Setor 09. Produtos Naturais e Toxinologia/Natural Products and Toxinology

09.001

Biological activity *in vitro* of a metalloproteinase from *Bothrops moojeni* snake venom. Gomes, MSR[1], Mendes MM[2], Hamaguchi[2], Homsi-Brandeburgo MI[2], Rodrigues VM[2] [1]UESB Química e Exatas, [2]UFU - Genética e Bioquímica

Introduction: The venoms of snake 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 protein 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 characterization *in vitro* of the biological activities of a metalloproteinase named BjmooF (Venom Fibrin(ogen)olytic Metalloproteinase of *Bothrops moojeni*) belonging to the class P-I SVMPs. **Methods:** The protease (BjmooF) was obtained by chromatography of ion-exchange DEAE-Sephacel and filtration Sephadex G-75.The enzyme was followed by biological activities *in vitro*: Fibrinogenolytic, inhibition of Fibrinogenolytic, fibrinolytic, Caseinolytic and TAME esterase activities was assayed as described by Rodrigues et al. (2000), With slight modifications. Coagulant activities on bovine plasma was assayed according to Assakura et al. (1992) and Hemolytic Activities (PLA_2), was measured by an indirect hemolytic assay on agarose gel, using washed mice erythrocytes and hen´s egg-yolk emulsion as substrate, as described by Gutierrez et al. (1988). **Results and Discussion:** The enzyme showed high proteolytic activity degrading intensity a,b-chains of bovine fibrinogen and casein with 5 µg of BjmooF were mixed (1:100 w/w) and the mixture was incubated at 37ºC for different time intervals (5, 10, 15, 30, 45 and 60min); The inhibition of Fibrinogenolytic activities was determined by incubating BjmooF (5mg), with 10mL EDTA, Aprotinin, 1,10-phenanthroline, β-mercaptoethanol and Leupeptin all 10mM. BjmooF was inhibited by EDTA and 1,10-phenanthroline. These tests revealed that BjmooF is a protease ion-dependent. According to fibrinolytic activity, 30mg of crude venom caused a similar action of 20mg of the enzyme, which means, a fibrinolytic activity increased by 50%. However, this enzyme showed no hemolytic activity, with 10, 20, 30 and 40mg after 24h of incubation in neither over night at 37º C; nor coagulation bovine plasma, nor TAME esterase activity. This assays suggest that metalloproteinase BjmooF belongs to class P-I of SVMPs. **References**: Assakura, *Compendium Biochemistry and Physiology,* V. 102; p. 727-732, 1992. Gutierrez, *Toxicon*, V. 26; p. 411-413, 1988. Rodrigues, *Archives of Biochemistry Biophysics*, V. 381; p. 213-224, 2000. **Financial support**: FAPEMIG, UFU, UESB.

09.002

Mechanisms underlying the cardiovascular effects of isoquercitrin - an active flavonoid of *Tropaeolum.majus* L. Gasparotto Jr A[1], Crestani S[2], Dias FD[3], Lourenço EL[1], Stefanello MEA[4], da Silva-Santos JE[5], Marques MCA[2], Kassuya CAL[2] [1]UNIPAR/UFPR - Farmacologia, [2]UFPR - Farmacologia, [3]UNIPAR - Farmacologia, [4]UFPR - Química, [5]UFSC - Farmacologia

Introduction: Flavonoids, such as isoquercitrin show markedly antihypertensive effects[1]. In addition, it has been shown that oral administration of a mixture of quercetin glycoside decreases the systolic pressure in spontaneously hypertensive rats (SHR)[2]. The aim of this study was to evaluate the mechanisms underlying the antihypertensive action of isoquercitrin (ISQ) and its structural analog kampferol (KPF), as well as the hydroethanolic extract (HETM) and the fraction (FPTM) obtained from *Tropaeolum majus*, an ISQ rich plant. **Methods:** Firstly, we evaluated the effects of HETM (10–300 mg/kg, p.o.), FPTM (12.5–100 mg/kg, p.o.), ISQ (0.5 - 4 mg/kg, i.v.) and KPF (0.5 - 4 mg/kg, i.v.) on the mean arterial pressure (MAP) of both normotensive and SHR rats. For this, the animals were anesthetized with ketamine/xilazyne (100/20 mg/kg, i.p.) and had polyethylene catheters inserted inside the left femoral vein and the right carotid artery for drug administration and MAP recording, respectively. To evaluate the role of the nitric oxide/guanylate cyclase pathway in our findings, different groups of animals were subjected to L-NAME infusion (a nitric oxide synthase inhibitor, 7 mg/kg/min), or ODQ (a guanylate cyclase inhibitor, 2 mg/kg) before the intravenous injection of ISQ (4 mg/kg). The angiotensin converting enzyme (ACE) activity was measured by indirect fluorimetry, in serum samples obtained from HETM, FPTM, ISQ, and KPF treated rats. All procedures were approved by the Institutional Ethics Committee of UFPR (authorization number 240). **Results:** The intravenous injection of ISQ (2 and 4 mg/kg), but not KPF, reduced the MAP by 16.4 ± 3.2 and 23.8 ± 3.2 mmHg (n = 6). Oral administration of HETM (100 mg/kg) and FPTM (50 mg/kg) reduced MAP by 9.37 ± 2.5 and 13.66 ± 3.1 mmHg in normotensive rats, and 10.33 ± 2.7 and 16.71 ± 2.9 mmHg in SHR rats. The basal MAP was 108.9 ± 2.5 and 134.4 ± 1.8 mmHg in normotensive and SHR animals, respectively; n = 6). The hypotensive effect induced by ISQ (4 mg/kg) was strongly reduced by L-NAME, as well as by ODQ (2 mg/kg). The oral treatment with HETM (100 mg/kg), FPTM (50 mg/kg), and ISQ (10 mg/kg) reduced the plasmatic ACE activity by 18 ± 3%, 26 ± 7% and 41 ± 3%, respectively. **Discussion:** Our results show that the hypotensive effects caused by the hydroethanolic extract of *Tropaeolum majus* (HETM), as well as its fraction (FPTM), may be associated with the high levels of the flavonoid isoquercetrin found in this plant. In addition, isoquercetrin-induced hypotension in rats is an event dependent of both stimulation of the nitric oxide/guanylate cyclase pathway and inhibition of angiotensin II generation by ACE. 1. Schramm D. D. *et al*. *J. Nutr. Biochemistry* 9: 560-566, 1998. 2. Emura K. *et al*. *J Nutr Sci Vitaminol* 53: 68-74, 2007. **Acknowledgements:** DEGPP/UNIPAR and CNPq.

09.003

Influência do mesocarpo do maracujá e da casca do abacaxi sobre a hipercolesterolemia em camundongos. Musial DC[1], Dall´Est, J.[1], Silva RD[1], Silva LT[1], Isolani, AP[1], Bracht L[2], Biazon, ACB[1] [1]Faculdade Integrado de Campo Mourão - Ciências Farmacêuticas, [2]UEM - Farmácia e Farmacologia

Introdução: A Hipercolesterolemia é um dos fatores de risco para as doenças cardiovasculares. Nos últimos anos, alguns pesquisadores mostraram a importância das dietas ricas em frutas e vegetais na prevenção da aterosclerose. A casca das frutas normalmente não tem aproveitamento pela maioria das pessoas. A casca do maracujá, por exemplo, representa um resíduo da indústria do suco do maracujá que vem sendo testada artesanalmente para a elaboração de alguns produtos. Diante destes fatos, o objetivo do presente trabalho foi o de avaliar a influência das cascas de maracujá e abacaxi sobre o perfil lipídico, glicêmico e sobre a atividade das transaminases plasmáticas de camundongos hipercolesterolêmicos. **Métodos**: Foram utilizados camundongos machos Swiss e todos os procedimentos utilizados foram previamente aprovados pelo Comitê de Ética em Experimentação Animal da Faculdade Integrado de Campo Mourão (protocolo nº 1507/07). A hipercolesterolemia foi induzida através de uma dieta hipercalórica constituída por ração padrão (37,5%), chocolate (25%), amendoim (25%) e bolacha maizena (12,5%). Os animais foram divididos em 4 grupos: Grupo controle (DC), grupo da dieta hipercalórica (DH), grupo da dieta hipercalórica tratado com mesocarpo de maracujá (DHM) e grupo da dieta hipercalórica tratado com casca de abacaxi (DHA). Foram utilizados dois protocolos experimentais: no protocolo profilático o tratamento com a dieta hipercalórica e com as cascas das frutas se iniciou ao mesmo tempo. Os animais receberam uma suspensão contendo a farinha das cascas das frutas por gavagem oral na dose de 1g/kg diariamente por 30 dias. No protocolo terapêutico, os animais foram submetidos previamente à indução da hipercolesterolemia com a dieta hipercalórica, por 4 semanas. Após este período, os grupos DHM e DHA foram ainda tratados diariamente por gavagem oral com uma suspensão contendo as cascas das frutas na dose de 1 g/kg. Os animais do grupo DH receberam apenas salina. Os animais do grupo DC receberam a dieta normocalórica padrão durante todo o período experimental. Após o período de experimentação, os animais foram submetidos à eutanásia e o sangue dos animais foi coletado e utilizado para análise de glicose, triglicerídeos, colesterol total e atividade das enzimas AST e ALT. **Resultados e Discussão:** O tratamento dos animais com dieta hipercalórica (DH) por 30 dias provocou um aumento significativo nos níveis plasmáticos de colesterol total e na atividade da AST de 43% ($p<0,05$) e 338% ($p<0,001$), respectivamente. O tratamento dos animais hipercolesterolêmicos com as cascas das frutas, em ambos os protocolos experimentais utilizados, não foi capaz de reduzir os níveis aumentados de colesterol total dos animais, nem de atenuar a atividade aumentada da AST. Os animais do grupo DHA, no protocolo profilático, apresentaram glicemia 96% maior que os grupos DC e DH ($p<0,05$). Nenhum dos outros parâmetros analisados foi alterado com o tratamento com a dieta hipercalórica ou com as cascas das frutas. Estes resultados mostram que o tratamento dos animais com as cascas de maracujá e abacaxi não provocaram mudanças significativas na concentração plasmática de colesterol, neste modelo. Apoio: FUNDADESP, Faculdade Integrado de Campo Mourão

09.004

Levantamento etnobotânico de plantas popularmente utilizadas como antimicrobianas no estado de Mato Grosso, Brasil. Santos KTJ[1], Martins DTO[2], Silva Junior IF[1] [1]UNIC - Farmácia, [2]UFMT - Ciências Básicas em Saúde

Introdução: A etnobotânica é uma área científica que estuda a relação que existe entre o Homem e as plantas e o modo como as populações usam os recursos vegetais. Neste contexto, os levantamentos etnobotânicos objetivam estudar o uso e o conhecimento do valor terapêutico das espécies vegetais pelos povos tradicionais e/ou contemporâneos, visando encontrar plantas que apresentem efetivamente uma atividade terapêutica e que consequentemente possibilitem a descoberta de novos fármacos. Identificou-se as principais espécies medicinais utilizadas popularmente no Estado de Mato-Grosso como antimicrobianas. **Métodos:** Todas as teses, dissertações e trabalhos de conclusão de curso encontradas na Biblioteca Central da UFMT, foram incluídas no estudo bibliográfico, seguindo os critérios: ter sido produzida entre 1986-2006 e possuir como tema principal: plantas medicinais utilizadas em Mato Grosso. Para seleção das espécies medicinais, elencou-se palavras-chaves relacionadas ao uso popular com finalidade antimicrobiana como por exemplo: infecções de pele, verminoses, diarréia, frieira, furúnculo, coceira, micoses, corrimento, antibióticos, infecção, infecção vaginal, pano branco, queda de cabelo, inflamação de mulher, impinge, antidiarréica, doença venérea, leucorréia, alopecia, gonorréia, eczemas, antisséptico, sarna, infecção urinária, feridas, sífilis, cicatrizante, cicatrização, dermatose, vitiligo, doenças de pele, urticária, erisipela, vermífugo, infecção de garganta, inflamações ginecológicas. **Resultados:** 11 exemplares bibliográficos atenderam os critérios estabelecidos. E nestes, foram encontradas 338 espécies vegetais, sendo que 58 destas, não relatavam a identificação botânica, apenas os nomes vulgares. Todas essas espécies relatavam uso popular como antimicrobiano. Destacarem-se as espécies: *Jatropha elliptica, Jacaranda brasiliana*, *Tabebuia heptaphylla, Macrosiphonia velame, Bowdichia virgilioides, Cassia occidentalis* e *Croton urucurana,* sendo as mais popularmente utilizadas em Mato-Grosso. Não houve prevalência de Famílias botânicas com propriedades antimicrobianas. As partes das plantas utilizadas com maior freqüência foram: entrecasca, folhas e raízes. Todos os farmacógenos de *Cassia occidentalis* são utilizados popularmente. Discussão: Os compostos vegetais com propriedades antimicrobianas ocorrem em todas as Famílias botânicas, porém as espécies vegetais pertencentes às famílias Euphorbiaceae, Bignoniaceae e Fabaceae, merecem maiores estudos etnobotânicos/etnofarmacológicos. Pelo fato de que algumas espécies reladas neste estudo possuírem sinonímias botânicas, é válido estudos farmacológicos futuros que comprovem o uso tradicional desta plantas medicinais, bem como a identificação botânica de algumas espécies vegetais ainda sem comprovação taxonômica. **Apoio financeiro:** UNIC/FAPEMAT

09.005

Atividade anti-inflamatória do extrato bruto hidroalcoólico das raízes de *Dioscorea multiflora***.** Sousa JMB, Guimarães CL, Darmarco ED, Magina MDA FURB - Ciências Farmacêuticas

Introdução: As raízes da *Dioscorea multiflora* Mart. ex Griseb são utilizadas na medicina popular da serra catarinense como analgésica e anti-inflamatória. Devido à similaridade morfológica, esta espécie é confundida com outras do gênero *Smilax spp.*. O presente trabalho teve por objetivos avaliar a atividade anti-inflamatória do extrato bruto hidroalcoólico das raízes de *D. multiflora* (EDM) no modelo de pleurisia e edema de pata em camundongos. **Métodos**: Os procedimentos experimentais foram avaliados e aceitos pelo Comitê de Ética de Experimentação Animal da FURB sob o número 23/08. Neste protocolo experimental, foram utilizados camundongos suíços machos (30-35 g) com acesso à água e ração *ad libitum.* **Pleurisia:** Em nossos experimentos, optamos por avaliar a primeira fase (4 h) do processo inflamatório induzido pela Cg. Antes da indução da pleurisia (Cg, 2%, 50μl, via intercostal), diferentes grupos experimentais foram tratados com diclofenaco, 50 mg/kg (i.p.) ou com EDM nas doses de 20, 50 e 200 mg/kg (i.p., 1 h antes da Cg). Passadas 4 h da administração de Cg, os animais foram eutanasiados (tiopental 400 mg/kg, i.p.), a cavidade torácica foi exposta, lavada com 2 ml de salina estéril e obtido o exsudato pleural para análise e comparação de seus resultados com o controle inflamado (apenas Cg, 2%). Deste foram analisados a celularidade total (câmara de Neubauer) e específica (coloração hematológica). **Edema:** foi induzido com Cg (2%, 50μl/pata). O edema foi avaliado através da diferença de peso (mg) entre a pata com Cg e a contralateral com salina estéril (50 μl/pata). Após 4 h da aplicação de Cg, os animais foram eutanasiados (tiopental 400 mg/kg, i.p), as patas excisadas e pesadas. Grupos tratados com diclofenaco (50 mg/kg, i.p) ou EDM (20, 50 ou 200 mg/kg, i.p), administrados 30 min antes da Cg, foram comparados ao controle. **Resultados: Pleurisia:** no grupo controle (N = 6) os leucócitos totais (LT) foram 16,32 ± 1,9 $.10^6$ havendo 2,28 ± 0,6 $.10^6$ leucócitos mononucleares (LM) e 14,04 ± 1,9 $.10^6$ leucócitos polimorfonucleares (LP). No grupo tratado com diclofenaco (N = 7) os LT foram 6,9 ± 0,7 havendo 1,5 ± 0,3 LM e 5,4 ± 0,6 LP. Nos grupos tratados com o EDM nas doses de 20, 50 ou 200 mg/kg obteve-se LT 8,3 ± 1,3 $.10^6$, LM 2,31 ± 0,4 $.10^6$ e LP 5,99 ± 1,4 $.10^6$; LT 6,2 ± 1,4 $.10^6$, LM 2,8 ± 0,3 $.10^6$ e LP 3,4 ± 0,6 $.10^6$; LT 4,3 ± 0,13 .106, LM 2,84 ± 0,9 $.10^6$ e LP 1,46 ± 0,25 $.10^6$; respectivamente. A análise estatística demonstrou haver significância (mínimo $P < 0,05$) entre o grupo controle e diclofenaco (teste “t” de Student) e, entre o controle e os grupos tratados (ANOVA, Teste de Student Newman Keuls). **Edema:** no grupo controle o edema foi de 89,9 ± 8,1 mg (N = 6) diferindo estatisticamente (teste “t”, $P < 0,05$) do grupo diclofenaco (61,5 ± 3,7 mg, N = 7) e, dos grupos tratados com o EBDM nas doses de 20, 50 ou 200 mg/kg, 64, 9 ± 7,3 mg, 58,0 ± 4,1 mg e 46,1 ± 8,5 mg, respectivamente (N de 6 a 9). **Discussão**: o diclofenaco inibe a migração leucocitária e o edema, devido ao bloqueio da síntese de prostaglandinas. Quanto ao EDM é preliminar sugerir o mesmo mecanismo de ação. Avaliações futuras podem predizer qual composto presente nas raízes de *D. multiflora* induz atividade anti-inflamatória. Os resultados sugerem que o EDM induz atividade anti-inflamatória em camundongos. **Apoio: FURB & PIBIC/CNPq.**

09.006

Relaxant effect induced by red wine of *Vale do São Francisco* (RWVSF) in mesenteric rings. Luciano MN, Ribeiro TP, Nascimento RJB, Silva MSF, Alustau MC, Oliveira EJ, Medeiros IA LTF-UFPB

Introduction: The mechanisms involved in the cardioprotector effect of red wine have not yet been completely elucidated but probably an endothelium-dependent vasodilation may play a significant role in this effect[1]. The aim of this study was to investigate the vasorelaxant effect induced by RWVSF in isolated superior mesenteric rings. All experiments were reviewed and approved by the Ethics Committee of Animal Experiments of the Laboratório de Tecnologia Farmacêutica of the Universidade Federal da Paraíba (0310/08). **Methods:** Red wines from Vale do São Francisco (Northeast Brazilian) were analyzed (ADSY – Adega do Vale, grape Syrah – vintage 2004; BOPS – Adega Boticelli, grape Petit Sirah – vintage 2006 and GASH – Adega Garziera, grape Shiraz - vintage 2005). The ethanol of RWVSF was evaporated under low pressure at 55ºC, until reduction of approximately 50% of the original volume. The liquid residue was lyophilized and frozen in -20ºC until the day of use. The quantification of quercetin, *cis*-resveratrol and *trans*-resveratrol was performed in an apparatus of high performance liquid chromatography with UV-visible detector (CLAE-UV-Vis). The isolated superior mesenteric rings (1-2 mm) were suspended by cotton threads for isometric tension recordings in a Tyrode's solution at 37°C and gassed with 95% O_2 and 5% CO_2, under a resting tension of 0.75 g. **Results:** The quantity (mg/mL) of *cis*-resveratrol, *trans*-resveratrol and quercetin found in the wines were respectively: ADSY (5.10± 0.0647; 0.20±0.0033; 1.00±0.0112); BOPS (4.57±0.0066; 0.24±0.0016; 1.78±0.0451) e GASH (8.32±0.0013; 0.34±0.0017; 2.16±0.0115). In isolated rat mesenteric artery rings, with intact endothelium, RWVSF (Log -5 to 2.5 mg/mL) induced concentration-dependent relaxation of the contractions induced by phenylephrine (10 mM) (E_{max} ADSY= 70.65±7.76%; E_{max} BOPS= 68.77±14.62%; E_{max} GASH= 92.25±4.47%). After endothelium removal the vasorelaxant effect elicited by RWVSF was significantly attenuated (E_{max} ADSY= 36.79±8.21%; E_{max} BOPS= 11.90±5.07%; E_{max} GASH= 33.30±4.47%). Similar results were obtained in the presence L-NAME 100 µM, a competitive antagonist of NOS (E_{max} ADSY= 18.45±4.07%; E_{max} BOPS= 19.43±4.95%; E_{max} GASH= 24.83±6.43%). **Discussion and Conclusion:** In the present study, we demonstrated that RWVSF induces an endothelium-dependent vasorelaxant effect, concentration-dependent, involving, in part, the eNOS. VITRAC *et al* (2005), in a study with twelve commercial red wines from southern of Brazil, showed that these wines contains about five times more *cis*-resveratrol than its *trans* isomer[2]. Our results apparently show that the vasorelaxation induced by RWVSF are related to the amount of *cis*-resveratrol in these wines. **References:** [1]Moura *et al.* J. Cardiovasc. Pharmacol. 44, 302, 2004. [2]Vitrac *et al.* J. Agric. Food Chem. 53, 5664, 2005. **Financial Support:** CNPq/CAPES/LTF-UFPB.

09.007

Avaliação da atividade cicatrizante do extrato etanólico de *Arctium lappa* em úlceras induzidas por ácido acético em ratos. Mota, L.[1], Allemand A[1], Potrich BP[1], Pizzolatti GM[2], Werner MFP[3], Andre E[4], Marques MCA[1] [1]UFPR - Farmacologia, [2]UFSC - Química, [3]UFSC - Farmacologia, [4] University of Ferrara - Experimental and Clinical Medicine

Introdução: A *Arctium lappa* L. é uma planta da família Asteraceae (Compositae), conhecida popularmente como bardana. Em estudos realizados no Departamento de Farmacologia da UFPR, DOS SANTOS *et al. J Pharm Pharmacol.* (2008) observou que extratos da bardana foram capazes de reduzir lesões gástricas por etanol ou por ácido acético com redução da secreção gástrica. O objetivo deste trabalho foi avaliar o mecanismo envolvido no efeito cicatrizante do extrato etanólico (EE) obtido das raízes da *Arctium lappa* L. em úlceras induzidas por ácido acético. **Métodos:** Ratas Wistar (250 g) em jejum de 18h foram anestesiadas para a indução das úlceras. Após a exposição do estômago, um cilindro de vidro de 6 mm de diâmetro foi colocado sobre a serosa do estômago, e dentro deste foi aplicado 500 µl de ácido acético 80%. Após 1 min, o ácido foi aspirado, o estômago foi lavado com salina e a parede abdominal foi suturada. Os animais foram tratados com o EE (10 mg/kg, vo) duas vezes ao dia, durante o 3º ao 9º dia após a indução da lesão. No 10º dia os animais foram sacrificados, seus estômagos retirados e fotografados e as áreas das lesões gástricas mensuradas (mm^2) através do software ImageJ. Foram realizadas dosagens de glutationa reduzida (GSH) e atividade das enzimas mieloperoxidase (MPO), N-acetil-β-D-glicosaminidase (NAG), superóxido dismutase (SOD), catalase (CAT) e glutationa S- transferase (GST), bem como do conteúdo de espécies reativas de oxigênio (ROS). Os protocolos experimentais foram aprovados pelo comitê de ética em experimentação animal da UFPR sob o número de licença 161. **Resultados:** O EE (10 mg/kg) diminuiu a área das lesões causadas pelo ácido acético em 65,61%. A indução da úlcera gástrica causou um aumento na atividade da MPO e NAG. Este aumento foi inibido pelo tratamento dos animais com EE em 62,55% e 36,81%, respectivamente. Em tecidos ulcerados, observou-se um aumento de ROS (51,4%)**,** o qual foi reduzido em 31,35% pelo tratamento com EE. Também foi observado uma redução nos níveis de GSH e GST nos tecidos ulcerados (98,61% e 50%, respectivamente) quando comparados ao grupo cotrole sem úlcera. O tratamento com EE foi capaz de reestabelecer em 30% os níveis de GSH e prevenir a redução de GST, quando comparado com os valores basais. Em estômagos lesionados também se observou um aumento da atividade da SOD (63,92%) o tratamento dos animais com EE preveniu em 37 % o aumento da atividade desta enzima. O tratamento não alterou os níveis de CAT. **Discussão:** Estes resultados demonstram uma ação gastroprotetora induzida pelo EE no modelo de úlcera crônica induzida pelo ácido acético. Este efeito parece, em parte, ser mediado por diminuição do processo inflamatório e pela ação antioxidante do extrato EE. Apoio Financeiro: Fundação Araucária.

09.008

Effects of *Piper carniconnectivum* in elevated plus-maze and forced swimming test in rats.

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Introduction: Anxiety and depression are serious brain disorders in today's society and highly prevalent in Latin American countries, including Brazil. Drugs of natural origin that possess beneficial effects on the central nervous system are emerging as promising alternative therapies to treat these affective disorders. *Piper carniconnectivum (Piperaceae),* popularly known as long pepper, grows in the Amazon forest and it is well known for its aromatic properties and the presence of chalcones and amides in its roots. The present study evaluated the effects of oral administration of a methanolic extract (ME) from *P. carniconnectivum* stems on locomotory activity, as well as on anxiety and depression in rats. **Methods:** Female Wistar rats (n=10 per group) were acutely treated with 0.5, 1, 10 or 100 mg/kg of ME by oral route (p.o.). The control group was treated with saline and two other groups received diazepam (1 mg/kg, DZP, a positive control for anxiolytic action) or fluoxetine (10 mg/kg, FXT, a positive control for antidepressant action). Spontaneous locomotor activity, anxiolytic and antidepressant properties were investigated by applying the open-field (OF), elevated plus-maze (EPM) and forced swimming (FS) tests, respectively. One-way ANOVA following the Tukey's test were used for statistical analysis.). All experiments were in accordance with our guidelines for the care of laboratory animals (UnBDOC nº 7779/2006). **Results:** All tested doses of ME showed no effects on rat locomotory behavior when evaluated by the OF test. In the EPM test, 1 mg/kg ME increased the percentage of frequency (40.63±2.50 vs 24.59±2.22) and 10 mg/kg ME increased time in the open arm entries (11.78±2.26 vs 4.60±0.80) compared to the control group, respectively. The results showed that DZP increased the percentage of frequency (38.81±0.99 vs 24.59±2.22) and time in the open arm entries (10.09±0.74 vs 4.60±0.80) compared to the control group. Statistical differences were not found among groups in the FST. The results showed that FXT reduced the time of immobility (59.40±3.42 vs 96.20±5.10) and increased swimming activity (65.30±4.17 vs 46.40±6.58) when compared to the control group treated with saline. **Discussion:** The results provided evidence, for the first time, that the ME from *P. carniconnectivum* stems exerts significant *in vivo* anxiolytic-like properties. Therefore, *P. carniconnectivum* may be a source of molecules useful for treating CNS disorders. **Acknowledgements:** We thank CAPES for a Ph.D. fellowship.

09.009

Cipura paludosa ethanolic extract reverts memory impairment in rats exposed to ethanol and/or methylmercury. Lucena GMRS[1], Porto FA[1], Coelho RC[1], Pinheiro WB[1], Azevedo MS[2], Campos EG[3], Ferreira VMM[1] [1]UnB - Ciências Farmacêuticas, [2]UNIR - Química, [3]UnB - Biologia Celular

Introduction: Alcohol and methylmercury (MeHg) are highly toxic to the central nervous system leading to neurological and developmental deficits in animals and humans. These effects are worsened during pregnancy. In this study, the effects of the ethanolic extract (EE) of *Cipura paludosa* on short- and long-term memory of rats exposed in utero and during the lactational period to MeHg and/or ethanol (EtOH) were assessed by the social recognition and step-down inhibitory avoidance tests. **Methods:** Pregnant Wistar rats were divided into two groups: one received tap water and the other EtOH 22.5% (w/v) for 21 days and another 21 days during breast-feeding. On the 15th day of pregnancy, each group was subdivided into two more groups which received tap water or 8 mg/kg MeHg (p.o.), totaling four groups: Control (C), EtOH (E), MeHg (M) and EtOH+MeHg (EM). Sixty days old adult offspring (n=10 per treatment) were used. Each group was treated with EE (1, 10 or 100 mg/kg p.o.), saline (S) or caffeine (CAF, 10 mg/kg p.o., positive control) for 14 days. All experiments were in accordance with our guidelines for the care of laboratory animals (UnBdoc 7779/2006). **Results:** EE (1, 10 or 100 mg/kg) or CAF treatment improved the short-term social memory when the same juvenile rats were re-exposed to the adult animals after a delay period of 120 min when compared to group C ($F_{(4,49)}$=27.23, p<0.001, One-way ANOVA). The animals of groups C, E, M and EM were unable of recognizing the juvenile rats (p>0.05, Tukey). In the M and EM groups EE significantly decreased the investigation time of the familiar juvenile in the forgetting procedure (exposure 120 min latter to adult rats), suggesting that EE facilitates short-term social memory (p<0,05). However, EE did affect the non-memory effect. EE (at all doses) or CAF also facilitated the step-down inhibitory avoidance short-term 1.5 h (H(16, N=170 =124.5; p<0.001) and long-term (24 h) memory (H(16, N=170 =119.3; p<0.001) evaluated after training (Kruskal-Wallis). However, the E group showed diminished step-down latencies during the acquisition of short- and long-term memory when compared with the respective control treated with saline (p<0.05) (Mann-Whitney test). This effect was reverted by treatment with EE at all doses (p<0.05). Diminished step-down latencies during the short- and long-term memory were also observed in the M and EM when compared with the respective controls, and EE also reverted the cognitive deficit, showing an increased in the animal's latencies in the step-down test. **Discussion:** Our results demonstrated that prenatal and lactational exposure to E and M caused behavioral changes and neurocognitive deficits and that EE protected against this deficit in the adult offspring from rats exposed to neurotoxicants. **Acknowledgements:** We thank CAPES for a Ph.D. fellowship.

09.010

Phytochemical study and evaluation of anti-inflammatory activity from the *Morus nigra* leaves. Leite Júnior JG[1], Padilha MM[1], Vilela FC[2], Silva MJD[1], Rocha CQ[1], Giusti-Paiva A[2], Silva GA[1] [1]UNIFAL - Farmácia, [2]UNIFAL - Ciências Biológicas

Introduction: The use of natural products with therapeutic properties is as ancient as human civilization. Most people living in developing countries are almost completely dependent on traditional medical practices to their needs for primary health care and higher plants are known to be the main source for drug therapy in traditional medicine. The *Morus nigra* belongs to the Morus genus and Moraceae family. This genus is known to contain phenolic compounds including varieties of flavonoids, coumarins and xantona. This study describes the chemical constituents isolated and anti-inflammatory evaluation of *Morus nigra* leaves grown in Brazil. **Methods**: The plant material was air dried at 40 °C and powdered. The dry powder was extracted previously with a 50% hydroethanolic solution, followed by percolation with methylene chloride at room temperature and the solvent was removed under reduced pressure. The *Morus nigra* hydroethanolic extract (MNHE) after suspending in a vehicle (1% carboxymethylcellulose sodium suspension in distilled water) was administered at doses of 30, 100 and 300 mg/kg. To evaluate the anti-inflammatory activity was carried out tests carrageenan-induced rat paw edema¹ and granulomatous tissue formation². It was used Wistar rats (n=8), which protocols were approved by UNIFAL-MG Animal Ethical Committee under the number 145/2007 and all drug tests were administered orally in an equivalent volume of 10 ml/kg animal body weight. Indomethacin (5 mg/kg) and dexamethasone (0,2 mg/kg) were used as reference drugs. Afterwards, the dichloromethane extract was fractionated through CC (in column chromatography) from silica gel using mixture of hexane and ethyl acetate (1:1) as eluents, allowing the isolation of 3 pentacyclic triterpenes. **Results:** Daily administration of MNHE (300 mg/kg, p.o) inhibited the formation of granulomatous tissue by 39,7% in comparison to the control group ($p < 0,05$). Moreover, the administration of dexamethasone reduced its formation by 72,2% when compared to the control group ($p < 0,001$). This result is quite similar to the one observed from the group treated with indomethacin, which inhibited edema formation by 66,9% ($p<0,001$). **Discussion**: The results of pharmacological tests showed that the extract of *M. nigra* (MNHE) shows anti-inflammatory effect and produced a significant reduction in swelling caused by carrageenin and inhibited the granulomatous tissue formation. The spectral data obtained from the isolated compounds were compared with the literature³ and concluded that they were the germanicol, β-sitosterol and acid betulin. ¹Carvalho, JC.; Sertie, JA.; Barbosa, MV.; Patrício, KC.; Caputo, LR.; Sarti, SJ.; Ferreira, L. P.; Bastos, JK. Journal of Ethnopharmacology. 64, 127, 1999. [2]Swingle, KF.; Shideman, FE. The Journal of Pharmacology and Experimental Therapeutics. 132, 608, 1972. [3]Mahato, S.B.; Kundu, A.P. *Phytochemistry*. 37, 1517, 1994. This work was developed by Universidade Federal de Alfenas with CAPES and FAPEMIG support.

09.011

Efeito do tratamento oral com extrato bruto de *Plectranthus neochilus* em modelos de pleurisia e lesão de mucosa estomacal. Calheiros AS, Souza CZ de, Sobreira, JGM, Azeredo JA Castro-Faria-Neto HC, Frutuoso V Fiocruz - Fisiologia e Farmacodinâmica

Introdução: Várias civilizações têm encontrado em suas florestas ricas fontes de substâncias bioativas capazes de atender às mais diversas necessidades terapêuticas. Nesse contexto encontramos a espécie *Plectranthus neochilus* (Pn), um tipo de boldo, largamente utilizada na medicina popular como analgésico, estimulante da digestão e no combate a azias. Neste trabalho tivemos como objetivo identificar o potencial antiinflamatório e antiulcerogênico do extrato bruto de Pn em modelos de pleurisia e lesão de mucosa estomacal. **Metodologia**: Para a realização da pleurisia (Licença CEUA 0260/05) camundongos Swiss receberam injeção intrapleural (i.pl) de carragenina (300ug/cavidade) ou LPS (250ng/cavidade), 1 h após tratamento, por via oral (p.o.), com 0,2mL de Pn (0,04/ 0,4 e 4 mg/kg), sendo a resposta inflamatória observada 4h ou 6h após o estímulo, respectivamente. A indução de lesão de mucosa foi realizada por Indometacina (20 mg/kg/0,2mL p.o) em ratos Wistar em jejum de 18h, tratados 1h antes com o extrato de Pn (500 mg/kg), sendo a intensidade de reação ulcerogênica determinada pelo número de lesões na mucosa estomacal 3h após indometacina. **Resultados**: Após injeção i.pl. de carragenina observou-se significativo recrutamento de leucócitos totais (de 1,07 ± 0,15 para 4,71 ± 0,44), caracterizado principalmente pelo influxo de neutrófilos (de 0,07 ± 0,03 para 2,70 ± 0,32). Animais tratados com o extrato bruto de Pn 0.4 e 4 mg/kg apresentaram discreta, porém significativa, redução no número de leucócitos totais (de 4,71 ± ,44 para 3,19 ± 0,25 e 3,41 ± 0,38). Por outro lado, observamos marcada redução no infiltrado neutrofílico em animais recebedores de extrato nas doses de 0.04, 0.4 e 4 mg/kg (de 2,70 ± 0,32 para 1,65 ± 0,23; 1,61 ± 0,17 e 1,72 ± 0,38). A mesma redução no número de neutrófios também foi observada após tratamento com Pn, na pleurisia indusida por LPS (de 0,62 ± 0,05 para 0,43 ± 0,08, 0,37 ± 0,05 e 0,35 ± 0,03). Nos ensaios de úlcera experimental se observou que animais recebedores de indometacina apresentaram elevado número de lesões na mucosa estomacal quando comparado ao grupo basal (de 5,0 ± 0,71 para 18,6 ± 3,61) e que o prévio tratamento com Pn promove expressiva diminuição do número de lesões de mucosa (de 18,6 ± 3,6 para 9,6 ± 3,3). Vale ressaltar que, o tratamento com o extrato bruto de Pn por si só não induz qualquer lesão na mucosa estomacal. **Discussão**: Nossos resultados demonstram uma significativa atividade anti-inflamatória para o extrato bruto de *Plectrantus neochilus*, marcado pela redução no número de neutrófios na cavidade pleural, sendo acompanhada de importante atividade citoprotetora da mucosa gástrica. Considerando-se os antiinflamatórios disponíveis atualmente e seus efeitos colaterais indesejáveis, torna-se de grande importância a busca de novos agentes potencialmente eficazes no tratamento de reações inflamatórias e que apresentem menos efeitos colaterais que os utilizados atualmente. **Apoio Financeiro**: CNPq, FAPERJ, IOC.

09.012

Effects of coffee on rat memory. Souza VYV[1], Lucena GMRS[2], Diniz JSV[3], Ferreira F[3], Campos EG[1] [1]UnB - Biologia Celular, [2]UnB - Ciências da Saúde, [3]UnB - Ciências Farmacêuticas

Introduction: Coffee as a beverage is widely consumed around the world. It has been linked to protective effects on various systems, including the central nervous system (CNS). Coffee phenolic compounds are reported to have antioxidant, anticarcinogenic, and antimutagenic effects. In the present study, we investigated the effects of coffee oral administration to rats on the short- and long-term memory using the step-down inhibitory avoidance test after acute exposure. **Methods:** Two months old female Wistar rats (n=10 per group) were used in this study. Coffee (1, 10 or 40 mg/kg; *Coffea Arabica*; commercial trade name *Prima Qualitá*), filtered water (control group; 10 mL/kg) or caffeine (CAF, 10 mg/kg, as positive control) were administered by oral route (p.o.) in rats evaluated in the step-down avoidance test. The test samples were given to the animals one hour before the training section (acquisition) or immediately after the training section (retention). The animals were evaluated for short- (1.5 h) and long-term memory (24 h). All experiments were in accordance with our guidelines for the care of laboratory animals (UnBDOC nº 7779/2006). **Results:** Treatment with coffee (1, 10 or 40 mg/kg) did not improved the latency during the acquisition of short- (H(3, N=40=5.0291; p=0.1697) and long-term memory (H(3, N=40=1.9593; p=0.5809), when compared to control group (Kruskal-Wallis test). However, coffee administration increased step-down latencies during the short (10 mg/kg) (H(3, N=40=9.8384; p=0.02) and long- (1 and 10 mg/kg) (H(3, N=40=9.9296; p=0.0192) term retention. The Mann-Whitney test indicated that the acute administration of coffee (1 or 10 mg/kg) or CAF (10 mg/kg), significantly increased the step-down latencies when compared to control group ($p \leq 0.01$), when analysed 1.5 or 24 h after the training session. **Discussion:** Our results agree with the literature which describes beneficial effects of coffee on cognitive functions and may be useful to compare different coffees in terms of effects on CNS. **Financial support:** FAPDF. **Acknowledgements:** We thank CNPq for the grant for the project.

