Session 09 − **Natural Products and Toxinology**

09.001

Leukotrienes, but not bradykinin and nitric oxide, are involved in paw edema induced by batroxase, a PI metalloproteinase isolated from *Bothrops atrox* snake venom. de Toni LGB[1], Figueiredo MJ[2], Sartim MA[1], Franco JJ[1], Cintra ACO[1], Souza GEP[3], Sampaio SV[1] [1]FCFRP-USP − Análises Clínicas, Toxicológicas e Bromatológicas, [2]FMRP-USP − Farmacologia, [3]FCFRP-USP − Física e Química

Introduction: The envenomation of Bothrops genus is characterized by hemorrhage, edema, pain, necrosis and systemic alterations like renal failure1. Metaloproteinases are responsible for the hemorrhagic activity present in a variety of snake venoms, mainly in Bothrops venoms, although it is involved in edema, inducing many inflammatory mediators, which cause plasma extravasations and vasodilatation2. We observed before that the ipl injection of batroxase, a metalloproteinase present in the *Bothrops atrox* venom at doses of 5, 10, 20 and 40 µg/paw induced a dose and time-dependent edematogenic response. Since 20 and 40µg/paw were almost equally effective in the edema induction (0.67 ± 0.04 and 0.77 ± 0.09, 1th h), 20 µg was selected for further experiments. It was also observed that histamine, serotonin but not prostaglandins are involved in this response3. In this step of the study it was investigated the participation of leukotrienes, nitric oxide and bradykinin on this response. **Methods:** The paw edema induced by intraplantar injection (ipl) of Batroxase was evaluated in male Wistar rats (180-200g) by plethysmometer (Ugo Basile, Italy) and expressed in milliliters (mL) of the difference between the left (Batroxase) and right (saline) paws during 6 hours. Rats were pre-treated with L-NAME, an inhibitor of nitric oxide synthesis, (5, 10 e 20 mg/kg, i.p.) 30 minutes before, MK-886, an inhibitor of leukotriene synthesis (1 and 5 mg/kg, s.c.), HOE-140, a B2 receptor antagonist (10 nmol, i.pl.) and DALBK, a B1 receptor antagonist, (100nmol, i.pl.), 15 minutes before. Control animals received saline. The experimental protocols were submitted and approved by the Ethical Committee of University of São Paulo, Campus of Ribeirão Preto, SP, protocol number 09.1.255.53.0. **Results:** The paw edema induced by Batroxase was insensible to L-NAME, DALBK and HOE-140 (0,71 ± 0,06 to 0,66 ± 0,04; 0,79 ± 0,10 to 0,75 ± 0,13; 0,79 ± 0,10 to 0,57 ± 0,08, 1th h, respectively). However MK-886 (5 mg/kg) were effective in reducing this response (0.874 ± 0.04 to 0.566 ± 0.07, 1th h, $P<0.05$). Conclusion: Leukotrienes seems to be involved in the first phase of edema induced by Batroxase. **References**: {1} Teixeira et al., Toxicon , 32, 419, 1994 {2} Galvão Nascimento et al., Toxicon, 30, 1, 2009. {3} De Toni, LGB et al., SBFTE, section 04, 4036, 2009. **Financial support:** FAPESP/CAPES/CNPq

09.002

Pharmacological characterization of a metalloproteinase from *Bothrops leucurus* snake venom. Gomes MSR[1], Queiroz MR[2], Mendes MM[2], Mamede CCN[2], Vieira SAPB[2], Gimenes SNC[2], Oliveira F[2], Rodrigues VM[2] [1]UESB − Química e Exatas, [2]UFU − Genética e Bioquímica

Introduction: Snake venoms are a mixture of complex proteins, many of which have biological activities, such as: hemorrhage, edema, pain, necrosis as well as disturbance in blood coagulation system. Snake venom metalloproteinases are sources of proteins with possible therapeutic applications. They are distributed in classes among P-I, P-II, P-III and PIV, according to organization of different domains. We reported the Pharmacological characterization of a metalloproteinase from Bothrops leucurus snake venom belonging to the class P-I SVMPs. **Methods:** The protease (BleucMP) was obtained by chromatography of ion-exchange DEAE-Sephadex A-25 and CM-Sepharose fast flow. This protease was homogeneous on SDS-PAGE and showed a single chain polypeptide of 23.4 kDa under reduced and unreduced 23.1 kDa condition. BleucMP showed proteolytic activity on fibrinogen, fibrin, but, no induce bleeding in the skin of mice and their proteolytic activity was inhibited by EDTA and 1,10-phenanthroline. This enzyme has the blood of mice incoagulable and plasma fibrinogen level showed a significant decrease. Analysis was also performed Clinic of biochemical tests (creatina kinase –CK-NAC, Creatinine, transaminases) and blood count (platelets, leukocytes) to better understand the action of this metalloproteinase, looking like a future therapeutic application in clinical disorders related to homeostasis. **Results and Discussion:** The enzyme was proteolytically active against bovine fibrinogen as substrate. When fibrinogen and BLeucMP were incubated at 37º C, the enzyme first cleaved the alpha-chain then the beta-chain and shows no effects on gamma-chains. BLeucMP was also able to degrade fibrin, when 10 µg of enzyme was incubated with fibrin at 37ºC for different time intervals. The analysis of digestion of fibrin and fibrinogen was performed on SDS-PAGE 12% (Gremski et al. 2007). The inhibition of Fibrinogenolytic activities was determined by incubating BleucMP with EDTA, Aprotinin, 1,10-phenanthroline, β-mercaptoethanol and Benzamidene all 10mM. BleucMP was inhibited by EDTA and 1,10-phenanthroline (Gomes et al. 2009), These tests revealed that BleucMP is a protease ion zinc-dependent. BLeucMP caused defibrinogenation when administered i.p. to mice, making the plasma uncoagulable. The fibrinogen levels were reduced to zero 2 h after injection. The clinical concentration of plaquestas, creatine, creatinine, aspartate aminotransferase and Glutamic pyruvic transaminase of the blood plasma of mice containing 10micrograms of crude venom or 50 micrograms of BLeucMP showed a low toxicity of the enzyme. BLeucMP was devoid coagulant and hemorrhagic activities with doses of up to 50micrograms.These properties suggest that BLeucMP belongs to class alpha-fibrinogenase snake venom and may have a clinical therapeutic application in cardiovascular and thrombotic disorders. The animal experiments were authorized by the committee ethics of the UFU. Protocol number 0082/2009. References: Gomes et al. *Toxicon* 53 (2009) pag. 24-32. Gremski et al. Toxicon 50 (2007) pag.120-134. **Financial support:** FAPEMIG, UFU, UESB.

09.003

Evaluation of the anti-hypernociceptive effect of the essential oil extracted from leaves of *Ugni myricoides* on inflammatory and neuropathic models of pain in mice. Rocha LR[1], Silva GF[1], Antonialli CS[1], Cechinel Filho V[2], Quintão NLM[1], Cicció, JF[3] [1]UNIVALI − Ciências Farmacêuticas, [2]NIQFAR-UNIVALI – Ciências Farmacêuticas, [3]Universidad de Costa Rica − Productos Naturales

Introduction: The myrtaceae family comprises a large number of plants, including at least 138 genera and approximately 3800 species. Several experimental studies have demonstrates the biological activity of extracts, essential oils, or fractions obtained from different species of this family. The aim of this study was to evaluate the anti-hypernociceptive effects of *Ugni myricoides* essential oil (EO) using experimental models of pain in mice. **Methods:** The pharmacological studies utilized female Swiss mice (25-30g) obtained from the University of Vale do Itajaí (UNIVALI). All procedures were approved by the Animal Ethics Committee of UNIVALI (protocol numbers 530/2008 UNIVALI). The number of animal used per group was 5-8. For the induction of the mechanical hypernociception, mice were submitted to the i.pl. injection of carrageenan (300 mg/paw), complete Freund adjuvant (CFA 20 mL/paw) or to the model of neuropathic pain induced by partial ligation of sciatic nerve (PLSN). The mechanical hypernociception was evaluated using the von Frey filament 0.6 g. **Results:** The oral treatment with *U. myricoides* EO (5-50 mg/kg) significantly inhibited the carrageenan-induced (300µg/paw) mechanical hypernociception, with maximal inhibition of 56 ± 6% at dose of 10 mg/kg. Similar results were obtained with animals injected with CFA (20 µl/paw) and pre-treated with *U. myricoides* EO (12,5, 25 or 50 mg/kg, p.o), with inhibitions of 56 ± 4%, 27 ± 2% and 57 ± 4%, respectively. It is know that the i.pl injection of CFA in mice produces sensorial changes in the contralateral hind paw after approximately 3 days; *U. myricoides* EO (10, 12,5 and 25 mg/kg, p.o) was effective in reducing mechanical hypernociception in the contralateral hind paw with inhibitions of 43 ± 8%, 62,5 ± 6% and 19 ± 2%, respectively. *U. myricoides* EO (5-50 mg/kg, p.o) also was capable of diminishing the nociceptive response induced by PLSN, with inhibitions of 60 ± 7%, 79 ± 6%, 68 ± 7% and 30 ± 5% at doses of 5, 10, 12,5 and 25 mg/kg, respectively. **Discussion:** This data demonstrates for the first time that the essential oil obtained from leaves of *U. myricoides* EO has relevant oral anti-hipernociceptive properties for persistent models of inflammatory and neuropathic pain in mice. *U. myricoides* EO has an efficacy comparable to that observed for indomethacin and gabapentin, drug used as positive control. The mechanisms through which *U. myricoides* OE exerts its anti-hypernociceptive actions remain currently unclear and require further investigation; however, it could well constitute a new and attractive alternative for the management of persistent inflammatory and neuropathic pain in humans. **Financial support:** CNPq, FAPESC, ProPPEC/UNIVALI, RIBIOFAR/CYTED/CNPq RT 0284.

09.004

Antidepressant-like effect of a supercritical carbon dioxide *Valeriana glechomifolia* extract. Müller LG[1], Salles LA[1], Betti AH[1], Stein AC[1], Sakamoto S[2], Quintas LEM[3], Bettero GM[3], Figueira R[3], Noel F[3], Von Poser GL[1], Rates SMK[1] [1]UFRGS − Ciências Farmacêuticas, [2]UFRGS − Farmácia, [3]UFRJ − Farmacologia

Introduction: Species of the genus Valeriana are traditionally used to treat mild sleep disorders and anxiety. Extracts from the roots of *V. officinalis* have long been used in traditional medicine and are the most well recognized herbal sedatives worldwide. The main active components are volatile oils and valepotriates. Among the native species, V. glechomifolia is the one with the highest valepotriate content (2.05%) (Silva et al., Planta Med 68:570, 2002). Considering that Hattesohl et al. (Phytomedicine 15:2, 2008) demonstrated the antidepressant action of V. officinallis, the aim of this study was to evaluate the effects of a supercritical carbon dioxide (SCCO2) V. glechomifolia extract (enriched in valepotriates) in predictive models of antidepressant activity and its effects on Na+/K+-ATPase activity, which has been related to the etiology of mood disorders, and acts on neuronal catecholamines uptake. **Methods:** Aerial parts of V. glechomifolia were submitted to SCCO2 at 40ºC, under 90 bar. The valepotriates (valtrate, acevaltrate, 1-β-acevaltrate, diavaltrate) were isolated by preparative thin layer chromatography. Mice (30-35g) were treated with the extract (1, 5 and 10 mg/kg, p.o.) 1h before the tail suspension test (TST) or 10 mg/kg before the forced swimming test (FST). The inhibitory effect of valepotriates on Na+/K+-ATPase activity was determined *in vitro* in rat kidney (containing the ouabain-resistant Na+/K+-ATPase a1 isoform) and brain hemispheres (containing mostly ouabain-sensitive a2/a3 isoforms) according to Fiske and Subbarow (J Biol Chem 66:375, 1925). The results were analyzed by one way ANOVA followed by Student-Newman-Keuls. All experimental protocols were approved by UFRGS Ethical Committee – 2007818. **Results and Discussion:** V. glechomifolia SCCO2 extract significantly reduced the immobility time in the FST (98.87 ± 18.42 s) as well as in the TST (141.62 ± 6.96; 116.7 ± 8.24; 106.9 ± 11.47 s; for 1, 5 and 10 mg/kg, respectively) as compared to control group (166.57 ± 4.47 and 152.3 ± 6.23 s for FST and TST, respectively). The extract activity was similar to the effect of imipramine (20 mg/kg v.o) in the FST (110.44 ± 7.05 s) and fluoxetine (30 mg/kg v.o.) in the TST (100.7 ± 6.65 s), indicating a potential antidepressant activity. Valepotriates inhibited Na+/K+-ATPase activity in a concentration-dependent fashion, with an IC50 ranging from 20 to 44 μM, without considerable difference between isoforms. Na+/K+-ATPase inhibition is involved in the regulation of neurotransmitter release in the peripheral and central nervous system. Interestingly, the therapeutic effect of some psychoactive drugs such as tricyclic antidepressants and selective serotonine reuptake inhibitors might include alterations of Na+/K+-ATPase activity (Zanatta et al., Braz J Med Biol Res 34(10):1265, 2001) Thus, these results suggest that valepotriates seem to represent new chemical entities with inhibitory Na+/K+-ATPase activity, as well as with antidepressant-like activity. Whether a correlation exists between these events is currently under investigation. **Financial support:** CNPq and CAPES.

09.005

Effects of dietary supplementation with a multimixture composed of oat bran, flaxseed, sesame and sunflower seed on renal function of diabetic rats. Damasceno DCF[1], Almeida IP[1], Sales ALCC[2], Teixeira JMR[1], Soares LFM[1], Santos Júnior JC[1], Cunha FVM[3], Soares MA[4], Martins MCC[1] [1]UFPI − Biophysics and Physiology, [2]UFPI − Nutrition, [3]Health, Human Science and Technologies Faculty − Physiotherapy, [4]UFPI − Biochemistry and Pharmacology

Introduction: Diabetes is a metabolic disease with complications involving damage in many organs, inclusively kidneys. Different food components, especially fiber rich compounds and polyunsaturated fatty acids have been tested as auxiliary resources in the treatment of diabetes. This study assessed the effects of dietary supplementation with oat bran, flaxseed, sesame and sunflower seeds on renal function of diabetic rats. **Methods:** Five days after induction of diabetes by streptozotocin (Sigma Chemical, USA, 40 mg/kg intravenously, citrate buffer pH=4.5), male Wistar rats (230 – 270 g) received daily, for 50 days: standard rat chow (Labina, Purina) enriched with oat, flaxseed, sesame and sunflower seed (M; n=10); standard rat chow (D; n=6); or standard rat chow plus subcutaneous insulin injection 3 U/animal (I; n=8) on alternate days. Animals were considered diabetic when their fasting blood glucose was higher than 250 mg/dL. The components of food supplementation were crushed and mixed with the standard diet at a ratio of 4 g of the mixture to 100 g of chow. Levels of glycemia, calcium, urea, creatinine, glycated hemoglobin (%), diuresis (mL/day), water ingestion (mL/day) and food intake (g/day), body weight (g) and kidney weight (g) were measured. Comparison between the groups was performed through analysis of variance and Tukey test. The study was approved by the Committee of Ethics and Research of NOVAFAPI (018/09). **Results:** No significant differences were shown between the groups in the following parameters: glycemia (mg/dL) pre-induction (D: 77.83 ± 4.01; M: 81.70 ± 1.85; I: 87 ± 3.47); glycemia post-induction (D: 406.17 ± 19.74; M: 419.20 ± 23.50; I: 367 ± 13.52) and final glycemia (D: 611.67 ± 43.65; M: 528.10 ± 55.55; I: 528.63 ± 52.95); water ingestion (D: 126.60 ± 5.17; M:123.10 ± 5.10 e I: 125.10 ± 9.10); diuresis (D: 83.81 ± 4.76; M: 77.79 ± 4.00 e I: 78.13 ± 6.30); food intake (D: 30.10 ± 1.15; M: 32.51 ± 1.84 e I: 35.62 ± 2.10) and calcium levels (mg/dL) (D:10.03 ± 0.52; M: 9.90 ± 0.27 e I: 9.62 ± 0.16). Kidney weight was significantly lower ($p<0,05$) for I group (0.93 ± 0.04) than for D (1.18 ± 0.05) and M (1.10 ± 0.04) groups. Serum levels of urea (mg/dL) were significantly higher for M (94.00 ± 4.01) than for D (79.60 ± 3.09) and I (70.20 ± 2.85), while creatinine levels (mg/dL) were significantly higher for I (0.77 ± 0.04) than for D (0.62 ± 0.03) and M (0.65 ± 0.04). Animals showed a propensity to weight loss, comparing body weight before and after the treatment: D (259.80 ± 5.06 and 185.80 ± 10.11), M (248.90 ± 3.78 and 179.00 ± 9.78) and I (238.50 ± 1.35 and 231.90 ± 10.43). **Discussion:** Dietary supplementation with oat bran, flaxseed, sesame and sunflower seed does not seems to improve the symptoms and the kidney function in streptozotocin induced diabetes. Additional studies are underway to evaluate possible renal histological changes indicative of a change in microarchitecture and/or renal vasculature. **Financial support:** UFPI. **Acknowledgments**: CENDOMED Laboratory

09.006

Dextran sulfate protected isolated rat heart from the cardiotoxic activity of *Bothrops jararacussu* venom. Martins VV, Ricardo HD, Machado MM, Tomaz MA, El-Kik CZ, Cons BL, Melo PA UFRJ − Farmacologia Básica e Clínica

Snakebites caused by *Bothrops* species induce local damage with edema, hemorrhage and myonecrosis. These effects are not completely understood and are poorly neutralized by antivenoms. Our group previously described the cardiotoxic effect of *B. jararacussu* venom in isolated heart preparation (Sifuentes et al., Toxicon, 2008). In this work we investigated the ability of dextran sulfate, a polyanion, to antagonize the cardiotoxic effect of *Bothrops jararacussu* crude venom. Adult male rats weighting 200-250g were anesthetized and their hearts were isolated, placed in the Langendorff preparation, bathed and continuously perfused (2-5 mL/min) with physiologic saline solution at 37°C to evaluate the cardiotoxicity. The cardiac tension and the electrocardiogram (EKG) were continuously recorded. We determinated the changes on cardiac frequency, interval PR, QRS complex, perfusion pressure and release of Creatine Kinase (CK). The venom of *B. jararacussu* (10 µg/mL) induced a gradual negative inotropic effect, decreased heart tension down to 0% in 15 minutes, and altered EKG waves, changing the others parameters markedly. The addition of dextran sulfate (100 µg/mL) diminished substantially the cardiotoxic effects, totally inhibiting the CK release induced by the venom. After the perfusion the hearts were removed from the Langendorff preparation, the ventricles sliced and stained with triphenyl tetrazolium chloride (TTC) 1%. The area without injury was marked with intense red, while the infarcted areas remained unstained. TTC staining showed large infarcted areas following exposure to the venom, and significant protection by dextran sulfate. Our study suggests that dextran sulfate is a remarkable antagonist of cardiotoxic effect of *B. jararacussu*. **Financial Support:** PRONEX, CNPq, CAPES, FAPERJ

09.007

Angiotensin-converting enzyme inhibition is involved in artemetin induced hypotension in rats. de Souza P[1], Gasparotto Júnior A[1], Crestani S[1], Silva RCMVAFda[1], Stefanello MEA[2], Marques MCA[1], da Silva-Santos JE[3], Kassuya CAL[4] UFPR − Farmacologia, [2]UFPR − Química, [3]UFSC − Farmacologia, [4]UFGD − Ciências da Saúde

Introduction: Our previous results showed that extract and semi-purified fractions obtained from *Achillea millefolium* L. (Asteraceae), popular use include the treatment of hypertension, were able to reduce the mean arterial blood pressure (MAP) of anesthetized rats (de Souza, P. unpublished results). Using a bio-guided approach we have characterized that the most active fraction obtained from A. millefolium presented as its main constituent the methoxylated flavonoid artemetin. The biological importance of these substances has been evidenced by their beneficial actions in several pathological conditions, being reported to have several activities in the cardiovascular system. Based on previous results, we investigate the possible hypotensive effect induced by artemetin, as well as its mechanism of action. **Methods:** Artemetin was isolated by chromatography techniques and it was identified by 1H and 13C NMR (200 MHz, CDCl3). Male Wistar rats received the oral treatment, 3 hours prior, with artemetin (1.5 mg/kg) or vehicle, or artemetin intravenously (0.15-1.5 mg/kg) and had the direct blood pressure measurement, where the anesthetized rats had the left carotid artery cannulated and connected to a pressure transducer coupled to a MacLab® recording system, and an application program (Chart, v 4.1) from ADI Instruments. To evaluate the hypotensive mechanism, we follow a trial of angiotensin-converting enzyme (ACE) in vivo, evaluating dose-response curves to angiotensin I (Ang I) and angiotensin II (Ang II), and response to bradykinin (BK). All procedures were approved by the Institutional Ethics Committee under protocol number 240. **Results and Discussion:** Artemetin reduced the MAP of rats both orally and intravenously administered, with changes about 8 ± 3 mmHg after 3 hours prior oral administration, and 5.6 ± 0.91 to 11.47 ± 1.5 mmHg after intravenous injection. The intravenous treatment of a single dose of artemetin (0.75 mg/kg) reduced the hypertensive response induced by Ang I in 37 ± 9 to 51 ± 7 %. The length of the hypertensive effect induced by Ang I was also reduced by artemetin in 25 ± 3 to 35 ± 9 % compared to control group. Artemetin wasn't able to increase significantly the hypotensive effect of BK, but the length of the hypotensive effect induced by it was significantly increased in 120 ± 29 % compared to control. To investigate the involvement of Ang II receptor, we administered artemetin in MAP changes induced by increasing doses of Ang II, but artemetin did not interfere in angiotensin II-induced hypertension in rats. Our study did show, artemetin may also be responsible for, the anti-hypertensive action attributed to A. millefolium, an effect, at least in part, mediated by angiotensin-converting enzyme inhibition. So further studies are needed in order to confirm the mechanism of action and to investigate others possibly involved mechanisms. **Acknowledgements:** CNPq and CAPES.

09.008

Antinociceptive effect of uliginosin B is mediated by the activation of dopaminergic and opioid systems. Stolz ED[1], Viana AF[2], Haas JS[2], Hasse DR[2], Von Poser GL[2], Costentin J[3], Do Rego JC[3], Rates SMK[2] [1]UFRGS − Neurociências, [2]UFRGS − Farmácia, [3]Université de Rouen − Neuro-psychopharmacologie Expérimentale

Uliginosin B (ULI) is the main phloroglucinol from Hypericum polyanthemum (POL), a vegetal species from South Brazil (Nör, et al. Biochem Syst Ecol. 32:517, 2004) that possesses antinociceptive effect in rodents (Viana et al. Braz. J. Med. Biol. Res. 36(5):631, 2003). The antinociceptive effect of ULI (90 mg/kg, i.p.) was also demonstrated. This effect was not blocked by naloxone in vivo and ULI did not affect [3H]-naloxone binding in mice brain as well (Heckler et al., FeSBE, 44.157:2005). However ULI inhibits dopamine, serotonine and norepinephrine reuptake in rat brain (Viana, UFRGS, 2007). The aim of this study was to further evaluate the antinociceptive effect of ULI by performing a dose response curve and pharmacological manipulations of different neurotransmitters systems in the hot-plate test as well as through [35S]GTPγS assays in brain membranes enriched in monoaminergic and opioid receptors. **Methods:** ULI was obtained from a n-hexane extract of aerial parts of POL by chromatographic **Methods:** and identified by 13CRMN and 1HRMN. ULI (5 – 45 mg/kg, i.p.) antinociceptive and motor coordination effects were evaluated in mice in the hot-plate (55 ± 1ºC) and on the rota-rod apparatus, respectively. The [35S]GTPγS binding was evaluated in striatum, hypothalamus, frontal cortex, and thalamus rat membranes incubated with ULI (10-6 – 10-10 M). [35S]GTPγS binding induced by dopamine (striatum), norephinephrine (hypothalamus), serotonin (frontal cortex) and DAMGO (thalamus) were performed as controls. Data from [35S]GTPγS binding, hot-plate and rota-rod tests were analyzed by ANOVA one or two factors RM followed by SNK post-hoc test. All experimental protocols were approved by UFRGS Ethical Committee – 2008195. **Results and Discussion:** ULI presented a dose dependent antinocicepitve effect in the hot-plate being the maximal effect reached at 15 mg/kg (19 ± 0.7s; $p<0.001$). Saline i.p. (11 ± 2.2s) and morphine 4 mg/kg i.p. (29 ± 2.2s; $p<0.001$) were used as control. Thus, this dose was chosen to continue the study. The effect of ULI was inhibited by pretreatment with sulpiride 10 mg/kg i.p. (14 ± 1.7s) and naloxone 2.5 mg/kg s.c. (14 ± 0.9s), dopamine D2-like receptor and opioid antagonists, respectively. ULI effect was not impaired by SCH 23390 15 µg/kg i.p. (dopamine D1-like receptor antagonist), pCPA 300 mg/kg i.p. (serotonin synthesis inhibitor), prazosin 1 mg/kg i.p. (α1 adrenergic receptor antagonist), MK-801 0.25 mg/kg i.p. (NMDA receptor antagonist) or rimonabant 1.5 mg/kg i.p (cannabinoid receptor antagonist). ULI did not affect the mice performance in the rota-rod and did not alter [35S]GTPγS binding in any brain structures evaluated. In conclusion ULI presents in vivo antinocicepitve effect at doses that does not compromise the motor coordination. This effect seems to be mediated by opioid and dopaminergic systems but not by the direct activation of monoaminergic and opioid receptors. **Financial support:** CNPq, CAPES/COFECUB 656/09.

09.009

Phytochemical analysis of ethanolic extract from *Terminalia cattapa* L. leaves and its correlation with gastroprotection. Silva LP[1], Angelis CD[1], Rinaldo D[2], Vilegas W[2], Hiruma-Lima CA[3], Toma W[4] [1]UNESP-Botucatu − Fisiologia, [2]UNESP-Araraquara − Química Orgânica, [3]UNESP-Botucatu, [4]UNISANTA − Farmácia

Introduction: *Terminalia catappa* L. (Combretaceae), popularly known as Chapéu-de-Sol, is a specie original of the East Indies and Oceania, and commonly used for afforestation along the entire Brazilian coast. It is popularly used for several diseases, such as liver injury, cramps, fever and diarrhea. Studies show that in *Terminalia catappa* leaves are present phenolic compounds, as: terpenes, flavonoids, chebulagic acid, quercetol and kaempferol, free diterpenes, triterpenes and catequin tannins. However, although there is a high incidence of scientific studies with this plants extracts, there is no report of anti-ulcer activity study performed with it, so this study aim to make a phytochemical analysis of the ethanolic extract (EtOHTc) and to correlate with gastroprotection. **Methods:** Phytochemical analysis: The chromatographic fingerprint of EtOHTc was established by HPLC. The mobile phase composition used was: water and acetonitrile, both with 0.05% trifluoroacetic acid. EZChrom Elite Data System software was used for both detector operation and data processing. The EtOHTc (2 mg) was dissolved in 2mL methanol, filtered through a 0.45-µm membrane polytetrafluoroethylene (PTFE) filter, resuspended in 3mL of water and 20µL was submitted to analysis by HPLC. Antiulcerogenic activity: Male Wistar rats or male Swiss mice were randomly divided into three groups (n = 7-10): EtOHTc (100 mg/kg), lansoprazole (30 mg/kg) as positive control or vehicle (saline, 10mL/kg) in the following protocols: NSAIDS/Bethanechol (Rainsford, 1978), HCl-Ethanol (Mizui, 1987), Ethanol (Robert, 1979), ischemia-reperfusion (Ueda, 1989), acetic acid-induced gastric ulcer (Takagi, 1979) and determination of gastric acid secretion (Shay, 1945). Results were expressed as mean ± S.E.M. Statistical significance was determined by one-way analysis of variance (ANOVA) followed by Dunnett's test, with the level of significance at * $p < 0.05$. All the procedures were approved by UNISANTA Ethics Committee of Research Center under protocol n° 53/07. **Results and Discussion:** The chromatographic fingerprint identified as the majority compounds hydrolysable tannins: gallic acid, ellagic acid and its derivates. The pharmacological assays demonstrated that EtOHTc reduced ulcer incidence: 58.1% (**p<0.01) in NSAIDS/Bethanechol; 70.76% (**p<0,01) in HCl-ethanol; 47.11% (**p<0,01) in ethanol-induced; 40.5% (**p<0,01) acetic acid-induced and 62.74% (**p<0,01) in ischemia-reperfusion. In determination of gastric acid secretion, there was an increase in pH values (**p<0,01), a decrease in ions H+ (**p<0,01) and in gastric secreted volume (*p<0,05). Studies demonstrated that poliphenolic compounds, as gallic acid, ellagic acid and derivates, can inhibit proton pump in parietal cells, protecting, so, the gastric mucosa from damages. Also, these compounds are described as natural antioxidants, scavenging free radical species, decreasing lipidic peroxidation, explaining, so, their antiulcerogenic activity. **References:** Andreo A.A. *J. Ethnopharmacol*. 107(3): 431, 2006. Chen P.S. Cancer Letters 152, 115-122, 2000. Gharzouli, K. Phytother. Res. 13, 42–45, 1999. Moraes, T. d. M. J. Ethnopharmacol. 120, 161-16, 2008. Mizui,T. Japanese J. Pharmacology, 44, 43, 1987. Murakami, S. Planta med. 57, 305-308, 1991. Murakami, S. Biochem. Pharmacol. 44,1947-51, 1992. Rainsford, K.D. Agents and Actions, 21: 316-319, 1978. Robert, A. Gastroenterology, v.77, p.433-443, 1979. Shay, H. Gastroenterol., 5:43-61, 1945. Szelenyi, I. Arch. Toxicol., 41(1): 99-105, 1978. Takagi, K. Japanese J. Pharmacology, 19, 418-426, 1969. Ueda, S. Scand. J. Gastroenterology, 162, 55-58, 1989. Financial support FAPESP: nº 07/59074-2

09.010

Anti-inflammatory effects of aqueous extract *Echinodorus macrophyllus* in mice air pouch model. Silva GP, Pinto FA, Vigliano MV, Leal NRF, Marques PR, Sabino KCC, Coelho MGP UERJ − Bioquímica

Introduction: *Echinodorus macrophyllus* (Kunth.) Mich, known as 'chapéu de couro' in Brazil, is a species from the Alismataceae family, used popularly to treat rheumatic and inflammatory diseases. This herb also possesses diuretic, depurative, laxative and uricosuric properties. Toxicological studies (Lopes, Toxic. Let., 116: 189, 2000), as well as immunoregulatory effects (Pinto, J. Ethnopharmacol., 111: 435, 2007) were previously described by our group from the aqueous extract of aerial parts of E. macrophyllus (EAEm). In this work we have evaluated the anti-inflammatory effects of EAEm in mice air pouch model. **Material and Methods:** The air pouch was induced by 5 mL of sterile air injection (s.c.) on the back of male SW mice (30-40 g). After 3 days, 3 mL of sterile air has been injected again to keep it. After six days each group (n=4) received intraperitoneal (i.p.) or oral (p.o.) treatment with EAEm (25 or 250 mg/kg) or controls indomethacin (10 mg/kg, p.o.) and vehicle (saline, i.p. or p.o.). One hour later 1 mL saline or carrageenan 1% sterile was injected into the pouch. After 4 h, the cavity was washed with NaCl 0.9%, EDTA 2 mM (1 mL), for determination of leukocyte numbers, final exudate volume and protein concentration. Cytospin preparations of exudates were stained with Panotic method for differential leukocyte count. Histological sections of tissue collected from different groups were fixed with 10% buffered formalin (pH 7.4) for 15 days and stained with HE and analyzed by MO. Results were expressed as mean ± SEM and compared using ANOVA and Dunnet's test. Experiments were performed in triplicate (Approved by CEA-IBRAG committee/protocol 05/2009). **Results and Discussion:** In air pouch model, were observed the following rates of inhibition of cell migration (expressed as million of cells): Indomethacin 2.50 ± 0.41 (84.01%); EAEm, p.o., at 25 mg/kg (8.99 ± 1.13; 38.47%) and 250 mg/kg (7.44 ± 1.41; 43.61%); EAEm, i.p., at 25 (4.93 ± 1.11; 66.44%) and 250 mg/kg (2.1 ± 0.36; 87.26%). The administration of EAEm at both process of treatment (p.o. or i.p) has reduced the leukocytes migration, when compared to the control group treated only with the vehicle (12.75 ± 1.72). However, the i.p treatment with EAEm reduced almost twice the cell migration. Regarding the differential count, the EAEm affected only the content of neutrophils. EAEm also reduced the total protein concentration in exudates mainly in the i.p. treatment. EAEm at 25 and 250 mg/kg showed 3.37 ± 0.55 and 2.05 ± 0.51 mg protein/mL, respectively, when compared to controls groups (Indomethacin 2.88 ± 0.64 mg/mL; Vehicle 5.48 ± 0.88 mg/mL). The histological analysis showed cellular infiltrate, mainly composed by polymorphonuclear leukocytes, and blood vessels throughout the inflamed dermis of animals treated with vehicle. Treatment with EAEm (25 or 250 mg/kg) (p.o. or i.p) reduced the leukocyte infiltrate and the vascularization area. In conclusion, all these findings support an anti-inflammatory potential suggested for this plant, however, further studies should be undertaken to elucidate the molecular mechanism of this response. In addition, phytochemical studies are already underway to identify active compounds in EAEm. **Financial support:** FAPERJ, CNPq and UERJ

09.011

Anxiolytic effect of the hydroalcoholic extract of *Lafoensia pacari* A. ST.-HIL. stem bark in mice. Galdino PM[1], Nascimento MVM[1], Sousa BF[1], de Paula JR[2], Costa EA[2] [1]UFG − Ciências Fisiológicas, [2]UFG − Farmácia

Introduction: In the Brazilian traditional medicine, *Lafoensia pacari* A. St.-Hil. (Lythraceae) has been referred for the treatment of different diseases, among them inflammation, gastric disturbs and central diseases. In previous results of our laboratory the hydroalcoholic extract from L. pacari stem bark (HEP) decreased the rearings, the number of invaded squares and the fecal bolus, and increased the immobility time in the open field test. HEP decreased the latency time to sleep and increased the sleep time induced by pentobarbital. The HEP does not change the rota rod test parameters. Continuing this study, this work evaluated the anxiolytic effect of this extract in mice. **Methods:** The stem barks of *L. pacari* were collected in the savannah region of Bela Vista, Goiás, and were authenticated by Prof. Dr. José Realino de Paula, a voucher specimen was deposited at the Herbarium of the UFG (27031/UFG). The hydroalcoholic extract of *L. pacari* (HEP) was obtained by maceration in 70% hydro-alcoholic solution, followed by filtration and evaporation (yield = 16.1% w/w). The anxiolytic activity was studied using the elevated plus maze (Lister, Psychopharm. v.92, p.180-185, 1987) and the light/dark box (LD) (Crawley & Goodwin, Pharmacol. Biochem. Behav., v.13, p.167-170, 1980) tests. The animal used were male Swiss mice (n = 9 per group) weighing 35-40 g from the Central Animal House of UFG. All experimental protocols were approved by the Ethic Commission of UFG (104/08). Results were expressed as means ± standard error of mean (SEM) and the statistical differences between experimental groups were detected by analysis of variance (ANOVA) followed by Student-Newman-Keuls test. **Results and Discussion:** In the elevated plus maze test, HEP at doses of 100, 300 and 1000 mg/kg did not alter the total entries in the open and closed arms, however, HEP at doses of 300 and 1000 mg/kg p.o. increased the percentage of entries from control value of 37.49 ± 3.69% to 54.14 ± 2.46% ($p<0.05$) and 52.37 ± 2.79% ($p<0.05$), respectively, as well as the percentage of permanence time in open arms from control value of 40.37 ± 6.16% to 61.88 ± 3.61% ($p<0.01$) and 60.78 ± 3.21% ($p<0.01$), respectively, and diazepam 1 mg/kg p.o. increased these parameters to 71.68 ± 5.871% ($p<0.001$) (percentage of entries) and 72.35 ± 4.94 % ($p<0.001$) (percentage of permanence time). In the light/dark box test, HEP at doses of 300 and 1000 mg/kg p.o. increased the time spent in the light area from control value of 104.88 ± 8.953 seconds to 143.50 ± 10.989 ($p<0.05$) and 151.50 ± 9.989 ($p<0.01$) seconds, respectively, as well as the total number of transitions from control values of 15.37 ± 1.700 to 23.25 ± 1.770 ($p<0.01$) and 25.00 ± 1.899 ($p<0.01$), respectively, and diazepam 1 mg/kg p.o. increased these parameters to 182.00 ± 11.594 seconds ($p<0.001$) (time spent in the light area) and 25.00 ± 2.215 ($p<0.01$) (total number of transitions). These data indicate that the extract of *Lafoensia pacari* A. St.-Hil. presented anxiolytic effect in the models tested. Acknowledgments The authors are grateful to Mrs. Ekaterina A.F.B. Rivera and Jackson Nascimento de Lima for technical assistance. **Financial support:** FUNAPE/UFG, PRPPG/UFG, CAPES and PIBIC/CNPq.

09.012

Effects of the hydroalcoholic extract of *Euterpe oleracea* Mart (açaí) on oxidative stress and endothelial dysfunction associated with 2-kidney, 1-clip hypertension. Costa CA[1], Oliveira PRB[1], Emiliano da Silva AF[1], Ognibene DT[1], Carvalho LCRM[1], Amaral TAS[1], Cordeiro VSC[1], Valença SS[2], Soares de Moura R[1], Resende AC[1] [1]UERJ − Farmacologia e Psicobiologia, [2]UFRJ − Farmacologia

Introduction: The Goldblatt hypertension 2-kidney, 1-clip (2K-1C) that resembles the renovascular hypertension in humans is associated with an endothelial dysfunction characterized by impairment of relaxation induced by vasodilator substances, such as acetylcholine. Among the main factors contributing to the etiology of endothelial dysfunction, great emphasis has been given to oxidative stress. Epidemiological studies suggest that polyphenols extracted from plants reduce the incidence of cardiovascular diseases. Recent results from our group show that the hydro-alcoholic extract of the stone of açaí (ASE) rich in polyphenols induces vasodilator and antihypertensive effects in different models of experimental hypertension (Rocha et al., Vascul Pharmacol. 2007;46(2):97-104; Rocha et al., J Pharmacol. & Toxicol. 2008;3:435-448). The aim of this study was to investigate the protective effects ASE (200 mg/kg/day) rich in polyphenols in Goldblatt hypertension 2-kidney, 1-clip (2K-1C) that is associated with an endothelial dysfunction and oxidative stress. **Methods:** The experiments were approved by the Ethics Committee of Animal Experiments of the UERJ (protocol: CEA/024/2010). Young male Wistar rats used to obtain 2K-1C and control rats (2K) received daily treatment with vehicle or ASE for 40 days, and systolic blood pressure (SBP, mm Hg) was measured by plethysmography. The vasodilator effect of acetylcholine (ACh, 1-100 pmol) was studied in mesenteric arterial bed (MAB) pre-contracted with norepinephrine (30 mM). Determination of oxidative damage was estimated by formation of thiobarbituric acid reaction substances (TBARS nmol/mg protein) in mesenteric arteries (MA) and plasma (P), and superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) activities (U/mg protein) by spectrophotometry. **Results:** SBP was increased in 2K-1C and treatment with ASE (232 ± 12 vs 137 ± 8) prevented the development of hypertension. The reduced vasodilator effect (% of relaxation) of ACh in 2K-1C rats was recovered by the ASE (45 ± 2 vs 62 ± 2). ASE decreased the levels of TBARS in 2K-1C rats (MA:1.5 ± 0.2 vs 0.3 ± 0.1 and P: 0,3 ± 0,01 vs 0,19 ± 0,005). The activities of SOD, CAT and GPx were lower in 2K-1C rats and ASE increased these activities (MA:14 ± 1.5 vs 24 ± 1.5 and P:73.9 ± 4.7 vs 135 ± 8.6; MA:0.8 ± 0.07 vs 2.1 ± 0.2 and P:0.2 ± 0.09 vs 1.1 ± 0.3; MA:0.06 ± 0.006 vs 0.12 ± 0.008 and P:0.0025 ± 0.0005 vs 0.006 ± 0.0005, respectively). **Discussion:** Our results demonstrated that chronic treatment with ASE prevents the development of hypertension and improves the vascular dysfunction in 2K, 1C rats. The reduction of antioxidant activity and increase in lipid peroxidation suggest involvement of a deficient mechanism of antioxidant defense which was reversed by ASE. **Financial support:** CNPq and FAPERJ.

