, 30 and 100 mg/Kg, orally, ID_{50}= 15 mg/Kg) dose-dependently inhibited methacholine-induced bronchoconstriction (35, 82 and 89 %, respectively) 3 h post-treatment, whereas mexiletine (10-100 mg/Kg) was inactive. **Conclusion**: These findings highlight the mexiletine analogue JME-209 as a promising molecular template for the control of respiratory diseases marked by bronchoconstriction and airflow obstruction, such as asthma and COPD. This effect is at least in part related to blockade of voltage-dependent Ca^{2+} channels and epithelium-derived smooth muscle relaxing factors. **Financial support**: PDTIS, FAPERJ and CNPq.

08.002 Quercetin targets senescent lung fibroblasts from idiopathic pulmonary fibrosis patients. Hohmann MS[1], Habiel DM[2], Coelho AL[2], Verri Jr WA[1], Hogaboam CM[2] [1]UEL – Ciências Patólogicas, [2]Cedars Sinai Medical Center – Pulmonary Medicine

Introduction: Idiopathic pulmonary fibrosis (IPF) is a fibrotic lung disease characterized by the presence of fibroblastic foci, which are rich in senescent and apoptosis-resistant fibroblasts. In models of pulmonary fibrosis, the accumulation of senescent cells contributes to a non-resolving wound healing response and persistent fibrosis. A recent report indicated that quercetin selectively "kills" senescent cells *in vitro* and promotes their clearance *in vivo*, suggesting that this flavonoid may ameliorate the burden of senescent fibroblasts and persistent fibrosis in IPF. **Aims**: To investigate the therapeutic potential of quercetin in reducing senescent IPF lung fibroblast resistance to apoptosis and modulating the expression of extracellular matrix (ECM) proteins. **Methods**: Fibroblasts were isolated from the lungs of normal (NL-F) or IPF patients with stable (IPF-S) or progressive (IPF-P) disease (n=4/group). Senescence was induced by serially sub-culturing the fibroblasts 10-15 times. Fibroblasts were treated with quercetin (50 µM) in the presence or absence of Fas ligand (FasL) (75 ng/mL) or TNF-related apoptosis-inducing ligand (TRAIL) (100 ng/mL). Apoptosis was assessed by caspase-3 activity, lactate dehydrogenase (LDH) release, cell viability, and altered cell morphology. Finally, the effect of quercetin on the expression of various apoptotic, inflammatory, and fibrotic mediators was assessed by qPCR and ELISA. **Results**: Senescent lung fibroblasts showed increased ß-galactosidase activity and up regulation of p21, p16, IL-6, and IL-8. IPF-S and IPF-P fibroblasts presented reduced expression of death receptor (DR) 4 and 5 and Fas compared to NL fibroblasts. Fibroblasts derived from patients with progressive disease expressed lower transcript levels of Fas relative to those with stable disease. FasL and TRAIL induced apoptosis in NL, but not in IPF fibroblasts. Quercetin did not induce apoptosis of proliferating or senescent cells. However, treatment with quercetin significantly increased FasL and TRAIL-induced apoptosis compared to FasL or TRAIL alone. Further, quercetin increased transcript expression of Fas and TRAIL but not DR4 or DR5. Finally, there was significant down regulation of type 1 collagen and fibronectin expression in senescent IPF fibroblasts. **Conclusions**: Quercetin may ameliorate fibrotic progression in IPF by reducing ECM deposition and the accumulation of senescent lung fibroblasts. **Acknowledgments**: CAPES, Cedars-Sinai Medical Center, CNPq, MedImmune. IRB approval number: Pro34067

08.003 Simvastatin protects against alendronate-induced gastric mucosal injury in mice. Carvalho NS, Souza LKM, Sousa NA, Araújo TSL, Silva MM, Silva IS, Costa DS, Lima Filho ACM, Almendra RB, Medeiros JVR UFPI – Farmacologia

Introduction: It has been reported that simvastatin, a statin commonly prescribed with anti-inflammatory and antioxidant effects have gastro protective effects in indomethacin and ethanol-induced gastric ulcers. However, the effects of simvastatin on gastric mucosal injury induced by alendronate already remain unexplored. **Aims**: This study investigated the rationale use of simvastatin in the treatment of gastric ulcer induced by alendronate. **Methods**: The present study was approved by the local ethics committee (protocol no.0066/10). Female rats were pretreated with vehicle or simvastatin (20 and 60mg/kg p.o). After 1 h, the rats were treated with alendronate (50mg/kg p.o). Simvastatin was administered once daily for 7 days and from the fourth day, alendronate was administered once daily for 4 days. On the last day of treatment, 4 h after alendronate administration, the animals were euthanized and their stomachs removed and gastric damage was measured. Samples of the stomach were fixed in 10% formalin immediately after its removal for subsequent histopathological assessment. Other samples were then weighed, frozen, and stored at 80 °C until assayed for glutathione (GSH) levels, malondialdehyde (MDA) concentration, myeloperoxidase (MPO) activity and cytokine levels. Another group was used to measure mucus and gastric secretion. **Results**: In the present study, our results showed that pretreatment with simvastatin (60mg/kg) orally prevented alendronate-induced macroscopic gastric damage. The results of microscopic analysis indicated that alendronate administration induced alterations in the gastric region characterized by intense hemorrhage, edema, epithelial cell loss and inflammatory cells. These changes were significantly prevented in rats treated with simvastatin. The treatment with alendronate (50mg/Kg) resulted in significantly decrease of the concentration of GSH (361.3±57.82 µg/g tissue) along with a concomitant increase the levels of gastric tissue MDA (113.3 ± 9.66 mmol/g tissue) in the stomachs of rats when compared to control treated with saline (*P<0.05). Pretreatment with simvastatin (60 mg/Kg) significantly increased GSH levels (610±88.49 µg/g tissue), and reduced significantly the concentration of gastric mucosal MDA (72.22±10.73 mmol/g tissue). In the evaluation of myeloperoxidase activity Simvastina (60 mg/kg) significantly reduced the MPO activity in stomach (2.164 ± 0.287 U/mg tissue) as compared to alendronate group (6,349±0,526 U/mg tissue). When measured levels of cytokines in the alendronate group, TNF-α and IL-1B levels, in that order, gave values of 2405 ± 264 pg/ml and 901.1±86.73 pg/ml, showing that increased levels of these cytokines in this group is statistically significant when compared to the saline group (*P<0,05). In the groups treated with simvastatin observed a reduction of TNF-α levels and IL-1β (1535±192.6 and 525.7±56.82) respectively. **Conclusion**: The study demonstrates the protective effects of simvastatin against alendronate-induced gastric ulceration. The properties of simvastatin warrant its safe use as drug antihyperlipidemic and gastroprotector to improve of bone health in combination with bisphosphonates. **Financial support**: CNPQ/FAPEPI

08.004 Gabapetin inhibits the production of free-radicals in colitis induced by Trinitrobenzene sulphonic acid (TNBS) in mices. Lima Filho ACM, Almendra RB, Batista JA, Silva IS, Carvalho NS, Junior JGD, Silva RO, Filgueiras MC, Barbosa ALR
UFPI – Farmacologia