09.013

Involvement of calcium in the vasorelaxant effect of the *Gochnatia polymorpha* ssp floccosa dichloromethane fraction in vascular smooth muscle of rats. da Silva RCMVAF[1], de Souza P[1], Crestani S[1], Batista R[2], Stefanello MEA[2], Marques MCA[1], Kassuya CAL[3]
[1]UFPR - Farmacologia, [2]UFPR - Química, [3]UFGD - Faculdade de Ciências da Saúde

Introduction: *Gochnatia polymorpha* ssp *floccosa* (Asteraceae) is known as “cambará” and is well recognized in Brazilian traditional medicine against diseases of respiratory airways. This study investigated the vasorelaxant effects of the dichloromethane fraction (DCM) obtained from ethanolic extract of the trunk of *G. polymorpha* ssp *floccosa,* and possible mechanism of action. **Methods:** The dried whole plant was ground and extracted with hexane and ethanol, successively. The crude extract was dissolved in a mix of ethanol-water 1:1 and submitted to extraction with dichloromethane. The dichloromethane fraction was submitted for phytochemical analysis. Male *Wistar* rats (200–250 g) were used in these experiments. Isolated aorta rings, with or without functional endothelium, were prepared according to the standard procedures previously described (Da Silva-Santos *et al.,* 2002). Tension was recorded via isometric force transducers coupled to a MacLab® recording system. After stabilization period (60 minutes) a curve of phenylephrine (1 μM) was obtained, and in the tonic phase of the contraction, cumulative concentrations of the DCM fraction was added (3-3000 μg/ml). For evaluation of the Calcium involvement, the methodology used was described by Rattmann, *et al.,* 2006. All procedures were approved by the Institutional Ethics Committee under protocol number 336. **Results and Discussion**: The phytochemical analysis showed the presence of abundant sesquiterpene lactones, diterpenes, triterpenes, coumarins and flavonoids in the DCM fraction. The maximum relaxation observed was of 43.2±7.0% obtained at concentration of 1000 μg/ml of DCM fraction in endothelium denuded aorta rings. To evaluate the effect of intracellular Calcium, the experiments were made in Calcium-free depolarizing nutritive solutions, DCM fraction (10-1000 μg/ml) reduced the contractions induced by phenylephrine with maximal reduction of 42%. Finally, to evaluate the effect of extracellular Calcium, denuded aorta rings were incubated with a depolarizing Ca^{2+}-free Krebs’ solution, DCM fraction (10-1000 μg/ml) reduced the contraction induced by $CaCl_2$ and maximal inhibition was 94±2%. The present study shows that DCM fraction obtained from *G. polymorpha* exhibit vasorelaxant activity in isolated aorta rings and this action was endothelium-independent with the main mechanism of action associated with extracellular calcium uptake and with intracellular calcium mobilization. So further studies are needed in order to study the compound(s) involved in this vasorelaxant effect. **Acknowledgements:** CNPq and CAPES. **References:** 1. Da Silva-Santos, J.E. *Shock* 17:70, 2002. 2. Rattmann, Y.D. *J Ethnopharm*, 104:328, 2006.

09.014

Anti-inflammatory activity of the butanolic fraction from *Gochnatia polymorpha ssp flocossa.* de Souza P[1], Piornedo RR[1], Lapa FR[1], Batista R[2], Stefanello MEA[2], Zampronio AR[1], Kassuya CAL[1] [1]UFPR - Farmacologia, [2]UFPR - Química

Introduction: *Gochnatia polymorpha* (Asteraceae) is known as “cambará” and is used in the folk medicine against respiratory diseases such as asthma. The aim of this study was to study the possible anti-inflammatory activity of the butanolic extract obtained from the barks of *G. polymorpha* in inflammation models in mice. **Methods:** The dried whole plant 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 ^{1}H. Male *Swiss* mice (25-35 g) received butanolic (BT) fraction (0.2-20 mg/kg, p.o.), or vehicle (10 ml/kg, p.o.) and after 1 h an injection of carrageenan (Cg, 300 µg) in the paw or Cg (1%/cavity) in the intrapleural cavity. Both paws were measured before and 0.5-4 h after induction of inflammation using a digital micrometer. Neutrophil migration was analyzed by measuring Myeloperoxidase (MPO) activity in the paw and the leukocyte numbers and protein exsudation were evaluated in the pleural cavity. All procedures were approved by the Institutional Ethics Committee under protocol number 336. **Results and Discussion**: The NMR ^{1}H analysis revealed that the crude extract and BT fraction contain phenolic compounds (probably clorogenic, cafeic and cinnamic acids) and flavonoids glycosides such as rutin. Oral administration of BT fraction significantly inhibited the paw oedema induced by Cg in a dose-dependent manner (inhibitions of 45±8, 82±7 and 83±4% for the doses of 2, 20 and 200 mg/kg, respectively, 2h after Cg injection). BT fraction also significantly reduced MPO activity 4 h after Cg injection (inhibitions of 12 ± 1%, 13 ± 1% and 14 ± 1%, for the doses of 2, 20 and 200 mg/kg, respectively. The oral administration of BT fraction significantly reduced the increase of total leukocyte number (52 ± 3%, 76 ± 6%, 80 ± 5%, 32 ± 11% at doses of 0.1, 1, 10 e 20 mg/kg, respectively), neutrophils (61 ± 7%, 73 ± 6%, 83 ± 1%, 57 ± 11% at doses of 0.1, 1, 10 and 20 mg/kg, respectively), mononuclear cells (64 ± 11%, 88 ± 6%, 74 ± 14% at doses of 0.1, 1 and 10 mg/kg , respectively) and also reduced the protein levels (31 ± 8%, 26 ± 6%, 44 ± 1%, 87 ± 13%, at doses of 0.1, 1, 10 e 20 mg/kg, respectively) induced by Cg injection in the pleural cavity. **Conclusion:** This study shows that the BT fraction from *G. polymorpha* exhibit an anti-inflammatory activity when used oral route. Also, these results may indicate that *G. polymorpha* can be of therapeutic interest against inflammatory respiratory disorders. However, further studies are necessary in order to evaluate the mechanism of action and possible toxicity. **Acknowledgements:** CNPq and CAPES.

09.015

Mikania laevigata decreases experimental periodontal breakdown. Campos-Júnior JC[1], da-Silva-Filho VJ[1], Vieira SM[2], Rodrigues IR[3], Uber-Bucek E[3], Napimoga MH[1], Benatti BB[5]
[1]UNIUBE - Biopatologia e Biologia Molecular, [2]COPE-INPA, [3]UNIUBE - Ciências Farmacêuticas, [5]UFMA - Periodontia

Introduction: The extract of *M. laevigata* (popularly known in Brazil as “guaco”) possesses anti-inflammatory properties. In the present study we tested the effects of *guaco* extract in a periodontitis experimental model in rats. We also investigated possible mechanisms underlying such effects. Periodontal disease was induced by a ligature placed around the mandible first molars of each animal. METHODS: All experimental procedures were approved by the Ethical Committee for Animal Research of the University of Uberaba (#001/2008). Male Wistar rats were divided into 4 groups: non-ligated animals treated with vehicle; non-ligated animals treated with “guaco” extract (10 mg/kg, daily); ligature-induced animals treated with vehicle and ligature-induced animals treated with *guaco* extract (10 mg/kg, daily). Thirty days after the induction of periodontal disease the animals were sacrificed and mandibles and gingival tissues removed for further analysis. RESULTS: Morphometrical analysis of alveolar bone loss demonstrated that *guaco*-treated animals presented a decreased alveolar bone loss and a lower expression of the activator of nuclear factor-κB ligand (RANKL) measured by immunohistochemistry. Moreover, gingival tissues from the guaco-treated group showed decreased neutrophil migration (MPO assay). DISCUSSION/CONCLUSION: These results indicate that *guaco* extract may be useful to control bone resorption during progression of experimental periodontitis in rats.

09.016

Lipid profile and blood glucose level of rats fed with yogurt containing extract of mate tea(*Ilex paraguariensis* ST. HILL.) and probiotics. Ril TF[1], Loch CR[2], Cichoski AJ[3], Valduga AT[4], Macedo SMD[1] [1]URI - Ciências da Saúde, [2]URI - Saúde Humana, [3]URI - Ciências Exatas e da Terra, [4]URI - Ciências Agrárias

Introduction: Many factors affect the quality of modern life, promoting the development of diseases. Therefore the production of food containing substances that help to improve health is something very important currently. In this context, the functional foods have health benefits such as the reduction of the incidence of various diseases and the maintenance of physical and mental well-being. Among this type of food, we have the probiotics, which are pure cultures of bacteria and, therefore, help maintaining health. Mate Tea has also been showing positive effects over health when used as mate and tea. The objective of this study was to evaluate the lipid profile and glucose level of rats subjected to the ingestion of yogurt containing extract of Mate Tea and probiotics for thirty days. Methods: Twenty-four rats were divided in three groups (n = 8/grupo); in group 1 (control), rats were treated with natural yogurt; group 2 was treated with yogurt containing extract of Mate Tea; and group 3, with extract of yogurt containing probiotic bacteria and Mate Tea. The animals were fed for 30 consecutive days by gavage with single dose of 1mL per day. This experimental protocol was approved by Ethics Committee of URI-Campus Erechim under number 024/PIA/09. Lipid profile was evaluated by the analysis of total cholesterol, HDL cholesterol, LDL cholesterol, and triglycerides; blood glucose level was evaluated by the test of seric glucose. Results and Discussions: Total cholesterol was 89.58±12,67mg/dL for group 1; 78.69±16.17mg/dL for group 2 and 87.79±24.30mg/dL for group 3; cholesterol HDL: group 1 had 67.64±40.53mg/dL; group 2, 60.46±16.13mg/dL and group 3, 59.85±22.62mg/dL; cholesterol LDL: group 1: 30.75±34.22mg/dL, group 2: 18.92±12.49mg/dL, and group 3: 13.95±14.22mg/dL; triglycerides: group 1: 104.23±45,19mg/dL; group 2: 128.62±69.16mg/dL and 104.46±57.10mg/dL, group 3. In the glucose test, group 1: 130.69±17.48mg/dL, group 2: 149.38±24.57mg/dL and group 3 138.54±27.04mg/dL. These results showed that yogurt with extract of Mate Tea did not promote changes on the majority of biochemical parameters studied in adult rats. There was also no sign of toxicity or death in rats that received this yogurt. However, there is a tendency of a decreasing in the levels of total cholesterol in animals that received yogurt with Mate Tea. **Reference**: SAAD I. M. SUSANA, Próboticos e Prébioticos: o estado da arte; *Revista Brasileira de Ciências Farmacêuticas*, vol. 42, n. 1, jan./mar., 2006 **Financial support**: BioTécnica and URI-Campus Erechim

09.017

Aspectos do mecanismo de ação antitumoral de fração proteolítica do látex de *Carica candamarcensis* em modelo murino de melanoma não-metastático. Figueiredo C[1], Lemos FO[1], Silva ACA[1], Viana CTR[1], Dittz D[1], Salas CE[2], Lopes MTP[1] [1]UFMG - Farmacologia, [2]UFMG - Bioquímica e Imunologia

Introdução: Resultados prévios têm demonstrado a eficácia de P1G10, fração proteolítica rica em cisteíno proteases, como antitumoral e antimetastático sobre diferentes modelos murinos[1,2]. Neste trabalho, passamos a investigar os possíveis mecanismos de ação envolvidos na atividade antitumoral sobre modelo de melanoma murino B16 F1. **Métodos:** Camundongos $C_{57}Bl_6$ machos (n=14) foram inoculados via *s.c.* com $5x10^5$ células B16F1/100mL. Após 4 dias, foram tratados com P1G10 5 mg/kg ou salina (controle), diariamente, via *s.c.*, por 15 dias. Após o sacrifício, os animais tiveram seus tumores removidos e processados para a determinação de hemoglobina (Hb), TGF β, TNFα, VEGF e da atividade de NAG[3,4]. A importância da atividade proteolítica na ação antitumoral, foi avaliada como descrito acima com a adição de um grupo que recebeu P1G10 5 mg/kg com a atividade proteolítica inibida por iodoacetamida (IAA), sendo determinada a massa tumoral. A avaliação da distribuição de P1G10 no tumor foi realizada através da administração de P1G10 marcada com ^{99m}Tc[5] via *s.c.*, diariamente, por 15 dias, em camundongos $C_{57}Bl_6$ machos (n=14) portadores de tumor B16F1. Os animais foram divididos em 3 grupos e sacrificados em tempos diferentes de tratamento (5, 10 e 15 dias). A taxa de captação de radioatividade no tumor foi medida em contador gama. **Resultados/Discussão:** Animais tratados P1G10-IAA mostraram tumores de massa semelhante (1,23 ± 1,21 g) ao grupo tratado com P1G10 5 mg/kg (1,05 ± 0,92 g), sugerindo que a atividade proteolítica não é fundamental para sua eficácia (controle – 2,36 ± 1,57, $p < 0,05$, ANOVA pós teste Bonferroni). No estudo da angiogênese, observamos que P1G10 reduziu a quantidade de Hb e VEGF (2,15 ± 1,19 rg/mg tumor e 1,4 ± 0,35 mg/mg tumor, respectivamente) em relação ao controle (7,90± 2,04 e 3,09 ± 0,80, respectivamente, $*p < 0,01$, Teste t de Student). Ao contrário, a atividade de NAG esteve aumentada para o grupo tratado (1,38 ± 0,27 OD/mg tumor) em relação ao controle (0,94 ± 0,40, $*p < 0,05$, Teste t de Student). As dosagens de citocinas TGF β e TNFα apresentaram valores semelhantes para ambos os grupos. Com o decorrer do tratamento com P1G10, se observa uma redução na captação de radioatividade (% cpm tumor/% cpm sangue), sendo no 5º dia de 0,86 ± 0,25, no 10º de 0,44 ± 0,12 e no 15 º de 0,39 ± 0,76. Este resultado corrobora com os obtidos sobre a atividade antiangiogênica, visto que, uma menor vascularização reduziria a quantidade de fração que atinge o tumor. **Conclusão:** Logo, se pode inferir que a atividade antiangiogênica de P1G10 deve estar envolvida na sua ação antitumoral, enquanto que a atividade proteolítica não é importante para esta ação. **Referências: 1**. FIGUEIREDO et al. *39º Cong Bras Farm Ter Exp,* 2008, 09119, R. Preto, 2. Viana et al, *40º Cong Bras Farm Ter Exp*, 2008, 09024, R. Preto, 3.PLUNKETT et al.,v.62,p.510-17,1990; 4. Bailey et al,v.162,327-34,1988, 5. Lemos et al, *40º Cong Bras Farm Ter Exp*, 2008, 09093, R. Preto. **Apoio Financeiro:** CNPq, FAPEMIG e CAPES. **Número do protocolo/CETEA-UFMG:** 103/2007.

09.018

O veneno de *Polybia occidentalis* apresenta ação antibacteriana *in vivo*. Quadros AU, Souza PF, Pittner E, Godoi, V, Shardosin AZ, Monteiro MC UNICENTRO Farmácia

Introdução: *Polybia occidentalis* é uma vespa social, pouco agressiva e bastante comum no Brasil. Atividades biológicas desencadeadas pelo seu veneno têm sido recentemente estudadas, tais como antibacteriana, fibrininogenolítica e antinociceptiva. Já é sabido, que o veneno de himenópteros estimula a liberação de mediadores importantes no recrutamento leucocitário, auxiliando assim na resolução de um quadro infeccioso. Por isto, esse trabalho teve como objetivo avaliar o efeito do veneno da vespa *Polybia occidentalis* no processo infecciosos induzido por *Staphylococcus aureus.* **Métodos e Resultados:** Infectou-se por via i.p camundongos BALB/c com *S. aureus,* sendo em seguida, tratados i.p com salina ou veneno (2,2 ou 4,4 µg/animal). Após 24 horas, os animais foram sacrificados e a cavidade peritoneal (c.p) lavada para as contagens total e diferencial das células e o cultivo bacteriano. Além disso, o fígado, baço e o coração foram retirados, macerados, centrifugados e o sobrenadante cultivado em ágar nutriente por 24 h a 37ºC, e as unidades formadoras de colônias (UFC) contadas. O tratamento tanto com 2,2 como com 4,4 µg de veneno nos animais infectados induziu principalmente a migração de mononucleares para o foco infeccioso (*S. aureus*/salina= 2 x 10^6; *S. aureus*/2,2µg de veneno= 15 x 10^6; *S. aureus*/4,4µg de veneno = 20 x 10^6céls/cav). Quanto a carga bacteriana, observou-se que os animais tratados com o veneno retinham a maior carga bacteriana no peritônio, fato que não foi observado nos animais tratados com salina (*S. aureus*/salina= 2 x 10^3; *S. aureus*/2,2= 3 x 10^3; *S. aureus*/4,4= 6 x 10^3 bactérias/órgão). Entretanto, a carga de *S. aureus* encontrada nos órgãos (fígado, baço e coração) dos animais tratados com o veneno foi extremamente baixa, quando comparado aos animais tratados com salina, cuja carga bacteriana foi elevadíssima nesses órgãos, principalmente o baço (*S. aureus*/salina= 3 x 10^4; *S. aureus*/2,2= 2 x 10^3; *S. aureus*/4,4= 2 x 10^2 bactérias/órgão). **Conclusão:** O veneno estimulou a migração de células mononucleares para o foco da infecção, o que pode ter auxiliado na retenção do agente infecciosos no peritônio, dados observados nos animais tratados com o veneno. Além disso, esse tratamento impediu uma maior disseminação do *S. aureus* por órgãos como baço, fígado e pulmão nesses animais infectados com a bactéria. **Apoio Financeiro**: CNPq, Fundação Araucária, UNICENTRO

09.019

Isolamento parcial de uma fosfolipase no veneno da vespa *Polybia occidentalis* com ação antibacteriana. Quadros AU[1], Pittner E[1], Shardosin AZ[1], Soares AM[2], Oliveira C[3], Marcussi S[2], Monteiro MC[4] [1]UNICENTRO Farmácia, [2]FCFRP - Análises Clínicas Toxicológicas e Bromatológicas, [3]USP - Farmácia, [4]UFPA - Farmácia/Microbiologia

Introdução: O veneno de vespas é composto por uma mistura de componentes bioquímicos e farmacologicamente ativos, especialmente enzimas e dentre elas fosfolipases (FL), responsáveis por promover a hidrólise de fosfolipídeos de membrana. Nos últimos anos, nosso grupo vem demonstrando que o veneno da vespa *P. occidentalis* tem ação fibrinogenolítica e antibacteriana *in vitro*, superior a melitina (*Apis mellifera*), e com 80% da ação da gentamicina, além de apresentar amplo espectro de ação, atuando tanto contra bactérias gram-positivas quanto gram-negativas de amostras de isolados clínicos e cepas padrão. Com isso, esse trabalho teve como objetivo avaliar a atividade fosfolipásica do veneno da *P. occidentalis e* correlacioná-la a ação antibacteriana observada *in vitro. E*m seguida, realizar um fracionamento do veneno e testar essas frações quanto as atividades fosfolipásica e antibacteriana. **Métodos:** Para obtenção das frações do veneno, 150 mg de veneno foram submetidos a cromatografia de exclusão molecular, em Sephadex G-75 com fluxo de 15 mL/hora. A leitura dos eluentes foi realizada em espectrofotômetro UV em 280 nm e em seguida as frações coletadas foram analisadas quanto a sua pureza em gel de SDS-PAGE 12%. A avaliação da atividade fosfolipásica do veneno bruto e das frações foi feita pelos testes de hemólise indireta em placa, além de que, com o veneno bruto também foram realizados os métodos de titulação potenciométrica e hidrólise de lipídios NBD. Quanto a ação antibacteriana, o veneno bruto e as frações foram testados, frente a *Staphylococcus aureus* ATCC 25923, pelos métodos de difusão em ágar e contagem de unidade formadora de colônias (UFC), respectivamente. **Resultados e Discussões:** Os ensaios mostraram a presença de uma FL no veneno, sendo que 50 µg de veneno foram capazes de induzir um halo hemolítico de até 0,9 cm, e a análise de titulação potenciométrica mostrou que 6,48 µg do veneno já foram capazes de hidrolisar até 75% dos lipídios quando comparados ao controle (veneno de *Bothrops jussu*). No ensaio de hidrólise de lipídios NBD, também se observou significativa hidrólise do lipídio, que foi potencializada na presença de cálcio, revelando um perfil cálcio dependente da enzima. A atividade antibacteriana do veneno revelou também um perfil cálcio dependente, visto que a inibição de 36% das UFC induzida por 22 ug de veneno foi potencializada na presença de cálcio 0,1 mM (94%). Por cromatografia de exclusão molecular foi possível obter 5 frações a partir do veneno bruto, cujo gel SDS-PAGE de cada uma delas revelou que a fração de maior pureza foi a 2, de alto peso molecular (cerca de 66 kD). O teste de hemólise indireta mostrou que 100 µg da fração 2 foram capazes de induzir um halo de hemólise de aproximandamente 1 cm, enquanto que as demais frações não apresentaram atividade significativa. Além disso, a fração 2 também foi a única a inibir o crescimento de *S. aureus in vitro,* apresentando um halo de inibição de 1,7 cm. **Conclusões:** No veneno de *P. occidentalis* foi observada a presença de uma fosfolipase, que após fracionamento se encontrava na fração 2, tendo alto peso molecular. Além disso, nossos dados sugerem que essa FL é a principal responsável pela atividade antibacteriana do veneno *in vitro*. **Apoio financeiro:** CNPq, Fundação Araucária e UNICENTRO

09.020

Efeito do tratamento com óleo essencial do Alecrim (*Rosmarinus officinalis* L.) sobre a quimiotaxia de leucócitos *in vitro.* Farinha TO, Fonseca JP, Anteguera AAC, Dantas JA, Nogueira de Melo GA, Caparroz-Assef SM, Bersani-Amado CA, Cuman RKN UEM - Farmácia e Farmacologia

Introdução: A espécie vegetal, *Rosmarinus offficinalis* L., conhecida popularmente como alecrim, tem sido utilizada na medicina popular para o tratamento de quadros febris, afecções hepáticas e das vias biliares, dispepsia, ansiedade, astenia, anorexia, cefaléia, bronquite crônica, asma brônquica e dores de origem reumática. Trabalhos têm demonstrado a atividade anti-inflamatória do extrato e do óleo essencial desta planta. **Objetivo:** Avaliar a atividade anti-inflamatória do óleo essencial do alecrim (OEA) sobre a quimiotaxia de leucócitos *in vitro*. **Métodos:** Os ensaios de quimiotaxia foram realizados em câmara de Boyden, utilizando-se filtros de nitrocelulose (poros de 8mm). Quatro horas após a injeção intraperitoneal de carragenina (200µg) em ratos machos Wistar, foram obtidos leucócitos a partir do exsudato peritoneal. Foi avaliada a viabilidade celular com azul de trypan e as células foram incubadas com OEA em diferentes concentrações (10^{-4}mL/mL,10^{-3}mL/mL ou 10^{-2}mL/mL) durante 30 min. No compartimento superior da câmara foi colocada a suspensão de células ($1x10^6$) e no inferior, a caseína (5%) como agente quimiotáxico. Após incubação em estufa de CO_2 por uma hora, os filtros foram retirados da câmara, fixados em etanol absoluto e corados com hematoxilina-eosina. O comportamento celular (quimiotaxia) foi avaliado por meio da contagem da distância percorrida através do filtro (µm) e o número de células migradas por microscopia óptica. Os procedimentos experimentais foram aprovados pelo Comitê de ética em Experimentação Animal / UEM (CEAE) e registrados sob n° 016/08. **Resultados:** O tratamento de leucócitos com diferentes concentrações de OEA inibiu significativamente a distância percorrida através do filtro ($p<0,05$): **Controle:** 81,23 ± 0,96mm; **$OEA_{10^{-4}mL/mL}$:** 63,68 ± 1,00* mm; **$OEA_{10^{-3}mL/mL}$:** 62,85 ± 1,23* mm; **$OEA_{10^{-2}mL/mL}$:** 50,82 ± 1,251* µL/mL. Somente o tratamento com OEA na dose de 10^{-2} µL/mL inibiu significativamente o número de células migradas ($p<0,05$): **Controle:** 28,60 ± 1, 965 cél.; **$OEA_{10^{-4}mL/mL}$:** 23,64 ± 1,262 cél.; **$OEA_{10^{-3}mL/mL}$:** 27,94 ± 1,564 cél.; **$OEA_{10^{-2}mL/mL}$:** 17,14 ± 1,365* cél. **Discussão:** Os resultados preliminares indicam que o OEA apresenta atividade inibitória sobre quimiotaxia de leucócitos avaliada a partir da distância percorrida e o número de células migradas. **Apoio Financeiro:** CAPES/CNPq/FADEC

09.021

Avaliação da atividade antiulcerogênica do extrato etanólico obtido a partir das folhas de *Terminalia catappa* Linn. (Combretaceae). Silva LP, Angelis CD, Toma W UNISANTA – Farmácia

Introdução: *Terminalia catappa* L. (Combretaceae), trata-se de espécie comumente utilizada para arborização ao longo de todo o litoral brasileiro. Popularmente conhecida como Amendoeira-da-Praia, Cuca e Chapéu-de-sol, vem sendo utilizada para o acometimento de males do trato gastrintestinal. O objetivo do trabalho foi avaliar a atividade antiulcerogênica do extrato etanólico (EtOH) obtido a partir de folhas de *Terminalia catappa* Linn em modelos de indução de úlcera gástrica em roedores. **Métodos:** *Processo extrativo e análise fitoquímica***:** Foi realizado procedimento de secagem em estufa a 50ºC por seis dias, seguida de maceração em etanol absoluto por sete dias, filtração e rotaevaporação. EtOH foi analisado qualitativamente e posteriormente submetido a ensaios farmacológicos. *Ensaios farmacológicos*: DAINE/Betanecol, HCl-Etanol, Etanol, isquemia-reperfusão e úlcera crônica após administração de ácido acético 30% e Ligadura do Piloro, sendo os dados obtidos submetidos à ANOVA com teste posteriori de Dunnet. Tais ensaios foram aprovados pelo Comitê de Ética da Universidade Santa Cecília com protocolo nº53/07. **Resultados:** Na análise fitoquímica qualitativa foi detectada presença de flavonóides pela coloração obtida, sendo, fluorescência verde na reação de Taubock, amarelo na reação de NaOH e verde nas reações de $AlCl_3$ e $FeCl_3$. Os ensaios farmacológicos demonstram redução da incidência de úlceras de 58,1% (**p<0.01) em DAINES/Betanecol; 70,76% (**p<0,01) em HCl-etanol; 47,11% (**p<0,01) no modelo de etanol em ratos; 40,5% (**p<0,01) no modelo de úlcera crônica e 62,74% (**p<0,01) em isquemia-reperfusão. No modelo de ligadura do piloro houve aumento dos valores de pH (**p<0,01), redução da concentração de íons H^+ (**p<0,01) e redução no volume gástrico secretado (*p<0,05). Todos os valores foram comparados em relação ao controle negativo (salina 0,9%). **Discussão:** Através da análise fitoquímica verificou-se a presença de flavonóides, que pelo resultado das reações podem ser da classe das flavonas e/ou flavonóis, fazendo parte desse grupo o canferol e a quercetina, que já haviam sido identificados em estudos anteriores dessa planta. Através dos ensaios farmacológicos verificou-se a atividade antiulcerogênica do extrato avaliado podendo ser sua atividade citoprotetora gástrica e/ou atividade anti-secretora. Tais atividades podem estar relacionadas à atividade antioxidante dos flavonóides, cuja literatura demonstra capacidade de seqüestro de radicais livres derivados do oxigênio, bem como, relação de tal mecanismo como citoprotetor e/ou antisecretor. **Referências Bibliográficas:** Andreo A.A. *J. Ethnopharmacol.* 107(3): 431, 2006. Chen P.S. *Cancer Letters* 152, 115-122, 2000. Mizui,T. *Japanese Journal of Pharmacology*, v.44, p.43, 1987. 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. **Apoio financeiro FAPESP: Processo nº 07/59074-2**

09.022

Diuretic effects of *Coix lacryma-jobi L*. (Poaceae). Boffo MA[1], Vieira LCD[1], Leme TSV[1], Cosmo MLA[1], Uchida DT[1], Lourenço EL[2], Kassuya CAL[3], Marques MCA[3], Gasparotto Júnior A[2] [1]UNIPAR - Farmacologia, [2]UNIPAR/UFPR - Farmacologia, [3]UFPR - Farmacologia

Introduction: *Coix lacryma* – jobi L. (POACEAE), popularly known in Brazil as "Conta de Lágrimas", Capim Miçanga" or "Capim Rosário". This species has been used by the brasilian folklore medicine as panacea for a great diversity of health problems. The part used as medicine is all the aerial part and habitually it is employed as an infusion or boiled[1]. Traditionally, this plant has been used as diuretic, however, few studies on biological activities have been carried out with *C. lacryma* , in order to confirm its assumed beneficial properties. Therefore, the present study was undertaken to verify the efficacy of the infusion and hydroethanolic extract (90:10) of the *C. Lacryma* (HECL) as diuretic drug in experimental rats. **Methods:** Six groups of rats (180-200g) were orally administered 5 mL/kg of the infusion (125, 250 and 500 mg/kg; n=6) and HECL (75, 150 and 300 mg/kg; n=6). One group of rats received orally 5mL/kg of hydrochlorotiazide (10 mg/kg). Control rats received the same amount of deionized water (5 mL/kg)[2]. Urine was collected in a graduated cylinder and its volume was recorded at 2h intervals for 8h. Cumulative urine excretion was calculated in relation to body weight and expressed as ml/100g body weight. Plasmatic and urinary electrolyte (Na^+ and K^+) concentrations were measured using a Jenway Corp. model PFP7 flame photometer. pH and conductivity were directly determined on fresh urine samples using a HI-8424 Hanna Instruments pH-meter and a LF-320 WTF conductivity meter, respectively. Density estimation was made by weighing with a Mettler AE163 (± 0.1mg) analytical balance on urine volume measured with a Nichiryo micropipette. Concentrations of creatinine and urea in plasma were analyzed using an automated chemistry analyzer (FRYKA Kaltetechnik-Ohmstraße 4, D-73730 Esslingen, Germany)[3]. All procedures were approved by the Institutional Ethics Committee of UNIPAR (authorization number 14957/2009). **Results:** The oral administration of 5 and 10% (corresponding to 250 and 500 mg/kg) of the infusion and 150 mg/kg of HECL increased significantly the urinary excretion when compared with untreated controls (4.08 ± 0.36**, 4.00 ± 0.32** and 3.47 ± 0.47* mL/100g/8hs, respectively; control group 2.44 ± 0.12 mL/100g/8hs). The HECL showed an interesting increase in sodium excretion, especially significant at 300 mg/kg with similar values to the groups that had received HCTZ (Control 100 ± 7.71 mmol/L; HEAM 126 ± 8.2 mmol/L *$p < 0.05$; and HCTZ 139 ± 4.2 mmol/L **$p < 0.01$). Urinary potassium, pH, conductivity and density, and plasmatic creatinine, urea, sodium and potassium determined at the end of the experiment (8 h), were not affected by any of the drugs tested. **Discussion:** The results suggest that infusion and HECL could present compound(s) responsible for diuretic activities with no signs of toxicity, and this diuretic action coud explain, at least in part, the ethnopharmacological uses of *C. lacryma*. 1. Ribeiro R A. *et al. J. Ethnopharmacology* 24: 19-29, 1996. 2. Benjumea D. *et al*. *J. Ethnopharmacology* 100: 205-220, 2005. 3. Gasparotto Jr A. *et al*. *J Ethnophamacology* 122: 517-522, 2009. **Acknowledgements:** DEGPP/UNIPAR

09.023

Potassium channel activation contributes to the vasorelaxant effect induced by warifteine in the rat aorta. Assis ACL[1], Araujo IGA[1], Lima RPC[1], Almeida MM[1], Silva DF[1], Marinho AF[1], Barbosa Filho JM[1], Cruz JS[2], Medeiros IA[1] [1]LTF-UFPB, [2]UFMG - Bioquímica e Imunologia,

Introduction: Warifteine, a bisbenzylisoquinoline alkaloid, was isolated from the leaves of *Cissampelos sympodialis* Eichl (Menispermaceae). This study was conducted to investigate the mechanisms by which warifteine causes vasorelaxation in the rat thoracic aorta. **Methods:** Rat aortic rings (2-4 mm) were suspended by platinum hooks for isometric tension recordings. Potassium current were recorded using the whole-cell configuration of the patch-clamp technique in freshly dissociated vascular myocytes isolated from rat aorta. All procedures were in compliance with Animal Research Ethics Committee (0905/07). **Results:** In rat aortic rings, with endothelium intact, warifteine (1 pM-10 μM) induced concentration-dependent relaxation of the contractions induced by norepinephrine (0.1 nM-100 μM) (pD_2=9.4±0.06, n=5) which was not attenuated after endothelium removal (pD_2=9.2±0.10, n=5). These results demonstrated that the vascular endothelium probably is not participating in the vasorelaxant response induced by warifteine. Therefore all the experimental protocols were carried out in endothelium-denuded aortic rings. Warifteine also induced relaxations (pD_2=9.2±0.19, n=8) in rings pre-contracted with prostaglandin F2alfa (1 μM-10 mM). In contrast, the relaxant activity of warifteine was nearly abolished in high- K^+ (80 mM) pre-contracted aortic rings. The vasorelaxant effect induced by warifteine was significantly atenuated when the vessels were pre-treated with K^+ channels blockers, such as KCl (20 mM), TEA (1, 3 and 5 mM), 4-aminopyridine (1 mM), glibenclamide (10 μM) (pD_2 = 6.7±0.63, n=5; 8.0±0.35, n=6; 6.5±0.17, n=5; 8.0±0.30, n=5; 8.3±0.2, n=7 and 8.1±0.1, n=5, respectively). Furthermore, $BaCl_2$ (1 mM), did not significantly affect the relaxant response to warifteine (pD_2=8.8±0.16, n=5). In vascular myocytes, warifteine (100 nM) significantly increased whole-cell potassium currents about 2-fold at 70 mV. **Conclusion:** Taken together, these data suggest that warifteine induces potent concentration-dependent relaxation in the rat aorta, which is, in part, mediated by activation of K+ channels. **Financial support**: CNPq/CAPES/FAPEMIG.

09.024

Topical anti-inflammatory and anti-hyperproliferative of *Combretum leprosum.* Silva CD[1], Mendes DAGB[1], Pietrovski EF[1], Santos ARS[2], Facundo VA[3], Otuki MF[1], Cabrini DA[1]
[1]UFPR - Farmacologia, [2]UFSC - Ciências Fisiológicas, [3]UNIR - Química

Introduction: The flowers of medicinal plant *Combretum leprosum* MART & EICHER (Combretaceae), found in the north of Brazil, presented an interesting anti-inflammatory effect on mouse skin when topically applied (Silva *et al*., 2008). The aim of this study was to evaluate the activity of the etanolic extrat (EE) of flowers from *C. leprosum* in a chronic skin inflammation in mice. **Methods:** Female Swiss mice (20-30g) were used. EE activity was valued in the animal model of skin multiple applications of croton oil. Croton oil (0.4 mg/ear) and EE (0.6 mg/ear) were dissolved in acetone (20 µL) and applied on the right ear of the mice. Croton oil was applied in an alternate manner for 9 days, the EE topical treatment (0.6 mg/ear, 2x/day) started after 4th day and the oedema (ear thickness) was measured daily. After chronic treatment, animals were sacrificed and samples were collected for histological and immunohistochemical analysis. Time course analysis was performed through acute ear oedema model induced by 12-O-tetradecanoylphorbol acetate (TPA). All animal procedures were approved by the Institutional Ethics of our University (n.296). **Results**: In the chronic model, EE and dexamethasone reverted oedema formation evidenced by the ears weight with inhibition of 63 ± 3% and 77 ± 2%, respectively. Histological analysis demonstrated that croton oil promoted an increase of epidermis thickness and both ethanolic extract and dexamethasone were effective reducing the epidermal hyperproliferation in 50 ± 4% and 70 ± 2%, respectively. Immunohistochemical analysis allowed the quantification of PCNA positive cells. The treatment with croton oil promoted an increase in the proliferative cells localized in the basal of epidermis and both extract and dexamethasone inhibited this increase in 27 ± 12% and 65 ± 6%, respectively. Time course analysis against TPA-induced ear oedema showed that the EE reduced oedema formation when treatment was performed six hours before (-6) until three hours after (+3) TPA, confirming its activity in an existing inflammatory process. However maximum inhibitory response was observed when the EE was applied simultaneously with TPA (90 ± 4% inhibition). **Discussion:** Our results suggest that the flowers of *C. leprosum* can be effective as a topical anti-inflammatory agent. Since it was able to reverse skin inflammatory and proliferative process, it could be considered as a new potential tool for the treatment of skin inflammatory diseases. However, it is necessary to continue the investigation about the efficacy and security of this plant. **References:** Silva, C.D. 40º Con Bras Farm Ter Exp, 2008. **Support:** Capes, CNPq and Fundação Araucária.