09.013

Gastroprotective effects of the trichloroethane fraction of *Piper tuberculatum* in rats. Burci LM[1], Pereira IT[1], da Silva LM[1], Baggio CH[1], Facundo VA[2], Rodrigues RV[2], Santos ARS[3], Marques MCA[1], Werner MFP[1] [1]UFPR − Farmacologia, [2]UNIR − Química, [3]UFSC − Ciências Fisiológicas

Introduction: *Piper tuberculatum*, popularly known as "jaborandi falso" or "pimento darta", is used in folk medicine as analgesic, sedative and antidote for snake bite. The genus Piper includes species used for gastric disorders; however, there are no studies about the gastroprotective effects of *P. tuberculatum*. Thus, the aim of the present study was to evaluate the gastroprotective properties of a trichloroethane fraction of *P. tuberculatum* (TFP). Materials and **Methods:** Female Wistar rats (180-250g) were deprived of food for 16 h prior to the experiments. Rats were orally treated with vehicle (water, 1 ml/kg), omeprazole (40 mg/kg) or TFP (10, 30 and 100 mg/kg) 1 h before intragastric administration of ethanol P.A. (0.5 ml). The animals were sacrificed 1 h after ethanol administration. The stomachs were removed and the area of ulceration (mm2) was measured by planimetry using the program Image Tool®. Gastric mucus content was measured by the Alcian Blue method. A pylorus ligature was performed in fasted animals and TFP (10, 30 and 100 mg/kg, i.d.), vehicle (water, 1 ml/kg, i.d.), omeprazole (40 mg/kg, p.o.) or atropine (1 mg/kg, s.c.) were administered in the moment of the ligature proceeding. The effects of TFP were also tested on gastric secretion induced by bethanechol (2.5 mg/kg, s.c.) or pentagastrin (0.4 mg/kg, s.c.). After 4 hours animals were killed, the gastric secretion was collected and its final volume and acidity were determined. Institutional Ethics Committee of the UFPR (CEEA approval number 446). **Results:** Oral pretreatment of animals with TFP (30 and 100 mg/kg) significantly reduced the gastric lesion induced by ethanol by 59 and 83%, respectively, compared to control group (17 ± 1.2 mm2). TFP (30 and 100 mg/kg) also enhanced the amount of mucus (when compared to injured control: 52 ± 3 mg Alcian Blue/g) of tissue) to 92 ± 3 and 106 ± 9 mg Alcian Blue/g of tissue. TFP (100 mg/kg, i.d.) reduced the gastric volume from 9.0 ± 0.5 ml in the control group to 6.0 ± 0.3 ml and reduced the acidity of gastric secretion by 47% (0,068 mEq[H+]/mL). Gastric secretion volume and total acidity were increased by bethanechol (25 and 26%, respectively) and by pentagastrin (33 and 33%, respectively). The i.d. administration of TFP (100 mg/kg) reduced both the gastric volume and total acidity stimulated by pentagastrin in 50 and 47% but did not inhibit the gastric secretion when stimulated by bethanechol. **Discussion:** Collectively, the present findings demonstrate that trichloroethane fraction of *P. tuberculatum* has gastroprotective effects in ethanol-induced ulcers with reduction of gastric secretion through endocrine pathways (CCK-gastrin receptors). **Financial support:** CNPq, Capes/Fundação Araucária

09.014

Analgesic and anti-inflammatory activity of *Anadenanthera macrocarpa* brenan. Silva, KO[1], Duarte JC[1], Souza EP[1], Cruz MP[1], Marques LM[1], Andrade MF[1], Dórea RSDM[1], Meireles VS[1], Yatsuda R[1], Napimoga MH[2], Clemente-Napimoga JT[2] [1]UFBA − Saúde, [2]UNIUBE − Saúde

Introduction: The *Anadenanthera macrocarpa* (Benth) Brenan (Fabaceae), popularly known as angico, is used as anti-inflammatory in the folklore medicine. In this study we evaluated in vivo the anti-inflammatory and antinociceptive activities of the bark crude hydroalcoholic extract (CHE) of *A. macrocarpa*. **Methods:** Fresh bark of angico was collected in August 2007 in the National Forest Contendas do Sincorá, BA, Brazil. The plant material was authenticated by Avaldo O. S. Silva, UESB, where a voucher specimen is maintained. The small pieces of bark was cleaned and dried in room temperature for 3 days. The dry stem bark (190 g) was submitted to maceration with ethanol (99.9ºGL) during 72h, filtered and this procedure was repeated 3 times. The extract was concentrated using rotary evaporator, obtaining 14,179 g of CHE. Male Balb-C mice were used (20–25 g; N=6-8) and the experiments were approved by the Ethics Committee of the University of Uberaba (protocol 0107/2009). The animals were pre-treated subcutaneous with CHE dissolved in ethanol 10% (v/v) and saline solution at 50, 100, 200 mg/kg, 30 min before of stimulation. The abdominal writhes induced by acetic acid (0.6%, i.p) were observed for 20 min and in formalin test, the number of right paw flinches was counted for 30 min. The both paws were removed and the weight was measured using an analytical balance. Paw inflammation was induced by i.pl. injection of carrageenan (Cg) (100µg/50µl saline) and hypernociception was evaluated using an electronic version of the von Frey test. The neutrophil migration was analyzed by measuring myeloperoxidase activity in the paw. The leukocyte migration induced by injection of Cg (500mg/cavity) into the peritoneal cavity was measured after 4h of Cg injection. Mice received CHE (100 mg/kg, s.c.) and after 2 h of Cg stimulus (500mg/ cavity), peritoneal exudates were recovered for cytokine measurement. Levels of TNF-a and IL-10 were determined by ELISA using protocols supplied by the manufacturer. The vascular permeability was also analyzed by Evans Blue (50 mg/kg) test. Additionally, the influence of CHE (100 mg/kg) on ICAM-1 expression was evaluated in the acute inflammation in mesenteric tissues induced by Cg injection and assessed by Western blot analysis. **Results:** The CHE of *A. macrocarpa* caused a significant inhibition of acid-acetic induced visceral pain at 50 and 100 mg/kg ($p<0.05$), and reduced the formalin-induced nociception on the inflammatory-phase (50 and 100 mg/kg, $p<0.05$), and also showed a significant decrease in the intensity of paw edema. The CHE reduced the Cg-induced edema formation and inhibited the neutrophils migration in to the peritoneal cavity at 100 and 200 mg/kg ($p<0.05$). The CHE group (177.57 ± 24.57) showed increased levels of IL-10 compared to Cg group (75.81 ± 19.78) and didn't modulate TNF-a levels. The CHE group (0.11 ± 0.07) also reduced the ICAM-1 expression compared to Cg group (0.52 ± 0.99) ($p<0.05$). CHE (100 mg/kg) also inhibited the mechanical hypernociception. **Discussion:** The CHE of *A. macrocarpa* shows antinociceptiva and anti-inflammatory activities at 100 mg/kg. The identification and isolation of bioactive components are in progress, which could elucidate the properties of A. macrocarpa. Support: PIBIC/CNPq, Universal/CNPq, CAPES and FAPESB.

09.015

Substances from the leaves of *Derris urucu* inhibit alpha-glucosidase. Pereira AC[1], Arruda MSP[2], Lemos VS[3], Côrtes SF[1] [1]UFMG − Farmacologia, [2]UFPA − Química, [3]UFMG − Fisiologia e Biofísica

Introduction: It is well known that diabetes mellitus is a common endocrine disorder, whose incidence is markedly increasing worldwide 1. Type 2 diabetes mellitus is the most prevalent 1, and its treatment goal is to reach normoglycemia to prevent later complications. Among the available glucose-lowering agents, alpha-glucosidase inhibitors are an important therapeutic option to delay the absorption of ingested carbohydrates, reducing the postprandial glucose and insulin peaks 2. The aim of this work is to identify alpha-glucosidase inhibitors from plant origin. In this context, we investigated the ethanolic extract of the Amazonian plant Derris urucu, also called timbó, its ethyl acetate fraction and 5 isolated substances for their potential to inhibit alpha-glucosidase using in-vitro model. **Methods:** The alpha-glucosidase inhibitory activity was determined by measuring the release of 4-nitrophenol from 4-nitrophenyl α-D-glucopyranoside (4-NPGP). The assay was performed in 96-well plate, containing 57 mM potassium phosphate buffer, pH 6.8, 0.1 mM reduced glutathione, 40 mU α-glucosidase (from Bacillus stearothermophilus) and plant material (0.1-1000 µg/mL), acarbose (reference drug 0.003-1.0 µg/mL) or potassium phosphate buffer (control). After preincubation, the assay was started by addition of 4-NPGP (0.85 mM), incubated for 30 minutes, 37°C. The reaction was stopped by sodium carbonate (0.1 M). The absorbance at 405 nm was measured in a microplate reader (Thermoplate) and the percentage of inhibition was calculated: (Acontrol - Asample)/Acontrol x 100%. The IC50 values were analyzed by one-way ANOVA and Newman-Keuls post-hoc analysis. **Results and Discussion:** The IC50 values for acarbose are significantly greater than the tested samples (Table 1). Among the tested samples, only the dihydroflavonol isotirumalin was not effective to inhibit alpha-glucosidase. The IC50 values for the ethanolic extract of D. urucu were similar to that obtained to the ethyl acetate fraction (Table 1). However, the dihydroflavonol urucuol and the stilbenes (S1, S3 and S4) were more potent than the ethanolic extract to inhibit alpha-glucosidase (Table 1). It´s known that stilbenes, like resveratrol, are an interesting agent in the treatment of diabetes mellitus 3. This suggests that the Amazonian plant D. urucu, can be source of substances to ameliorate this disorder. Table 1: pIC50 values for the ethanolic extract of D. urucu, ethyl acetate fraction (EAF), urucuol, S1, S2, S3 and acarbose. Extract EAF Urucuol S1 S3 S4 Acarbose 3.96 ± 0.22 4.30 ± 0.16 4.84 ± 0.07 4.72 ± 0.22 4.88 ± 0.21 5.02 ± 0.09 8.20 ± 0.21 data represent media ± SEM, n=5. **Financial support:** FAPEMIG and CNPq 1 Yamabe N. et al. J Med Food 12 (4), 714–721, 2009. 2 Stuart AR. Chemical Reviews 104, 1255–1282, 2004. 3 Su HC. Am J Physiol Endocrinol Metab 290:1339-1346, 2006.

09.016

Effects of an extract obtained from fruits of *Euterpe oleracea* mart. (Açaí) on experimental metabolic syndrome in C57BL/6 mice. Oliveira PRB[1], Costa CA[1], Bem GF[2], Cordeiro VSC[3], Carvalho LCRM[5], Souza MAV[5], Lemos Neto M[5], Soares de Moura R[2], Resende AC[1] [1]UERJ − Farmacologia e Psicobiologia, [2]UERJ − Farmacologia, [3]UERJ

Introduction: Previously we have shown that a hydro-alcoholic extract of the stone of *Euterpe oleracea* Mart., a fruit from the Amazon region commonly known as "Açaí", exerts significant nitric oxide-dependent vasodilator effect, an antioxidant and antihypertensive actions (Rocha et al., Vascul Pharmacol. 2007;46(2):97-104; Rocha et al., *J Pharmacol. & Toxicol*. 2008;3:435-448). This study was designed to determine the protective effects of açaí stone extract (ASE, 300 mg/kg/day) in C57BL/6J mice fed a high-fat diet (HF) that delineate components of metabolic syndrome. **Methods:** The experiments were approved by the Ethics Committee of Animal Experiments of the UERJ (protocol: CEA/025/2010). C57BL/6 mice at 6 weeks of age were fed a control diet and received water (CD; 10% fat) or ASE (CD+ASE) and a HF (60% fat) that received water (HF) or ASE (HF+ASE) for 12 weeks. The vasodilator effect of acetylcholine (ACh, 0.1-1000 nmol) was studied in perfused mesenteric arterial bed of the mice pre-contracted with norepinephrine (30 mM). Body weight, plasma total cholesterol, triglyceride, glucose, insulin levels and oxidative damage were determined and the insulin resistance measured by HOMA index. Oral glucose tolerance test (OGTT) was also assessed by oral administration of glucose (2g/kg) at time 0 and measurement of glucose at different times. **Results:** Vasodilator response to ACh (% relaxation) was reduced in HF (HF: 32 ± 3.0 vs CD: 6.9 ± 2.7; $p<0.05$) and ASE restored the response (65 ± 7.3). Increased body weight (g) was observed in HF (HF:41.6 ± 0.9 vs CD:22.9 ± 0.5, $p<0.05$) and reduced by ASE (31.2 ± 0.4). Plasma triglyceride (mg/dl) was increased in HF (HF 55.7 ± 6.5 vs CD: 31 ± 3.8; $p<0.05$), as well as, total cholesterol (HF:125 ± 7.9 vs CD: 73 ± 4.8; $p<0.05$) and ASE normalized these values (31 ± 5.6 and 79 ± 9.2, respectively; $p<0.05$). Glucose levels (mmol/dl) were increased in HF (HF:10.2 ± 0.2 vs CD:6.3 ± 0.1; $P<0.05$), as well as, insulin (HF: 25.9 ± 2.4 vs CD:11.5 ± 1.0 mU/mL; $p<0.05$) and ASE reduced these values (8.8 ± 0.3 and 17.9 ± 1.2, respectively; $p<0.05$). Insulin resistance (HOMA Index) was observed in HF (HF:1.1 ± 0.1 vs CD:0.3 ± 0.02) and reduced by ASE (0.7 ± 0,1). Glucose intolerance was observed by OGTT (area under the curve) in HF group (HF: 1685 ± 79.4 vs CD: 1092 ± 22.7) and was reduced by treatment with ASE (1335 ± 37.7). ASE decreased the levels of malondialdehyde (MAD; nmol/mg protein) in HF (1.3 ± 0.1 vs 0.7 ± 0.1). **Discussion:** We have demonstrated that long-term administration of the hydro-alcoholic extract of the stone of *Euterpe oleracea* Mart.(ASE) protect C57BL/6J mice fed HF from obesity, endothelial dysfunction, hypercholesterolemia, hyperglicemia and insulin resistance. The nitric oxide synthesis and antioxidant action induced by ASE may contribute to these benefic effects of ASE. **Financial support:** CNPq and FAPERJ.

09.017

The antinociceptive effect of triterpene 3beta, 6beta, 16beta-trihydroxylup-20(29)-ene against acute and chronic pain in mice: the involvement of glutamatergic system.

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Introduction: According to our previous results1, we hypothesize that the antinociceptive effect of 3beta, 6beta, 16beta-trihydroxylup-20(29)-ene (TTHL) obtained from the flowers of *Combretum leprosum* against acute and chronic pain involves the modulation of glutamatergic system, via NMDA and metabotropic receptors. Thus, in the present study we evaluate the effect of TTHL against acute and chronic model of nociception and verified the involvement of glutamatergic system by binding studies. **Methods:** Swiss mice of both sexes were used (25-35g; N=8) and the experiments were approved by the Institutional Ethics Committee under the protocol PP0162. To investigate the antinociceptive effect of TTHL against acute nociception model, TTHL was given orally (p.o. 30 mg/kg) or intrathecally (6.5nmol/5ml, co-injected). The model of acute nociception was induced by intrathecal (i.t.) injection of NMDA and trans-ACPD (ionotropic and metabotropic glutamatergic agonists, respectively) in mice. To evaluate the effect of TTHL against chronic model of nociception, mice were submitted to a partial nerve sciatic ligation (PNSL) and treated with TTHL, acutely and chronically by p.o. route (30 mg/kg). To confirm the involvement of glutamatergic system in the antinociceptive effect of TTHL, [3H]Glutamate binding experiments were carried out in brain membranes (without cerebellum) to evaluate glutamate binding to receptors and transporters, in absence or presence of sodium, respectively. To verify the displacement of [3H] Glutamate by TTHL, the membranes were incubated with TTHL in a concentration range of 0.01-1mM. **Results and Discussion:** TTHL administered orally (p.o. 30 mg/kg) or intrathecally (6.5nmol/5ml, co-injected) caused a marked inhibition of the nociceptive responses induced by i.t. injection of NMDA (NMDA:191 ± 21.3/TTHL[i.t.]:59 ± 16.7; TTHL[p.o.]:59.1 ± 7.6), trans-ACPD (trans-ACPD:256.4 ± 21.5/TTHL[i.t.]:86.2 ± 18.4; TTHL[p.o.]:146.4 ± 13.9), with inhibitions of 69 ± 4%; 69 ± 9% and 67 ± 8%; 43 ± 5% for p.o. and i.t. routes, respectively. Moreover, TTHL given orally (30 mg/kg) reversed the mechanical allodynia induced by PNSL (59 ± 12%). Binding experiments indicate that TTHL was able to displace [3H]Glutamate to its receptor and glutamate uptake binding sites, with EC50 of 0.06(0.04-0.09)mM and 0.07(0.05-0.10)mM, respectively. Together, these results demonstrated that TTHL presents significant antinociceptive effect against acute and chronic pain, and provides experimental evidence for the involvement of glutamatergic system (via NMDA and metabotropic glutamate receptors) in this effect. References: 1) Longhi-Balbinot et al., European Journal Pharmacol. 623: 30; 2009. Supported by: CAPES, CNPq, UFSC

09.018

Antiproliferative activity of extracts from leaves of fruit trees. Begnami AF[1], Figueira GM[2], Pereira B.[2], Ruiz ALTG[2], Carvalho JE[2], Rehder VLG[2] [1]FOP-UNICAMP, [2]CPQBA-UNICAMP

Introduction: Natural products represent an important source of knowledge in the discovery of new anticancer drugs and infectious diseases. About 67% the effective drugs used in chemotherapy are derived from natural products. The search for anticancer drugs through the sorting of extracts and active principles obtained from natural sources enabled the discovery and development of a variety of chemotherapeutic drug currently used to treat cancer. The aim of this study was to evaluate the antiproliferative activity of crude dichloromethane extracts of leaves of 37 fruit species against nine human tumor cell lines, as well as to identify their major constituents. **Methods:** Samples were collected during the month of March 2009 at the experimental field CPQBA / UNICAMP, including the following families: Anacardiaceae, Annonaceae, Arecaceae, Celastraaceae, Fabaceae, Juglandaceae, Malpighiaceae, Meliaceae, Myrtaceae, Proteas, Rhamnaceae, Sapindaceae, Sapotaceae and Urticaceae. Dried leaves were submitted to dynamic extraction with dichloromethane resulting in crude dichloromethane extracts (EETFs) that were analyzed by thin layer chromatography (TLC) and Gas Chromatographic-Mass Spectrometry (GC-MS). All extracts were submitted to an "*in vitro*" antiproliferative assay using nine human cancer lines, kindly donated by National Cancer Institute, EUA: [glioma (U251) ,breast (MCF-7), ovarian expressing multiple drugs resistance phenotype (NCI/ADR-RES), kidney (786-0), lung (NCI-H460), ovarian (OVCAR-03), colon (HT-29), prostate (PC-3) and leukemia (K562)]. The EETFs were tested at concentrations ranging from 0.25 to 250 µg / mL, using doxorubicin as positive control. Forty-eight hours after the treatment, cell growth was assessed by determination of total proteins by Sulforhodamine B and from concentration-effect curves was assessed the activity of the extracts, as well as TGI (total growth inhibition concentration)a. **Results and Discussion:** Among the extracts under study was observed the activity concentration dependent for Annonaceae and Fabaceae with selectivity for OVCAR-03; Anacardiacea, Celastraceae and Malpighiacea for U251; Sapotaceae for MCF-7, NCI/ADR-RES and Myrtaceae for cell line U251, OVCAR-03, MCF-7 and NCI/ADR-RES. The GC-MS analysis of the most active extracts showed the presence of sesquiterpenes, triterpenes and vitamin E such as more frequent compounds. The results allowed the identification of some of the most promising extracts, which will be object of future studies phytochemicals. *Bibliographic Citation*: a Holbeck SL. European J Cancer.40:785-93.2004. **Acknowledgments**: CAPES.

09.019

Gastric antisecretory activity of an ethanolic extract of *Arctium lappa* L. in rats. da Silva LM[1], Pereira IT[1], Mendes DAGB[1], Pizzolatti MG[2], Werner MFP[3], Andre E[4], Marques MCA[1] [1]UFPR − Farmacologia, [2]UFSC − Química, [3]UFSC − Farmacologia, [4]UFRN − Biofísica e Farmacologia

Introduction: *Arctium lappa* L. is a member of the Asteraceae (Compositae) family popularly known as ”bardana”. In previous studies, we have reported a significant gastroprotective effect of this plant in gastric ulcer induced by acetic acid in rats. However, the mechanism by which *Arctium lappa* causes this action has not been completely clarified. Thus, this study evaluated the gastric antisecretory activity of ethanolic extracts from roots of *Arctium lappa* L. **Methods:** The ethanolic extract of *Arctium lappa* L. root was used to evaluate its gastric antisecretory activity in the pylorus-ligated rat model at dose of 10 mg/kg (stimulated by bethanechol - 2,5 mg/kg, histamine - 20 mg/kg and pentagastrine – 0,4 mg/kg). *In vitro* experiments with isolated stomach were also conducted to assess the effect of the extract (0,1; 0,3 and 1 mg/ml) on muscarinic and histamine receptors in gastric mucosa. All procedures using animals were approved by the Ethics Committee on Animal Experiments of UFPR (protocol number 576). **Results:** The ethanolic extract of *Arctium lappa* L. roots showed significant antisecretory activity as evidenced by decreased the volume and basal acid secretion in gastric juice in 49.8% e 64%, respectively. The volume and acid secretion were also reduced to baseline levels in the pylorus-ligated rats stimulated by bethanechol and histamine, but not by pentagastrin. The muscular contraction of isolated rat stomach tissue induced by acetylcholine was antagonized, in a concentration-dependent manner by ethanolic extract of *Arctium lappa* L. roots. However, the histamine induced gastric relaxation remained unchanged by ethanolic extract of *Arctium lappa* L. Together, these results suggest that the gastroprotective effects of ethanolic extract of *Arctium lappa* appear to be due to reduction of gastric acid secretion through inhibition of muscarinic receptors. **Financial support:** CAPES e Fundação Araucária.

09.020

Gastroprotective and antioxidant effects of ethanolic extract of *Arctium lappa* L. on acetic acid-induced ulcers in rats. da Silva LM.[1], Crestani S[1], Burci LM[1], Pizzolatti MG[2], Werner MFP[1], Andre E[3], Marques MCA[1] [1]UFPR − Farmacologia, [2]UFSC − Química, [3]UFRN − Biofísica e Farmacologia

Introduction: *Arctium lappa* L. (Asteraceae), popularly known as ”bardana” is used in folk medicine as diuretic, depurative, digestive stimulant and in dermatological conditions. This study evaluated the healing property of ethanolic extract of *Arctium lappa* L (EET) on chronic gastric ulcer induced by acetic acid and investigated mechanisms that may underlie this effect. **Methods:** The chronic gastric ulcer was induced by acetic acid (80%) and the treatment with EET was performed once daily for seven days. The effect of EET on the inflammatory process was evaluated through the microvascular permeability, myeloperoxidase (MPO) and N-acetilglucosaminidase (NAG) activity. Immunohistochemistry analysis of proliferating cell nuclear antigen (PCNA) was performed to evaluate the effects of the extract on ulcer healing. The antioxidant activities (superoxide dismutase – SOD and catalase – CAT), levels of lipid hydroperoxides (LOOH), inhibition of 2,2- diphenyl-1-picrylhydrazyl (DPPH) radical scavenging, intracellular free radicals and the amount of non-proteic sulfhydryl groups (GSH) and gastric mucus were also evaluated. All procedures using animals were approved by the Ethics Committee on Animal Experiments of UFPR (protocol number 576). **Results and Discussion:** Oral administration of EET (1, 3, 10 and 30 mg/kg daily for seven days) reduced the chronic gastric ulceration induced by acetic acid by 29.2%, 41.4%, 59.3% and 38.5%, respectively. The immunohistochemistry analysis showed an increase in PCNA-immunoreactivity gastric cells of animals treated whit EET (10 mg/kg). Moreover, treatment with EET (10 mg/kg) significantly reduced the vascular permeability, inhibited the MPO and NAG activity in 64.2% 65.3% and 20.3%, respectively. EET restored the SOD activity, partially prevented the decreasing of GSH levels and reduced LOOH levels to 60, 57 and 45.5%, respectively, but not affect the increase of CAT activity in ulcerated stomachs, and did not alter the levels of gastric mucus in the ulcerated stomach. *In vitro* EET inhibited DPPH radical and pretreatment of animals with EET (10 mg/kg, v.o.) reduced in 59% the free radical generation when compared to control ulcerated group. Collectively, these results clearly demonstrate the potent gastric healing effect of EET and suggest that the antiulcer activity is due in part to its anti-inflammatory and antioxidant action. **Financial support:** CAPES and Fundação Araucária.

09.021

Crotalus durissus terrificus: hepatic effects of snake venom in rats. da Silva JG[1], Soley BS[1], Gris V.[1], Rocio AAP[2], Cadena SMSC[2], Eler GJ[3], Bracht A[3], Dalsenter PR[1], Acco A[1] [1]UFPR − Farmacologia, [2]UFPR − Bioquímica e Biologia Molecular, [3]UEM − Bioquímica

Introduction: Snake venoms present different action mechanisms because of their complex composition, represented mainly by toxins and enzymes. This work aimed to investigate the effects of the *Crotalus durissus terrificus* (Cdt) venom in the liver, focusing in its effects on oxidative stress and hepatic metabolism. **Methods:** Wistar rats were inoculated intraperitoneally (i.p.) with saline (control) or three different doses of Cdt venom. After 3 or 6 hours the following parameters were analyzed: (a) hepatic function through plasmatic levels of alanine aminotraferase (ALT) and aspartate aminotransferase (AST); (b) oxidative stress, analyzed by levels of lipoperoxidation (LPO) and gluthatione (GSH), and by activities of catalase (CAT) and glutatione S-transferase (GST); and (c) the metabolism of alanine (2.5 mM) in the isolated perfused liver. Also, *in vitro* mitochondrial oxygen consumption was measured in incubation with succinate or glutamate plus malate. The experiments were conducted following the recommendation of the Brazilian Law 6638 for the scientific management of animals, and the Institutional Animal Ethics Committee approved all procedures (CEEA, certificate 280). **Results:** Plasma activities of ALT and AST, and hepatic GST and CAT presented significant elevation in rats inoculated with 300 µg×kg-1 Cdt venom, after 6 h and 3 h, respectively. Liver lipoperoxidation was enormously increased by venom doses of 100, 200 and 300 µg×kg-1, while glutathione S-transferase was not change, both parameters analyzed 3 h after venom inoculation. The venom decreased the oxygen consumption by intact mitochondria in about 20% in incubations with both tested substrates. Six hours after i.p. venom administration, perfused livers from rats inoculated with 1,500 µg×kg-1 of venom showed increased production of lactate, pyruvate and ammonia, while production of glucose and urea and oxygen consumption did not change significantly in the presence of alanine as metabolic substrate. Discussion. These results demonstrate that the Cdt venom can produce acute changes in the liver functions. The mechanisms are related to the unbalance in the redox homeostasis and to the impairment of the mitochondrial functions. Consequently, the venom can also modify liver metabolism. **Financial Support**. CAPES and Post Graduate Program in Pharmacology of UFPR.

09.022

Lonomia obliqua venom-induced pro-inflammatory profile in endothelial cell *in vitro* and increased leukocyte trafficking *in vivo*. Nascimento-Silva V[1], Rodrigues GS[1], Moraes JA[1], Cyrino FZ[2], Bouskela E[2], Guimarães JA[3], Barja Fidalgo TC[1] [1]UERJ − Farmacologia, [2]UERJ − Fisiologia, [3]UFRGS − Farmacologia

Introduction: Caterpillar envenomation has been emerged as health issue in southern Brazil. *Lonomia obliqua* victims present a hemorrhagic syndrome that can progress to acute renal failure, intracranial hemorrhage and death. In vivo and *in vitro* studies have shown that the venom contains several toxins with procoagulant, anticoagulant and antithrombotic activities. We have demonstrated that endothelial cells (EC) exposed to *L. obliqua* caterpillar bristle extract (LOCBE) display an activated profile with increased expression of pro-inflammatory molecules. In a follow up of these studies we have further investigated the effects of LOCBE on the activation of the transcription factor, NFκB, and its contribution to regulate the expression of pro-inflammatory cytokines, inducible enzymes and matrix metalloproteinases (MMP) expression. Additionally, we studied the effects of the venom on the leukocyte traffic in vivo by intravital microscopy.. **Materials and Methods:** LOCBE was obtained as described (Bohrer et al., *Toxicon* 49:663, 2007). Cell viability was assessed by Trypan blue exclusion. EC were incubated in the absence or in the presence of LOCBE (3µg/mL) for different periods of time. An immunofluorescence microscopy analysis was used to detect NFkB activation. Protein levels were detected by western blot analysis and mRNA was analyzed by RT-PCR. Leukocyte trafficking was evaluated in hamster cheek pouch prepared for intravital microscopy (COBEA CEA/215/2007). **Results:** The exposition of EC to non-hemorrhagic concentrations of LOCBE promoted increased expression of cyclooxygenase (COX)-2, nitric oxide synthase (NOS)-2 and also of mRNA and protein expression of MMP-2 and MMP-9. Western blot and immunofluorescence microscopy analysis showed that LOCBE activated NFkB pathway in EC, characterizing the inflammatory response associated to many vascular pathologies. Intravital microscopy assays shows that LOCBE (1 and 3ug/ml nM: non-hemorrhagic concentrations), did not affect vascular permeability but induces inflammatory response on the vascular bed, increasing leukocyte rolling and adhesion, slowing the flux and gradually leading to stasis in some confluent venules of hamster cheek pouch. **Discussion:** The inflammatory properties of caterpillar venom, LOCBE, have been frequently addressed, although few works have associated it to the severity of envenomation. We show that the pro-inflammatory properties of the venom are responsible not only for the local effects, but also leads to systemic vascular disturbances. LOCBE, at low, non-hemorrhagic concentrations activates endothelial cells, activates NFkB pathway inducing COX-2 and NOS-2 and also increasing MMP-2 and MMP-9 expression, that will contribute to local inflammation and endothelial disturbance, affecting leukocyte trafficking and microcirculation perfusion. These findings contribute to the elucidation of the biochemical properties of the venom and could explain some important aspects of the envenoming and their effects on the vascular biology. **Financial support:** FAPERJ, CNPq, SR-2/UERJ.

09.023

Gastroprotective effect of *Terminalia fagifolia* ethanolic extract. Soares GFS, Sousa OT, Souza AES, Oliveira AC, Nunes PHM, Martins MCC UFPI − Biofísica e Fisiologia

Introduction: *Terminalia fagifolia* is a Combretaceae popularly used in the state of Piaui, Brazil, to treat intestinal and stomach disorders (Freire, F.M.T., BID/CNPq/UFPI 160, 1992). Nevertheless, other species of the same genus have been reported to show gastroprotective and anti-ulcerogenic effects (Nunes, P.H.M. et al Pharmazie 64:58, 2009). The present study has been approved by the Ethics Committee of Federal University of Piaui (Protocol Nº 042/09) and was carried out to investigate the protective effect of *Terminalia fagifolia* ethanolic extract (TFEE) on acute experimental model of gastroprotective activity in female rats. **Methods:** Groups of six pylorus ligated (Shay, H., Gastroenterology 5:43, 1945) female rats (150-250 g) were intraduodenally administered distilled water (5 mL/kg, control), ranitidine (60 mg/kg, standard) and TFEE (500 mg/kg). Animals were sacrificed 4h after treatment and the stomachs were removed and gastric juice solution was collected. The content (mL), pH and total acidity (mEq/h) of gastric juice were determinated. Additionally, glandular segments from the stomachs were excised for determination of the gastric wall mucus content (mg/g) by the Alcian Blue method (Corne, S.J., J Physiol 242:116, 1974) and of the nonprotein sulfhydryl compounds (NPSH) concentration (mM/g) by the Sedlak and Lindsay method (Anal Biochem 24:192, 1968). **Results:** TFEEa and ranitidineb provoked significant increase ($p<0.05$) in the pH (a: 3.76 ± 0.39 and b: 5.79 ± 0.66) and reduction in the content (a: 1.2 ± 0.21 and b: 1.5 ± 0.22) as well as in the total acidity of gastric juice produced (a: 13.19 ± 4.33 and b: 5.83 ± 3.13) compared to the control (pH: 2.22 ± 0,22; content: 3.0 ± 0.17 and total acidity: 69.77 ± 5.51). There were no significant modification of the gastric wall mucus content and of the NPSH concentration in the animals treated with TFEE (c: 34.47 ± 2.83 and d: 46.11 ± 2.45) or with ranitidine (c: 38.97 ± 2.32 and d: 42.44 ± 3.73) compared to the animals of the control group (c: 36.07 ± 4.42 and d: 48.17 ± 1.80). Discussion and Conclusion: This study provides evidence that the TFEE possesses a gastroprotective effect, which is related to the inhibition of the gastric acid secretion without an increase of mucosal defensive factors, such as mucus an NPSH compounds. Support: UFPI. Keywords: *Terminalia fagifolia*; gastroprotective effect; gastric wall mucus.

09.024

Biological activity of hydroalcoholic fraction of *Herissantia crispa* (L.) Brizicky. Dias GEN, Mota KSL[1], Lima IO[1], Pereira, FO[1], Viana WP[1], Teles YCF[1], Lima EO[1], Diniz MFFM[1], Souza MFV[1], Batista LM[1] [1] DCF-UFPB

Introduction: The diarrhoea is the biggest cause in mortality of children in the world (Pickering, Seminars in Pediatric Infectious Diseases, 15, 71, 2004). This disease can be caused by several pathogens, like species of *Salmonella* and *Shigella*, *Escherichia coli*, *Listeria monocytogenes*. The use of antimicrobial agents in treatment of diarrhea has been limited, because of collateral effects and bacterial resistance (Silveira et al., Quím. Nova, 29, 844, 2006). Therefore, the aim of this study was to investigate the antibacterial activity, to determine a CL50, and anti-diarrhoeal action of the hydroalcoholic fraction of *Herissantia crispa* of the Malvaceae family. **Methods:** The antibacterial action of hydroalcoholic fraction was evaluated using the bacteria: E. coli (Classica; ATCC-1105; ATCC-10536; ATCC-18739), *Listeria monocytogenes* (ATCC–7664), *Pseudomonas aeruginosa* (ATCC-25853), *Shigella flexineri* (MM-412), *Shigella sonnei* (LM-07), *Shigella enterocolitica* (ATCC-6017), Salmonella spp (LM-08), *Staphylococcus aureus* (ATCC-13150) and *Staphylococcus epidermidis* (ATCC-12228) it was used the microdiluition technique (NCCLS, 2000; Cleeland, Antibiotics in Laboratory Medicine, 739, 1991). The bioassay with *Artemia salina* Leach was used to determine a CL50, To investigate the anti-diarrhoeal action of hydroalcoholic fraction (62,5, 125, 250, and 500 mg/kg, v.o.), it was used the diarrhea induced by ricinus oil in mice, n=6 (Awouters et al., J. Pharm. Pharmacol., 30, 41, 1978). The results of anti-diarrheal activity were expressed in mean ± S.D. and analyzed by ANOVA/Dunnett´s test, with the level of significance $p < 0,05$. Number of Ethical in Animal Research license is 705/06. **Results:** The hydroalcoholic fraction showed CL50 > 1000 µg/mL. The hydroalcoholic fraction (10 mg/mL) inhibited the growth of E. coli (ATCC-10536), *Pseudomonas aeruginosa* (ATCC-25853), Shigella enterocolitica (ATCC-6017), and S. Aureus (ATCC13150), corresponding 33% of the yeasts tested; the dose 5 mg/mL inhibited the growth *Pseudomonas aeruginosa* (ATCC-25853), S. enterocolitica (ATCC-6017), and S. Aureus (ATCC13150) corresponding 25%; the dose de 2,5 mg/mL inhibited the growth Shigella enterocolitica (ATCC-6017), and S. Aureus (ATCC13150), corresponding 16,7% of the yeasts tested. The hydroalcoholic fraction (62,5, 125, 250 and 500 mg/kg) no reduced the faeces number (6,7 ± 1,5; 5,7 ± 0,5; 7,0 ± 1,3; 5,8 ± 0,7), just loperamide (2 mg/kg) reduced the faeces number (0,0 ± 0,0), when it was compared with control group saline 0,9% (7,2 ± 1,5). Conclusion: The results suggest that hydroalcoholic fraction of H. crispa has low toxicity and antibacterial activity. **Financial support:** CNPq-LTF-UFPB

09.025

Effect of methanolic extract and fractions from *Davilla elliptica* leaves (Dilleniaceae) on MMPs in *Bothrops jararaca* envenomation and inflammation. Nishijima CM[1], Delella FK[2], Bruni FM[3], Rodrigues CM[4], Vilegas W[4], Lopes-Ferreira M[5], Felisbino S[6], Hiruma-Lima CA[7] [1]UNESP-Botucatu − Fisiologia, [2]UNESP-Botucatu − Morfologia, [3]IBu − Toxinologia Aplicada, [4]IQ-UNESP-Araraquara − Química Orgânica, [5]IBu − Imunopatologia, [6]UNESP, [7]UNESP-Botucatu

Introduction: Inflammatory reaction and hemorrhage are the main characteristics of tissue injury in bothropic envenenomation. These reactions are triggered by venom proteins known as´´ snake venom metalloproteinases´´ (SVMP) that in addition to possessing Zn2+ dependent enzymatic activity activated endogenous matrix metalloproteinases (MMPs). MMPs play role in remodelation and homeostasis of extracellular compounds and during inflammatory process promoted leukocytes migration from vessels to tissue by removing structural proteins such as collagen. Although antivenom prevents systemic reactions, it is not efficient to prevent tissue injury in site of bite. So, beside anti-inflammatory effect, substances that exert inhibition of metalloproteinase are important therapeutic targets in complementary treatments to envenomation bothropic. **Methods:** This study examined changes of *D. elliptica* on MMP-2 and MMP-9 activities by intradermal injection of *B. jararaca* venom using zymography analyses. Anti-inflammatory activity from *D. elliptica* was evaluated by intravital microscopy. Mice (Swiss male, 18–22 g) were divided into two groups of 12 animals each. One group received vehicle and other group received tannins fraction from *D. elliptica* (30 mg/kg) by intraperitoneal route and 30 min later all mice were anesthetized with sodium pentobarbital and underwent surgery to expose the cremaster muscle. An initial leukocyte count was performed, and then 1 µg of LPS was applied topically to the cremaster muscle. The number of rolling leukocytes in post-capillary venules was recorded for a period of 30 min at predetermined fixed point. The results were expressed as mean ± standard error and the differences between groups were determined by analysis of variance. Significant differences were analyzed by the student's t-test (for two groups) with $p < 0.05$ considered significant. In zymography assay, differences equal or more than 50% were considered as significant. **Results and Discussion:** The extract and tannins fraction were able to decrease the activation of MMP-9 activity induced by venom in 50% and 65%, respectively. The tannin fraction was also able to decrease in 85% the activity of pro MMP-2 in relation to venom. Due to decrease of MMP-9 activity caused by extract and tannin fraction, there was not observed necrosis in dermis of animals that receive the mixture venom + extract and venom + tannin fraction. The decrease in leukocyte rolling induced by tannin fraction is one of mechanisms of action of *D. elliptica*. Intravital microscopy also shows a significant inhibition in rolling of leukocyte in cremaster muscle challenged with LPS in mice pretreated with tannin fraction from *D. elliptica* (56 ± 3 and 28 ± 1 leukocytes rolling in animal pre-treated with vehicle or fraction, respectively). These results suggest that the active form from MMP-9 is closely related to necrosis by *B. jararaca* and that condensed tannins are responsible for anti-inflammatory effect of this vegetal species, both by reducing the rolling of leukocytes and decreased activity of MMP-9 activity during the inflammatory process caused by *B. jararaca*. No of ethical protocol: 18/05 CEEA. **Acknowledgment**: BIOTA, proc. 2007/57377-8

09.026

Evaluation of anti-inflammatory activity of butanolic fraction from *Dioscorea scabra* Humb. & Bonpl. ex Willd. Hank A[1], Beduschi MG[1], Darmarco ED[2], Sousa JMB[1], Magina MDA[3], Guimarães CL[5] [1]FURB − Medicina, [2]FURB − Farmácia, [3]FURB − Ciências Farmacêuticas

Introduction: The genus Dioscorea has the largest number of representatives of the family Dioscoreaceae, especially in the South American tropics. It is estimated to occur in Brazil between 150 and 200 species of Dioscorea. In southern Brazil, Campo Belo, Santa Catarina, the roots of D. scabra specie, known as “Cará”, are used in the form infusion in throat affections and gout as anti-inflammatory and analgesic. The aim of this study was to study the possible anti-inflammatory activity of butanolic extract obtained from the roots of D. scabra. **Methods:** The plant roots were grounded and macerated with ethanol 70%, to afford the crude hydroalcoholic (CE). After filtration, the CE was concentrated under low pressure, resuspended in water and partitioned with dichloromethane, ethyl acetate and butanol (BT), yielding the corresponding fractions. Groups of male Swiss mice (30-35 g) were pretreated with butanolic (BT) fraction (5, 20 and 50 mg/kg, i.p.), after 4 h an injection of carrageenan (Cg, 2%, 20µl/paw) in the paw (oedema mice method) or Cg (2%, 50 µl/cavity) in the intrapleural cavity (pleurisy mice method). The control group received 10 ml/kg, i.p. vehicle (saline) 30 min before the Cg administration. In both models, the inflammatory parameters were evaluated 4 h before induction of inflammation with Cg. The volume of paw oedema was analyzed on plethysmometer. Total leukocytes counts were performed in a Neubauer chamber, while cytospin preparations of pleural lavage fluid were stained by the May-Grünwald-Giemsa technique for the differential leukocyte counts, which were performed under an oil immersion objective. Results was presented as mean ± S.E.M of at least 6 animals, and analyzed statistically by one-way ANOVA, and differences between groups were assessed using the Student-Newman-Keuls post-test ($P < 0.05$). All procedures were approved by the Institutional Ethics Committee under protocol number CEUA - 011/09. **Results and Discussion:** Intraperitoneal administration of BT fraction significantly inhibited the paw oedema induced by Cg in a dose-dependent manner (inhibitions of 37.5 ± 6.7%, 31.6 ± 2.3% and 15.8 ± 1.9% at doses of 5, 20 and 50 mg/kg – control values 42.2 ± 1.7%). In pleurisy the BT fraction significantly reduced the increase of total leukocyte number (11.0 ± 1.0x106, 3,6 ± 0.8x106 and 6.6 ± 3.0x106 – control 11.7 ± 0.5x106) and neutrophils (8.5 ± 0.7x106, 1.9 ± 3.4x106 – control 7.9 ± 0.4x106) and did not reduced the mononuclear cells (2.5 ± 0.5x106, 2.9 ± 0.9.106, 4.9 1.5x106 – control 3,8 ± 0.3x106) at doses of 5, 20 and 50 mg/kg. These preliminary results showed that the butanolic fraction from roods of D. scabra have promising anti-inflammatory activity in mouse models of inflammation. However, further studies are necessary in order to evaluate the mechanism of anti-inflammatory action. **Financial support:** PIBIC/CNPq and FURB.