Introduction: Gabapentin (GBP) is a structural isomorphic amino acid neurotransmitter GABA, which belongs to the class of anticonvulsant drugs, generates anti-hyperalgic effect and antinociceptive. Data obtained in a number of experimental models of neuropathic pain and inflammatory hyperalgesia shows that GBP has an effective anti-nociceptive or anti-hyperalgesic action, in addition to being an anti-convulsant. In humans, GBP has become increasingly popular as a treatment for chronic neuropathic pain. Clinical studies have shown that GBP is an effective analgesic in different types of neuropathic pain syndromes, such as diabetic neuropathy, postherpetic neuralgia, and trigeminal neuralgia. **Aims**: Knowing that the GBP can reduce some conditions of the inflammatory response, the aim of this study is to test whether GBP is able to inhibit production of free-radical. **Methods**: Male Swiss mice (25-35 g) were sourced by the Central Animal Facility of the Federal University of Piauí (FUP). The animals were housed at 25±2 °C under a 12: 12-h light/dark cycle, and food and water were supplied ad libitum until 18 hours before of experiments. The experiments were conducted in accordance with current established principles for the care and use of research animals of COBEA (Brazilian College of Animal Experimentation) and were approved by the ethics committees in research of FUP, protocol nº: 011/15. For the analyses, the animals was pretreated with GBP 15 mg/kg or Dexamethasone 1 mg/kg, 1 hour before of the colitis induction and after treated with the same drugs each 24 hour during 3 days. The colitis was induced using 20 mg of Trinitrobenzene sulphonic acid (TNBS) in alcohol 50%, a technique introduced by Mac Pherson and Pfeiffer (1978). 1 hour after 3rd day, the animal were anesthetized and sacrificed by cervical dislocation, colon samples were removed for assess Malondialdehyde (MDA) concentration and Levels of Glutathione (GSH) was measured using the method described previously for Sedlak, J. (J. Biochem 24, 1968) with modifications. **Results**: The effect of Gabapentin on MDA Levels in the colitis induced by TNBS (179,465 ± 27,39 nmol/g of tissue) shows significantly increased the levels of MDA compared to the group that received only saline (58,51 ± 5,493 nmol/g of tissue). However, the group pretreated with GBP 15 mg/kg (84,13 ± 14,93 nmol/g of tissue) and dexamethasone 1 mg/kg (91,88 ± 13,81 nmol/g of tissue) had significantly reduced MDA levels compared to the untreated group. In the same way, the group treatment with TNBS (37,41 ± 3,488 µg/g of tissue) decreases the levels of GSH compared to the saline group (131,62 ± 9,504 µg/g of tissue). However, It was observed that the group treatment with GBP (87,34 ± 21,22 µg/g of tissue) inhibited the consumption of GSH levels compared to the untreated group. **Conclusion**: Ours results indicate that Gabapetin has significant antioxidant effect against colitis induced by TNBS in rats. In addition, the mechanism of protection may be related to decreases free radical production and lipid peroxidation. **Financial support**: CNPQ and FAPEPI

08.005 Gastroprotective activity and related mechanisms of *p*-Cymene (*p*-isopropyltoluene). Paulo LL, Sales IRP, Formiga RO, Nascimento RF, Machado FDF, Lima GRM, Sobral MV, Batista LM – UFPB

Introduction: *p*-Cymene (*p*-isopropyltoluene) is a monoterpene present in essential oils of various species. Studies with this compound have already demonstrated anti-inflammatory activity (BONJARDIM, Z Naturforsch C, v. 67, p. 15, 2012), as well as data from the literature shows that other monoterpenes such as menthol (ROZZA, Biol Chem Interact, v. 206, p. 272, 2013) has gastroprotective activity. **Aims**: This study aimed to evaluate the gastroprotective activity of *p*-cymene and anti-secretory and cytoprotective mechanisms related to this activity. **Methods**: Albino Wistar rats (*Rattus norvegicus*), weighing 180-250 g, fasted for 24 hours were pre-treated pathway oral (p.o.) with the negative control (5% Tween 80 solution), positive control (carbenoxolone 100 mg/kg or cimetidine 200 mg/kg) and *p*-cymene in four different doses (25, 50, 100 and 200 mg/kg) or its best dose for the assessment of mechanisms of action (200 mg/kg). After the pre-treatment, the animals were submitted to ethanol or containment of gastric juice-induced gastric ulcer. To evaluate the mechanisms of action, biochemical parameters of the gastric juice were analyzed after oral and intraduodenal administration of *p*-cymene. Mucus adhered to the gastric mucosa quantification was performed using alcian blue as the colorimetric marker and the effect of blocking with N-nitro-L-arginine-methyl-ester (L-NAME) (70 mg/kg – i.p.), N-ethylmaleimide (NEM) (10 mg/kg – i.p.) and indomethacin (30 mg/kg – p.o) in the gastroprotective activity of *p*-cymene to evaluate the role of nitric oxide, sulfhydryl groups and prostaglandins, respectively. **Results**: In the ethanol-induced gastric lesions, *p*-cymene, at doses of 25, 50, 100 and 200 mg/kg protected the gastric mucosa in 66, 91, 99 and 99% ($p<0.001$) respectively when compared to the negative control group. In the ulcers induced by restraining of the gastric juice (pylorus ligation) *p*-cymene (200 mg/kg) induced a protection of gastric mucosa when administered intraduodenally (i.d.) and orally (p.o.) in 45% and 51% ($p<0.001$) respectively when compared to the negative control. Also, *p*-cymene when treated orally increased pH, decreased the concentration of H^+ ions and did not alter volume of gastric content. In the same model, *p*-cymene administration (i.d.) did not change any of these parameters. In the evaluation of cytoprotective mechanisms of action, it has been found that the gastroprotective effect of *p*-cymene does not involve an increase of the mucus adhered to the mucosa. However, its effect is related to the participation of sulfhydryl groups, nitric oxide and maintenance of prostaglandin levels. **Conclusions**: Thus, *p*-cymene presents gastroprotective activity observed in different models of acute ulcer induction and this effect involves cytoprotective and local neutralizing mechanisms. **Acknowledgements**: CNPq/CAPES/CCS/PgPNSB/UFPB. **Research approval by the Animal Research Ethical Committee**: 0110/13.

08.006 Extracellular cAMP-adenosine pathway and carbachol synergistically increase airway smooth muscle contraction. Pacini ESA, Godinho RO Unifesp-EPM – Farmacologia

Introduction: The β_2-adrenoceptor agonists are important drugs used in the treatment of chronic obstructive pulmonary disease and asthma. It is well known that β_2-adrenoceptor activation promotes airway smooth muscle (ASM) relaxation through the adenylyl cyclase/cyclic AMP (cAMP) signaling pathway. In many tissues, in addition to its classical second messenger function, cAMP may also have an extracellular third messenger activity, which is secondary to its efflux and the sequential metabolism to AMP and adenosine by ecto-phosphodiesterase and ecto-5'-nucleotidase, respectively. Nevertheless, the existence and relevance of so-called "extracellular cAMP-adenosine pathway" in airway function is unknown. **Aims**: In the present study, we evaluate the existence of the extracellular cAMP-adenosine pathway in the respiratory system by analyzing the functional role of extracellular cAMP and its metabolite adenosine in the rat ASM contraction. **Methods**: Tracheal rings obtained from adult male Wistar rats were mounted in an organ bath containing carbogenated Krebs-bicarbonate solution at 37°C, under optimal resting tension. Tracheal rings were first challenged with 1 µM carbachol, then rinsed and after 30 min the tissues were subjected to two different protocols. First Protocol: the trachea were incubated with a) 300 µM adenosine, b) 300 µM cAMP alone or in the presence of c) uridine, an adenosine uptake inhibitor, d) EHNA, an adenosine deaminase inhibitor or e) CGS-15943, a non-selective adenosine receptor antagonist. Second Protocol: The tracheas were incubated with a concentration of carbachol (CCh) that caused 30% of maximum contraction (EC_{30}). After stabilization of CCh response, 300 µM cAMP was added either alone or in the presence of a) uridine plus EHNA or b) CGS-15943. The isometric contraction forces were expressed as mean ± S.E.M. (n = 4-12). All values were normalized and presented as percentage of the response induced by 1 µM CCh. **Results**: In resting tension, both cAMP and adenosine induced rat tracheal ring contraction, but had different magnitudes (3.5±0.5% versus 32±8.7%). The inhibition of either the adenosine uptake or the adenosine deaminase increased by 3.4-fold and 4.7-fold the cAMP-induced contraction, respectively. In the presence of CGS-15943, the contractile response to 300 µM cAMP was inhibited by 79% (4.0±1.2% versus 0.85±0.24%), indicating the involvement of adenosine receptors on cAMP effect. In EC_{30} CCh pre-contracted trachea, 300 µM cAMP induced an additional contraction of 21±3.0%, which was 6-fold higher than the one observed in resting tension. Pre-incubation of tracheal rings with EHNA plus uridine increased by 2.4 fold the synergic effect of cAMP on CCh-induced contraction (from 19±4% to 46±2%). On the other hand, CGS-15943 reduced by 94% the stimulatory effect of 300 µM cAMP on CCh-pre-contracted trachea. **Conclusions**: The present results indicate a prominent function of the "extracellular cAMP-adenosine pathway" in the modulation of ASM contraction. The greater effect of cAMP in CCh-pre-contracted tracheas suggests that cholinergic system and extracellular cAMP synergistically increase the force of contraction in the ASM. **Financial Support**: CAPES, CNPq and Fapesp. Animal Ethics Committee: CEUA #9987150714