09.025

Efeito cicatrizante do extrato hidroalcoólico de *Salvia officinalis* em úlceras induzidas por ácido acético em ratos. Allemand A[1], Potrich BP[1], Mota, L.[1], Freitas CS[1], Baggio CH[1], Mendes DAGB[1], Santos AC[1], Werner MFP[2], Andre E[3], Pizzolatti MG[4], Marques MCA[1], Otuki MF[1] [1]UFPR - Farmacologia, [2]UFSC - Farmacologia, [3]University of Ferrara - Experimental and Clinical Medicine [4]UFSC - Química

Introdução: As folhas da *Salvia officinalis* são bastante conhecidas por suas propriedades antioxidantes e anti-inflamatórias (POECKEL et al, 2008). Este trabalho tem como objetivo verificar o potencial cicatrizante do extrato hidroalcóolico de *S. officinalis* (EHS) em úlceras já estabelecidas. **Métodos:** Ratas (250g) em jejum de 18h foram anestesiadas para a exposição do estômago. Sobre a serosa foi aplicado um cilindro de vidro de 6mm de diâmetro, dentro deste foi injetado 500µl de ácido acético 80%. Após 1 minuto o ácido foi aspirado, o estômago lavado com salina e a parede abdominal suturada (OKABE, et al, 1971). Os animais foram divididos em 3 grupos de tratamento(2x ao dia, via oral): grupo controle lesado (0,1 mL/100g), grupo omeprazol (40 mg/kg) e grupo EHS. Após 7 dias de tratamento os animais foram sacrificados, seus estômagos retirados e as áreas das lesões mensuradas (mm^2) com auxílio de uma régua milimetrada. Foram realizadas dosagens de glutationa reduzida (GSH), atividade das enzimas mieloperoxidase (MPO) *in vivo* e *in vitro*, N-acetilglucosaminidase (NAG), superóxido dismutase (SOD), catalase (CAT), além da quantificação intracelular de radicais livres (RL), o qual foi determinado pela medida da fluorescência emitida pela sonda DCFH e a quantificação da formação de hidroperóxidos durante a peroxidação lipídica. Os procedimentos com animais foram aprovados pelo Comitê de Ética da UFPR sob o número de protocolo 318. **Resultados:** Os grupos tratados com EHS (0,003; 0,03; 0,3 e 3,0 mg/kg) e omeprazol apresentaram uma redução de 18%, 30%, 49% , 65% e 55% na área das lesões, respectivamente, comparado com o grupo controle. O tratamento com EHS (3,0 mg/kg) conseguiu restabelecer à níveis basais o aumento das enzimas MPO, NAG e SOD provocado pela ácido acético. Essa mesma dose não foi capaz de reverter a diminuição de GSH e CAT. *In vitro*, a incubação com EHS (1,0; 10,0 e 30,0 µg/mL) reduziu a atividade enzimática da MPO em 13%, 19% e 23% respectivamente. O aumento de 60% na produção de RL no grupo lesado foi completamente restabelecido no grupo tratado com EHS (3,0 mg/kg). A peroxidação lipídica foi reduzida em 38% no grupo tratado com o EHS. **Conclusão:** O tratamento com EHS (3,0 mg/kg) foi efetivo na resolução da úlcera. Um fator importante para este efeito do EHS pode ser a diminuição da infiltração de neutrófilos, observada através da redução da atividade da MPO *in vivo.* A redução nos níveis de radicais livres e conseqüentemente da atividade da enzima antioxidante SOD e peroxidação lipídica também indicam uma redução na infiltração de neutrófilos capazes de liberar esses radicais. A redução da MPO *in vitro* pelo EHS sugere o seqüestro de H_2O_2, substrato para esta enzima. Esses resultados sugerem potente efeito cicatrizante do EHS em úlceras gástricas induzidas por ácido acético, que parece decorrer da atividade seqüestradora de radicais livres além da inibição do processo inflamatório.**Referências:** POECKEL et al, *Biochem. pharmacol*. v.7 6, p. 91, 2008; OKABE, *Am J Dig Dis*. v.16, p.277, 1971. **Financiamento**: CNPq e Fundação Araucária.

09.026

Evaluation the action mechanism involved in gastroprotective of the hydroalcoholic fraction of *Herissantia crispa* (L.) *brizicky* in animals: the role of sulphydryls compounds and nitric oxide. Dias GEN, Mota KSL, Lima IO, Teles YCF, Sousa MFV, Batista LM LTF-UFPB - Ciências Farmacêuticas

Introduction: Plant extracts are among the most attractive sources for developing new drugs in the treatment of gastric ulcers. *Herissantia crispa* is a specie which belongs to the Malvaceae family. It was chosen by the quimiotaxonomic criterion that points this specie as rich in flavonoid. The hydroalcoholic fraction of *H. crispa* showed gastroprotective action in animal models (ethanol, non-steroidal antiinflamatory and stress). The aim of this work is to evaluate the action mechanism of the gastroprotective activity of the hydroalcoholic fraction of *H. crispa.* **Materials and methods:** Male Wistar rats (180-250g) were used. The experimental model used to determinate the action mechanism was ethanol-induced gastric lesions in NEM-pre-treated rats, n=5 or 8 animals (Matsuda et al., Life Sciences, 65, 27 1999) and ethanol-induced gastric lesions in L-NAME-pre-treated rats, n=5 or 8 animals (Sikiric et al., J Pharmacol, 332, 23 1997). The results are expressed as the mean ± S.D. Statistical significance was assessed by one-way analysis of variance, followed by Dunnett and Tukey-Kramer tests for multiple comparisons. The level of significance was $p<0.05$. Number of Ethical in Animal Research license is 705/06. **Results:** The action of hydroalcoholic fraction (62,5 mg/kg) to NEM-pre-treated rats (278,3 ± 45,51) was different with the saline-pre-treated rats (187,8 ± 50,87). The effect of hydroalcoholic fraction (62,5 mg/kg) to L-NAME-pre-treated rats (168 ± 32) did not cause significant differences when compared with the saline-pre-treated rats (173 ± 54). **Discussion:** Based on those results it can be concluded that the mechanism involving the gastroprotective action the hydroalcoholic fraction of *H. crispa* does not depend of the nitric oxide way, but depends of the sulphydryls compounds. **Financial Support:** CNPq/LTF/UFPB

09.027

Mechanisms of action of *Cipura paludosa* ethanolic extract on memory in rats. Lucena GMRS[1], Diniz JSV[2], Ferreira F[3], Porto FA[4], Pinheiro WB[2], Santos ARS[5], Campos EG[6], Azevedo MS[7], Ferreira VMM[1] [1]UnB - Ciências da Saúde, [2]UnB - Ciências Farmacêuticas, [3]FS-UnB, [4]UnB - Farmácia, [5]UFSC - Ciências Fisiológicas, [6]UnB - Biologia Celular, [7]UNIR - Química

Introduction: Previous studies from our laboratory showed protective effects of *Cipura paludosa* ethanolic extract (EE) against rat memory dysfunctions. In this study we investigated the mechanisms of action of EE on short- and long-term memory. **Methods:** Male Wistar rats (n=10 per group) were pre-treated by intraperitonal route (i.p.) with one of the following substances: MK801 (0.01 mg/kg, a NMDA non-competitive antagonist), atropine (1 mg/kg, a non-selective muscarinic antagonist), mecamylamine (5 mg/kg, a selective nicotinic α3β4 antagonist), L-NAME (2 mg/kg, a nitric-oxide synthase inhibitor) or vehicle (10 mL/kg). After 30 min the animals were treated acutely by oral route (p.o.) with EE (100 mg/kg) or saline (10 mL/kg). One hour later, they were submitted to the step-down inhibitory avoidance test and the short- (1.5 h) and long- (24 h) term memory were evaluated after the training session. All experiments were in accordance with our guidelines for the care of laboratory animals (UnBdoc 67849/2005). **Results:** Treatment with EE improved memory processes in rats submitted to the inhibitory avoidance test in the short- and long-term memory when compared to the control group treated with saline ($H(3, N=40=29.6; p \leq 0.0001)$, analysed by Kruskal-Wallis test. Additionally, in the Mann-Whitney test this effect was blocked by i.p. treatment of rats with MK 801 (0.01 mg/kg, $p<0.05$), mecamylamine (5 mg/kg, $p<0.05$) or L-NAME (2 mg/kg, $p<0.05$). However, the facilitator effect of EE was not blocked by treatment with atropine (1 mg/kg, $p<0.05$). **Discussion:** Our results demonstrated that EE improved the memory of rats in the inhibitory avoidance test. EE seemed to produce this effect by modulation of the L-arginine-nitric oxide pathway, and also by glutamatergic and cholinergic systems. **Acknowledgements:** We thank CAPES for a Ph.D. fellowship.

09.028

Dicksonia sellowiana induces endothelium-dependent relaxations mediated by a redox-sensitive Src- and Akt-dependent activation of eNOS in the porcine coronary artery. Rattmann YD[1], Anselm E[2], Kim J-H[2], Dal-Ros S[2], Miguel OG[3], Auger C.[2], Chataigneau T.[2], Santos ARS[4], Schini-Kerth V. B.[2] [1]UFPR - Farmacologia, [2]Université de Strasbourg - Biophotonique et de Pharmacologie, [3]UFPR - Farmácia, [4]UFSC - Ciências Fisiológicas

Introduction: Several epidemiological studies have indicated that regular consumption of fruits and vegetables rich in polyphenols is associated with a reduced mortality from coronary heart diseases. The present study examined whether polyphenols contained in a standardized hydroalcoholic extract of *Dicksonia sellowiana* leaves (HEDS) enhance the endothelial formation of nitric oxide (NO), a major vasoprotective factor, and, if so, to characterize the underlying mechanism. ***Methods:*** Left coronary artery rings were prepared from porcine hearts and suspended in organ chambers for the determination of changes in isometric tension. The phosphorylation level of Src, Akt and endothelial NO synthase (eNOS) was assessed by Western blot analysis in cultured coronary artery endothelial cells. The formation of reactive oxygen species (ROS) and the level of phosphorylated eNOS at Ser 1177 were determined in sections of porcine coronary artery using dihydroethidine and a specific antibody, respectively, by confocal microscopy. The procedures were approved by the Research Ethics Board of the UFPR (number 287). ***Results:*** HEDS induced endothelium-dependent relaxations, which were markedly reduced by L-NA, an eNOS inhibitor, and slightly by charybdotoxin (CTX) plus apamin (APA), two inhibitors of EDHF-mediated responses, whereas they were abolished by the combination of L-NA, CTX plus APA. HEDS-induced relaxations were also reduced by calmidazolium, a calmodulin inhibitor, but not by KN-93, a selective CaMKII inhibitor. In addition, they were markedly reduced by MnTMPyP (a membrane permeant mimetic of superoxide dismutase, SOD), polyethyleneglycol-catalase (PEG-catalase, a membrane permeant analogue of catalase), PP2 (an inhibitor of Src kinase), and by wortmannin (an inhibitor of the PI3-kinase). HEDS caused the sustained phosphorylation of Akt and eNOS at Ser1177 in endothelial cells, these effects were markedly reduced by MnTMPyP, PEG-catalase and inhibitors of PI3-kinase. ***Discussion:*** The present findings indicate that HEDS strongly induced endothelium-dependent relaxations of coronary artery rings, which were predominantly mediated by NO. They further indicate that HEDS caused eNOS activation by phosphorylation through the redox-sensitive activation of the Src/PI3-kinase/Akt pathway and possibly also via a calmodulin-dependent pathway in endothelial cells. ***Acknowledgment:*** This study was supported in part by a fellowship from CAPES.

09.030

Protective effects laticifer proteins from *Calotropis procera* in sepsis induced by cecal ligation and puncture model. Oliveira RSB[1], Freitas LBN[2], Figueiredo IST[2], Pinheiro RSP[2], Matos MPV[1], Lima Filho JVM[3], Ramos MV[1], Alencar NMN de[2] [1]UFC - Bioquímica e Biologia Molecular, [2]UFC - Fisiologia e Farmacologia, [3]UFRPE - Departamento Biologia

Introduction: The latex of *C. procera* has been extensively used in folk medicine. Many studies describe interesting properties on immune responses displayed by latex molecules as anti-inflammatory, healing and anti-cancer activities. In this study the protective effect of laticifer proteins (LP) was evaluated during a lethal experimental infection using the cecal ligation and puncture (CLP) model. **Methods**: Animal handling and experimental protocols were registered on the Institutional Ethics Committee under number 24/09. Sepsis was induced in male Swiss mice (25-30 g) through CLP model. Briefly, mice treated or not with LP (LP-CLP 5, 10 and 25 mg/kg; i.p.) 24 hours before the surgery were anesthetized i.m. with 2% xilazine chloridate and 10% ketamine chloridate. A 1 cm midline incision was made on the anterior abdomen, and the cecum was exposed and ligated below the ileo-cecal junction. The cecum was punctured 1 transverse time with a 18 G1/gauge needle and squeezed under sterile conditions. All groups were analyzed for survival rate, assessed daily for 7 days. The neutrophil migration to the peritoneal cavity was evaluated at 4 and 24 h after surgery in LP-CLP 10 mg/kg, sham and CLP groups. The content of tumor necrosis factor-α (TNF-α) and interleukin-1-beta (IL-1β) in the supernatant of the macrophages in medium containing LP (500 µg/ml) was estimated *in vitro* by the ELISA immunoassay. **Results and Discussion**: No death was observed on Sham-operated animals. CLP mice succumbed until 24 hours after surgery. Animals pre-treated with LP showed 30% (5 and 25 mg/kg) and 40% (10 mg/kg) survival at day 7 after surgery ($p < 0.05$). Relevant neutrophil migration was observed in LP-CLP 10 mg/kg at both 4 and 24 h after surgery, compared with that in sham and CLP groups. Interestingly, in the CLP animals, despite the high degree of infection, the neutrophil migration toward the peritoneal cavity was not statistically different from that observed in the sham-operated animals at either 4 or 24 h after surgery. The incapacity of the CLP animals to restrict the infection in the peritoneal cavity may be due to the impairment of neutrophil migration to the infection focus in these animals. On the other hand, in LP-CLP 10 mg/kg mice, in which an impairment of neutrophil migration was not observed, the bacterial infection was restricted to the peritoneal cavities. Moreover, cultured macrophages pretreated with LP and stimulated with LPS did not modify TNF-α secretion by cells, but released significantly less IL-1β ($p < 0.05$). The data support that LP reduces mortality and prevents the neutrophil migration failure provoked by the infectious focus and down-regulates the pro-inflammatory cytokine IL-1β level. Keywords: *Calotropis procera*, sepsis, laticifers, proteins. Supported by CNPq, CAPES, RENORBIO and IFS.

09.031

Effects of natriuretic peptide isolated from *Crotalus durissus cascavella* venom on blood pressure. Evangelista JSAM[1], Morais GB[1], Silveira JAM[1], Evangelista JJF[2], Brito, RMG[1], Gomes, A. S.[2], Santos LFL[1], Nascimento NRF1[3], Toyama MH[3], Souza MHLP[2], Martins AMC[4], Monteiro HSA[5] [1]UECE - Medicina Veterinária, [2]UFC - Fisiologia e Farmacologia, [3]IB-UNICAMP, [4]UFC - Análises Clínicas e Toxicológicas

Introduction: *Crotalus durissus cascavella* is a snake that is usually found in the scrublands of northeast Brazil. The components of its venom may have effects on the vascular system. The aim of the present study was to investigate the vascular effects of the natriuretic peptide isolated from the venom of *Crotalus durissus cascavella* (NPCasca). **Methods:** Male Wistar rats, weighing 250-300g, were anesthetized with 50 mg/kg pentobarbital, and thereafter, the right carotid artery was cannulated with a polyethylene tube (PE50) and the systemic blood pressure was recorded directly using a pressure transducer connected to a polygraph. The mean arterial blood pressure was recorded continuously, and after a 30min equilibration period the test and control substances were injected by a cannula implanted in the jugular vein. NPCasca (0.1, 0.3 mg/mL) was injected at 15min intervals and compared with isovolumetric injection of saline. Nitrite concentrations were determined after the infusion of NPCasca in the blood pressure assay by the colorimetric Griess method. The results were expressed as means ± SEM. The data were analyzed using Student's t-test and analysis of variance (ANOVA) followed by the Bonferroni test. The level of significance was set at *$p < 0.05$. **Results:** The mean arterial pressure (MAP) showed a dose-dependent decrease after an infusion of the natriuretic peptide isolated from *Crotalus durissus cascavella* venom in doses of 0.1 mg/mL and 0.3 mg/mL; (Control=125±2.1mmHg; $NPcasca_{(0.1mg/mL)}$=100±4.7*mmHg;$NPcasca_{(0.3mg/mL)}$=75±3.5*mmHg). A significant increase in the production of nitrite (mmol) was observed after infusion of *NPcasca* at a dose of 0.1 mg/mL (Control= 20± 2.8µmol; *NPcasca*= 100±5.3*µmol). **Discussion:** In our work, we observed a decrease in heart rate and breathing as well as in mean arterial pressure in rats treated with the natriuretic peptide from *Crotalus durissus cascavella* venom. de MESQUITA *et al*., (Am. J. Trop. Med. Hyg 44 (3), 345–353, 1991) showed the hypotensive activity of *Crotalus atrox*. Lately, a bradykinin-potentiating peptide (BPP) product of a gene coding for an CEI/BPP-Ctype natriuretic peptide (CNP) precursor has been isolated from crotaline venom (HIGUCHI *et al.,* Physiol. C Toxicol. Pharmacol 144 (2), 107–121, 2006). In conclusion, the natriuretic peptide, NPCasca, isolated from *Crotalus durissus cascavella* venom has vascular effects. This natriuretic peptide showed a hypotensive effect in the arterial pressure assay, along with increased nitrite production, suggesting a vasoactive action. **Financial Support:** CAPES, CNPq and FUNCAP. **License of the ethics committee with the use of animals:**107/07-Federal University of Ceará; 08670084-7-State University of Ceará.

09.032

Anti-inflammatory effects of marine algae in mouse antigen-induced arthritis. Santos AG[1], Costa VV[2], Amaral FA[2], Coelho FM[2], Sachs D[3], Valadão DF[1], Morcatty TQ[2], Teixeira MM[2], Souza DG[2] [1]UFBH - Microbiologia, [2]UFMG - Bioquímica e Imunologia, [3]FMRP-USP - Farmacologia

Introduction: Rheumatoid Arthritis is a common human autoimmune disease that affects approximately 1% of the world population. It is characterized by chronic inflammation of the synovial joints, infiltration by blood-derived cells, increasing of cellular mediators and consequently articular pain. In the present work, we investigate the effect of a new compound, *Lithothamnium calcareum,* a red marine algae rich in calcium, as an alternative therapy for this disease. **Methods:** This project was previously approved by CETEA/UFMG on number access 166/06. Wild type male C57/BL6 mice (WT) was used. Antigen-induced Arthritis (AIA) was induced by administration of antigen (mBSA) into the knee joint of previously immunized mice. For treatment, *Lithothamnium calcareum* was given by gavage (200 µL/animal - twice/day) in different quantities and times before the challenge in the knee. Hypernociception was measured by a digital analgesimetro (Insight mod. EFF-301). Sample of periarticular tissue were removed for cytokines and chemokines (ELISA) analysis and neutrophil quantification by evaluation of mieloperoxidase activity (MPO). A joint lavage (BSA 3%; 10µL) was been to evaluate the cell infiltration on articular space, which was performed total cell (Neubauer clamber) and differential count (Cytospin3 - Shandon). Treatment with CACO3 was given to a group of animals to evaluate the role o calcium in this model and to compare with algae treatments. Knee tissue was collected for histological analyses using the method of HE. **Results:** Treatment with *Lithothamnium calcareum* reveals an anti-inflammatory response on different doses (1, 10 and 100 mg/kg) and times (3, 5, 10 days) used. The dose of 100 mg/kg/ twice a day reveal the best results. In next experiments, we used the best time and dose. This treatment decreases cell infiltration to knee cavity, the levels of mieloperoxidase and the production of the chemokines KC and MIP-2 in periarticular tissue. Also, hypernociception was reduced in the group that received algae treatment. In another experiment, we evaluate the effects of the calcium (CACO3) in equivalent quantities presented in algae. We not observed any effect of calcium in all evaluated parameters. Histological analyses revealed lesser damage in knee after treatment with algae. **Discussion:** *Lithothamnium calcareum* reveals an anti-inflammatory and anti-hypernociceptive response in an experimental model of AIA. It is possible that this algae act hindering the neutrophil migration for the knee cavity and periarticular tissue. It was supported by the lower levels of related chemokines (KC and MIP-2). Although, this effects are not caused by the calcium, suggesting that another compound present in this algae is responsible by anti-inflammatory effects. These compounds are being purified. More experiments will be conducted to test the possible effects of the other purified compounds from these algae in this model. Financial Support: CNPq and CAPES.

09.033

Composition and antibacterial activity of extracts of *Aloysia triphylla* (L'Herit.) Britton obtained by supercritical fluid extraction. Parodi TV[1], Baldisserotto B[1], Heinzmann BM[2], Oliveira JV[3], Minozzo M[3], Popioslki AS[3], Vargas AC[4], Krewer C[4] [1]UFSM - Fisiologia e Farmacologia, [2]UFSM - Farmácia Industrial, [3]URI - Ciências Agrárias, [4]UFSM - Medicina Veterinária Preventiva

Introduction: *Aloysia triphylla* (L'Herit.) Britton is used both as spice in foods and as phytomedicine to treat infections and other diseases. The genus *Aeromonas* comprises a group of Gram-negative, facultatively anaerobic bacteria that are pathogenic for aquatic and terrestrial animals and have also been associated with a wide spectrum of infectious diseases in humans and animals. **Methods**: This work investigated antibacterial activity of extracts obtained from the leaves of *Aloysia triphylla* against *Aeromonas hydrophila* by supercritical fluid extraction, a method which offers many important advantages as low energy cost and organic solvents consumption. The extracts were obtained using three temperatures (30, 50 and 70 ºC) and three pressure levels (100, 150, 200 bar) and their analyses were performed by GC/MS and GC/FID. Antibacterial activity was tested against *Aeromonas hydrophila* as described in CLSI M7-A4 protocol to obtained Minimum Bactericidal Concentration. **Results and Discussion**: Minimum Bactericidal Concentration of the extract obtained *Aloysia triphylla* by the fluid supercritical method against *Aeromonas hydrophila* were the following: 1339.3 µg/mL (30 ºC and 100 bar); 1674.1 µg/mL (30 ºC and 200 bar); 3125 µg/mL (50 ºC and 150 bar); 4464.3 µg/mL (70 ºC and 100 bar); 1562 µg/mL (70 ºC and 200 bar). In relation the yield of extraction the data showed that the highest extraction yield was obtained at 70^{o}C and 200 bar. However, the best extract regarding antibacterial activity optimization was that obtained at 30^{o}C and 100 bar at concentration of 1339.3 µg/mL. The results allow concluding that *Aloysia triphylla* presented moderate antibacterial activity against *Aeromonas hydrophila*. **Acknowledgments and Financial Support**: CAPES, CNPq.

09.034

In vitro inhibition of acetylcholinesterase by myrsinoic acid A. Filippin FB[1], Gazoni VF[1], Meyre-Silva C[1], Yunes RA[2], Malheiros A[1], De-Souza MM[1], Burger C[1] [1]NIQFAR-CCS-UNIVALI Farmácia, [2]UFSC - Química

Introduction: The acetylcholinesterase enzyme (AChE) is an attractive target for the rational drug design and for the discovery of mechanism based inhibitors because of its role in the hydrolysis of the neurotransmitter acetylcholine (ACh). AChE inhibitors are the most effective approach to treat the cognitive symptoms of Alzheimer's disease (AD) and other possible therapeutic applications in the treatment of Parkinson's disease, senile dementia, among others. Some AChE inhibitors like galanthamine and tacrine are approved for the treatment of AD, but these drugs have limitations for clinical use. Myrsinoic acid A (MAA) is a compound derivate from benzoic acid, which was isolated from *Rapanea ferruginea* (Myrsinaceae). **Methods:** MAA was isolated from fruits of *R. ferruginea* by chromatography methods. MAA (2-60 µg) was spotted onto the TLC plate. Migration was conducted with hexan:ethyl acetate (6:4). The plate was sprayed with an AChE solution (6.67 IU/mL) and pre-incubated at 37ºC/20 min. TLC was revealed using Fast Blue salt and naphtyl acetate as reagent. For the *in vitro* AChE activity, male Wistar rats (2 months) were used in accordance with Ethics Committee (Univali, number 126/2008). The animals were killed under anesthesia by decapitation. The brain was quickly removed dissected, weighed and homogenized in 10 volumes of 10 mM Tris-HCl, pH 7.2, containing 160 mM sucrose. The homogenate was subjected to centrifugation (1000 g/10 min at 4 °C). The supernatant obtained was stored at 20 °C until the time of enzymatic assays. The same protocol was followed for the preparation of the hippocampus where it was homogenized in 20 volumes of Tris-HCl 10mM. The specific activities of AChE in the hippocampus and total brain were determined by spectrophotometric method of Ellman et al. (*Biochem. Pharmacol.,* v.7, p. 88, 1961). The possible inhibitory effect of MAA was evaluated at 22, 33 and 44 µM. **Results and Discussion:** TLC with bioautography approaches were used in screening for anticholinesterase properties of the extracts and isolated molecules. In this case, MAA inhibited AChE in 8-60 µg. MAA inhibited AChE activity in a dose-dependent manner in total brain and hippocampus.In brain total, the inhibition caused by AMA at a concentration of 44 µM was 67.86% in total brain (IC_{50} 35 µM) and 76.99% in the hippocampus ((IC_{50} 36.25 µM)). This study showed that MAA, isolated from *R. ferruginea* inhibit AChE in brain tissue, especially hippocampus. AChE inhibitors have been studied for neuroprotective action and lowering of b-amyloid. This study may be useful for the prevention of the development or progression of AD. **Financial support:** PMUC/FAPESC

09.035

Evaluation of *Bauhinia forficata* tea on glibenclamide pharmacodynamic in diabetes induced by streptozotocin. Campos G[1], Burguer C[2], Meyre-Silva C[3], Oliveira AE[4], Ferreira RA[1] [1]UNIVALI, [2]UNIVALI - Farmacologia, [3]NIQFAR-UNIVALI, [4]UNIVALI - Farmácia

Introduction: The interaction of herbs with drugs is well known. Both pharmacokinetic and pharmacodynamic interaction have been reported when herbs are administrated concomitantly with drugs. Management of type 2 diabetes mellitus usually involves combined pharmacological therapy to obtain adequate blood glucose control and treatment of concurrent pathologies associated with it. Apart from combining two or more hypoglycaemic drugs in the treatment of diabetes, some patients and even physicians recommend use of antidiabetic herbs along with oral hypoglycemics. *Bauhinia forficata* Link, is a widely used herb in the traditional medical systems in Brazil. Considering the wide use of *B. forficata* tea together hypoglycemiant agents by diabetic patients, the present study was investigate the relationship of this plant with glybenclamide (GLY), that is the main medicine used to threat type 2 diabetes. **Methodology:** The dried leaves of *B. forficata* (2 or 4 g) were submitted to infusion using 100 mL of purified water (90º C) during 10 minutes. Male albino Wistar rats (160-210 g) were used for the investigations (CEP/UNIVALI, 09/2007). A group of animals received streptozotocin (STZ) 60 mg/kg, i.p. to induce diabetes. The diabetic animals were divided in 5 groups: **i**) saline (3mL/kg, v.o.); **ii**) tea 2 g/100 mL in place of drinking-water; **iii**) tea 4g/100 mL in place of drinking-water**; iv**) tea 2 g/100 mL + GLY (5 mg/kg, v.o.) and **v**) tea 4 g/100 mL + GLY (5 mg/kg, v.o.). The fasting plasma glucose was measured after 15 and 30 days of treatment. During the treatment, daily tea drinking was measured to verify if the association of GLY and tea modified the liquid intake. At the end of treatment, liver was removed and hepatic glycogen was measured. **Results and discussion:** The hyperglycemia induced by STZ was significantly attenuated by *B.forficata* tea (4 g/100 mL) with 70.72% of decrease of glucose level in 30 days of treatment in this group. However when animals were treated with tea and GLY (2 or 4 g/100 mL), plasmatic glucose level doesn't change in comparison to diabetic control. At the end of experiment, the glycemia for group **i** (control) was 474.0 ± 78.0 and for group treated (groups **iv** and **v)** were 343.4 ± 17.2 mg/dL and 372.3 ± 12.8, respectively. The intake of tea did not change during the treatment period for treat groups. Hepatic glycogen for group **i** was 0.42 ± 0.081 mg/g hepatic tissue. For all treat groups, hepatic glycogen decreased, reflecting the framework caused by diabetes. For group **iii** and **v,** hepatic glycogen was 0.16 ± 0.01 and 0.165 ± 0.01 mg/g hepatic tissue respectively. Many herbal medicines possess antioxidant properties, which play an important role in therapeutics and are often administered in combination with therapeutic drugs, raising the potential of herb-drug interaction. In this study, the results suggest that *B. forficate* tea when combined with glibenclamide during treatment period (30 days) doesn't change the glucose levels in the animals. **Financial support:** Artigo 170-ProPPEC/UNIVALI, FAPESC.

09.036

Aqueous fraction from *Averrhoa bilimbi* L. reduces the calcium sarcolemmal current in guinea pig left atrium. Santos ACO, Caldas, APD, Conde-Garcia, EA, Vasconcelos CML
UFS - Fisiologia

Introduction: *Averrhoa bilimbi* L. is known in Brazil as "bilimbino", "biri-biri", "caramboleira amarela" or "limão de caiena". It was used by folk medicine to treat hyperlipidemia, fever, mumps, and diabetes. **Methods**: hydroalcoholic crude extract (EBH) was obtained by macerating dry leaves in water:ethanol (1:1, v/v, 10 days). The aqueous fraction (FAq) was prepared by dissolving EBH in deionized water. The insoluble residue was discarded by filtration. Experiments were conducted in guinea pig left atria maintained in organ chamber (5 ml, Tyrode, 27 ± 0.1 ^{o}C; stretched to 1 gf, stimulation: 2 Hz; 400 V; 0.5 ms). Atrial contraction force was measured isometrically (HP FTA10 force transducer). Electrical signals were amplified (HP 8805B amplifier) and digitalized (DATAQ DI400) before stored in computer. Concentration-effect curves concerned to the inotropic effect of FAq (50-4000 mg/l) were obtained before and after adding atropine sulfate (1.5 mM) to the bath. Concentration-effect curves of $CaCl_2$ (0.6 – 8.0 mM) were also obtained before and after adding of FAq (2000 mg/l). **Results e Discussion:** FAq reduced the atrial force (EC_{50} = 430 ± 110 mg/l, n = 6). The effect was concentration-dependent and disappeared partially during the washout. FAq (2000 mg/l) reduced in 88 % the atrial force. The time of contraction measured at 50 % of the force amplitude increased 42 % and the relaxation time determined at 80 %, 50 %, and 20 % were reduced in 23 %, 20 %, and 19 %, respectively. Atropine (1.5 mM), an antagonist of the muscarinic receptors, shifted rightward the concentration-effect curve of FAq increasing EC_{50} to 825 ± 170 mg/l (n = 4, $p < 0.05$). FAq also shifted to the right the $CaCl_2$ concentration-effect curve increasing EC_{50} from 1.4 to 2.8 mM (n = 4, $p < 0.05$). The results allow the following conclusions about the myocardial inotropic effects of FAq: 1) It reduces the atrial contractility; 2) It shortens the relaxation phase of myocardial contraction; 3) Its contractile effect can be explained by the activation of muscarinic receptors and by blocking the sarcolemmal calcium channels. **Financial Support**: ELETROBRÁS, FAPITEC/SE, CNPq, UFS.

09.037

Pharmacological and chemical characterization of *Zingiber sp*. collected in the Amazon state. Santos DR[1], Pinheiro CCS[2] [1]INPA - Farmacologia, [2]COPE-INPA

Introduction: The Zingiberaceae family is the biggest of the Zingiberales order, possess 53 sorts and more than 1.200 native species of tropical regions and some of these are cultivated in Brazil and are well known as ginger. They grow in shadings or half-shadings habitat, rich in humus and presents economic value for supplying foods, aromatic condiments, staple fibers and paper. The study of this family aroused the interest in the search of new species, so new assays could be carried through and contribute in the search of information. This study comes with the intention to make the identification of the botanical species chosen and to characterize chemistry and pharmacologically this plant that we'll call *Zingiber sp*., in order to verify and prove its possible therapeutic action. **Methods:** The vegetal material (rhizomes) of the *Zingiber sp*. was collected in rural area of Careiro Castanho/Manaus/AM. The vegetal extracts were made by maceration, with *Zingiber sp*. dry rhizomes and the extraction was made by sequence of solvent of increasing polarity: Dichloromethane (DCM), Methanol (MeOH) and Water (H_2O). For the accomplishment of the pharmacologic assays the following tests had been used: General Activity Test, Acute Toxicity, Writhing Test and Hot Plate. For these tests, we used MeOH Extract in groups of mice (n=5), administered oral and intraperitoneal. In the sequence, we carried through Paw Oedema test and Analgesimeter, using MeOH Extract in groups of rats (n=5, v.o.). For analysis, the answers had been analyzed through parametric and non parametric tests, using the statistical program GraphPad Prism 4.0. **Results and Discussion:** The effects observed in the General Test, 1000, 1500 and 2000 mg/kg, had been: reduction of the motility, exploratory activity, muscular tonus loss, respiratory difficulty, piloerection and sleepiness. The animals control (Saline 0,9%+Tween 20%) had not presented similar effects. The results had shown evident signals of SNC compromising, indicative of a possible analgesic activity. The acute toxicity test using 1000, 1550 and 2000 mg/kg doses made possible to determine the DL50 of the MeOH extract, whose lethal dose corresponds to 1550 mg/kg. In acetic acid-induced writhing test, was verified that (500, 1000 and 1500 mg/kg i.p.) the treat mice had presented total elimination of the writhing if compared with the control group. The treatment also was efficient in the 500, 1000 and 1500 mg/kg v.o., eliminating significantly the writhing. Through the Hot Plate (500, 1000 and 1500 mg/kg i.p.) we observed that the test had been sufficiently significant showing an increase of latency period(s) in all the tested doses, fact that was evidenced in lesser scale in the treatment v.o., since only the 1500 mg/kg dose presented significance. For the Paw Oedema test v.o. was used doses of 500, 1000 and 1500 mg/kg. The two last ones had been the doses with better performance, with significant reduction of oedema. Finally, in the Analgesimetro test v.o., we verified that the doses that demonstrated better efficiency had been 1000 and 1500 mg/kg. With these results, we confirm that our drug has analgesic and anti-inflammatory activity, being necessary more studies and additional models to consider adjusted doses, without or with the minimum of collateral effect.

09.038

Acute anti-inflammatory potential of hexanic fractions from *Pterodon polygalaeflorus.*

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Introduction: The genus *Pterodon* comprises few species widely distributed over central region of Brazil. Their seeds are commercially available at the medicinal flora market being largely used for their pharmacological properties. Alcoholic extracts made from these seeds are used in folk medicine as anti-rheumatic, anti-inflammatory (sore throat) and analgesic preparations, when ingested by oral route in small quantities at regular intervals. The aim of this work was to study the anti-inflammatory effects of hexanic fractions from *Pterodon polygalaeflorus* Benth. seeds using an acute inflammation model. **Material and methods:** The paw edema model was induced in male SW mice (25–35 g b.w., n= 5/group). One hour before the administration of carrageenan (50 µL in left hind paw, i.p.), different doses of Ppg fractions (100 µL), prepared in ethanol 15% with 1.25% Tween-20 (vehicle) were administrated. One group received the vehicle (control) and another was treated with the control drug indomethacin (10 mg/kg b.w.). After one hour of carrageenan injection, the edema was evaluated in plethysmometer until 4 h. The edema inhibition was evaluated at the peak of inflammation (3 h). The animals were killed and the paws removed for histological analysis (HE). All procedures were approved by CEA-IBRAG committee/protocol 05/2009. **Results and discussion:** *P. polygalaeflorus* fractions exhibited anti-inflammatory activity in the paw edema model. The inhibitions observed after 3 h of edema induction were: 67.2 ± 24.8% (0.02 mg/kg) and 54.4 ± 24.2 (0.2 mg/kg) for Ppg fraction I; 62,1 ± 32,42 (0.02 mg/kg) and 67,4 ± 35,8 (0.2 mg/kg) for Ppg fraction II; 65,8 ± 14,3 for Indomethacin. No effect was exhibited by Ppg fraction III. Histological analysis of control group (vehicle) showed intense inflammatory infiltrate in the paws. Groups treated with both doses of Ppg fractions I and II exhibited reduction of vasodilation and of leukocyte infiltrate (neutrophils). In summary, the hexane extract fractionation resulted in two active fractions exhibiting important acute anti-inflammatory activity. **Financial support:** FAPERJ, CNPq.