09.027

Antibacterial activity of the *Byrsonima gardneriana* A. Juss. Dias GEN, Leite ATJ, Pereira FO, Rolim TL, Lima EO, Tavares JF, Batista LM DCF-UFPB

Introduction: *Byrsonima gardneriana* is a specie which belongs to the malphigiaceae family. It was chosen by the quimiotaxonomic criterion that points this specie as rich in flavonoid. The aim of this study was to investigate the Antimicrobial activity of the ethanolic extract (EtOHE) and the phase ethyl acetate (AcOEt) obtained of the leaves of *Byrsonima gardneriana*. **Methods:** The antimicrobial action of EtOHE was evaluated using the yeast: Candida albicans (ATCC-76615; ATCC-6958), Candida tropicalis (LM-37), Candida tropicalis (ATCC-13803), *Microsporum canis* (ATCC-13185), Trichophyton rubrum (LM-640), *Aspergillus fumigatus* (ATCC-40640) and Aspergillus flavius (LM-247) and the bacteria: *Escherichia coli* (Classica; ATCC-1105), *Pseudomonas aeruginosa* (ATCC-25853), *Staphylococcus aureus* (ATCC-6538) and *Staphylococcus epidermidis* (ATCC-12228) it was used the disc diffusion method (Sahin, et al., Food Control., 15, 7, 2004; Cleeland; Squires, Antibiot. in Lab. Med. 1991). and to evaluate the antibacterial activity of the EtOHE and AcOEt using the bacteria: E. coli (Classica; ATCC-1105; ATCC-10536; ATCC-18739), Listeria monocytogenes (ATCC–7664), *Pseudomonas aeruginosa* (ATCC-25853), Shigella flexineri (MM-412), *Shigella sonnei* (LM-07), Shigella enterocolitica (ATCC-6017), Salmonella spp (LM-08), *Staphylococcus aureus* (ATCC-13150) and *Staphylococcus epidermidis* (ATCC-12228) it was used the microdilution technique (NCCLS, 2000; Cleeland, Antibiotics in Laboratory Medicine, 739, 1991) **Results:** The EtOHE (10 mg/mL) only showed activity against bacteria type; E. coli (ATCC-11105) zones of inhibition 16 mm, *Staphylococcus aureus* (ATCC-6538) 16 mm ,and *Staphylococcus epidermidis* (ATCC-12228) 18 mm. In the microdilution technique EtOHE (10mg/mL) inhibited the growth of 100% of the yeasts tested; the dose 5 mg/mL inhibited the growth E. coli (Classica; ATCC-1105; ATCC-10536; ATCC-18739), S. enterocolitica (ATCC-6017), Salmonella spp (LM-08), L. monocytogenes (ATCC–7664), S. aureus (ATCC-13150) and S. epidermidis (ATCC-12228) corresponding 75%; and the dose de 2.5 mg/mL inhibited the growth E. coli (ATCC-10536), Salmonella spp (LM-08) and S. aureus (ATCC-13150) corresponding 33% of the yeasts tested. The AcOEt (10, 5, 2.5, 1.25 mg/mL) inhibited the growth of 100% of the yeasts tested; the dose 0.63mg/mL inhibited the growth E. coli (ATCC-10536), P. aeruginosa (ATCC-25853), Salmonella spp (LM-08), S. enterocolitica (ATCC-6017), S. flexineri (MM-412), S. sonnei (LM-07), L. monocytogenes (ATCC–7664), and S. epidermidis (ATCC-12228) corresponding 67%; the dose de 0.32 mg/mL inhibited the growth of E. coli (ATCC-10536), Salmonella spp (LM-08), S. enterocolitica (ATCC-6017), S. flexineri (MM-412), S. sonnei (LM-07) and L. monocytogenes (ATCC–7664) corresponding 50%; and the dose de 0.16 mg/mL inhibited the growth of E. coli (ATCC-10536) and L. monocytogenes (ATCC–7664) corresponding 17% of the yeasts tested. Conclusion: The results suggest that EtOHE and AcOEt have antibacterial activity. **Financial support:** CNPq-LTF-UFPB

09.028

Effect of sub-chronic treatment with psychollatine in the mice light/dark paradigm. Passos CS[1], Both FL[1], Steffen VM[1], Kerber VA[2], Henriques AT[1] [1]UFRGS − Ciências Farmacêuticas, [2]UFPR − Farmácia,

Introduction: Psychollatine is a monoterpene indole alkaloid, isolated from Psychotria *umbellata* leaves1, which displayed analgesic and anxiolytic properties in mice models2,3. The mechanism of the acute anxiolytic action seemed unusual, since reversed by previous treatment with serotonin and glutamate antagonists (ritanserine and MK801, respectively)2. The aim of this study was to evaluate the anxiolytic effect of sub-chronic administration of psychollatine. **Methods:** Male CF1 mice (48) acquired from FEPPS (RS, Brazil), weighing 30 – 50 g at testing, were housed in groups of 4 – 8 per cage and maintained under a 12-h cycle (lights on at 07:00 a.m. and lights off at 07:00 p.m.) in a temperature/humidity-controlled environment (22 ± 1ºC/60 ± 5%). Food and water were freely available. All procedures were carried out in accordance with institutional policies on the handling of experimental animals (ethics committee approval # 2007682), which follow NIH guidelines. Mice were randomly assigned in four different groups: saline (SAL; n = 12), polyethylene glycol 20% (PEG 20%; n = 11), diazepam 1.0 mg/kg (DZP 1.0 mg/kg; n = 12), and psychollatine 7.5 mg/kg (PSY 7.5 mg/kg; n = 13) and treated during 15 days, once a day. The anxiety levels were evaluated in the light/dark paradigm 30 minutes after the last treatment. The apparatus consisted of a rectangular wood box (46 x 27 x 30 cm), divided into one small (18 x 27 cm) and one large (27 x 27) areas, with a door opening (7.5 x 7.5 cm) in the center of the separation. The small compartment was painted in black and light-free, whereas the large one was white and brightly lit with two 60 W cold light sources. Each animal was individually placed in the center of the bright compartment (facing away from the door) and the following parameters were evaluated for 5 minutes: latency to the first crossing from one compartment to other, time spent in the light compartment and the number crossings between the light and dark compartments. The test was performed in a quiet and darkened room (red bulb), and mice were kept in this room for at least 2 h before the session. **Results:** Locomotion was not affected by sub-chronic treatment with DZP 1.0 mg/kg or PSY 7.5 mg/kg. Contrary to what was observed with the acute treatment, neither PSY 7.5 mg/kg nor DZP 1.0 mg/kg displayed anxiolytic effects in the light/dark test after sub-chronic treatment (time spent in the light area, PSY 7.5 mg/kg and DZP 1.0 mg/kg 112.5 ± 10.80 s and 137.0 ± 17.05 s, respectively, compared to SAL 138.3 ± 10.89 s and PEG 105.1 ± 11.44 s, respectively). The data show that the anxiolytic effect of acute PSY 7.5 mg/kg is not maintained after two weeks treatment with the smallest active dose. These data could suggest that drug-induced receptor plasticity (down regulation) could be in place. **Financial Support:** CNPq and CAPES. 1V.A. Kerber, J. Nat. Prod. 71: 697 (2008). 2F.L. Both, J. Nat. Prod. 68: 374 (2005).3F.L. Both, J. Nat. Prod. 69: 342 (2006).

09.029

Mechanisms underlying the diuretic effects of isoquercitrin − an active flavonoid of *Tropaeolum majus* L. Gasparotto Júnior A[1], Gasparotto, FM[2], Leme TSV[2], Lourenço EL[1], Stefanello MEA[3], Silva Santos, JE[4], Kassuya CAL[5], Marques MCA[6] [1]UNIPAR/UFPR − Farmacologia, [2]UNIPAR − Farmacologia, [3]UFPR − Química, [4]UFSC − Farmacologia, [5]UFGD − Farmacologia, [6]UFPR − Farmacologia

Introduction: Flavonoids, such as isoquercitrin show markedly hypotensive and antihypertensive effects1. In addition, it has been shown that oral administration of flavonols rich plant induces an important diuretic effect2,3. The aim of this study was to evaluate the mechanisms underlying the diuretic action of isoquercitrin (ISQ), as well as the hydroethanolic extract (HETM) and the fraction (TMLR) obtained from *Tropaeolum majus*, an ISQ rich plant. **Methods:** Firstly, we evaluated the effects of T. majus infusion (2.5–10 %, p.o.), HETM (75–300 mg/kg, p.o.), TMLR (25–100 mg/kg, p.o.) and ISQ (5-10 mg/kg, p.o.) on the diuretic activity of both normotensive and SHR rats. Controls groups received the same amount of deionized water (5 mL/kg, p.o.) or spironolactone (50 mg/kg, p.o.). After 1-8 h (acute activity) or daily per seven days (chronic activity), the urine volume was measured. Additionally, sodium, potassium, chloride, bicarbonate, conductivity, pH and density were estimated from a pooled urine sample of each pair of rats at the end of the experiment. Plasmatic concentration of electrolytes, urea and creatinine were measured on day 7. The angiotensin converting enzyme (ACE) activity and aldosterone levels was measured by indirect fluorimetry and ELISA, respectively, in serum samples obtained from HETM, TMLR, ISQ treated rats. To evaluate the role of the bradykinin, prostaglandin and nitric oxide (NO) pathway in our findings, different groups of animals were subjected to HOE-140 (1.5 mg/kg, i.p. 15 minutes prior), Indomethacin (5 mg/kg, p.o. 1 h prior) and L-NAME (60 mg/kg, p.o. 1 h prior) before the treatments with HETM, TMLR or ISQ. The erythrocytary carbonic anhydrase and renal Na/K/ATPase activity also they had been determined. All procedures were approved by the Institutional Ethics Committee of UFPR (authorization number 240). **Results:** After one single dose of the HETM (300 mg/kg), TMLR (100 mg/kg) and ISQ (10 mg/kg) urine volume was significantly increased after 6 h of the treatments (70 ± 08%, 93 ± 15% and 99 ± 11%, respectively) remaining even 24 h. Daily administration of the HETM, TMLR and ISQ produced significant increased urinary volume and excretion of sodium starting on day 4 until day 7. The diuretic effect induced by HETM, TMLR and ISQ was strongly reduced by HOE-140, as well as by indomethacin or L-NAME (~ 65%). The oral treatment with HETM (300 mg/kg), TMLR (100 mg/kg) and ISQ (10 mg/kg) reduced the plasmatic ACE activity by 18 ± 3%, 26 ± 7% and 41 ± 3%, respectively, as well as the plasmatic concentrations of aldosterone. In the same way, the renal Na/K/ATPase activity was significantly reduced by all treatments (~ 30%), while urinary and plasmatic concentrations of others parameters were not affected. **Discussion:** Our results show that the diuretic effects caused by the hydroethanolic extract of *T. majus* (HETM), as well as its fraction (TMLR), may be associated with the high levels of the flavonoid isoquercetrin found in this plant. In addition, this effect may be an event dependent of inhibition of angiotensin II generation by ACE with consequent aldosterone reduce level and low activity of renal Na/K/ATPase, as well as by bradykinin-dependent stimulation of the NO and prostaglandin pathway. 1. Schramm D. D. et al. J. Nutr. Biochemistry 9: 560-566, 1998. 2. Wright C. I. et al. J Ethnopharmacology 114: 01-31, 2007. 3. Gasparotto Junior A. et al. J Ethnopharmacology 122: 517-522, 2009. **Acknowledgements:** DEGPP/UNIPAR and CAPES.

09.030

Anti-nociceptive and antiedematogenic effect of *Argyrovernonia harleyi* (H. Rob) Macleish hydroalcoholic extract on writhing test. Silva, AAR[1], Val DR[2], Souza RB[2], Araújo EB[2], Ribeiro KA[2], Brayner MMB[3], Chaves HV[4], Maia MBS[5] [1]UFC-Sobral − Odontologia, [2]UFC-Sobral, [3]UFC − Fisiologia e Farmacologia, [4]UFC, [5]UFPE − Fisiologia e Farmacologia

Introduction: *Argyrovernonia harleyi* (Asteraceae) is found on Xingó region (semi-arid area) in Northeast of Brazil, and recognized by local population as a traditional herb used to manage gastric-related complications. The aim of this study was to evaluate the effects of hydro alcoholic extract (HAE) of aerial parts (leaves and flowers) from *A. harleyi* in models of nociception (writhing test in mice) and inflammation (Carrageenan-induced rat paw edema). **Methods:** The experimental protocols used in this study were approved by the Institutional Animal Care and Use Committee of the Federal University of Ceará (UFC), Fortaleza, Brazil (protocol number 21/09) in accordance with international guidelines (NIH publication No. 85-23, revised 1985). Acetic acid (0.1 ml/10 g body weight of a 0.6% v/v solution) was injected i.p. on control (saline), indomethacin (5 mg/kg; s.c.) or HAE (100 or 400 mg/kg, p.o.) pretreated mice (n=6/group). The intensity of nociception was quantified by the number of writhes occurring between 0 and 30 min after stimulus injection. 4h after acetic acid injection cell influx were assessed in the peritoneal exudate. Carrageenan (500 µg; 0.1 ml) were injected s.c. into the right hind paw of rats. Saline, dexamethasone (1 mg/kg, s.c.) or HAE (100 or 400 mg/kg, p.o.) were administered 1h before acetic acid injection. Paw volume was measured by plethysmometer (PANLAB). Measures were made in zero (before), 1, 2 and 3h after edema induction. Edema was calculated by paw volume variation (ΔV) between each time interval and initial paw volume (t=zero). Data were analyzed using one-way ANOVA followed by Bonferroni's test ($P<0.05$). **Results:** HAE (100 or 400 mg/kg) administered p.o. to mice inhibited ($P<0.05$) the number of writhes (19.4 ± 5 or 10.5 ± 3), when compared to acetic acid group (52.7 ± 7). Only HAE (100 mg/kg) reduced significantly ($P<0.05$) cell influx (0.76 ± 0.2 x 103 cells/mm3), compared to saline group (1.92 ± 0.4 x 103 cells/mm3). However, HAE (100 or 400 mg/kg) did not result in paw edema relief. **Discussion:** The local irritation provoked by acetic acid in the intraperitoneal cavity triggers a variety of mediators, such as bradykinin, substance P, and prostaglandins, especially PGI2, as well as some cytokines such as IL-1β, TNF-α, and IL-8. These mediators activate chemosensitive nociceptors that contribute to the development of inflammatory pain. HAE (100 mg/kg; v.o.) of *A. harleyi* was able to reduce the writhings, but not able to prevent carrageenan-induced edema in rodents. The mechanism whereby the HAE produce this effect is yet unclear. Our data do not allow to reach a final conclusion whether the reduced writhing movements result of reduced motor function or to peripheral mechanisms. Further studies, however, are needed to elicit the mechanism(s) involved on the prevention of nociception sensitization. Di Rosa, M., et al. J. Pathol. v. 104, p. 15, 1971. Koster, R et al. Fed Proc v. 18, p. 412, 1959. Vane, J.R. and Botting, R. Inflamm. Res. v. 44, p. 1, 1995. **Financial support**: CNPq

09.031

Modulation of T lymphocyte and eosinophil functions *in vitro* by natural tetranortriterpenoids isolated from *Carapa guianensis* Aublet. Ferraris FK[1], Rodrigues R[2], Silva VP[2], Figueiredo MR[2], Penido C[1], Henriques MGMO[1] – [1]Farmanguinhos-FIOCRUZ − Farmacologia Aplicada, [2]Farmanguinhos-FIOCRUZ − Química de Produtos Naturais

Introduction: We have previously described the anti-allergic activities of a pooled fraction of tetranortriterpenoids (TNTPs) containing 6α-acetoxygedunin, 7-deacetoxy-7-oxogedunin, andirobin and methyl angolensate isolated from the seeds of *Carapa guianensis* (Penido C. et al., Inflamm. Res. 54: 295, 2005; Penido C. et al., Int. Immunopharmacol. 6: 109, 2006) in different in vivo models. In the present study, we performed *in vitro* studies in order to elucidate the mechanisms by which TNTPs present their anti-allergic effects and to identify the bioactive compound(s) present in such fraction. **Methods:** & **Results:** Here, we show that *in vitro* incubation of eosinophils with the pooled TNTP fraction (50 mg/ml, 1 h before stimulation) impaired the adhesion of eosinophils to tumor necrosis factor-α (TNF-α)-primed tEND.1 endothelial cells. Furthermore, the pooled TNTP fraction, as well as with each one of the five isolated TNTPs: 6a-acetoxygedunin (TNTP1), 7-deacetoxy-7-oxogedunin (TNTP2), andirobin (TNTP3), gedunin (TNTP4) and methyl angolensate (TNTP5) (50 mg/ml, 1 h before stimulation), impaired CCL11/eotaxin-mediated chemotaxis (maximal inhibition of 68% by TNTP5). By contrast, pooled TNTPs failed to inhibit adhesion and chemotaxis of T lymphocytes. However, TNTPs were able to impair anti-CD3 monoclonal antibody-induced T cell proliferation and the expression of CD25 and CD69. In accordance with the impairment of T cell proliferation and expression of CD25, pre-incubation of cells with TNTPs impaired IL-2 production. These data suggest that TNTPs prevent T cell activation. Moreover, pretreatment of splenocytes with the pooled TNTP fraction (50 mg/ml), as well as with each one of the five isolated TNTPs (50 mg/ml), inhibited ovalbumin (OVA; 10 µg/well)-induced *in vitro* production of CCL5 (maximum inhibition of 92% by TNTP3) and CCL11 (was inhibited at the same intensity by all TNTP tested, ~84% - 90%). TNTPs (except TNTP 1) also impaired nuclear factor-kB (NFkB) nuclear translocation in OVA (10 µg/well)-challenged splenocytes from previously sensitized C57BL/6 mice (CEUA, Fiocruz; license n. L-0004/08). Conclusion: Taken together, these results demonstrate that the anti-allergic effects of TNTPs isolated from C. guianensis might rely on their ability to inhibit eosinophil migration, as well as the activation of T lymphocytes, which is shared by the five isolated TNTPs. Supported by: CNPq, FAPERJ and FIOCRUZ.

09.032

Evaluation of the antiulcer activity of the extract obtained from rhizomes of *Typha domingesis* Pers (Typhaceae). Molina L[1], Ornelas FGI[1], Toma W[1] [1]UNISANTA − Farmácia

Introduction: Brazil is the largest country with the world´s plant genetic diversity, with over 55,000 species cataloged, a total of 350,000 and 550,000. Among these species is the species *Typha domingensis* Pers. Popularly known as cattails. That it is a perennial herb, Rhizomatous, aquatic, with stems cylindrical, reaching up to three meters tall, native to South-America. *Typha domingensis* Pers has been used as purifying natural aquatic environments, to absorb heavy metals may contribute to the environmental sanitation. Ethnopharmacological data obtained in the village in Itaoca Itanhaém-SP show that the Tupi-Guarani Indian tribe that lives there *Typha domingensis* used for the treatment of inflammatory and gastric problems. Few phytochemical and pharmacological studies related to this plant. However, this project aims to probable antiulcer activity of ethyl acetate fraction obtained from the extract of *Typha domingensis* and the presence of phenolic compounds giving them the likely antiulcer activity. **Methods:** After collection and species identification by the Herbarium of University Santa Cecilia, the rhizomes of *Typha domingensis* remained in an oven for 7days at 45°C. After this process they were crushed and subjected to extraction by soaking in hexane in proportion 50g rhizome/300ml solvent for 7days. Another soaking for more than 7 days was performed with acetone-water (7:3). The extract was subjected to liquid-liquid partition with ethyl acetate, n-butanol and water, getting 3fractions submitted to rotatory evaporator at 60°C. The ethyl acetate fraction was subjected to phytochemical analysis, according to the observations of colors in the samples, we confirm the presence of polyphenolic compounds (COSTA, A. F. Pharmacognosy experimental part III, 2000).Albino Wistar rats (180-220g) were acclimated under ideal conditions in the vivarium of the University Santa Cecilia. All procedures were submitted to the Ethics Committee on Animal Experiments of the core research (ECE) of the University under protocol n°10/2010. Groups of Wistar rats, after 24 hours fasting, were treated orally with lansoprazole 30 mg/kg (positive control, n=8), 10 ml/kg 0.9% saline (negative control, n=8) and 100 mg/kg of the ethyl acetate fraction of *Typha domingensis* (n=8) (Robert, A. et al. Cytoprotection by prostaglandins in rats. Gastroenterology. 77, p.433-43, 1979). After 50 min of treatment, the gastric injury was induced by oral administration of 1ml of absolute ethanol. The animals were sacrificed 1 hour after administration of the injurious agent, and the stomachs removed for counting of the ulcerogenic lesions. RESULTS The results of the ethyl acetate fraction of *Typha domingensis* showed significant antiulcer activity. The negative control of ulcerogenic lesion area (1.372 ± 0.2450 mm2), the positive control (lansoprazole) and the extract of *Typha domingensis* showed, respectively, (0.9820 ± 0.2694 mm 2 with $p > 0.05$ compared with negative control) and (0.7366 ± 0.2013 mm2 with ** $p < 0.01$ compared with negative control). The results were statistically analyzed using analysis of variance (ANOVA) with Dunnett´s post test and support software GraphPasd Instat®. **Discussion:** The results serve as a basis for future studies aiming to use this plant in patients with gastric ulcer, serving as support for the pharmaceutical industry in producing new drugs for this pathology.

09.033

Comparative study of different portions and extract from *Byrsonima intermedia* (leaves) A. Juss against disturbances gastrointestinal in rodents. dos Santos RC[1], Sannomiya M[2], Rodrigues CM[2], Vilegas W[2], Hiruma-Lima CA[1] [1]IB-UNESP-Botucatu − Fisiologia, [2]IQ-UNESP-Araraquara − Química Orgânica

Introduction: *Byrsonima intermedia* (Malphiguiaceae) is a medicinal plant used in folk medicine as an antiulcer and healing agent against gastritis. The present work investigated the ability of aqueous (AcoAq 100 mg/kg – p.o.) and ethyl acetate (AcoEt 100 mg/kg – p.o.) portions obtained from polar extract of B. intermedia (BiMeOH 500 mg/kg – p.o) leaves to prevent gastric ulcer and diarrhea in vivo. **Methods:** The preventive action of BiMeOH, AcoAq or AcoEt were evaluated in experimental models in male Wistar rats (n=7, 180-220 g) that simulated the disease in human gastric mucosa by oral administration of absolute ethanol (1mL/animal) or NSAID (non-steroidal anti-inflammatory drug– indomethacin 50 mg/kg). Male Swiss Mice (n=7,25-35g) were used for evaluation of the activity anti-diarrheal by oral administration of castor oil (0.2ml/animal).The antidiarrheal effect of BiMeOH and portions AcoAq or AcoEt were evaluated. **Results and Discussion:** BiMeOH presented effective gastroprotection (4.4 ± 4.4 mm) reducing significantly ($p<0.5$) the ulcer area compared with the control (212.8 ± 49.8 mm) when the harmful agent was the absolute ethanol. This gastroprotective action of BiMeOH was completely reversed by the sulfhydryl blocker (147.3 ± 42.2mm) indicating that the protective effects of BiMeOH were dependent from this factor. BiMeOH also presented decrease the formation of the lesions in the presence of the NSAID (12.8 ± 6.9 mm) when compared to the negative control group (60.4 ± 5.2 mm). The gastroprotective action of AcoAq or AcoEt was completely reversed by the sulfhydryl blocker (317.2 ± 52.6 and 247.2 ± 18.4mm, respectively). AcoAq also showed effective gastroprotective action in the presence of AINE by reducing significantly the gastric lesions provoked by this harmful agent (26.2 ± 5.9mm). This same results was not observed for AcoEt (44.2 ± 12 mm) when compared to negative control group (60.4 ± 5.2 mm). This reduction of lesions presented by AcoAq was accompanied by the maintenance of the levels of myeloperoxidase (763.4 ± 55.7 unit of myeloperoxidase/g of tissue) compared with Sham animals (which no received the harmful agent) (690.1 ± 67.7 unit of myeloperoxidase/g of tissue) and are diminished in comparison with the negative control group (1448 ± 238.0 unit of myeloperoxidase/g of tissue). The anti-diarrheal action of BiMeOH was verify by reduced number of feces liquidate (0.9 ± 0.6), as well as it delayed begin them (167.0 ± 30.8 min) when compared to the negative group controls (3.9 ± 0.5 for number from feces and 74.1 ± 16.3 to retard in minutes of the evacuations). This same was observed for AcoAq, that reduced the number of feces liquidate (0.8 ± 0.5) and delayed the emergence of them (169.6 ± 23.6 min) without AcoEt presented some alteration for those parameters. These results corroborate with the popular indication of B. intermedia for disturbances of gastrointestinal tract, once the polar extract of the leaves was capable to inhibit the formation of gastric lesions in different models of damage agents, also it showed a dependence of sulfhydryl compounds for this action. Both portions were effective to prevent acute injuries however AcoAq demonstrated a gastroprotection more effective against different harmful agents. BiMeOH also demonstrated important anti-diarrheal effect. The group of the data suggests that AcoAq is more efficient to produce the same activity observed in BiMeOH suggesting that present chemical compositions in this portion are the responsible for the general activity of the extract of *Byrsonima intermedia*. Number of experimental protocol: 18/05 CEEA. Acknowledgements and **Financial support:** Biota/FAPESP (proc 2009/54761-7)

09.034

Croton grewioides Baill. shows antidiarrhoeal activity in mice. Silva ADS[1], Silva, KM[1], Lima LO[1], Silva-Junior V[2], Silva PCB[3], Medeiros VM[3], Costa VCO[3], Tavares JF[3], Silva MS[4], Cavalcante FA[1] [1]ICBS-UFAL, [2]UFAL − Nutrição, [3]LTF-UFPB, [4]UFPB − Química

Introduction: Croton genus stands out as the second largest of the Euphorbiaceae family, with about 1200 species distributed in tropical and subtropical regions. Croton grewioides Baill. presents oneself as a shrub that grows naturally in forests and pastures interspersed with rocky outcrops, has a strong aroma of cinnamon noticeable even dried material. Based on chemotaxonomic criteria, coupled with the fact that several species of Croton have antidiarrhoeal activity, we decided to investigate a possible antidiarrhoeal activity of ethanol extract obtained from aerial parts of C. grewioides (CG-EtOH) in mice. **Methods:** Pharmacological behavioral screening and determination of the LD50: were used mice treated with saline plus cremophor p.o. (10mL/kg) or CG-EtOH (2500 and 5000 mg/kg p.o. or 1000 and 2000 mg/kg i.p.), and several behavioral parameters were evaluated and quantified the number of deaths (24, 48 and 72h). Castor oil-induced diarrhoea: mice (n=6) were divided into negative control (saline plus cremophor 10 mL/kg), positive control (loperamide 10 mg/kg) and test groups (CG-EtOH). Diarrhoea was induced by oral administration of 0.01 mL castor oil/animal gram 30 min after the above treatments. During an observation period (4h), the total number of faecal output and number of wet faeces excreted by the animals were recorded. Normal intestinal transit: mice were divided into groups (n=6). Group 1 received saline plus cremophor p.o., group 2 was administered atropine 2 mg/kg p.o. (positive control) and the others groups were administered CG-EtOH p.o. After 30 min, standard charcoal meal (0.01mL/animal gram) were given to mice orally. Mice were sacrificed 30 min after administration of charcoal meal and the small intestine immediately isolated. Intestinal fluid accumulation: mice were divided into groups (n=6). Group 1 received saline plus cremophor p.o. and the others groups were administered CG-EtOH p.o. After 30 minutes, was administered 2mL of castor oil/animal. 30 min later, the mice were euthanized, and the fluid volume was measured. All the experimental protocols were approved by Ethical Committee in Research of UFAL (Protocol 010489/2009-15). **Results and Discussion:** CG-EtOH did not show lethality orally in mice tested. In contrast, intraperitoneal the extract showed lethality only at a dose of 2000 mg/kg in females. CG-EtOH produced a significant antidiarrhoeal activity ($p<0.05$), both the frequency of defaecation (ED50=157.7 ± 41.0 mg/kg), and liquid faeces (ED50=112.8 ± 14.4 mg/kg) in mice. This effect of the extract was similar to the standard drug, loperamide that produced a maximal inhibition of 97.7%. This effect may be related to an inhibition of muscle contractility and motility, since CG-EtOH (125 mg/kg) was able to inhibit the intestinal transit by charcoal meal (65.1 ± 3.7%), and inhibit of intestinal fluid content induced by castor oil (ED50=85.7 ± 18.7 mg/kg). The treatment of the diarrhoeal aims at, among other objectives, to increase resistance to flow (segmental contraction, decrease propulsion and peristalsis) and to increase mucosal absorption or to decrease secretion. In this context, the results of this study suggest that the CG-EtOH possesses antidiarrhoeal activity, however other studies must be carried out to elucidate the mechanisms involved in these activity. **Financial support:** PIBIC/ CNPq/FAPEAL/UFAL

09.035

Evaluation of the toxicity and gastroprotective activity of the ethanolic extract from leaves of *Xylopia langsdorffiana* A. St.-Hil. & Tul. (Annonaceae). Montenegro CA, Lima GRM, Pessoa DR, Viana WP, Castello Branco MVS, Tavares JF, Batista LM LTF-DCF-UFPB

Introduction: *Xylopia langsdorffiana* A. St-Hil.&Tul., known as "pimenteira da terra", is a tree with 5-7 m high and his choice for the study was based on chemotaxonomic criteria. The toxicological study of natural products is useful for ensuring security in the investigation of pharmacological properties, among them the antiulcer activity. The aim of this study was to determine a CL50, to investigate the acute oral toxicity and gastroprotective activity of ethanolic extract from leaves of *X. langsdorffiana* (Xl-EtOH). **Methods:** In the lethality test with brine shrimp was used 25 mg of eggs of Artemia saline which were incubated in seawater (pH 8-9 and 29°C) with artificial light for 24 hours to obtain occlusion of cysts and larvae. The extract was diluted in seawater and was added 5 mL of different concentrations of Xl-EtOH in tubes containing 13-15 nauplii. The surviving larvae were counted to determine the LC50. In the study of acute oral toxicity, a single dose of Xl-EtOH (2000 mg/kg) was administered in a group of twelve mice (six males and six females). The animals (six males and six females) that received the tween 80 solution 12% served as controls. After treatment, the parameters of behavior were observed for 30, 60, 90, 120, 180, 240 minutes, 24 h, 48 h and 72 h. Food and water consumption were evaluated in both sexes, within 14 days. At the end of the period the number of survivors was recorded to determine the LD50, was estimated body weight of mice, then the animals were sacrificed, organs were weighed and macroscopic changes in the organs were observed. The antiulcer assay was performed using the HCl/ethanol-induced ulcer model in mice. The animals were treated with Xl-EtOH, carbenoxolone (100 mg/kg) or the tween 80 solution 12% (n=5-7). The ulcerogenic index (IU) is expressed in mean ± S.D and were compared using ANOVA followed by Dunnett´s, $p<0.05$. The experimental protocols were approved by the Institutional Committee for Ethics in Animal Research of LTF/UFPB registered under 0106/09. **Results and Discussion:** The test of lethality of brine shrimp showed that the Xl-EtOH has bioactivity suggesting the presence of important bioactive substances in it, because the LC50 (658.4 µg/mL) was less than 1000 µg/mL. In acute toxicity test showed that a single dose of Xl-EtOH (2000 mg/kg) induced hyperactivity and irritability only female mice and this effect was reversible in the third hour after oral administration. During the 14 days of observation there were no deaths. In the group of male mice that received Xl-EtOH was observed an increase in water consumption. The increase in food consumption of females which received the Xl-EtOH was demonstrated when compared with the tween group and we observed an increase in body weight of females compared to the beginning of the protocol. Xl-EtOH not induced macroscopic changes in the organs of mice. The gastric damage induced in the model of HCl/ethanol, Xl-EtOH (62.5, 125, 250 and 500 mg/kg) and carbenoxolone, significantly reduced the IU for 115 ± 19.86, 99.83 ± 21.35, 78 ± 16.56, 62.57 ± 19.22 and 92.5 ± 33.61, respectively, in comparison with tween 161.3 ± 37.59. These results suggest that the Xl-EtOH has low toxicity and displays gastroprotective activity in the HCl/ethanol-induced ulcer model. **Financial support:** CNPq/CAPES/LTF/UFPB

09.036

Investigation of gastroprotective effect and 50 lethal concentration of the ethanolic extract from *Combretum duarteanum cambess* (Combretaceae). Lima GRM, Montenegro CA, Almeida CLF, Pessoa DR, Moreira MMB, Castello Branco MVS, Tavares JF, Batista LM LTF-DCF-UFPB

Introduction: The Combretaceae family has about 13 gender and 500 species which are distributed in tropical environments, especially in Africa and Asia (Stace, Flowering Neotropics, 110, 2004). These species are ornamental, some are used empirically by population, and others have been already evaluated their pharmacological effects. *Combretum duarteanum Cambess* can be found in some countries from Southern American such as Bolivia, Paraguay and Brazil. In Paraiba state (Brazil), this specie usually occurs in Caatinga biome (Loiola, Acta bot. Bras, 330, 2009). Based on the pharmacological effects, this work aims to evaluate the gastroprotective activity and the toxicity by 50 lethal concentrations (LC50) of the ethanolic extract (EtOHE) obtained from the *C. duarteanum* leaves. **Methods:** The toxicity was evaluated by the brine shrimp lethality assay (McLaughlin, Drug. Inf. J., 32, 513, 1998). *Artemia salina L*. eggs were incubated in artificial seawater at 28°C for 24 h, with continuous side illumination (40-W lamp) in order to obtain the larvae. After their hatching, the eggs' nauplii were collected and treated with different concentrations in DMSO, 10-1000 µg/ml of EtOHE. 24h later the LC50 was determined, being made in three replications. (Meyer, Planta Med., 45, 31, 1982). The gastroprotective activity was investigated using 0.03M HCl/60% ethanol solution which induced to the gastric ulcer in male Swiss mice (Mizui, *J. Pharmacol*., 33, 939, 1983). After 24h of fasting, the animals (n=5-7) were treated using 10 ml/kg of the following reactants: EtOHE (62.5, 125, 250 and 500 mg/kg) or carbenoxolone (100 mg/kg) or 12% Tween-80 solution. The ulcerative index (UI) was determined and expressed as mean ± S.D using ANOVA followed by Dunnett´s Test ($p<0.05$). The experimental protocols were approved by the Institutional Committee for Ethics in Animal Research of LTF/UFPB and they were registered under 0211/09. **Results and Discussion:** The brine shrimp test is a screening of bioactive or toxic actions of plants and derivates. In this test, EtOHE was bioactive with LC50 868.2 µg/mL. In HCl/ethanol-induced ulcer model, the EtOHE (62.5, 125, 250 and 500 mg/kg) and carbenoxolone (100 mg/kg) reduced significantly the UI for 101.6 ± 18.17, 102 ± 11.02, 73.60 ± 12.56, 60.60 ± 12.01 and 98.40 ± 29.46 when compared to the negative control 153.6 ± 42.45, respectively. These results suggest that the EtOHE from Combretum duarteanum was lethal for Artemia salina which indicates the presence of important bioactive substances, besides presenting gastroprotective effect in ulcer induction models. **Financial support:** CAPES/CNPq/LTF/UFPB

09.037

Mechanisms involved in the antinociceptive effect of the ethanolic extract from the leaves of C*eltis iguanaea* (jacq.) Sargent (Ulmaceae). Nascimento MVM[1], Lino RC[1], Sousa BF[1], Florentino FI[1], Galdino PM[1], Couto, RO[2], Paula JR[2], Costa EA[1] [1]ICB-UFG, [2]UFG − Farmácia

Introduction: *Celtis iguanaea* (JACQ.) Sargent (ULMACEAE) is a small specie, thorny and with flexible branches and native in the Cerrado. The leaves are used in body aches, rheumatism and colic. Previous data showed that the ethanolic extract from the leaves of "esporão-de-galo" (EEEG) decrease the acetic acid-induced writhing and the animals pain reactivity in both phases of formalin test (FeSBE, res. 36,005, 2009). The objective of this work is to continue the study of the mechanisms involved in antinociceptive effect of EEEG. **Methods:** The EEEG was obtained by maceration of the leaf dry powder in ethanol (96 ºGL), followed by filtration and concentration under reduced pressure (yield = 6% w/w). The animals used were adult male Swiss mice (Ethic Commission of UFG, protocol nº 106/08). The antinociceptive mechanisms were evaluated in capsaicin-induced pain model, where groups of mice (n = 7) were treated with vehicle (10 mL/kg p.o.), EEEG (0.1, 0.3 and 1.0 g/kg p.o.), morphine (10 mg/kg s.c.) or (capsazepine 10 mg/kg i.p.). One hour after the treatments by p.o. or 30 minutes by s.c. and i.p. routs, the animals received 50 µL of capsaicin solution (1.6 µg/paw) and the licking time was measured between 0 and 5 minutes after the application of the phlogistic agent. In the tail flick test, the pain threshold was measured using the Insight® analgesimeter, assessing the spinal analgesic effect of the treatment with morphine or EEEG, being recorded the reaction time and the intensity of thermal stimulation. Results were expressed as mean ± SEM of the values in percentages relative to controls. **Results and Discussion:** In the capsaicin-induced pain, the EEEG at doses of 0.3 and 1.0 g/kg decreased the licking time from 100.00 ± 8.54% (55.57 ± 4.75 s control) to 53.21 ± 3.63 and 43.50 ± 7.28%, respectively. The treatment with capsazepine decreased to 50.11 ± 9.23% and morphine to 16.11 ± 14.32%. These data suggest that EEEG at these doses showed antinociceptive activity related to peripheral vanilloid receptors, and this effect was similar to the selective antagonist of these receptors (capsazepine). In the tail flick test, the treatment with EEEG (1.0 g/kg) increased the latency to pain reactivity from 97.39 ± 8.43% (control) to 131.49 ± 8.15%, 60 minutes after the treatment. 90 minutes after the treatment with EEEG at doses of 0.3 and 1.0 g/kg increased this latency from 79.61 ± 9.3% (control) to 100.86 ± 9.92 and 126.96 ± 9.53%, respectively. These doses also increased the resistance of animals' thermal intensity. EEEG 1.0 g/kg increased the thermal stimulation from 53.67 ± 1.78 °C (control) to 66.00 ± 2.79 °C and from 56.86 ± 2.12 °C (control) to 68.17 ± 2.84 °C after 60 and 90 minutes of treatment, respectively, and EEEG 0.3 g/kg increased to 57.00 ± 1.4 °C after 90 minutes of treatment. The analgesic effect observed in the tail flick test suggests a spinal action. In conclusion, the antinociceptive effect of EEEG should involve peripheral mechanisms through vanilloid receptors and/or spinal mechanisms as assessed in capsaicin and tail flick tests. **Financial support:** CAPES, CNPq, FAPEG, FUNAPE/UFG.

09.038

Effects of amblyomin-X on tumor growth, endothelial cell migration, adhesion and secretion. Dias RYS[1], Drewes CC[1], Hebeda CB[1], Simons SM[2], Chudzinski-Tavassi AM[2], Farsky S[1] [1]FCF-USP Análises Clínicas e Toxicológicas, [2]IBu − Laboratório de Bioquímica

Introduction: Amblyomin-X is a recombinant protein inhibitor of serinoprotease isolated from the salivary gland of Amblyoma cajennense. Previous data have shown that systemic administration of Amblyomin-X decreases in vivo installation and development of tumors. The mechanisms are not completely elucidated and therefore, here we investigated the role of Amblyomin-X on in vivo new vessels formation and tumor growth, and *in vitro* endothelial cell migration, adhesion and secretion. **Methods:** In vivo angiogenesis was studied using dorsal chambers implanted on male Swiss mice after anesthesia. Saline or Amblyomin-X treatment (10,100 and 1000ng/10mL) was topically applied each 48 hours in the absence or presence of murine melanoma B16F10 lineage (1x105 cells), which was injected into microcirculatory beds of the subcutaneous tissue. Numbers of vessels and tumor growth were quantified in images obtained before and at 8th day after beginning of treatments using Axio Vision software. *In vitro* investigations were performed using microcirculatory endothelial cell lineage (T- end lineage). The cell migration was investigated by a mechanic lesion in the confluent T-end culture by a cell scraper before adding Amblyomin-X (10 or 100 and 1000ng/well) or /and VEGF (100ng/mL) to the wells. Number of migrating cells into lesioned area was quantified 12 h after the treatment. PGE2 and NO were quantified in the supernatant of this endothelial cell culture, 24 hours after beginning of treatments. Endothelial cell adhesion was evaluated on Matrigel® system. 5 x 104 cells per well were allowed to adhere on Matrigel® for 30 min at 37ºC and adhered cells were quantified after staining with 0,1% Crystal Violet (20% methanol; v/v). Data were obtained by spectrophotometry (l = 600nm). **Results:** In vivo Amblyomin-X treatment significantly reduced the number of vessels in the subcutaneous microcirculation (10ng = 21.7%, 100ng/10mL= 35,7% and 1000ng/10mL= 36,8% vs the first day of treatment) and the tumor growth (1000ng/10mL= 88,8% vs the first day of treatment). Amblyomin-X treatment reduced endothelial cell migration into the lesioned area only in the absence of VEGF and independently PGE2/NO secretion (10ng/10mL = 16,4%, 100ng/10mL= 23,1% and1000ng/10mL= 26,8% vs control). And marked inhibited endothelial cell adhesion on Matrigel® (100ng/10mL = 46% and 1000ng/10mL= 48% vs control). **Discussion:** Based on these findings, local application of Amblyomin-X reduces the number of microcirculatory vessels and tumor growth in vivo and the mechanisms may involve impairment on endothelial cell migration and adhesion. Future investigations will be carried out to clarify the intracellular pathways involved in this process. **Financial support:** CAPES, FAPESP grant 08/57850-8.Brazilian College of animal experimentation (COBEA; Protocol number: 212, 19/02/ 2009).