08.007 Involvement of TRPV1 receptor in plasma extravasation in trachea and bronchi of rats treated with angiotensin-converting enzyme inhibitor. Oliveira JRJM, André E UFPR – Farmacologia

Introduction: Patients receiving angiotensin-converting enzyme inhibitors (ACEI) as therapy for hypertension and heart failure report adverse effects such as cough and angioedema. The mechanism is unclear, although a role for bradykinin (BK) has been postulated. Studies have shown that BK can activate indirectly transient receptor potential vanilloid 1 (TRPV1). **Aims**: This study aimed to evaluate the involvement of TRPV1 in plasma extravasation caused in the airways after acute treatment with captopril, an ACEI. **Methods**: Male Wistar rats (200-250g) anesthetized (ketamine 50 mg/kg and xylazine 10 mg/kg intraperitoneally) had their trachea exposed and were for intratracheal (i.t) pre-treatment with Capsazepine (CPZ, TRPV1 antagonist) or HOE140 (bradykinin B2 receptor antagonist) or their respective vehicles (veh). The association of CPZ plus HOE140 was also performed (i.t). Captopril intravenous (i.v.) and/or capsaicin (CPS) i.t. as well as their respective veh and Evans blue dye injection (30 mg/kg,i.v.) was administered 15 minutes after antagonists pre-treatment. Ten minutes after, animals were transcardially perfused with saline (0.9% NaCl) and trachea (T) and bronchi (B) were removed, weighed and kept in 1 ml of formamide for 24 hours (in dark and room temperature). The amount of Evans blue dye extravasated was measured spectrophotometrically at 620nm and expressed in μg/g of tissue. **Results**: Evans blue dye extravasation was increased after CPS administration (1 nmol/100 μl i.t.) in T (103.6 ± 8.6 μg/g) and in B (97.2 ± 5.6 μg/g) compared to veh (21.8 ± 3.7 and 17.2 ± 2.6 μg/g respectively; $P<0.05$). This effect was inhibited by pretreatment with CPZ (100 nmol/100 μl i.t.; % inhibiton of 49 ± 6% in T and 37 ± 10% in B; $P<0.05$). In another series of experiments captopril (2.5 mg/kg i.v.) also induced plasma extravasation in T (75.5 ± 14.5 μg/g) and in B (52.9 ± 6 μg/g) as compared to veh (24.7 ± 5.8 and 13 ± 2.7 μg/g respectively; $P<0.05$). This effect was inhibited by pretreatment with CPZ (100 nmol/100 μl i.t.; % inhibition: 57 ± 4% in T and 48 ± 5% in B; $P<0.05$). The plasma extravasation induced by captopril (55.7 ± 9.7 in T and 47 ± 6.5 μg/g in B) was also inhibited by Hoe 140 (10 nmol/100 μl i.t.) which corresponding to 61 ± 8% and 42 ± 6% of reduction, respectively; $P<0.05$). Administration of captopril low doses (0.25 mg/kg i.v.) or CPS (0.5 nmol/100 μl i.t.) which do not caused, *per se,* plasma extravasation when associated induced plasma extravasation in T (128.5 ± 12 μg/g) and in B (114 ± 13 μg/g) compared to veh (27.6 ± 3 and 25.7 ± 5.8 μg/g, respectively; $P<0.05$). This response was reduced by pretreatment with CPZ (100 nmol/100 μl i.t.; 49 ± 11% in T and 46 ± 10% in B of reduction; $P<0.05$). **Conclusion**: These preliminary results suggest that plasma extravasation induced by captopril on airway occur, in part, by TRPV1 indirect modulation possibly via stimulation of bradykinin receptors. **Financial support**: This study was supported by CAPES and CNPq. **Animal research ethical committee**: The protocols were approved by Ethics Committee of UFPR (process number 800/2014).

08.008 Gastric healing properties of a medicinal plant in threat of extinction: *Persea willdenovii* **Kosterm.** Somensi LB, da Silva LM, Boeing T, Cury BJ, Andrade FS
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Introduction: *Persea willdenovii* Korstem (Lauracea) popularly known as Pau de Andrade is a medicinal plant widely used in Santa Catarina for the treatment of ulcers and gastritis. However, this species is currently endangered in some Brazilian states in consequence to exploratory use. **Aims**: Based on popular use and motivated by the need to search for new treatments, the aim of this study was to evaluate pre clinically the gastric healing activity of the hidroalcoholic extract from the bark of *P. willdenovii* (HEPW). **Methods**: Fasted female Wistar rats were treated with vehicle (water, 1 ml/kg, p.o), carbenoxolone (CBX: 200 mg/kg, p.o) or HEPW (30 - 300 mg/kg, p.o), 1 hour before administration of 80% ethanol (5 ml/kg, p.o) or indomethacin (80 mg/kg, p.o). In another set of experiments, rats were orally pretreated with dichloromethane- (59.2 mg/kg), ethyl acetate- (16.6 mg/kg) or aqueous-fraction (177.7 mg/kg) from HEPW. The doses of fractions were based in its yield and the effective dose of HEPW. Chronic ulcer was induced in rats by 80% acid acetic and animals were treated with vehicle (water, 1 ml/kg, p.o), omeprazole (20 mg/kg, p.o), or HEPW (300 mg/kg) twice a day for 7 days. The ulcer area was measured at the final of treatment and sample were processed for histological, histochemical and biochemical analysis. Antisecretory properties were evaluated using pylorus ligature model. Acute toxicity also was evaluated. In addition, evaluation of prokinetic properties of HEPW (30-100 mg/kg, p.o) was undertaken in mice (approval number in CEUA: 02/15p). **Results and Conclusions**: Administration of HEPW (300 mg/kg) and CBX reduced the gastric lesion induced by ethanol in 59% and 97%, respectively; and induced by indomethacin in 75% and 60%, respectively, when compared with ulcerated vehicle group (148 ± 14.9 mm^2 and 12 ± 0.8 mm^2, respectively). Moreover, dichloromethane fraction reduced the ethanol- induced ulcer in 68% and indomethacin- induced ulcer in 60%. In chronic assay, HEPW and omeprazole reduced by 40% and 49% the ulcer area compared to the vehicle group (128 ± 12.1 mm^2). The gastric healing effect promoted by HEPW was accompanied by increase in mucin (PAS stained), GSH levels, SOD and CAT activities; and by reduction in MPO activity in stomach tissue. In addition, HEPW does not promote changes in volume, pH, total acidity or pepsin activity in pylorus ligated rats. In relation to the gastrointestinal motility, HEPW did not provoke alterations in intestinal transit or gastric emptying. Besides, is important to mention that the oral administration of HEPW did not produce any sign of acute toxicity in the animals. Phytochemical trials are being carried out to identify active compounds. Taken together, these findings contribute to the validation of the medicinal use of bark from *P. willdenovii* and show that the strengthening of protective factors of gastric mucosa, such as mucus layer and antioxidant defenses, is involved in healing effects of HEPW. However, it is worth noting that, emergency measures for sustainable management in the cultivation and exploitation of *P. willdenovii* should be taken so that this plant continues to contribute in Brazilian folk medicine. **Financial support**: CNPQ, CAPES, FAPESC. **Approval number CEUA**: 02/15p.