09.039

Anti-allergic properties of gedunin: inhibition of T-lymphocyte activation and migration. Ferraris FK, Penido C, Henriques MGMO Farmanguinhos-FIOCRUZ - Farmacologia Aplicada

Introduction: The discovery of drugs for the treatment of inflammatory allergic such as, asthma, allergic rhinitis and sinusitis is a very important subject in human health. It is well described that T-lymphocytes are crucial cells in coordinating the maintenance of the inflammatory response in allergic disease. We have previously described that a pool of 5 tetranortriterpenoids (TNTPs), isolated from the seeds of *Carapa guianensis*, presents anti-allergic effects in different *in vivo* models (Penido, C., *Inflamm. Re*s. 54: 295, 2005; Penido, C., *Int. Immunopharmacol*. 6: 109, 2006). However, the precise mechanisms underlying the anti-allergic activities of isolated TNTPs remaines to be elucidated. **Objective**: Considering the central role of T lymphocytes in the pathogenesis of allergic diseases, in the current study we investigated the effects of one of the TNTPs, gedunin, in T lymphocyte population in a model of allergic pleurisy and in *in vitro* assays. **Methods and Results**: The intra-peritoneal (i.p.) pretreatment with gedunin (0.5 mg/kg) in previously sensitized C57BL/6 mice (CEUA, Fiocruz; licence n. L-0004/08) impaired total leukocyte and eosinophil influx into pleural cavities of ovalbumin (OVA, 12.5 µg/cavity)-challenged mice. In accordance with such results, ELISA assays showed decreased levels of CCL11/eotaxin and IL-5 in in the pleural cavities of gedunin pretreated mice 24 h after OVA intra-thoracic (i.t.) stimulation. *In vivo* pre-treatment with gedunin (0.5 mg/kg, i.p.) blocked pleural T lymphocyte $CD69^{+}/CD25^{+}$ influx. Likewise, gedunin pretreatment (50 µg/ml *in vitro*, 1 h before stimulation) downregulated CD69 and CD25 expression ($P<0.001$) on cell surface of isolated T lymphocytes 24 hours after α-CD3 mAb (10µg/ml) stimulation *in vitro*. Pre-treatment with gedunin (50 µg/ml) also inhibited the *in vitro* production of the eosinophilotactic chemokines, RANTES/CCL5 (88.6% of inhibition) and CCL11 (44.5% of inhibition), by OVA (10µg/well)-stimulated splenocytes recovered from previously sensitized C57BL/6 mice. Moreover, gedunin impaired (~100% of inhibition) splenocyte proliferation as well as blocked interleukin-2 production (73.6% of inhibition) induced by α-CD3 mAb. Gedunin inhibitory effects seem to be dependent on NFkB activation, since *in vitro* pre-treatment of splenocytes with this compound impaired NFkB nuclear translocation. P values ≤ 0,05 were regarded as significant. **CONCLUSION**: Our *in vivo* and *in vitro* results provide evidence that gedunin might contribute to the treatment of allergic inflammatory diseases. **Supported by** CNPq and Farmanguinhos/FIOCRUZ.

09.040

Acetic extract of *Mentha x. villosa* Hudson leaf produces atrioventricular blockage in isolated guinea pig heart. Brandão WB[1], Dantas, RN[2], Britto, RM[2], Vasconcelos CML[2], Silva BA[1], Conde-Garcia, EA[2] [1]LTF-UFPB Ciências Farmacêuticas, [2]UFS - Fisiologia

Introduction. *Mentha x. villosa* Hudson (*M.villosa*) is known in Brazil as "hortelã-miúda" or "hortelã-de-panela". Its acetic extract reduced the contractility of the guinea pig left atrium (AE). Muscarinic receptors as well as potassium channels participate in the mechanism of action of the inotropic effect (FESBE XXIII Reunião Anual, Res. 44.028, 2008). On the other hand, the extract abolished the Bowditch phenomenon, suggesting it could act by reducing the inward calcium current (Anais do VIII Congresso Sergipano de Cardiologia, Resumo TLP 11, p.26, 2007). The present study deals with the effects of acetic extract on the electrocardiogram of the isolated guinea pig heart. **Methods.** To prepare the acetic extract of *M. villosa,* leaves were extracted in Soxhlet apparatus by using the following solvents: hexane, acetone, ethanol, and acetic acid. The experiments were carried out in the guinea pig (*Cavia porcellus*) isolated heart. Animals were previously injected with heparin (100 UI/kg) and half an hour after that they were sacrificed by a blow applied on the skull (DORIGO et al. Cardiovasc Drugs Ther, 4:1477-1486, 1990). The heart was promptly removed and mounted in a constant flow Langendorff apparatus (4ml/min, 34±0.1ºC), where it remained under perfusion with Tyrode solution. This solution was thoroughly filtered in Millipore filter (mesh 0.45 mm) to avoid microembolism. The hearts were oxygenated (carbogen mixture: 95% O_2 + 5% CO_2) and electrically stimulated (Digitimer DS2, D4030). That biological preparation was maintained into 50 ml of Tyrode and its electrical signals were recorded by Ag/AgCl/NaCl 1.0 M electrodes disposed into the bath solution. The electrical signals were amplified (HP8811B Amplifier, HP7754A), digitalized (DATAQ DI-205, DI-400, Windaq Pro), and stored in computer. The experiments were performed in the following conditions: 1) control (perfusion with Tyrode); 2) test (Tyrode plus 50, 100 or 200 mg/L of acetic extract); 3) washout. In spontaneous beating preparations the effect of acetic extract on the heart rate was evaluated. Experiments were performed before and after blocking the muscarinic receptors with atropine sulfate (1 mM). **Results and Discussion.** Our results showed that the acetic extract (50 to 200 mg/l) depresses the conduction of the electrical impulse throughout the atrioventricular node, leading to 2nd and 3rd degrees of atrioventricular block (AVB). Furthermore, during perfusion with the acetic extract, some ventricular extrasystolic activity could be recorded. This extract reduced the spontaneous heart rate from 167 ± 11 to 145 ± 6 bpm (15 %, n = 3, $p < 0,05$), but such effect disappeared during washout. Atropine sulfate (1mM) avoided that bradycardia. **Conclusion.** These depressant effects on both sinusal and atrioventricular nodes producing bradycardia and/or AVB can be explained by the reduction of the calcium inward currents promoted by the acetic extract. **Apoio financeiro:** ELETROBRÁS, FAP/SE, CNPq, UFS. **Número da Licença do Comitê de Ética:** (UFS-Processo 04/07)

09.041

Antiulcer activity of the hydroalcoholic fraction of *Herissantia crispa* (L.) Brizicky in acetic acid induced ulcer in rats. Dias GEN[1], Mota KSL[2], Lima IO[1], Matias WN[1], Teles YCF[1], Souza MFV de[3], Batista LM[1] [1]UFPB - Ciências Farmacêuticas, [2]LTF-UFPB, [3]UFPB - Ciências da Saúde

Introduction: *Herissantia crispa* (L.) Brizicky (Malvaceae) was collected in Pedra da Boca, Araruna, Paraíba. Although it has not popular indication, this specie was chosen according quimiotaxonomic criteria, because this plant belongs to family that is rich in triterpenes, flavonoids, flavonoid heterosides, essential oils, sesquiterpenelactones and fatty acids (COSTA et al., Quím Nova, 32, 48, 2009). *H. crispa* showed gastroprotective action against gastric ulcers induced by ethanol in the rats, stress (restriction and cold) and non steroidal anti-inflammatory (piroxicam) in mice (data not show). The aim of this work was to evaluate the action of the hydroalcoholic phase of *H. crispa* in gastric ulcers induced by acetic acid. **Materials and methods**: The aerial parts of *H. crispa* were dried, powdered and macerated with methanol at room temperature. It was suspended in methanol:water (7:3) and submitted at partitions with hexane, chloroform, ethyl acetate and n-butanol, obtaining the respective phases, besides hydroalcoholic phase (Costa et al., *Quím Nova*, 32, 48, 2009). The experimental model used to evaluate the capacity of hydroalcoholic phase of *H. crispa* (flavonoid heterosides) in accelerate the healing of gastric ulcers was acetic acid model. Male Wistar rats (180-250g), n=7 or 10, were used and the lesions were induced by acetic acid 30% at subserosal layer (TAKAGI et al., Jap J Pharmac, 19, 418, 1969). One day after, the animals were treated, orally, with saline or negative control (10mL/kg), cimetidine (100 mg/kg) and the hydroalcoholic phase of *H. crispa* (62,5 mg/kg) and the treatment period was fourteen days. Following this, the ulcerative area (UA) and toxicity parameters (body and organs weight, water and food consumption and biochemical and hematological parameters) were determined. The results were expressed as the mean ± S.D. Statistical significance was assessed by one-way analysis of variance, followed by Dunnett and Tukey-Kramer tests for multiple comparisons. The level of significance was $p<0.05$. Number of Ethical in Animal Research license is 705/06. **Results:** The action of hydroalcoholic phase 62,5 mg/kg (20,5 ± 5,8 mm^2) and cimetidine 100 mg/kg (24,4 ± 5,4 mm^2) decreased the gastric lesions compared with saline group (44,3 ± 12 mm^2). The hydroalcoholic phase of *H. crispa* did not alter the wet of rats, of the organs, and the biochemistry parameters, for example alanine aminotransferase (ALT), aspartate aminotransferase (AST), urea and creatinine. The hemoglobin and hematocrit was increased in the group that received hydroalcoholic phase of *H. crispa* compared with negative control and the other hematological parameters did not alter. **Discussion:** The hydroalcoholic phase of *H. crispa* protected the stomach against lesions induced by acetic acid and the plant did not show the toxic action, because it did not alter the wet of animals, of the organs and the biochemistry parameters. **Financial Support:** CNPq/LTF/UFPB

09.042

Antidepressant-like effect and phytochemical study of *Lafoensia pacari* A. ST.-HIL. ethanolic extract and fractions. Galdino PM[1], Nascimento MVM[1], Sampaio BL[2], de Paula JR[3], Costa EA[4] [1]UFG - Ciências Fisiológicas, [2]UFG - Farmácia, [3]UFG - Farmacognosia, [4]UNIFESP - Farmacologia

Introduction: In the search for new molecules useful for the treatment of neurological disorders, worldwide medicinal plant research has progressed constantly, demonstrating the pharmacological effectiveness of different plant species in a variety of animals models. *Lafoensia pacari* A. St.-Hil. (Lythraceae), has been referred in Brazilian traditional medicine for the treatment of different diseases, among them inflammation, gastric disturbs and central diseases. This work evaluated the antidepressant-like effects of the ethanolic extract of *L. pacari* (PEtExt) and its fractions on 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 PEtExt was obtained by maceration in 70% hydro-alcoholic solution, followed by filtration and evaporation (yield = 16.1% w/w). PEtExt (32 g) was dissolved in 300 mL of methanol/water (7:3), and partitioned successively with hexane, chloroform and ethyl acetate. The yields of the hexanic (HexF), chloroformic (ChloF) ethyl acetate (EAF) and methanolic/water (MetF) fractions were 1.05, 2.56, 31.43 and 64.96% (w/w), respectively. The phytochemical screening of PEtExt and ChloF were performed by the methods of Ikhiri *et al.* (Ikhiri, Intern. J. Pharmacog. 30, 251, 1992). The confirmation of the chemical constituents presents in PEtExt and ChloF were performed by thin-layer chromatography (TLC) in silica gel plates. All experimental protocols were approved by the Ethic Commission of UFG (104/08). The antidepressant activity was studied using forced swimming test (FST) (Porsolt, Nature 266, 730, 1977), and the motor activity using the open field test (OFT) (Archer**,** Anim. Behav. 21, 205, 1973). PEtExt 1.0 g/kg, p.o. were administered acutely and PEtExt 0.1, 0.3, and 1.0 g/kg/day p.o. for 21 days. The fractions were administered p.o. for 21 days. Imipramine (IMI) 15 mg/kg/day, p.o. was used as the control positive. **Results and Discussion:** Phytochemical screening had shown the presence of saponins, flavonoids, tannins and triterpene in the PEtExt, and the presence of flavonoids, triterpene, tannins in ChloF, confirmed by TLC. On the FST, the acute administration of PEtExt did not alter the immobility time, and PEtExt 0.1, 0.3 and 1.0 g/kg, p.o. for 21 days decreased the immobility time from 143.00 ± 10.99 to 83.2 ± 9.36, 70.0 ± 14.73 and 60.6 ± 15.40, respectively, and IMI to 88.60 ± 11.89. On the OFT, the treatment with PEtExt for 21 days did not alter the parameters evaluated. Only ChloF 50 mg/kg/day decrease the immobility time from 235.00 ± 6.18 to 147.6 ± 8.97 and IMI to 191.2 ± 8.45. These data indicate that the extract of *Lafoensia pacari* A. St.-Hil. possesses antidepressant-like properties in mice without affecting the motor activity and after de partition only the CholF has the active constituents of the crude extract. **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.

09.043

Effect of *in vitro Crotalus durissus terrificus* snake venom and crotoxin on neutrophil functions. Lima TS, Iritus ACC, Sampaio SC, Cirillo MC Instituto Butantan - Fisiopatologia

Introduction: Previous works showed that *Crotalus durissus terrificus* snake venom (CdtV) modulates macrophage function, inhibiting the spreading and phagocytic activity but increasing the oxidative burst of these cells. In addition, crotoxin (CTX), the main component of the venom, was reported to inhibit this phagocytic activity. Recently, CdtV was shown to inhibit carrageenan-induced inflammatory response and phagocytosis by neutrophils. Despite these evidences, the component of CdtV responsible for phagocytosis inhibition is still unknow. Moreover, the effect of crude CdtV in other important functions of neutrophils, like the reactive oxygen species production, has not been investigated yet. The aim of this study was to investigate the effect of CTX on phagocytosis activity, via C3b receptor, and the effect of crude CdtV on hydrogen peroxide (H_2O_2) production, both by neutrophils obtained by carrageenan-induced peritonitis. **Methods:** Neutrophils were obtained from peritoneal cavity of male Wistar rats (170g) (Institutional Animal Care Committee at Butantan Institute, protocol number 407/07) 4h after the intraperitoneal (i.p.) administration of carrageenan (cg) (4.5 mg/kg). Phagocytosis of opsonizaded zymosan was evaluated after *in vitro* treatment with CTX. For this treatment, cells ($1.2x10^6$ cells/mL) were incubated (1h) with RPMI 1640 medium (control) or with RPMI 1640 medium containing CTX (0.02, 0.04, 0.08, 0.16 and 0.32 µg/mL). Reactive oxygen species production was analyzed by H_2O_2 production. For this assay, cells ($4x10^5$ cells/mL) were incubated (1h) with RPMI 1640 medium (control) or with RPMI 1640 medium containing CdtV (0.25, 0.5 and 1.0 µg/mL), and H_2O_2 production was evaluated by phenol red oxidation method. **Results:** *In vitro*, CTX significantly reduced the phagocytic activity of neutrophils in the following concentrations: 0.02 µg/mL: 24% (cg+CTX: 86.6±7.7; $p<0.05$), 0.04 µg/mL: 26% (83.3±11.7; $p<0.01$), 0.08 µg/mL: 27% (87.2±8.4; $p<0.05$). However, CTX at 0.16 and 0.32 µg/mL did not alter the phagocytic activity of neutrophils. For reactive oxygen species production, crude CdtV, at all concentrations, did not alter H_2O_2 production by neutrophils. **Discussion:** These results show that CTX inhibits phagocytosis in neutrophils, as has been described for macrophages. However, CdtV did not stimulate H_2O_2 production in neutrophils, unlike in macrophages. Considering the important role of neutrophils in inflammation, the data presented herein contribute to the characterization of anti-inflammatory effect of the CdtV, particularly of the CTX, recently described. These data reinforce the role of CTX as a new approach to control inflammatory diseases. **Supported by** FAPESP and CAPES.

09.044

Renal heme-oxygenase activity and expression in rats treated with *Bothrops alternatus* snake venom. Linardi A[1], Rennó AL[2], Cardoso KC[2], Franco-Penteado CF[3], Hyslop S[2] [1]FMSCSP/UNICAMP - Fisiologia/Farmacologia, [2]UNICAMP - Farmacologia, [3]UNICAMP - Hemocentro

Introduction: Heme-oxygenase (HO) mediates the degradation of heme, with the formation of biliverdin IX, iron and carbon monoxide (CO) that can modulate renal blood flow, diuresis and natriuresis, partly via CO-mediated activation of the guanylate cyclase-cGMP signaling pathway. The main isoforms of HO are HO-1 (inducible) and HO-2 (constitutive). HO-1 is protective in heme- and non-heme-mediated models of renal failure, possibly through its antioxidant, anti-inflammatory, anti-proliferative, anti-apoptotic, immunomodulatory and vasorelaxant actions. Since envenoming by *Bothrops* snakes can cause acute renal failure (ARF), in this work we examined the effect of *Bothrops alternatus* venom on renal HO activity and expression. **Methods:** Male Wistar rats (~250 g) were injected with *B. alternatus* venom (0.8 mg/kg, i.v.) and 1, 3, 6, 24, 48 and 72 h and 7 and 15 days post-venom the rats were killed with an overdose of isoflurane and the kidneys were removed, frozen in liquid nitrogen and stored at -80^{o}C until processed for HO activity and gene and protein expression analysis (by real-time PCR and immunohistochemistry, respectively). The experimental protocols were approved by an institutional Committee for Ethics in Animal Experimentation (CEEA/UNICAMP, protocol no. 681-1). **Results:** Treatment with venom significantly increased HO activity (pmol of bilirubin/mg/h) 3 h (3471$\pm$782; mean$\pm$S.D.), 7 days (3114$\pm$792) and 15 days (3140$\pm$908) post-venom when compared to saline-treated (control) rats (2349$\pm$237) (n=6 each; $p<0.05$; ANOVA followed by Dunnet´s test). Quantitative real-time PCR revealed a significant increase ($p<0.05$) in HO-1 gene expression (arbitrary units) 3 h (0.10$\pm$0.05) and 6 h (0.17$\pm$0.03) post-venom when compared to control rats (no expression detected) (n=4 each). Immunohistochemistry also showed an increase in HO-1 expression in renal cortex and medulla at 3 and 6 h and 7 and 15 days post-venom. In contrast, there were no significant alterations in the gene and protein expression of HO-2 after envenoming when compared to control rats. **Discussion:** These results indicate that *B. alternatus* venom increases renal HO activity, probably as a result of the enhanced expression of HO-1. Although the role of HO in venom-induced ARF remains to be established, the enhanced expression and activity seen here could be part of a renoprotective mechanism to counterbalance venom-induced damage. **Financial Support**: CNPq, FAPESP.

09.045

Avaliação da atividade antinociceptiva do extrato aquoso de *Stephanolepis hispidus.*

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Introdução: Grande parte dos medicamentos existentes atualmente no mercado e largamente utilizados pela população é composta por substâncias isoladas ou sintetizadas a partir de extratos de produtos naturais. O ambiente marinho é uma fonte rica de produtos naturais biologicamente ativos. Além disso, diversas comunidades pesqueiras do litoral brasileiro relatam o uso da pele de peixe-porco com finalidade terapêutica. Neste trabalho, nós avaliamos a atividade analgésica do extrato bruto aquoso da pele do peixe *Stephanolepis hispidus* (EAPP), popularmente conhecido como peixe-porco, que é o mais incidente nas regiões costeiras do litoral brasileiro. **Métodos**: Camundongos Swiss-Webster (20-25 g) foram tratados por via i.p. com EAPP (1, 10 ou 100 mg/kg) 1h antes da indução dos protocolos experimentais de nocicepção utilizados. Neste trabalho, foram analisados a constrição abdominal induzida por ácido acético (0,8%); a hiperalgesia inflamatória induzida pela administração de carragenina intraplantar (50µg/pata) analisada no sistema de placa quente modificada e a nocicepção de origem neurogênica analisada no sistema de placa quente. Todos os procedimentos estão licenciados junto a CEUA/Fiocruz, sob a Licença número 0260/05**. Resultados e Discussão**: Preliminarmente observamos que o extrato bruto aquoso da pele de *S. hispidus* foi capaz de inibir a resposta nociceptiva dos animais de forma concentração dependente no modelo de contorção, com um percentual máximo de inibição da resposta de 74%, o que foi confirmado em todos os outros modelos de dor utilizados. Além disso, observou-se que a atividade antinociceptiva de *S. hispidus* foi completamente impedida pelo tratamento prévio dos animais com naloxona no modelo de contorção induzida por ácido acético, sugerindo um possível papel opióide para o composto. Estes resultados demonstram que o extrato da pele de *S. hispidus* possui substâncias capazes de suprimir a nocicepção tanto inflamatória quanto neurogênica. Além disso, estes dados podem auxiliar na validação do uso popular deste produto. Apoio Financeiro: FAPERJ / Fiocruz

09.046

Valeriana officinalis as a potent antioxidant against lipid hydroperoxides and reactive oxygen species formation *in vitro.* Sudati JH, Fachinetto R, Pereira RP, Barbosa NBV, Rocha JBT UFSM - Química

Introduction: *Valeriana officinalis L.* (Valerian) is widely used as a traditional medicine to improve the quality of sleep [1]. Although *V. officinalis* have been well documented as promising pharmacological agent; the exact mechanisms by which this plant acts is still unknown. Limited literature data has been indicated that *V. officinalis* extracts can exhibit antioxidant properties against iron in hippocampal neurons *in vitro*. In this study, the protective effect of *V. officinalis* on lipid peroxidation (LPO) and against reactive oxygen species (ROS) formation was investigated. **Methods:** A standard tincture of *V. officinalis* (10 g of valerian roots per 100 ml of ethanol) was obtained from Bio extracts (São Paulo, Brazil) and tested at concentrations of 0-40 µg/ml. LPO was determined in cortex of rat's brain as described [2]. Ferrous oxidation/xylenol orange assay (FOX) is based on the oxidation of Fe^{2+} by lipid hydroperoxides at acid pH in the presence of the Fe^{3+} complexing dye and xylenol orange (Sigma). Samples were homogenized (1:20 w/v) in a 100% cold (4°C) methanol. The homogenate was then centrifuged at 1,000g, for 10 min at 4°C. The supernatant was incubated with *V. officinalis* (0-40 µg/ml) in the presence and absence of Fe^{2+}/EDTA (100 μM) and then it was used for LPO determination (λ = 580 nm). Cumene hydroperoxide (CHP; Sigma) was used as standard. For measurements of ROS production, slices from cortex of rat´s brain were incubated (2 h, 37°C) in a buffer containing artificial cerebrospinal fluid and *V. officinalis* (0-40 µg/ml) in the presence and absence of quinolinic acid (QA) (1 mM), in a final volume of 2 ml. At the end of incubation, slices were homogenized and an aliquot of 1 ml was collected in order to read the ROS production. About 10 µM of 2',7'-dichlorofluorescein diacetate (DCHF-DA) was added to supernatants and samples were read after 1 h. The DCF fluorescence intensity emission was recorded at 520 nm (with 480 nm excitation) as described [3]. The protocol of this study was approved by Ethic Commission of UFSM (0089.0.243.000-07). **Results:** Fe^{2+}/EDTA produced an increase on cortical lipid oxidation when compared with basal condition ($p<0.05$) and *V. officinalis* diminished the Fe^{2+}/EDTA pro-oxidant effect ($p<0.05$). The incubation of brain cortical slices with QA (1 mM) caused an increase in ROS production when compared to basal conditions ($p<0.05$) and *V. officinalis* blocked the pro-oxidant effect of QA ($p< 0.05$). **Discussion:** Fe^{2+} can catalyze one-electron transfer reactions that generates reactive oxygen species, such as the reactive $OH^{\cdot -}$, which is formed from H_2O_2 through the Fenton reaction increasing LPO. We also tested the ability of QA in inducing DCFH-DA oxidation in a model using cortical slices where the cells are more preserved than in brain homogenate since the toxic action of QA involves the participation of NMDA receptors. *V. officinalis* reduced ROS production induced by this pro-oxidant agent in cortical slices and also reduced the increase of LPO caused by Fe^{2+}/EDTA. Results emphasize the protective properties of *V. officinalis* against these neurotoxic insults and indicate that *V. officinalis* could be used in several models of neurotoxicity. **Acknowledges:** CAPES/CNPq. [1] Sateia MJ; *Sleep* 23:243; 2000. [2] Montserrat JM; *Toxicol*. 45:177; 2003. [3] Pérez-Severiano P; *Neurochem Int* 45:1175; 2004.

09.047

Avaliação da citotoxicidade do extrato bruto metanólico de *Bauhínia cheilantha* (Bong.) Steud. e suas frações. Lima CE[1], Corrêa AJC[2], Costa MCCD[2], Aguiar JS[3], Nascimento SC[3], Rodrigues MD[3] [1]UNICAP - Biologia, [2]UNICAP - Ciências Biológicas, [3]UFPE Antibióticos

Introdução: *Bauhinia cheilantha* (Bong.) Steud. (Caesalpiniaceae) conhecida popularmente como mororó, é uma espécie encontrada na Caatinga e amplamente utilizada na medicina popular por apresentar ação hipoglicemiante. É uma espécie de expressiva importância local sendo usada na produção de remédios tradicionais com ação anti-inflamatória, antidiabética, antireumática, sedativa e para distúrbios digestivos. O intenso uso da espécie para fins terapêuticos, nos motivou a investigar acerca de seus aspectos toxicológicos. **Métodos:** A partir das folhas da espécie foi produzido, por maceração em repouso, o extrato bruto metanólico, do qual foram obtidas partições com hexano, diclorometano, acetato de etila e butanol. Cada fração obtida foi evaporada e posteriormente armazenada (7ºC), para determinação da atividade citotóxica. As linhagens celulares HEp-2 (derivada de tumor da laringe humana) e NCI-H292 (carcinoma de pulmão humano) utilizadas nos testes, foram obtidas da seção de culturas de células do Instituto Adolfo Lutz (SP) e mantidas de acordo com o protocolo do Departamento de Antibióticos da UFPE. A atividade citotóxica foi avaliada segundo o protocolo do Instituto Nacional do Câncer, pelo método colorimétrico do MTT (brometo [3-(4,5-dimetil (tiazol-il)-3,5-difenil] tetrazólio). Uma suspensão celular com 10^5células/mL foi distribuída em placas de cultura com 96 poços, que foram incubadas a 37ºC, em atmosfera úmida (5% de CO_2), durante 24h. Após este período a substância teste foi adicionada as placas (22µL/poço), que foram reincubadas a 37ºC por 72h, quando então, foi adicionado a cada poço 25µL de MTT (5mg/mL). As placas foram mantidas por duas horas na estufa, e a seguir, foi adicionado 100µL de DMSO a cada poço. A leitura óptica foi realizada em leitor automático de placas (595nm) e a CI_{50} foi determinada a partir de uma regressão linear, relacionando-se o percentual de inibição com o logaritmo das concentrações testadas, admitindo-se ($p<0,01$), para a reta obtida. Extratos brutos com valores de CI_{50} menor ou igual a 30µg/mL foram considerados citotóxicos e para a vincristina usada como padrão, valores de CI_{50} menor ou igual a 4 µg/mL foram considerados significativos. Em ambos os casos, utilizou-se como base o protocolo do Instituto Nacional do Câncer-USA. (GERAN, Canc.Chemo.Reports. v.3, p.1, 1972; ALLEY, C. Research, v.48, p.589, 1988; PEREIRA, T. Jorn. Exp. Clin. Medc. v.19, p. 47, 1994). **Resultados e Discussão:** A literatura não dispõe de estudos acerca das ações toxicológicas atribuídas ao uso terapêutico da espécie. O estudo da citotoxicidade de *B. cheilantha*, portanto é inédito, e mostrou os seguintes resultados frente as células HEp-2: fração hexânica com CI_{50} igual a 35 µg/mL e fração diclorometano com CI_{50} igual a 42 µg/mL. Frente a linhagem NCI-H292 os resultados foram: CI_{50} igual de 64µg/mL para a fração hexânica e CI_{50} 78µg/mL para a fração diclorometano. Os resultados obtidos evidenciaram uma relativa toxicidade para as folhas da espécie, o que justifica cautela na utilização da mesma para fins terapêuticos. **Apoio Financeiro:** PIBIC/UNICAP

09.048

Antileishmanial activity of aqueous extractive solutions from *Hyptis pectinata, Stryphnodendron adstringens* and *Pfaffia glomerata.* Queiroz AC[1], Dias TLMF[2], Matta CBB[2], Cavalcante-Silva, LHA[2], Porfírio, APR[2], Cupertino-Silva YK[2], Souza ET[2], Nunes MP[3], Martins MV[4], Alexandre-Moreira MS[2] [1]UFAL - Farmácia, [2]UFAL - Farmacologia e Imunidade, [3]FIOCRUZ - Imunologia, [4]FIOCRUZ - Imunoparasitologia

Introduction: Leishmaniasis is a widespread parasitic disease caused by protozoan parasites of the genus *Leishmania*. The disease is endemic in some tropical areas of the world and in underdeveloped countries, directly affecting about 2 million people annually worldwide. These observations prompted us to investigate the leishmanicidal activity of plants *Hyptis pectinata, Stryphnodendron adstringens* and *Pfaffia glomerata*, popularly known as ‘‘Sambacaitá”, “Barbatimão” and “Meracilina”, respectively. These plants were selected through data gotten in ethnopharmacological studies in three endemic areas of Alagoas, Brazil. **Methods**: The aqueous extractive solutions from plants were prepared the same as the people used in endemic areas of Alagoas. For experiments, cell line of macrophages J774 were infected with promastigote forms of *Leishmania amazonensis* at a parasite: macrophage ratio of 10:1 for 4 h at room temperature in 24-well culture plates, and then the plates were washed with Hanks. All cultures were done in complete medium instead of FCS, and the cultures were kept for 3 days 37 °C, 7% CO_2. After 3 days of incubation, promastigote forms burdens were microscopically assessed. Cytotoxic effects against promastigote forms were also evaluated. For assessing the activity of compounds against the amastigote stage of the parasite, were realized infection model in coverglass. The cell mammalian viability was determined using the LDH test. The aqueous solutions from plants were tested at concentration 100 µg/ml in all experiments, except in assay to evaluate activity against intracellular forms which were used the concentration of 10 µg/ml. *P. glomerata* was only evaluated in the experiments of cytotoxic against promastigote and macrophages. The Ethical Committee of Federal University of Alagoas (N º 014869/2006-86) approved all experimental protocols described in this study. **Result and Discussion:** The aqueous extractive solutions from *H. pectinata* and *S. adstringens* demonstrated high order of *in vitro* leishmanicidal activity in the concentration used against promastigotes of *L. amazonensis* proceeding of macrophages culture, presenting percentages of inhibition of growth of 81.9% and 90.9%, respectively. The plants *H. pectinata, S. adstringens* and *P. glomerata* present direct activity against extracellular forms, observing itself percentages of inhibition of growth of 85.0%, 90.4% and 88.7%, respectively. The plants *H. pectinata* and *S. adstringens* also significantly diminished the number of amastigotes, presenting percentages of inhibition of 55.5% and 41.2%, respectively. Moreover, all the plants weren´t able to reduce the viability of macrophages. Based on the data, we can conclude that the plants exhibited considerable leishmanicidal activity. **Acknowledgements:** CNPq, FAPEAL, Ministério da Saúde, Secretaria de Estado da Saúde de Alagoas and IM-INOFAR.

09.049

Spinal antinociception evoked by the triterpene 3b, 6b, 16b-trihidroxilup-20(29)-ENE in mice: evidence for the involvement of the glutamatergic system via NMDA and metabotropic glutamate receptors. Longhi-Balbinot DT[1], Gadotti VM[1], Martins DF[1], Facundo VA[2], Santos ARS[1] [1]UFSC - Ciências Fisiológicas, [2]UNIR - Química

Introduction: The present study aims to investigate the possible involvement of the spinal glutamatergic system, in the antinociception caused by triterpene 3b, 6b, 16b-trihidroxilup-20(29)-ene (TTHL) in mice. **Methods**: Swiss mice of both sexes were used (25-35g; N=6-8). Experiments were performed after approval by the Institutional Ethics Committee under the protocol: 23080.003593/2008-84. In order to investigate the participation of the glutamatergic system, nociception was induced by i.t. injection (site = 5ml) of Glu (175 nmol/site), AMPA (135 pmol/site), NMDA (450 pmol/site), kainate (135 pmol/site), trans-ACPD (10 nmol/site). Furthermore, to clarify the involvement of NMDA and metabotropic glutamate receptors in the antinociceptive effect of TTHL, we associated sub-effective doses of MK-801(1 nmol/site; non competitive NMDA antagonist) plus TTHL (6.5 nmol/site) or (RS)-MCPG (30 nmol/site; non-selective group I/group II metabotropic glutamate receptor antagonist) plus TTHL (6.5 nmol/site) that were i.t. administered just before NMDA (450 pmol/site) or trans-ACPD (10 nmol/site). **Results:** TTHL injected by i.t. route (6.5-218 nmol/5ml) also caused significant and dose-dependent reduction of nociception induced by i.t. injection of Glu (Glu: 122±5.3; TTHL 65.5 nmol/i.t: 61±5.2; ID_{50}: 54.5 (51.2-57.8) nmol/site and inhibition of 51±6%). Moreover, TTHL (65.5 nmol/i.t., co-injected) caused a marked inhibition of the nociceptive responses induced by i.t. injection of NMDA (NMDA: 156±7.3/TTHL: 33±7.5), trans-ACPD (trans-ACPD: 209.6±15/TTHL: 46.3±13.5), with inhibitions of 81±7; 79±7, respectively, but had no effect on AMPA (AMPA: 37.1±2.5/TTHL: 37.1±5.5) and kainate (kainate: 153.3±28.3/TTHL:140.6±27.5)-induced nociceptive response. It was demonstrated that the association of sub-effective doses of TTHL (6.5 nmol/site, i.t.) with MK-801(1 nmol/site, i.t.; non-competitive NMDA antagonist) or with (RS)-MCPG (30 nmol/site, i.t.; non-selective group I/group II metabotropic glutamate receptor antagonist) produced a synergistic antinociceptive effect on pain induced by NMDA (NMDA: 128.7±13.6; MK-801+TTHL: 37±4.7) or trans-ACPD (t-ACPD: 192.6±15.4; (RS)-MCPG+TTHL: 54.8±13.3), with inhibitions of 73±5% and 78±7, respectively. **Discussion:** Together, these results provide an experimental evidence for the involvement of the spinal glutamatergic system (NMDA and metabotropic glutamate receptors) in the antinociceptive action caused by TTHL in mice. **Supported by**: CAPES, CNPq, UFSC.

09.050

Anti-inflammatory and anti-helycobacter activity of extracts and isolated compounds from *Solanum cernuum Vell*: 24-OXO-31-norcycloartanone and cicloeucalenone.

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Introduction: The species *Solanum cernuum* Vell is commonly known as 'panacéia' and 'braço preguiça.' It is used for the treatment of ulcer, liver diseases, skin affections, hemorrhages, to stimulate sweat, as a depurative, antioneoplastic and diuretic. In the past study from our research group we did the screening of the dichloromethane extract and we established the presence of a homologous series of alkanes (C25-C34), triterpenoids and the xanthophyll lutein. The terpenoid fraction was mainly composed of cycloeucalenone and 24-oxo-31-norcycloartanone that were isolated for the first time from Solanum genus, beside β-sitosterol. Both compounds demonstrated activity in against the lung tumor cell line NCI-H460 [1]. The aim of this work is evaluate *in vitro* anti-inflammatory, bactericid and anti-helicobacter pylori activity. Methods: The oven-dried (45 ºC) and powdered leaves (600 g) were extracted three times with dichloromethane by maceration. The residue was washed three times with ethanol 95%. Both solvents were evaporated under reduced pressure to obtain the dichloromethane (EBD) and ethanol (EBE) extracts. The writhing test with acetic acid 1,5% and the ear edema induced by croton oil 2,5% on male Swiss mice evaluated with the EBD and EBE (Protocol nº A18/CEP/2008). Human sirtuin-1 activity (deacetilase) was measured by commercial kit (Biomol Res. Lab.) and it was assayed for cycloeucalenone and 24-oxo-31-norcycloartanone compounds. Anti-Helicobacter activity was evaluated by minimum bactericidal concentration (MBC) in broth microdilution method plus plate inoculation against 3 reference strains, H. pylori 26695 (ATCC 700392), J99 (ATCC 700824) and SS1 (Sidney Strain 1). The fractions from dichloromethane extract were twofold diluted ranging from 120 to 7,5 mg/ml. All assays were performed in duplicate using amoxicillin as positive internal control. **Results:** Both extracts were effective on the inhibition of abdominal writhing in relation of the control group dipyrone (93,8%), EBE 600 mg/kg (61,9%), EBE 300 mg/kg (26,4%), EBD 100 mg/kg (38,1%); EBD 300 mg/kg (61,5%); EBD 600 mg/kg (74%) $p<0,05$. In the ear edema test, the EBE 10% inhibited 20,3%. Bactericidal activity was showed by 18 fractions with values up to 15 mg/ml against three *H. pylori* strains, including SS1 that is traditionally used for *in vivo* assays. None of the compounds assayed significantly modified sirtuin-1 activity. **Conclusions:** EBD and their fractions showed have antinociceptive and bactericidal activities more pronounced than EBE. The cycloeucalenone and 24-oxo-31-norcycloartanone are the main constituents of the EBD and it is possible that they should be responsible for this activities. More studies are necessary to understand it. *Support:* AECID and Universidade de Sorocaba. [1] R. Grando, M., et. al., Z. Natur. C, 63c, 507, 2008.