09.039

Healing properties of bark extract of *Tabebuia avellanedae* in chronic gastric ulcer induced by acetic acid in rats. Pereira IT[1], Burci LM[1], da Silva LM[1], Baggio CH[1], Andre E[2], Pizzolatti MG[3], Marques MCA[1], Werner MFP[4] [1]UFPR − Farmacologia, [2]UFRN − Farmacologia, [3]UFSC − Química, [4]UFSC − Farmacologia

Introduction: *Tabebuia avellanedae* is popularly known as “ipê-roxo” and has been used in folk medicine as an anti-inflammatory and in the treatment of ulcers, bacterial and fungal infections. Previous studies demonstrated that the crude extract of *Tabebuia avellanedae* reduced the gastric lesion induced by ethanol and acetic acid, increased gastric mucus and inhibited the H^+/K^+/ATPase enzymatic activity *in vitro* (Twardowschy et al., *J. Ethnopharmacol*., 118, 455, 2008). On light of these data, we investigated additional gastroprotective effects of the hydroalcoholic extract from the barks of *Tabebuia avellanedae* (EHT). **Materials and Methods:** Female Wistar rats (180-250g) were anaesthetized, the stomach was exposed and 500 µl of 80% acetic acid was injected into a glass cylinder of 6 mm on the serosa of the stomach. The animals were orally treated with vehicle (water, 1 ml/kg), omeprazole (40 mg/kg) or EHT (30, 100 and 300 mg/kg) twice a day for 7 days. On the day following the last administration, the animals were sacrificed, the stomachs were removed and the extent of the gastric lesion was measured as the total injured area (mm^2). Hematoxylin-eosin stain was used for histopathological examination. Immunohistochemical expression of PCNA (Proliferating Cell Nuclear Antigen) was performed to evaluate the effects of the extract on the healing of ulcerated gastric mucosa. Mucin histochemistry using periodic acid Schiff (PAS) was performed to evaluate the expression of mucin glands in the gastric mucosa (score range 0-3). The experimental procedures were previously approved by the Institutional Ethics Committee of the UFPR (CEEA approval number 437). **Results:** The treatment of animals with omeprazole (positive control) and EHT (100 and 300 mg/kg) significantly reduced the gastric lesion induced with acetic acid by 48, 44 and 36%, respectively, compared to control group (140 ± 13 mm2). The histopathological evaluation demonstrated a partial gastric healing of stomachs treated with EHT (100 and 300 mg/kg). EHT (100 and 300 mg/kg) also increased the PCNA-immunoreactivity by 61 ± 5 and 120 ± 10 when compared to injured control (27 ± 4 cells in proliferation). Mucin expression increased with EHT treatments (100 and 300 mg/kg) to 2-3 and 1-3 score ranges, respectively, when compared to control ulcerated group (score range 0-2). **Discussion:** The oral administration of EHT for 7 days accelerated the healing of gastric ulcers induced by acetic acid in rats, probably due to epithelial cell proliferation. The gastric cell proliferation could be confirmed by PCNA immunohistochemistry and the ulcer healing by histological and mucin assays. Collectively, these results demonstrate the potent antiulcerogenic activity of the crude extract of *Tabebuia avellanedae*. **Financial support:** CNPq, Capes, Fundação Araucária

09.040

Subfraction of *Pterodon pubescens* seeds oil induces apoptosis of leukemic cells by inducing Apaf-1 gene expression. Martino T, Pereira MF, Dalmau SR, Silva MCC, Coelho MGP, Sabino KCC UERJ − Bioquímica

Introduction: *Pterodon pubescens* is a Brazilian plant known as “Sucupira Branca”, which its seeds extract is popularly used to treat rheumatoid arthritis. Previous studies had shown that seeds oil (OPp) presents anti-inflammatory, analgesic and antiarthritic effects (Cardoso CC, Pak J Biol. Sci., 11:2308,2008). OPp also inhibited leukemic cells (K562) proliferation as its hexanic subfraction 5 (SF5). In this work, we compared the cytotoxic effects of SF5 between different tumor cells as A549 (lung), MCF7 (breast), PC3 (prostate) and the leukemic cells Jurkat (lymphocytic) and K562 (myelocytic). **Methods:** The cell proliferation (2.5 x 105cells/mL) was determined by MTT assay, for 24h in absence and presence of SF5. Apoptosis was studied by flow cytometry (cell shrinkage). Apaf-1 mRNA expression was analyzed by RT-PCR, where the cells were treated in different times with SF5 20 µg/mL. The DNA fragmentation was measured by agarose gel electrophoresis. Results/**Discussion:** SF5 (20 µg/mL, 24h) inhibited 87% ($p < 0.05$) of Jurkat cells proliferation and 32% ($p < 0.05$) of PC3 cells, while inhibited only 6.7% ($p > 0.05$) of K562 cells, 6.6% ($p > 0.05$) of A549 cells and 1.6% ($p > 0.05$) of MCF7 cells. SF5 treatment (36h) not only was more efficient on inhibition of lymphocytic leukemia cell proliferation but it also induced them into apoptosis. SF5 treatment increased ($p < 0.05$) the number of Jurkat apoptotic cells (reduced size) from 10.2 + 2.3 % (control) to 36.8 + 4.4%, while it did not altered ($p > 0.05$) the number of K562 apoptotic cells, showing 3.3 + 1.7% for non-treated cells and 4.6 + 0.3% for treated ones. On the other hand, K562 cells was also sensitive to higher SF5 concentration (50 µg/mL), showing 13.3% ($p < 0.05$) of apoptotic cells. Methotrexate (20 µg/mL), used as a positive control drug, induced ($p < 0.05$) both cells to apoptosis. Another hallmark of apoptosis in Jurkat cells by SF5 treatment was shown by DNA fragmentation (ladder). SF5 also induced increase of Apaf-1 mRNA expression on Jurkat cells. An exciting result was that SF5 (20 µg/mL, 24h), which markedly inhibited Jurkat cells proliferation (87%), did not inhibited the proliferation of human peripheral blood mononuclear cells stimulated by concanavalin A. Conclusion: This work shows that the lymphocytic leukemia cell line Jurkat is more sensitive to SF5 than the other tumor cells tested, that SF5 is not toxic to human mononuclear cells and suggests that SF5 induces Jurkat cells into apoptosis by the mitochondrial pathway, mediated by apaf-1 expression and apoptosome assembly. Support: UERJ, FAPERJ, CNPq, CAPES.

09.041

Antinociceptive effects of (1→3),(1→6)-linked b-glucan isolated from *Pleurotus pulmonarius* in models of acute and neuropathic pain in mice. Baggio CH[1], Freitas CS[1], Werner MFP[2], Martins DF[3], Mazzardo L[3], Smiderle FR[4], Sassaki GL[4], Iacomini M[4], Marques MCA[1], Santos ARS[5] [1]UFPR − Farmacologia, [2]UFSC − Farmacologia, [3]UFSC − Fisiologia, [4]UFPR − Bioquímica, [5]UFSC − Ciências Fisiológicas

Introduction: Previous studies showed the antinociceptive activity of β-glucan (GL) isolated from mushroom *Pleurotus pulmonarius* on nociceptive responses induced by chemical models. This study evaluated the possible mechanisms of action involved on antinociceptive effect of a (1®3),(1®6)-linked b-glucan (GL) in a model of acute and neuropathic pain. **Methods:** Swiss mice (30-40 g, n= 8-12/group) both sexes were used (Ethics committee number: 303/UFSC). The GL was evaluated in glutamate model of pain or by nociceptive response following intrathecal (i.t.) administration of agonists of AMPA (25 ng/site, 1 min), NMDA (74.3 ng/site, 5 min), kainate (23.5 ng/site, 4 min), trans-ACPD (1.9 µg/site, 15 min), SP (135 ng/site, 6 min), IL-1β (0.1 pg/site, 15 min) and TNF-a (1 pg/site, 15 min) in mice. In another set of experiments, it was evaluated the role of medullar astrocytes on GL (30 mg/kg, i.p.) antinociception after formalin test. The neuropathic model of pain was induced by partial ligation sciatic nerve (PSNL) and the mechanical allodynia were recorded before and after treatment with GL (30 mg/kg, i.p., twice a day). **Results:** Intraperitoneal administration of GL inhibited the glutamate-induced licking with ID50 of 0.34 mg/kg and inhibition of 96 ± 3%. The treatment of animals with GL (1 mg/kg, i.p.) inhibited the nociception induced by intrathecal injection of NMDA, AMPA, kainate and IL-1b in 67 ± 13, 89 ± 11, 74 ± 9 and 75 ± 7%, respectively, but not the nociceptive response induced by trans-ACPD, SP and TNF-a. The treatment with GL (30 mg/kg, i.p.) also reduced the GFAP immunoreactivity of astrocytes from spinal cord after the formalin-induced nociception Moreover, GL (30 mg/kg, i.p.) also reduced the mechanical allodynia caused by partial sciatic nerve ligation (PSNL) for 2 h, with inhibition of 47 ± 10% observed 0.5 h after the treatment. When given chronically (twice a day) for 7 days, GL reversed the mechanical allodynia caused by PSNL (inhibition of 45 ± 13% to 60 ± 8%). Of note, GL did not affect the locomotor activity of mice on open field model with doses which possess antinociceptive effect. **Discussion:** Collectively, present findings show that GL is effective in inhibiting acute and neuropathic pain in mice through mechanisms that involved the inhibition of ionotropic glutamate receptors, pro-inflammatory cytokines, IL-1b pathway and medullar astrocytes activation. **Financial support:** CAPES, CNPq, FAPESC, UFSC, UFPR.

09.042

Effect of p-cymene obtained from *Citrus latifolia* Tanaka essential oil on *in vitro* leukocytes chemotaxis. Kummer R[1], Fachini FC[1], Silva CFE[1], Freitag A[1], Silva EL[2], Grespan R[1], Bersani-Amado CA[1], Cuman RKN[1] [1]UEM − Farmácia e Farmacologia, [2]UEM − Química

Introduction: The fruits of the genus Citrus (Rutaceae) have appreciable content of essential oil, being the main constituents: limonene, a-pinene, b-pinene, p-cymene, g-terpinene, linalool, nerol e citral. Studies have demonstrated the biological activity of essential oils, such as: anti-inflammatory, anthelmintic, anti-infective, antinociceptive and immunomodulatory properties. However, there is not study demonstrating the effect of p-cymene (CYM), a terpenoid, on leukocytes chemotaxis. This way, we evaluated the effect of CYM, on the *in vitro* chemotaxis activity of leukocytes. **Methods:** The fruits were purchased in the city of Maringa-PR. The essential oil of the fruits of Tahiti lime (Citrus latifolia Tanaka) was extracted from distillation in Clevenger apparatus. The CYM was identified by GC-MS and RMN. To evaluate the effect of chemotaxis, leukocytes were isolated from the peritoneal cavity of mice, 4 hours after injection with zymosan (1mg/cavity, i.p). The cell number was adjusted to 1x106 cells /ml in RPMI/ BSA 0.1% and pretreated with CYM (3, 10 or 30 µg/ml) for 30 min. Then, leukocytes were allowed to migrate toward fMLP (10-6M) or RPMI 1640 medium alone as control of migration in the chemotaxis Boyden chamber (48 wells; Neuro Probe, Inc., Cabin John, MD–USA). The cells were incubated at 37 °C, 5% CO2 for 1h. Following incubation, the membrane was washed and stained using the Instant Prov (Newprove). Leukocytes were counted by optical microscope, five fields (1000X) in each well. The results were expressed as the number of leukocytes per field. Data were presented as mean ± SEM of 3 separate experiments. The means from different treatments were compared by ANOVA with Tukey's correction. Statistical significance was set at $p \leq 0.05$. The protocol (number 041/2008) regarding this study was approved by the ethical commission of ethics in animal research (CEAE/State University of Maringá). **Results and Discussion:** The pretreatment with CYM in all tested doses inhibited fMLP-induced leukocytes migration (CYM3µg/ml: 5.12 ± 0.92*; CYM10µg/ml: 3.6 ± 0.48*; CYM30µg/ml: 3.6 ± 0.71*) when compared with leukocytes stimulated with fMLP without pretreatment (10.9 ± 1.24). Additionally, the leukocytes migration stimulated with medium alone, negative control, was the 1.2 ± 0.08 cells. Our study provides evidence that CYM at tested doses inhibits *in vitro* leukocytes chemotaxis, suggesting that CYM can be seen as a potential therapeutic immunointervention in the inflammatory diseases. Supported by: CNPq; CAPES. **Acknowledgements:** Jaílson Araújo and Célia Miranda.

09.043

Effect of eugenol treatment on renal parameters after renal ischemia and reperfusion in mice. Damião MJ[1], Victor ML[1], Fonseca JP[1], Bersani-Amado CA[1], Rilson JP[1], Giannocco G[2], Câmara NOS[3], Cuman RKN[1] [1]UEM − Farmácia e Farmacologia, [2]UNIFESP − Endocrinologia, [3]ICB-USP

The ischemia (I) is related to the interruption of blood supply in oxygen and nutrients during a determined period of time. In the ischemic injury after renal reperfusion (R) where the blood flow is restored in the ischemic tissue, an increased uremia is observed. Eugenol is the main constituent from clove essential oil (*Eugenia caryophyllata* Thunb). It has been demonstrated many biological activities for this oil, such as: immunostimulant, anti-inflammatory and antinociceptive. In this work the effect of Eugenol (EUG) treatment on the renal function was evaluated in experimental models of renal ischemia and reperfusion in mice. **Methods:** The protocol regarding this study was approved by the ethical commission of ethics and in animal research. (041/2008/CEAE/UEM). Male Swiss mice (20 to 28g) were anaesthetized with ketamine (100 mg/kg; i.p.) and xylazin (10 mg/kg, i.p.). The animals were submitted at the renal pedicle unilateral I/R procedure during 45 minutes, according to KELLY et al.,1996. The experimental groups were: a) eugenol (100, 200 or 400 mg/kg, once a day); b) Sham: animals submitted to I/R procedure; and c) control: non-isquemic animals receiving saline. The animals were treated by gavage during 48 h. The mice were euthanized seric creatinine and urea determination. The results were expressed as average ± epm, and were statistically analyzed by ANOVA ($P \leq 0.05$). **Results and Discussion:** serum creatinine: no significant differences were observed for creatinine determination in all groups tested: control (0.34 ± 0.01mg/dl); Sham (0.41 ± 0.01 mg/dl); EUG 100 mg/kg (0.43 ± 0.01) and EUG200 mg/kg (0.41 ± 0.01). However, after EUG400 mg/kg dose, an increased creatinin level was observed (0.63 ± 1.16* mg/dl ;P<0,01) suggesting a possible renal toxicity of Eug in high doses. Serum Urea: after 48hs of EUG treatment urea levels were reduced when compared to sham group. Control: 52.73 ± 6.71 mg/dl); Sham: 66.27 ± 5.64 mg/dl; EUG100 mg/kg (38.7 ± 3.59* mg/dl); EUG200 mg/kg (46.9 ± 2.33mg/dl). In the other hand, urea levels after EUG400 mg/kg treatment were similar to that in the control group (61.9 ± 11.2* mg/dl, P<0.05). The eugenol treatment at dose of 100 mg/kg, restored the renal function evaluated by urea excretion observed to be increased in uremic mice. However, EUG treatment after I/R showed an important renal toxicity effect at 200 and 400 mg/kg doses, observed by creatinine and urea increased levels. **Apoio Financeiro**: CAPES, CNPq, Fundação Araucária Kelly et al, 1996. Ischemia/reperfusion injury in transplantation. in Transplantation Biology: cellular and molecular aspects. Editors: Tilney NL, Strom TB,Paul LC, eds. Pag: 257-274.

09.044

Cardiovascular activity *Hancornia speciosa* ethanolic extract in a model of hypertension induced by nitric oxide synthesis inhibition in rats. Silva MDA[1], Serra CP[2], Grabe-Guimarães A[2], Guimarães HN[3], Braga FC[4] [1]CiPharma-UFOP, [2]DEFAR-UFOP, [3]UFMG − Engenharia Elétrica, [4]FaFar -UFMG

Introduction: Inhibition of angiotensin converting enzyme (ACE) is an effective therapeutic target in hypertension. Several natural products are reported as ACE inhibitors *in vitro*, despite this, reports in the literature from studies of ACE inhibitory activity in animal models are scarce. Pharmacological studies have shown that the desired therapeutic activity is more pronounced in crude extracts than in an isolated active component. The vasodilator effect of the ethanolic extract of leaves from Hancornia speciosa Gomes was evaluated in superior mesenteric artery rings and produced a concentration-dependent vasodilatation induced by ACE inhibition (Serra, C. P. 2004). The aim of this study was to evaluate the effects of the crude extract of H. speciosa on cardiovascular parameters in rats with acute hypertension induced by Nω-nitro-L-arginine methyl ester (L-NAME). **Methods:** Leaves of H. speciosa were dried at 400C, reduced to powder, extracted with ethanol by sonication and solvent was removed. The extracts solutions were dissolved in saline solution and dimethylsulfoxide (DMSO) (95:5). All the procedures were approved by the UFOP Ethical Committee under number 2009/11. Male Wistar rats (270 ± 20 g) were divided into 3 groups that previously received L-NAME (60 mg/kg, i.v.) and after 20 minutes received : 1) saline+DMSO solution, 2) 30 mg/kg H. speciosa extract, 3) 100 mg/kg H. speciosa extract. The animals were anesthetized with sodium pentobarbital (60 mg/kg) and had catheters inserted into a femoral artery and vein, in order to obtain arterial pressure (AP) signal and for drugs administration, respectively. Electrodes were inserted subcutaneously for electrocardiogram (ECG) recording (DII). After the i.v. administration of extract or control solution, the signals were obtained for more 30 minutes. **Results:** The systolic AP (SAP) and diastolic AP (DAP) increased 28.8% and 50.8% after the L-NAME administration. Both doses of H. speciosa reduced the AP soon after the administration and this effect was not maintained. The maximum reduction was observed after 10 seconds and was 18.4% and 20.8% for SAP at 30 mg/kg and 100 mg/kg, respectively. For DAP it was observed a maximum reduction of 23.6% and 30.9% at 30 mg/kg and 100 mg/kg. There was no significant changes of heart rate. **Discussion:** These preliminary results indicate that the extract of H. speciosa has hypotensive activity. Considering the short term response observed in this model of hypertension, higher doses or another administration via of the extract and also other in vivo animal models could be evaluated in order to confirm the ACE inhibition activity already shown *in vitro*. References: Serra, C. P. 2004. Estudo de Espécies Vegetais com Potencial Atividade Anti-Hipertensiva: Desenvolvimento e Validação de Ensaio *in vitro* de Inibição da ACE e Avaliação da Atividade Vasodilatadora. PhD Thesis in Pharmaceutical Sciences – UFMG. Supported by: UFOP, FAPEMIG, CNPq.

09.045

Investigation of toxic and antidiarrhoeal activities of ethanol extract of aerial parts from *Xylopia langsdorffiana* A. St. Hil. & Tul. (Annonaceae) in mice. Silva KM[1], Silva ADS[1], Lima LO[1], Clementino-Neto J[1], Silva PCB[2], Medeiros VM[2], Costa VCO[2], Tavares JF[2], Silva MS[2], Cavalcante FA[1] [1]ICBS-UFAL, [2]LTF-UFPB

Introduction: *Xylopia genus* belongs to the Annonaceae family. Several members of this family are known for their edible fruits and medicinal properties. *Xylopia langsdorffiana* is popularly known in Northeast Brazil as "pimenteira da terra" (TAVARES, Z. Naturforsch, 62, 742, 2007). Plants of Xylopia genus are native to Brazil, where they are used popularly as antidiarrheal agent, insecticide, parasiticide, and against snake bite (CORREA, Dicionário de plantas úteis do Brasil e das exóticas cultivadas, p. 315, 1984). Studies show that some diterpenes isolated from *Xylopia* present spasmolytic effect. Thus, we decided to investigate a possible toxic effect and antidiarrhoeal activity of ethanol extract obtained from aerial parts of *X. langsdorffiana* (XL-EtOH) in mice. **Methods:** Pharmacological behavioral screening and determination of LD50: were used mice treated with saline p.o. (10 mL/kg) or XL-EtOH (2500 and 5000 mg/kg p.o. or 1000 and 2000 mg/kg i.p.), and several behavioral parameters were evaluated and quantified the number of deaths (24, 48 and 72h). Castor oil-induced diarrhoea: mice (n=6) were divided into negative control (saline 10 mL/kg), positive control (loperamide 10 mg/kg) and test groups (XL-EtOH). Diarrhoea was induced by oral administration of 0.01 mL castor oil/ animal gram 30 min after the above treatments. During an observation period (4h), the total number of faecal output and number of wet faeces excreted by the animals were recorded. Normal intestinal transit: mice were divided into groups (n=6). Group 1 received saline p.o., group 2 was administered atropine 2 mg/kg p.o. (positive control) and the others groups were treated with XL-EtOH p.o. After 30 min, standard charcoal meal (0.01mL/animal gram) were given to mice orally. Animals were sacrificed 30 min after administration of charcoal meal, the small intestine immediately isolated and determined the distance traveled by the marker. Intestinal fluid accumulation induced by castor oil: mice were divided into groups (n=6). Group 1 received saline p.o. and the others groups were administered XL-EtOH p.o. After 30 min, was administered 2 mL of castor oil/animal. 30 min later, the mice were euthanized, and the fluid volume was measured. All the experimental protocols were approved by Ethical Committee in Research of UFAL (Protocol 010489/2009-15). Results and discussion XL-EtOH did not induce behavioral changes, or induce death of animals showing absence of acute toxicity. XL-EtOH produced a notable antidiarrhoeal activity, when inhibiting significantly ($p < 0.05$), both the frequency of defecation (ED50=180.2 ± 28.4 mg/kg) as well as the wetness of the faecal droppings (ED50=124.5 ± 6.5 mg/kg) in mice. The effect of the extract (250 mg/kg) was similar to that of the standard drug, loperamide, which produced a maximum inhibition of 95%. This effect of the extract may be related to an inhibition of muscle contractility and motility, since XL-EtOH (125 mg/kg) was able to inhibit the intestinal transit by charcoal meal (55.3 ± 3.0%), and inhibit of intestinal fluid content induced by castor oil (ED50=65.5 ± 9.2 mg/kg). Thus, we conclude that the antidiarrhoeal effect of XL-EtOH involves both changes intestinal motility and secretion.

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09.046

Investigation of topical anti-inflammatory activity of *Vochysia bifalcata*. Silva CD[1], Mendes DAGB[1], Soley BS[1], Ferreira BGA[2], Zuffellato-Ribas KC[2], Otuki MF[1], Cabrini DA[1] [1]UFPR − Farmacologia, [2]UFPR − Botânica

Introduction: Many species of Vochysia (Vochysiaceae) are popularly used in South America for the treatment of inflammatory illness. Studies have been done to support its use and show anti-inflammatory and antioxidant activities (Weniger, B. Phytother. Res. 19:75, 2005; Gomes, R.C. *J. Ethnopharmacol*. 121:466, 2009) . The aim of this study was to evaluate the activity of the crude extract of leaves from *V. bifalcata* in a model of skin inflammation in mice. **Methods:** Female Swiss mice (20-30g) were used. Extract activity was valued in the animal models of croton oil or phenol-induced ear oedema. Thus, the increase of the ear thickness (μm) was measured with a digital micrometer (Great MT-045B) before and 6 h and 1 h after application of croton oil (0.4 mg/ear) or phenol (10%), respectively. Phlogistic agents, as well as extract (0.03 to 1 mg/ear) or dexamethasone (0.1 mg/ear), as a positive control, were dissolved in 20 μL of acetone and applied on the inner right ear. After 24 h from application, animals were killed, and ear tissue samples (6 mm) were collected to evaluate the myeloperoxidase (MPO) activity, which indicates the neutrophil migration, or to histological analysis. All animal procedures were approved by the Institutional Ethics Committee (n.390). **Results:** Extract from *V. bifalcata* leaves and dexamethasone inhibited inflammatory parameters in both croton oil and phenol-induced oedema models. Extract reverted croton oil-induced oedema formation in a dose-dependent manner with DI50=0.32 (0.26-0.39) mg/ear and maximum inhibition of 68.8 ± 3% (1.0 mg/ear), as well as MPO activity with DI50= 0.28 (0.18-0.38) mg/ear and inhibition of 60.7 ± 3% (1.0 mg/ear). Dexamethasone used as a positive control inhibited 68.3 ± 3.9% (0.1 mg/ear) and 64.9 ± 2.2% (0.1 mg/ear) of oedema and cell migration, respectively. Histological analysis allowed quantification of leukocyte migration and both extract and dexamethasone were effective reducing cell migration in 34.5 ± 2.5% (1.0 mg/ear) and 52.7 ± 0.3% (0.1 mg/ear), respectively. Extract and dexamethasone also reduced phenol-induced oedema formation in 83.6 ± 10% (0.6 mg/ear) and 78.4 ± 10% (0.1 mg/ear), respectively **Discussion:** These results suggest that *V. bifalcata* is a potential topical anti-inflammatory agent. Since it reverted skin inflammation induced for two different phlogistic agents, it should be considered as a new potential tool for the treatment of skin inflammatory diseases. Further investigation is necessary to elucidate the mechanism and support efficacy and security of this plant. Support: CAPES, CNPq and Fundação Araucária.

09.047

Anti-inflammatory effect of latex proteins (LP) isolated from *calotropis procera* in 5-fluorouracil-induced oral mucositis in hamsters. Freitas APF[1], Almeida RA[2], Cerqueira GS[2], Alencar NMN[2], Brito GAC[3], Ribeiro RA[2], Ramos MV[4], Vale ML[2] [1]UFC − Medicina Clínica, [2]UFC − Fisiologia e Farmacologia, [3]UFC − Morfologia, [4]UFC − Bioquímica

Introduction: Mucositis induced by antineoplastic drugs is an important, dose-limiting, and costly side effect of cancer therapy. Generally, 100% of patients submitted to cancer chemotherapy develop oral mucositis and 30% of the patients with oral mucositis of grade 3 and 4 have to suspend the chemotherapeutics treatment. Calotropis procera (CP) is the laticiferous plant belonging to the Asclepiadaceae family. It is widely distributed in Asia, Africa and South America, and abundant in the Northeast of Brazil. Relevant properties have been detected in protein fraction (LP) isolated from latex of Calotropis procera. The anti-inflammatory activities of the proteic fraction of CP were studied in oral mucositis model, in male Hamsters. **Methods:** This work was submitted to Animal Ethical and Research Committee of the Federal University of Ceará (protocol number 036/10). Oral mucositis was induced by two intraperitoneal (i.p) administrations of 5-FU on the first and second days of the experiment (60 and 40 mg/kg, respectively) in male hamsters. LP was i.p injected 24h before and 24h after mechanical trauma of the cheek pouches. At the day 10, after initial 5-FU injection, the animals were sacrificed and tissues from the cheek pouches were harvested. The anti-inflammatory activity was evaluated by myeloperoxidase (MPO) activity, macroscopical and histopathological analysis of cheek pouche tissue and corporal weight. **Results:** LP significantly inhibited macroscopical and histopathological parameters when compared to non treated group with maximum effect in macroscopic scores reaching 75% for the dose of 5 mg/kg ($p<0,001$) and 66% of maximum effect (5 mg/kg) at the histopathological evaluation ($p<0,001$). The MPO activity was also significantly inhibited by LP in 91% at the same dose ($p<0,001$) and also inhibited the lost weight of 5-FU induced-oral mucositis. **Discussion:** Protein fraction from Calotropis procera latex showed anti-inflammatory effect that was demonstrated by the mascroscopically and histopathological results and also has an inhibitory activity upon neutrophil migration demonstrated by MPO activity. These preliminary data show an important inhibitory effect that should be explored in another pre-clinical assay in order to obtain sufficient results to elaborate a possible clinical trial. **Financial support:** CNPq and CAPES

09.048

Evaluation of antimicrobial activity and preliminary phytochemical profile of *Hyptis suaveolens* L. Poit. Jesus NZT[1], Mota FD[2], Silva Junior IF[3], Tavares JF[4], Batista LM[5]
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Introduction: Bacteria and fungi are microorganisms able to develop adaptive biochemical mechanisms which confer resistance to drugs. This fact has encouraged the search for new substances with mechanisms of action capable of promoting the effective treatment of infections. The objective was to evaluate the preliminary phytochemical profile and assess the antimicrobial activity of ethanol extract of Hyptis suaveolens. **Methods:** Hyptis suaveolens was collected in the Mato Grosso Pantanal (Pirizal District) and were identified by the Institute for the Study of Flora Plantarum. The ethanol extract of aerial parts was obtained by maceration and subsequently concentrated by rotary evaporation. The preliminary phytochemical analysis was performed according to Matos (1988). The antimicrobial activity of H. suaveolens was evaluated by agar diffusion method on Müller-Hinton and Sabouraud broth using disks impregnated with the extract at a dose of 1.000µg/disco. Chloramphenicol 30 mg / disc, fluconazole 25 mg / disk, and amphotericin B were used as standards. For the tests we selected bacteria and yeasts ATCC: *Staphylococcus aureus* 25923, *Enterococcus faecalis* 29212, *Klebsiella pneumoniae* 13833, *Pseudomonas aeruginosa* 27853, *Escherichia coli* 25922, *Candida parapsilosis* 40058, *Candida albicans* 10231, *Candida krusei* 6258 and *Candida glabrata* 90030. The plates were incubated at 37 $_{o}$C and after 18h, the inhibition of growth was performed. **Results and Discussion:** Preliminary phytochemical H. suaveolens revealed the presence of alkaloids, polyphenols, tannins, saponins and coumarins. The extract was active for Candida albicans (10mm), Candida glabrata (10mm) and Candida krusei (13mm). The results showed antifungal activity of Hyptis suaveolens L. Poit has not been demonstrated its antibacterial activity against the strains studied. **Financial support:** FAPEMAT Thanks: Instituto Plantarum for studies of the flora

09.049

Acute toxicity and gastric cytoprotective effect of *Argyrovernonia harleyi* (H. ROB) Macleish in mice. Silva AAR[1], Bezerra MM[3], Aguiar, JA[2], Chaves, HV[4], Ribeiro, KA[5], Pereira, KMA[4], Maia, MBS[6] [1]UFC − Pharmacology Laboratory, [2]UFC − Medicine, [3]UFC − Biotechnology, [4]UFC − Pharmacology Laboratory, [5]UVA − Pharmacology, [6]UFPE − Pharmacology and Toxicology of Bioactive Products

Introduction: *Argyrovernonia harleyi* (Asteraceae) is found in the Xingó region (semi-arid area) in Northeast of Brazil, and recognized by local population as a traditional herb used to manage gastric-related complications The aim of this study was to evaluate both acute toxicity and gastric cytoprotective effect of hydroalcoholic extract (HAE) of the aerial parts (leaves and flowers) from *A. harleyi* **Methods:** The experimental protocols used in this study were approved by the Institutional Animal Care and Use Committee of the Federal University of Ceará (UFC), Fortaleza, Brazil (protocol number 21/09) in accordance with international guidelines (NIH publication No. 85-23, revised 1985). HAE (10 to 2000 mg/kg, i.p. or 50 to 3000 mg/kg, p.o.) was administered to mice (n=10 animal/group) as a single dose. The control group was treated with saline (15mL/kg p.o.) Animals were observed over a 48-h period for toxicity signs and mortality. Gastric ulcers were induced in 24-h fasted mice by indomethacin (40 mg kg, s.c.). Groups of mice (n=7 animals/group) were treated with HAE (100, or 400 mg/kg; p.o.), omeprazole (30 mg/kg; p.o.) or saline 1h before indomethacin, and were euthanatized 6h after ulcerogenic procedure. The stomachs were removed and opened along its greater curvature and the ulcer index was evaluated. The stomachs were histologically processed and microscopically evaluated for gastric damage. The percentual of inhibition was calculated in relation to control group. **Results:** In acute toxicity study, the HAE at the highest oral dose (3000 mg/kg) caused neither death nor any observed adverse symptoms. No toxicity was observed in animals treated with HAE (10, 100 or 500 mg/kg; i.p.). However, intraperitoneal injection of HAE at doses of 1000 mg/kg or 2000 mg/kg caused 20% and 90% of mortality, respectively. The results showed that the comparison of ulcer index averages between control and HAE (100 or 400 mg/kg.) treated groups showed significantly ($P<0.001$) inhibition (71.73 and 76.72%, respectively) of gastric lesions. Microscopic analysis showed significant difference between control [gastric lesion median: 2(1-2); inflammatory cells presence median: 2(1-3)] and HAE (100 mg/kg) [gastric lesion median: 1(0-1); inflammatory cells presence median: 1(0-1)] groups. **Discussion:** The results suggest that the HAE of aerial parts of *A. harleyi* has a wide margin of safety when administered orally, that is too important to its popular traditional use, and showed a significant cytoprotective effect against indomethacin-induced gastric lesions, that confirm its folk utilization. New studies are necessary to elucidate the cytoprotective mechanism, still unclear, considering that exists many routes to protection, as well as increase of blood flow, carbonic anhydrase blockade, prostaglandins synthesis inhibition and others. Mota, JMSC, et al. Dig Dis Sci, v. 52, p. 119, 2007. Olinda, TM, et al. Phytomedicine, v. 15, p. 327, 2008. **Financial support:** Funcap and CNPq.

09.050

(+)-cordiaquinone J triggers both death receptor-dependent apoptosis and necrosis by oxidative stress pathway in leukemia cells. Marinho-Filho JDB[1], Araújo AJ[1], Bezerra DP[2], Montenegro RC[3], Pessoa C[1], Diniz J[3], Viana FA[4], Pessoa ODL[4], Silveira ER[4], Moraes MO[1], Costa-Lotufo LV[1] [1]UFC − Fisiologia e Farmacologia, [2]UFAL, [3]UERN − Química, [4]UFC − Química Orgânica e Inorgânica

Introduction: (+)-Cordiaquinone J is a 1,4-naphthoquinone isolated from the roots of Cordia leucocephala that has antifungal and larvicidal effects. However, the cytotoxic effects of (+)-cordiaquinone J have never being explored. **Methods:** The cytotoxicity of (+)-Cordiaquinone J was tested against HL-60 (leukemia), MDA-MB-435 (melanoma), SF-295 (brain), and HCT-8 (colon) human cancer cell lines by MTT assay. The inhibition of proliferation was also determined by trypan blue dye exclusion assay and DNA synthesis, based on the reduction of BrdU incorporation, using HL-60 as model. To further investigate the mechanisms involved in the cytotoxic activity, the effect of (+)-Cordiaquinone J on the differential morphology were also analyzed through May-Grünwald Giemsa and acridine orange/ethidium bromide (AO/EB) staining of treated cells. DNA fragmentation, cell cycle analysis, phosphatidyl serine externalization, mitochondrial depolarization, caspases activation and inhibition and measurement of ROS were performed by flow cytometry in HL-60 cell line, using doxorubicin and/or β-lapachone as a positive control. **Results and Discussion:** In the present study, the effect of (+)-cordiaquinone J on tumor cells viability was investigated, showing IC50 values in the range of 2.7–6.6 μM in HL-60 and SF-295 cells, respectively. Studies performed in HL-60 leukemia cells indicated that (+)-cordiaquinone J (1.5 and 3.0 μM) reduces cell viability and 5-bromo-2-deoxyuridine incorporation after 24 h of incubation. (+)-Cordiaquinone J showed rapid induction of apoptosis, as indicated by phosphatidylserine externalization, caspase activation, DNA fragmentation, morphologic changes, and rapid induction of necrosis, as indicated by the loss of membrane integrity and morphologic changes. The DNA fragmentation after 3 hours of incubation was inhibited after the use of specific inhibitors of caspases 3 / 7, 8, but was not inhibited after the use of inhibitors of caspase 9. The effects of mitochondrial depolarization and loss of cell membrane integrity was not altered when incubated with inhibitors of caspases. (+)-Cordiaquinone J altered the redox potential of cells by inducing the depletion of reduced GSH intracellular content, the generation of reactive oxygen species and the loss of mitochondrial membrane potential. However, pre-treatment of cells with N-acetyl-lcysteine abolished most of the observed effects related to (+)-cordiaquinone J treatment, including those involving apoptosis and necrosis induction. Supported by: CNPq, CAPES, BNB, CNPq/Neoplasias, IM-INOFAR, FUNCAP, FINEP e InCb.

09.051

Anti-inflammatory activity of anethole obtained from *Foeniculum vulgare* Miller var. *vulgare* Miller essential oil. Domiciano TP[1], Ritter AMV[1], Silva EL[2], Dantas JA[1], Caparroz-Assef SM[3], Cuman RKN[1], Bersani-Amado CA[1] [1]UEM − Farmácia e Farmacologia, [2]UEM − Química, [3]UEM − Inflamação

Introduction: Plants have been used in traditional medicine around the world for hundreds of years. In recent decades, the biological properties of plants have been studied whereas a possibility for using the essential oils and their components for diseases treatment is also evaluated. The wild fennel, Foeniculum vulgare Miller var. vulgare (Miller), Apiaceae, a medicinal and aromatic plant is typical of the Mediterranean region. Its fruit is commonly used as natural medicine for digestive disorders, besides being used for food and flavored liqueurs. The main constituent from fennel essential oil is trans-anethole (ANT) which has carminative, eupeptic, expectorant, anti-spasmodic, antibacterial, antifungal and anti parasital activities. The plant has been also indicated as anti-inflammatory medicine since studies carried out in acute and chronic inflammatory diseases were developed. In this study, we evaluated the effect of anethole on acute inflammatory response by carrageenan-induced pleurisy method. **Methods:** The experimental protocol was approved by the Ethics Committee for Animal Experimentation (CEAE/UEM041/2008). Fasting male Wistar rats received by gavage anethole (ANT: 62.5, 125, 250 and 500 mg /kg), indomethacin (IND: 5 mg/kg) or vehicle (CON: water), 1 hour before carrageenan injection into pleural cavity. Four hours after inflammatory stimuli the pleural inflammatory exudates volume and the number of migrated leukocytes were determined. **Results and Discussion:** A reduction in the volume of pleural exudates was observed after IND (0.35* ± 0.01ml) and ANT treatment: 62.5 mg/kg (0.78 ± 0.07mL); 125 mg/kg (0.59 ± 0.8mL); 250 mg/kg (0.40* ± 0.06mL) and 500 mg/kg (0.40* ± 0.05mL) in a dose-dependent manner ($p<0.0001$) when compared to the control group (0.73 ± 0.04mL). The number of leukocytes migrated into pleural cavity was also reduced after IND (42250x103* ± 3750cells/mm3) and ANT (62.5 mg/kg (56458x103 ± 2586cells/mm3); 125 mg/kg (46833x103 ± 4417cells/mm3); 250 mg/kg (39786x103* ± 4440 cells/mm3) and 500 mg/kg (41643x103* ± 3596cells/mm3) treatments ($p<0.004$) when compared to the control group (66500x103 ± 8183cells/mm3). Data showed that ANT has an anti-inflammatory activity whereas antiedematogenic effect and an inhibitory leukocyte migration were observed. **Financial support:** CNPq, Fundação Araucaria, UEM.

09.052

Evaluation of antimicrobial activity of ethanol extract of the aerial parts of *Nanuza plicata* (Mart.) L. B. Smith & Ayensu. Tenório JAB[1], Mendes JM[1], Falcão HS[1], Lima EO[2], Marculino DMM[1], Tavares JF[1], Batista LM[1], Montes RC[1] [1]UFPB − Ciências Farmacêuticas, [2]UFPB − Micologia

Introduction: Due to the increasing resistance of microorganisms, it is necessary to search for new antimicrobial agents that are more effective and provide security to users, in this perspective the use of medicinal plants has an important role. The plant species *Nanuza plicata* (Mart.) L. B. Smith & Ayensu of the Veloziaceae family is used in folk medicine as anti-inflammatory and tonic, popularly known as “canela d’ema”. Objectives: Evaluate the antimicrobial activity of the ethanol extract of the aerial parts of the *Nanuza plicata* (EEtOH). **Methods:** The antimicrobial activity of the EEtOH was evaluated using the bacteria: *Staphylococcus aureus* (ATCC-6538), *Staphylococcus epidermidis* (ATCC-12228), *Escherichia coli* (ATCC-11105, ATCC-18739, ATCC-10536), Samonella spp. (LM-08), *Shigella enterocolitica* (ATCC-6017), Shigella flexineri (MM-412), *Shigella sonnei* (LM-07) Yersinia enterocolitica (ATCC-9610) and Listeria monocytogenes (ATCC-7664) and fungal species: *Candida albicans* (ATCC - 76485, LM – 16-F, LM – 42V, LM – 958, LM -18 F), *Candida tropicalis* (ATCC - 13803, LM – 708, LM – 15V), *Candida krusei* (LM - 08, LM – 12 V) e *Candida guilliermondii* (LM – V 70, LM - 01). The microdiluition technique was used to determine the minimum inhibitory concentration (MANN, j. app. microbiol., 84, 538, 1998) doses were investigated at concentrations 10, 5, 2.5, 1.25, 0.625 and 0.32 mg/mL. **Results and Discussion:** The EEtOH (10 mg/mL) inhibited the growth of all bacterial strains and all fungal strains investigated corresponding inhibition rate 100%.At the concentration 5 mg/mL, the EEtOH inhibited the growth of Salmonella spp (LM 08) and *Yersinia enterocolitica* (ATCC 9610) strains corresponding 18,2% of the bacterial strains tested and inhibited the growth of all fungal strains except C.albicans (LM-18F) corresponding 96,7% of the yeasts tested. The EEtOH (2.5 mg/mL) inhibited the growth of *C. albicans* (ATCC-76485, LM-18F) strains corresponding 16,7% of the yeasts tested. In the others concentrations the EEtOH did not inhibit the growth of microorganisms. Conclusion: The results suggest that ethanol extract of the aerial parts of the *Nanuza plicata* has antibacterial activity and antifungal activity in different doses, the better dose was 10 mg/mL showing activity for both microorganisms. **Financial support:** CNPq. **Acknowledgments**: LTF-UFPB.