08.009 Pre-clinical evaluation of intestinal anti-inflammatory activity of three Brazilian medicinal species: *Achyrocline satureoides*, *Maytenus robusta* and *Rubus imperialis*. Farias JAM[1], da Silva LM[1], Somensi LB[1], Cury BJ[1], Santin JR[1], Niero R[1], Andrade SF[1] [1]Univali – Pharmaceutical Sciences

Introduction: Ulcerative colitis (UC) is a chronic inflammatory disease characterized by diffuse inflammation of the rectal and colonic mucosa. The etiopathology is related to an abnormal colonic immune response and interactions between genetics, colonic gut flora and environmental factors. However, due to the complexity of etiology and potentially serious adverse effects, treatment options for UC are relatively limited. Therefore, new treatment options, including herbal formulations, can change the current therapeutic situation. **Aim**: The purpose of this study was to examine the intestinal anti-inflammatory therapeutic effect of extracts from Achyrocline satureioides, Maytenus robusta and Rubus imperialis, three Brazilian medicinal species with anti-inflammatory, anti-nociceptive and anti-ulcer reported effects. **Methods**: Colitis was induced in Swiss female mice by addition of 3% (w/v) DSS in drinking water over 7 days. A non-colitic group not received DSS. Colitic mice were treated once a day for 9 days with vehicle (water, 1mL/kg), hydro alcoholic extract of A. satureoides inflorescences (HEAS, 100 mg/kg, p.o), hydro alcoholic extract of M. robusta leaves (HEMR, 100 mg/kg, p.o) or hydro alcoholic extract of aerial parts of R. imperialis (HERI, 100 mg/kg, p.o) once a day for 9 days. The treatments started simultaneously with the DSS administration. The effects of extracts on signs of colitis were then determined. Colon, liver and spleen weight also were measured. Besides, histological changes, levels of mucin, neutrophil influx by myeloperoxidase (MPO) assay and oxidative parameters were determined in the colon tissue. **Approval number CEUA**: 033/14p. **Results**: As expected, colitic mice treated with vehicle exhibited marked colitis clinical symptoms, including weight loss, reduced colon length, intense diarrhea and gross retal bleeding. In other hand, treatment with HEAS or HEMR, but not HERI, attenuated these symptoms and also suppressed the MPO accumulation in colon tissues. Supporting these findings, treatment with HEAS or HEMR markedly attenuated DSS-induced pathological changes in colon, such as loss of epithelial barrier, decrease in the number of crypts, goblet cells and mucin content and submucosal edema. Moreover, the decrease in colon and the increase on spleen weight induced by DSS were prevented by HEAS or HEMR administration. In addition, availability of the antioxidant GSH and the SOD activity were decreased in colon of colitic mice treated with vehicle and normalized to basal levels in colon of mice treated with HEAS or HEMR. **Conclusion**: Our results suggest anti-inflammatory effect of HEAS and HEMR, but not HERI, at colorectal sites and provided experimental evidence about the potential of A. satureoides inflorescences and M. robusta leaves such as a source of therapeutic recourse for treatment of UC. Effect of HEAS and HEMR on levels of pro-inflammatory cytokines induced by DSS is being evaluated in colon tissues to better understand the observed effects.. **Financial support**: CNPQ, CAPES, FAPESC.

08.010 JME-209 II: An orally active mexiletine analogue exhibiting anti-inflammatory actions in experimental models of Acute Respiratory Distress Syndrome and Chronic Obstructive Pulmonary Disease. Oliveira MTP[1], Coutinho DS[1], Carvalho KIM[1], Bernardi A[1], Xavier RF[2], Silva ET[3], Silva PMR[1], Costa JCS[4], Martins MA[1] [1]Fiocruz – Inflammation, [2]Fiocruz – Cellular Communication, [3]Fiocruz – Organic Synthesis

Introduction: Lung diseases are responsible for the death and suffering of millions of people worldwide. Triggered by several factors, lung diseases activate an inflammatory response characterized by inflammatory cell influx, endothelial cell damage and compromised alveolar function that leads to respiratory failure. The acute respiratory distress syndrome (ARDS) and chronic obstructive pulmonary disease (COPD) are examples of serious respiratory diseases, which do not have satisfactory treatment. Nebulized lidocaine has raised interest as an alternative therapy for respiratory diseases, but airway irritation, a side effect inherently linked to the anesthetic property of this drug, is a drawback. Our research group has recently identified new analogues of the orally active local anesthetic mexiletine, which have a higher capacity to relax respiratory smooth muscle contraction compared to the prototype, in spite of presenting a highly attenuated capacity to block sodium channels. **Aim**: The objective of this study was to evaluate the anti-inflammatory potential of JME-209, a mexiletine analogue, in experimental murine models of ARDS and COPD. **Materials and Methods**: To mimic ARDS, male mice A/J (18-20g) were nasally instilled with LPS (25µg/ 25µl), and pretreated orally with JME-209 (30 and 100 mg/kg) 1 h before. After 18 h, Airway hyper-reactivity (AHR) to methacholine aerosol was evaluated by invasive whole body plethysmography. To access the migration of inflammatory cells, the animals underwent bronchoalveolar lavage. For COPD protocol male mice C57BL/6 were exposed to smoke from four cigarettes three times a day with intervals of at least 3 h for 5 days. The animals were treated with JME-209 (30 and 100 mg/kg) orally 1 h prior to the first cigarette smoke (CS) provocation. Analyses were carried out 24 h after the last CS exposure. All experimental procedures were approved by the Committee on Use of Laboratory Animals of Oswaldo Cruz Foundation (license LW 23/10). **Results**: Pretreatment with JME-209 (30 and 100 mg/kg) significantly reduced LPS-induced AHR as assessed by lung resistance (70 and 81%) and elastance (56 and 84%). We also observed a significant decrease in the total leukocytes (44 and 56%) and neutrophils (47 and 62%) recovered from the bronchoalveolar fluid (BAL) following LPS. The exposure of animals to CS caused a significant increase in the number of leukocytes recovered from the BAL fluid. The treatment with JME-209 (30 and 100 mg/kg) reduced infiltration of total leukocytes (79 and 100%) and neutrophils triggered by CS exposure. **Conclusion**: These results reinforce the interpretation that JME-209 is an orally active mexiletine analogue, which present a great potential for therapeutic interference in inflammatory respiratory diseases, such as acute lung injury and COPD, with marked advantages over local anesthetic agents such as lidocaine. **Financial support**: FIOCRUZ, FAPERJ, CNPq and INCT-INOFAR.