09.051

Mechanisms underlying the antinociceptive effect of triterpene 3-beta, 6-beta, 16-beta-trihydroxylup-20(29)-ene in mice. Longhi-Balbinot DT[1], Lanznaster D[1], Silva MD[1], Facundo VA[2] [1]UFSC - Ciências Fisiológicas, [2]UNIR - Química

Introduction: Based on our previous results[1], the present study examined the antinociceptive effect of 3b, 6b, 16b-trihydroxylup-20(29)-ene (TTHL) obtained from the flowers of *Combretum leprosum* in chemical behavioral models of nociception and investigates some of the mechanisms underlying this effect. **Methods**: Swiss mice of both sexes were used (25-35g; N=6-8) and the experiments were approved by the Institutional Ethics Committee under the protocol 23080.003593/2008-84. We investigate the antinociceptive effect of TTHL given by oral (p.o) route (0.01-10 mg/kg), against writhing test induced by acetic acid (0.6%, i.p). Additionally, peritoneal exudates were removed to assess the extravasation (measured by Evans Blue leakage) and total cells migration. We also investigated the effect of TTHL (3-300 mg/kg, p.o) in the formalin (2.5%/20µl) and glutamate (Glu; 20nmol/paw, i.pl)-induced nociception in the mouse paw. The time course of the antinociceptive action of TTHL (30 mg/kg, p.o) was evaluated in the nociceptive response induced by i.pl Glu 1, 2, 4, 8 and 10 h after treatment. To investigate the involvement of the opioid and serotonergic systems in the antinociceptive effect of TTHL, mice were pre-treated with naloxone (1 mg/kg, i.p), and after 20 min they were injected with TTHL (30 mg/kg, p.o), morphine (2.5 mg/kg, s.c) or vehicle (10ml/kg, p.o); or mice were pre-treated with PCPA (an inhibitor of serotonin synthesis, 100 mg/kg, i.p) once a day for 4 consecutive days. 20 min after the last administration of PCPA, mice received TTHL (30 mg/kg, p.o), morphine (2.5 mg/kg, s.c) or vehicle (10ml/kg, p.o). For both systems, the algesic responses to i.pl Glu were recorded 30, 60, 60 min after morphine, TTHL or vehicle injection, respectively. **Results:** The results are expressed as mean ± SEM, ID_{50} values and inhibition, respectively. TTHL (0.01-10 mg/kg, p.o) caused a significant and dose-dependent inhibition of acid-acetic (AA) induced visceral pain (AA: 34±3/TTHL10: 11±9, 0.15 mg/kg; 69±3%) and total cells migration (AA:5±1/TTHL10: 2±0.3; 3.1 mg/kg; 59±9% of inhibition) but not Evans blue leakage (AA:0.7±1/TTHL10: 0.4±1). Also, TTHL (3-100 mg/kg, p.o) evoked dose-dependent inhibition of the Glu-induced nociception (Glu:263±10; TTHL30: 116±9, 19 mg/kg, 56±3% of inhibition) and of both phases of formalin (F)-induced pain (1ª phase: F:87±8/TTHL300: 32±5; 108 mg/kg; 63±5%; 2ª: F:263±25/TTHL30: 128±34; @30 mg/kg; 51±9%). When Glu was injected at different time points after TTHL treatment, we verified that TTHL had a peak of response after 1 h (Glu:334±35.1; TTHL30: 208.3±21.7; 59±6% of inhibition) that lasted up to 6 h (Glu:330±38.4; TTHL30: 208.3±38.7; 37±6%). The pre-treatment of mice with naloxone (N) and PCPA (P), given 20 min beforehand, reversed the antinociception caused by morphine (M) and TTHL (30 mg/kg, p.o) when analyzed against i.pl. Glu-induced pain (Glu:264.9±24.8; M:81.1±14.4; N+M: 212.5±29.2; TTHL:120.3±17.3; N+TTHL:232.4±27; Glu:164±8; M:54±11; P+M:114±9; TTHL:103±12; P+TTHL:179±21). **Conclusion:** TTHL produced significant antinociception against several models of chemical pain through mechanisms that involve an interaction with the opioid and serotonergic systems. **References**: 1) Pietrovski et al., *Pharmacol. Biochem. Behav.* 83: 90; 2006. **Supported by:** CAPES, CNPq, UFSC

09.052

Evaluation of leishmanial activity of extracted from aqueous solutions *Stryphnodendron adstringens, Aloe vera and Ruta graveolens*. Matta CBB[1], Queiroz AC[1], Dias TLMF[1], Cavalcante-Silva, LHA[1], Muniz G[1], Aquino AB[1], Porfírio, APR[1], Martins MV[2], Nunes MP[3], Alexandre-Moreira MS[1] [1]UFAL - Farmacologia e Imunidade, [2]FIOCRUZ - Imunoparasitologia , [3]FIOCRUZ - Imunologia

Introduction: Leishmaniasis is an infectious parasitic disease transmitted to humans by phebotomines, and is caused by several species of protozoa of the genus *Leishmania*. Is an endemic disease in the tropical and subtropical regions of the world. Currently, it is found in all Brazilian states, under different epidemiological profiles, and a serious public health problem. Furthermore, it is a neglected disease of disinterest for the pharmaceutical industry. The chemotherapy to this disease is not always effective and can cause several side effects. Studies have demonstrated the use of plants popular in the treatment of leishmaniasis, that led us to assess the leishmanicidal activity of three plants, used with this intention, in endemic areas of the state of Alagoas in Brazil. **Methods**: From an ethnobotanical study were identified some plants used to treat leishmaniasis: *Ruta graveolens*, *Chenopodium ambrosioides* and *Aloe Vera*. With them was prepared an aqueous extract (tea) similarly made by users. For experiments, macrophages of the lines J774 were infected with promastigote forms of *Leishmania amazonensis* a ratio of 1:10 for 3 h at room temperature in culture plates, and then were washed with Hanks. All cultures were done in complete medium, instead of FBS, and the cultures were incubated for 3 days 37 °C, 7% CO_2. After that, promastigote forms burdens were microscopically assessed. Cytotoxic effects against the parasite was also evaluated. For assessing the activity of compounds against the amastigote stage of the parasite, were realized infection model in coverglass. The macrophages viability was determined using the LDH test. Pentamidin was used as standard drugs for positive controls and all experiments were performed in triplicate. The aqueous solutions from plants were tested in the concentration of 100, 10 and 1 µg/ml in all experiments, except in assay to evaluate activity against intracellular forms which were used the concentration 10 µg/ml. All experimental protocols described in this study were approved by the Ethical Committee of Federal University of Alagoas (Nº 014869/2006-86). **Result and Discussion:** The extraction solutions tested showed high leishminicidal activity against forms of *L. amazonensis* proceeding of macrophages culture. The percentages of inhibition of the extracts of *Aloe Vera*, *Ruta graveolens* and *Chenopodium ambrosioides* were: 82,95% 74,37% and 87,42%, respectively. The activity of plants *Aloe Vera* and *Ruta graveolens* against intra and extracellular forms of the parasites were evaluated. The percentages of inhibition were:26,52% and 41,73%, for intracellular forms, and 94,2%, 97,36%, for extracellular forms, respectively. Furthermore, no plant was shown to be toxic to macrophages. We conclude that the plants showed significant leishmanicidal activity. **Acknowledgements:** CNPq, FAPEAL, Ministério da Saúde, Secretaria Estadual de Saúde de Alagoas and IM-INOFAR.

09.053

Antiulcer effect of epoxy-carvone. Siqueira BPJ, Barboza, RR, Sousa DP, Batista JS UFS - Fisiologia

Introduction: The epoxi-carvone is a monoterpene present in essential oils of several plants, such as *Carum carvi* and *Mentha x villosa* (Arruda et al, Rev. Bras. Farmacogn, v.16, p.307, 2006). It is a ketonic derivative from carvone which has an epoxy group instead of alpha- and beta-unsaturation in the carvone. Since recent studies have shown that several compounds with alpha- and beta-unsaturated ketonic group present antiulcer effects, the present study evaluated if the epoxi-carvone also presents this effect. **Methods**: Epoxy-carvone antiulcer effect was evaluated in ethanol- and indomethacin-induced ulcers in Wistar male rats. In ethanol-induced ulcers, animals were divided in 5 groups (n=10) and were orally treated with vehicle (tween80, 1mL/100g), ranitidine (50 mg/kg) or with epoxy-carvone (10, 30 or 50 mg/kg). After 60 min, the rats were orally treated with absolute ethanol (0.4mL/kg) and, 30 min thereafter, were euthanatized and the stomachs was removed for quantification of ulcer index (UI) according to Alkofahi e Atta, J. Ethnopharmacol., v.67, p.341, 1999. The same protocol was used in indomethacin-induced ulcers, but ethanol was replaced by indomethacin (50 mg/kg) and the animals were euthanatized 6 hours after administration of indomethacin. In other experiments set, rats were pre-treated with indomethacin (10 mg/kg, s.c.) 30 min before the administration of the epoxy-carvone (10 mg/kg) in ethanol-induced ulcer test. The experimental protocols were approved by the Ethical Committee of this institution under number 26/08. **Results and Discussion**: Epoxy-carvone at the doses of 10, 30 and 50 mg/kg reduced the ethanol-induced ulcers formation with UI values of 32±11, 37±9 and 48±10, respectively, in comparison to control group (UI = 112±19). In the indomethacin-induced ulcers, a significant reduction of UI values was only observed at the doses of 10 and 30 mg/kg (UI = 6.7±1.2 and 8.8±1.8), respectively, compared to control group (UI = 16.9±3.2). The pre-treatment with indomethacin (10 mg/kg, s.c.) did not prevent the gastroprotetor effect of epoxy-carvone (10 mg/kg) in ethanol-induced ulcers. **Conclusion**: These results permit us to conclude that the epoxy-carvone presents antiulcer effect in both ethanol- and indomethacin-induced ulcers. Moreover, the gastroprotetor effect of epoxy-carvone in ethanol-induced ulcer does not appear to involve increase in the synthesis of prostaglandins. Additional experiments are still necessary to characterize the action mechanism of this compound. **Financial Support**: FAPITEC, UFS.

09.054

Anti-inflammatory activity of the methanolic fraction isolated from the ethanol extract of leaves of *Spiranthera odoratissima* A. ST. Hillaire (manacá). Florentino FI[1], Barbosa DBM[2], Nascimento, M. V. M[2], Matos LGM[3], Galdino PM[4], Sousa B F[5], Lino RC[4], Paula JR[6], Costa EA[6] [1]ICB-UFG, [2]UFG - Fisiologia, [3]DCIF-UFG, [4]UFG - Ciências Fisiológicas, [5]UFG - Farmácia, [6]UNIFESP - EPM - Farmacologia

Introduction: *Spiranthera odoratissima* A. St. Hillaire*,* popularly known as Manacá, is a species found in Cerrado in the state of Goiás used in folk medicine, in the form of tea or bottle, to treat pain, rheumatism or stomachache. Some results demonstrated the anti-nociceptive and anti-inflammatory activities of root and leaves extracts of this plants (Matos L.G et al, *Phytother.Res.*18, 963-966, 2004). The objective of this work was evaluate the anti-inflammatory effect of the purified fraction of the methanol phase (FM) of the ethanol extract of leaves of Manacá using different methodologies. **Methods:** The leaves of Manacá were collected in Bela Vista-Go and a voucher specimen was deposited in the Herbarium of UFG (n° 24,330). The leaves desiccated and crushed were macerated during 7 days with ethanol (EtOH) 96°GL. The macerate was rotaevaporated until dryness, then was dissolved in methanol (MeOH): water (7:3) and filtered on celite, and partitioned successively with hexane and chloroform, resulting in three phases: hexanic (HF), chloroformic (CF), and methanolic (FM) phase. FM was submitted to molecular filtration in Sephadex LH-20 eluted with MeOH. The fractions of 10-28 (Fr10-28) isolated were grouped based on their RFs obtained by CCD, using EtOH: Acetic acid (8:2) as mobile phase, the analgesic activity was evaluated by acetic acid-induced abdominal writhing test in mice (Koster et al.1959). The anti-inflammatory effect was evaluated by the method of pleurisy in mice (VINEGAR et al., 1973) and croton oil-induced ear oedema test in mice (Zanini et al, 1992). The animal was male *Swiss* mice (n = 7 per group) weighing 35-40 g. All the experimental protocol was approved by the Ethical Committee for Animal Research of UFG (number 102/08). **Results:** On acetic acid-induced abdominal writhing test, the previous treatments v.o. with FM 150 and 500 mg/kg reduced the number of abdominal writhes from 70.2 ± 4.4 (control group) to 49 ± 6.3 and 30.5 ± 11.1, respectively. On pleurisy, the previous treatment (v.o) with FM (150 and 500 mg/kg) or Fr_{10-28} (20 and 40 mg/kg), reduced the number of leukocyte/mL x 10^6 migrated for the pleural cavity from 6.03 ± 0.61 (control group) to 3.78 ± 0.36, 2.97 ± 0.67, 3.74 ± 0.307, 1.71 ± 0,224, respectively. The previous treatment with Fr_{10-28} (20 mg/kg v.o) reduced the ear oedema from 17.8 ± 0.8 mg to 14.0 ± 0.4 mg, as well as the Evan´s blue concentration in the pleural exsudate from 4.41 ± 0.46 µg/mL to 2.08 ± 0.50 µg/mL. **Conclusion:** The results obtained show that the methanolic fraction has anti-inflammatory activity. This activity should be responsible for the analgesic effect seen in the acetic acid-induced abdominal writhing test. The anti-inflammatory action of *Spiranthera odoratissima* A. St. Hillaire was maintained in the isolated fraction Fr_{10-28} obtained by molecular filtration in Sephadex. **Financial Support:** CNPq/PIBIC, FUNAP/UFG.

09.055

Evidence for the involvement of TRPV1 and PKC on the antinociceptive effect of 3,4,5-trimethoxydihydrocinnamic acid obtained from *Piper tuberculatum.* Lanznaster D[1], Longhi-Balbinot DT[1], Rodrigues RV[2], Facundo VA[3], Santos ARS[1] [1]UFSC - Ciências Fisiológicas, [2]UNIR - Medicina, [3]UNIR - Química

Introduction: In a previous study we demonstrated that the 3,4,5-trimethoxydihydrocinnamic acid (TMDC), obtained from Ethyl Acetate fraction of *Piper tuberculatum* dried fruits, significantly inhibits both hyperalgesia and nociceptive responses induced by intraplantar (i.pl) and intrathecal (i.t) injection of bradykinin (BK), respectively[1]. Considering the involvement of TRP receptors and PKC in the nociceptive actions of BK[2,3,4], the aim of this study was to investigate the possible involvement of TRPV1, TRPM8 and TRPA1 channels and PKC on the antinociceptive effect of TMDC. **Methods:** Swiss mice (25-35g) and Wistar rats (250-350g) were used. Experiments were approved by the Institutional Ethics Committee, under the protocol 23080.003593/2008-84. Animals were pretreated with TMDC (0.0001 – 10 mg/kg) by i.p. route 30 min beforehand. Thermal (Hargreaves method) and mechanical (Randall-Selito test) hyperalgesia were induced in rats by an i.pl. injection of PMA (100 µmol/paw), a PKC activator. The nociceptive response was induced by an i.pl. injection of 20 µl of capsaicin (TRPV1 agonist, 1.6 µg/paw), menthol (TRPM8 agonist, 1%) and cinnamaldehyde (TRPA1 agonist, 10 nmol/paw). **Results:** TMDC reversed both thermal and mechanical hyperalgesia induced by PMA, with inhibitions of 61 ± 8% and 46 ± 10%, respectively. The values observed were: Vehicle (V): 14.9 ± 3.8; PMA: 3.2 ± 1.3; TMDC 1 mg/kg: 10.4 ± 2.1 for thermal; and V: 631.1 ± 184.2; PMA: 88.0 ± 23.7; TMDC 10 mg/kg: 340.0 ± 121.4 for mechanical hyperalgesia. Furthermore, TMDC significantly inhibited the nociception induced by capsaicin (Control (C): 54.6 ± 14.9; TMDC 1 mg/kg: 20.4 ± 10.2), with inhibition of 63 ± 6% and DI50 value of 0.29 (0.17-0.49) mg/kg. Otherwise, TMDC was not able to inhibit the nociception induced by menthol (C: 267.4 ± 69.7; TMDC 10 mg/kg: 196.7 ± 72.6) and cinnamaldehyde (C: 82.0 ± 23.4; TMDC 10 mg/kg: 65.7 ± 20.4). **Conclusion:** Together, present results demonstrated that TRPV1 and PKC-signalling pathways, but not TRPA1 and TRPM8 channels, are involved on the antinociception induced by TMDC. Moreover, we suggest that an involvement between BK, TRPV1 and PKC could contribute to this antinociceptive effect. However, additional experiments are necessary to confirm this hypothesis. **References**: 1) Lanznaster et al. I Congress IBRO/LARC of Neuroscience from Latin American (Neurolatam), 2008. 2) Mizumura et al., Neurosci. Lett. 237: 29, 1997. 3) Bandell et al., Neuron 41: 849, 2004. 4) Katanosaka et al., Neurosci. Res. 62: 168, 2008. **Financial support**: CAPES, CNPq, UFSC

09.056

Comparative study of antiophidian properties of aqueous extracts *Myrsine guianensis* and *Jatropha elliptica.* Alves LM[1], Brito CD[1], Alves IS[1], Silva TDS[1], Vieira SAPB[2], Homsi-Brandeburgo MI[2], Hamaguchi A[2], Rodrigues VM[2], Mendes MM[2], Izidoro LFM[2] [1]UFU-FACIP, [2]UFU - Instituto de Genética e Bioquímica

Introduction: The venoms from snakes are probably the most complex of all venoms animals, containing a diverse mixture of enzymes and non-enzymatic toxins, which may perform different pharmacological activities. The venoms from Viperidae and Crotalidae snakes induce relevant hemostatic and hematological alterations, in addition to several manifestations, which may lead to fatal consequences. Long the plants are used by humans as food and treatment of diseases. The interest in the study of plants as a form of alternative therapies is occupying an increasingly significant position in ethnopharmacological studies. *Myrsine guianensis* and *Jatropha elliptica* are species of plants used in folk medicine for the treatment of snakebite. **OBJECTIVE:** The objective of this study was to evaluate the inhibition of the coagulant, hemorrhagic **(Comitê de Ética na Utilização de Animais-CEUA, protocolo 08-2008)** and phospholipasic activities induced by the venom of *Bothrops pauloensis* through the aqueous extracts of *Myrsine guianensis* and *Jatropha elliptica*. **Methods:** The aqueous extracts were prepared with the leaves of *Myrsine guianensis* and the root of *Jatropha elliptica* which were washed with deionized water and homogenized for 15' at room temperature and then filtrated. The filtrate was centrifuged and the supernatant was lyophilized and stored at -20°C. The inhibitions of the coagulant, hemorrhagic and phospholipasic activities induced by the venom were assayed with incubation by 30' to 37ºC in three ratios: 1:5, 1:10 and 1:50 (w/w; venom/extract). PLA_2 enzymatic activity was measured by an indirect hemolytic assay. Coagulant activity of venom on bovine plasma and the time to clot the plasma solutions was recorded (in seconds). For hemorrhagic activity, Swiss male mice received two minimum hemorrhagic doses (MDH) of venom of *Bothrops pauloensis*, combined or not with the extract. **Results:** The inhibition of coagulant activity by *Myrsine guianensis* was statistically significant in the proportions of 1:5, 1:10 and 1:50 (w/w; extract/venom) with 95%, 100% and 100% of inhibition respectively, while as extract of *Jatropha elliptica* was 70%, 85% and 100% in the same ratios. Phospholipase A_2 activity in the extract of *Jatropha elliptica* the results were not significant and the best result was in the proportion of 1:50 with 30% of inhibition, already the extract of *Myrsine guianensis* inhibited 72% in the proportion of 1:5 and 75 % in the proportion of 1:10. The extract of *Myrsine guianensis* was able to inhibit 100% of the hemorrhagic activity at all concentrations tested and the extract of *Jatropha elliptica* inhibit 70% in the proportion of 1:5 and 100% at ratios of 1:10 and 1:50. **Discussion**: According to the results conclude that the extracts of *Myrsine guianensis* and *Jatropha elliptica*, have active compounds capable of inhibiting some toxics effects induced by snake venom *Bothrops pauloensis*. Furthermore, these inhibitors can be used as molecular models for development of new therapeutical agents in treatment of ophidian accidents. **Financial supported: UFU and FAPEMIG**

09.057

Effect of eye drops of essential oil of *Cordia verbenacea* on inflammatory corneal angiogenesis. Fechine FV, Borges EV, Moraes MEA, Moraes MO UFC - Fisiologia e Farmacologia

Introduction: The anti-inflammatory properties of the essential oil of *Cordia verbenacea* (EOCv) have been studied in different experimental models. Moreover, its anti-inflammatory activity is due mainly to two sesquiterpene compounds, identified as alpha-humulene and trans-caryophyllene. Thus, this study was aimed to investigate the effect of topical EOCv on corneal neovascularization using a model of inflammatory corneal angiogenesis. **Methods:** Experiments were performed in accordance with protocol (Nº 112/07) approved by the Ethical Committee in Animal Research of UFC. Twenty three male New Zealand rabbits were submitted to a punctual cauterization in the superior periphery of the left cornea using a circular piece of filter paper, 3 mm of diameter, soaked in NaOH 1M solution. The animals were randomly allocated into four groups: Control (n=5), treated with 0.5% carboxymethylcellulose sodium solution (5 mg/mL – vehicle); Diclofenac (n=6), treated with 0.1% diclofenac sodium solution (1 mg/mL); EOCv0.5% (n=6) and EOCv1% (n=6), which were treated with 0.5% (5 mg/mL) and 1% (10 mg/mL) EOCv, respectively, diluted in the vehicle. Eye drops were instilled into the conjunctival sac (40 µL) three times daily during 21 days. Evaluations were done on days 3, 6, 9, 12, 15, 18, and 21, post cauterization. During these days, digital images of the cornea were captured in a standard fashion. Angiogenic response was measured using a software which was developed specifically for this purpose (SQAN – Angiogenesis Quantification System). It calculated the following parameters: Neovascularization Area (NA), Total Vascular Length (TVL), and Number of Blood Vessels (NBV). Based on NA parameter, it was calculated the Angiogenesis Mean Rate (AMR) and the Inhibitory Effect (IE) of each treatment in relation to Control on day 21. **Results and discussion:** In this model, the neovascular response observed in Control group followed a biphasic pattern: proliferation (between days 0 and 12) and maturation (from days 12 to 21). Analyzing the temporal pattern of NV, TVL and NBV, it was observed that their values in the treated groups were lower than in the Control group during all evaluations, although statistically significant differences have been found only at the end of the study. Such parameters were significantly reduced in EOCv0.5% group only on day 21 ($P<0.05$), while in EOCv1% group the angiogenic response was inhibited on days 18 ($P<0.05$) and 21 ($P<0.05$). Thus, AMR of both EOCv0.5% (0.032 ± 0.012 mm^2/day) and EOCv1% (0.035 ± 0.030 mm^2/day) were significantly lower ($P<0.05$) than Control (0.089 ± 0.046 mm^2/day), mainly caused by the reduction of vascular growth on the second half of the experiment. However, in Diclofenac group, only NBV was significantly reduced ($P<0.05$) on day 21, so that AMR value (0.051 ± 0.018 mm^2/day) was not significantly different from Control. The parameter IE summarized the efficacy of the tested drugs. Thus, compared to Control group, IE of EOCv0.5%, EOCv1% and Diclofenac were 53.20%, 52.70% and 36.86%, respectively. **Conclusion:** Eye drops of both 0.5% EOCv and 1% EOCv inhibit partially inflammatory corneal angiogenesis and their effects are observed mainly during the second half of angiogenic process. **Financial support:** CNPq, CAPES, FINEP, DECIT/MS, InCB.

09.058

Avaliação da atividade citotóxica de *Erythroxylum caatingae* Plowman (Erythroxylaceae).

Aguiar JS[1], Rodrigues MD[1], Cruz ACN[1], Oliveira SL[2], Tavares JF[2], Silva MVB[2], Silva MS[2], Silva TG[1], Nascimento SC[1] [1]UFPE - Antibióticos, [2]UFPB - Tecnologia Farmacêutica

Introdução: A família Erythroxylaceae compreende quarto gêneros (*Erythroxylum*, *Aneulophus*, *Nectaropetalum* e *Pinacopodium*) e cerca de 240 espécies com distribuição pantropical, tendo seus principais centros de diversidade e endenismo na Venezuela, Brasil e Madagascar (Loiola et al., 2007). O gênero *Erythroxylum* é caracterizado pela presença de alcalóides tropânicos, taninos, terpenos e fenilpropanóides (Zuanazzi et al., 2001). Devido à utilização de alcalóides isolados de plantas na medicina contra o câncer, nesse trabalho investigamos a atividade citotóxica da fase acetato caule de *Erythroxylum caatingae* frente às linhagens NCI-H292 (Carcinoma de pulmão humano) e HEp-2 (Carcinoma de laringe humana). **Métodos:** A atividade citotóxica foi feita através do método colorimétrico do MTT [3-(4,5-dimetiltiazol-2-il)-2,5-brometo de difeniltetrazólio] (Mosmann, 1983; Alley et al., 1988). Uma suspensão celular com 10^5células/mL foi distribuída em placas de cultura com 96 poços, incubadas a 37ºC, em atmosfera úmida (5% de CO_2), durante 24h. Em seguida a substância teste (50, 25, 12,5 e 6,25µg/mL) foi adicionada às placas (22µL/poço), que foram reincubadas a 37ºC por 72h, quando então, foi adicionado 25µl de MTT (5mg/mL). As placas foram mantidas por duas horas na estufa, após este período, foi adicionado 100µl de DMSO a cada poço. A leitura óptica foi feita em leitor automático de placas (595nm). Os resultados foram avaliados com base na CI_{50}, a concentração que reduziu em 50% o crescimento celular em relação aos controles não tratados. **Resultados:** A fase acetato do caule de *E. caatingae* apresentou citotoxicidade frente as linhagens celulares HEp-2 e NCI-H292, com CI_{50} = 25,2*µ*g/mL e CI_{50} = 17,3*µ*g/mL, respectivamente. **Conclusão:** Devido aos resultados citotóxicos promissores apresentados em ambas as linhagens celulares, futuras investigações em outras linhagens celulares serão feitas como também os estudos da atividade hemolítica e/ou apoptótica e o isolamento e avaliação dos constituintes responsáveis por tal atividade. **Citação Bibliográfica**: Alley, M. C., Cancer Res., 48, 589, 1988. Loiola, M. I. B., Acta Bot. Bras., 21, 473, 2007. Mosmann, T. J. Immunol. Methods, 65, 55, 1983. Zuanazzi, J. S. A., Biochem. System. Ecol., 29, 819, 2001. **Apoio Financeiro:** CNPq.

09.059

Atividade antitumoral do extrato etanólico das folhas de *Scoparia dulcis* L. Cruz ACN, Campos IA, Oliveira TB, Aguiar JS, Silva TG UFPE - Antibióticos

Introdução: S*coparia dulcis* L. pertence à família Scrophulariaceae e é conhecida no Brasil como vassourinha. É uma planta nativa na América Tropical, hoje com larga distribuição no mundo. No Brasil é utilizada na medicina popular no tratamento do trato respiratório, trato gastrointestinal, distúrbios hepáticos e como anti-inflamatória (Mesía-Vela et al., 2008). Estudos mostraram que o ácido scopadúlcico B isolado da espécie apresentou citotoxicidade em algumas linhagens tumorais (Hayashi et al., 1992). Apesar de existirem vários estudos descritos na literatura sobre os constituintes químicos e atividades biológicas desta espécie, não encontramos estudos sobre atividade antitumoral *in vivo* em Sarcoma 180. Este trabalho teve como objetivo estudar a atividade antitumoral do extrato etanólico das folhas (EEF) de *S. dulcis* frente ao Sarcoma 180. **Métodos:** Para avaliação da atividade antitumoral, foram utilizados camundongos fêmeas albinos Swiss (*Mus musculus)* procedentes do Biotério do Departamento de Antibióticos da UFPE, com faixa etária de 60 dias, pesando 25±5g, separados em grupos de oito animais por gaiola. Os animais foram mantidos à temperatura ambiente 23±2ºC sob o ciclo dia/noite natural (12h luz e 12h escuro), com água e alimento *ad libitum* durante o experimento. Células do tumor ascítico de Sarcoma 180 (suspensão de $5x10^6$células/mL) foram inoculadas subcutaneamente na região axilar dos animais sadios. Após 24h do implante, foi iniciado o tratamento. Os grupos testes receberam, por via i.p., EEF nas doses de 100 e 300 mg/kg, enquanto o grupo controle recebeu o veículo, por sete dias consecutivos. Os produtos foram solubilizados em solução fisiológica a 0,9 % contendo 2% de cremofor. Vinte e quatro horas após o término do tratamento, os animais foram pesados e eutanasiados em câmera de CO_2 para a extirpação da massa tumoral. Os tumores foram dissecados, pesados e a inibição tumoral foi avaliada (Machon et al., 1981). Esse estudo foi aprovado pelo Comitê de Ética em Experimentação Animal da Universidade Federal de Pernambuco sob o processo número 23076.012173/2007-77. **Resultados:** O extrato etanólico das folhas de *S. dulcis* na dose de 100 mg/kg não apresentou inibição tumoral, porém a dose de 300 mg/kg inibiu o crescimento tumoral em 45,6% (0,81±0,39g) em relação ao controle (1,49±0,29g). **Conclusão:** Devido a ausência de efeitos tóxicos visíveis após a administração do EEF, serão testadas doses maiores bem como serão feitos estudos histopatológicos nos principais órgãos dos animais tratados. Se esses estudos apresentarem ausência de efeitos tóxicos, *S. dulcis* poderá ser um candidato a fitoterápico para tratamento do câncer. **Citação Bibliográfica:** Hayashi, K., Phyto. Res., 6, 6, 1992. Mesía-Vela, S., J. Ethnopharm., 111, 404, 2007. Machon Z., *Arch. Immunol. Ther. Exp*.**,** 1981, 29, 217, 1981. Ribeiro-Costa, R. M., J. *Microencapsul*., 21, 4, 371, 2004.
Apoio Financeiro: CNPq/UFPE

09.060

Antioxidant, vasodilatator and hypotensive effects of a standardized hydroalcoholic extract of *Dicksonia sellowiana* Presl. HooK (Dicksoniaceae). Rattmann YD[1], Sanches[2], Furian, AF[3], Paludo KS[4], Schneider Oliveira, M.[3], Crestani S[1], Lapa, F. R.[1], Miguel OG[5], Franco, CRC[6], Mello CF[3], Cadena SMSC[2], da Silva-Santos JE[7], Marques MCA[1], Santos ARS[8] [1]UFPR - Farmacologia, [2]UFPR - Bioquímica e Biologia Molecular, [3]UFSM - Fisiologia e Farmacologia, [4]UNIFESP - Medicina, [5]UFPR - Farmácia, [6]UFPR - Biologia Celular, [7]UFSC - Farmacologia, [8]UFSC - Fisiologia

Introduction: *Dicksonia sellowiana* (Presl.) Hook, a plant native to Central and South America, popularly known as “Xaxim”, is currently under clinical tests in Brazil against asthma, but has been associated with beneficial effects on cardiovascular system. **Methods:** We have thus investigated the vasodilatory, hypotensive and antioxidant effects of the standardized hydroalcoholic extract of *D. sellowiana* leaves (HEDS) through *in vivo* and *in vitro* tests. All the procedures were approved by the Research Ethics Board of the UFPR (number 287). **Results:** In phenylephrine-contracted rat aortic rings, HEDS caused a complete relaxation which was fully prevented by endothelium removal, L-NAME (a nitric oxide synthase inhibitor), ODQ (a guanylate cyclase inhibitor ODQ), charybdotoxin (CTX; a large and intermediate-conductance calcium-activated potassium channel blocker), or atropine (a muscarinic receptor antagonist), and partially inhibited by indomethacin (a cyclooxygenase inhibitor), KT 5730 (a PKA inhibitor) and apamin (APA; a small conductance calcium-activated potassium channel blocker inhibitors). In addition, HEDS caused hypotension in anaesthetized rats, an event also inhibited by atropine, but not pyrilamine (a histaminic H_1 receptor antagonist). The HEDS (0.1 to 100 µg/mL) exhibited a strong scavenging effect against all reactive species tested (•DPPH, $O_2^{\cdot-}$, ·OH and H_2O_2), and protected cultured endothelial cells against H_2O_2-induced oxidative stress, entirely via catalase-independent mechanisms. In addition, HEDS exerted a protective effect against lipidic peroxidation in rats. **Discussion:** Taken together, our results reveal that the standardized hydroalchoolic extract from *D. sellowiana* contains substances which display vasodilatory, hypotensive and antioxidant properties both *in vivo* and *in vitro*. The mechanisms responsible for the vascular and hypotensive effects of HEDS involve activation of muscarinic receptors, stimulation of the nitric oxide-guanilate cyclase pathway, and opening of calcium-activated potassium channels in rats. Moreover, HEDS presents an important scavenger activity against free radicals, which can contribute for the vasodilatatory and hypotensive effects by preservating the endogenously produced NO. Therefore, our study discloses that HEDS, and preparations obtained from *D. sellowiana,* may be usefull to improve the management of several pathological conditions related to endothelial dysfunction. **Financial Support:** CAPES, CNPq, FAPESC and FINEP.

09.061

Atividade antinociceptiva dos extratos hexânicos obtidos a partir das cascas de caule e das folhas de *Clusia nemorosa* May: evidência do mecanismo de ação. Ferro JNS[1], Silva JP[1], Barros BS[1], Agra IKR[1], Silva OBS[1], Oliveira FM[2], Conserva LM[2], Barreto E[1] [1]UFAL - Genética e Biologia Molecular, [2]IQB-UFAL

Introdução: *Clusia nemorosa* May, conhecida popularmente por "orelha-de-burro", é uma planta que pertence à família Clusiaceae e possui ampla distribuição pelo nordeste brasileiro, sendo utilizada na medicina popular para diferentes finalidades. Anteriormente, demonstramos que o extrato haxânico da casca do caule de *C. nemorosa* revelou importante atividade antinociceptiva (Ferro e col., SBFTE, p.72, 2008). Dando continuidade aos nossos estudos, no presente trabalho objetivamos investigar o possível mecanismo de ação envolvido na atividade antinociceptiva dos extratos hexânicos obtido a partir da casca do caule e das folhas de *Clusia nemorosa*. **Métodos**: Camundongos Swiss (18-22 g, n=6) de ambos os sexos foram pré-tratados por via intraperitoneal (i.p.) com salina ou extrato hexânico da casca do caule (EHC) ou folha (EHF) de *C. nemorosa* e, após 1 h, foram submetidos ao teste de contorção abdominal induzida pelo ácido acético (0,6 %). Neste teste, cada contorção foi registrada de maneira cumulativa durante 10 minutos após injeção i.p. de ácido acético. Os antagonistas naloxona (5 mg/kg), metoclopramida (1 mg/kg) e ioimbina (0,1 mg/kg) foram injetados (i.p.) 1 h antes da administração dos extratos. Os resultados foram expressos como média ± erro padrão da média e analisados estatisticamente através do Teste 't' de Student. Valores de $P < 0,05$ foram considerados significantes. Todos os experimentos foram aprovados e realizados de acordo com as normas do Comitê de Ética Institucional (Licença nº 23065.12614/2006-89). **Resultados**: As contorções abdominais observadas no grupo tratado com salina foram de 34,8 ± 1,8 contorções. O tratamento com EHC reduziu de forma dose dependente a nocicepção induzida pelo ácido acético, apresentando uma DI50 de 47 ± 3,8 mg/kg. Este efeito antinociceptivo foi revertido de modo significativo pelo pré-tratamento com metoclopramida (de 10,2 ± 1,8 para 22,0 ± 2,2 contorções), mas não por naloxona (15,0 ± 3,1 contorções) ou ioimbina (16,0 ± 4,9 contorções). Em outro grupo experimental, o tratamento com EHF também inibiu a nocicepção induzida pelo ácido acético com DI50 de 62 ± 5,6 mg/kg. Entretanto, esta antinocicepção não foi alterada pelo pré-tratamento com metoclopramida (9,7 ± 2,5 contorções) ou naloxona (17,5 ± 2,4 contorções), mas sim pelo tratamento com ioimbina (de 13,5 ± 1,0 para 28,1 ± 4,2 contorções). **Discussão**: Em conjunto, estes resultados indicam que os extratos hexânicos obtidos a partir da casca do caule e das folhas de *Clusia nemorosa* possuem ações antinociceptivas por um mecanismo que parece não estar relacionado ao sistema opióide. Além disso, estes resultados indicam que o extrato da casca do caule parece exercer efeitos antinociceptivos mediados por receptores dopaminérgicos, enquanto que a atividade antinociceptiva do extrato da folha mostra-se mediada por receptores alfa2-adrenérgicos. **Apoio Financeiro**: CNPq, CAPES e FAPEAL.

09.062

Avaliação do efeito antialérgico do extrato hexânico da casca de *Clusia nemorosa* Mey em camundongos. Farias JAC de[1], Ferro JNS[1], Silva JP[1], Barros BS[1], Agra IKR[1], Silva-Filho BF[1], Silva LAF[1], Oliveira FM[2], Conserva LM[2], Barreto E[1] [1]UFAL - Genética e Biologia Molecular, [2]IQB-UFAL

Introdução: Em estudos anteriores mostramos que o extrato hexânico da folha de *Clusia nemorosa* (Clusiaceae) foi capaz de suprimir o recrutamento de neutrófilos em modelos de inflamação aguda e crônica (Farias e col., SBFTE, p. 58, 2008). Neste trabalho avaliamos a atividade do extrato hexânico da casca (EHC) de *Clusia nemorosa* sobre o recrutamento de eosinófilos no modelo de pleurisia alérgica induzida por ovoalbumina em camundongos. **Métodos:** Camundongos Swiss machos (18-22 g, n=6) foram sensibilizados com ovoalbumina (OVA, 50 mg) e $Al(OH)_3$ (5 mg) e após 14 dias desafiados com OVA (12 µg/cav). O pré-tratamento com EHC (10 e 100 mg/kg, i.p.) ocorreu 1 h antes do desafio alérgico, sendo a celularidade avaliada no tempo de 24 h após estímulo. A expressão de genes eosinofilotáticos dos leucócitos recuperados da cavidade pleural após o desafio alérgico foi avaliada por RT-PCR em tempo real. Os valores foram expressos como média ± E.P.M. e $P<0,05$ foram significantes. Todos os experimentos foram aprovados e realizados de acordo com as normas do Comitê de Ética Institucional (Licença nº 23065.12614/2006-89). **Resultados:** No tempo de 24 h após o desafio antigênico animais alérgicos exibiram um seletivo acúmulo de eosinófilos no espaço pleural (2,21 ± 0,15 x 10^6 cél/cav) quando comparado aos controles estimulados com salina (0,01 ± 0,02 x 10^6 cél/cav). O tratamento com EHC nas doses de 10 e 100 mg/kg suprimiu de modo significativo o influxo de eosinófilos (1,07 ± 0,21 e 0,81 ± 0,12 x10^6 cél/cav, respectivamente). Leucócitos recuperados do lavado pleural de animais alérgicos estimulados após OVA exibiram um aumento relativo na expressão de IL-5 (de 0,10 ± 0,03 para 1,35 ± 0,03 IL-5/b-actina), fenômeno semelhante foi observado na expressão de CCL11 (de 0,06 ± 0,02 para 0,44 ± 0,07 CCL11/b-actina). O tratamento com EHC (100 mg/kg, i.p.) inibiu de modo significativo a expressão relativa de RNAm para IL-5 e CCL11 (0,8 ± 0,01 e 0,1 ± 0,05 RNAm/b-actina, respectivamente). **Discussão:** Os resultados demonstram que o extrato hexânico da casca de *Clusia nemorosa* possui propriedade de inibir o recrutamento de eosinófilos no modelo experimental de inflamação alérgica, fenômeno que parece ser relacionado com a supressão de mediadores eosinofilitáticos. Estudos estão em andamento para esclarecer os princípios ativos responsáveis pela atividade estudada. **Apoio Financeiro:** CNPq, CAPES e FAPEAL.