09.053

Evaluation of the effects of jatobá juice concentrating on the glucemic control, lipid profile and liver function of diabetic rats. Almeida IP[1], Damasceno DCF[1], Sales ALCC[2], Teixeira JMR[1], Soares LFM[1], Santos Júnior JC[1], Amorim VR[3], Silva AFS[3], Assis RC[3], Martins MCC[1] [1]UFPI − Biophysics and Physiology, [2]UFPI − Nutrition, [3]UFPI − Biochemistry and Pharmacology

Introduction: Jatoba (*Hymenaea courbaril* L.) is a characteristic legume of Brazilian "cerrado" that contains a high level of fibers. Moreover, the astilbina founds on the jatoba may have antioxidant properties and protective effect of the hepatic function. This study evaluated the effects of the jatoba juice concentrating administration on the glucemic control, lipid profile and hepatic function of diabetic rats. **Methods:** Five days after induction of diabetes by streptozotocin (Sigma Chemical, USA, 40 mg/kg intravenously, citrate buffer pH=4.5), male Wistar rats (230 – 270 g) received daily, for 50 days: standard rat chow (Labina, Purina) and jatoba juice concentrating 10% 0,5 mL/100g (J; n=8); standard rat chow (D; n=6); or standard rat chow plus subcutaneous insulin injection of 3 U/animal (I; n=8) on alternate days. Animals were considered diabetic when their fasting blood glucose was higher than 250 mg/dL. Levels of glycemia, aspartate aminotransferase (AST), alanine aminotransferase (ALT), albumin (ALB), total proteins (PT), triglycerides (TG), total cholesterol (CT) and HDL-cholesterol (HDL) were measured by colorimetric method. Comparison between the groups was performed through analysis of variance and Tukey test. The study was approved by the Committee of Ethics and Research of NOVAFAPI (018/09). **Results:** No significant differences were shown between the groups in the following parameters: initial body weight (D: 234.0 ± 5.80; J: 231.31 ± 4.02; I: 231.8 ± 3.63); food intake (C: 30.09 ± 1.15; J: 29.56 ± 2.33; I: 35.52 ± 2.18 g/day); ALB (D: 3.48 ± 0.1; J: 3.18 ± 0.1; I: 3.63 ± 0.39); ALT (C: 254.6 ± 40.95; J: 181.5 ± 20.18; I:194.0 ± 33.65); AST (D:165.3 ± 28.2; J:165.6 ± 15.75; I:212.2 ± 52.27); PT (C:5.617.0 ± 0.26; J:6.01 ± 0.2; I:5.64 ± 0.2). The J group in comparison with I and D groups presented levels significantly lower ($p< 0.05$) in this parameters: CT (D: 61 ± 3.78; J: 44.5 ± 5.12; I: 70.5 ± 2.67); TG (D: 91.0 ± 7.93; J: 36.2 ± 7.5; I: 98.83 ± 15.97); and HDL (D: 22.67 ± 1.02; J: 31.75 ± 2.02; I: 30.79 ± 1.7). The HDL levels were significantly higher ($p<0.05$) in the groups treated with insulin in relation with the D group. The final body weight was significantly lower ($p<0.05$) in J group comparing with D and I groups (D: 185.83 ± 10.11; J: 158.1 ± 9.01; I: 213.93 ± 10.43). In the J group was a significantly reduction in the differences of initial and final glucemic levels (261.75 ± 52.95 versus 351 ± 16.91), but this wasn't observed in the other groups. Discussion The treatment with jatoba juice concentrating showed that the hypoglycemic and hypolipidemic effects on diabetics animals. However, the reductions observed on the serum levels of lipids were considered excessive, especially in HDL levels. There weren't observe alterations in the hepatic function. Additional studies are underway to evaluate possible hepatic histological changes indicative of hepatic toxicity of jatoba. **Financial support:** UFPI Acknowledgments: CENDOMED Laboratory

09.054

Inhibitory effects of ginger (*Zingiber officinale* Roscoe) essential oil on *in vivo* and *in vitro* leukocytes migration. Grespan R, Nogueira de Melo GA, Fonseca JP, Farinha TO, Dantas JA, Miranda CR, Bersani-Amado CA, Cuman RKN UEM − Pharmacy and Pharmacology

Introduction: The *Zingiber officinale* Roscoe, popularly known as ginger, has been used for pain, inflammation, arthritis, urinary infections and gastrointestinal disorders. In our laboratory, we have demonstrated the anti-inflammatory and antinociceptive effects of ginger essential oil (GEO) in animal models. However, there is not study demonstrating the GEO effect on neutrophil chemotaxis. This way, we evaluated the effect of GEO on the *in vitro* and in vivo leukocytes recruitment. **Methods:** The GEO was extracted from distillation in Clevenger apparatus. Intravital microscopy was used to evaluate the rolling, adhesion, and migration of leukocytes in the microcirculation of spermatic fascia in situ pretreated or not with GEO. To evaluate the effect of GEO on leukocytes chemotaxis, neutrophils were isolated from the peritoneal cavity of mice, 4 hours after injection with zymosan (1mg/cavity, i.p). The cells (1x106 cells /ml) were pretreated or not with GEO (10-4, 10-3 or 10-2 µl/ml) for 30 min and stimulated with casein (50 mg/ml) or RPMI 1640 medium alone in the Boyden chamber. The cells were incubated at 37 °C, 5% CO2 for 1h and membrane was stained using the Instant Prov (Newprove). Leukocytes were counted and the results were expressed as the number of leukocytes per field. Data were presented as mean ± SEM of 3 separate experiments. The means from different treatments were compared by ANOVA with Tukey's correction ($p \leq 0.05$). The experimental protocol (number 041/2008) regarding this study was approved by the ethical commission of ethics in animal research (CEAE/State University of Maringá). **Results and Discussion:** Oral administration of the GEO (200–500 mg/kg) reduced the rolling and leukocyte adherence after 2 hours of carrageenan injection (100 mg) into scrotal chamber. The number of leukocytes migrated to the perivascular tissue 4 hours after the irritant stimulus was also diminished. The pretreatment with GEO in all tested doses (10-4, 10-3 or 10-2 µL/ml) inhibited casein-induced leukocytes migration (35.89 ± 4.33, 30.67 ± 0.7 and 35.85 ± 3.83, respectively) when compared with leukocytes stimulated with casein without pretreatment (72 ± 2.24). Our study provides evidence that GEO at tested doses inhibits *in vitro* and in vivo leukocytes migration, suggesting that GEO can be seen as a potential therapeutic immunointervention in the inflammatory diseases. Supported by: CNPq; CAPES.

09.055

Hypoglycemic effect of *Terminalia catappa* Linn. in alloxan-induced diabetic rats. Ferreira AKB[1], Costa DL[2], Tenório EP[1], Oliveira DA[1], Santana AEG[2], Humberto MMS[2], Grillo LAM[1], Ribeiro EAN[1] [1]ESENFAR-UFAL, [2]IQB-UFAL

Introduction: *Terminalia catappa* L. originating in Malaysia is a tree of tropical coast regions (1) found in the Brazilian coast including in the state of Alagoas. In Brazil it is used in folk medicine to treat respiratory diseases, to fight dysentery and diarrhea (2). A pharmacological evaluation showed antidiabetic activity of aqueous extracts of leaves (3). This study was aimed to investigate the hypoglycemic effect of ethanol extract from the stem bark of *Terminalia catappa* (TCEE). **Methods:** The Diabetes was induced with alloxan (40 mg/kg). Diabetes was identified by measuring fasting plasma glucose levels 7 days after alloxan injection. Animals with plasma glucose levels > 190 mg/dL were selected and used for this study. The male Wistar rats were randomly divided in the four (n = 5 rats per group): Group I (non-diabetic control), group II (untreated diabetic control), group III (diabetic control treated with insulin, 4UI s. c.) and Group IV (diabetic treated with TCEE, 200 mg / kg orally for daily for 15 days). The study was approved by the Ethics Committee of the Federal University of Alagoas (010151/2008-82). **Results:** Group I showed blood glucose values of 89.2 ± 5.4 which did not change during the protocol. The treatment of group IV caused a significant drop in blood glucose (335.7 ± 49.5 and 157.8 ± 35.9 ** mg / dL, respectively) compared with group II (401.3 ± 57.60 and 554 ± 6.93 * mg / dL). Insulin was used as reference and showed significant hypoglycemic effect (479.7 ± 17.0 and 203.0 ± 9.8 *** mg / dL, respectively). Significance being p, then * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Discussion: The findings showed that the TCEE has beneficial effects, in reducing the elevated blood glucose level of alloxan-induced-diabetic rats. **Financial support:** CNPq and PPSUS-MS. **Acknowledgements:** FAPEAL, CNPq, UFAL, PPSUS-MS. **References:**

1. Fan, Y. M. et al., Fitoterapia, v. 75, p. 253, 2004. 2. Cruz, G. L. Dicionário das plantas úteis do Brasil, 2ª ed. p. 48, 1982. 3. Ahmed, S. M. et al., Iranian J Pharmacol & Therapeutics, v. 4, p 36, 2005.

09.056

Fraction from *Calotropis procera* Latex shows anti-inflammatory effects on the pathogenesis of irinotecan-induced intestinal mucositis in mice. Alverne SM[1], Bitencourt FS[1], Figueiredo JG[2], Luz PB[1], Lima-Júnior RCP[1], Ramos MV[3], Cunha FQ[4], Ribeiro RA[1], Alencar NMN[1] [1]UFC − Fisiologia e Farmacologia, [2]UFC − Bioquímica e Biologia Molecular, [3]UFC − Bioquímica, [4]FMRP-USP

Introduction: The intestinal mucositis (IM) is a side effect of irinotecan (CPT-11), used to treat colorectal cancer. There is an incidence of IM associated with severe diarrhea in up to 25% of patients. However, the clinical management of these side effects is still partly ineffective. *Calotropis procera* (CP) is a plant found in Africa, Asia and South America, being so abundant in the Northeast of Brazil and has made an antidiarrheal potential in animal models. Thus, we aimed to evaluate the anti-inflammatory effects of a non-dialyzed proteic fraction of latex from CP in CPT-11-induced IM. **Methods:** Animal handling and experimental protocols were registered on the Institutional Ethics Committee under number 24/09. Swiss mice (n = 10, 23 ± 2g) were treated for 4 days with saline (Sal, 5 ml/kg, i.p.) or CPT-11 (75 mg/kg, i.p.). In other experimental groups, CP (5, 10 and 20 mg/kg/day, i.v.) was administered for 6 days, 30 min before the CPT-11. On the 7th day, we evaluated the diarrhea and total leukocyte count (x103/mL). After sacrifice, the duodenum was collected for measurement of myeloperoxidase activity (MPO) (neutrophils/mg tissue). Regarding statistics we used ANOVA/Bonferroni's test or Kruskal-Wallis/Dunn. $P<0.05$ was accepted. **Results and Discussion:** Latex from CP did not change ($P>0.05$) leukopenia induced by CPT-11 at doses tested (5: 3232 ± 134.0; 10: 3273 ± 123.6 20: 3222 ± 141.2, respectively) vs group that received only CPT-11 (2900 ± 83.8; Sal: 4971 ± 237.8). Latex from CP decreased significantly ($P<0.05$) scores for diarrhea and MPO levels only at 5 mg/kg (diarrhea: 1 [0-2]; MPO: 1.29 ± 0.54) when compared with CPT11 (diarrhea: 3 [2-3]; MPO: 7.06 ± 1.5, respectively). These findings demonstrate anti-inflammatory activity of latex from Calotropis procera in the model of IM induced by CPT-11. New approaches are being undertaken to elucidate the possible mechanism of action involved. **Acknowledgements:** CNPq and CAPES.

09.057

Ability of fucosylated chondroitin sulfate to inhibit muscle damage induced by *Bothrops jararacussu* crude snake venom. Machado MM[1], Strauch MA[1], Tomaz MA[1], Cons BL[1], Branco AMC[1], Martins VV[1], Mourão PAS[2], Melo PA[1] [1]UFRJ − Farmacologia Básica e Clínica, [2]UFRJ − Bioquímica Médica

Introduction: Snakebites by *Bothrops jararacussu* snake induces intense local tissue damage. The venom contains a complex mixture of enzymes and small peptides. Phospholipases A2 are enzymes present in the venom which are responsible for a wide range of activities, such as myotoxicity, oedema, anticoagulant, hemolytic, neurotoxic and cardiotoxic effects (Kini, Toxicon 45, p.1147, 2005). Some polyanions have been shown to present antivenom properties against this venom (Melo et al., Toxicon 31, p.285, 1993). A new natural polyanion polysaccharide, named Fucosylated Chondroitin Sulfate (FucCS), has been isolated from the body wall of the sea cucumber Ludwigothurea grisea, and it is involved in many biological activities (Borsig et al., JBC 282 (20), p.14984, 2007). We assessed the ability of FucCS to antagonize the muscle damage induced by *B. jararacussu* crude venom. **Methods:** *In vitro* CK assays were performed with isolated mouse extensor digitorium longus muscle bathed with venom alone (25 µg/mL) or incubated with FucCS (1-50 µg/mL). In vivo experiments were performed by i.m. venom injection alone or preincubated with FucCS and the plasma CK activity was evaluated before and 2 hours after injection (1 mg/kg). We also studied the effects of pre- and posttreatment with FucCS (10 mg/kg). The proteolytic, phospholipase, collagenase and hyaluronidase activities were measured using turbidimetric and colorimetric **Methods:** Histological sections were performed in EDL muscle after crude venom perimuscular injection. All experiments were approved by the Committee of Animal Use of the Rio de Janeiro Federal University (DFBCICB 026). **Results:** *In vitro* myotoxicity was completely neutralized by FucCS (30 and 50 µg/mL). It was observed that FucCS inhibits 75% of proteolytic venom activity and phospholipase venom with IC50 = 10 mg/mL, in concentration-dependent manner. Incubation of FucCS with the venom eliminates the increase of plasma CK, in vivo, but pre and post-treatment were ineffective. The oedema was reduced in presence of FucCS (1 mg/kg) and the muscle EDL was preserved (1 and 10 mg/kg). **Discussion:** FucCS was capable to inhibit venom activities related to tissue damage. Although the plasma CK levels did not reduce in the pre- and post-treatment (actually these values were raised) we believe that this occurred because of the stasis caused by the venom, besides slow CK washout from plasma. These results indicate that FucCS presents activity against *Bothrops jararacussu* venom and we believe that this antivenom activity may be due to the interaction of FucCS with positively charges toxins present in this snake venom, like others polyanions. Our study suggests that Fucosylate Chondroitin Sulfate is a new inhibitor of myotoxicity induced by *B. jararacussu* crude venom. **Financial Support:** CNPq, CAPES, FAPERJ and PRONEX.

09.058

Evaluation of the toxicity of the ethanol extract of aerial parts of *Nanuza plicata* (Mart.) L. B. Smith & Ayensu.Tenório JAB[1], Falcão HS[1], Viana WP[2], Dias GEN[1], Batista LM[1], Diniz MFFM[1], Tavares JF[1] [1]LTF-UFPB − Ciências Farmacêuticas, [2]UFPB − Ciências da Saúde

Introduction: The indiscriminate use of the medicinal plants can lead to serious health problems for people, is needed to determine its efficacy, safety and quality. Thus, we choose the plant species *Nanuza plicata* (Mart.) L. B. Smith & Ayensu (Veloziaceae), it is popularly known as "canela d'ema", and used in folk medicine like infusion from leaves for the treatment of inflammation. **Objectives**: Evaluation of the acute oral toxicity and determination of the LC50 and LD50 of the EEtOH. **Methods:** The acute oral toxicity study was performed in mice, a single dose of 2000 mg/kg of EEtOH was orally administered to male and female groups (n=10), after 6 h fast. Animals receiving the vehicle (Tween 80 9%) served as control. After treatment, the behavior parameters were observed, during 30, 60, 90, 120, 180 and 240 minutes in the first day and once daily in the following 13 days. At the end of the period, the number of survivors was recorded for the determination of the LD50. The organs weight, food and water consumption were evaluated in both sexes (ALMEIDA et al., Rev. Bras. Farmacog., v.80, 72-76, 1999). To brine shrimp (A. salina) lethality test, the EEtOH was diluted in vehicle and then it was added 5 mL of different concentrations in tubes containing 13 to 15 nauplii. The set was incubated at artificial light for 24 h and then the survivors larvae were counted to determine the LC50 (PARRA et al., Phytomed. V.8, 395-400, 2001). All results were statistically analyzed with the software GraphPad Prism 4. This protocol was approved, having the license number 707/06. **Results:** The brine shrimp assay was performed three times and the LC50 of these three tests were higher than 1000 µg/mL (Data not shown). In the acute oral toxicity assay, the EEtOH induced irritability in the first, second and third hours after oral administration in the mice. During the 14 days of observation no deaths were showed. The results of water consumption were (mL ± SD): Control Female (CF) (76.0 ± 3.3), EEtOH Female (EF) (87.6 ± 4.7); Control Male (CM) (67.0 ± 1.8), EEtOH male (EM) (62.5 ± 1.4). The results of food consumption were (g ± SD): CF (85.4 ± 7.7), EF (78.6 ± 3.9); CM (76.0 ± 3.3), EM (87.6 ± 4.7). The results of organs weight were (g ± SD): Spleen, CF (0.380 ± 0.008), EF (0.380 ± 0.015); CM (0.255 ± 0.024), EM (0.151 ± 0.008); Heart, CF (0.137 ± 0.003), EF (0.129 ± 0.004); CM (0.156 ± 0.006), EM (0.158 ± 0.014); Liver, CF (1.77 ± 0.09), EF (1.59 ± 0.03); CM (1.92 ± 0.1), EM (1.82 ± 0.04); Kidney, CF (0.174 ± 0.012), EF (0.177 ± 0.011); CM (0.493 ± 0.040), EM (0.435 ± 0.025). Conclusion: In the present work, EEtOH did not induce changes in the organs weight, water and food consumption of the mice of both sexes when compared with controls. The LC50>1000 µg/mL and LD50 > 2000 mg/kg showed low toxicity in A. salina and mice. But, in the organs macroscopic analysis, black spots were observed with blackened areas across the surface of the liver and spleen. Liver cyst formation has been identified which displayed gelatinous contents. Furthermore, all organs of the animals showed significant weakness compared to the control group. All these observed parameters were potentiated in female mice. This toxicity may be related to the formation of a toxic metabolite being necessary more extensive toxicological studies for the determination.
Financial support: CNPq-LTF-UFPB.

09.059

Fish oil supplementation on motor and cognitive side effects of typical antipsychotics in psychiatric patients. Bürger ME[1], Cardoso PM[2], Reckziegel P[2], Pase CS[2], Emanuelli, T[3], Santos DB[4], Cunha A[5], Rocha JBT[4] [1]UFSM − Farmacologia, [2]UFSM − Fisiologia e Farmacologia, [3]UFSM − Tecnologia e Ciência dos Alimentos, [4]UFSM − Química, [5]UFSM − Neuropsiquiatria

Introduction: Typical antipsychotics are related to oxidative stress and brain damages (Sivrioglu et al., Prog NeuroPsychopharmacol Biol Psych 31, 1493-2007), leading to extrapyramidal symptoms (EPS) and cognitive dysfunction development (Bressan et al., Schizophr Res 56, 31-2002; Saeedi et al., Schizophr Res 85, 222-2006). EPS are manifested by minor changes such as tremors, dystonias, acathisia and parkinsonism, or may even reach a more severe form known by tardive dyskinesia (TD), observed through disabling repetitive involuntary movements. This search was authorized by the commission of search ethical (CONEP) under number 243. **Methods:** We evaluated the effects of fish oil (FO) supplementation in psychiatric patients with movement disorders and on ongoing typical neuroleptic treatment. After baseline EPS evaluation (by AIMS-Abnormal Involuntary Movement Scale), global cognitive function (by MMSE-Mini-Mental State Examination), DNA damage and blood collection, the subjects were supplemented either with FO (3g of n-3 FAs) or placebo. FO safety were monitored through evaluations of lipidemias and fasting glucose, as well as prothrombin time (PT) and partial thromboplastin time (PTT). Assessments were done at baseline and 4, 8, and 12 weeks thereafter. **Results:** After 3 months of supplementation, the EPS were reduced and the cognitive function was not changed by FO intake in relation to the baseline. FO caused no difference in lipidemias either; however, fasting glucose was reduced in all evaluations and the PT increased in the last week. DNA damage in leukocytes was not changed by FO. **Discussion:** Our results showed the benefits of FO supplementation on EPS in patients neuroleptic-treated and the low risk of side effects reinforces this potential therapeutic, but the PT should be monitored during chronic treatment with FO. **Financial support:** “Enxoval program” (Pró Reitoria de Pós Graduação e Pesquisa (PRPGP)-UFSM); FIPE (UFSM); PROAP (PRPGP, UFSM). Nº comitê de ética em pesquisa (CONEP/MS) nº processo 23081.015702/2006-34 Certificado de Apresentação para Apreciação ética (CAAE): 0128.0.243.000-06

09.060

Anti-inflammatory activity of the hydroalcoholic extract and fractions from *Gochnatia polymorpha ssp floccosa* in mouse air pouch model. Piornedo RR[1], Kassuya CAL[2], Zampronio AR[1], Stefanello MEA[3], Strapasson RLB[3] [1]UFPR − Farmacologia, [2]UFGD − Ciências da Saúde, [3]UFPR − Química

Introduction: *Gochnatia polymorpha* (Asteraceae) is known as “cambará” and is used in folk medicine against respiratory diseases such as asthma. The aim of this study was to investigate the possible anti-inflammatory activity of the hydroethanolic extract (HE), dichloromethane fraction (DCM) and butanolic fraction (BT) in mouse air pouch model. **Methods:** The dried trunk bark of *G. polymorpha* was grounded and extracted with hexane and ethanol, successively. The crude ethanol extract was dissolved in ethanol-water 1:1 and submitted to extraction with dichloromethane, ethyl acetate and butanol, sequentially. The crude extract and butanolic fraction were analyzed by NMR 1H. Mice were injected with 4 ml of sterile air on day 0, a second injection of 2 ml was performed on day 3. On day 6, animals received the oral treatment with extract (30, 100 and 300 mg/kg), BT (20 mg/kg) or DCM (50 mg/kg) fractions, vehicle or dexamethasone (0.5 mg/kg, i.p.). After one hour carrageenan (0.25 ml of a 0.1% solution) was injected into the pouch to promote an inflammatory response. Four-hours after carrageenan stimulus, pouches were washed with 2 ml of heparinized (10 UI/ml) PBS. Leukocytes that migrated were counted on a Neubauer chamber. Lavage was centrifuged and protein levels were evaluated in the supernatants, as plasmatic extravasation indicative. All procedures were approved by the Institutional Ethics Committee (CEEA number 336). **Results and Discussion:** The oral administration of HE (30, 100 and 300 mg/kg) inhibited the carrageenan-induced leukocyte migration into the air pouch (37.2 ± 12.5%, 62.6 ± 5.0% and 54.3 ± 6.8%, respectively) as well as protein extravasation (47.9 ± 12.5%, 51.7 ± 15.2% and 60.9 ± 13.7%, respectively) compared to vehicle. DCM (50 mg/kg), but not BT fraction (20 mg/kg), inhibited leukocyte infiltration into the pouch (29.5 ± 10.6%). However, both DCM and BT decreased significantly the protein levels in the supernatants (52.4 ± 15.0, and 58.2 ± 11.2%, respectively). Dexamethasone inhibited both leukocyte infiltration (76.7 ± 5.5%) and protein extravasation (63.6 ± 12.9). Phytochemical studies from DCM fraction indicated the presence of 11, 13-dihydrozaluzanin C. Conclusion: The oral administration of HE, BT and DCM obtained from *G. polymorpha* exhibited anti-inflammatory activity probably due to different active compounds since HE and DCM fraction reduced cell migration and protein extravasation while BT reduced only protein extravasation. **Acknowledgements:** CNPq and CAPES.

09.061

In vitro chlorogenic acid inhibits adhesion molecules expression and inflammatory mediators secretion in neutrophils. Bolonheis SM[1], Hebeda CB[1], Belinati KD[1], Lopes NP[2], Farsky S[1] [1]FCF-USP − Análises Clínicas e Toxicológicas, [2]FCFRP−USP − Física e Química

Introduction: *Solidago chilensis* Meyen (*Solidago chilensis*; SC) is a medicinal herb, popularly used in the south of Brazil due to its anti-inflammatory, antimicrobial, antineoplasic, analgesic and antipyretic effects (Bader et al., Pharmazie, v.55, p. 72, 2000; Vila et al., Planta Medica, v. 68, p. 164, 2002; Chaturvedula et al., Bioorg. Med. Chem., v.12, p.6271, 2004; and Apati et al., J. Pharm. Pharmacol., v.58, p. 251; 2006). We have previously demonstrated that systemic and topic administration of the hydro alcoholic extract of the aerial parts (stems, leaves, and inflorescences) of SC in male Wistar rats, markedly reduced leukocyte recruitment into the inflammatory focus (Tamura et al., J. *Ethnopharmacology*, v.122, p.478, 2009). Chlorogenic acid (CGA) present in SC may be responsible for these anti-inflammatory actions (Krakauer, *Immunopharmacol*. *Immunotoxicol*., v.24, p. 113, 2002). Therefore, here we investigated the effects of CGA on adhesion molecules expression and inflammatory mediators secretion by neutrophils, important cells in the innate inflammatory response. **Methods:** Neutrophils were obtained from male Wistar rats 4 h after intraperitoneal injection of oyster glycogen (1%, 10ml). Cells were incubated with culture medium (RPMI 1640 with 10% FBS; control) or with CGA (25; 50; 100 or 1000 mM; dissolved in the culture medium), during 1 or 18 h in the absence or presence of LPS (5 mg/ml). Expressions of the adhesion molecules L-selectin and b2integrin were quantified by flow cytometry. Levels of nitrite and cytokines were quantified in the supernatant of cells, by Griess reaction and enzyme linked immunosorbent assay (ELISA), respectively. The experiments were conducted according to the Ethics Committee in Animal Experiments n.53/2008 – Number Protocol – 276. **Results:** CGA, which did not present cytotoxicity at concentrations here used, per se impaired the b2integrin expression (1000uM = 26% of reduction). In presence of LPS, CGA inhibited the L-selectin cleavage (around 25%); reduced the expression of b2integrin (1000uM = 83%) and NO secretion (1000uM = 32%); inhibited IL-1b production (50uM = 27%) and did not modify TNF-a levels in the supernatant. **Discussion:** Based on these findings, our data suggest that CGA exerts its anti-inflammatory effects modulating adhesion molecules expression and secretion of inflammatory mediators, which are important for the control of leukocyte recruitment into the inflammatory focus. **Financial support:** Capes.

09.062

Topical effect of crude hydroalcoholic extract from *Psychotria nuda* (Cham. & Schltdl.) Wawra leaves in skin inflammation model. Mendes DAGB[1], Soley BS[1], Ferreira BGA[2], Zuffellato-Ribas KC[2], Otuki MF[1], Cabrini DA[1] [1]UFPR − Farmacologia, [2]UFPR − Botânica

Introduction: Psychotria is the largest genus of Rubiaceae family, predominantly in tropical areas, and its species are rich sources of nectar and fruit for animals. There are no reports in literature about the biological activity of the specie Psychotria nuda (Cham. & Schltdl.) Wawra. Thus, the aim of this study was to evaluate the anti-inflammatory activity of crude hydroalcoholic extract from leaves of P. nuda (EHP). **Methods:** Female Swiss mice (25-30 g) were used for experimental procedure (n = 5-10). Acute inflammatory process was induced by single topical application of croton oil (0.4 mg/ear). Mice were topically treated with EHP (0.03, 0.1, 0.3, 0.6 and 1 mg/ear) or dexamethasone (0.05 mg/ear). The chronic inflammatory process was induced by multiple applications of croton oil (0.4 mg/ear) for 9 days on alternate days. Topical treatment with EHP (1 mg/ear) or dexamethasone (0.05 mg/ear) started on the 5th day of experiment. Edema was measured by the increasing of ear thickness, measured 6 h after croton oil application in the acute model and daily in chronic model. Samples of ear tissue (6 mm of diameter) from acute and chronic model were collected, weighed and analyzed using the following parameters: histology, myeloperoxidase (MPO) and N-acetyl-β-D-glucosaminidase (NAG) enzymatic activity. Procedures have been approved by Institutional Ethics Committee under the number nº 390/UFPR. **Results:** In the acute model, single application of croton oil induces maximum formation of ear edema after 6 h and MPO activity after 24 h. Control group show increased of ear thickness (0.127 ± 0.01 mm), MPO activity (278 ± 40 mDO/biopsy) and in the number of inflammatory cells (48.5 ± 1.4 cells/field). EHP dose dependent reduced the edema formation and MPO activity with an ED50 of 0.47 (0.40 - 0.55 mg/ear) and 0.13 (0.09 - 0.18 mg/ear), respectively. Highest tested dose (1 mg/ear) inhibited edema formation in 71.6 ± 1.3%, MPO activity in 73.4 ± 0.7% and inflammatory cells in 37 ± 2.1%, when compared to control. Dexamethasone inhibited edema formation in 72.7 ± 3.4%, MPO activity in 65 ± 2.2% and number of inflammatory cells in 52.7%, when compared to control. In chronic model, multiple applications of croton oil induced formation of ear edema that started at 6 h after the first dose and increased within days of treatment. In the last day (9th) control group show increased in ear thickness (0.180 ± 0.02 mm), in ear weight (19.15 ± 0.7 mg), in MPO activity (613 ± 131 mDO/biopsy) and in NAG activity (125 ± 7.6 mDO/biopsy) when compared to naive group. EHP (1 mg/ear) reduced edema formation in 67 ± 7.3%, ear weight in 25%, MPO activity in 79 ± 4.0%, but was not able to reduced NAG activity, when compared to control. Dexamethasone inhibited edema formation in 98.3 ± 1.7%, ear weight in 39.7%, MPO activity in 97.3% and NAG activity in 29.8 ± 3.5%, when compared to control. **Discussion:** Results indicate that EHP showed an interesting topical anti-inflammatory activity, reduction edema and leukocytes migration, in acute and chronic skin inflammation in mice. Still, other inflammatory parameters will be evaluated in order to investigate the possible mechanism of action, as well as the phytochemical analysis. Support: REUNI, CAPES and CNPq.

09.063

Ethanol crude extract of *Erythroxylum caatingae* induces relaxant effect in the guinea-pig trachea. Santos HAS[1], Oliveira SL[2], Tavares JF[2], Ribeiro LAA[3], Lima JT[3] [1]UNIVASF − Medicina, [2]UFPB − Tecnologia Farmacêutica, [3]UNIVASF − Ciências Farmacêuticas

Introduction: the smooth muscle has central role in the control and regulation of several physiologic functions in many body organs, such as control of vascular tonus, gastrointestinal motility, airway air flow, uterine tonus and others. Given the physiologic importance of that muscle, a drug with potential in interfere with the mechanisms that lead to contraction or relaxation of smooth muscles could be very useful against several pathophysiological processes that involves contraction of that muscle. The Erythroxylum coca is the one most studied species in the Erythroxylum family and has a notorious importance in the biological and chemical fields. However, so far, a few pharmacological studies were realized about others species of the Erythroxylaceae family such as *E. caatingae*. The aim of this work was to evaluate the relaxant effect of the ethanol crude extract obtained from *E. caatingae* (Ec-EtOH) in isolated guinea-pig trachea. **Methods:** the guinea-pigs were euthanized by cervical dislocation, in accordance of the proceedings approved by Ethics Committee on Animal Research of UNIVASF (protocol 023240408), the chest was opened and dissected and the trachea was removed and cleaned out. The organ was segmented into rings and suspended individually by stainless steel rods in the organ bath chambers (10 mL), containing Krebs solution at 37° C bubbled with carbogen gas mixture, under a pre-load tension of 1 gF. The contractions and relaxations of the tracheal rings were recorded through the force transducers coupled to a digital data acquisition system. The muscarinic agonist carbachol (10-6 M) was used to evoke tonic contractions of tracheal rings. Were used tracheal rings with and without epithelium. The presence of epithelium was confirmed when the rings reached relaxations above 50% in response to addition of arachidonic acid (AA) while the rings without epithelium presented no relaxation in response to AA. **Results:** the Ec-EtOH (3-750 ug/mL) relaxed the trachea in concentration-dependent manner in the presence (EC50=30.8 ± 2.4 ug/mL) and absence (EC50=10.0 ± 1.6 ug/mL) of functional epithelium with maximal effect (Emax=100%) attained at 500 ug/mL in both cases (n=3). The Ec-EtOH also relaxed the guinea-pig trachea in its basal tone (pre-load tension of 1gF) in the concentration-dependent manner presented a EC50 of 162.3 ± 23.5 ug/mL and Emax=100% attained at 500 ug/mL (n=3). That effect was compared to those obtained by isoprenaline (10-6 M) as control. **Discussion:** the results show that the species contains special metabolites responsible for the observed pharmacological effects in the isolated guinea-pig trachea. The difference in the EC50 values presented by Ec-EtOH, in the presence and absence of epithelium, suggests a possible modulation of Ec-EtOH relaxant effect by epithelium-derived factors, such as prostanoids. **Financial support:** CNPq, CAPES and UNIVASF.

09.064

Effect of LED treatment on muscular edema and mionecrose induced by *Bothrops jararaca* venom. Bulgarelli[1], Barbosa AM[2], Lima CJ[3], Zamuner SR[4] [1]UNIVAP − Fisiologia e Inflamação, [2]UNIVAP − Pesquisa e Desenvolvimento, [3]UNICASTELO − Instrumentação Optobiomédica, [4]UNINOVE − Ciências da Reabilitação

Introduction: Envenoming induced by snakes from *Bothrops* sp. induce local pathological alteration characterized by intense hemorrhage, mionecrose, edema and pain. The most effective treatment for Bothrops snakebites is antivenom (AV) therapy. However, this procedure does not reverse the local damage caused by the venom1. Other alternatives being investigated with the purpose of diminishing the local myotoxicity of bothropic snakebites that include the photobiostimulation therapy. The objective of the present study was to investigate the effectiveness of the Light Emittion Diode (LED) therapy to revert the edema formation and mionecrose, caused by *Bothrops jararaca* snake venom. **Methods:** Male Swiss mice had been injected with *B. jararaca* venom (2 mg/kg) in the right gastrocnemius muscle. To measure the muscle edema, 50 ml of *B. jararaca* venom was injected into the right gastrocnemius muscle of mice, at the same time the contralateral muscle received the same volume of a sterile saline solution. After mice were euthanized (3 or 24 h after venom injection), gastrocnemius muscles were dissected out. Both muscles were weighed, and edema was expressed as the percentage of the weight increase of the venom-injected muscle compared to the contralateral muscle. Mionecrose was evaluated through the quantification of the creatine kinase (CK) activity at 3 h and the histological analysis 3 and 24 h, after venom injection. The animals had been treated with the LEDs (infrared LED, power of 120 mW, λ 945 nm, density of energy of 4 J/cm ², time of irradiation of 38 s and area of 1.2 cm2; Red LED, power of 110 mW, λ 635 nm, density of energy of 4 J/cm ², time of irradiation of 41 s and area of 1,2 cm2) applied: immediately and 2 h after the venom injection. **Results:** Edema muscular caused by *B. jararaca* venom was significantly reduced by two treatments used: Red LED caused a reduction of 20 and 11% and infrared LED: 29 and 19%, at 3 and 24 h respectively, when compared with the animals treated with venom. The treatment with the LED did not modify the mionecrose caused by the venom. **Discussion:** In this work the photobiostimulation therapy with infrared LED and red LED was capable to reduce muscular edema caused by the total venom of *Bothrops jararaca*, however, mionecrose was not affected by this treatment. In this way, the therapy with the LED can contribute, at least in part, as an alternative to the current treatment, not effective against local effect. 1.Camey, K.U. et al., Toxicon, 40 (5): 9-501, 2002. Aprovado pelo Comitê de Ética em Pesquisa (CEP) da UNIVAP n°. A23/CEP/2008. **Financial Support**: Fundação Valeparaibana de Ensino

09.065

Modulation of gene expression in melanoma cells by treatment with crotamine. Moura AB[1], Yonamine CM[1], Pellegrino R[2], Oliveira EB[3], Yamane T[4], Lapa AJ[1], Hayashi MA[1] [1]UNIFESP − Farmacologia, [2]UNIFESP − Psicobiologia, [3]FMRP-USP − Biochemistry and Immunology, [4]CBA − Bioquímica e Biologia Molecular

Introduction: Crotamine is a polypeptide consisting of 42 amino acid residues, in which 11 residues are basic (9 lysines and 2 arginines), and 6 cysteines form 3 disulfide bonds. The presence of three disulfide bonds confers to crotamine a high compactness, stability, and an overall structural similarity to b-defensin 2, an antimicrobial peptide from the human epithelia. We have shown that crotamine is able to cross the cell membrane and to transport genes and/or other molecules to the interior of cells and also to the nucleus with a higher specificity for proliferating cells. Therefore, we have suggested the employment of this molecule for disease diagnosis and drug delivery. Up to 1 μM, crotamine is innocuous to normal cells (e.g., human fibroblasts and murine embryonic stem cells), but lethal for tumorigenic CHO-K1 cells (Hayashi et al., Toxicon 52: 508, 2008). One biochemical reason for the action of this peptide as antitumor is based on the differential lipid composition of tumor cells (Rádis-Baptista et al., J. Braz. Chem. Soc. 19: 211, 2008), although the presence of proteoglycans in the cell surface had also been shown to be essential for the uptake of this peptide (Nascimento et al., JBC 282: 21349, 2007). The co-localization of crotamine with tumor cells suggests that crotamine is a potential marker of proliferating tumor cells, and it might be also a good candidate for specific targeting and delivery of drugs into proliferating cells (Nascimento et al., Mol. Therap. submitted, 2010). The effects of chronic treatment with crotamine on B16F10 melanoma tumors was also studied in living animals for 21 days demonstrating a specific and selective cytotoxic activity against aggressive and fast growing types of tumor (Pereira et al., Cancer Res. submitted, 2010). However, the molecular mechanism underlying the cytotoxic effects of crotamine on tumor cells is still not completely clear. **Methods:** The effect of crotamine treatment in gene expression of B16F10 cells maintained in culture was evaluated by microarray after for 24 hours treatment with concentrations of crotamine considered toxic (10 μM) or sub-toxic (1 μM) to this cell line. The panel of activated or repressed genes compared to untreated control was analyzed for the two treatment conditions. **Results and Discussion:** The expression of genes involved in regulation of DNA replication, cell proliferation and apoptosis was already observed for 1 μM crotamine treatment, while genes involved in mitosis, DNA repair, regulation of cell proliferation, and DNA replication had their expression changed only for treatments with 10 μM of crotamine. The up and down regulation of each specific gene of interest suggested to be modulated by the treatment with different concentrations of crotamine is under confirmation by real-time PCR. We believe that this study will highlight some potential pathways targeted by crotamine, which may help us to better understand the mechanism of action of crotamine on tumor cells at molecular level. Financial support by FAPESP and CNPq.

09.066

Preliminary biochemical and pharmacological characterizations of *Rhinela icterica* toad venom. Pesamosca ME, Freitas TC, Franco JL, Dal Belo CA UNIPAMPA

Introduction: A highly diverse array of small peptides has been found in the venom of animals. These natural toxins are precious templates that, because of their limited range of molecular targets, can be used in studies aimed to develop new drugs. In this work we have examined the *Rhinela icterica* toad venom (RITV) in order to verify its preliminary pharmacological activities in cockroach semi-isolated heart. **Methods:** The *in vitro* inhibition of AChE was evaluated according to the assays described by Ellman et al. (1961) with some modifications (Franco et al. 2009). Animals had their heads removed and homogenized in cold phosphate buffered saline (pH 7.0) and centrifuged at 1000 x g for 3 minutes. The supernatant was used for determination of AChE activity. The cockroach semi-isolated heart preparation (Baumann and Gersch, 1982) was used to evaluate the pharmacological effects of RITV. Briefly, adult cockroaches (*Phoetalia pallida*) were anesthetized with ether until immobile and placed ventral side up. The lateral margins of the abdomen were cut along each side, and the ventral abdominal body wall was peeled up to expose the viscera. The viscera were then carefully moved aside to expose the heart underneath, and heart frequency was monitored during 30 min by using a stereoscope microscope. Statistical protocols were applied when necessary, by using non parametric Student t test. This project was approved by the Committee for Ethics in Animal Use (CEUA, UFSC, protocol number 23080.026023/2004-32). **Results:** The biochemical analysis of RITV demonstrated that the venom has anticholinesterase activity. When cockroaches head homogenates were incubated with RITV (1, 2 and 3μg.μl-1) an inhibition of about 35-65% of the enzyme activity was found ($n=3$, $p<0.05$). The addition of RITV at cockroach semi-isolated heart preparation induced a concentration and time-dependent mode of action. In this set of experiments, RIVT (1, 2, 4 and 8μg.g-1) induced a progressive negative chronotropic effect that initiated after 15min and was maximum (50$\pm$4 beats.min-1) for the highest dose at 30 min incubation ($n=6$, $p<0.05$). The washing of preparations with insect saline was ineffective to reverse the inhibitory effect. **Discussion:** The general toxicity associated with toad poisoning in mammals results primarily from the binding of the cardiotonic steroids to the membrane-bound sodium potassium pump (Na+/K+-ATPase) (Lim et al., 1997; Chi et al., 1998). In this work we demonstrated that the *Rhinela icterica* venom is cardiotoxic for cockroach cardiovascular preparation. The cardiotoxicity is associated to the heart negative chronotropic effect that can be partly mediated by the venom-induced anticholinesterasic activity. References Ellman G.L. et al. Biochem. Pharmacol 7 (2) 88 1961. Franco J.L. et al. Toxicol Lett. 2009 187(3):137-43, 2009 Baumann E. and Gersch M. Insect Biochem. 12 (1) 7, 1982. Lim T.H et al. Toxicon 35 293, 1997. Chi H.T. et al., 1998. Hum. Exp. Toxicol.17, 343–346. **Financial support:** Universidade Federal do Pampa-UNIPAMPA Acknowledgments: The authors thank Mr. Alcindo Gonçalves for helping with venom collection.