08.011 D-cysteine protects gastric mucosa by an independent mechanism of Cystathionine γ-Lyase and D-amino acid oxidase. Araújo TSL[1], Souza LKM[2], Nicolau LAD[3], Costa DS[4], Sousa NA[1], Sousa FBM[1], Carvalho NS[4], Silva IS[4], Pacífico DM[1], Medeiros JVR[1,2,4] [1]UFPI – Biotecnologia, [2]UFPI – Ciências Biomédicas, [3]UFC – Farmacologia, [4]UFPI – Farmacologia

Introduction: Hydrogen sulphide (H_2S) is known to be produced from L-cysteine by cystathionine β-synthase (CBS) and cystathionine γ-lyase (CSE). Recently was discovered a novel pathway which involves D-Amino acid oxidase (DAO) that uses D-cysteine to produce H_2S. A previous study reveals that administration of D-cysteine protects cerebellar neurons and kidney (Shibuya, N., Nat. Commun., v. 4, p. 1366, 2013). However there are no studies elucidating the role of the DAO in the protective effect of H_2S in gastric mucosa. **Aim**: The aim of the study was to investigate the gastroprotective effect of D-cysteine against ethanol-induced gastric damage in mice and if this effect is due to increase of H_2S production. **Methods**: Mice (Swiss, 25-30g) were treated orally with saline (group 1), L-cystein (100 mg/kg; group 2) or D-cystein (100 mg/kg; group 3). Groups 4 and 5 were pretreated with propargylglycine (PAG, CSE inhibitor, 100 mg/kg, *p.o.*) and indole-2-carboxylate (I2CA, DAO inhibitor, 100 mg/kg, *p.o.*), respectively, and thirty minutes later received D-cystein. After 30 min, ethanol 50% (2.5 ml/kg, *p.o.*) was administered. After 1 h, they were sacrificed and their stomachs removed and opened. The lesions were measured using a computer planimetry program (Image J) and samples of each stomach were fixed in 10% formalin for histopathological assessment. Other samples were then analyzed for glutathione (GSH) and malonyldialdehyde (MDA) levels. Measurement of H_2S in gastric tissue was performed using an ion-selective electrode on a Fisher Accumet Model 10 pH meter following the manufacturer's directions. **Results**: Ethanol 50% administration induced mucosal gastric damage (17.73 ± 2.92 lesion mm^2). However, pretreated with L-cystein and D-cystein (100 mg/kg, *p.o.*) prevents, significantly ($P < 0.05$), ethanol-induced lesions (5.17 ± 1.86 and 5.02 ± 1.41 lesion mm^2, respectively). Histological analysis revealed that pretreatment with L-cystein and D-cystein decreased hemorrhagic damage, edema and the loss of epithelial cells caused by ethanol administration. L-cystein and D-cystein also reduced MDA and kept the GSH levels. Pretreatment of D-cystein-treated group with PAG and I2CA had no subsequent effect on gastroprotector effect of D-cystein (7.02 ± 2.32 and 4.15 ± 0.98 lesion mm^2, respectively), however, pretreatment with D-cystein increased H_2S levels (0.52 ± 0.02 µmol/g) as well as L-cystein (0.62 ± 0.04 µmol/g) when compared with untreated group (0.25 ± 0.05µmol/g). **Conclusion**: Taken together, these results shown that D-cysteine had a protective effect against ethanol-induced gastric damage and this effect could be by H_2S production by other mechanism independent of CSE and DAO. However, further tests are needed to evaluate this possibility. **Financial Support**: CNPq and FAPEPI. **CEP**: This study was approved by the local Ethics Committee (nº 0066/10).

08.012 Sulphated polysaccharides extracted from *Gracilaria birdiae* reduces parameters inflammatory of the mucositis induced by 5-fluorouracil (5-FU) in mice. Almendra RB, Teles RHG, Costa MS, Magalhães DA, Lima Filho ACM, Batista JA, Coelho ML, Lima GM, Carvalho NS, Silva IS, Macêdo WBS, Barbosa ALR, Filgueiras MC UFPI – Farmacologia

Introduction: Marine resources have attracted attention because of its bioactive compounds, especially in regard to the development of new drugs. The sulfated polysaccharide (PLS) in algae contain substances with potential pharmaceutical and biomedical. Recent studies have shown that PLS extracted from *Gracilaria birdiae* demonstrated antioxidant and anti-inflammatory activities. The use of antineoplastic agents for treatment of cancer patients has important side effects such as dyspeptic syndrome and intestinal mucositis. **Aims**: Knowing that mucositis induced by 5-fluorouracil chemotherapy (5-FU) leads to inflammation of the intestinal mucosa and causes changes in its operation, it's necessary to check the possible anti-inflammatory activity of sulfated polysaccharides extracted from red seaweed *Gracilaria birdiae* about mucositis. **Methods**: The present study was approved by the local ethics committee (protocol n° 036/12). We used male Swiss mice, coming from the Central Animal Facility of the Federal University of Piauí. Intestinal Mucositis was induced in the experimental day 7 after pretreatment with sulphated polysaccharides(PLS) with a single intraperitoneal dose of 450 mg / kg (body weight) of 5-FU, followed by euthanasia on the day 10. The 5-fu group was subjected to intestinal mucositis induction on the day 7 without pretreatment with PLS, followed by euthanasia on day 10. The group without 5-FU-induced mucositis received the same volume of PBS (Phosphate-Buffered Saline), also intraperitoneally, followed by euthanasia at 10 days. After the sacrifice of animals, jejunum and ileum samples were then weighed, frozen, and stored at -80 °C until assayed for glutathione (GSH) levels, malondialdehyde (MDA) concentration, myeloperoxidase (MPO) activity. **Results**: The treatment of jejunum and ileum with 5-fluorouracil (450 mg/Kg) resulted in significantly decrease of the concentration of GSH (66,57 ± 6,512 µg/g tissue/ 239,2 ± 34,12 µg/g tissue) along with a concomitant increase the levels of tissue MDA (654,9 ± 98,05 mmol/g tissue/ 504,8 ± 97,04 mmol/g tissue) in the jejunum and ileum of rats, respectively, when compared to control treated with saline (*P < 0.05). Pretreatment of jejunum and ileum with sulfated polysaccharides (90 mg/Kg) significantly increased GSH levels (181,4± 27,26 µg/g tissue/ 601,5±13,20 µg/g tissue) respectively, and reduced significantly the concentration of gastric mucosal MDA (134,8±5,160 nmol/g tissue/ 233,1±98,39 nmol/g tissue). In the evaluation of myeloperoxidase activity, sulfated polysaccharides (90 mg/kg) significantly reduced the MPO activity in jejunum and ileum (6,887 ±3,684 U/mg tissue/ 37,07±14,70 U/mg tissue) as compared to 5-FU group (36,36±11,69 U/mg tissue/ 82,40±22,67 U/mg tissue). **Conclusion**: This study demonstrates the protective effects of sulfated polysaccharides extracted from red seaweed *Gracilaria birdiae* against 5FU-induced instestinal mucositis. The properties of sulfated polysaccharides warrant its safe use as drug antioxidant activity and anti-inflammatory activities to improve the health and the prognosis of the disease. **Financial support**: **CNPQ/FAPEPI**

08.013 Gastroprotective effect of diminazene aceturate: role of ACEII/Ang(1-7)/MAS pathway in gastric injury models in mice. Souza LKM[1], Nicolau LAD[2], Araújo TSL[2], Costa DS[2], Sousa NA[2], Sousa FBM[2], Silva IS[2], Pacífico DM[2], Medeiros JVR[1] – [1]UFPI – Ciências Biomédicas, [2]UFPI