09.063

Propriedade antialérgica do extrato aquoso da casca de *Bowdichia virgilioides* (Fabaceae). Agra IKR[1], Silva JP[1], Barros BS[2], Silva OBS[2], Carvalho VF[5], Filho BFS[1], Silva LAF[1], Frutuoso V[3], Barreto E[1] [1]UFAL - Genética e Biologia Celular, [2]UFAL - Biologia Celular e Molecular, [3]FIOCRUZ - Fisiologia e Farmacodinâmica

Introdução: Em estudos anteriores demonstramos que o extrato aquoso da casca de *B. virgilioides* (EABv) mostrou-se capaz de suprimir o recrutamento leucocitário *in vivo* (Silva e col., 40º SBFTE, 2008, p. 57). Neste trabalho objetivamos investigar a atividade antialérgica do EABv em camundongos ativamente sensibilizados. **Métodos:** Camundongos Swiss machos (18-22 g, n=6) foram ativamente sensibilizados com ovoalbumina (OVA, 50 mg) e $Al(OH)_3$ (5 mg) e após 14 dias desafiados com OVA (12 µg/cav). O pré-tratamento com EABv (10, 50 e 100 mg/kg, i.p.) ocorreu 1 h antes do desafio alérgico. Nos tempos de 1 e 24 h após estímulo alérgico foram avaliados a exsudação protéica e o recrutamento leucocitário, respectivamente. A expressão de genes eosinofilotáticos dos leucócitos recuperados da cavidade pleural após o desafio alérgico foi avaliada por RT-PCR em tempo real. Para avaliar o efeito do EABv sobre a reatividade dos mastócitos, fragmentos de tecido subcutâneo obtido de animais ativamente sensibilizados foram estimulados *in vitro* com OVA (50 mg/ml). Os resultados foram expressos como média ± E.P.M. foram $P<0,05$ significantes. Todos os experimentos foram aprovados e realizados de acordo com as normas do Comitê de Ética Institucional (Licença nº 23065.12614/2006-89). **Resultados:** No tempo de 1 h após o estímulo antigênico, animais alérgicos exibiram um marcado acúmulo de proteína para a cavidade pleural (de 1,78 ± 0,1 para 10,5 ± 0,7 mg de proteínas/cav), fenômeno que foi inibido de maneira dose-dependente por EABv. Animais alérgicos exibiram no tempo de 24 h após estímulo um significativo acúmulo de eosinófilos no espaço pleural (2,86 ± 0,6 x 10^5 cél/cav) quando comparado aos controles estimulados com salina (0,2 ± 0,01 x 10^5 cél/cav). O tratamento com EABv suprimiu de modo significativo o influxo de eosinófilos apenas nas doses de 50 e 100 mg/kg (0,78 ± 0,4 e 0,27 ± 0,1 x10^5 cél/cav, respectivamente). O desafio *in vitro* do tecido subcutâneo de animais alérgicos foi capaz de induzir a liberação significativa de histamina quando comparado ao controle (de 117,9 ± 6,3 para 262, 8 ± 27,3 histamina (ng)/tecido), fenômeno que foi inibido após pré-incubação com 10 ou 50 mg/ml de EABv (202, 1 ± 13,9 para 112,1 ± 3,0 histamina (ng)/tecido, respectivamente). Leucócitos recuperados do lavado pleural de animais alérgicos estimulados após OVA exibiram um aumento na expressão de IL-5 (de 0,04 ± 0,001 para 1,35 ± 0,01 IL-5/b-actina), fenômeno semelhante foi observado na expressão de CCL11 (de 0,06 ± 0,001 para 0,87 ± 0,1 CCL11/b-actina). O tratamento com EABv (50 mg/kg) inibiu de modo significativo a expressão de RNAm para IL-5 e CCL11 (0,8 ± 0,1 e 0,4 ± 0,01 RNAm/b-actina, respectivamente). **Discussão:** Em conjunto, estes resultados demonstram que *B. virgilioides* possui substâncias com propriedades de inibir parâmetros da resposta alérgica, tais como extravasamento plasmático, desgranulação de mastócito e recrutamento de eosinófilos. Além disso, o extrato aquoso da casca de *B. virgilioides* apresenta uma potente atividade anti-eosinofílica, aparecendo como uma alternativa para terapia antialérgica. Estes dados podem auxiliar na validação do uso popular desta planta. Estudos estão em andamento para esclarecer os princípios ativos responsáveis pela atividade estudada. **Apoio Financeiro:** CNPq, CAPES e FAPEAL.

09.064

Therapeutic potential of *Melissa officinalis* in the treatment of anxiety- and depression-like behaviors in rats: comparative effects with anxiolytic and antidepressive drugs. Oliveira LAB[1], Oliveira J[1], Pereira LR[1], Rodrigues KF[1], Souza FHA[1], Pires FR[1], Carneiro FP[1], Sousa JB[1], Lucena GMRS[2], Ferreira VMM[2] [1]UnB - Medicina, [2]UnB - Ciências da Saúde/Ciências Farmacêuticas

Introduction: *Melissa officinalis* (MO), popularly known as lemon balm, has been used predominantly in brain-related disorders, having sedative, hypnotic, analgesic, neuroleptic and mnemonic properties. Notably, different presentations of this herb are reported to have a lower excitability, stress and anxiety levels in rodent and human models. Therefore, the present study analyzed the behavioral effects of MO ethanolic extract in the behavioral deficits of sepsis surviving rats. **Methods**: Female Wistar rats (n=8 animals/group) were obtained from the Animal Facility, in accordance with the recommendations of ethical committee for animal care (UnB doc 33887/2009). All experiments were carried out at the Pathology Laboratory/Faculty of Medicine. The animals were anesthetized i.p., using a mixture of ketamine (80 mg/kg) and xylazine (10 mg/kg), to allow exposure of the cecum, which was squeezed to extrude a small amount of feces from the perforation site, which was later placed back into the peritoneal cavity. All animals were returned to their cages after administration of ceftriaxone (30 mg/kg) + clindamicine (25 mg/kg). MO ethanolic extract (30 or 100 mg/kg) was administered by gavage, for one week after sepsis induction. On the last day, one hour after MO administration, the animals were submitted to the open field (OF), elevated plus-maze (EPM) and forced swimming (FS) tests. **Results**: ANOVA, followed by Tukey's test, showed that the locomotion was not significantly altered by treatments in the EPM and OF tests. In the EPM test, the percentage of open arm time (MO 30 mg/kg; 38.63±4.08; MO 100 mg/kg: 29.92±4.68) of rats that received subchronic MO extract were significantly higher than sham-operated animals treated with vehicle (3.28±1.12), and the response levels were similar to those of the diazepam group (22.90±4.89). **Discussion**: Several active components, present in dried leaves or in the essential oil obtained from lemon balm are thought to carry the psychotherapeutic potential of MO, including monoterpenoid aldehydes, flavonoids, polyphenolic compounds (rosmarinic acid) and monoterpene glycosides. Regardless of which active component or mechanisms of action were responsible for the effects, the investigated extract possesses similar anxiolytic-like properties of the MO, comparable to that of diazepam. These psychoactive properties, along with the safety profile of the lemon balm's may provide a pharmacological alternative for specific psychiatric disorders. **Acknowledgements:** Pathology Laboratory, Faculty of Medicine/UnB, for financial and technical support.

09.065

Short and long-term memory following subchronic administration of *Melissa officinalis* extract in sepsis-surviving rats. Amorim Campos V[1], Lôbo RE[1], Volpe Jr JF[1], Rodrigues ED[1], Neves NT[1], Lessa LA[1], Carneiro FP[1], Sousa JB[1], Lucena GMRS[2], Ferreira VMM[2] - [1]UNB - Medicina, [2]UnB - Ciências da Saúde/Ciências Farmacêuticas

Introduction: It has been suggested, on the basis of a number of herbs in the improvement of memory, that *Melissa officinalis* might provide natural treatment, given some protection against the putative aetiological free radical damage in cognitive impairment. Despite its long history as a putative memory enhancer, it was considered important to investigate the cognitive effects of *Melissa officinalis* ethanolic extract in sepsis-surviving rats. **Methods**: Female Wistar rats (n=8 animals/group) were obtained from the Animal Facility, in accordance with the recommendations of ethical committee for animal care (UnB doc 33887/2009). All experiments were carried out at the Pathology Laboratory/Faculty of Medicine. The animals were anesthetized i.p., using a mixture of ketamine (80 mg/kg) and xylazine (10 mg/kg), to allow exposure of the cecum, which was squeezed to extrude a small amount of feces from the perforation site, which was later placed back into the peritoneal cavity. All animals were returned to their cages after administration of ceftriaxone (30 mg/kg) + clindamicine (25 mg/kg). *Melissa officinalis* ethanolic extract (30 or 100 mg/kg) was administered by gavage, for one week after sepsis induction. On the last day, one hour after *Melissa officinalis* administration, the animals were submitted to the step-down inhibitory avoidance test. **Results**: Kruskal-Wallis test revealed a significant effect of the treatment with *Melissa officinalis* extract (100 mg/kg) in the animal's latencies during the short- ($H(4, N=40)=29.4728$; $p=0.0001$) and long-term memory ($H(3, N=40)=29.4768$; $p=0.0001$) of the retention test session. The Mann-Whitney test indicated that the sepsis group, significantly decreased the animal's latencies during the short- ($p<0.05$) and long-term memory ($p<0.05$), performed 1.5 h or 24 after the training session, respectively), when compared to the sham-operated animals. **Discussion**: Several active components, present in dried leaves or in the essential oil obtained from lemon balm are thought to carry the psychotherapeutic potential of *Melissa officinalis*, including monoterpenoid aldehydes, flavonoids, polyphenolic compounds (rosmarinic acid) and monoterpene glycosides. Regardless of which active component or mechanisms of action were responsible for improvement of memory in sepsis-surviving rats, these issues need further investigations. **Acknowledgements:** Pathology Laboratory, Faculty of Medicine/UnB, for financial and technical support.

09.066

Ensaio *in vivo* para atividade anticâncer de frações ativas de *Psidium guaja*va L. sobre o Tumor Sólido de Ehrlich. Rizzo LY[1], Longato GB[2], Seno FZ[1], Tinti SV[1], Ruiz ALT G.[1], Foglio M[3], Carvalho JE[1] [1]CPQBA-UNICAMP - Farmacologia e Toxicologia, [2]IB-UNICAMP, [3]CPQBA-UNICAMP - Fitoquímica

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 chemotherapics that are currently used in cancer treatment. This project evaluates the *in vivo* anticancer activity of active fractions from *Psidium guajava* L., a tropical tree popularly known as guava and traditionally used for its antiparasitic activity. The dry plant material (leaves) was submitted to the process of hot extraction with dichloromethane and ethanol (95%), leading to the obtention of crude extracts. The most active extract went through the 1st fractioning process through the filtrating column process. The 2nd fractioning process was held with the most active fraction, through the classic column method, and all the fractions obtained in this process were evaluated *in vitro* for its potential anticancer activity. *In vitro* essays were held in nine human cancer lines, donated by *National Cancer Institute*, EUA: K562 (leukemia), MCF-7 (breast), NCI/ADR-RES (breast cancer resistant to multiple drugs), NCI-H460 (lung), UACC62 (melanoma), PC-3 (prostate), HT-29 (colon), OVCAR-03 (ovary) and 786-0 (kidney). The most active fraction was evaluated *in vivo* against the Solid Ehrlich Tumor, in Balb/C female mice. Saline was used as a negative control, doxorubicin as a positive control, and three doses of the active fraction (10, 30 and 50 mg/kg) were used as treatments, with 7 mice per group. Ehrlich cells were inoculated in the right posterior leg, in D0, at the inoculation rate of 2,5 x 10^6cells per animal. Animals were treated every 3 days (D3, D6, D9, D12, D15, D18) and were sacrificed at D21. The volume of the tumor leg and the weight of each animal was verified each day. By the end of the experiment, the organs (tumor leg, brain, uterus, stomach, kidneys, spleen, liver, lung and heart) were weighted and collected. Graphics were done with the following analysis: animals weight variation, tumor volume variation, relative tumor weight and relative organs weight, for each group individually, and statistics were done through the Duncan´s test ($p<0,05$). The tested active fraction from *P. Guajava* L. presented significant anticancer activity in all evaluated doses (10, 30 and 50 mg/kg), showing an interesting potential to reduce the Solid Ehrlich Tumor. The organs weight showed an interesting pattern, mainly for the uterus, suggesting a possible hormone dependent relation, but further studies are necessary to confirm this hypothesis. Histopathology tests are currently under development with the collected organs to see if any significant changes were observed in this *in vivo* essay. This experiment was approved under the protocol number 1810-1, by the CEEA/Unicamp and is part of a master project supported by Fapesp and Cnpq..

09.067

Habilidade da suramina em antagonizar as atividades citotóxicas da melitina. El-Kik CZ, Fernandes FFA, Fonseca TF, Gaban GA, Borges PA, Martins V, Melo PA UFRJ - Farmacologia Básica e Clínica

O veneno de *Apis mellifera* é uma mistura de compostos como proteínas, peptídeos e moléculas orgânicas de baixo peso molecular. O efeito tóxico do veneno é atribuído principalmente à presença de melitina que corresponde a 40-60% do peso do veneno. A melitina tem atividade citotóxica, induz hemólise, cardiotoxicidade e miotoxicidade. Sua propriedade de diminuir a tensão superficial da membrana plasmática lhe confere uma potente ação destrutiva sobre as membranas biológicas.Avaliamos a habilidade da suramina, um inibidor do veneno bruto de A. mellifera , em antagonizar as atividades da melitina. Camundongos suíços adultos (20-25 g) foram utilizados nos experimentos. O extravasamento de plasma foi avaliado utilizando um marcador visual, o azul de Evans. A injeção intradérmica da melitina (0,5 µg/g) induziu intenso extravasamento de plasma na região injetada (630.5±30) e foi comparada com animais controle, que receberam apenas injeção de PSS (420,55±22). O efeito da melitina foi reduzido para (533±41,32) quando pré-incubado om 30 µg/g de suramina. O edema de coxa induzido por 0,3 µg/g de melitina foi inibido quando pré incubado com suramina em doses crescentes (1 - 30 µg/g) em 31% e 36% respectivamente. Para avaliação da miotoxicidade *in vivo*, animais receberam injeção i.m. de melitina (0,3 µg/g) sozinha ou pré-incubada com suramina (1, 10 e 30 µg/g). Após 2 horas foram retiradas amostras de sangue para análise do CK plasmático. A suramina (30 µg/g) foi capaz de inibir cerca de 50% o efeito miotóxico da melitina. Sendo a suramina uma molécula polianiôntica, seus efeitos podem estar relacionados à interação de suas cargas com os policátions presentes na molécula de melitina, impedindo que haja lesão das membranas biológicas. Apoio Financeiro: CAPES, FAPERJ, CNPq -PRONEX.

09.068

Antinociceptive, anti-inflammatory and antioxidant activities of *Sideroxylum obtusifolium.*

Araujo Neto V, Bomfim RR, Oliveira VOB, Passos AMPR, Oliveira JPR, Camargo E, Estevam CS, Thomazzi SM UFS Fisiologia

Introduction: *Sideroxylum obtusifolium* Roem & Schult. (Sapotaceae) is a plant with antinociceptive and anti-inflammatory activities used in folk medicine. In Northeastern Brazil it is known as "quixabeira". In order to evaluate the actions of this plant, studies were performed on antinociceptive, anti-inflammatory, and antioxidant activities. **Methods:** The hydroalcoholic extract (HE) of *S. obtusifolium* inner bark was used in the following experiments. *S. obtusifolium* inner bark was collected in August 2006, in the Poço Redondo county, Sergipe State (09º80'S, 37º68'W). The plant was identified by Prof. Carlos Dias da Silva Júnior (Federal University of Sergipe) with voucher number ASE 8717. The dried inner bark of *S. obtusifolium* (2.8Kg) were powdered, extracted by maceration at room temperature with 90% ethanol for 5 days (459,6g). Swiss mice (20-30g) and Wistar rats (120-180g) of both sexes were obtained from the Central Animal House of the Federal University of Sergipe and complied with the guidelines on animal care of the Ethics Committee for Animal Use in Research (CEPA/UFS 47/07). The animals were pre-treated with *S. obtusifolium* HE (100, 200, or 400 mg/kg) orally 60 min before of stimulation (n = 6/group). The abdominal writhes were observed for a period of 20 min and in formalin test the time that the animal spent licking or biting its paw was measured during the first phase (0-5 min) and the second phase (20-25 min) of the test. The time elapsed until the appearance of reactions to the thermal stimulus, such as lifting or licking of the paws was recorded as an index of nociception and measurements were performed at time 0, 30, 60, 90, and 120 min after the first thermal stimulus. The anti-inflammatory activity was studied using the paw edema model induced by 1% carrageenan and the volume of the paw was at the time 0 and the intervals of 1, 2, 3, and 4h. The leukocyte migration was induced by injection of carrageenan (500mg/cavity, 500mL, i.p.) into the peritoneal cavity of rats and 4h after carrageenan injection the total cells were counted. The quantitative analysis of antioxidant activity was based on the scavenging of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical by monitoring the decrease in absorbance at 515nm. **Results:** Oral treatment with the HE of *S. obtusifolium* elicited inhibitory activity ($p<0.01$) on acetic acid-induced abdominal writhes at 200 (49.7%) and 400 mg/kg (61.3%), and reduced the formalin-induce nociception on the inflammatory-phase (100, 200, and 400 mg/kg, $p<0.001$), however it did not elicit any inhibitory effect on hot-plate test. The HE reduced the carrageenan-induced edema formation and inhibited the neutrophil migration into the peritoneal cavity at 100, 200, and 400 mg/kg ($p<0.001$). The HE of *S. obtusifolium* react with the DPPH radical and reduce the same by 90.79%, and exhibited IC_{50} value of 25.39 ± 0.78 µg/mL. **Discussion:** The HE of *S. obtusifolium* shows antinociceptive, anti-inflammatory, and antioxidant activities. The identification and isolation of bioactive components are in progress, which could elucidate the properties of *S. obtusifolium*. **Support:** PIBIC/CNPq/FAPITEC/UFS, Universal/CNPq, PROCAD/NF/CAPES and PAIRD/UFS.

09.069

Pharmacological properties of *Lippia gracilis*. Mendes SS[1], Bomfim RR[1], Alves PB[2], Blank AF[3], Estevam CS[1], Antoniolli AR[1], Thomazzi SM[1] [1]UFS - Fisiologia, [2]UFS - Química, [3]UFS - Engenharia Agronômica

Introduction: *Lippia gracilis* Schauer (Verbenaceae) is a plant with antinociceptive, anti-inflammatory, and antimicrobial properties used in folk medicine. In Northeastern Brazil it is known as “alecrim do campo”. In order to evaluate the actions of this plant, studies were performed on antinociceptive, anti-inflammatory, and antioxidant activities. **Methods:** The essential oil (EO) of *L. gracilis* leaves was used in the following experiments. *L. gracilis* was collected in August 2004, in the Tomar de Geru county, Sergipe State (11º19’S, 37º55’W). A voucher specimen was deposited in the Herbarium of the Federal University of Sergipe under number ASE 9205. The EO of *L. gracilis* was obtained from the dried leaves by hydrodistillation and analyzed by GC/MS. Swiss mice (20-30g) and Wistar rats (120-180g) of both sexes were obtained from the Central Biotery of the Federal University of Sergipe and complied with the guidelines on animal care of the Ethics Committee for Animal Use in Research (CEPA/UFS 11/08). The animals were pre-treated with *L. gracilis* EO (50, 100, or 200 mg/kg) orally 60 min before of stimulation (n = 6/group). The abdominal writhes were observed for a period of 20 min and in formalin test the time that the animal spent licking or biting its paw was measured during the first phase (0-5 min) and the second phase (20-25 min) of the test. The anti-inflammatory activity was studied using the paw edema model induced by 1% carrageenan and the volume of the paw was at the time 0 and the intervals of 1, 2, 3, and 4h. The leukocyte migration was induced by injection of carrageenan (1%, 250mL, i.p.) into the peritoneal cavity of mice and 4h after carrageenan injection the total cells were counted. The quantitative analysis of antioxidant activity was based on the scavenging of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical by monitoring the decrease in absorbance at 515nm. **Results:** Oral treatment with the EO of *L. gracilis* elicited inhibitory activity ($p<0.01$) on acetic acid-induced abdominal writhes at 100 (44.1%) and 200 mg/kg (34.5%), and reduced the formalin-induce nociception on the inflammatory-phase (50, 100, and 200 mg/kg, $p<0.001$). The EO reduced the carrageenan-induced edema formation and inhibited the neutrophil migration into the peritoneal cavity at 100 and 200 mg/kg ($p<0.05$). The OE of *L. gracilis* react with the DPPH radical and reduce the same by 32.08%, and exhibited IC_{50} value of 93.78 ± 5.32 µg/mL. **Discussion:** The OE of *L. gracilis* shows antinociceptive and anti-inflammatory activities. The identification and isolation of bioactive components are in progress, which could elucidate the properties of *L. gracilis*. **Support:** RENORBIO/CNPq and Universal/FAPITEC.

09.070

Blockade of calcium channels is involved in the relaxing action of monoterpene epoxy-limonene in guinea pig ileum. Santos SS, Andrade LN, Sousa DP, Batista JS UFS - Fisiologia

Introduction: We have shown that the monoterpene epoxy-limonene presented more potent spasmolitic action than its chemistry analog rotundifolone in guinea-pig ileum (Sousa et al, Z. Naturforsch., 63c, p. 808, 2008). Since the action mechanism of epoxy-limonene is not yet known, the present study aimed to characterize the mechanism of the relaxant activity of this terpene. METHODS: The voltage-dependent calcium and potassium channels participation in the relaxing response of epoxy-limonene was investigated in guinea pig isolated ileum. Concentration-response curves to calcium were obtained in the absence and presence of verapamil (0.01mM) and epoxy-limonene at the concentrations of 0.1mM, 0.57mM (EC_{50} value obtained by Sousa et al, 2008) and 1mM. The involvement of potassium channels was investigated by comparing the relaxing activity of epoxy-limonene (0.1 mM) in bethanecol pre-contracted ileum in the absence and presence of 1mM tetraetylammonium (TEA). In both protocols, the ileum was mounted in isolated organ bath containing Tyrode nutritive solution. At the calcium protocol, a free calcium Tyrode solution was used. The experimental protocols were approved by the Ethical Committee of this institution under number 19/08. **Results and Discussion**: the calcium-induced contraction was fully blocked by verapamil. On the other hand, 0.1 mM epoxy-limonene did not produce significant shift on calcium curve. The calcium EC_{50} values were 1.1mM (IC 95% = 0.6-2.1 mM) in the absence and 1.2mM (IC 95% = 0.5-3.0mM) in the presence of epoxy-limonene. However, at the concentrations of 0.57 and 1mM, epoxy-limonene produced right shift on calcium curve with reduction in the maximal response, suggesting the involvement of the blockade of voltage-regulated calcium channel. The pre-treatment with TEA did not inhibit the relaxation produced by 1mM epoxy-limonene, indicating that potassium channels activation is not involved in the relaxant action of epoxy-limonene. **Conclusion**: the results obtained suggest that the relaxant action of epoxy-limonene in guinea pig ileum involves voltage-dependent calcium channels blockade, but not involve potassium channels activation. **Financial Support**: FAPITEC/SE, UFS.

09.071

Inhibition of enzymatic and biological activities induced by *Bothrops* venom by triacontil *P*-coumarate isolated from *Bombacopsis glabra* vegetal extract. Mendes MM[1], Paula VF[2], Correia SJ[2], Moreira BO[2], Gomes MSR[2], Izidoro L F M[1], Vieira SAPB[1], Homsi-Brandeburgo M I[1], Hamaguchi[1], Rodrigues V M[1] [1]UFU - Genética e Bioquímica, [2]UESB - Química

Introduction: The use of plant extracts as antidote against animals venoms is an old practice, mainly envenomations for snakes, besides also may used as supplemental of serum therapy. Snake venoms are a complex mixture of proteins involved in a series of events that depends on the synergic action of these molecules. The present study explores the ability of an active compound isolated from bark of *Bombacopsis glabra* to inhibit the harmful effects of *Bothrops pauloensis* snake venom. **Methods:** The root of *B. glabra* was collected and dried. The material was triturated and then submitted to successive cold extractions with hexane. The distillation of the solvents was accomplished in rotating evaporator. The material was fractioned in silica eluted witch hexane:AcOEt (9:1). Triacontil *P*-Coumarate (PCT) isolated from *B. glabra* vegetal extract was previously incubated with *B. pauloensis* venom in the ratios 1:1 and 1:5 (w/w; venom/PCT) for 30 min at 37°C before the tests. Coagulant activity of *B. pauloensis* venom on bovine plasma and the time to clot the plasma solutions was recorded (in seconds). PLA_2 activity was determined using an indirect hemolytic assay in a gel plate containing egg yolk, $CaCl_2$ and agar. For inhibition of the hemorrhagic activity 2 Minimum Hemorrhagic Doses was injected intradermally in the dorsal region of Swiss mice. After 150 minutes the animals were anesthetized and sacrificed and the hemorrhagic area of halos in the skins was exanimate and measured. The experimental protocol was approved by the Committee of Ethics for the Use of Animals of Federal University of Uberlândia (Minas Gerais-Brazil) protocol number 027/08. **Results and Discussion:** The PCT was able to neutralize around 45% of coagulant activity induced by *B. pauloensis* venom in the ratio of 1:5 (w/w; venom/PCT). In the phospolipasic and hemorrhagic activities the PCT induce the inhibition of 16.14%, 16.7% and 50.73%, 76.35% in the ratios 1:1 and 1:5 (w/w; venom/PCT), respectively. Our results show that PCT is a compound able to antagonize the some activities induced by venom. In this way, PCT may provide new complementary alternative to treatments for ophidian envenomations. **Financial support:** CAPES, UFU and CNPq

09.072

Analysis of the relationship between concentrations of lead in blood and in serum of pregnant women and in umbilical cord. Amaral JH[1], De Rezende V[2], Barbosa Jr F[3], Quintana S[4], Gerlach RF[5], Tanus-Santos JE[1] [1]FMRP-USP - Farmacologia, [2]UNICAMP - Farmacologia, [3]FCFRP-USP - Análises Clínicas, Toxicológicas e Bromatológicas, [4]FMRP-USP - Ginecologia e Obstetrícia, [5]FORP-USP - Morfologia

Pregnancy may have great effects on lead (Pb) toxicokinetics and thus affect Pb toxicity. This is particularly important for pregnant women and their fetus because pregnancy is associated with intense bone remodeling, thus leading to increased Pb mobilization from bone tissues. Indeed, pregnancy has been associated with increased serum Pb concentrations (Pb-S), which is the Pb fraction that is able to cross biological barriers such as the placenta. In this regard, maternal Pb-S may be more relevant to fetal Pb exposure than maternal whole blood lead (Pb-B). However, there is no information on how maternal Pb-S and Pb-B relate with fetal Pb-S and Pb-B. The objective of this work was to examine whether there is any relationship between %Pb-S/Pb-B ratio in maternal samples and the Pb concentrations in their respective umbilical cord. We studied 120 blood samples drawn from pregnant women and 120 blood samples drawn from their respective umbilical cord. Pb concentrations were measured by inductively coupled plasma mass spectrometry (ICP-MS). We found statistical differences between the medians of Pb-B and Pb-S in pregnant women and umbilical cords, but not in %Pb-S/Pb-B. The values were respectively ($p<0.0001$; $p=0.0041$ and $p=0.2756$). Positive correlations were found when the same parameters were analyzed among pregnant women and umbilical cords, and they were respectively (Pb-B, $r=0.5714$; Pb-S, $r=0.3902$; %Pb-S/Pb-B, $r=0.3767$). These results show that maternal %Pb-S/Pb-B ratio is very similar to %Pb-S/Pb-B ratio of the umbilical cords, helping to ensure that the intervention measures can be taken as soon as possible if it is understood, for example, as an increase intake of calcium for pregnant and monitoring neurological development of newborns. Ethics committee: 10314/2004. Gulson, B.L., Pregnancy increases mobilization of lead from maternal skeleton. *J. Lab. Clin. Med*., 130(1): p. 51. 1997. Smith, D., The relationship between lead in plasma and whole blood in women. *Environ. Health Perspect*., 110(3): p. 263. 2002. Barbosa, F. Jr., A critical review of biomarkers used for monitoring human exposure to lead: advantages, limitations, and future needs. *Environ. Health Perspect*., 113(12): p. 1669. 2005. Bergdahl, I.A., Plasma-lead concentration: investigations into its usefulness for biological monitoring of occupational lead exposure. *Am. J. Ind. Med*., 49(2):93. 2006. Montenegro, M. F., Assessment of how pregnancy modifies plasma lead and plasma/whole blood lead ratio in ALAD 1-1 genotype women. *Basic Clin. Pharmacol Toxicol*., 102(4):347. 2008. **Financial Support:** CNPq, CAPES, FAPESP

09.073

Anticancer activity of dichloromethanic extract and fractions from *Piper regnellii* leaves. Longato GB[1], Tinti SV[2], Ruiz ALTG[2], Foglio M[3], Carvalho JE[2] [1]IB- UNICAMP Biologia Celular e Estrutural, [2]CPQBA-UNICAMP Farmacologia e Toxicologia, [3]CPQBA-UNICAMP Fitoquímica

Introduction: For many centuries, plants have provided a rich source of therapeutic agents and bases for synthetic drugs. Despite the great development of organic synthesis, about 25% of prescribed drugs are still derived from plant sources[a]. *Piper* genus has high commercial and medicinal importance[b]. In folk medicine, *P. regnellii* (Miq.) C.DC. var *regnellii* is used for parasitic and infectious diseases treatment[c,d]. This work aimed the evaluation of *in vitro* and *in vivo* anticancer activity of crude extract and fractions obtained from *P. regnellii* leaves. **Methods:** *P. regnellii* dried leaves were successively extracted with hexane and dichloromethane, resulting in dichloromethanic crude extract (DCE). DCE was fractioned by column chromatography, eluted with hexane/dichloromethane, providing 11 fractions (F). *In vitro* activity of DCE and its fractions (0,25 to 250 µg/mL) were evaluated in 9 human tumor cell lines: breast (MCF-7), lung (NCI-H460), melanoma (UACC-62), prostate (PC-3), kidney (786-0), colon (HT-29), ovarian (OVCAR-03), ovarian expressing multiple drugs resistance phenotype (NCI/ADR-RES) and leukemia (K562). Anticancer activity *in vitro* was determined by total growth inhibition (TGI)[e]. *In vivo* activity was evaluated by Ehrlich cancer cells ($2x10^6$ cell/50 µL) implanted on mice right footpad (BalbC, male). Animals were treated with EBD every 72h (100, 300 and 1000 mg/kg, ip, n=10). Positive control was doxorubicin chloridrate (3 mg/kg, ip, n=10). Before each treatment footpad volume was measured by a plethysmometer. At 13^{th} day, all animals were sacrificed and both footpad were collected and weighted. This experiment is in agreement with CEEA/Unicamp (protocol 1466-1). Data were analyzed for statistical significance by ANOVA followed by Tukey's test ($p < 0.05$). **Results:** DCE was active for prostate, ovarian, lung, kidney and melanoma (TGI = 10.97, 12.05, 20.39, 21.93 and 26.45 µg/mL, respectively). Compounds responsible for DCE activity were found in fractions F6, F7, F8 and F10 for prostate; in F7 and F8 for ovarian; in F6 for lung; in F6 and F7 for kidney and in F6, F8, F9 and F10 for melanoma. F6 was also active for breast and colon, suggesting that DCE minority molecules are responsible for these activities. Anticancer activity *in vivo* was determined by tumor growth inhibition, measured by tumor weight (mg): 49% for doxorubicin (372 ± 26; saline: 724 ± 34), 40% for 100 (431 ± 41), 69% for 300 (232 ± 40) and 66% for 1000 mg/kg (245 ± 30). At higher doses, EBD showed toxic effects with deaths and reduction of body weight in 5%, while doxorubicin reduced in 12%. **Discussion:** Anticancer activity of *P. regnellii* extract and fractions observed in these experimental models suggests the participation of different compounds with distinct action mechanisms. These results are very consistent with literature[b], demonstrating the huge potential of Piperaceae family as a new drug source. Further investigations are in progress to identify active principles and action mechanisms involved in this antitumor activity. a. Rates SMK. [a]*Toxicon* 39: 603, 2001; [b]Parmar VS. *Phytoch* 46: 597, 1997; [c]Felipe DF. *La Am J Pharm* 27: 618, 2008; [d]Luize PS. *Biol Pharm Bull* 10: 2126, 2006; [e]Shoemaker RH. *Nat Rev Cancer* 6: 813, 2006; Financial Support: Fapesp, Capes and CNPq.

09.074

Molecular cloning of a truncated hyaluronidase from *Bothrops pauloensis* venom gland. Castanheira LE[1], Amaral LO[1], Rodrigues RS[1], França JB[2], Cardoso TM[1], Fonseca FPP[3], Otaviano AR[1], Silva FH[3], Hamaguchi[1], Homsi-Brandeburgo M I[1], Rodrigues V M[1] [1]UFU - Genética e Bioquímica, [2]FCFRP-USP - Análises Clínicas Toxicológicas e Bromatológicas, [3]UFSCar - Genética e Evolução

Intoduction: Ophidic accidents represent a serious health problem in tropical countries, where *Bothrops* genus is responsible for the highest indexes of bites. This kind of poisoning is characterized by local effects, although some systemic effects may occur due to the action of enzymes which degrade the extracellular matrix of the victims, like hyaluronidase, in order to spread the main toxins of the venom. This work had as aim identify and analyze the sequence of a truncated hyaluronidase from *Bothrops pauloensis* venom gland transcriptome. **Methods:** The sequence corresponding to truncated hyaluronidase was obtained from a transcritopme of *Bothrops pauloensis* venom gland, using the universal M13F. It was analyzed all amino acid higly conserved by the alignment with two hyaluronidases-like from *Echis carinatus* and *Bitis* arietans, focusing in those probably involved in catalytic activity. We also analyzed probable glycosylation sites by the software NetNGlyc. **Results:** The truncated sequence codifies 157 amino acids for the mature protein, called Bp-Hyase, with a predicted pI of 9,6. Bp-Hyase was aligned with *Bitis arietans* e *Echis carinatus*, resulting in 124 conserved amino acids which include Asp/Glu83, Asp 127, Glu/Asp137, maybe related to the action of hyaluronidase with the breakdown of its substrate. Finally, Asn151, Asn152 and Ser153 represent a probable site of glycosylation. **Discussion:** Asp/Glu83, Asp 127 and Glu/Asp137 represent acid amino acids important for the catalysis of hyaluronate, the subtrate of hyaluronidase, which are highly conserved in snake hyaluronidase. In addition, Asn151, Asn152 and Ser153 represent a point of glycosylation supposed to be necessary for regulation of protein conformation and the stabilization of intramolecular folding, retaining enzymatic activity. This is the first description of a hyaluronidase sequence from brazilian snake venoms. The analysis of highly conserved amino acids shows the catalytic action of hyaluronidase, what is related to the its importance for bothropic poisoning, since it degrades the extracellular matrix and further the diffusion of the main toxins of the venom. **Financial Support:** CNPq, FAPEMIG, UFU.

09.075

Cardiovascular effects induced by EHRA in normotensive and pulmonary hypertensive rats. Gomes MAS[1], Magalhães DMS[1], Araujo IGA[1], Alustau MC[1], De Assis, KS[1], Oliveira Junior FA[2], Cavalcante KVM[1], Barbosa Filho JM[1], Dias KLG[1], Medeiros IA[1], Correia NA[2]
[1]LTF-UFPB, [2]UFPB - Fisiologia e Patologia

Introduction: *Ruellia asperula* is a plant belonging to *Acanthaceae* family popularly known as candeia. It is used in the folk-medicine to treat bronchitis, uterine inflammation and flu. The aim of this study was to evaluate the effect of the hydroalchoolic extract of *Ruellia asperula* (EHRA) on Arterial Pressure (AP) and Heart Rate (HR) in normotensive and pulmonary hypertensive rats. **Methods:** Male Wistar rats weighing 200 to 300 g were used in this study. All protocols used in this study were approved by the CEPA/TF (protocol nº 0207/08). The animals had polyethylene catheters implanted into the abdominal aorta and inferior vena cava to data recordings and administration of drugs, respectively. Experiments were performed 24 hours after the surgery. In model of pulmonary hypertension rats were randomly given a subcutaneous injection of either, 60 mg/kg monocrotaline (MCT), or 0.9% saline and assigned to receive oral administration of 0.9% saline or EHRA (100 mg/kg, *per os*). Thus the animals were divided into three groups: Saline group (n=06), MCT group (n=12), MCT group treated with oral EHRA (EHRA group, n=04). Hemodynamic measurements were performed 4 weeks after MCT injection. **Results and discussion:** In normotensive rats, EHRA (1, 5, 10, 20 and 40 mg/kg , i.v., randomly) injections produced hypotension (% PAM = -4.40 ± 1.14; -11.3 ± 0.96; -13.6 ±1.54; -15.7 ± 1.6 and -16.8 ± 1.6 , respectively) accompanied by a tachycardic responses (% HR = 2,5 ± 1,3; 11.0 ± 1.8; 13.6 ± 1.4; 19.2 ± 2.0 and 17.1 ± 1.3, respectively). EHRA treatment (100 mg/kg, oral administration two times a day, during 28 days) completely inhibited the installation of the pulmonary hypertension in the MCT model. In addition, EHRA did not alter the pulmonary arterial pressure in normotensive rats after acute intravenously administration. In conclusion**,** taken together, these results suggest that EHRA induce in normotensive rats hypotension and tachycardic effects and was able to inhibit the installation of pulmonary hypertension, in the MCT model. Financial Support: CNPq, CAPES, LTF.