09.067

Evaluation of antinociceptive property of ethanolic extract of *Sidastrum micranthum* (A. St.-Hil.) Fryxell. Villa JKD[1], Marinho DG[2], Dias DM[1], Ramiro JB[1], Scherrer JV[1], Faccim AG[1], Almança CCJ[3], Marinho BG[1] [1]UFES − Medicina Veterinária, [2]ICB-UFRJ − Farmacologia e Química Medicinal, [3]FAFIA − Farmácia

Introduction: The “malva preta”, “guaxuma” or “guaxima” (*Sidastrum micranthum* (A. St.-Hil.) Fryxell) occurs in Central and South America. It was identified and stated by Dr. Paul A. Fryxell from Rancho Santa Ana Botanic Garden, California, USA. In Brazil, this plant occurs predominantly in Northeast. Its leaf prepared by infusion, it’s utilized by the local populations for the treatment of cardiopathies and pulmonary disorders. The oral use was related to pain treatment. The objective of this work was to evaluate and confirm the antinociceptive property from the extract of *Sidastrum micranthum*. **Methods:** The dry leafs were grated at grinder and extracted with ethanol 70%. The use of animals in this work was approved by the CEUA-UFES, and received the number 002/2009. Male Swiss mice (20-25g, n=6-8) were used in the acetic acid (2%, intraperitoneal) induced abdominal contortions, in the licking response induced by formalin (2.5%, intraplantar) and tail flick test. Animals received oral administration of *S. micranthum* ethanolic extract at doses of 1 to 100 mg/kg, 1h before experiments. **Results:** Pre-treatment of mice with 50 and 100 mg/kg of extract inhibited in 27% and 42% the acetic acid-induced contortions, respectively. It was observed dependent-dose effect. Pre-treatment with 100 mg/kg inhibited in 25% the 1st phase of licking response induced by formalin and pre-treatment with 50 and 100 mg/kg inhibited in 40% and 54% the 2nd phase of licking response, respectively. Pre-treatment with 100 mg/kg obtained an increase of 99% compared to baseline, at the time of 60 minutes. **Discussion:** Ethanolic extract from *S. micranthum* showed a significant antinociceptive activity at doses of 50 and 100 mg/kg. This effect was observed in all models tested. The results obtained suggest effect on inflammatory and non-inflammatory pain.

09.068

Endothelium-dependent and independent relaxation of rat aortic ring by crude extracts and fractions from *Scutia buxifolia*. Silva RCMVAF[1], Crestani S[1], Boligon AA[2], Athayde ML[2], Santos ARS[3], Marques MCA[1], Kassuya CAL[1], da Silva-Santos JE[4] [1]UFPR − Farmacologia, [2]UFSM − Farmácia Industrial, [3]UFSC − Ciências Fisiológicas, [4]UFPA − Farmacologia Experimental e Pré-clínica

Introduction: *Scutia buxifolia* Reiss (Rhamnaceae), known as “coronilha”, is used in folk medicine against cardiovascular diseases, such as hypertension. However, its pharmacological properties have been scarcely studied, and there are no data showing the effects of this plant on vascular system. In this study, we evaluated the ability of crude extracts prepared from leaves and bark of *S. buxifolia* and its dichloromethane (DCM), butanolic (BuOH) and ethyl acetate (AcOEt) to relax *in vitro* rat aortic rings. **Methods:** Male Wistar rats (200-250 g) were used in these experiments. The animals were anesthetized and killed before removal of their thoracic aorta, which was cut in 3-4 mm rings and mounted in baths coupled to isometric transducers connected to a MacLab® recording system. The aortic rings were kept in Kreb’s nutritive solution at 37 °C, aerated with 95% O2/5% CO2. Experiments were performed using vessels with (E+) and without (E-) functional endothelium. The functionality of endothelium was confirmed when the addition of acetylcholine (ACh; 1 μM) in preparations pre-contracted by phenyleprhine (PE; 1 μM) did result in relaxation ≥ 90%. A resting period of 60 min was allowed between each set of exposition to drugs. Both E+ and E- aortic rings were contracted by PE (1 μM), and in the tonic phase of this contraction, exposed to cumulative concentrations of the ethanolic extract obtained from bark (EEB) and leaves (EEL) of *S. buxifolia* (3-300 μg/ml), as well its DCM, BuOH and AcOEt fractions (3-30 μg/ml). All procedures were approved by the Institutional Ethics Committee under protocol number 372. **Results and Discussion:** Addition of EEB and EEL (100 μg/ml) in PE-contracted E+ vessels resulted in a maximal relaxation of 59.5 ± 12.8 and 82.1 ± 8.2%, which was reduced to 47.4 ± 6.1 and 40.9 ± 8.6% in E- aortic rings, respectively (n = 6-10). Except to DCM fraction from leaves, all other fractions were able to concentration-dependently relax E+ contracted by PE in a range of 50 to 95%. For instance, the AcOEt (50 μg/ml) and BuOH (30 μg/ml) fractions from leaves of *S. buxifolia* promoted a maximum relaxation of 89.3 ± 4.2 and 95.1 ± 4.8%, respectively (n = 7). Removal of endothelium did not abolish these effects, but significantly reduced it by 50%. In addition, the DCM fraction from bark (at 30 μg/ml) was able to relax both E+ and E- aortic rings by 83.1 ± 10.9 and 89.1 ± 5.5, respectively (n = 4-6). Conclusion: Our data provide the first evidence that both crude extracts and fractions obtained from *S. buxifolia* (either leaves or bark), a plant popularly used against cardiovascular diseases, are able to cause relaxation of *in vitro* isolated aortic rings, by mechanisms dependent and independent of endothelial function. We are currently investigating how *S. buxifolia* is acting on both endothelial and smooth muscle cells to induce vascular relaxation. **Financial support:** CNPq and CAPES.

09.069

Anti-inflammatory and antinociceptive activity of the ethanolic extract from *Sinningia leucotricha*. Botelho A[1], Verdan ML[2], Stefanello MEA[2], Kassuya CAL[3], Zampronio AR[1]
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Introduction: The genus Sinningia (Gesneriaceae) is popularly used for its anti-inflammatory and antipyretic properties but there are no studies that support the popular use. In this study we evaluated the anti-inflammatory and antinociceptive activity of the ethanol extracts obtained from the leaves and tubers of Sinningia leucotricha (Hoehne) H. E. Moore (EESl) popularly known as “Rainha do Abismo”. **Methods:** Swiss male mice were treated with EESl (3-300 mg/kg) or vehicle orally 1h before the noxious stimulus. Carrageenan (Cg, 300µg) was injected into the hind paw. Oedema was evaluated using digital micrometer, the mechanical allodynia using von Frey filaments and cell migration through the test of myeloperoxidase. The antinociceptive activity was also evaluated using the formalin (2.5% 20µl/paw) and the hot plate test. We also evaluated fractions acetate (F1) and butanol (F2) obtained from EESl in the paw oedema test (6 mg/kg). All protocols were previously approved by UFPR’s Ethical Committee on Animal Use (Nbr. 387). **Results:** EESl from the tubers but not from leaves, inhibited the oedema induced by Cg in a dose-dependent manner at doses of 3, 10 and 30 mg/kg (0, 23 and 42.4% respectively, 2h after Cg injection) similarly to the positive control indomethacin (42%). EESl (30 mg/kg) significantly reduced the oedema formation at all times tested (0.5 to 4h) The EESl (30 mg/kg) also decreased 47% to myeloperoxidase activity. Altogether, these results suggest that EESl possess anti-inflammatory activity. The EESl did not modify the first phase of nociceptive behavior evoked by formalin but significantly reduced the phase II (inflammatory) of this response (10 and 30 mg/kg with inhibition of 53% and 60% respectively). These same doses also reduced (59% and 66% respectively) the mechanical allodynia 2 hours after the injection of Cg. The EESl 30 mg/kg did not alter the latency time of the animals on the hot plate test. These results suggest that the EESl is acting peripherally, and not in the central nervous system, reducing the sensitization of nociceptive fibers that occur during inflammation. Fraction F1 (6 mg/kg) reduced 35% the oedema induced by Cg. **Discussion:** These results show that EESl possess anti-inflammatory and antinociceptive activity which corroborates the popular use of this plant. The antinociceptive activity seems to be related to a peripheral effect. The anti-inflammatory activity seems to be present in the acetate fraction that is going to be submitted to further studies for the isolation of active compound(s). **Financial support:** CNPq and Fundação Araucária

09.070

Preliminary evaluation of wound healing activity of the aqueous extract from stem bark of *Bowdichia virgilioides*. Agra IKR, Santos TC, Smaniotto S, Barreto E UFAL − Biologia Celular

Introduction: Previous results from our group demonstrated anti-inflammatory and immunomodulatory effects of aqueous extract from B. virgilioides (AEBv) (Agra IKR et al., Anais do 40º Congresso da SBFTE, v.1, p.71, 2008). The present study was undertaken to provide a basic set of data on the healing effect of aqueous extract of stem bark of *Bowdichia virgilioides*. **Methods:** Male Swiss mice were used (18-20 g). The excision (1 cm of diameter) in the depilated dorsal interscapular region was used in order to assess the effect of aqueous extract of stem bark of the Bowdichia virgilioides, called AEBv, on wound healing in mice. Four groups of mice (4 in each group) were wounded under anaesthesia; the skin was surgically incised in a circular manner (the circular incision in reference measuring 1 cm in diameter) and afterwards daily treated for 10 days in a following manner: Group 1: the control group treated with saline solution (0.9%, NaCl) by intraperitoneal route; Group 2: treated with AEBv (10 mg/kg/day) topically; Group 3: treated with AEBv (10 mg/kg/day, i.p.) and Group 4: treated with dexamethasone (1 mg/kg/day, i.p.). The animals were sacrificed on day 10 post wounding and the area of wound was measured monitored periodically by a camera (Sony Cyber Shot, Dsc w80). Wound healing efficacy was measured by determining the morphological parameters. The excised wounds were collected on day 10 by freezing and dully stained for histopathological analysis. The morphology was analyzed by hematoxylin and eosin (HE) and the content of collagen was quantified by Masson´s staining. All procedures were previously approved by the Ethics Committee on Animal Experiments of UFAL (Protocol 23065.12614/2006-89). **Results:** Treatment of the wounds with topical AEBv enhanced significantly the rate of wound contraction reducing to 0.07 ± 0.09 cm2 of its original size (2.28 cm2) on day 10, which represented a 97 % reduction in the wound area. In addition, mice treated with AEBv by intraperitoneal route exhibited an expressive reduction to 0.2 ± 0.09 cm2 (92 % reduction). The histological study of the granulation tissue obtained on day 10 from the animals treated with AEBv showed well organized bands of collagen, fibroblasts and few inflammatory cells. Whereas the granulation tissue obtained from controls showed disorganized collagen fibers and few fibroblasts. **Discussion:** The reported observations suggest, for the first time, that *B. virgilioides* aids wound healing in the mice model. Thus, aqueous extract of stem bark of *Bowdichia virgilioides*, might be considered as a new wound-healing agent suitable for use in both veterinary and human medicine practice. Further studies are in progress to isolate the active constituents and also study its mechanism of action. **Financial support:** CNPq, FAPEAL, CAPES.

09.071

Antioxidant activity of dichloromethane fraction of *Baccharis trimera* and its effects on murines macrophages. Freitas GM, Gayer CRM, Coelho MGP, Sabino KCC UERJ − Bioquímica

Introduction: *Baccharis trimera* (Asteraceae), popularly known as “carqueja”, is a plant found in the South America, which has been used to treat gastrointestinal and liver diseases, angina, diabetes and inflammatory processes (Torres, Phytochemistry 55: 617, 2000). The aim of this work was to assess the antioxidant activity of dichloromethane fraction of *Baccharis trimera* and study its effects on murines macrophages. **Material and Methods:** The ethanolic extract (EEBt) was obtained from the aerial parts of the plant. The EEBt was fractionated by liquid-liquid partition with hexane (HEX fraction), dichloromethane (DCM fraction) and ethyl acetate (EtAc fraction). The crude extract and fractions were analyzed by HPLC-DAD and GC-MS. Their flavonoids content was determined through the reaction with AlCl3 by 30 min followed by reading absorbance in 425 nm. The antioxidant activity was determined by mixturing the samples with DPPH for 30 min, followed by absorbance determination in 517 nm. The plant cytotoxicity was assayed by the MTT reduction test on the macrophagic cell line RAW 264.7. The cells (2x105/mL) were treated with different concentrations of EEBt and DCM fraction for 24 h and the cytotoxicity was evaluated for the last two hours of culture. The RAW 264.7 cells (5x105 cells/mL) were stimulated with LPS (1 µg/mL) and treated simultaneously with different concentrations of DCM fraction by 24 h for nitric oxide production. Nitrite concentration was determined by Griess reaction. **Results and Discussion:** The EEBt analysis by HPLC-DAD showed the presence of peaks I and II (retention times of 17.29 and 17.981 min). The partition with hexane (HEX fraction) concentrated the compounds of peak II, with addition of substances with higher retention time and the DCM fraction concentrated the compounds of peak I. EtAc fraction profile was similar to DCM, but with significantly lower intensity. The GC-MS analysis showed the presence of compounds with low volatility in the EEBt and DCM and EtAc fractions, whereas the HEX fraction presented both compounds with low and high volatility. The content of flavonoids showed to be higher ($p<0.05$) in DCM. The DPPH assay demonstrated significant antioxidant activity for the EEBt and DCM and EtAc fractions ($p<0.05$) which may be related to their high content of flavonoids. DCM fraction inhibited ($p<0.05$) the nitric oxide production by RAW264 macrophage cells in a concentration-dependent mechanism ($p<0.05$), showing no cytotoxic effect, except for 100 µg/mL. EEBt also showed no cytotoxic activity to these cells. This work showed increased inhibition of nitric oxide production by fractioning of EEBt and suggests that this effect added to the demonstrated antioxidant action may be contributing to the anti-inflammatory effects of *Baccharis trimera* popular preparations. **Financial support:** UERJ, CNPq and FAPERJ.

09.072

Pulsed therapeutic ultrasound effects on skeletal muscle damage induced by *Bothrops jararacussu* snake venom. Tomaz MA[1], Saturnino-Oliveira J[2], Machado MM[1], Cons BL[1], Calil-Elias S[3], Martinez AMB[4], Melo PA[1] [1]UFRJ − Farmacologia Básica e Clínica, [2]UESC − Microscopia Eletrônica, [3]UFF − Farmácia e Administração Farmacêutica, [4]UFRJ − Embriologia e Histologia

Introduction: We have investigated the injury induced by *Bothrops jararacussu* crude venom on mice extensor digitorum longus (EDL) muscle, as well as the influences of Pulsed Therapeutic Ultrasound (PTU) and antibothropic polyvalent antivenom (PAV) on muscle regeneration. **Methods:** Animals (5 per group) received perimuscular injection of venom (1,0 µg/g) and one hour later they were treated with PTU (3 MHz - 0,3 W/cm2 for one minute). One group received the ultrasound treatment, just like the other, plus one injection, by intravenous route, of antibothropic polyvalent antivenom (1.0 µg/g) 15 min after the venom injection. The negative control group received an injection of PSS in the right limb. Isolation and processment for light microscopy were performed 3 and 28 days after perimuscular injections. In our experiments we used isolated muscles from Swiss adult mice weighing 25.0 ± 5.0 g and adhered to the guidelines for animal care prepared by the Committee on Care and Use of Laboratory Animal Resources, National Research Council, USA, which is adopted by our University Committee (CEUA-UFRJ, number DFBCICB 022). **Results:** Muscle fibers were completely degenerated by venom injection; PTU and its association with PAV protected some of these fibers, while muscles that received no treatment showed regeneration with disorganized fascicules with fibers presenting reduced diameter. In the acute phase of the process (3rd day), treated muscles showed angiogenesis and reduced inflammatory reaction. Moreover, by the 3rd day the venom had induced a decrease of 76,7 ± 2,2 % in muscle CK content, while treatment with PTU, PAV and association induced decreases of 20.1 ± 6,0, 61.4 ± 4.5 and 11,2 ± 3,8 %, respectively (both values are significantly different from the venom group – ANOVA). Venom also led to deficient motor activity, assessed by the time the animal was able to keep walking on the Rota-Rod®. Control mice were able to walk as long as 120 seconds, against 56.7 ± 11.3 seconds of venom group. PTU (97,4 ± 3,9 s) and PTU + PAV (109,8 ± 6,3 s) were both able to increase mice standing on Rota-Rod® (both values are significantly different from the venom group – ANOVA), while PAV alone was not (32,0 ± 5,3 s). CONCLUSION: Our data have shown the ability of PTU to improve, both structurally and functionally, the regenerated muscle following injury induced by *B. jararacussu* venom. **Financial support:** CAPES, CNPq, PRONEX and FAPERJ

09.073

Study of the neuropharmacological activity of the compound GB-2a obtained from *Rheedia gardneriana*. Santos ECS[1], Marques de Carvalho RS[1], Cechinel-Filho V[2], De Lima TCM[1] [1]UFSC − Farmacologia, [2]NIQFAR-UNIVALI

Introduction: *Rheedia gardneriana* (Guttiferae), an Amazonian tree widely distributed in Brazil and popularly known as "bacopari", is used in folk medicine to treat inflammatory diseases of the urinary tract, arthritis and pain. The phytochemical analysis of this plant indicates the presence of constituents like biflavonoids, steroids, triterpenes, xanthones as putative responsible for some pharmacological activities. However, so far the study of a putative central effect of this plant has not been undertaken. Our aim in the present study was to evaluate the anticonvulsant and hypnosedative effects of the compound GB-2a, a biflavonoid isolated from the leaves of *Rheedia gardneriana* in mice. **Methods:** Female Swiss mice were intracerebroventricularly (i.c.v.) pretreated with the GB-2a (0.002 - 2000 pmol) or vehicle (DMSO 1%). The hypnosedative activity was evaluated by the potentiation of ethyl ether induced-hypnosis and recorded as the duration of sleep. The anticonvulsant activity was evaluated in the pentylenetetrazol-induced convulsions (PTZ – 80 mg/kg), recording the latency to first seizure, the severity of induced convulsions and latency to death. All protocols described herein were approved by a local ethical committee (23080.08007244/006-70/CEUA/UFSC). **Results:** Analysis of variance (ANOVA) followed by Dunnett´s test showed that only the dose of 0.2 pmol caused a significant increase ($F_{7,64} = 2.9220$, $p = 0.01022$, ANOVA) of sleep duration by 125%. However, in the evaluation of its possible anticonvulsant this dose was found to be ineffective, since it was unable to prevent the episodes of convulsion and/or the deaths induced by PTZ. **Discussion:** Our data suggest a hypnosedative effect to the compound GB-2a, however, no anticonvulsant activity was observed and but more studies are needed in order to elucidate its putative mechanism of action. **Financial support:** CNPq e CAPES

09.074

Effects of chronic treatment with aqueous extract of *Cuphea balsamona* L. on the lipid profile of rats submitted to a high-cholesterol diet. Baracho NCV[1], Brügger PG[2], Camanducaia DSM[2], Sanches AIF[2], Sanches RS[2] [1]FMIT − Farmacologia e Bioquímica, [2]FMIT − Medicina

Introduction: *Cuphea balsamona* L. is a plant from central and south America, popularly known as “seven bleedings” and it has been used as an hypocholesterolemic agent by people in general. This study has the aim of evaluate the effects of chronic treatment with aqueous extract of *Cuphea balsamona* L., “sete sangrias”, on the lipid profile of rats submitted to a high-cholesterol diet. **Methods:** This study was approved by the Research Ethics Committee of the Faculty of Medicine of Itajubá under protocol 013/08 and received financial support from FAPEMIG. Were used twelve young adults, male Wistar rats, weighting 200 to 250g and age between 60 and 90 days. They were submitted to a high-cholesterol diet for 45 days and, later, randomizing into three groups, which received during 45 days, by gavage, the following treatments: 1) Control group (n=4): 1mL of distilled water; 2) *Cuphea balsamona* L. 50mg/L (n=4): 0,4mL/100g; 3) *Cuphea balsamona* L. 100mg/L (n=4): 0,4mL/100g. The animals were kept in plastic cages with water and high-cholesterol food *ad libitum* and submitted to a twelve-hours day-night cycle. The animals’ euthanasia was made with aspirative puncture of left ventricle, after the anesthesia with Ketamine (50 mg/kg) and Xylazine (25 mg/kg) by intraperitoneal way. **Results:** Compared to Control Group, the treatment with Cuphea Balsamona L. 50 mg/L has produced a significant reduction in blood levels of total cholesterol (857,81 ± 56,22 vs 500,0 ± 108,25; $p< 0,05$), triglycerides (173,80 ± 63,35 vs 80,95 ± 27,49; $p<0,05$), HDL (69,32 ± 3,34 vs 38,65 ± 1,03; $p<0,05$), VLDL (34,75 ± 12,67 vs 16,31 ± 5,36; $p<0,05$) and LDL(753,73 ± 55,17 vs 445,16 ± 101,71; $p< 0,05$). Compared to Control Group, the treatment with Cuphea Balsamona L. 100 mg/L has produced a significant reduction in blood levels of triglycerides (173,80 ± 63,35 vs 61,90 ± 22,67 ; $p<0,05$), HDL (69,32 ± 3,34 vs 48,28 ± 7,33; $p<0,05$), VLDL (34,75 ± 12,67 vs 12,37 ± 4,53; $p<0,05$) and LDL (753,73 ± 55,17 vs 623,72 ± 92,08; $p< 0,05$) and a tendency to significance in reducing blood levels of total cholesterol (857,81 ± 56,22 vs 684,37 ± 98,22; $p= 0,0567$). **Discussion:** The data suggest that the chronic treatment with aqueous extract of *Cuphea balsamona* L. in concentrations of 50mg/L and 100mg/L produce important changes on lipid profile. There were no significant differences comparing the treatment groups among themselves, showing that its action don’t appears to be dose-dependent. The mechanism by which this effect is produced is not well understood, since in literature there are still no studies that describe its possible hypocholesterolemic effect. **Financial support:** FAPEMIG and PDIC-FMIt. 1- Baracho NCV. O tratamento crônico com geléia de *Citrullus vulgaris* L. produz diurese e diminuição da pressão arterial. In: Faculdade de Medicina de Itajubá: Anais da 28ª Semana Médica 2008. Itajubá: FMIt; 2008. p. 8. 2- Souza Neto JL. Effects of simvastatin in abdominal sepsis in rats. Acta Cir Bras. 2006; vol.21 Page 8. 3- Jaldin RG. O processo aterosclerótico em artérias de coelhos submetidos a dieta suplementada com gema de ovo: modelo experimental de baixo custo. J. Vasc Bras. 2006; vol.5 Page 247. 4- Lemus I. Diuretic activity of an Equisetum bogotense tea (Platero herb): Evaluation in healthy volunteers. J ethnopharmacol. 1996; vol.54 Page 55.

09.075

Evaluation of antinociceptive property of ethanolic extract of *Buddleja brasiliensis*.

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Introduction: The “barbaço”, “barbasco” or “calção de velho” (*Buddleja brasiliensis*) occurs in various regions of Brazil. Its tea extracted from leafs or roots is utilized by local populations to many purposes: anti-inflammatory, analgesic, haemostatic, sedative and others. The objective of this work was to evaluate and confirm the antinociceptive property from the extract of *Buddleja brasiliensis*. **Methods:** The dry leafs were grated at grinder and extracted with ethanol 70%. The use of animals in this work was approved by the CEUA-UFES, and received the number 002/2009. Male Swiss mice (20-25g, n=6-8) were used in the acetic acid (2%, intraperitoneal) induced abdominal contortions, in the licking response induced by formalin (2.5%, intraplantar) and tail flick test. Animals received oral administration of *B. brasiliensis* ethanolic extract at doses of 1 to 100 mg/kg, 1h before experiments. **Results:** Pre-treatment of mice with 50 and 100 mg/kg of extract inhibited in 25% and 35% the acetic acid-induced contortions, respectively. It was observed dependent-dose effect. Pre-treatment did not inhibit expressively the 1st phase of licking response induced by formalin and pre-treatment with 50 and 100 mg/kg inhibited in 22% and 42% the 2nd phase of licking response, respectively. Pre-treatment with extract did not obtain significant increase in relation with baseline in the tail flick test. **Discussion:** Ethanolic extract from *B. brasiliensis* showed a significant antinociceptive activity at doses of 50 and 100 mg/kg. It was not observed significant effect in the 1st phase of licking response induced by formalin and in the tail flick test. The results obtained suggest effect on inflammatory pain.

09.076

Antidiarrheic activity of hydroalcoholic extract from barks of *Astronium fraxinifolium* schott in mice. Serikava SMO[1], Kushima H[1], Hiruma-Lima CA[1], da Silva VC[2], Vilegas W[3] [1]IB-UNESP-Botucatu − Fisiologia, [2]IQ-UNESP − Química Orgânica, [3]UNESP-Araraquara − Química Orgânica

Astronium fraxinifolium Schott. (Anacardiaceae) is a Cerrado specie popularly used for the treatment of skin problems, allergies and diarrhoeas. The objectives of this study were to evaluate the pharmacological effects of the hydroalcoholic extract obtained (HDA) from barks of A. fraxinifolium in the experiments of diarrhea induced by castor oil, of intestinal motility and intestinal fluid accumulation. In the evaluation of anti-diarrhoeal activity, mice (25-35g) were randomly divided in three experimental groups (n=5) and were fasted for 20 h and orally treated with saline 0,9% (negative control), Loperamide (50 mg/kg, positive control) or extract of A. fraxinifolium (500 mg/kg). After 30 minutes, all animals received orally 0,2 mL castor oil and were kept in individuals cages covered with absorbent paper. The time of the first watery evacuation and the number of watery, semisolid and solid evacuations were noted for each animal. The excrements were classified of the following form: value 1 was attributed to the normal evacuations (stool 1), value 2, to the semisolid (stool 2) and value 3 for the watery evacuations (stool 3). For each group the index of evacuations (IE) was calculated (IE (group) = 1 x (nº stool 1) + 2 x (nº stool 2) + 3 x (nº stool 3)). In the evaluation of the extract effect on motility, mice (25-35 g) were starved for 6 hours and were orally pre-treated with saline, extract of A. fraxinifolium (500 mg/kg) or subcutaneous atropine at dose of 5 mg/kg (positive control). After 30 minutes, all animals received orally 10% of active coal in volume of 10 mL/kg. Passed 30 minutes all animals were sacrificed and the small intestine was dissected out from the pylorus to the caecum. The ratio of total distance traveled by charcoal and the total length of small intestinal was calculated to post analyses. The effect of extract on intestinal fluid accumulation was evaluated in mice starved for 6 h and pre-treated orally with saline, extract of A. fraxinifolium (500 mg/kg) or morphine (10 mg/kg, s.c.). After 1 hour, all animals received 0,2 mL castor oil and passed 30 minutes all animals were sacrificed and theirs small intestine were excised and intestinal fluid accumulated were measured. The statistic analysis was the ANOVA followed by Dunnett's post hoc test. The values for $p < 0,05$ were considered the minimum significance. An expressive antidiarrhoeal effect was observed with the administration of A. fraxinifolium extract, demonstrated by the reduction of 85% in the evacuations index (2,2 ± 1,0) and by the delay of 140% (240 ± 0,0 min) in the time of the first watery evacuation when compared to negative control (IE=14,4 ± 2,1; Time of watery evacuation beginning = 100 ± 18,0 min). The intestinal transit was delayed (20%) by treatment with extract (59,64 ± 4,7) when compared to saline group(75,19 ± 4,1) but the volume of intestinal fluid wasn't altered by the treatment with extract ($p > 0,05$). We can concluded that the extract has a visible antidiarrhoeal effect and a potential action on the gastrointestinal transit reduction. Ethics committee protocol number: 18/05 and 74/08 **Financial support:** FAPESP, nº 2008/58179-8

09.077

Evaluation of the anti inflammatory cutaneous effect of *Croton brasiliensis*. Silva MO[1], Prudente AS[1], Conserva LM[2], Cabrini DA[1], Otuki MF[3] [1]UFPR − Farmacologia, [2]IQB-UFAL, [3]UEPG − Ciências Farmacêuticas

Introduction: The genus Croton (Euphorbiaceae) is the second largest in Euphorbiaceae family, distributed predominantly in the America continent (Secco, 1992). There are about 700 species widely distributed in Brazil. Many of these species are known in the Brazilian Northeast region as 'capixingui' and 'marmeleiro', and have been used in folk medicine for a large number of applications as healing, carminative and to treat intestinal colic, being this last action scientifically proven (Palmeira and Conserva, 2005). Previous phytochemical investigations show that this genus possesses alkaloids, flavonoids, triterpenoids, and a large number of diterpenoids (Barbosa et al, 2003). The aim of this study was to test Croton brasiliensis (CB) for an anti-inflammatory activity. Methodology: Female Swiss mice (25-30 g, N = 5-8) were used and ear edema was induced by topical application of 12-O-tetradecanoilforbol acetato (TPA) (2.5 µg/ear). Thickness (µm) of the ear was measured before and 6 h after application of TPA, using a digital micrometer (Great MT-045B). The air dried powdered leaves and stems were extracted with acetone and EtOH. The crude extract were suspended in MeOH-H2O (3:2) solution and extracted successively with C6H14, CH2Cl2 and EtOAc. The fractions then were on chromatographed on silica gel column. Drugs were dissolved in 20 µl of acetone and applied on the inner ear of mice shortly after the phlogistic agent. 24h after the application of TPA, the animals were sacrificed and samples (6 mm circles of the right ear tissue) were collected for activity evaluation of myeloperoxidase (MPO), as indicative of neutrophil migration. Samples of the ears (6 h) were processed for histological analysis. These protocol experiments were conducted with approval of UFPR ethics committee under registration number 315. **Results:** Four fractions of CB were tested, but, only one showed significant inhibition. Several experiments based on the fraction 1 obtained from CB (CBF1) were conducted. The CBF1 was able to inhibit dose-dependently the edema induced by TPA with a Imax of 66.2 ± 1.6% (dose 0.6 mg/ear) and ED50 of 0.091 (0.06126 to 0.1364) mg/ear. The activity of MPO was also reduced by CBF1 application in 78.5 ± 4% (dose 0.6 mg/ear) and with DE50 of 0.127 (0.07532 to 0.2173) mg/ear. Histological analysis showed that treatment with CBF1 caused a reduction of edema and cellular infiltration caused by TPA in a dose of 0.6 mg/ear, as well as the positive control (dexamethasone). **Discussion:** The results suggest that CBF1 possesses anti-inflammatory action when applied topically, altering the edema and leukocytes infiltration. New experiments are being conducted to study the mechanisms involved in these activities. Support: CAPES, CNPq and REUNI Bibliography: Barbosa, P.R. et al. Biochem. Syst. Ecol. 31, 307, 2003. Palmeira, S. F. and Conserva, L. M. Two clerodane diterpenes and flavonoids from Croton brasiliensis. Journal of the Brazilian Chemical Society 16 1420, 2005. Secco, R. S. S. *Croton ascendens* (Euphorbiaceae), a new liana from Eastern Amazonia. Novon 2: 252, 1992.

09.078

Crotoxin modifies intracellular signaling involved in phagocytosis by neutrophils. Lima TS[1], Sampaio SC[1], Della-Casa MS[2], Cirillo MC[1] [1]IBu − Fisiopatologia, [2]IBu − Imunopatologia

Introduction: Phagocytosis is an essential process of the innate immune response. This process is initiated by the binding of particle ligands to a variety of receptors at the cell surface. This triggers a diversity of signaling pathways that are commonly initiated by tyrosine phosphorylation and that lead to rearrangement of the actin cytoskeleton and consequently phagosome formation. Previous studies showed that *Crotalus durissus terrificus* venom (CdtV) inhibits the phagocytic activity of macrophages and neutrophils and that crotoxin (CTX), the main component of the CdtV, is responsible for this effect. In macrophages, CTX cause reorganization of the actin cytoskeleton and inhibition of phosphotyrosine. Considering that the signaling pathways for phagocytosis both in macrophages and neutrophils have some differences and that the mechanisms involved in the inhibitory effect of CTX on phagocytosis by neutrophils is still unknown, the aim of this study was to investigate the *in vitro* effect of CTX on tyrosine phosphorylation and actin polymerization on nascent and mature phagosome. **Methods:** Neutrophils were obtained from peritoneal cavity of male Wistar rats (CEUAIB, protocol 705/10) 4h after the intraperitoneal administration of carrageenan (4.5 mg/kg). Neutrophils (1x106 cells/mL) were incubated (1h) with CdtV (0.5 µg/mL) or CTX (0.08 µg/mL) and then submitted to phagocytosis of opsonized zymosan for 5 or 15 min. Then, neutrophils were fixed and permeabilized. Cells were post-fixed and rehydrated with of non-immune goat serum. Incubation with primary antibody against phosphotyrosine was performed overnight. Then, cells were incubated with the secondary antibody FITC-labeled and stained with rodhamin-phalloidin. Nuclei were stained with DAPI. Slides were mounted and observed in a confocal microscopy. **Results:** The qualitative analysis by confocal microscope showed that incubation of neutrophils with CTX induced a marked reduction in staining of phosphotyrosine and F-actin in neutrophils during phagocytosis at 5 min, when compared to the controls. At the same way, when phagocytosis was performed for 15 min, CTX reduced the content of F-actin, in relation to controls. The same effect was observed when neutrophils were incubated with crude CdtV. **Discussion:** During phagocytosis, the engulfment of the particle begins with the nascent phagosome formation, which occurs at 5 min and gets complete in approximately 15 min with maturation of the phagosome. At nascent phagosomes an increase in tyrosine phosphorilation and actin polymerization is observed, this polymerization leads to phagosome maturation. Unlike what occurs in macrophages, our results demonstrate that CTX inhibits tyrosine phosphorilation and consequently actin polymerization. The results presented herein may contribute to explain the inhibitory effect of CdtV, particularly CTX, on phagocytosis by neutrophils. Furthermore, taking into account the importance of these phagocytes on inflammatory response, these results contribute to the elucidation of the mechanisms involved in the anti-inflammatory effect of CTX that has been reported in the literature. Supported by FAPESP, CAPES and INCTTOX.

09.079

Anxiogenic-like effect of repeated administration of *Passiflora alata* aqueous extract in rodents in the elevated plus maze. Braga A[1], Fenner R[1], Betti AH[1], Stolz ED[2], Hasse DR[3], Gosmann G[1], Rates SMK[1] [1]UFRGS − Ciências Farmacêuticas, [2]UFRGS − Neurociências, [3]UFRGS − Psicofarmacologia Experimental, [6]UFRGS − Ciências Farmacêuticas

Introduction: The Passiflora genus is widely used in folk medicine because its supposed sedative and tranquilizer properties, *P. incarnata* is extensively used in Europe (Dhawan A., J. Ethnopharmacology v.94, p.1, 2004), whereas *P. edulis* and *P. alata* are employed by food and pharmaceutical industries in Brazil (Barbosa, P.R., J Med Food, v.11, p.282, 2008) and P. alata is described in Brazilian Pharmacopoeia (Brazilian Pharmacopoeia. 3rd ed.,p. 839,1977). However, there are few studies about its chemical and pharmacological aspects. In *P. alata*, saponins are the major metabolites and flavonoids were found in minor concentrations (Reginatto, F.H.,J.Braz.Chem.Soc. v.12, p.32, 2001). Anxiolytic and sedative effects of acute treatment with P. alata aqueous and hydroethanolic extracts have been reported in rodents (Petry, R.D., Phytother. Res. v.15, p.162, 2001; Barbosa, P.R., J Med Food, v.11, p.282, 2008; Provensi, G., Lat.Am.J.Pharm. v.27, p.845, 2008). Recently Elsas et al (Phytomedicine, In Press, 2010) suggested that repeated treatment with P. incarnata induces an anxiogenic effect in mice. As far as we know there are no reports about the evaluation of the effects of repeated administration of P. alata. The main objective of this study was to evaluate the anxiolytic effect of 14 days treatment with an aqueous extract (2.4% flavonoids) from leaves of P. alata (PA) in rats and mice. **Methods:** The animals were divided in five groups and treated daily (once time, 14 days) as follows: saline (10 mL/kg, p.o.), diazepam (2 mg/kg, p.o.) and P. alata aqueous extract (300 mg/kg, p.o.). The effects of human handling were evaluated in a parallel group, which did not receive any treatment (SHAM group). The animals behavior was assessed in the elevated plus maze test 1 h after the last treatment (14th day). All the experiments were previously approved by Ethical Committee of UFRGS Protocols 2005453. The data from elevated plus-maze were analyzed by ANOVA one way followed by Student-Newman-Keuls. **Results and Discussion:** Rats treated with PA exhibited a significant ($p < 0.05$) decrease in time spent in the open arms (40,6 ± 39,6s) when compared to control group (121,8 ± 72,4s), and an increase in time spent in the closed arms (210,7 ± 21,8s) when compared to control group (103,9 ± 52,3s). Mice treated with PA exhibited a significant ($p < 0.05$) decrease in time spent in the open arms only (49,31 ± 33,6s) when compared to control group (104,95 ± 46,0s). The number of entries in both arms as well as the total entries were not altered neither in rats, PA (9,9 ± 3,8) when compared to control group (11,8 ± 2,4) nor in mice, PA (14,5 ± 4,7) when compared to control group (19,3 ± 3,3), SHAM animals presented the same behavior that saline group. These results suggest that repeated administration of PA induced an anxiogenic effect instead of causing anxiolytic effect. This assumption deserves further studies.

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09.080

Bioassay-guided fractionation of the marine sponge *Polymastia janeirensis* for anticancer and anticoagulant activity. Biegelmeyer R[1], da Frota Jr. MLC[2], Andrade JMM[1], Carraro JLF[3], Zanotto-Filho A[2], Lorenzi R[2], Mothes B[3], Moreira JCF[2], Henriques AT[1] [1]UFRGS − Farmacognosia, [2]UFRGS − Bioquímica, [3]Fundação Zoobotânica − Ciências Naturais

Introduction: The marine environment has been reported to be a rich source of biologically active compounds. Among all marine organisms, sponges are one of the most promising sources of new bioactive metabolites1. Previous works about marine sponge Polymastia janeirensis, carried out in our research group, showed promising results, especially in the cancer area. This work demonstrated that sponge extracts reduced human glioma cell viability. The lower concentration (10 µg/ml) induces oxidative cell death through a caspase-9-apoptotic pathway and it has a selective cytotoxic effect on glioma cell line compared to a normal cell culture2,3. Thus, in view of the possibility to obtain new active metabolites, the objective of this work was to evaluate the initial fractions of the extract of marine sponge Polymastia janeirensis. Considering that many kinds of cancer are associated with hypercoagulation problems, it was also an objective the experimental condition optimization to evaluate the anticoagulant activity. **Methods:** To obtain the initial fractions of the sponge P. janeirensis, the raw methanol extract was partitioned with other solvents, obtaining the aqueous, ethyl acetate and hexane fractions. Cell viability was assessed using MTT (3-(4,5-dimethiazol-zyl)-2-5-diphenyltetrazolium bromide) assay. To anticoagulant method4 it was optimized the time to defreeze the blood plasma, time incubation and method to evaluation the results. **Results and Discussion:** For anticoagulant experiment it was standardized a defrosting time of one hour. This is an important parameter because over time the plasma begins to lose its viability, altering the kinetic profile of blood coagulation. Furthermore, it should be considered that plasma of different individuals can form clots with different fibers. Therefore, it is important to assess the results across half time for the clotting (time at ½ maximum). Ethyl acetate and hexane fractions demonstrated inhibit the growth of glioma cells at concentrations of 1, 10, 50, 100. and 250 µg/ml. These same fractions also showed alter the coagulation cascade, in order to the increased clotting time. The license number of the ethics committee: 09-551. **Acknowledgements:** CNPq (Ed.70/2008) for financial support and Hospital de Clinicas de Porto Alegre (HCPA), for the blood plasma. 1D. Sipkema et al., Mar. Biotechnol., 7, 142 (2005); 2M.L.C. da Frota Jr et al., Invest New Drugs, 27, 13 (2009); 3M.L.C. da Frota Jr et al., Invest New Drugs, 27, 440 (2009); 4M.E. Andrades et al., Chem-Biol. Interact., 180, 478 (2009).