Introduction: In the gastrointestinal tract, ethanol can induce acute hemorrhagic lesions, their excessive intake may result gastritis frames. In current research, the route of the Angiotensin Converting Enzyme II (ACEII) was related to several beneficial effects in the body, including the gastroprotection. Recently, Diminazene Aceturate (DIZE), a trypanosomicide used in animals, was described as a possible activator ACEII (Kuriakose, S.; Int immunopharmacol, v.21, p.343, 2014). **Aim**: Evaluate the gastroprotective effect of DIZE way ACEII/Ang(1-7)/MAS on ethanol- and acetic acid-induced gastric lesions in mice. **Methods**: To assess the gastroprotective effect of DIZE against ethanol-induced acute injury, mice (25-30g) were pretreated orally with saline (control), DIZE (0.7, 7 and 20 mg/kg) or omeprazole (10mg/kg). After one hour ethanol 50% (0.5ml/25g) was administered. One hour after animals were sacrificed, the stomach removed and immediately opened for analysis. Gastric damage was measured (Image J®). To evaluate the role of ACEII/Ang(1-7)/MAS pathway, animals were pretreated with A-779 (antagonist MAS receptor; 5mg/kg, *i.p*.) and after 30 minutes DIZE (7mg/kg) or saline were administered. The others steps were similar to those described above. Tissue samples were removed for microscopic analysis, MDA and GSH. Mucus and gastric secretion tests were performed. For analysis of DIZE effects in wound healing against acetic acid-induced chronic damage, animals were anesthetized and laparotomy was performed, the stomach was exposed and the acetic acid 40% (100 µl/1min) was administered in the serous. Treatment with saline, DIZE, A-779, A-779 + DIZE or OMP was performed the 2nd to 7th day after the injury. The animals were sacrificed of the seventh day and the stomach removed and opened for analysis. Samples were removed for histological analyzes and MPO activity. The concentration of Ang(1-7) was measured using ELISA in both lesions. **Results**: Ethanol-induced damage was reversed by DIZE pretreatment (20 and 7 mg/kg, inhibition of 90 and 94%, respectively), as well as OMP (88% inhibition) ($P<0.0001$). A-779 reversed the protective effect of DIZE. DIZE (7 mg/kg, better dose) elevated GSH levels and decreased the MDA concentration when compared with untreated group (403.3 ± 23.9; 187.8 ± 13.3 mg/NPSH/g and 166.4 ± 9.9; 327.0 ± 44.8 nmol/g, respectively). A-779 administration reversed these biochemical parameters when compared with control group (279.9 ± 9.1, 382.2 ± 38.7 mg/NPSH/g, and 481.1 ± 39.0, 169.4 ± 7.4 nmol/g, respectively). DIZE elevated mucus levels (58.9 ± 3.3 alcian Blue µg/g), decreased gastric secretion ($p<0.0001$) and decreased MPO activity (2.3 ± 1.7 U/mg). DIZE, also, decreases acetic acid-induced gastric injury and A-779 reversed this effect (64%; 0% inhibition, respectively) ($P<0.0001$). The pre-treatment and treatment with DIZE decrease inflammatory cell infiltration, edema formation and loss of epithelial cells. Furthermore, DIZE elevated Ang(1-7) levels in both injury models ($P<0.0001$). **Conclusions**: The DIZE promotes gastroprotective effect on acute and chronic injury model, acting through ACEII/Ang(1-7)/MAS axis. **Financial Support**: CNPq. **CEP**: Approved by local ethics committee, Nº0066/10.

08.014 Gastroprotective potential of the *Artocarpus heterophyllus* Lam. (jackfruit) seeds in Mice. da Rosa RL, Almeida CLB, da Silva LM, Cechinel-Filho V, Andrade SF Univali – Pharmaceutical Sciences

Introduction: folk medicine all parts of *Artocarpus heterophyllus* are used to treat several diseases, including dyspepsia, diarrhea, skin diseases, diabetes, wound healing and to improve digestion. However, the gastroprotective potencial of *A. heterophyllus* has not been evidenced in the literature. **Aim**: The aim of this study was to investigate the potential gastroprotective of crude methanolic extract (CME) of A. heterophyllus seed and identify the possible mechanism of action. **Methods**: Experimental acute gastric ulcer induced by absolute ethanol (5 ml/kg) or indomethacin (80 mg/kg, p.o) in mice pretreated whit vehicle (1 ml/100 g, p.o) or CME (50 - 250 mg/kg) was employed. To verify the involvement of nitric oxide and nonprotein sulfhydryl compounds in the gastroprotection, mice were pretreated with N^G-nitro-l-arginine methyl esters (l-NAME, 70 mg/kg, s.c) and N-ethylmaleimide (NEM, 10 mg/kg, s.c) prior CME treatment. Moreover, CME acid antisecretory effect was verified by pylorus ligation in mice, wherein volume, pH, total acidity and peptic activity of gastric content, and adhered mucus levels were determined. The gastric healing activity of CME (250 mg/kg, p.o) in installed 80% acetic acid- induced chronic gastric ulcer also was evaluated in mice. Carbenoxolone (200 mg/kg, p.o) or cimetidine (20 mg/kg, p.o) was used as positive controls in this study. **Results**: The CME showed gastroprotective activity, which can be observed in both models of acute gastric ulcer, evidenced lesion area from CME- treated group significantly lower ($p < 0.01$) compared to vehicle-treated rats. In addition, the cure rate evoked by CME (125 or 250 mg/kg, p.o) is close to the found in the cimetidine (100 mg/kg, p.o) group in both acute models. In addition, we showed that administration of L-NAME or NEM reversed the gastroprotective effect of CME against ethanol. Interestingly, CME (50, 125 or 250 mg/kg, i.d) did not alter the pH or total acidity of gastric acid secretion, but decrease the volume of gastric juice secreted in 40, 63 and 52%, respectively, and increase the gastric mucus adhered levels in mice up to 29%, when compared to vehicle group (0.3 ± 0.02 ml and 5 ± 0.66 mg Alcian Blue/ g of tissue, respectively). Furthermore, the healing gastric effect of the extract also was verified by reduction in 78% in area of lesion in stomachs of CME-(250 mg/kg, p.o) treated group compared to vehicle- treated ulcerated group (6.7 ± 1 mm^2). Phytochemical analysis demonstrated the presence of different phenolic compounds (flavonoids), terpenes and steroids in CME. **Conclusion**: Taken together, these results evidenced the gastroprotective potential of seed from A. *heterophyllus* fruits and show that this effect is related to different and complementary mechanisms, including the involvement of oxide nitric, sulfhydryl groups and mucus barrier, which are protective mechanisms of gastric mucosa. **Financial support**: CNPQ, CAPES, FAPESC. **Approval number in CEUA**: 019/13.

08.015 Antidiarrheal activity of *Maytenus erythroxylon* **Reissek (Celastraceae) in mice.** Formiga RO, Sales IRP, Nascimento RF, Lima GRM, Quirino ZGM, Tavares JF, Batista LM – UFPB