09.076

Avaliação da citotoxicidade extratos brutos e frações de *Alpinia zerumbet* (PERS.) B. L. Burtt. & R. M. SM. Corrêa AJC[1], Lima CE[2], Costa MCCD[1], Aguiar JS[3], Nascimento SC[3], Rodrigues MD[3] [1]UNICAP - Ciências Biológicas, [2]UNICAP - Biologia, [3]UFPE - Antibióticos

Introdução: *Alpinia zerumbet* (Pers.) B. L. Burtt. & R. M. Sm. (Zingiberaceae), originaria da Ásia, é conhecida como colônia e utilizada popularmente como diurética, antihipertensiva e febrífuga. Devido ao grande uso popular da espécie, neste estudo objetivou-se avaliar a citotoxicidade do extrato metanólico do rizoma e de frações produzidas a partir dele sobre células HEp-2 e NCI-H292. **Métodos:** Para o estudo, foi coletado o rizoma de plantas cultivadas de maneira padronizada no Laboratório de Fitoterapia do Instituto de Pesquisas Agropecuárias – IPA. Foram produzidos extratos brutos acetônico e metanólico por maceração em repouso à exaustão. A partir do extrato metanólico, foram obtidas as frações hexânica, diclorometano, acetato de etila e butanólica. Cada fração, depois de evaporada, foi armazenada (7ºC), para realização dos testes de citotoxicidade. As linhagens celulares HEp-2 (derivada de tumor da laringe humana) e NCI-H292 (obtidas de carcinoma de pulmão humano) utilizadas nos testes, foram obtidas da seção de culturas de células do Instituto Adolfo Lutz (SP) e mantidas de acordo com o protocolo do Departamento de Antibióticos da UFPE. A atividade citotóxica foi avaliada segundo o protocolo do Instituto Nacional do Câncer, pelo método colorimétrico do MTT. Uma suspensão celular com 10^5células/mL foi distribuída em placas de cultura com 96 poços, que foram incubadas a 37ºC, em atmosfera úmida (5% de CO_2), durante 24h. Após este período a substância teste foi adicionada as placas (22 µL/poço), que foram reincubadas a 37ºC por 72h, quando então, foi adicionado a cada poço 25µL de MTT (5mg/mL). As placas foram mantidas por duas horas na estufa, após o quê, foi adicionado 100µL de DMSO a cada poço. A leitura óptica foi realizada em leitor automático de placas (595nm) e a CI_{50} foi determinada a partir de uma regressão linear, relacionando-se o percentual de inibição com o logaritmo das concentrações testadas ($p<0,01$). A vincristina foi usada nos testes como padrão. Extratos brutos e frações com valores de CI_{50} menor ou igual a 30µg/mL foram considerados citotóxicos e para o padrão valores de CI_{50} menor ou igual a 4µg/mL foram considerados significativos. (GERAN, Canc.Chemo.Reports. v.3, p.1, 1972; ALLEY, C. Research, v.48, p.589, 1988; PEREIRA, T. Jorn. Exp. Clin. Medc. v.19, p. 47, 1994). **Resultados e Discussão:** A fração hexânica do rizoma de A. zerumbet mostrou-se altamente citotóxica frente as células HEp-2 com CI_{50} igual a 12 µg/mL e frente as células NCI-H292 com CI_{50} igual a 21µg/mL. A fração diclorometano mostrou-se fracamente citotóxica frente a células HEp-2 com CI_{50} igual a 125µg/mL e com citotoxicidade alta frente as célula NCI-H292 com CI_{50} igual a 29µg/mL. Os resultado citotóxicos encontrados para a fração hexânica do rizoma, corroboram com os estudo de Costa et. al. que em 2007 já havia referido uma citotoxicidade fraca para o extrato hexânico bruto frente a linhagem celular NCI-H292 com CI_{50} de 59µg/mL. **Apoio Financeiro:** PIBIC/UNICAP

09.077

A new acid PLA_2 from *Bothrops pauloensis* venom gland transcriptome. Ferreira FB[1], Rodrigues RS[1], Souza DLN[2], Otaviano AR[1], Hamaguchi A[1], Homsi-Brandeburgo MI[1], Rodrigues VM[1] [1]UFU - Instituto de Genética e Bioquímica, [2]UFU - Instituto de Biologia

Introduction: The phospholipase A_2 (PLA_2, E.C. 3.1.1.4) superfamily is defined by enzymes that catalyze the hydrolysis of the *sn-2* bond of phosphoglycerides. Most PLA_2s from the venom of *Bothrops* species are basic proteins, which have been well characterized both structurally and functionally, however, little is known about acidic PLA_2s from this venom. Nevertheless, it has been demonstrated that they have high catalytic activity and show the ability to inhibit platelet aggregation. In addition, they can produce any toxic effects as myotoxicity, edema and myonecrosis. **Methods**: To further understand the function of these proteins, we have isolated by cDNA that encodes an acidic PLA_2 (Asp49), named BPr-TXI, from venom gland transcriptome of *Bothrops pauloensis*. The total RNA extraction from the *Bothrops pauloensis* venom gland. was carried out by using the TRIZOL reagent. The primers were designed based on the N-terminal sequence determined for the toxins previously isolated and the C-terminal by multiple alignments with other toxins of snake venom (sequences deposited in NCBI -Genebank/SwissProt. The product of PCR was cloned in *p-GEM-T Easy Vector System,* Promega® and the gene was sequencing in *MegaBace 1000* (Amersham Biosciences) automatic sequencer. **Results:** The full-length nucleotide sequence of 420 bp encodes a predicted gene product with 139 amino acid with theoretical 13,649 kDa, with significant sequence similarity to many other phospholipase A_2 from snake venoms. **Discussion:** This enzyme is a isoform of Bp-PLA_2 isolated from the *Bothrops pauloensis* snake venom. Analysis of the toxic and pharmacological activities of this recombinant protein will be conducted for elucidation of the structure–function relationships of these toxins of biotechnological interest. **Support**: CNPq and FAPEMIG.

09.078

NAPHtoquinone isohemigossypolone from *Bombacopsis Glabra*, a inhibitor of phospolipasic activity of *Bothrops pauloensis* snake venom. Gimenes SNC[1], Amaral LO[1], Mendes MM[1], Paula VF[2], Correia SJ[2], Moreira BO[2], Gomes MSR[2], Hamaguchi A[1], Homsi-Brandeburgo MI[1], Rodrigues VM[1] [1]UFU - Genética e Bioquímica, [2]UESB - Química

Introduction: Animal venoms including snake venoms are complex mixtures of proteins. A group of enzymes very important present in snake venom are the phospholipases A_2. They hydrolyse phospholipids realizing fatty acids and lysophospholipids. These enzymes are responsible for many effects, such as neurotoxicity, miotoxicity and cytotoxicity. Many plants are used in popular medicine to treat snake bite envenomations. Medicinal plant extracts are a rich source of nature inhibitors and pharmacologically active compounds, have been shown to antagonize the activity of some venoms and toxins, including PLA_2 enzymes. **Methods:** This study shows the ability of the naphtoquinone Isohemigossypolone (ISO) isolated from *Bombacopsis glabra* to neutralize the Phospholipasic activity induced by *Bothrops pauloensis* snake venom in the ratios 1:5 and 1:10 (w/w), under incubation for 30 min at 37°C. Phospholipasic activity was determined using egg yolk suspension as substrate and the released free fatty acids were potentiometrically titrated (uEq.NaOH/min/mg) with NaOH (0.1208N). **Results and discussion:** The results shows that in presence of ISO, PLA_2 enzymes presents in the snake venom are inhibited in around 26% in the ratio 1:10 (w/w). Studies such as indicate the greater importance of evaluating the products derived from plants. The presence of PLA_2 inhibitory proteins opens the possibility to search for vegetal inhibitors with therapeutic purposes such as increase the ability of antivenenins to neutralize snake venom myotoxic effects. **Financial support:** Capes and UFU

09.079

Vasorelaxant effect induced by the hydroalchoolic extract of *Ruellia asperula* in rat superior mesenteric rings. Gomes MAS[1], Carvalho EM[1], Araujo IGA[1], Alustau MC[1], De Assis KS[1], Oliveira Junior FA[2], [1]Guedes DN[2], Dias KLG[1], Medeiros IA[1], Correia NA[2] [1]LTF-UFPB, [2]UFPB - Fisiologia e Patologia

Introduction: *Ruellia asperula* is a plant belonging to *Acanthaceae* family popularly known as candeia. It is used in the folk-medicine to treat bronchitis, uterine inflammation and flu. The purpose of the present study was to evaluate the mechanisms underlying the vascular effect induced by the hydroalchoolic extract of *Ruellia asperula* (EHRA) in mesenteric artery rings. **Methods:** All protocols used in this study were approved by the CEPA/LTF (protocol nº 0207/08). Mesenteric rings (1-2 mm) were obtained and suspended by cotton threads in organ baths, maintained at 37 ºC and gassed with carbogenic mixture, pH 7.4, under resting tension of 0.75 g. Statistical analyses was performed by student's t-test and ANOVA one way. **Results and discussion:** In isolated rat mesenteric artery rings with intact endothelium, EHRA (1 - 300 µg/mL) induced concentration-dependent relaxation of the contractions evoked by phenylephrine, PHE, (10 µM) [EC_{50} = 51.47 (44.48 – 59.56 CI), µg/mL, E_{max} = 98.8 ± 10.68%, n = 4]. The relaxant effect induced by EHRA was not attenuated by removal of the vascular endothelium [EC_{50} = 50.34 (44.07 - 57.51 CI) µg/mL, 98.43, E_{max} = 98.43 ± 5.65%, n=6, suggesting that the vascular endothelium, probably, is not participating in the vasorelaxant response induced by EHRA. Therefore, all the experimental protocols were carried out in endothelium-denuded mesenteric rings. In preparations pre-incubated with KCl 20 mM, the vasorelaxant activity against PHE induced contractions was not altered in comparison with results with PHE alone [EC_{50} = 61.74 (55.41 – 68.79 CI) µg/mL, E_{max} = 105.76 ± 7.39%, n = 6], indicating that k^+ channels probably, are not participating in this effect. EHRA (1 - 300 µg/mL) relaxed the sustained contractions induced by KCl 80 mM [EC_{50} = 44.65 (40.32 – 49.44) µg/mL n = 4) and E_{max} = 98.34 ± 2.67 %], this effect was not significantly different from those obtained in the presence of PHE 10 µM. In depolarizing nominally without Ca^{2+} medium, EHRA (100 and 300 µg/mL) inhibited $CaCl_2$-induced contractions. EHRA (1 – 300 µg/mL) also induced concentration-dependent relaxation on the contractions elicited by the L-type Ca^{2+} channel agonist, S (-)-Bay K 8644 [EC_{50} = 101.3 (93.08 –110.1) µg/mL and E_{max} = 106.69 ± 11.93 %, n=5]. Together, these results suggest a possible participation of the L-type Ca^{2+} channel in the vasorelaxant effects evoked by EHRA. On the other hand, EHRA did not alter the transient contractions induced by PHE (10 µM) in a medium without calcium, neither modify the contraction induced by sodium orthovanadate, a potent inhibitor of protein tyrosine phosphatase. Moreover, EHRA caused relaxation of the contractions evoked by PHE (10 µM) in the presence of KCl 60 mM and nifedipine (1 µM), suggesting that, in addition to the calcium influx inhibition, another mechanism can be involved in the vasorelaxant effect induced by EHRA. In conclusion, these results suggest that EHRA exerts an endothelium-independent relaxant effect and this effect may be due, in part, to Ca^{2+} influx inhibition through voltage operated calcium channels. Financial Support: CNPq, CAPES, LTF.

09.080

Antitumor activity of constituents from *Xylopia langsdorffiana* against sarcoma 180 cells.

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Introduction: The genus *Xylopia* (Annonaceae) comprises about 160 species (MAAS, P. J. M., Rodriguésia, 80, 65, 2001). *Xylopia langsdorffiana* St-Hil. & Tul. is a tree, 5-7 m high and popularly known in Northeast Brazil as "pimenteira da terra" (TAVARES, J. F., *Z.* Naturforsch, 62, 742, 2007). Various terpenoids are attractive natural compounds as therapeutic agents for the treatment of cancer (KONDOH, M., *J. Pharmacol. Exp. Ther*., 311, 115, 2004). We have previously reported that different substances of *X. langsdorffiana* showed antitumor activity *in vitro* by inducing cell differentiation and apoptosis on leukemia cells. In this study, we investigated the antitumor activity of three products of *X. langsdorffiana*: *ent*-7α-acetoxytrachyloban-18-oic acid (XLC-1), *ent*-7α-hydroxytrachyloban-18-oic acid (XLC-3) and Essential Oil from leaves (E.O.X). Sarcoma 180 cell line was used to assess the antitumour activity *in vitro* of these products. **Methods:** Sarcoma 180, also known as tumor of Crocker, was cultured in RPMI-1640 medium supplemented with 10 % fetal bovine serum (FBS), penicillin (100 IU/mL), and streptomycin (100 *μ*g/mL) in a humidified atmosphere with a 5 % CO_2 incubator, at 37 °C. Cytotoxicity was assessed by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) reduction (MOSMANN, T., *J. Immunol. Meth*., 65, 55, 1983). The cells were plated (2.10^5 cells/well) in 96-well plates and treated with different concentrations (0 – 500 μg/mL) of XLC-1, XLC-3 and E.O.X, dissolved in DMSO 2 %, for 24 h. After the treatment, 10 μL was removed for the Trypan blue exclusion test to evaluate the survival (%). Then, 10 μL of the tetrazolium compound MTT (5 mg/mL) was added for 4 h at 37 °C and 5 % CO_2. The cells were lysed and solubilized by the addition of 50 μL of SDS (Sodium Dodecyl Sulfate) (10 %) in 0.01 N HCl. The absorbance of each well was determined at 590 nm using an Microplate Reader. Survival (%) was calculated relative to the control. The IC_{50} values (concentration that produced 50 % inhibition on the parameter evaluated) were calculated after expressing the results as a percentage of the controls by nonlinear regression with confidence interval 95%. **Results:** Sarcoma 180 cells were treated with XLC-1, XLC-3 e E.O.X for 24 h as described. All compounds inhibited the growth of sarcoma 180 cells in a concentration-dependent manner. The IC_{50} values, for MTT reduction, were 54.58, 103.92 and 211.84 *μ*g/mL, respectively. For Trypan blue exclusion test, the IC_{50} values for these products were 54.34, 100.70 and 206,38 *μ*g/mL, respectively. **Discussion:** The present work reports the antitumor activity of three products of *Xylopia langsdorffiana*. Using those viability tests, XLC-1 was the most toxic diterpene to sarcoma 180 cells. Changes in metabolic activity or interactions in the reduction to formazan can give large changes in MTT results while the number of viable cells is constant. The values of IC_{50} obtained by the MTT were supported by values of trypan blue exclusion assay, which is a visual test, confirming the *in vitro* antitumor activity of XLC-1, XLC-3 and E.O.X. **Acknowledgement:** The authors would like to express their sincere thanks to CAPES/CNPq for the financial support.

09.081

Estudo comparativo da ação antinociceptiva das frações polares obtidas das cascas e das folhas de *Persea cordata* Meisn. (Lauraceae) em modelos farmacológicos específicos.

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Introdução: A *P. cordata* Meisn. é uma planta arbórea, cuja casca é utilizada popularmente para afecções de pele, úlcera gástrica e gastrite. Estudos prévios revelaram significativos efeitos farmacológicos da P. cordata em diferentes modelos experimentais *in vivo* e *in vitro*. (Santos et al., 2008; Schlemper *et al*., 2000,2003;2007; Pereira *et al*., 1998; Silva, 1997; Martins & Oliveira 1997; Mello & Gil, 1996;). O objetivo deste trabalho foi comparar a atividade biológica da fração acetato de etila (AEP) da casca e da fração de acetato de etila (FAEF) das folhas de *P. cordata*, investigando o efeito antinociceptivo em testes de dor induzida quimicamente. **Métodos:** Camundongos suíços (25-30 g) foram tratados pelas vias intraperitoneal (i.p.) e via oral (v.o.)com as frações AEP e FAEF, 1 hora antes da administração do irritante. O estímulo doloroso foi induzido por uma solução de ácido acético 0,6% via i.p. e as contorções abdominais foram observadas por um período de 20 minutos. Este trabalho foi aprovado no Comitê de ética da UDESC sob protocolo 1.27/2008 **Resultados:** A AEP (1 a 30 mg/kg, i.p.), apresentou significante efeito inibitório nesse modelo de dor com inibição máxima (IM) de 96,58 ± 3,43% e a DI_{50} de 5,53 (1,64-18,65) mg/kg. Ao ser administrada pela v.o., a AEP (100 a 800 mg/kg) inibiu significativamente a dor induzida pelo irritante químico, com valores de DI_{50} de 325,91 (286,66-370,53) mg/kg e IM de 89,77 ± 5,43% em relação ao grupo controle. Quando administrada via i.p. (100 a 1000 mg/kg) a FAEF apresentou IM de 71 ± 8% e DI_{50} de 438,79 (310,07-620,94) mg/kg. Já quando a FAEF foi administrada v.o. (100 a 1000 mg/kg) houve a inibição das contorções abdominais causadas por ácido acético com valores de IM de 59 ± 9% e DI_{50} de 593,15 (374,86-938,58) mg/kg. **Discussão:** Os resultados sugerem que a AEP e FAEF apresentam significante efeito analgésico inespecífico nos modelos utilizados, observados tanto pela via sistêmica como pela via oral, sendo a AEP mais potente e eficaz que o FAEF, tanto na administração i.p. como v.o. O estudo sugere que em diferentes partes anatômicas da planta existem composto(s) químico(s) ativo(s) que poderia(m) ser utilizado(s) como um potencial fármaco ou fitoterápico para o combate de dores de origem periférica. **Auxílio financeiro:** FAPESC

09.082

Anti-inflammatory action of ethanol extract and its substances obtained of plants from an anacardiaceae family specie leaves using *in vivo* assays. Costa TEMMC[1], Chagas MSS[1], Heringer AL[2], Figueiredo MR[2], Henriques MG[1], Rosas EC[1] - [1]FIOCRUZ - Farmacologia Aplicada, [2]FIOCRUZ - Tecnologia em Fármacos / Produtos Naturais

Introduction: Plants from Anacardiaceae family occur from Pernambuco to Rio Grande do Sul. Its leaves and stem bark are used in folk medicine against fever, cystitis, bronchitis, flu and general inflammations. Previous results obtained in our laboratory with the ethanol extract of stem bark from this specie indicated an anti-inflammatory effect. The aim of this study was to evaluate the anti-inflammatory action of the ethanol extract and its substances obtained from leaves (EFl) in the pleurisy and arthritis models. **Methods:** Pleurisy was induced by an intra-thoracic injection of zymosan (100µg/cavity) or carrageen (300 µg/cavity) in animals oral pre-treated with EFl (12,5 - 400 mg/kg), substances: GA, MG, PG, its MIX (100mg/mL) and diclofenac (100 mg/kg). Controls animals received an equal volume of sterile saline. Pleural wash was used to evaluate total and differential leukocyte count. Supernatant was collected to analyze protein extravasation, cytokines IL-8 (KC) and IL-6 production by ELISA and LTB4 by EIA. Joint inflammation was induced by intra articular (i.a.) injection of zymosan (500 mg/cavity in 25 ml sterile saline). Knee-joint swelling was evaluated by measurement of the transverse diameters of left knee joints using a digital caliper. Knee synovial cavities were washed to realize total and differential leukocyte counts. Animals were killed by an excess of carbon dioxide. The experiments were realized under approval of Committee on Ethical Use of Laboratory Animals of Fundação Oswaldo Cruz (licence n. L0052/08) and according with the recommendations of International Association for the Study of Pain. Results was presented as mean± S.E.M. and analyzed statistically by one-way ANOVA, and differences between groups were assessed using the Student–Newman–Keuls post-test ($p<0.05$). **Results:** The pre-treatment with EFl in dose-response way (12.5-400 mg/kg reduced the total leukocyte influx in the doses of 100, 200 and 400 mg/kg in pleurisy induced by zymosan in mice. The pre-treatment with EFl (100 mg/kg) was able to inhibit the neutrophil influx and protein extravasation in the pleurisy induced by carrageen or zymosan in mice and rats. EFl, the purified substances and MIX showed an anti-inflammatory effect on pleurisy induced by zymosan, reducing the LTB4, KC and IL-6 production. In the arthritis model, the EFl also inhibited total leukocyte and neutrophil influx reducing the oedema caused by the i.a. injection of zymosan. **Discussion:** The results suggest the anti-inflammatory activity to EFl and showing a great potential to a new phytomedicine. **Supported by:** PDTIS/FIOCRUZ/FAPERJ.

09.083

Anti-inflammatory action of the ethyl acetate fraction, a plant from anacardiaceae family.

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Introduction: Plants from Anacardiaceae family occurs in Brazilian coastline, being common in near rivers and are employed in herbal medicine in many countries on inflammatory diseases. Recently we demonstrated that ethyl acetate ST fraction inhibits the allergic pleurisy and paw edema induced by ovalbumin in sensitize mice and HPLC analysis revealed that gallic acid (GC), methyl gallate (MG) and 1,2,3,4,6-pentagalloylglucose (PG) are the major aromatic components of the fraction (Cavalher-Machado et al, 2008). The aim of the present work was to investigate the anti-inflammatory activity of leaves ethyl acetate ST fraction using *in vitro* and *in vivo* models under license L0052/08 (CEUA/FIOCRUZ). **Methods:** The citotoxity activity was evaluated by MTT assay. ST fraction was added in each well at concentrations from 0.5 to 500 *m*g/ml. To evaluate the production of nitric oxide, mice peritoneal macrophages were incubated with different concentrations of ST fraction in the presence of LPS (30 ng/mL). After 4h, 12 or 24 hours, the supernatants were collected to detect nitric oxide, analyze cytokine and PGE_2 production. Swiss mice received oral pre-treatment with the ST fraction (100 mg/kg) 1 hour before intraplantar (i.pl.) or intrathoracic injection of zymosan (500µg/paw or 100µg/cavity) or carrageen (300µg /paw or 300µg/cavity). The volume of paw edema was analyzed on plethysmometer 4h after stimulus and the thoracic cavity was washed with PBS/EDTA, the total and differential of leukocytes were performed. Results was presented as mean± S.E.M. and analyzed statistically by one-way ANOVA, and differences between groups were assessed using the Student–Newman–Keuls post-test ($p<0.05$). **Results:** The ST fraction (500 *m*g/ml) inhibited the nitric oxide production (87%), IL-6, KC, TNF-a (100%) and PGE_2 (89%).production. The oral pre-treatment with ST fraction in dose-response way (6.25-200 mg/kg) reduced the paw edema induced by zymosan in the all doses; however the same fraction inhibited the paw edema induced by carrageen in the doses of 100 and 200 mg/kg. The ST fraction (100 mg/kg) reduced the migration of total leukocytes, neutrophils and protein extravasation on inflammatory focus in the pleurisy induced by zymosan, and inhibited the migration of total leukocytes and neutrophils by carrageen. **Discussion:** This work showed that ST fraction has an inhibitory effect to macrophage stimulated *in vitro* and migration of leukocytes and protein extravasation at inflammatory site. Our results suggest an anti-inflammatory activity to ST fraction. **Supported by: PDTIS/FIOCRUZ**.

09.084

Evaluation of the inhibition of the toxic effects of the venom of *Bothrops pauloensis*, by extracts of juice of *Jatropha curcas* and leaves of *Polygonum hydropiperoides.* Brito CD[1], Alves LM[1], Alves IS[1], Silva TDS[1], Vieira SAPB[2], Rodrigues VM[2], Mendes MM[2], Izidoro LFM[2], Homsi-Brandeburgo M I[2], Hamaguchi A[2] [1]FACIP-UFU, [2]UFU - Genética e Bioquímica

Introduction: The interest in the study of plants as a form of alternative therapies is occupying an increasingly significant position within the medical. One way to exploit the therapeutic potential of plants is their use against snakebite. The venoms from snakes are complex mixture of proteins with potential to cause local effects in the victim as bleeding, necrosis, edema and others. *J. curcas* and *P. hydropiperoides* are species of plants used in folk medicine for the treatment of snakebite. **Objective:** The objective of this study was to compare the anti-snake venom potential of *J. curcas* and *P. hydropiperoides* extracts aqueous that were tested for the inhibition of the activities coagulant, hemorrhagic **(Comitê de Ética na Utilização de Animais-CEUA, protocolo 08-2008)** and phospholipasic induced by the venom of *Bothrops pauloensis.* **Methods:** The aqueous extract was prepared with juice *J. curcas* and the leaves of *P. hydropiperoides* which were washed with deionized water and homogenized and then sieved. The filtrate was centrifuged and the supernatant was lyophilized and stored at -20°C. The inhibitions of the activities coagulant, hemorrhagic and phospholipasic induced by the venom were assayed with incubation by 30' to 37ºC in three ratios: 1:5, 1:10 and 1:50 (w/w; venom/extract). PLA_2 enzymatic activity was measured by an indirect hemolytic assay on agarose gel, using washed mice erythrocytes and hen´s egg-yolk emulsion as substrate. Inhibition of coagulant activity of venom on bovine plasma was assayed testing venoms solutions incubated with vegetal extract and added immediately on 0,1ml plasma. The time to clot the plasma solutions was recorded. For the realization of hemorrhagic activity, Swiss male mice received two minimum hemorrhagic doses (MDH) of crude venom of *Bothrops pauloensis* combined or not with the vegetal extract. Three hours after injection, mice were killed, and the hemorrhagic area of their skin was measured. **Results:** Inhibition of coagulant activity with the extract of *J. curcas* was 100% in the ratios of 1:10 and 1:50, while *P. hydropiperoides* was 100% in ratio 1:50 respectively. Phospholipase A_2 activity was able inhibited 73.5% and 76.4% in the diameter of the halo in the ratio 1:5 and 1:10 by extract *J. curcas*, for extract *P. hydropiperoides* was 17.8% and 27.3% respectively. The extract of *J. curcas* was able to inhibit 100% of the hemorrhagic activity at all ratios tested, while extract *P. hydropiperoides* inhibited 100% only in the ratio of 1:50. **CONCLUSION:** According to the results we conclude that the extracts of *J. curcas* and *P. hydropiperoides* active compounds are able to neutralize some toxic effects induced by snake venom *Bothrops pauloensis*, where the extract of *J. curcas* presents a greater antiophiadian potential. Furthermore, these inhibitors purified can be used as molecular models for development of new therapeutical agents in treatment of snakebites. **Financial Supported: UFU and FAPEMIG.**

09.085

Anti-inflammatory activy of *Acanthospermum hispidum* DC (Asteraceae). Duarte T.[1], Rodrigues MD[2], Colaço W[1], Silva TG[2], Albuquerque JFC[2] [1]UFPE - Energia Nuclear, [2]UFPE - Antibióticos

Introduction: *Acanthospermum hispidum* DC (Asteraceae, is a plant known as the name of espinho de cigano or carrapicho de cigano, in northeastern Brazil in others regions is know by carrapicho-de-carneiro, belongs to the Asteraceae family. This specie is included in the concept of weeds plants (Lorenzi, 2008). It is an annual plant, herb, erect, dense downy stem, its height varies between 30 and 100 cm. Its reproduction is by seed, which is protected by a small thorny capsule, flows in almost all brasiliens regions. It is a weed of annual and perennial agricultural crops. The classification of this plant was realized at the Agricultural Research Institute (Instituto de Pesquisas Agrárias, IPA) and received the number 69580. The material used in this experiment were grown in a greenhouse of the Department of Nuclear Energie-UFPE, and collected 85 weeks after planting in order to study its anti-inflammatory activity. **Methods:** The aerial parts were dried in oven at 37ºC, cut into small pieces and ground. After this procedure was extracted with ethanol, and evaporated in rotary evaporator until dryness. The ethanol extract was subjected to acute toxicity test and after the evaluation of cell migration. The experimental protocol was approved by the Ethics Committee of UFPE (nº 23076.004788/2005 - 68). Male swiss albino mice were used, weighing between 20-25g from animals house of Department of Antibiotics. Groups of six animals were used. The oral toxicity acute was performed according to OECD protocol 423. Fixed doses to 2000 mg/kg were tested. To evaluate the anti-inflammatory activity, the animals were treated with the ethanolic extract by oral route sixty minutes before the induction of inflammation. The standard group received indomethacin (10 g/kg oral route) and the control group received the vehicle (10% of propylene glycol in saline solution). After, the animals were submitted to injection of 0.25 mL of a carrageenan solution (1%) in the peritoneal cavity. Four hours after induction, the animals were killed by exposure to CO_2. Immediately, the peritoneal cavity was washed with 2 mL of saline solution containing 3µM of EDTA and the liquid collected. The counting polymorphonuclear leukocytes (PMNL) in the exudates was performed in an automatic cell counter (Micros 60).The results were expressed as average of the total number of leukocytes per cavity ± standard deviation (SD) of the groups. **RESULTS AND Discussion:** In the test of acute toxicity in higher dose (2000 mg/kg) no animal has died. The alcoholic extract of *Acanthospermum hispidum*, at a dose of 200 mg/kg inhibited by 40% the migration of polymorphonuclear leukocytes ($6.3\pm0.8 \times 10^6$ cell/cavity) in the control group ($10.5\pm2.6 \times 10^6$ cell/cavity). The indomethacin was used as standard inhibiting cell migration in 47.3% ($5.3\pm0.5 \times 10^6$ cell/cavity). The results were significant for $P < 0.05$. These preliminary results showed that the ethanolic extract of the *Acanthospermum hispidum* shows promising anti-inflammatory activity with low toxicity, requiring the continuity of study, testing other doses of the extract of different partitions in order to find out the real fraction that displays this activity. **References**: LORENZI, H. Plantas daninhas do Brasil: terrestres, aquáticas, parasitas e tóxicas. 4ª ed. Nova Odessa, SP: Ed. Plantarum, 2008. 640p. **Financial support**: CNPq

09.086

Pharmacological and chemical characterization of *Piper purusanum* (Piperaceae) collected in the Amazon state. Souza JO[1], Júnior OLP[2], Pinheiro CCS[3] [1]INPA - Farmacologia, [2]INPA - Fitoquímica, [3]COPE- INPA

Introduction: The Piperaceae family is composed for a great variety of species, being common in diverse localities, many times dominating edge or the inferior bushes stratum. The biggest sort of this family (Piper) includes more than 1000 species, and the Brazilian forests shelters about 283 and they can reach a height of 2,20 to the 5,50 meters and contain 472 seeds on average (Figueiredo, *P. Biol. Piper. Spec*. *South*. *Bra*. *Ann. Bot.,*85:455,2002). Since Piper sort presents in its majority species with pharmacological action, together with the etno knowledge, the study of *Piper purusanum* presents excellent importance for the evidence of this activity. Once few studies are being developed regarding this one, it was really necessary to carry through new studies and tests to its respect. **Methods:** The vegetal material (leaves) of *Piper purusanum*, was collected in the rural area of Tarumã Mirim/Manaus/AM. The extraction was made by sequence of solvent with increasing polarity: Hexane, Acetate of etila and Water. For the accomplishment of the pharmacological assays the tests that had been used were General Activity Test, Acute Toxicity, Writhing Test and Hot Plate all using hexane extract in groups of mice (n=5), administered oral and intraperitonial, Paw Oedema test and Analgesimeter used rats (n=5; v.o.) (Lapa, *Mét. Aval. Ativ. Farm. Plan. Med.,*1:97,2005). The analysis of the answers of the pharmacological assays was evaluated through parametric and non parametric tests, using the statistical program GraphPad Prism 4.0. The statistical significant values will be considered level of 5% ($p < 0,05$). **Results and Discussion:** In the General Activity Test we could observe an acceptable analgesic activity when administered oral and intraperitonially. They presented sleepiness, and passenger depression with an average recovery time after five minutes of the application. In contrast, the test of acute toxicity did not present significant effect, being able then to be considered as a no toxic extract. In the Hot Plate test was observed that the i.p. administration predominated, once all the used dosages had been expressive, demonstrating an increase of the latency period(s), fact evidenced only in the 1500 mg/kg v.o. dose. In the played tests, Writhing Test was who presented the better income, demonstrating a reduction of at least 50% of the acetic acid (1%) initial action, since the lesser dose of the extract (500 mg/kg). Again the intraperitonial administration presented better effectiveness in comparison with oral. In the biggest dose of i.p. we could determine a total elimination of the writhing. In the other tests, analgesimeter and Paw oedema was used only oral applications, the biggest doses had presented a gradual reduction of the paw inflammation tax. Confirming that the applicability of the hexanic extract possess analgesic activity. With these results, it's necessary deeper studies in the search of the adjusted dose without the presence of collateral effect, as well as, to consider the accomplishment of pharmacological assays to examine the activity of watery and acetate of etila extracts, to determining an active conformity of the drug. I take advantage to thanks INPA, as well all the people that had participated in the accomplishment of this work.

09.087

Role of amblyomin-x on angiogenesis and endothelial cell migration. Dias RYS[1], Drewes CC[1], Hebeda CB[1], Chudzinski-Tavassi AM[2], Farsky S[2] [1]FCF-USP - Análises Clínicas e toxicológicas, [2]Instituto Butantan - Bioquímica

Introduction: Amblyomin-X is a recombinant protein inhibitor of serinoprotease, originally isolated from the salivary gland of *Amblyoma cajennense*. It has been demonstrated that amblyomin-X inhibits factor X activation, induces apoptosis in various lines of human or murine melanoma, reduces phagocytic activity by peritoneal macrophages and decreases the formation of tumors *in vivo*, by unknown mechanism. Since angiogenesis is one of the mechanisms involved on tumor growth and endothelial cell is closely related to formation of new vessels, here we aimed to investigate the role of Amblyomin-X on angiogenesis *in vivo* and on *in vitro* endothelial cell migration. **Methods:** *In vivo* angiogenesis was studied using dorsal chambers implanted at Male Swiss mice after anesthesia (ketanime/xylazine). Saline (control) or amblyomin-X treatment (10 or 100ng/10mL) was topically applied each 48 hours. Numbers of vessels were quantified in images obtained before and at 8th day after beginning of treatments. Microcirculatory endothelial cell lineage (T-end lineage; RPMI 1640 medium, 10% of fetal bovine serum, 37°C, 5% CO_2). After cell confluence, a mechanic lesion in the culture was done by a cell scraper cells and amblyomin-X (10 or 100ng/well) were added to the wells. Cell migration was monitored at 2, 4 or 6 hours after incubations by the number of cells migrating into the lesion. Results obtained were analyzed by *t* test or one-way ANOVA with Tukey post-test. All the experiments were conducted according to Ethics Committee in Animal Experiments n° 053/2008 - Protocol n° 211. **Results**: Amblyomin-X at a dose of 10ng and 100ng/10mL significantly reduced the number of new vessels in the skin microcirculation in 31,7% and 42,7%, respectively, in comparison to the first day of treatment. In control animals, the number of vessels was not modified by topic application of saline during all period of treatment. Amblyomin-X (10ng or 100ng/well) did not alter endothelial cell migration into the lesion focus during all period of time monitored. **Discussion**: Data present here show that Amblyomin-X, a recently expressed molecule, impairs new vessels formation without any stimulus, independently of impairment of cell migration. Future investigations will be carried out to clarify the mechanisms involved in this process. **Financial Support**: FAPESP (Project 08/57850-8; 08/56072-1) and Capes.

09.088

Cardiovascular effects induced by *Attalea excelsa* Mart. ethanolic extract in rats. Medeiros AAN[1], Medeiros FA[1], Queiroz TM[2], Medeiros MAA[2], Oliveira AC[2], Medeiros IA[2] [1]IEPA-DF, [2]LTF-UFPB

Introduction: *Attalea excelsa* is an Amazonian species popularly known by urucurí. The pharmacological effects induced by the ethanolic extract of *Attalea excelsa* (EAE) on the cardiovascular system were studied in rats using a combined *in vivo* and *in vitro* approach. **Methods:** Male Wistar rats (250-300 g) were anesthetized and the abdominal aorta and inferior vena cava were cannulated for pressure recordings and administration of drugs. Mesenteric rings (1-2 mm) were suspended by cotton threads for isometric tension recordings in Tyrode's solution at 37ºC and gassed with a 95% O_2 and 5% CO_2, under resting tension of 0.75g. All protocols were approved by the Ethics Committee in Animal Research of LTF/UFPB (n. 0603/07). **Results:** In non-anaesthetized rats, EAE (5, 10, 20, 40 and 60 mg/kg i.v.) injections produced hypotension (-3.7±1.2; -6.1±2.3; -8.5±1.3; -9.9±1.6 and -11.2±1.8%, respectively) and tachycardia (3.8±1.7; 4.0±1.6; 3.8±2.0; 3.7±3.1 and 12.4±2.7%, respectively) (n=5). In PHE pre-contracted rings, EAE (1-1000 mg/mL) induced a concentration-dependent relaxation in both intact (EC_{50} = 172.3 ± 36.9, E_{max} = 100 ± 0.0%) or endothelium-denuded mesenteric rings (EC_{50} = 166,7±31,4, $E_{máx}$ = 92,2±7,1%) with the same potency and effectiveness (n=6). These results suggest that EAE acts by an endothelium-independent mechanism. Subsequent experiments were performed in preparations without endothelium. In preparation pre-incubated with KCl 20 mM, the vasorelaxant activity of EAE was not changed (EC_{50} = 108.1 ± 10.7 and $E_{máx}$ = 95.9 ± 4.4%). EAE relaxed with the same potency rings pre-contracted with KCl 80 mM (E_{max} = 97.1 ± 1.5%) or with Phe (E_{max} = 92.2 ± 7.1%). Furthermore, in a Ca^{2+} free medium, EAE antagonized $CaCl_2$-induced contractions in a concentration-dependent manner. EAE (1-1000 mg/mL) induced concentration-dependent relaxation against contractions elicited by the L-type Ca^{2+} channel agonist, S(-)-Bay K 8644 ($E_{máx}$ = 128.8±5.8%, n=8). In depolarized nominally without calcium medium EAE did not alter transient contractions induced by caffeine (20 mM) and had a slight influence on those induced by Phe (10 mM). In rat isolated atrium, EAE produced negative inotropic and chronotropic effects. Electrophysiological studies on A7r5 cells EAE (100 mM) inhibited Ba^{2+} current through the Ca_VL1.2 **Conclusion:** In conclusion, the results suggested that the hypotensive effect of EAE is probably due to its vasorelaxant action that seems to involve the inhibition of Ca^{2+} influx through voltage-operated Ca^{2+} channels, leading to the reduction in $[Ca^{2+}]_i$ vascular smooth muscle cells. **Financial Support:** CNPq/LTF/UFPB/IEPA.