09.081

Biomonitoring of *Coutarea hexandra* in topic model of inflammation in mice. Prudente AS[1], Lima SF[2], Conserva LM[2], Cabrini DA[1], Otuki MF[3] [1]UFPR − Farmacologia, [2]IQB-UFAL, [3]UEPG − Ciências Farmacêuticas

Introduction: *Coutarea hexandra* is a tree that occurs from Guyana to São Paulo (Brazil). In the Northeast of Brazil, this specie is known as "quina-quina", in Minas Gerais as "murta do mato" and in São Paulo as "quina-quina-branca" or "quina-do-Para" and "amora da floresta". Bark tea is used as an abortifacient, diuretic and to combat pain and inflammation (Abner et al., 2004, Lucena et al., 2006). The methanolic extract and the isolated compound 5,7,2´, 5´-tetra acetoxiphenylcoumarin-4 presented antimicrobial activity (Araújo et al., 1988). Despite the extensive popular use of C. hexandra as analgesic and anti-inflammatory therapy, there are no studies concerning its efficacy. Thus, a biomonitoring fractionation starting with the ethanolic extract was investigated for the anti-inflammatory action. Methodology: Female Swiss mice (25-30 g, N = 5-8) were used and ear edema was induced by topical application of croton oil (0.4 mg/ear). Thickness (mm) of the ear was measured before and 6 h after application of croton oil using a digital micrometer (Great MT-045B). The extracts, fractions, sub-fractions and compounds obtained from C. hexandra were dissolved in 20 µl of acetone and applied on the inner ear of mice shortly after the croton oil. Dexamethasone (0.05 mg/ear) was used as a positive control. All experiments were submitted to the ethics committee of Universidade Federal do Paraná under nº 315 **Results:** The ethanol extract of leaves was able to inhibit ear edema in 41.13 ± 4.45% and 56.81 ± 9.02% at doses of 0.6 and 1 mg/ear, respectively. The partition using solvents of different polarities resulted in the partition dichloromethane which presented an inhibition of 52.44 ± 5.78% of edema formation. The following fractions AcOEt-MeOH (1:1)/ AcOEt, Hex-AcOEt (1:1)/ CH2Cl2, AcOEt/CH2Cl2 and /AcOEt (0.6 mg/ear) obtained from the partition dichloromethane, when compared to control group, were able to inhibit in 37.32 ± 6.06%, 38.87 ± 5.15%, 38.20 ± 7.80% and 41.04 ± 5.78%. From both fractions there were obtained following the 4 sub-fractionations: Hex-AcOEt 1:1/Hex-AcOEt 8:2/CH2Cl2; Hex-AcOEt 7:3/Hex-AcOEt 1:1/CH2Cl2; Hex-AcOEt 1:1/AcOEt/CH2Cl2 and AcOEt/AcOEt/CH2Cl2 (0.6 mg/ear), and all caused reduction of ear edema caused by croton oil in 7.30 ± 2.07% 35.20 ± 2.96% 42.25 ± 3.23% and 50.59 ± 1.45%, respectively. The next step involved the isolation of four compounds from the AcOEt/AcOEt/CH2Cl2, and all show inhibition of edema, but the most effective was a phenylcoumarin (5-Hidroxi-7-metoxi-4-(p-hidroxifenil)cumarina) with inhibition of 60.96 ± 2.55% and ED50 of 0.0866 (0.03164 to 0.2373) mg/ear. **Discussion:** The anti-inflammatory biomonitoring activity of Coutarea hexandra lead us to a compound with an interesting efficiency. New experiments are being conducted in order to confirm the anti-inflammatory effect and its possible mechanism of action. Support: CAPES, CNPq and REUNI. Bibliography: Abner, C. A distribuição geográfica da família Rubiaceae Juss. Flora Brasiliensis de Martius. Rodriguésia 55, 47, 2004. Araújo, C.C. Avaliação da atividade antimicrobiana do composto 5,7,2´,5´ tetraacetoxi-4-fenilcumarina obtido a partir de Coutarea hexandra (Rubiaceae) Revista de Microbiologia 19, 177, 1988. Lucena, J.E.X. Efeito antinociceptivo e anti-inflamatório do extrato aquoso da entrecasca de Coutarea hexandra Schum. (Rubiaceae). Revista Brasileira de Farmacognosia 16, 67, 2006.

09.082

Preliminary investigation on the pharmacological activities of *Araucaria angustifolia* hydroalcoholic extract in insects. Lucho APB[1], Corrêa MS[2], Franco J[3], Dal Belo CA[1]
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Introduction: The extracts of Araucária angustifolia have been used traditionally as ecotoparasiticide against ticks. In this work we have assayed the hydroalcoholic extract of A. angustifolia (HEAA) in order to analyze the phytochemical constitution, insecticide and pharmacological activities in insect models. **Methods:** Fresh leaves of A. angustifolia were used to prepare the hydroalcoholic extract by using conventional extraction protocols. To the phytochemical analysis aliquots of the (HEAA) and standards were spotted onto thin-layer (0.3 mm thick) silica gel plates (Merck®) and the separated spots were visualized with diphenylboric acid 2-aminoethyl ester (NP) and polyethylene glycol 4000 (PEG). Lethal activity was accessed using three cockroaches (Phoetalia pallida) injected with different doses of HEAA (Kagabu et al. 2007). The minimum dose at which two or three insects were considered killed in 24h was taken as the minimum lethal dose (MLD). Cockroach semi-isolated heart preparation (CSHP) (Baumann and Gersch, 1982) was used to evaluate the pharmacology of HEAA. Briefly, after anesthesia the heart was exposed and bathed with 200ml insect saline (0.15 M). Heart frequency was monitored during 30 min by using a stereoscope microscope. When necessary statistical analysis was applied using non parametric Student “t” test. This project was approved by the Committee for Ethics in Animal Use (CEUA, UFSC, protocol number 23080.026023/2004-32). **Results:** The chromatographic profile of HEAA showed the presence of flavonoids with running factor values similar to the standard quercetin. The MLD for HEAA was 800mg.g-1 ($n=3$, $p<0.05$). It was observed that before death, the animals showed motor incoordination and hyperactivity disorders. At CSHP the application of HEAA (4, 8, 16 and 32mg.g-1) induced a concentration and time-dependent effect. When the maximum dose (32mg.g-1) was applied at this preparation, there was a decrease of the heart rate by (30 ± 1.beats-1) in 30 min recordings ($n=6$, $p<0.05$). After washout of preparation with insect saline, a complete recovery of heart frequency was seen. **Discussion:** In this work we demonstrated the insecticide and cardiotoxic activity of A. angustifolia extracts in cockroaches. The phytochemical analysis of HEAA showed the presence of the flavonoid quercetin. The later compound is associated with the insecticide activity induced by botanical extracts (Ateyyat and Abu-Darwish, 2009). Furthermore, in cockroaches, the control of heart rate is related to the cholinergic nerves alongside to the cardiovascular system (Collins and Miller, 1977). Quercetin is also related to the inhibition of acetylcholinesterases in insects (Ateyyat and Abu, 2009). In conclusion, insecticide and cardiotoxic activities induced by A. angustifolia hydroalcoholic extracts may be associated with interactions of this compound with insect cholinergic sites. References Ateyyat MA, Abu MS. Spanish J. Agricultural Res. 7(1), 160. 2009 Baumann E. and Gersch M. Insect Biochem. 12 (1) 7, 1982. Collins C, Miller T. J. Exp. Biol. 67, 1. 1977 Kagabu S. et al. J. Agric. Food Chem. 55, 812. 2007.
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09.083

Amblyomin-X impairs VEGF-induced new vessels formation by altering endothelial cell functions. Drewes CC[1], Dias RYS[1], Hebeda CB[1], Simons SM[2], Chudzinski-Tavassi AM[2], Farsky S[1] [1]FCF-USP − Análises Clínicas e Toxicológicas, [2]IBu − Bioquímica

Introduction: Amblyomin-X is a recombinant protein, inhibitor of serineprotease, initially isolated from the salivary gland of Amblyoma cajennense. Our data have shown that Amblyomin-X inhibits in vivo tumors development, at least partially, by reduction of the number of new vessels formation in the microcirculatory beds. Vascular endothelial growth factor (VEGF) is involved in the angiogenesis. Here we investigated the mechanism of Amblyomin-X on in vivo VEGF induced-new vessels formation. **Methods:** Angiogenesis was studied using: i) dorsal chambers implanted on male Swiss mice anaesthetized (ketamine/xylazine; 80/40 mg/kg); and ii) chorioallantoic membrane assay (CAM). In the dorsal chambers, PBS (control), Amblyomin-X (10 or 100ng/10mL) or VEGF (10ng/10µL) was topically applied at each 48h and the number of vessels was quantified in images obtained before and at 8th day after beginning of the treatments. In the CAM, Ringer solution (control), Amblyomin-X (100ng/10mL) or VEGF (0,25ng/10µL) was topically applied at each 24h for 3 days and the number of vessels was quantified in images obtained at 24h after the last treatment. Using flow cytometry, endothelial cell (t-End lineage; 1,25x105) proliferation was evaluated after treatment with PBS (control), Amblyomin-X (1, 10, 100 or 1000ng/mL) or VEGF (50ng/mL), which was monitored at 0, 24, 48 and 72h after the treatments, using carboxyfluorescein succinimidyl ester (5µM) and PECAM-1, VCAM-1, ICAM-1 and β3 integrin expressions by t-End were quantified 8h after PBS (control), Amblyomin-X (100ng/10mL) or VEGF (10ng/10mL) treatment. The experiments were conducted according to the Ethics Committee in Animal Experiments n° 053/2008 - Protocol n° 211. **Results:** Simultaneous topical administration of Amblyomin-X and VEGF reduced the formation of new vessels in both, dorsal subcutaneous microcirculation (10ng/10mL:49 ± 4% and 100ng/10mL:54 ± 4%) and in CAM (42 ± 5%). The Amblyomin-X treatment, which did not presented cytotoxicity, simultaneously incubated with VEGF, reduced the endothelial cell proliferation at 48h (1ng/mL:80 ± 8%, 10ng/mL:40 ± 8%, 100ng/mL:53 ± 20% and 1000ng/mL:121 ± 40%) and 72h (10ng/mL:109 ± 12%, 100ng/mL:108 ± 7% and 1000ng/mL:115 ± 4%). VEGF per se induced expression of adhesion molecules of PECAM-1, VCAM-1, ICAM-1 and β3 integrin (36 ± 12%, 48 ± 15%, 37 ± 14%, 50 ± 15%, respectively) in comparison to control cells. Amblyomin-X (100ng/mL), significantly reduced these expressions (PECAM-1:26 ± 3%, VCAM-1:30 ± 7% and ICAM-1:45 ± 6%), exception to β3 integrin. **Discussion:** Findings obtained here show that in presence of the growth factor, Amblyomin-X impaired endothelial cell proliferation and expression of adhesion molecules, specifically, molecules from Ig superfamily, responsible for the homotypic endothelial cell interactions. Altogether, these data contribute to elucidate the mechanism by which Amblyomin-X reduces in vivo new vessels formation. Future investigations will be carried out to clarify the mechanisms involved in this process. **Financial support:** FAPESP (Project 08/57850-8; 08/56072-1).

09.084

Ability of suramin to antagonize permeability alterations induced by honey bee venom.

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In this work we evaluate the ability of suramin, a polyaniont, to antagonize damage induced by *Apis mellifera* crude venom as lethality, hematocrit, vascular permeability, edema and citotoxicity in endothelial cells. Lethality of bee venom was evaluated by measured of death animals. Suramin (30 µg/g) reduce circa of 100% the mortality induced by bee venom when injected before, after or with the venom. Two hours after venom injection the hematocrit of the same animals were measured with the animals under anesthesia, and the blood collected from the orbital plexus. The venom induced an hemoconcentration that was antagonized by suramin (30 µg/g) at the preincubation and pre treatment protocols. The plasma extravasation was assessed by using a i.v. injection of a visual marker, Evans blue and measured the absorbance which was express in arbitrary units. Intradermical injection of bee venom (1 µg/g) induced intense plasma extravasation in the local injection (690.5 ± 23) and was compared with control animals that received only PSS injection (448.55 ± 35.34). The effect of crude venom was reduced to 499.75 ± 44.35; 500 ± 37; 607.7 ± 37 respectively when was preincubated, pre and post treated with suramin (30 µg/g). Paw edema induced by injection of 0.3 µg/g of bee venom was inhibited by suramin (10 µg/g) treatments. In the preincubation and pre treatment the inhibition was 30% and 33% respectively. In the post treatment protocol, suramin was administrated 5 minutes after venom injection, and inhibited circa of 20% the edematogenic effect of A. mellifera venom. Primary cultures of microvascular endothelial cells were obtained from rat cremaster muscle microcirculation according to a method described previously (Silva et al., 2007). Cells culture were incubated per 1 hour with venom (0,01-10 µg/ml) alone or with suramin (0,1-1 mM). After incubation sobrenadant was collected and LDH measured. Data show that suramin 10 mM inhibit completely the citotoxic activity of bee venom. The protective effect of suramin could be result from its polyaniontic properties and its effect seems to be due to the interaction of its charges with the many polycations present in the bee venom. These data are indicative that suramin has a protective effect against damage caused by bee venom components. License of Ethics Committee: DFBCICB 027 **Financial support:** CAPES, FAPERJ, CNPq -PRONEX.

09.085

Purification and characterization of hyaluronidase from venom of the Brazilian spider *Vitalius dubius* (Araneae, Theraphosidae). Sutti R, Tamascia ML, Hyslop S UNICAMP − Farmacologia

Introduction: Hyaluronidase (HYase) is a ubiquitous enzyme in animal venoms and contributes to the diffusion of venom from the site of inoculation. Arthropod venoms are a particularly rich source of HYase. In this work, we purified and partially characterized HYase from the venom of Vitalius dubius (Araneae, Theraphosidae), a large theraphosid found in southeastern Brazil. **Methods:** Venom (10 mg samples) obtained by electrical stimulation of adult male and female V. dubius was initially fractionated by gel filtration on Superdex 75 (1 cm x 30 cm) equilibrated with 0.1 M sodium acetate, pH 6, containing 0.15 M NaCl, at a flow rate of 1 ml/min. The elution profile was monitored at 280 nm and 1 ml fractions were collected and assayed for HYase by a turbidimetric method. The fractions with hyaluronidase activity were pooled and applied to an affinity column of HiTrap Heparin-Sepharose (5 ml) equilibrated with 0.01 M sodium acetate, pH 6. The column was washed with the same buffer and proteins were eluted with a linear gradient of NaCl (0-1 M). The active fractions were pooled and assessed for purity by SDS-PAGE. The pH optimum, heat stability, zymography and neutralization by commercial arachnid antivenom were assessed by conventional techniques. **Results:** HYase was purified in two chromatographic steps with a specific activity of 146 turbidity reducing units (TRU)/mg (venom: 36 TRU/mg; purification factor of ~4). The enzyme eluted before the salt gradient in ion exchange chromatography. HYase had a molecular mass of 43 kDa by SDS-PAGE that was unaffected by b-mercaptoethanol. Zymography in gels containing hyaluronic acid indicated the presence of only one isoform with the same molecular mass detected in SDS-PAGE. The pH optimum was 4-5, with optimal activity occurring at 37°C. HYase was stable at up to 60°C, but rapidly lost activity at higher temperatures; the enzyme was stable in 0.1 M sodium acetate, pH 6, containing 0.15 M NaCl at 4°C and maintained activity after several freeze-thaw cycles. The concentration of NaCl (0.05-1 M) did not influence the activity. The enzyme showed greater activity towards hyaluronic acid compared to chondroitin sulphate and was completely neutralized by polyspecific arachnid antivenom raised against brown spider (Loxosceles spp.), banana spider (*Phoneutria nigriventer*) and yellow scorpion (*Tityus serrulatus*) venoms, but not by antivenoms to caterpillar (*Lonomia obliqua*) venom, scorpion (*Tityus serrulatus* and *Tityus bahiensis*) venoms or snake venoms (Bothrops, Crotalus and Micrurus species). **Conclusions**: The two-step purification used here yielded pure HYase with characteristics similar to those described for other venom HYases. The neutralization by arachnid antivenom, but not scorpion antivenom, indicates that this enzyme shares antigenic epitopes with similar enzymes in other spider venoms, but has little cross-reactivity with venoms from non-spider arachnids. **Financial support:** CNPq, FAPESP

09.086

Effect of oral treatment with crude extract of *Plectranthus neochilus* in models of nociception and injury of the stomach mucosa. Calheiros AS[1], Souza[1], Azeredo JA[2], Castro-Faria-Neto HC[2], Frutuoso VS.[2] [1]FIOCRUZ – Imunofarmacologia, [2]FIOCRUZ − Fisiologia e Farmacodinâmica

Introduction: Various civilizations have found in their forests rich sources of drugs able to meet many different therapeutic needs. In this context we find the species Plectranthus neochilus (Pln), a type of "boldo", widely used in folk medicine as analgesic, stimulating of digestion and heartburn combat. Therefore, we aimed to identify the potential analgesic and ulcerogenic effects of the crude extract of Pln in models of pain evaluation used capsaicin and formalin stimulus, and experimental ulcer. **Methods:** To perform de capsaicin model (CEUA License 033/09) Swiss mice were pretreated with vehicle (saline/p.o./0,2mL/animal), morphine (10 mg/kg) or Pln (100 mg/kg,/p.o.) 1h before the intraplantar (i.pl.) administration of capsaicin (1,6 µg/paw, 20 µl) in the ventral surface of the right hind paw. The time spent licking the injected paw was recorded with a chronometer for 5 min following capsaicin injection and was considered as a nociceptive behavior. In the formalin test animals received i.pl. injection of formalin (2.5%/paw, 20 µl – v/v) into the dorsal right hind paw. One hour before formalin injection, animals was treated with vehicle (saline, p.o., 0,2mL/animal), morphine (10 mg/kg/intraperitoneal – i.p.), dipyrone (200 mg/kg/p.o) or Pln (0,4 e 4 mg/kg, p.o.) and the time spent licking or biting the injected paw was recorded during the periods of 0–5 min (early phase) and 15–30 min (late phase). Induction of injury of the mucosa was performed by Indomethacin (20 mg/kg/0, 2mL po) in rats fasting for 18h, treated 30 minutes prior to the extract of Pln (500 mg/kg), and intensity of ulcerogenic reaction determined by the number of lesions in the stomach lining after 3h of indomethacin administration. **Results:** After i.pl. injection of capsaicin, a significant reduction of the licking response was observed in animals treated with Pln or morphine (36,0 ± 3,77 or 13,17 ± 6,75) compared to control (70,83 ± 14,97). The formalin test show that Pln (4 mg/kg) caused significant inhibition of both early (0–5 min) and late (15–30 min) phases of formalin-induced licking. The maximal inhibition values observed were 38,45% and 54,01% for the first and second phases, respectively. In tests of experimental ulcer, the animals that receiving indomethacin showed a high number of injuries in the stomach lining when compared to baseline (from 5.0 ± 0.71 to 18.6 ± 3.61) and that prior treatment with Pln promotes significant decrease in the number of mucosal lesions (from 18,6 ± 3,6 to 9,6 ± 3,3). It is noteworthy that the treatment with the crude extract of Pln alone does not induce any lesions in the stomach mucosa. **Discussion:** A major use of analgesic / anti-inflammatory drugs is related to its side effects which mainly involve the damage to the stomach lining. it is thus of great importance to seek new effective analgesic agents and have fewer side effects than those used today. The present study demonstrates that Pln administered orally induced analgesic effects in chemical models of neurogenic and inflammatory nociception and is accompanied by important cytoprotection activity of the stomach lining. Additional studies are, however, necessary to investigate the exact mechanism involved in the antinociceptive effects of Pln. **Financial support:** CNPq, FAPERJ, IOC.

09.087

Hecogenin-induced gastroprotection against acute gastric lesions: role of prostaglandins and K^+ channels. Neves KRT[1], Cerqueira GS[1], Siqueira RMP[1], Rocha NFM[1], Freitas APF[2], Vasconcelos SMM[1], Leal LKAM[3], Rios ERV[1], Macedo DS[1], Viana GSB[1], Moura BA[1], Sampaio LRL[1], [1]UFC − Fisiologia e Farmacologia, [2]UFC − Ciências Médica, [3]UFC − Farmácia

The effect of hecogenin (3beta-Hydroxy-5alpha-spirostan-12-one; 5alpha-Spirostan-3beta-ol-12-one), a bioactive component from Agave sisalana folk extract was investigated against gastric damage induced by absolute ethanol (96%, 0.2 ml/animal). The aim of this work was to evaluate the gastroprotective action of hecogenin on ethanol-induced ulcer model in mice, in order to investigate the pharmacological mechanisms involved. The study was submitted to Ethic Committee in animal research of UFC (protocol number: 36/09). **Methods:** Swiss mice, weighing 25-35g, were administered with hecogenin (30 and 90 mg/kg, p.o) and 60 min after ulcer was induced using ethanol, 0.2 mL/animal, p.o. The gastroprotective mechanism was analyzed only at the 90 mg/kg dose with animals pretreated with Glibenclamide (10 mg/kg i.p.), a KATP channel blocker, or indomethacin (10 mg/kg p.o.), a prostaglandins synthesis inhibitor. **Results and Discussion:** The oral administration of hecogenin in both doses was able to protect the gastric mucosa from ethanol-induced lesion in 78.8 and 71.2%, respectively, with $p<0,001$. The gastroprotective effect of hecogenin was significantly antagonized by glibenclamide and indomethacin, suggesting an activation of KATP channels in its gastroprotection, besides, be also associated with stimulation of endogenous prostaglandins. **Financial support:** CAPES and CNPq

09.088

Antinociceptive effect of the hydroalcoholic extract of *Salvia officinalis* in mice. Rodrigues MRA[1], Kanazawa LKS[1], dos Santos FC[1], Neves TLM[1], Pereira IT.[1], Burci LM[1], Santos ARS[2], Pizzolatti GM[3], Baggio CH[1], Werner MFP[1] [1]UFPR − Farmacologia, [2]UFSC − Ciências Fisiológicas, [3]UFSC − Química

Introduction: Salvia officinalis L., popularly known as sage, belongs to Lamiaceae family and has been used as a traditional herbal medicine against a variety of diseases, including gastric disturbances and inflammatory processes. Recently, our group have demonstrated that the hydroalcoholic extract of Salvia officinalis L. (HE) protect the gastric mucosa against ethanol- and acetic acid-induced gastric lesions (Mayer et al., Fitoterapia, 80, 421, 2009). Here, we investigated the pharmacological actions of HE obtained from the leaves of S. officinalis in chemical behavioral models of nociception in mice. **Methods:** Dried leaves of S. officinalis were extracted by percolation with 85% ethanol to obtain the HE. The antinociceptive HE effects were evaluated on three experimental models, namely glutamate (1 µmol/paw), formalin (2.5%/paw) and acetic acid (0.6%, 0.2 ml/10 g, i.p.) tests. Swiss female mice (20-30 g) were treated with HE (1-300 mg/kg) by oral route one hour before the administration of algogens. Each procedure was performed with two repetitions. The experimental procedures were approved by the Ethics Committee on Animal Experiments of UFPR (443). **Results:** HE only at a dose of 30 mg/kg reduced the glutamate-induced nociception by 51% when compared to control (148 ± 19 s). At the end of the experiment (15 min), HE 1 and 3 mg/kg partially reduced the glutamate-induced edema in 35 and 24%, respectively. The treatment with HE 10 and 100 mg/kg reduced the phase II of formalin test by 41 and 52%, respectively (control 131 ± 8 s). Moreover, opioidergic mechanisms are not involved in the antinociception caused by HE in the formalin test, since it was not affected by the non-selective opioid antagonist naloxone. In addition, HE (3-300 mg/kg) did not inhibit the number of abdominal constriction and the infiltration of leukocytes in acetic acid-induced visceral nociception. **Discussion:** These preliminary experimental results suggest that the hydroalcoholic extract of Salvia officinalis has an antinociceptive activity and a partial anti-inflammatory effect in mice. **Financial Support:** CNPq, CAPES e Fundação Araucária

09.089

Antiophidic activity of the Amazon plant *Humirianthera ampla* and its compounds lupeol and sitosterol. Strauch MA[1], Azevedo SM[2], Ricardo HD[3], Lemos BC[3], Tomaz MA[1], Machado MM[3], Melo PA[1] [1]UFRJ − Farmacologia Básica e Clínica, [2]UNIR − Química Orgânica, [3]UFRJ − Farmacologia e Química Medicinal

Introduction: Despite being so far the only therapy officially prescribed for snakebites, specific or polyvalent antivenoms are not always available in some remote places, and present limited efficacy in antagonizing some venom activities, so that sometimes the standard treatment is replaced by folk medicine. We have notice that the use of plants against snakebite of the popular culture is common in Brazil, mainly in the Amazon area. One of these plants is named Humirianthera ampla (HA) and in this work we have investigated its antiophidic activities in different experimental protocols against *Bothrops jararacussu*, *Bothrops atrox* and *Bothrops jararaca* venoms. **Methods:** We studied the effects of the crude extract of HA and compounds on the phospholipase, proteolytic, pro-coagulant, hemorrhagic, edematogenic and myotoxic activities induced by venoms. Myotoxicity was assessed in vivo by plasma creatine kinase (CK) activity in mice, and *in vitro* by the rate of CK release from mouse isolated extensor digitorum longus muscle exposed to venom. The hemorrhagic activity was evaluated by intradermal injection of the venoms alone or pre-incubated with the extract of HA and compounds in mice, as well as by the timing of blood clotting. Antiproteolytic activity was performed by using azocasein as substrate, while phospholipase activity was determined by using as substrate a suspension of chicken egg yolk. **Results:** The extract of HA and compounds inhibited the proteolytic and phospholipase activities of the venoms. In the study of the hemorrhagic activity, B. atrox venom was completely inhibited by the extract in a dose of 300 mg/kg. The extract and compounds showed anti-myotoxic activities in vivo and *in vitro* against the venom of *B. jararacussu*. HA extract also decreased edematogenic and pro-coagulant activities of *B. jararacussu*, B. atrox and *B. jararaca* crude venoms. Conclusions: Our data indicate that the extract of HA and compounds present relevant antiophidic activities, demonstrating that some information about popular culture plants should be investigated. **Financial support:** CAPES, CNPq, PRONEX and FAPERJ

09.090

Evaluation of anti-inflammatory activity of crude extract from *Sapium glandulatum (Vell.)*. Soley BS[1], Mendes DAGB[1], Ferreira BGA[2], Zuffellato-Ribas KC[2], Otuki MF[3], Cabrini DA[1] [1]UFPR − Farmacologia, [2]UFPR − Botânica, [3]UEPG − Ciências Farmacêuticas

Introduction: Sapium glandulatum (Vell) Pax (Euphorbiaceae) is popularly known as "leitera". In Brazil it is distributed mainly from south to southeast. Representatives from this genus are frequently used in folk medicine due anti-inflammatory and analgesic properties. This study aims to evaluate topical and oral effect of crude extract from S. glandulatum (EBSG) on anti-inflammatory activity in models of inflammation induced by 1) croton oil 2) 12-O-tetradecanoilforbol acetate (TPA) and 3) carrageenan. **Methods:** Inflammatory process was topically induced at right ear of male Swiss mice (20-30 g, n = 5-6) by administration of croton oil (0.4 mg/ear) or TPA (2.5 mg/ear). To evaluate systemic effect of extract, paw edema was induced in the hind paw by intra-plantar administration of carrageenan (300 mg/paw). Animals were divided into groups and topically treated on ear with EBSG in different doses (0.03, 0.1, 0.3, 0.6 and 1.0 mg/ear) or dexamethasone (0.1 mg/ear), diluted in 20 µl of acetone and orally treated (1, 10 and 100 mg/kg). Edema formation was assessed by the difference between the thickness before and 6 h after application of phlogistic agent. After 24 h from induction of edema, animals were euthanized and a sample tissue (6 mm) was removed for evaluate myeloperoxidase enzymatic activity (MPO) and for histological preparation. All procedures were approved by the Ethics Committee for Animal Experimentation (CEEA), Federal University of Paraná, Protocol 390.**Results:** Topical application of EBSG was able to inhibit formation of ear edema caused by croton oil in dose-dependent manner, with ED50 = 0.45 ± 0.02 mg/ea and inhibition of 67.8 ± 1.4%, while dexamethasone inhibited 71.9 ± 3.4%. In assessing the MPO activity, we observed a dose-dependent inhibition, with reduction of 76.7 ± 0.7% in treated group with 1 mg of EBSG and 65.0 ± 2.2% in dexamethasone group, when compared with control group. To verify if EBSG was able to inhibit enzyme MPO activity or promotes reduction in cell migration, we evaluated direct effect (*in vitro*) of EBSG (0.01, 0.03, 0.1, 0.3, 1.0, 10, 30, 100 and 300 mg/mL) on MPO activity. *In vitro* evaluation of MPO showed that EBSG was able to reduce enzymatic activity in 81.9 ± 4.1% (300 mg/ml). In histological sections, EBSG reduced number of inflammatory cells in 86.1 ± 0.8%, while dexamethasone inhibited 90.3 ± 0.3%. When EBSG was orally administered caused dose-dependent inhibition of ear edema induced by topical application of TPA and paw edema induced by carrageenin. Dose of 100 mg/kg, caused reduction on ear edema formation of 34 ± 4.4% and of 35.9 ± 6.5% on paw edema formation, both compared to the control group. **Discussion:** Results indicate that crude extract of S. glandulatum has topical and oral anti-inflammatory activity, since it promoted the inhibition of edema formation, cell migration and myeloperoxidase activity. The results are very promising for the continuation of this work, since there are no studies on therapeutic properties and phytochemical characteristics of S. glandulatum. **References**: PANTHONG, A. Anti-inflammatory activity of the alkaloid bukittinggine from Sapium baccaatum. Planta Médica, New York, v. 64, p. 530-535, 1998. **Support**: Capes, CNPq, Fundação Araucária

09.091

Hypericum polyanthemum and its main phloroglucinol derivative uliginosin B present synergistic effect with different antidepressant drugs in the forced swimming test in mice. Stein AC[1], Centurião FB[1], Haas JS[1], Viana AF[2], Do Rego JC[3], Costentin J[3], Von Poser GL[2], Rates SMK[2] [1]UFRJ − Farmácia, [2]UFRGS − Ciências Farmacêuticas, [3]Université de Rouen − Neuropsychopharmacologie Expérimentale

Introduction: Using a chemotaxonomic approach to the search for bioactive molecules in natural products, the study of H. polyanthemum demonstrated antidepressant-like effect of both ciclohexane extract as its main phloroglucinol derivative uliginosina B (Viana, Thesis, 2007). It has already been demonstrated by our group that the lipophilic fraction of Hypericum caprifoliatum and its main phloroglucinol derivative (HC1) display antidepressant-like activity in rodents and inhibits the sinaptossomal uptake of dopamine, noradrenaline and serotonin without interacting with their respective neuronal carriers (Viana et al., Neuropharmacol. 49, 1042, 2005). Thus, this study aimed to investigate the antidepressant-like effect of H. polyanthemum (POL) and uliginosin B (ULI), and the possible involvement of the dopaminergic, serotonergic and noradrenergic system in this activity. **Methods:** POL, ULI, bupropion, fluoxetine and imipramine were testedin the forced swimming test (FST) (Porsolt et al., Eur J Pharmacol 47: 379, 1978) these substances were administered by p.o. route to adult male CF1 mice that had their immobility time assessed during 6 minutes. Then the subeffective doses of POL ciclohexane extract (45 mg/kg) or ULI (5 mg/kg) were co-administered with subeffective doses of bupropion (3 mg/kg), a dopamine reuptake inhibitor; fluoxetine (15 mg/kg), a serotonergic reuptake inhibitor and imipramine (10 mg/kg), a noradrenergic reuptake inhibitor, or vehicle (1mL/100g, p.o.). All the treatments were performed one hour before the test. All results are cited as mean immobility time + SEM (n = 8). Statistical analysis was performed by one-way ANOVA followed by Student-Newman-Keuls test for comparison with control group. The protocols were approved by UFRGS Research Ethical Committee (project number 01-588). **Results and Discussion:** We observed that when animals were treated only with subbeffective doses of POL, ULI or the antidepressants, the values were not significantly different from the saline group (POL- 196.87 ± 15.51, ULI- 223.25 ± 19.42, saline: 221.60 ± 8.78, bupropion- 187.25 ± 20.40, fluoxetine- 169.62 ± 12.09 and imipramine- 185.62 ± 19.61). While all the associations of subeffective doses for both POL and ULI, were positive, i.e. the immobility time of mice was reduced when compared to the saline group ($p<0.001$). The values obtained from associating subeffective doses of POL with antidepressants sub-doses were: bupropion- 98.87 ± 15.76 s; fluoxetine- 108,71 ± 19,68 and imipramine- 80,50 ± 17,08, and the values of the associations for ULI were: bupropion- 78,66 ± 15,94; fluoxetine- 101,50 ± 21,69 and imipramine- 125,87 ± 15,59. These results demonstrate a potential synergistic effect between POL or ULI with the three systems studied, dopaminergic (bupropion), serotonergic (fluoxetine) and noradrenergic system (imipramine). **Financial support:** CNPq, CAPES

09.092

Cardiovascular effects of *Syzygium cumini* L. Skeels fruit extract in rats. Tenório EP[1], Ferreira AKB[1], Oliveira DA[1], Aquino PGV[2], Araújo-Júnior JX[1], Santana AEG[2], Ribeiro EAN[1] [1]ESENFAR-UFAL, [2]UFAL − Química e Biotecnologia

Introduction: Syzygium cumini (L.), popularly known as jambolão, is a plant known for its various pharmacological actions such as: activities antidiabetic, antidiarrheal, antioxidant and antimicrobial properties (1). This study evaluated the cardiovascular effects induced by ethanol extract of the fruits of Syzygium cumini (SCEE) in rats. **Methods:** Normotensive Wistar rats and spontaneously hypertensive rats (270-300 g) were anesthetized with sodium pentobarbital and polyethylene catheters were inserted into the abdominal aorta and inferior vena cava for measurements of blood pressure and drug administration, respectively. The experiments were performed 24 hours after surgery. The study was approved by the ethics committee of the Federal University of Alagoas (010151/2008-82). **Results:** In non-anesthetized normotensive (n = 6), administration of the SCEE (0.5; 1; 5; 10; 20 and 30 mg/kg; i.v., randomly) induced hypotension (-16 ± 2, -12 ± 3, -16 ± 3, -18 ± 3, -18 ± 2 and -16 ± 2 mmHg) and changes in heart rate (33 ± 6; 21 ± 9, -12 ± 7, -25 ± 9,-15 ± 4 and -4 ± 1 bpm). Both responses were not attenuated after blockade with L-NAME (20 mg/kg, iv). In SHR non-anaesthetized rats (n = 6), SCEE (0.5; 1; 5; 10; 20 and 30 mg/kg; i.v., randomly) induced hypotension (-17 ± 2, -16 ± 3, -13 ± 3, -14 ± 3, -16 ± 2 and -20 ± 5 mmHg) and bradycardia (-10 ± 6; -5 ± 1, -6 ± 1, -30 ± 6, -8 ± 1 and -14 ± 2 bpm). **Discussion:** The results showed that the extract produced a hypotensive response in both normotensive rats and in hypertensive rats. The L-NAME was also not capable of significantly changing SCEE-induced hypotension in rats normotensive, suggesting that NO appears not be participating of this effect. **Financial support:** CNPq, FAPEAL and PPSUS-MS. **Acknowledgements:** FAPEAL, CNPq, UFAL, PPSUS-MS. **References**:

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09.093

Subchronic toxicity of a proteolytic fraction from *C. candamarcensis* latex: qualitative histopathological analysis. Villalba MIC[1], Bilheiro RP[2], Salas CE[3], Cassali, G. D.[4], Vasconcelos A[4], Tagliati CA[5], Lopes MTP[2] [1]UFMG − Fisiologia e Farmacologia, [2]UFMG − Farmacologia, [3]UFMG − Bioquímica e Imunologia, [4]UFMG − Patologia Geral, [5]UFMG − Farmácia

Introduction: Our research group has been involved in the biochemical, pharmacological and toxicological characterization of a proteolytic fraction (P1G10) from Carica candamarcensis latex. In previous studies, P1G10 has shown a variety of effects upon pathophysiological systems, such as mitogenesis in mammalian cells, angiogenesis, antitumor and antimetastatic effect, gastric wound healing and cicatrization of skin injuries. Based on these findings, our group proceeded to determination of the toxicological patterns. The histopathological analysis reported in this abstract, which followed the instructions of the Organization for Economic Cooperation and Development (OECD, guideline n. 408, Paris, 1998), are part of the Subchronic Toxicity Assays performed to P1G10. **Methods:** The histopathological studies were conducted in Wistar rats (protocol n. 91/09, CETEA/UFMG), both male and female, 8 weeks old. Groups of 10 animals, 5 male and 5 female, were randomly separated to be given each a different test dose of P1G10 (10, 50, 100 or 300 mg/kg) or vehicle, p. o. daily for 90 days. All groups were kept in a controlled environment with free access to food and water. After the treatment period, the animals were anesthetized by i. p. route, euthanized and the selected organs (heart, spleen, liver, kidneys, adrenal glands, lungs, stomach, small intestines (Peyer's patch) and reproductive organs) were collected. All organs were weighed and submitted to macroscopical analysis. Histological slices of 5 mm were submitted to the hematoxylin and eosin staining protocol, as well as to terminal deoxynucleotidyl transferase-mediated dUDP nick-end labeling (TUNEL) assay, which aims at the detection of possible apoptotic bodies in the liver. **Results and Discussion:** In all animals treated with P1G10 in the doses of 10, 50 e 100 mg/kg, the analysis of the selected organs revealed no difference in the weight and in the macroscopical aspect, neither in the histopathological evaluation, compared to controls. However, 70% of the animals that were treated with 300 mg/kg died between the 30th and the 40th days after the beginning of the treatment. The remaining animals showed lung alterations, such as hepatization with thickening of the interalveolar septa with mixed inflammatory infiltration, characterizing interstitial pneumonitis. In the liver, apoptotic bodies were found, which was confirmed by the TUNEL assay. These findings suggest that P1G10 produces lung toxicity if given in repeated high doses (300 mg/kg), pointing to the lung as a more susceptible organ to toxic effect in the subchronic treatment. Our results also showed that P1G10 produced traces of apoptosis in the liver in the higher dosage tested. According to the European Medicines Agency (EMEA, 2009) the clinical dosage of a test substance must be at least 10-fold lower than the one found toxic in repeated doses. Based on our findings and in the international recommendations, dosages lower than 30 mg/kg of P1G10 might be considered safe in future clinical trials. **Financial support:** CNPq, CAPES and FAPEMIG.

09.094

Effect of salvia (*Salvia officinalis*) hydroalcoholic extract on the topic anti-inflammatory response in mice. Lopes VM, Fonseca JP, Melo GAN, Damião JM, Freitag A, Amado CAB, Cuman RKN UEM − Farmácia e Farmacologia

Introduction: Salvia officinalis L. (Lamiaceae) is an aromatic and medicinal plant commonly known in Brazil as Salvia. The plant has been used as condiment and in folk medicine to treat various ailments, from gastrointestinal discomfort until infection diseases. In this work the topical anti-inflammatory activity of hydroalcoholic extract of Salvia officinalis (EHS) was evaluated. **Methods:** Salvia officinalis leaves were collected from the Herbarium of the State University of Maringá. The shade-dried powder was extracted directly with 90% ethanol (hydro-alcoholic extract), concentrated under reduced pressure and lyophilized. The dexametasone (standard anti-inflammatory drug) was topically applied at mice ear one hour before inflammatory stimuli (croton oil). The animal's right ear were topically treated with EHS (5; 2.5; 1.25; 0.625 and 0.312 mg/ear), croton oil (5% v/v) and dexametasone (DEX)(0,1 mg/ear). All drugs were diluted or dissolved in acetone (vehicle). Eight animals were used for each group. Four hours after, the ear weight (edema volume) and the myeloperoxidase (MPO) activity were evaluated according to the technique of Bradley & Priebat (1982). MPO is an enzyme present in the intracellular granules of neutrophils, and can be used as a marker for the influx of polymorfonuclear leucocytes into inflamed tissues. The MPO activity was evaluated in the supernatant of homogenates of the ear sections. The ear tissue was placed in potassium phosphate buffer, ph 6.0l containing 0,5% hexadeciltrimethyl ammonium bromide (HDTMA) in a homogenizer. The supernatant was added to a 96-wells microplate, followed by addition of a potassium phosphate buffer solution containing o-dianisidine dihydrochloride and 1% H_2O_2. The enzyme activity was determined by measuring the optical absorbance (460 nm) ELISA reader. The results were statistically analyzed using one-way ANOVA followed by Turkey test. The difference were considered significant at $P<0.05$*. The protocol regarding this study was approved by the ethical commission of ethics and in animal research (041/2008/CEAE-UEM). **Results and Discussion:** After topical administration of croton oil an increased ear edema was observed. However, a reduced ear edema reduction was observed after EHS and DEX treatments when compared to that of control group: croton oil: 0.02 ± 0.0006*g; EHS5mg 0.013 ± 0.0003*g; EHS2.5 mg 0.01 ± 0.0003*g; EHS1.25mg 0.013 ± 0.00004*g; EHS0.625mg 0.014 ± 0.00006*g and EHS0.312mg 0.017 ± 0.0006g); DEX: 0.01 ± 0.001*g. A reduced MPO activity was observed in after DEX and EHS treatment group treated with croton oil. (Croton oil 0,172 ± 0,012; EHS5mg 0.093 ± 0.005*; EHS2.5 mg 0.084 ± 0.005*; EHS1.25mg 0.078 g ± 0.0075*; EHS0.625mg 0.11 ± 0.008* and EHS0.312 mg 0.15 ± 0.022 when compared to that observed for the control group. Conclusion: The topical anti-inflammatory effects of EHS can be related to the inhibition of edema formation and leukocyte chemotaxis stimuli. Supported by: Capes/CNPq Reference: BRADLEY, P.P.; PRIEBAT, D.A.; CHRISTENSEN, R.D.; ROTHTEIN, G. Measurement of cutaneous inflammation: stimulation of neutrophil content with an enzyme marker. The Journal of Investigative Dermatology, v.78, n.3, p206-209, 1982.