Introduction: In recent years, there has been a significant increase in the interest of Maytenus species, mainly in Brazil. This genus has shown promising results in pharmacological tests in the gastrointestinal tract, primarily attributed to the presence of compounds such as flavonoids, pentacyclic triterpenes, sesquiterpenes, steroids, tannins and alkaloids (Niero, R., Curr. Pharm. Des., 17, 1851, 2011). The species *Maytenus erythroxylon* Reissek was selected for this study based on chemotaxonomic criteria. **Aims**: To evaluate the antidiarrheal activity, effects on gastrointestinal motility and to determine the mechanism of action of ethanol extract obtained from the aerial parts from the plant (EEtOH-Me). **Methods**: For the experimental protocols, male Swiss mice were used (n = 5-7), weighing 25-35 g and pre-treated orally with vehicle (10 mL/kg), saline solution 0.9% (negative control), loperamide 5 mg/kg (positive control) and EEtOH-Me (62.5, 125, 250 and 500 mg/kg). The antidiarrheal activity was evaluated using the diarrhea induced by castor oil model (Awouters, F., J. pharmacol., 30, 1978) and the assessment of possible effects on gastrointestinal motility determined by the gastric emptying and intestinal transit protocols (Scarpignato, S., Pharmacodyn., 246, 1980 and Stickney, J. Exp. Biol. Med., 101, 1959). For the evaluation of potential anti-secretory mechanisms, it was used the enteropooling induced by castor oil essay, using the best dose of EEtOH-Me (Ezeja, M.I., Int. J. Toxicol. Pharmacol., 2, 40, 2010). The results were analyzed using Kruskal-Wallis test followed by Dunn's (median, maximum and minimum - non-parametric data) and ANOVA followed by Dunnett's test (mean ± standard deviation - parametric data). All results were considered significant when $p<0.05$. **Results**: The castor oil-induced acute diarrhea model showed that all tested doses of EEtOH-Me 62.5, 125, 250 and 500 mg/kg possess antidiarrheal activity, with respective evacuation index 8 (11, 5) and 62% of diarrhea inhibition ($p<0.05$), 7 (8, 6) and 66% ($p<0.05$), 6.5 (7, 3) and 69% ($p<0.05$) and 4 (5, 3) and 80% ($p<0.001$), when compared with negative control group 21 (25, 19). In the evaluation of effects on the gastrointestinal motility, the EEtOH-Me (250 and 500 mg/kg) reduced gastric emptying in 55 ($p<0.01$) and 37% ($p<0.001$), respectively. Moreover, the extract in all tested doses decreased the percentage of intestinal transit in 57, 49, 41 and 35% ($p<0.001$), respectively. In the castor oil-induced enteropooling, the EEtOH-Me in its best dose (500 mg/kg) reduced intestinal fluid in 0,6429 ± 0,1272, with 51% of fluid inhibition ($p<0.001$), when compared to the negative control group (1,325 ± 0,2053). **Conclusions**: Thus, the results of this study showed that the EEtOH-Me presents antidiarrheal activity that might be related to antimotility and antisecretory mechanisms. However, subsequent studies are needed to elucidate the complete mechanisms of action involved in the antidiarrheal effect. Acknowledgment: CNPq/CAPES/UFPB. Research approval by the Animal Research Ethical Committee (UFPB): 0105/14

08.016 Hydrogen sulfide reduces inflammation in acute pancreatitis induced by common bile duct obstruction in mice. Santos-Oliveira A[1], Santana DG[1], Muscara MN[2], Costa SKP[2], Camargo EA[1] [1]UFS – Physiology, [2]USP – Pharmacology

Introduction: Acute pancreatitis is an inflammatory disease of the pancreas that involves a complex interplay of many mediators. Hydrogen sulphide (H2S) is a modulator of the inflammatory process and some studies have demonstrated its involvement in the pathophysiology of pancreatitis in a controversial way. **Aim**: This study aimed to investigate the role of H2S in acute pancreatitis induced by common bile duct obstruction (CBDO) in mice. **Methods**: Male Swiss mice (20-30 g, n = 8/group) were submitted to acute pancreatitis induction by CBDO. Mice were treated with a H2S donor, Lawesson´s Reagent (RLw, 30 umol / kg, i.p.), an inhibitor of H2S production (PAG, 50 mg/kg, i.p.) or vehicle (saline) 1 h before and 12 h after induction. After 24 h, animals were killed and blood and tissue were collected to determine the serum amylase levels, myeloperoxidase (MPO) activity in the pancreas and lung, total and differential leukocyte count in peripheral blood, pancreatic edema index and histological analysis in pancreas. Results were expressed as mean ± SEM, and analyzed by ANOVA followed by Tukey´s test. P values <0.05 were considered as significative. **Results**: The administration of RLw reduced serum amylase levels (7,955 ± 2,421 U / dL, p<0.001) in comparison to vehicle-treated group (33,547 ± 1,925 U / dL), which did not occur with the treatment with PAG (34,704 ± 3,293 U / dL). The pancreatic MPO activity was also reduced by treatment with RLw (4.58 ± 1.51 UMPO / mg of tissue, p<0.05) in comparison to vehicle-treated group (13.85 ± 3.15 UMPO / mg of tissue). In the same way, lung MPO activity was inhibited by RLw treatment (8.35 ± 0.45 UMPO / mg of tissue, p<0.001) when compared to the group treated with vehicle (19.15 ± 1.93 UMPO / mg of tissue). On the other hand, the administration of PAG did not change MPO activity in the pancreas (12.57 ± 3.00 UMPO / mg of tissue), although it reduced the MPO activity in lung (12.73 ± 1.46 UMPO / mg of tissue, p<0.05). Regarding to pancreatic edema index, treatment with RLw caused a decrease in this parameter (3.17 cells / mL ± 0.22, p<0.01), which was not observed by the administration of PAG (4.41 ± 0.28), in comparison to the group treated with vehicle (4.68 ± 0.43). Also, total leukocyte counts in peripheral blood were not affected by the treatment with RLw (6.84 ± 1.35 cells / mL) or PAG (9.11 ± 0.92 cells / mL), when compared to vehicle-treated group (11.84 ± 1.88 cells / mL), as well as polymorphonuclear cell counts (3.13 ± 0.77; 2.84 ± 0.36; 3.07 ± 0.51 cells / mL respectively for RLw, PAG and vehicle groups). However, the mononuclear cell counts were reduced by treatment with RLw (3.70 ± 0.62, p<0.05), but not PAG, in comparison to vehicle group (8.77 ± 1.54 cells / mL). Qualitative histological analysis revealed that the treatment with RLw reduced the leukocyte infiltration, acini destruction and hemorrhagic/necrotic areas in pancreas, which did not occur with the treatment with PAG. Conclusions: These data suggest that donation of H2S by RLw can reduce the severity of acute pancreatitis induced by CBDO in mice. **Financial support**: CAPES and CNPq.

08.017 Rutin reduces abdominal hyperalgesia and pancreatic inflammation in acute pancreatitis induced by L-Arginine in mice. Teixeira DF[1], Camargo EA[1], Abreu FF[1], Souza ACA[1], Costa SKP[2], Muscará MN[2], Teixeira SA[2], Oliveira JP[1] [1]UFS – Ciências Fisiológicas, [2]USP – Farmacologia