09.095

Spasmolytic action of *Solanum agrarium* sendtner (Solanaceae) involves blockade of L-type calcium channels on guinea-pig ileum. Oliveira GA[1], Correia ACC[1], Santos RF[1], Agra MF[1], Silva TMS[2], Silva BA[1] [1]LTF-CCS-UFPB − Ciências Farmacêuticas, [2]DQ-UFRPE

Introduction: *Genus Solanum* belongs to the Solanaceae family. This genus is well represented in Brazil and is widely distributed from north to south in diverse phytogeographic regions (Roe, Brittonia, p. 239, 1972). In the Northeast of Brazil, many Solanum species, commonly known as “jurubeba”, are widely used in folk medicine (Agra; Bhattacharyya, Royal Botanic Gardens, p. 341, 1999). Solanum agrarium Sendtner is known popularly as “babá”, “gogóia”, and “melancia da praia”. In a preliminary study, Santos et al. (Iniciados, 9 ed, p. 98, 2003) demonstrated that crude ethanolic extract of aerial parts from S. agrarium (SA-EtOH) showed non-selective spasmolytic activity on guinea-pig ileum. Therefore, we decided to investigate the spasmolytic action mechanism of SA-EtOH on guinea-pig ileum. **Methods:** The extract SA-EtOH was obtained from aerial parts of Solanum agrarium Sendtner and was gently provided by collaborators in phytochemistry. Guinea-pig ileum was suspended in organ bath containing modified Krebs solution (pH 7.4) at 37° C, gassed with 95% O2 and 5% CO2 mixture and resting tension of 1 g. Isotonic contractions were recorded on a smoked drums through levers coupled to kymographs. Isometric contractions were registered through force transducer coupled to amplifier, which was connected to a microcomputer. All experimental protocols were carried out according to “Pharmacological techniques for the *in vitro* study of intestinal smooth muscle” (Daniel et al., Journal of Pharmacological and Toxicological **Methods:**, v. 45, p. 141, 2001). All the experimental protocols were approved by Ethical Committee in Animal Research of LTF/UFPB (Protocol 0605/09). **Results:** SA-EtOH (9- 750 µg/mL) inhibited the cumulative concentration-response curves to CCh and histamine, and these were shifted to the right, in a non-parallel manner, with reduction of the maximal effect (Emax), suggesting a noncompetitive antagonism. SA-EtOH (0.1 – 750 µg/mL) relaxed of significant and concentration dependent manner the ileum pre-contracted with 40 mM KCl (EC50 = 17.4 ± 3.5 µg/mL), 10-6 M CCh (EC50 = 119.4 ± 24.3 µg/mL) or 10-6 M histamine (EC50 = 18.7 ± 4.6 µg/mL), suggesting that SA-EtOH extract could be acting on voltage-gated Ca2+ channels (Cav). This assumption was confirmed by observation that SA-EtOH (9 – 750 µg/mL) antagonized the CaCl2 induced contractions in the depolarizing medium without Ca2+. The finding that SA-EtOH (0.1 – 750 µg/mL) relaxed (EC50 = 187.2 ± 27.3 µg/mL) guinea-pig ileum pre-contracted with S-(-)-BAY K8644 suggests that the Ca2+ channel subtype involved is the CaV1. As the SA-EtOH was more potent in relaxing the organ pre-contracted with KCl than by S-(-)-Bay K8644, it is suggestive of indirect blockade of the Cav1. **Discussion:** These results suggest that the spasmolytic action mechanism of SA-EtOH extract no involves muscarinic or histaminergic receptors, but an indirect blockade of the Cav1. Supported by: CNPq, CAPES, LTF/UFPB.

09.096

Hydroxydihydrocarvone, a monoterpene derivative, decreases carrageenan-induced inflammation in rodents. Camargo E, de Souza DP UFS− Fisiologia

Introduction: Hydroxydihydrocarvone (HC) is a synthetic intermediate obtained by hydration of the monoterpene (R)-(-)-carvone, that has a structural similarity with monoterpenes found in many plant-derived essential oils. A previous study showed that HC exerts significant depressant and antinociceptive effects on the central nervous system of mice (De Sousa DP et al; Biol. Pharm. Bull.; vol 29, pg 811, 2006). The aim of the present study was to investigate the possible anti-inflammatory activity of orally administered HC. **Methods:** All experimental protocol was approved by the Ethics Committee of this institution (CEPA/UFS, number 47/2010). Carrageenan -induced paw edema (0.5 mg/paw; 0-4 h) and MPO activity in male Wistar rats (150-200 g), as well as, carrageenan-induced leukocyte migration (4 h) to peritoneal cavity of Swiss mice (25-30 g) were evaluated. Animals were treated with HC (50-200 mg/kg) or vehicle (Tween 80 at 5%) 1 h before carrageenan injection into paws or peritoneal cavities. Dexamethasone (2 mg/kg)-pretreated animals were used as controls for both paw oedema and peritonitis experiments. Data are presented as mean ± S.E.M. of n=5-8 animals. Statistical analyses were performed by using ANOVA followed by Bonferroni's t-test. $P<0.05$ was taken as significant. **Results and Discussion:** HC significantly decreased the area under curve of carrageenan-induced rat paw edema at 100 and 200 mg/kg (1.8 ± 0.2 and 1.6 ± 0.2 mL.h, respectively) when compared with vehicle -treated animals (3.0 ± 0.1 mL.h). Additionally, MPO activity in rat paws was decreased by the treatment with HC at 200 mg/kg (2.7 ± 1.9 UMPO/mg of tissue), when compared with vehicle-treated animals (11.3 ± 1.1 UMPO/mg of tissue). The other doses used of HC (50 and 100 mg/kg) did not significantly affect the rat paw MPO activity. Carrageenan-induced neutrophil recruitment to mice peritoneal cavity was significantly reduced by HC at doses of 100 or 200 mg/kg (4.0 ± 0.5 and 4.9 ± 0.6 x106 cell/cavity), but not 50 mg/kg, when compared with vehicle group (8.3 ± 0.2 x106 cell/cavity). Total and mononuclear counts in mice peritoneal cavity were not significantly affected by HC treatment. As expected, treatment of animals with dexamethasone also decreased either the rat paw oedema/MPO activity or mice leukocyte migration to peritoneal cavity. These findings demonstrate that orally-administered HC exerts anti-inflammatory actions in rats and mice. We suggest that this molecule can be of value to treat inflammation or permit the development of other structurally similar molecules with anti-inflammatory potential. **Financial support**: FAPITEC/SE and CNPQ.

09.097

Evaluation of anethole obtained from *Foeniculum vulgare Mill* essential oil on renal ischemia and reperfusion in mice. Fonseca JP[1], Lopes VM[1], Damião MJ[1], Pinheiro RJ[1], Giannocco G[2], Bersani-Amado CA[1], Cuman RKN[1] [1]UEM − Farmácia e Farmacologia, [2]USP − Fisiologia e Biofísica

Anethole (ANE) is the main constituent from fennel essential oil (Foeniculum vulgare Mill.). It has been demonstrated many biological activities for this oil, such as: dispsia, gastrointestinal disorders, antispasmodic and anti-inflammatory. The ischemia (I) is related to the interruption of blood supply in oxygen and nutrients during a determined period of time. In the ischemic injury after renal reperfusion (R) where the blood flow is restored in the ischemic tissue. In this work the effect of anethole treatment on the renal function was evaluated in a renal ischemia/reperfusion experimental model in mice. **Methods:** The protocol regarding this study was approved by the ethical commission of ethics and in animal research (041/2008-CEAE/UEM). Male Swiss mice (20 to 28g) were anaesthetized with ketamine (100 mg/kg; i.p.) and xylazin (10 mg/kg, i.p.). The animals were submitted at the renal pedicle unilateral I/R procedure during 45 minutes, according to KELLY et al.,1996. The experimental groups were: a) anethole (200 mg/kg, once a day); b) Sham: animals submitted to I/R procedure; and c) control: non-ischemic animals receiving saline. The animals were treated by gavage during 12, 24 e 48 hs. The mice were euthanized and both seric creatinin and urea were determined. The results were expressed as average ± S.E.M., and were statistically analyzed by ANOVA ($p \leq 0.05$). **Results:** seric creatinin: Data showed significant differences in creatinin levels after ANE treatment when compared to sham group: Sham: 12h: 0.23 ± 0.02mg/dl; 24h: 0.53 ± 0.02* mg/dl, ($p<0.001$); 48 h: 0.41 ± 0.01mg/dl; Control: 0.34 ± 0.01mg/dl; and ANE: 12h: 0.42 ± 0.03*mg/dl, $p<0.0001$; 24h: 0.35 ± 0.02*mg/dl; $p<0.0001$; 48h: 0.34 ± 0.02mg/dl. Seric urea: An increased urea levels were observed after 12h of ANE treatment, whereas no significant differences were observed after 24 and 48 h of treatment: Control: 66.27 ± 5.64mg/dl; Sham 12h: 38.24 ± 6.63mg/dl; 24h: 56.75 ± 3.75mg/dl; 48h: 52.73 ± 6.71 mg/dl; ANE200 mg/kg:12h: 82,21 ± 2,26*mg/dl,$p<0.0001$; 24h: 52,95 ± 3,13mg/dl; and 48h: 46,52 ± 1,65mg/dl. Conclusion: Anethole treatment during 24 and 48 h has a protective effect in renal ischemic mice. Supported by: Capes/CNPq Reference: Kelly KJ, Bonventre JV. Ischemia/reperfusion injury in transplantation: Tilney NL, Strom TB, Paul LC, editors. Transplantation biology cellular and molecular aspects. Philadelphia: Lippincott-Raven; 1996. pp. 257-274

09.098

Inhibitory effects of *Combretum leprosum* Mart. & Eicher, *Protium heptaphyllum* March and *Copernica prunifera* on glycated hemoglobin. Piauilino CA[1], Sales Filho HLA[1], Sousa VR[1], Ayres MCC[2], Carvalho AA[2], Chaves MH[2], Brito SMRC[1] [1]NPPM-UFPI, [2]UFPI − Química

Introduction: the non-enzymatic glycation of proteins is a process related to chronic hyperglycemia and long-term complications of Diabetic patients, such as retinopathy, cataract, atherosclerosis, neuropathy, nephropathy which induce to a smaller life expectancy. Anti-glycation agents can contribute to prevention of chronic diabetic complications acting on different mechanisms, including antioxidant actions which may protect against free radicals derived via autoxidative glycation, glycoxidation and AGEs (Ahmed, N. Diabetes Res. Clin. Pract. (67): 3, 2005). In the research reported here, we investigate the effects of three plants on the formation *in vitro* of glycated hemoglobin comparing with commercial flavonoids (quercetin and rutin). The hydroalcoholic fraction (HAF) obtained from the partition of the ethanolic extract of barks of Combretum leprosum Mart. & Eicher (mofumbo, voucher specimen no 10.557, Combretaceae), α-amirin and β-amirin mixture was extracted from the resin of Protium heptaphyllum March (almêcega, voucher specimen no 18.247, Burseraceae) and Carnaubadiol was obtained from the commercial wax of Copernica prunifera (Arecaceae). **Methods:** blood from 5 rats (Animal ethics committee 03A/08) was collected into tubes containing EDTA and centrifuged at 3,000 rpm for 5 min. Red cell suspension was filtered through cotton wool. The red blood cells were washed thrice with cold 0.15 M sodium chloride solution and centrifuged at 3,000 rpm, 5 min each time. Red blood cells suspension was diluted with phosphate buffer (0.01 M, pH 7.4) and lysed with CCL4 at a ratio of 1:2:0.5 (v/v/v). The tubes were centrifuged at 3,000 rpm, 5 min (Asgary, S. Pharm. Act. Helvet., (73): 223, 1999). Concentration of supernatant hemoglobin was 8g dL-1 detected spectrophotometrically using assay kit based on method of potassium ferricyanide and potassium cianyde reaction. The control tube was incubated with 1 mL of supernatant hemoglobin (8 g dL-1), 1 mL of phosphate buffer (0.01 M, pH 7.4), glucose (50 mM) and gentamycin (20 mg dL-1) for 48 h in the dark at room temperature. HAF (50 mg mL-1), α-amirin and β-amirin mixture (50 mg mL-1), carnaubadiol (50 mg mL-1), quercetin (50 mg mL-1) or rutin tubes (50 mg mL-1) were incubated under the same conditions of controls tubes. After incubation, samples were treated with boric acid (1 M) to remove the unstable fraction. The amount of glycated hemoglobin (%HbA1c) was determined by ion exchange chromatography (Lino, C. S. Am. J. Pharm. Toxic, (2): 178, 2007). **Results and Discussion:** data represent mean ± SD, *p<0,05. The results showed that HAF, carnabaudiol, resin and amyrins of P. heptaphyllum, quercetin and rutin revealed inhibitory effects on the formation of HbA1c (% of total Hb) *in vitro*, described as follow: Control tubes (8,74 ± 0,50), Quercetin (4,25 ± 0,28*), Rutin (6,32 ± 0,2*), Resin (5,97 ± 0,62*), α e β-Amyrins (5,22 ± 0,22*), Carnaubadiol (4,2 ± 0,15*), HAF (5,54 ± 0,7*). The action of triterpenoids α and β-amirins in proteic glication can be related with antioxidant effect (Oliveira, F. A., J. Ethnopharmacol.,(98): 103, 2005). More investigation of antioxidant action and AGEs production may be evaluated as a future perspective. Support by CAPES, FINEP, CNPq.

09.099

Involvement of potassium channels and cyclic nucleotides in the tocolytic action of Labdane-302 on rat uterus. Travassos RA, Macedo CL, Santos RF, Oliveira GA, Silva ACL, Carreiro JN, Ferreira TF, Tavares JF, Silva BA LTF-UFPB − Ciências Farmacêuticas

Introduction: *Xylopia langsdorfiana* A. St.-Hil. & Tul. (Annonaceae) species is popularly known in northeast Brazil as "pimenteira da terra" (CORREA, M. P., Dicionário de plantas úteis do Brasil e das exóticas cultivadas, p. 315, 1984). The labdane-type diterpene identified as 8(17),12E,14-labdatrien-18-oic acid (labdane-302), isolated from hexanic phase of the crude ethanolic extract of the stem bark of X. langsdorfiana showed spasmolytic effect on rat uterus through modulation of K+ channels that, indirectly, can block the CaV-channels leading to that effect. There are evidences that any diterpenes, such as forskolin, modulate indirectly opening potassium channels by phosphorylation via PKA (WELLMAN et al., Journal of Physiology, v. 507, p. 117 1998; WINKLHOFFER et al., Neuropharmacology, v. 44, p. 829, 2003). Substances capable of increasing the content of cyclic nucleotides has its effect relaxing potentiated by inhibition of phosphodiesterasis (PDE) in various tissues, due to an accumulation of the total content of these nucleotides (BENDER; BEAVO, Pharmacological Reviews, v. 58, p. 488, 2006; LUGNIER, Pharmacology & Therapeutics, v. 109, p. 366, 2006). About the tocolytic action, labdane-302 relaxant effect was decreased in the presence of CsCl, a non-selective K+ channels blocker (TRAVASSOS et al., SBFTE, 2008). Thus, we aim to verify which subtypes of potassium channels could be involved and the possible involvement of cyclic nucleotides in relaxation induced by labdano-302. **Methods:** To investigate the mechanism of action tocolytic, the uterus was suspended in organ bath containing Locke Ringer solution (pH 7.4) at 32 °C, gassed with 95 % O2 and 5% CO2 mixture and resting tension of 1 g. Isometric contractions were registered through force transducer coupled to amplifier, which was connected to a microcomputer. All experimental protocols were carried out according to “Pharmacological techniques for the *in vitro* study of the uterus" (Crankshaw, v. 45, p. 123, 2001). All the experimental protocols were approved by Ethical Committee in Animal Research of LTF/UFPB (Protocol 0705/09). **Results and Discussion:** The relaxation induced by diterpene was not reduced significantly (n = 5) by 3 x 10-5M glibenclamide (KATP blocker). However, the relaxant potency of labdane-302 was reduced about 3 folds in the presence of 100 mM apamine (SKCa blocker) (pD2= 3.8 ± 0.03, n = 5) in a concentration dependent manner. In the presence of aminophylline (PDE inhibitor) the relaxant potency of labdane-302 was increased (pD2 = 7.8 ± 0.1, n = 5) about 320 folds in a significant and concentration-dependent manner. The results suggest that tocolytic effect of labdane-302, involves SKCa and BKCa activation leading to blockage of CaV, besides the participation of cyclic nucleotides. Supported by: CAPES, CNPq, LTF/UFPB

09.100

Involvement of K^+ channels on spasmolytic effect of the fraction of the total alkaloids from *Solanum paludosum* Moric. root bark on guinea-pig ileum. Silva ACL[1], Monteiro FS[1], Martins IRR[2], Travassos RA[4], Santos RF[2], Agra MF[1], Basílio IJLD[2], Bhattacharyya J[2,] Silva BA[1], [1]LTF-UFPB − Ciências Farmacêuticas, [2]LTF-CCS-UFPB

Introduction: *Solanum paludosum* Moric. (Solanaceae) is herbaceous species, known popularly as "jurubeba-roxa" in the Northeast of Brazil. (AGRA, M.F., Royal Botanic Gardens, p. 341, 1999). Chemical studies of the root bark of this species showed the presence of steroid alkaloids and its glycosides (BASÍLIO, Dissertação, 2008), and about pharmacological studies of the fraction of total alkaloids from root bark (FAT-SP) has shown spasmolytic activity on guinea-pig ileum. We observed that FAT-SP relaxes in a more potent manner pre-contracted ileum by carbachol, when compared with histamine and KCl. Since the maximum relaxing effect of FAT-SP was attenuated when the organ was pre-contracted by KCl 40 mM, we decided to study the mechanism of spasmolytic action observed in isolated guinea-pig ileum, investigating the involvement of potassium channels. **Methods:** Guinea-pig ileum was suspended in organ bath containing modified Krebs solution (pH 7.4) at 37° C, gassed with 95 % O2 and 5 % CO2 mixture. Isometric contractions were recorded. All the experimental protocols were approved by Ethical Committee in Animal Research of LTF/UFPB (Protocol 0111/09). **Results:** FAT-SP spasmolytic effect (EC50 = 37.2 ± 3.8 µg/mL, n = 5) was attenuated significantly in the presence of CsCl 5mM (EC50 = 307.6 ± 55.5 µg/mL, n = 5), a non-selective blocker of K+ channels. We decided to investigate which subtypes of K+ channels participate in this FAT-SP response. Interestingly, the FAT-SP relaxation effect was reduced significantly by TEA+ 1mM (EC50 = 79.3 ± 11.4 µg/mL, n = 5), blocker of BKCa, and apamin 100 nM (EC50 = 78.8 ± 10.9 µg/mL, n = 5), blocker of SKCa. **Discussion:** According to obtained results, we can suggest that in functional level, the spasmolytic effect of FAT-SP on guinea-pig ileum seems to involve a non-selective activation of K+ channels (BKCa and SKCa), and probably FAT-SP is indirectly blocking the calcium channels due to a positive modulation of the potassium channels, thus leading to relaxation of smooth muscle. However further studies are necessaries to investigate the participation of other potassium channels (KATP, KIR, Kv) in mechanism of spasmolytic action of FAT-SP. Financial supported: CNPq, CAPES, LTF/UFPB

09.101

Evaluation of the activity of the crude extract, fractions and isolated compounds obtained from the leaves of *Chrysophyllum cainito* against sensory changes present in experimental models of clinical pain in rodents. Meira NA[1], Quintão NLM[1], Cechinel Filho V[1], Klein Jr LC[2], Martin Z[3], Rodriguez LMP[3] [1]NIQFAR-UNIVALI – Ciências Farmacêuticas, [2]UNIVALI − Produtos Naturais e Substâncias Bioativas, [3]CICY − Biotecnologia

Introduction: Preliminary analysis of the crude methanolic extract (CME) of leaves of Chrysophyllum cainito performed by our group, it was demonstrated that this specie presents significant antinociceptive activity in the model of mechanical hypernociception induced by carrageenan-injection. In the literature, has been described that the species has diuretic, antioxidant, antipyretic, antitussive and anti-tumoral activity. So there is the need for further investigation about the effect of this plant in front of chronic pain processes, including the identification of the active principle responsible for the antinociceptive property and the possible mechanism of action. Methodology: Swiss female mice were used (25-35g, n = 6-8). The animals were pretreated intraperitoneally with the CME of Chrysophyllum cainito leaves (3, 10 and 30 mg/kg) or saline (negative control). After 30 minutes, mice were injected with carrageenan (300 mg/paw), LPS (100 ng/paw) or PGE2 (0.1 nmol/paw). After that the animals were evaluated using the electronic von Frey in different time point. All the procedures were approved by the Animal Ethics Committee of UNIVALI (protocol number 008/10 UNIVALI). **Results:** The CME of Chrysophyllum cainito leaves, when administered intraperitoneally 30 minutes before the i.pl. injection of carrragenina, was able to significantly inhibit the mechanical hypernociception in a dose dependent manner, with maximal inhibition of 74.4 ± 12.2%. This extract was also capable to diminish the mechanical sensitization induced by PGE2 or LPS, with maximal inhibition of 54.4 ± 11.6% and 81.09 ± 5.2%, respectively. Conclusion: These results demonstrate that the significant antinociceptive effect of CME of Chrysophyllum cainito leaves in different models of mechanical hypernociception in mice (carrageenan, PGE2 and LPS). This data confirms, in part, the folk use of Chrysophyllum cainito to treat painful processes. **Financial Support:** CNPq, FAPESC-SC, ProPPEC/UNIVALI.

09.102

Gastroprotective activity of chloroform and aqueous fractions obtained of hydroalcoholic extracts of *Brassica oleracea* L. var. *acephala* DC. Lemos M, Santin JR, Oliveira AP, Klein LC, Niero R, Andrade SF NIQFAR-CCS-UNIVALI

Introduction: In recent years, there is an increase of work correlating the foods and their biologically active components on human health. The digestive tract is responsible for processing of food and absorption of nutrients. The pathophysiology of gastric ulcer has focused on an imbalance between factors in the stomach, factors offensives (gastric acid secretion) and defensive (gastric mucosal integrity) of the mucosal. Brassica oleracea L. var. acephala DC (Brassicaceae) is known in Brazil as “couve”, and is popularly used in the form of juice for the treatment of ulcers and gastritis. In our laboratory, we tested the gastroprotective activity of hydroalcoholic extract from leaves of B. oleracea L. var. acephala DC, showed significant gastroprotective activity in addition to decrease the acidity of the secretion and increase the amount of mucus in the stomach. Seeking possible metabolites responsible for the gastroprotective activity, the extract fractionated with solvents of increasing polarity, obtaining the hexane (FH), chloroform (FC), ethyl acetate (FAc) and water (FAq), which were parse for their potential gastroprotective. **Methods:** Swiss mice were used throughout this study (25-35g, n=6). The gastroprotective assays was evaluated by using models of acute gastric lesions induced by ethanol/HCl (MIZUI, Jpn J Pharmacol., 33, 939, 1983), nonsteroidal anti-inflammatory drug (NSAID)/betanechol (RAINSFORD, Biochem. Pharmacol., 29, 1281, 1980), with some modifications. All the procedures used in the present study were approved by the Animal Ethics Committee of UNIVALI (Protocol numbers 369/09a UNIVALI). **Results:** The ethanol/HCl-induced ulcer model, it was observed that the treatment with 50 mg/kg of FC and FAq; 30 mg/kg omeprazole significantly cure ratio (51.60 ± 10.19; 43.20 ± 11.68 and 72.14 ± 8.83, respectively), compared with the control group. The FH and FAc did not show gastroprotective activity. Seeking to evaluate the dose response, evaluated FC and FAq in doses of 25, 50 and 100 mg/kg. The doses of 50 and 100 mg/kg FC showed a cure ratio of 54.71 ± 1.72 and 61.18 ± 2.95, respectively; 50 and 100 mg/kg to a cure ratio 43.78 ± 2.90 and 68.57 ± 1.17, respectively, when compared with omeprazole (79.50 ± 2.63) and control group. The dose of 25 mg/kg was inactive. In the NSAID/betanechol-induced ulcer model, a cure ratio in 41.88 ± 4.87 and 63.27 ± 5.65 for the FC; 34.88 ± 2.04 and 61.58 ± 5.33 for the FAq, respectively, when compared with cimetidine 100 mg/kg (71.18 ± 1.98) and control group. The dose of 25 mg/kg was inactive. **Discussion:** The survey of secondary metabolites in foods is a promising field in search of functional combinations, preventive or curative. Results presented tend to confirm the use ethnopharmacological of Brassica oleracea L. var. acephala DC, demonstrating that the metabolites present probably act in synergy. Additional experiments are being conducted to determine the mechanisms involved in gastroprotection and isolation of the substances responsible for gastroprotection.

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Antinociceptive effects of *Rheedia longifolia* Planch & Triana aqueous extract and its fractions. Nascimento DD[1], Siqueira AM[1], Costa NF[1], Bérenger ALR[2], Castro-Faria-Neto HC[1], Figueiredo MR[2], Frutuoso VS[1] [1]IOC-FIOCRUZ − Imunofarmacologia, [2]Farmanguinhos-FIOCRUZ − Produtos Naturais

Introduction: Earlier studies with aqueous extract from leaves of Rheedia longifolia (Clusiaceae family), demonstrated important antinociceptive effects, with low toxicity. A fractionation of this extract was realized using different solvents (hexane, dichloromethane, ethyl acetate, butyl alcohol and purified water). Among these fractions, ethyl acetate fraction (RhFAcEt) was the one that presented the best activity in murine models of inflammatory pain, while butanolic fraction (RhFBuOH) prevented neurogenic pain. *In vitro* analyses suggest the inhibition of purinergic receptor P2X7 is one of this action mechanism, however the mechanism of inhibition of neurogenic pain is still unknown. The aim of this study was evaluate RhFAcEt e RhFBuOH antinociceptive effects. **Methods:** Indomethacin-induced ulcerogenesis model in rats: rats were treated with R. longifolia extract (10 mg/kg) orally or via intraperithoneal (i. p.) 1 hour before receiving indomethacin (20 mg/kg) or saline orally. Animals were killed after 6 hours and number of ulcers in the stomachs was counted. Capsaicin-induced nociceptive: mice were stimulated with capsaicin in the right hind paw (1.6 µg/paw) one hour after the treatment with the R. longifolia extract and its fractions (1 mg/kg, p.o.). The time licking spent was counted for 5 minutes. Acetic acid writhing test: mice received i. p. injection of 0.8% acetic acid. Animals were treated p.o. with fractions or vehicle one hour before the stimulus, writhing numbers was counted for 10 minutes. Morphine (10 mg/kg, i.p.), diclofenac (50 mg/kg, p.o.) and naloxone (10 mg/kg, s.c.) were used as standard drugs. Tail flick: mice tails were exposed to a focused beam of light from a 45-W projection bulb. The stimulus was terminated when a withdrawal response occurred. Changes in withdrawal response latency were evaluated 30, 60, and 90 min after treatment with RhFBuOH (10 mg/kg, p.o.). All experiments had been performed in accordance to the Fiocruz Council on Animal Care (CEUA) under number 033/09. **Results and Discussion:** Treatment with R. longifolia did not induce injury on gastric mucosa, interestingly, it was able to significantly reduce the occurrence of gastric ulcer induced by indomethacin. RhFBuOH was the best one in inhibiting licking time in the capsaicin model, in relation to the other fractions. In the acetic acid writhing test, all fractions showed significant analgesic effect. RhFAcEt and RhFBuOH decreased significantly the number of abdominal constrictions (31.5 ± 4.30, 35.5 ± 4.69, respectively) in relation to the control group (58.9 ± 6.07, n≥6). When animals were pretreated with naloxone, RhFAcEt effect was not changed (30.5 ± 5.03, n≥6), but RhFBuOH had its effect reversed (47.8 ± 3.57, n≥6) suggesting that this fraction act through opioid receptors. On the other hand, RhFBuOH failed in increasing the latency time in the tail flick model. The analgesic effect of R. longifolia appears to be result of a combination of different substances that are acting by distinct mechanisms of action. RhFBuOH had no effect in the tail flick model in spite of inhibits the licking in the capsaicin assay. The explanation for that apparent contradiction is still unclear. **Financial support:** CNPQ.

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Hypertension and oxidative stress associated with development of fetal programming: influence of extract from *Vitis vinifera* grape skin. Emiliano da Silva AF[1], Costa CA[2], Bem G[2], Carvalho LCRM[3], Boaventura GT[4], Soares de Moura R[3], Resende AC[1] [1]UERJ − Farmacologia e Psicobiologia, [2]UERJ − Farmacologia e Psicobiologia, [3]UERJ − Farmacologia, [4]UFF − Nutrição Dietética

Introduction: Most of the basic components of metabolic syndrome such as hypertension, dyslipidemia and altered glucose homeostasis seem to be associated with oxidative stress. In contrast, epidemiological and experimental studies have suggested that cardiovascular risk factors may be partly attributed to environmental influences in which the individual lives. Thus, maternal nutrition should influence the outcome of pregnancy and determine the health of the fetus (McArdle HJ et al. Placenta. 27(13)A:S56-60, 2006). The aim of this study was to evaluate the effect of a Vitis vinifera grape skin extract (GSE) on hypertension and oxidative stress observed in adult offspring (female) rats whose mothers were subjected to a high-fat diet (HF) during lactation. **Methods:** The experiments were approved by the Ethics Committee of the UERJ (CEP/HUPE – CAAE:0018.0.228.000-07). We used Wistar adult offspring female rats (3 and 6 months) divided into four groups: control group (was offered control diet), high-fat group (was offered a HF with 24% fat), control group + GSE (was offered concurrently with the control diet, GSE at a concentration of 200 mg/kg, diluted in the bottle) and high-fat diet + GSE group (was offered concurrently with the HF, the GSE at a concentration of 200 mg/kg). Measurement of systolic blood pressure (SBP, mm Hg) was performed by plethysmography. Blood glucose levels (mmol/dl) were measured with a glucometer (Accu–Chek Active). Determination of oxidative damage was estimated by formation of thiobarbituric acid reaction substances (TBARS nmol/mg protein) in plasma and superoxide dismutase (SOD) and catalase (CAT) activities (U/mg protein) by spectrophotometry. **Results:** The SBP was increased in high-fat group with 3 and 6 months (149+5 and 167+2; respectively) compared to control (113+3 and 136+4) and control + GSE (117+3 and 100+1) and treatment with GSE prevented the development of hypertension (high-fat+GSE: 100+4 and 133+1.5). The glucose levels were significantly increased in high-fat group with 3 and 6 months (138+2 and 86+1; respectively) compared to control (98+5 and 74+2), control + GSE (102+2 and 74+2) and GSE reduced the values to control levels (high-fat+GSE: 88+3 and 76+1). The levels of TBARS were increased in high-fat group with 3 and 6 months (0.35+0.01 and 1.2+0.02; respectively) compared to control (0.22+0.02 and 0.8+0.01), control + GSE (0.12 +0.01; 0.78+0.14) and significantly reduced by GSE (high-fat+GSE:0.11+0.01 and 0.8 +0.05). In high-fat group with 3 and 6 months the activities of SOD (12+4 and 15+1, respectively) and CAT (0.13+0.03 and 0.21+0.03, respectively) were lower than that observed in control groups (SOD:57+2 and 55+2; CAT:0.24+0.03 and 0.71+0,06) and treatment with GSE restored the activities of the antioxidant enzymes (high-fat + GSE; SOD:55+5 and 53+3; CAT:0.3+0.02 and 0.5+0.08). **Discussion:** The results suggest that GSE has antioxidant effect and protects adult females, whose mothers were subjected to high-fat diet during lactation, against the development of hypertension and hyperglycemia. **Financial support:** CNPq and FAPERJ.

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Comparative study between the effect of aqueous extract of B*ixa orellana L*. and sinvastatin on lipidic profile and blood pressure of hypertensive rats and submitted to a high-cholesterol diet. Reis MLA, Baracho NCV, Ferreira MRC FMIT

High blood pressure and hypercholesterolemia are considered high-cost pathologies to the Health System.1 According to population sayings, Bixa orellana L. ´s aqueous extract has antihypertensive and hypocholesterolemic properties.2 Nowadays, Sinvastatin is the classic patterned treatment to hypercholesterolemia. **Methods:** This study received financial support from FAPEMIG. It was approved by the Ethics and Research Committee of the Medicine School of Itajubá under the register number 15/2008. It obeys the Federal law 6.638 and the Brazilian Animal Experimentation College (COBEA). Thirty male wistar rats were used weighing 200 to 250 grams and allocated in three distinct groups: 1)Control (N=10); 2) Bixa orellana L (n=10); 3)Sinvastatin (n=10). For hypertension induction were used the modified Grollman's method and hypercholesterolemia the rats were to a high-cholesterol diet for 30 days.3 The animals were underwent to gavages administration on daily doses for 45 days. It contained: water to control group, extract of Bixa orellana L.´s seed to treatment group (15mg/ml) and Sinvastatin to the third group(10 mg/kg). Average mean arterial pressure (MAP) was measured in all animals by tail plethismography in a period of 45 days. **Results:** For Statistics calculations was used Student´s T test with a significance level of 95%($p<0,05$).4 The averages of arterial blood pressure levels were not significant when Sinvastatin treatment was compared to Bixa orellana L. On the other hand, the treatment with Bixa orellana L. , when compared to control group, showed a significant reduction on: total cholesterol (enzymatic method) (171,4 ± 8,2 vs 120,6 ± 19,5 mg/dl - $p<0,01$); LDL-cholesterol (precipitation and enzymatic **Methods:**) (107,7 ± 11,8 vs 23 ± 20,6 mg/dl - $p<0,05$); VLDL-cholesterol (Friedwald calculation) (35 ± 7,4 vs 22,96 ± 3,3 mg/dl - $p<0,05$); triglycerides (enzimatic method) (175,4 ± 37 vs 114,8 ± 16,8 mg/dl - $p<0,004$); HDL-cholesterol (precipitation and enzymatic **Methods:**) (28,6 ± 2,5 vs 36,8 ± 5,2 mg/dl - $p<0,01$). How expected sinvastatin, when compared to control group, had a significant decrease in the levels of: total cholesterol (189,8 ± 22,5 vs 129 ± 22 mg/dl - $p<0,01$); LDL-cholesterol (117,3 ± 27,9 vs 69,5 ± 17,1 mg/dl - $p<0,01$); VLDL-cholesterol (39,2 ± 8,1 vs 28 ± 6,1 mg/dl - $p<0,01$). When the effect of Bixa orellana L. extract was compared to Sivastatin effect, there was significance in the levels of: HDL-cholesterol (36,8 ± 5,2 vs 28,6 ± 2,2 mg/dl - $p<0,01$) and triglycerides (114,8 ± 16,8 vs 165,9 ± 37,3 mg/dl - $p<0,01$). **Discussion:** The treatment with Bixa orellana L. showed itself more effective than Sinvastatin to reduce triglycerides rates and to increase HDL-cholesterol. When compared to control group, both were more effective to reduce VLDL-cholesterol, LDL-cholesterol and total cholesterol. References: 1. Fuchs Sc, Petter Jg, Accordi MC, Zen VL, Pizzol Ad, Moreira LB, et al. Establishing the prevalence of hypertension. Influence of sampling criteria. Arq Bras Cardiol. 2001; 76: 445-52. 2. Yunes RA, Pedrosa RC, Cechinel Filho V. Fármacos e fitoterápicos: a necessidade de desenvolvimento da indústria de fitoterápicos e fitofármacos no Brasil. Quím. Nova. 2001; 24(1): 148-52. 3. Jaldin RG, Filho HAF, Siqueira JL, Yoshida WB. O processo aterosclerótico em artérias de coelhos submetidos a dieta suplementada com gema de ovo: modelo experimental de baixo custo.Art. Vasc. Bras. 2006; 5 (4): 247-56. 4. Arango HG. Bioestatística: teórica e computacional: testes paramétricos. 2 ed. Rio de Janeiro: Guanabara Koogan 2005. p.253-272.

09.106

Study of the acute toxicity of *Eugenia brasiliensis* Lamarck and *Eugenia beaurepaireana* (Kiaerskou) Legrand extracts on mice. Lemes EV[1], Cabrini DA[2], Otuki MF[2], Pizzolatti MG[3], Brighente IMC[3], Magina MDA[4], Beirith A[5] [1]FURB − Physiotherapy, [2]UFPR − Pharmacology, [3]UFSC − Chemistry, [4]FURB − Pharmaceutical Sciences, [5]FURB − Natural Sciences

Introduction: The use of medicinal plants to treat, healing or prevention of disease is one of the most ancient medical practices of the humanity. The concept of “natural” contributes to increase of plant medicinal use in last decades. For many people, this concept means “absence of chemical products”, that can cause some damage or represents danger. Due to great diversity of medicinal plants in its vegetation, the Brazilian flora is considered as one of the richest substance sources with pharmacological activity. However, many times, the pharmacological properties of the plants do not have scientific validation. The plants of the Eugenia genus possess several therapeutic properties described in specialized literature; however, there are no data in literature about the possible toxic effect of these plants. In virtue of the therapeutic applications of this plant in the popular medicine, the present study it has as objective to investigate the acute toxicity of Eugenia brasiliensis and Eugenia beaurepaireana extracts. **Methods:** The experiments were conducted in mice, being 5 males in each group, created in the Central Bioterium of the Regional University of Blumenau. For evaluation of the acute toxicity, groups of animals had been treated dealt with extracts in the doses 250, 500, 1000 or 2000 mg/kg and evaluated during the next 24 following hours. The hypocratic parameters analyzed were: number of abdominal contortions, palpebral ptosis, muscular movement, tremors, convulsions, secretions, hair standing and others. Were also evaluated the biochemical and hematological parameters: amino alanine transferase (ALT), number of plaques and leukocytes. After the withdrawal of the blood the animals had been submitted the laparotomy for macroscopic evaluation of the kidneys, spleen, liver, lung and heart, that had been washed with physiological solution and weighed in analytical scale. The numbers of approval of Animal Use Ethic Committee were 102/09 and 106/09. **Results and Discussion:** None of the evaluated parameters was modified by the treatment with Eugenia brasiliensis or Eugenia beaurepaireana demonstrating that, at least acutely, taking in consideration the evaluated parameters, the plants had not presented toxicity in mice. **Financial support:** Regional University of Blumenau.

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Evaluation of the anti-inflammatory and antinociceptive activities of the leaf and stem of *Costus spiralis* (Jacq.) Roscoe (Costaceae). Campesatto-Mella E[1], Araújo MV[1], Silva AKD[1], Santos DLF[1], Delatorre P[2], Rocha BAM[3] [1]UFAL - Farmacologia, [2]UFPB - Biologia Celular, [3]UFC – Bioquímica.

Objective: Evaluation of the potential anti-inflammatory and antinociceptive effect of the ethyl acetate (FAcF), methanol (FMF) and the leaf hexane (FHC) fractions as well as chloroform (FCC), ethyl acetate (FAcC) and methanol (FMC) from stems of *C. spiralis* (Jacq.) Roscoe (Costaceae). Experimental models of formalin-induced nociception and zymosan-induced peritonitis were used. **Methods**: The experiments were performed in adult male and female Swiss mice (20 – 30 g) provided by colony breeding of BIOCEN – UFAL. All experimental procedures were approved by the Ethical Committee – UFAL (license: 006443/2005-78). Inflammatory models consisted of nociception induced by formalin (Hunskaar; Pain, 30; 103, 1987) and peritonitis induced by zymosan (Leite et al.; J*ournal of Leukocyte Biology.,* 82:630-637, 2007). All compounds and standards were administrated orally, (100 µmol/kg), 40 min before the stimuli. Results were expressed as mean ± standard error of mean and analyzed by t test in tutorial Prism ® ($p < 0.05$ and $p < 0.01$). **Results**: In the formalin test, the FAcF, FMF, FHC, FCC, FAcC and FMC did not inhibit the neurogenic phase of formalin-induced nociception as compared to the positive control. In the case of the inflammatory phase, FMF, FAcF, FHC and FCC significantly decreased the time to licking (69.63 ± 7.25%; $p < 0.01$), 79.55 ± 8.17%; $p < 0.01$; 48.08 ± 2.15%; $p < 0.01$, 66.11 ± 5.20%; $p < 0.01$), respectively. Indomethacin induced 70.1% ± 15.8; $p < 0.01$) inhibition of the inflammatory phase of formalin stimulation. To evaluate the anti-inflammatory activity of the fractions, peritonitis induced by zymosan was used. The positive control animals showed increased numbers of leukocytes in the peritoneal cavity 21.32 ± 2.88 x 10^6 cells/mL (mean ± SEM), a phenomenon reduced by treatment with indomethacin (2.28 ± 8.85 x 10^6 cells / mL). The fractions FAcF, FMF, FAcC and FMC, significantly inhibited the leukocyte migration (8.8 ± 1.76 x 10^6, 8.4 ± 0.87 x 10^6 11:24 ± 1:2310^6, 7.88 ± 0.81 x 10^6 cells/mL ($p < 0.01$), respectively. **Conclusion**: Our results show that the fractions FMF, FAcF, FHC and FCC *C. spiralis* presented antinocipeptive and anti-inflammatory effects on formalin and zymosan-induced peritonitis in mice. **Financial support)**: INCT-INOFAR (573.564/2008-6), CNPq, FAPEAL, UFAL.