Introduction: About 20% of cases of acute pancreatitis (AP) can be severe and cause hospitalization or death and the treatment of this condition is still inefficient to control the pancreatic inflammatory process and pain. In this context, rutin is a natural flavonoid with potential to treat AP, by considering its anti-inflammatory and antioxidant activities**. Aim**: This study investigated the possible anti-inflammatory and antinociceptive effects of rutin on experimental AP induced by L-arginine administration to mice. **Methods**: Adult male Swiss mice (n=6-7), were used in this study. All experiments were approved by Ethics Committee in Animal Research (CEPA/UFS, 43/2012). For the induction of AP, mice received 2 injections of L-arginine (8%, 4 g/kg, i.p., with an interval of 1 h). The control group received the same volume of saline (0.9%) instead of L-arginine. Mice submitted to AP induction were treated with rutin (75 mg/kg, p.o.) or vehicle (saline) after 24, 36, 48 and 60 h of the first injection of L-arginine. The control group received vehicle at the same time points. After 72 h of the first L-arginine injection, the serum concentrations of amylase, lipase, C reactive protein (CRP) and IL-6, as well as pancreatic myeloperoxidase (MPO) activity and edema index, were measured. Abdominal hyperalgesia was measured through the reduction of withdrawal threshold of the stimulus applied to the abdominal region with electronic von Frey (in g) at 1 h before (basal) and 72 h after induction. Data were expressed as mean ± SEM and analyzed by one-way ANOVA/Bonferroni's test. **Results**: Injection of L-arginine induced acute pancreatitis in mice, characterized by development abdominal hyperalgesia, increased serum amylase ($p<0.001$), lipase ($p<0.001$), CRP ($p<0.001$) and IL-6 ($p<0.001$) concentration, pancreatic MPO ($p<0.001$) and edema index ($p<0.001$) in comparison with saline-injected group. The abdominal hyperalgesia observed in vehicle-treated group (reduction of 5.97±3.00 g in the intensity of stimulus) was decreased ($p<0.05$) by the treatment with rutin (reduction of 3.31±1.67 g). Treatment with rutin reduced ($p<0.001$) the serum concentration of amylase (403.80±29.92 U/L), lipase (463.80±15.34 U/L), CRP (1.54±0.19 U/L) and IL-6 (9.29±0.24 pg/mL), when compared with vehicle-treated group (1,223.00±153.80 U/L, 1,293.00±78.63 U/L, 5.91±0.23 U/L and 36.04±4.33 pg/mL, respectively). The pancreatic MPO activity was also inhibited ($p<0.001$) by the treatment with rutin (0.18±0.06 UMPO/mg of tissue), when compared with the control group (1.84±0.26 UMPO/mg of tissue). Pancreatic edema index (wet/dry weight) was reduced ($p<0.05$) by treatment with rutin (3.40±0.06) when compared with the vehicle group (4.43±0.33). **Conclusion**: These results show that rutin reduce abdominal hyperalgesia and this may be linked to its anti-inflammatory effects in AP induced by L-arginine, which are suggestive that this flavonoid is of interest for the development of studies or future approaches for the treatment of AP. **Financial support**: CAPES, FAPITEC/SE and CNPq.

08.018 Evaluation of gastroprotective activity and mechanism of action of allantoin in different experimental ulcer models. Silva DM[1], Martins JLR[2], Oliveira DR[1], Oliveira TS[1], Ghedini PC[1], Costa EA[3] [1]UFG, [2]Centro Universitário Unievangélica, [3]UFG – Farmacologia

Introduction: Gastric ulcer, a benign lesion of gastric mucosa, affects a lot of people worldwide. The etiopathogenesis of gastric ulcer is associated with stress, use of alcohol and non-steroidal anti-inflammatory drugs (NSAIDs) among others. Efforts are being made in the search for new and suitable antiulcer agents of plant origin. Previous studies have shown evidences of gastroprotective property of ethanolic root extract of *Memora nodosa*. The allantoin, a purinic derivative founded in this organic extract was suggested to be responsible for the gastroprotective property of this specie. **Aims**: The present study sought to evaluate gastroprotective activity of allantoin using different agents that commonly attack gastric mucosa. **Methods**: Albino *Swiss* mice, weighing 30-35g, maintained on standard conditions of temperature (23 ± 1°C) and illumination (12-h light/12-h dark cycle) were used. The gastroprotective activity of this compound was investigated using models of gastric ulcers induced by ethanol, stress and indomethacin. Quantification of mucus content and measurement of catalase activity were carried out. **Results and Conclusions**: In ethanol induced gastric ulcer, allantoin (60 mg/kg p.o.) elicited 80% of reduction in ulcerated area. The same dose of allantoin reduced index of lesion from 11.90 ± 0.93 to 6.62 ± 0.49 (in acute lesions induced by stress) and from 15.35 ± 0.81 to 7.09 ± 0.96 (in acute lesions induced by indomethacin). In the quantification of gastric mucus, allantoin prevented mucus depletion (34.77 ± 4.14) as compared to control group with lesion (19.75 ± 2.53). Allantoin restored the activity of catalase enzyme from 403.7 ± 15.1 (control group with lesion) to 499.9 ± 16.38 nmol/mg of protein in ethanol induced gastric lesion. In conclusion, the present work showed the gastroprotective activity of allantoin in different models of gastric ulcer. These effects might be related to increase in mucus content and in the catalase activity. As the exact gastroprotective mechanisms of allantoin are still unclear, future research will be focused on the investigation of the underlying mechanism. **Financial Support**: CNPq, CAPES, FAPEG. Processing number for approval by CEUA (“Comitê de Ética no Uso de Animais”): CEUA UFG 038/14.

08.019 Ethanol-impaired hepatic and gastric function: benefits with *Baccharis trimera* extract. Lívero FAR[1], Silva LM[1], Ferreira DM[1], Beltrame OC[2], Werner MFP[1], Acco A[1] [1]UFPR – Farmacologia, [2]UFPR – Medicina Veterinária

Introduction: An estimated 2 billion people consume ethanol worldwide and 76.3 million have ethanol-related disorders. Among then, gastric and hepatic lesions are frequent in alcoholics. These lesions deserve special attention because the absence of specific treatment. The aim of this work was to investigate the influence of the hydroethanolic *Baccharis trimera* extract (HEBT) in both lesions induced by ethanol, gastric ulcer and alcoholic fatty liver disease (AFLD), using mice as the animal disease models. **Methods**: AFLD was induced in mice with 10% ethanol and 6% low-protein diet for 6 weeks, and in the last 15 days the animals were treated with vehicle (tween + water) or 30 $mg.kg^{-1}$ HEBT. The anti-ulcerogenic effects of 30 $mg.kg^{-1}$ HEBT were evaluated on acute gastric ulcers induced by P.A. ethanol. Mice fed with water and norm-protein diet, treated with vehicle, composed the "basal" group. In both models, treatments were performed once a day, by gavage. After, the animals were anesthetized and stomach, liver and blood were collected for the following analyses: GSH and hydroperoxides (LOOH) levels were measured as gastric and hepatic oxidative stress parameters; ALT and AST plasmatic levels indicated the liver function; plasmatic and hepatic levels of triglycerides (TG) were performed to demonstrate AFLD; hepatic gene expression of CYP2E1, SCD1 and Nrf2 were assessed (relatively to 18S gene) to check pathways of ethanol, lipid and oxidative metabolism, respectively; and gastric and hepatic histology were performed to check cell damage. **Results**: In AFLD model, ethanol administration associated with a low-protein diet increased plasmatic and hepatic TG levels by 130% and 52%, respectively. Elevation also occurred in ALT and AST levels by 182% and 51%, respectively; hepatic LOOH increased 23% and GSH levels 262%, compared with the basal group. Regardless gene expression, ethanol increased 7-fold the SCD1 and 2.5-fold the CYP2E1 RNA level. Treatment with HEBT revert totally the elevation in TG and AST levels and decreased ALT levels by 92%. HEBT was also able to reverse the accumulation of hepatic TG triggered by ethanol and low-protein diet. The histology confirmed these results, showing a protective effect of HEBT by preventing hepatic triglycerides accumulation. Additionally, HEBT reduced SCD1 and CYP2E1 gene expression to basal levels, and also increased significantly Nrf2 expression. In stomach, HEBT presented therapeutic efficacy in preventing ethanol-induced ulcers. Administration of HEBT significantly reduced the lesion area by 79% compared with the basal group. The administration of ethanol P.A. increased LOOH and decrease GSH levels by 409% and 69%, respectively, compared with the non-lesioned group. Interesting, HEBT also normalized LOOH and GSH levels. **Conclusions**: Our results indicate that HEBT has both gastroprotective and hepatoprotective activity against ethanol-induced lesions in animal models. The mechanisms involve the regulation of oxidative stress in both organs. HEBT also act modulating the expression of genes involved in ethanol metabolism, lipids metabolism and oxidative response. HEBT can be a promise therapy against gastro-hepatic diseases developed by the ethanol consumption. **Financial support**: REUNI-CAPES, CAPES and Fundação Araucária. **CEUA-UFPR**: The institutional committee for the animal care (# 619 and 810) approved all the procedures.