09.089

Inibição de atividades do veneno de *Bothrops leucurus* pela suramina e substâncias planejadas (LASSBio 448). Cons, BL[1], Calil-Elias S[2], Fernandes FFA[3], Tomaz MA[3], El-Kik CZ[3], Ricardo HD[3], Strauch MA[3], Machado MM[4], Borges PA[5], Lima LM[6], Melo PA[3] [1]UFRJ - Farmacologia e Química Medicinal, [2]FF-UFF - Farmácia e Administração Farmacêutica, [3]UFRJ - Farmacologia Básica e Clínica, [4]FMC-UFRJ - Farmácia / Departamento de Farmacologia Básica e Clínica, [5]UFRJ - Farmacologia, [6]LASSBio-UFRJ - Farmácia

Introdução: Os acidentes ofídicos por serpentes do gênero *Bothrops* são comuns em todo país e especificamente na região cacaueira baiana, onde ocorre grande incidência pela serpente *B. leucurus*. Essa serpente é bem adaptada a plantações de cacau que é quase sempre mixada com a Mata Atlântica. Investigamos os efeitos deste veneno em camundongos e *in vitro*, e o antagonismo destes efeitos pela suramina e a substância sintética LASSBio 448. Métodos: Testamos os efeitos da suramina e LASSBio 448 nas atividades fosfolipásica, proteolítica, hemorrágica e miotóxica *in vitro* / *in vivo* do veneno de *B. leucurus.* A atividade fosfolipásica foi determinada através da adaptação do método turbidimétrico (Marinetti, Biochim Biophys Acta, v. 3, p. 554, 1965), utilizando como substrato solução de gema de ovo de galinha. A atividade proteolítica foi testada usando o substrato azocaseína (Garcia, Arq Biochem Biophy, v. 188, p. 315, 1978) na concentração de 10µg/mL do veneno. A atividade miotóxica *in vitro* (Melo e Suarez-Kurtz, Braz J Med Biol Res, v. 21, p. 545, 1988), avaliando o aumento da atividade de creatina kinase do músculo *extensor digitorum longus* após a exposição ao veneno (25mg/mL), sendo este perfundido isolado ou pré-incubado com suramina e LASSBio 448 (1-30µM). Já *in vivo* foi determinada a atividade CK plasmática (Melo e Suarez-Kurtz, Braz J Med Biol Res, v. 21, p. 545, 1988 e Melo & Ownby, Toxicon, v. 37, p. 199, 1999) nos protocolos de pré-incubação e pós-tratamento com Suramina (1 – 30 mg/kg) e LASSBio 448 (1 – 30 mg/kg). Foi também avaliada a atividade hemorrágica (Melo, Toxicon, v. 32, p. 595, 1994), nos protocolos de pré-incubação e pós-tratamento com Suramina (1-30 mg/kg) e LASSBio 448 (1-30 mg/kg). Protocolo de animais: DFBCICB 022. **Resultados**: A suramina (30 µM) e o LASSBio 448 (300 µM) inibiram a atividade fosfolipásica em 100% e 40%, respectivamente. Na atividade proteolítica a Suramina (30µM) apresentou inibição de 30% e o LASSBio 448 (300µM) nenhuma; na miotoxicidade *in vitro* a suramina (30µM) foi capaz de antagonizar 100%; a atividade *in vivo* foi diminuída pela suramina (30 mg/kg) em cerca de 90%. Na atividade hemorrágica a Suramina (10 e 30 mg/kg) inibiu em 65 e 45 %, respectivamente e LASSBio 448 (10 e 50 mg/kg) inibiu em cerca de 90 e 45%, respectivamente. Conclusão: Os resultados mostram que a Suramina pode ser um possível adjuvante na terapia antiofídica, e LASSBio 448 apresentou um perfil de inibição parcial dos efeitos provocados pelo veneno de *Bothrops leucurus*. Suporte Financeiro: FAPERJ, CNPQ, PRONEX

09.090

Assessment of the anti-inflammatory action of hydroalcoholic extract from *Schinus terebinthifolius Raddi.* Pereira FMS[1], Costa TEMMC[1], Heringer AL[2], Figueiredo MR[2], Henriques MGMO[1], Rosas EC[1] [1]FIOCRUZ - Farmacologia Aplicada, [2]FIOCRUZ - Tecnologia em Fármacos / Produtos Naturais

Introduction: Anacardiaceae family species is widely used as anti-inflammatory, analgesic e antipyretics. The aim of this work was to evaluate the anti-inflammatory effect of the hydroalcoolic extract from Anacardiaceae family species (EFI) on paw oedema induced by zymosan (100 µg/paw) and inflammatory mediators in mice. **Methods:** Male Swiss mice were stimulated with an intra-plantar injection of zymosan (100 µg/pata) 1 h or 15 min after the treatement with the hydroalcoolic extract (100 mg/kg) or with the following inflammatory mediators antagonists: Promethazin (10 mg/kg), HOE140 (100nmol/kg), WEB2170 (8 mg/kg), indomethacin (10 mg/kg) and metisergide (5 mg/kg),. The animals were also stimulated with an intra-plantar injection of the following inflammatory mediators: histamine (100µg/pata), Bradikynin (3nmol/pata), PAF (1µg/pata) or serotonin (100µg/pata) in the paw 1 h or 15 min after the treatment with the hydroalcoolic extract. The oedema was measured in a Plethysmometer 7140, Ugo Basile, in the specific time for each administrated substance. All experiments were realized under approval of Committee on Ethical Use of Laboratory Animals of Oswaldo Cruz Foudation (licence n. L0052/08) and according with the recommendations of International Association for the Study of Pain. **Results:** The intraplantar injection of zymosan induced an oedema that was measured in different times. It was observed that prometazine reduced the oedema 1h after the stimulus, HOE 140 was able to reduce the paw volume 2 h after the stimulus and WEB 2170 inhibited significantly the oedema 2 and 4 h after zymosan-induced paw oedema. The oral administration of Hydroalcoolic extract (100 mg/kg) inhibited the paw oedema induced by zymosan 1, 2 e 4 h after stimulus. It was also able to prevent the oedema induced by histamin (100 µg/paw) and serotonin (100 µg/paw) 30 min after stimulus, however it did not inhibited the oedema induced by bradikynin and PAF **Discussion:** The results suggest a participation of histamine (1 h after the stimulus), bradikynin (2 h after the stimulus) and PAF (2 and 4 h after the stimulus) at the first 4 hours of the paw oedema induced by zymosan. The present data appoint to an anti-inflammatory effect of the hydroalcoolic extract from plants of the Anacardiaceae family and suggests an anti-histaminic effect. **Supported by: CNPQ**

09.091

Atividade antiproliferativa de extrativos da madeira de *Vatairea paraensis* em cultura de células tumorais humanas. Jankowsky L[1], Jorge MP[2], Ruiz ALTG [3]Santana, MA[4], Carvalho JE[4], Moreno Junior H[5] [1]CPQBA/FCM-UNICAMP - Farmacologia, [2]CPQBA/FCM - UNICAMP, [3]UNICAMP - CPQBA, [4]SFB/MMA - Produtos Florestais, [5]FCM-UNICAMP Farmacologia

Introdução: Desde seus primórdios o homem utiliza de extrativos da madeira tais como pigmentos e resinas, em diversas atividades. O avanço nos estudos dos metabólitos secundários de plantas, visando principalmente seu uso medicinal; facilita a procura de novas moléculas bioativas extraídas de madeira, ou dos resíduos gerados na sua industrialização, colaborando para o uso mais racional de matéria prima abundante e agregando valor ao manejo florestal sustentável. **Metodologia:** A madeira de *Vatairea paraensis*, previamente moída, foi submetida à extração a quente com diclorometano (produzindo o extrato bruto diclometânico EBD) e, na sequência, com etanol 95% (originando o extrato bruto etanólico EBE). O EBD foi submetido a fracionamento em coluna analítica de fase estacionária, obtendo-se nove frações, que foram analisadas por técnicas espectroscópicas de infravermelho e ressonância magnética nuclear. Amostras dos extratos e suas frações, nas concentrações entre 0,25; 2,5; 25 e 250 µg/mL foram testadas em cultura de células tumorais humanas, nas seguintes linhagens: pulmão (NCI-H460), mama (MCF-7), melanoma (UACC-62), rim (786-0), ovário (OVCAR-03), próstata (PC-3), ovário com fenótipo de resistência a múltiplos fármacos (NCI/ADR-RES), cólon (HT-29) e leucemia (K-562). Como controle positivo foi utilizada a doxorubicina. Após 48h do tratamento, o crescimento celular foi avaliado através da dosagem de proteínas totais pelo método de sulforrodamina B e, a partir das curvas concentração-efeito, foi avaliada a atividade dos extratos, assim como calculado o TGI (total growth inhibition) (Holbeck at al., 2004). **Resultados:** Entre os dois extratos brutos somente o EBD apresentou atividade anticâncer *in vitro* com seletividade para as linhagens de NCI-H460 (TGI=0,27 µg/mL), OVCAR-03 (TGI=0,31 µg/mL), HT-29 (TGI=0,44 µg/mL), NCI-ADR/RES (TGI=2,93 µg/mL) e 786-0 (TGI=4,09 µg/mL). O formato do gráfico de atividade é muito semelhante ao obtido com a doxorrubicina. O fracionamento do EBD resultou em duas frações enriquecidas em compostos, F2 e F6, cujas análises espectrométricas identificaram uma antracenediona (F2) e um antracenotriol (F6). A fração F2 com baixa potência foi seletiva para a linhagem de próstata PC-3 (TGI=54,49 µg/mL), enquanto a fração F6 para as de NCI-ADR/RES (TGI=0,71 µg/mL), OVCAR-03 (TGI=11,04 µg/mL) e MCF-7 (TGI=23,94 µg/mL). **Discussão:** Os resultados obtidos revelaram que a atividade anticâncer *in vitro* do EBD da *Vatairea paraensis* é conseqüência da presença de derivados do antraceno. A semelhança estrutural entre a doxorrubicina e os princípios ativos identificados, bem como o perfil de atividade demonstrado sugerem mecanismo de ação semelhante. A inibição da topoisomerase II é um dos mecanismos de ação propostos para a doxorrubicina. **Financiadores**: CAPES e CNPq. Holbeck, SL. *European J. of Cancer*. 40: 785-93. 2004.

09.092

Involvement of oxidative stress in apoptosis induced by an atisane diterpene from *Xylopia langsdorffiana* (Annonaceae) on HL60 cells. Gadelha PS[1], Pita JCLR[1], Castello Branco MVS[1], Anazetti MC[2], Frungillo L[2], Tavares JF[3], Silva MS[3], Diniz MFFM[1], Haun M[2], Melo PS[5] [1]UFPB - Ciências Farmacêuticas, [2]UNICAMP - Bioquímica, [3]UFPB - Tecnologia Farmacêutica, [4]LTF-UFPB, [5]UNICAMP/METROCAMP - Bioquímica

Introduction: *Atisane diterpenes* are rare in the Annonaceae family and constitute a group of compounds little-studied biologically thus far. An atisane diterpene was isolated from *Xylopia langsdorffiana* leaves, *ent*-atisane-7a,16a-diol, named xylodiol (Tavares, J. F., Z. Naturforsch, 62, 742, 2007). We have previously reported that xylodiol inhibits cell growth and induce differentiation and apoptosis on HL60 cells. A role for oxidative stress in the induction of apoptosis is suggested by the observations that low levels of reactive oxygen species (ROS) induce apoptosis whereas antioxidants such as N-acetylcysteine (NAC) inhibit cell death (Chandra, J., Blood, 102, 4512, 2003). The ability of oxidative stress to provoke apoptosis through massive cellular damage has been associated with lipid peroxidation and alterations in proteins and nuclei. Additionally, ROS generation occurs following the treatment of cells with various agents, including chemotherapeutic drugs (Kannan, K., Pathophysiology, 7, 153, 2000). Mitochondria are a source of ROS during apoptosis and reduced mitochondrial membrane potential leads to increased generation of ROS and apoptosis (Huang, Y.-T., Food Chem Toxicol, 44, 1261, 2006). In this study, we investigated the involvement of oxidative stress in apoptosis induced by xylodiol. **Methods:** The extent of xylodiol-induced lipid peroxidation was determined by the reaction of thiobarbituric acid (TBA) with malondialdehyde (MDA), a product formed by lipid peroxidation (Salgo, M. G., Arch. Biochem. Biophys. 15, 482, 1996). To assess cell viability and the protective effect of reduced glutathione (GSH) and N-acetylcysteine (NAC), HL60 cells were seeded (3 x 10^5 cells/mL) in 96-well plates containing 1 mM of GSH or 1 mM of NAC, and incubated with different concentrations of xylodiol for 72 h. Cell viability was determined by MTT reduction (Anazetti, M. C., Toxicology, 188, 261, 2003). **Results:** The treatment with 50, 100 and 150 µM of xylodiol for 24 and 48 h led to a marked increase of percentage of TBARS formed. The TBARS production increased by about 70 % and 80 % after 24 and 48 h of exposition to 100 µM of xylodiol, respectively. To examine whether the cytotoxic effect induced by xylodiol in HL60 cells was due to the generation of lipid peroxidation products, the cell viability was determined in the presence of the antioxidants. GSH and NAC could partly block the cytotoxity of xylodiol. **Discussion:** Based on results obtained, we speculated that generation of ROS could be an important factor in xylodiol-induced apoptosis. However, the ability of the antioxidants to inhibit only partly the cytotoxic effects of the xylodiol in HL60 cells provide evidence that ROS are intermediates of xylodiol-induced apoptosis. This work was supported by the Brazilian agencies CAPES and FAPESP.

09.093

Evaluation of gastroprotector effect of the essential oil of *Hyptis martiusii* Benth. (Lamiaceae) in Wistar rats. Caldas GFR[1], Silva JBR[2], Leite VR[2], Costa, LJL[2], Lafayette SSL[2], Costa JGM[3], Wanderley AG[2] [1]UFPE - Farmácia, [2]UFPE - Fisiologia e Farmacologia, [3]URCA - Ciências Biológicas e da Saúde

Introduction: *Hyptis martiusii* Benth. is a endemic and abundant species in northeastern of Brazil, popularly known as "cidreira-do-mato", this species provides an essential oil. Antitumor, cytotoxic, antimicrobial and insecticidal activities have been identified; however no pharmacological *in vivo* activity has so far been reported according to a literature survey. The aim of the study was evaluating the gastroprotector effect of essential oil from leaves of *H.martiusii* (OEHM), on gastric lesions induced by absolute ethanol and HCl/ethanol. **Methods:** Males Wistar rats (250-350g) were used in either protocols, divided into five groups (n=6), which were fasted for 24h, but given water *ad libitum* prior of the treatments. Animals were treated orally with OEHM (1% Tween in distilled water as vehicle) in doses 100, 200, 400mg.kg^{-1}, vehicle (10ml.kg^{-1}) and pantoprazole (40mg.kg^{-1}). Following a 60 min period, groups for each protocol, received absolute ethanol (70%, 1mL/100g) and a 0.3M HCl/60% ethanol solution (HCl/ethanol, 1mL/150g), by gavage for gastric ulcer induction. Thirty minutes after the administration of the harmful agent, the animals were sacrificed and their stomachs were removed. The gastric lesion was measured by planimetry. The experimental protocols were approved by Ethics Committee of the UFPE, under license n° 007764/2009-94. Data are expressed as mean±s.e.m. of lesioned area (%) in relation to the total area of gastric corpus injured. Differences between groups were analyzed by ANOVA and Tukey's test. **Results:** The animals pre-treated with OEHM (200 and 400mg.kg^{-1}) induced a significant reduction in the area of gastric lesions by ethanol (12.4 ± 5.0 and 3.7 ± 1.7% versus control 37.5 ± 8.0%, respectively) corresponding to an inhibition index of 66.9 and 90.2%. In the models by HCl/ethanol all doses reduced significantly the area of gastric lesions (3.5 ± 1.6; 2.4 ± 1.4 and 1.6 ± 0.6% versus control 17.1 ± 2.7%), corresponding to an index of inhibition of 79.5; 85.7 and 9.5% respectively. **Discussion:** These findings indicate that *H. martiusii* has a gastroprotective property that needs further elucidation regarding its action mechanism. However, the chromatography analysis of the essential oil showed the presence mono and sesquiterpenes (Araujo et al, J. Agric. Food. Chem, v. 51, p. 3760, 2003), terpenoids compounds of remarkable therapeutic properties, which allows us to suggest that these substances are probably involved in the gastroprotector activity observed. Financial Support: CAPES

09.094

Avaliação da ação antimicrobiana de galhos de *jatobá* comercializado no Mercado Municipal de Campo Grande, MS. Maldonado KS[1], Schwab L[1], Gimenes AHG[1], Garcia DCB[1], Oliveira EJT[1], Tomazoni E[1], Nascimento CCC[1], Arantes TS[1], Mariano YY[1], Negrete CL[1], Oliveira RF[2], Yano M[2] [1]UCDB - Farmácia, [2]UCDB - Biotecnologia

Introdução: A livre comercialização de plantas medicinais na área urbana é uma atividade corriqueira em muitas cidades, sendo essa prática geralmente realizada informalmente, transmitindo-se o conhecimento popular junto à ela. O jatobá *(Hymenaea courbaril L.)* é uma àrvore originalmente encontrada na Amazônia e Mata Atlântica brasileiras, onde ocorre naturalmente desde o Piauí até o Norte do Paraná, na floresta latifoliada semidecidual, e também encontrada no Cerrado na espécie *H. stigonocarpa*. O jatobá apresenta eficácia no tratamento de uma ampla variedade de tumores, infecções cutâneas, bronquites, tosses, coqueluches, bem como atividade como vermífugo. Este trabalho teve como objetivo avaliar a atividade antimicrobiana do extrato do galho de jatobá adquirido no Mercado Municipal de Campo Grande, MS. **Métodos:** Os galhos de jatobá foram adquiridos no Mercado Municipal de Campo Grande, MS, moídos e preparado o extrato bruto etanólico do galho por maceração estática. O extrato foi filtrado e seco no rotaevaporador e, em seguida, preparadas alíquotas de 0,25g do extrato. Após seco, o extrato foi ressuspendido em 2,5 mL de solução salina a 0,9% e preparadas as seguintes concentrações: 100%, 50% e 25%. A avaliação da atividade antimicrobiana foi realizada *in vitro* utilizando-se os seguintes microrganismos: *Staphylococcus aureus* ATCC 6538, *Pseudomonas aeruginosa* ATCC 9027, *Klebsiella pneumaniae* ATCC 700603 e *Candida albicans* ATCC 10231. Para os testes, foram utilizados discos estéreis de papel de 6 mm de diâmetro, impregnados com 20 µL de cada concentração (100%, 50% e 25%). Após secagem, os discos foram colocados em placas de Petri com meio Ágar Mueller-Hinton, para as bactérias e Ágar Batata Dextrose, para o fungo, onde foram inoculados os microrganismos em solução padronizada. Numa placa de Petri, além dos discos de extrato, foram colocados também um controle negativo (solução salina a 0,9%) e um controle positivo (penicilina para *S. aureus*, gentamicina para *P. aeruginosa*, tetraciclina para *K. pneumoniae* e itraconazol para *C. albicans*), sendo os testes realizados em triplicata. As placas foram incubadas em estufa a 37°C por 24 horas. Após o período de incubação, os resultados foram lidos para a verificação da presença ou não de halos de inibição (mm). **Resultados e Discussão:** O extrato bruto etanólico de galhos de jatobá mostrou atividade frente a *S. aureus* nas três concentrações testadas, com os seguintes halos: 9 mm na concentração de 100%, 8 mm na concentração de 50% e 7 mm na concentração de 25%. Esse resultado demonstra a importância do extrato de galho de jatobá frente a esse microorganismo Gram positivo, o qual é patogênico, sendo um dos mais comuns encontrados no meio ambiente e o mais virulento do seu gênero, causador de infecções na pele e na região da nasofaringe geralmente por pequenos cortes **(PIBIC/UCDB e CNPq)**.

09.095

Effect of tetranortriterpenoids isolated from *Carapa guianensis* in murine models of lung allergic inflammation Figueiredo A[1], Ferraris FK[1], Tappin MRR[2], Henriques MGMO[1], Penido C[1] [1]Farmanguinhos-FIOCRUZ - Farmacologia Aplicada, [2]Farmanguinhos -Fiocruz - Química de Produtos Naturais

Introduction: Allergic diseases, such as asthma, are accompanied by a chronic inflammatory response, characterized by increased vascular permeability, edema formation and accumulation of leukocytes, markedly T lymphocytes and eosinophils. Products of natural origin are widely recognized as an important therapeutic alternative for presenting various pharmacological activities. Previous results obtained in our laboratory demonstrated that the oil extracted from the seeds of *Carapa guianensis* Aublet presents an important anti-allergic activity in murine models of ovalbumin (OVA)-induced allergic response in paw, ear and pleura of previously sensitized mice (Penido C., *Inflamm. Res*. 54:295, 2005; *Int. Immunopharmacol*.6:109, 2006). Similarly, a group of five tetranortriterpenoids (TNTPs; 6α-acetoxygedunin, 7-deacetoxy-7-oxogedunin, andirobin, methyl angolensate and gedunin) isolated from this oil mimicked its effects *in vivo* and *in vitro*. **Objective**: To evaluate the anti-allergic effect of TNTPs in *in vivo* models of allergic pleurisy and allergic lung inflammation. **Methods**: Allergic pleurisy was induced by an intrathoracic (i.t.) injection of OVA (12.5 mg/cav). Lung inflammation was achieved by 5 intra-nasal (i.n.) instillations of OVA (50 µg) every other day. Both stimulations were given 14 days after sensitization (5 mg $Al[OH]_3$ 50 µg OVA, s.c.) of BALB/c mice (18 - 25 g). TNTPs were given orally (p.o.; 100 mg/kg) or intraperitoneally (i.p.; 0.5 mg/kg). Dexamethasone (dexa) was administered i.p. (10 mg/kg) or i.n. (1 mg/kg). All treatments were given 1 h before or after OVA stimulation. (License L-0004/08, CEUA). **Results**: Mice submitted to allergic pleurisy showed increased numbers of total leukocytes in pleural cavities 24 h after OVA challenge, due to migration of mononuclear cells and eosinophils. TNTP p.o. and i.p. pre-treatments, as well as dexa (i.p.) pre-treatment, inhibited i.t. OVA-induced pleural accumulation of total leukocytes and eosinophils. $CD3^+$ and $\gamma\delta^+$ T lymphocytes also accumulated in inflamed pleura of OVA-challenged mice; however TNTP treatment failed to impair such phenomenon. It is interesting to note that, even though TNTPs failed to change T cell counts, such treatment inhibited OVA-induced increase in the percentage of $CD69^+$ $CD3^+$ and $\gamma\delta^+$ T lymphocytes in the pleural space. It is noteworthy that the post-treatment with TNTPs (1 h after challenge, p.o.) was also able to reduce pleural eosinophil accumulation. Mice submitted to the model of lung allergic inflammation also presented a significant increase in total leukocyte and eosinophil counts in lung tissue of OVA-challenged mice 24 h after OVA i.n. instillation. Pre-treatment with dexa (i.n. or i.p.) and TNTPs (i.p.) impaired total leukocyte and eosinophil recruitment into inflamed lung. **Conclusion**: TNTPs obtained from *C. guianensis* present a significant anti-allergic activity in different models of murine airway inflammation, impairing leukocyte influx into inflamed pleura and lung. Further studies will be carried out in order to contribute for the scientific knowledge of the therapeutic properties of such plant species. P values£0.05 were regarded as significant. **Financial Support**: FAPERJ, Farmanguinhos, FIOCRUZ.

09.096

Na^+/K^+-ATPase activity and expression in cultured Madin-Darby canine kidney cells treated with *Bothrops alternatus* snake venom: modulation by catalase. Linardi A[1], Nascimento JM[2], Miyabara E[3], Cardoso KC[4], Rocha e Silva TAA.[1], Moriscot AS[3], Collares-Buzato CB[5], Hyslop S[4] [1]FCMSCSP/UNICAMP - Fisiologia / Farmacologia, [2]UNICAMP - Bioquímica, [3]ICB-USP Biologia Celular e Desenvolvimento, [4]UNICAMP - Farmacologia, [5]UNICAMP - Histologia e Embriologia

Introduction: The ion pump Na^+/K^+-ATPase is widely expressed in renal tubules and has an important role in modulating sodium reabsorption, renal function and homeostasis of the extracellular compartment. Since *Bothrops* snake venoms cause renal damage that can lead to acute renal failure, in this study we investigated the effect of *Bothrops alternatus* venom on Na^+/K^+-ATPase in cultured Madin-Darby canine kidney (MDCK) epithelial cells and the influence of catalase and superoxide dismutase (SOD) on Na^+/K^+-ATPase activity and expression. **Methods:** Cultured MDCK cells (J.M. Nascimento *et al., Biochem. Cell Biol.*, **85:**591-605, 2007) were incubated with 10 µg of venom/ml, and after 1, 3 and 6 h Na^+/K^+-ATPase activity was assayed based on the detection of inorganic phosphate. Gene and protein expression of the α_1 subunit was assessed by quantitative real-time PCR and immunofluorescence, respectively. **Results:** Venom significantly decreased ($p<0.05$; ANOVA and Bonferroni test) Na^+/K^+-ATPase activity (µmol/min/mg) after 1 h (0.49±0.16; mean±S.D.), 3 h (0.51±0.14) and 6 h (0.24±0.09) when compared to control cells (0.91±0.33) (n=4 each). Treatment with catalase (100 U/ml; 30 min before venom) progressively restored this activity 1 h (0.59±0.13), 3 h (0.66±0.25) and 6 h (0.79±0.25) post-venom (activity with catalase alone: 0.69-0.79 µmol/min/mg), whereas SOD (40 U/ml) did not. There was a significant decrease ($p<0.05$) in gene expression of the catalytic α_1 subunit 1 h (0.01±0.01; arbitrary units), 3 h (0.24±0.08) and 6 h (0.46±0.22) (n=3 each) post-venom, when compared to control cells (1.0±0.17). Immunofluorescence also revealed a significant decrease in α_1 subunit protein expression ($p<0.05$) 1 h ($1.41±0.66 \times 10^6$; arbitrary units) and 3 h ($1.62±0.49 \times 10^6$) post-venom compared to control cells (4.22±1.24 at 1 h; $4.47±1.46 \times 10^6$ at 3 h); protein expression was restored by catalase 1 h (3.36±1.15) and 3 h (3.00±0.74) after venom, when compared to control with catalase alone (3.56±1.84 at 1 h and $4.13±1.52 \times 10^6$ at 3 h). **Discussion**: These results indicate that *B. alternatus* venom alters Na^+/K^+-ATPase activity and expression in MDCK cells. This downregulation may be mediated by enhanced H_2O_2 production since catalase, which degrades H_2O_2, attenuated the reduction in Na^+/K^+-ATPase activity and expression. **Financial Support**: CNPq, FAPESP.

09.097

Mitochondrial condensation, but not swelling, is involved in xylodiol-induced apoptosis in hl60 cells. Pita JCLR[1], Castello Branco MVS[1], Viana WP[2], Anazetti MC[3], Frungillo L[3], Tavares JF[4], Silva MS[4], Diniz MFFM[1], Haun M[3], Melo PS[5] [1]UFPB - Ciências Farmacêuticas, [2]UFPB - Ciências da Saúde, [3]UNICAMP - Bioquímica, [4]UFPB - Tecnologia Farmacêutica, [5]UNICAMP/METROCAMP - Bioquímica

Introduction: Xylodiol, an atisane diterpene was isolated from the *Xylopia langsdorffiana* (Annonaceae) leaves, and characterized as a new diterpene, *ent*-atisan-7α,16α-diol (Tavares, J. F., Z. Naturforsch, 62, 742, 2007). We have previously reported that xylodiol inhibits cell growth and induce differentiation on human leukemia cell lines (HL60, U937 and K562). Anticancer drugs act by interfering with proliferation or by inducing apoptosis. Mitochondrial changes in apoptosis include the opening of permeability transition pore (MPTP) that causes the dissipation of inner transmembrane potential (DY_m), matrix swelling and outer membrane disruption, thus leading to the release of apoptogenic factors (Philchenkov, A. J., Cell. Mol. Med., 8, 432, 2004). In this study, we investigated the apoptosis-inducing effects of xylodiol to HL60 cells in mitochondrial level. **Methods:** Apoptotic cells were detected using an ApoDETECT^{TM} Annexin V-FITC kit. After the addition of propidium iodide, the cells were analyzed by flow cytometry. For the detection of mitochondrial swelling, the mitochondria were isolated from HL60 cells after treatment with xylodiol (Schneider, W. C., J. Biol. Chem., 183, 123, 1950). FACS analysis, using the fluorescent mitochondrial probe JC-1, was uses to verify if xylodiol dissipated the mitochondrial membrane DY in HL60 cells, indicating the opening of the permeability pore. Cell viability and the protective effect of cyclosporine A was determined by MTT reduction (Anazetti, M. C., Toxicology, 188, 261, 2003). **Results:** Xylodiol induced apoptosis and secondary necrosis in concentration and time-dependent manners in HL60 cells treated with 50, 100 and 150 μM of xylodiol for 12, 24, 48 and 72 h. At 72 h, the percentage of Annexin V- and PI-positive cells (late stages of apoptosis and/or necrotic cells) reached about 81%. It was observed an increase of mitochondrial swelling by about 15% after treatment of the HL60 cells with 50 μM of xylodiol for 12 and 24h. In contrast, it was observed a decrease of mitochondrial swelling (6,4 – 26,7%) in cells treated with 100 and 150 μM of xylodiol. A significant decrease in the ratio of red fluorescence to green fluorescence was evident after exposure of HL60 cells to xylodiol (50, 100 and 150 μM), showing a loss of DY_m. **Discussion:** HL60 cells treated with xylodiol showed biochemical changes characteristic of apoptosis, including Annexin V staining and loss of DY_m. However, the present study shows condensation rather than swelling of mitochondria in HL60 cells. It was described that the condensation of mitochondria and the reduction in DY_m are downstream of apoptogenic factors release, such as cytochrome c. In addition, it was proposed that the formation of condensed mitochondria occurred downstream of caspase activation (Zhuang, J., Cell Death and Differentiation, 5, 953, 1998). The observation that CSA (MPT inhibitor) did not protect HL60 cells from cytotoxic effects of the xylodiol corroborate with the hypothesis that the loss of DY_m and mitochondrial condensation are late events in the xylodiol-induced apoptosis. This work was supported by the Brazilian agencies CAPES and FAPESP.

09.098

Brine shrimp toxicity of Euphorbiaceae species. Viana WP[1], Gadelha PS[2], Pita JCLR[2], Medeiros VM[3], Tavares JF[3], Silva MS[3], Castello Branco MVS[2], Diniz MFFM[2] [1]UFPB - Ciências da Saúde, [2]UFPB - Ciências Farmacêuticas, [3]UFPB - Tecnologia Farmacêutica

Introduction: Medicinal plants has no doubt played a central role in the search for development of new drugs (HEINRICH, M., Phytother. Res., 14, 478, 2000). The Euphorbiaceae family includes approximately 290 genera and 7500 species distributed in all tropical and subtropical regions of the globe, especially in America and Africa. In Brazil, there are 72 genera and about 1100 species distributed in all vegetation types. Pharmacological studies performed with crude extracts and isolated compounds of Euphorbiaceae species showed cytotoxic activity against cancer cells Hep-G2 (hepatocellular carcinoma), MDA-MB-231 (breast adenocarcinoma) and A-431 (carcinoma epidermoid) (SETZER, W. N., Fitoterapia, 71, 195, 2000). In order to establish the toxicity of new natural products, many tests may be used, as the brine shrimp test (*Artemia salina*), which was developed to detect bioactive compounds on plant extracts (MEYER, B. N., Planta Med., 45, 31, 1982; NICK, A., J. Ethnopharmacol., 49, 147, 1995). Toxicity to brine shrimp *Artemia salina* is considered to be well correlated to antitumor activity (MEYER, B. N., Planta Med., 45, 31, 1982). In addition, *Artemia salina* could be a test organism in the search for compounds having the ability to protect against superoxide-mediated toxicity (MATTHEWS, R. S., Free Radic. Biol. Med., 18, 919, 1995). **Methods:** To evaluate the toxicity of ethanolic extract of *Sapium obovatum* (SO-EtOH), *Croton grewioides* (CG-EtOH), *Pera leandrii* (PL-EtOH) and *Acalypha muticaulis* (AM-EtOH), it was used the brine shrimp (*A. salina*) lethality test. 25 mg of eggs of *A. salina* were incubated in sea water (pH 8-9 and 29 ^{o}C) at artificial light during 24 h for occlusion of cysts and obtaining of the larvae. Samples were dissolved in dimethylsulphoxide (DMSO) and diluted with sea water and then 5 mL of each sample in different concentrations (10-1000 µg/mL) was added in tubes containing 10 nauplii. Three replications were done for each concentration and the experiment was repeated three times. The control group was prepared with the solvent and *A. salina.* The set was incubated at artificial light for 24 h and then the survivors larvae were counted to determine the LC_{50} (Lethal Concentration 50 %) (MEYER, B. N., Planta Med., 45, 31, 1982; PARRA, A. L., Phytomedicine, 8, 395, 2001). **Results:** In the present study, the toxicity of the SO-EtOH, CG-EtOH, PL-EtOH and AM-EtOH by the brine shrimp lethality test was examined. This bioassay was performed three times and the LC_{50} of tests were 259.6 µg/mL, 265.7 µg/mL, 168.4 µg/mL and higher than 1000 µg/mL, respectively. **Discussion:** These results show that the extract of *Acalypha muticaulis* showed low activity against *Artemia salina*. The higher bioactivity focused on extracts of *Sapium obovatum, Croton grewioides* and *Pera leandrii*, the latter being the most active, suggesting the presence of bioactive substances. These findings provide a direction for the study of extracts, fractions and substances obtained of these species in the search for new active biologically molecules. **Acknowledgement:** The authors would like to express their sincere thanks to CAPES/CNPq for the financial support.

09.099

Antidiarrhoeal activity of the roots from *Solanum asterophorum* Mart. (Solanaceae) in mice. Silva PCB[1], Vasconcelos MA[2], Silva KM[2], Lima, L. O.[2], Silva ADS[2], Leite, J. A.[3], Silva TMS[4], Cavalcante FA[2] [1]FANUT-UFAL, [2]ICBS-UFAL, [3]LTF-UFPB, [4]DQ-UFRPE

Introduction: *Solanum* genus belongs to the Solanaceae family. Economically, it is one of the most important families, including numerous ornamental, edible, spicy, medicinal, narcotic, and poisonous species. *Solanum* is well represented in Brazil and is widely distributed from north to south in diverse phytogeographic regions. Many of the species are endemic in the country, and are commonly known as "jurubeba". In the Northeast of Brazil, some *Solanum* species are widely used in folk medicine. *Solanum asterophorum* Mart. is a shrub popularly known as "jurubeba-de-fogo" and its roots are popularly used in the treatment of liver diseases. Many *Solanum* species have showed spasmolytic activity and, among them some also showed antidiarrhoeal activity. Based on the ethnomedical and chemiotaxonomic criteria we decided to investigate a possible antidiarrhoeal activity of the methanol extract obtained from roots of *Solanum asterophorum* (Sast-$MeOH_R$) in mice. **Methods:** *Castor oil-induced diarrhoea***:** mice were weighted and divided into negative control (saline), positive control (loperamide 10 mg/kg) and test groups (Sast-$MeOH_R$ 250, 500 or 750 mg/kg), containing four mice in each group. Each animal was placed in an individual cage, the floor was lined with blotting paper and changed every hour. Diarrhoea was induced by oral administration of 0.4 mL castor oil/mice 30 min after the above treatments. During an observation period for 3h, the total number of faecal output and number of wet faeces excreted by the animals were recorded. *Normal intestinal transit***:** animals were divided into 4 groups of 6 animals each. Group 1 received saline 10 mL/kg, p.o., group 2 were administered atropine 2 mg/kg p.o. (positive control) and group 3 were administered Sast-$MeOH_R$ 125, 250 or 500 mg/kg p.o. (test groups). After 30 min, standard charcoal meal (0.4 mL/mice) were given to mice orally. Animals were sacrificed 30 min after administration of charcoal meal and the small intestine immediately isolated. All the experimental protocols were approved by Ethical Committee in Research of UFAL (Protocol 027241/2008-11). Results: The Sast-$MeOH_R$ produced a notable antidiarrhoeal activity in the study, when inhibiting significantly ($P < 0.001$), both the frequency of defaecation as well as the wetness of the faecal droppings in mice. The effect of the extract (750 mg/kg) was similar to that of the standard drug, loperamide (10 mg/kg), which produced a maximum inhibition of 100 %. However, this effect of the extract not may be related to an inhibition of muscle contractility and motility, since Sast-$MeOH_R$ was unable to inhibit the intestinal transit by charcoal meal, unlike from atropine (2 mg/kg) that inhibited 54,5 ± 4,4 %. **Discussion:** 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. The results obtained in this study suggest that the Sast-$MeOH_R$ possesses antidiarrhoeal activity, however other studies must be carried out to elucidate the mechanisms involved in these activity. **Financial support**: PIBIC/UFAL/FAPEAL.

09.100

Investigation of spasmolytic activity of crude ethanolic extract from *Acalypha multicaulis* Müll. Arg. (Euphorbiaceae). Silva ACL, Travassos RA, Sousa NM, Martins IRR, Oliveira GA, Carreiro JN, Santos RF, Tavares JF, Silva BA[3] CCS-DCF-LTF-UFPB

Introduction: Euphorbiaceae family is the sixth largest in the world and is represented by 300 genera and about 7500 species (CRONQUIST, Columbia University Press, v.55, 1981). The genus *Acalypha* has 450-500 species (CRONQUIST, Botanical Garden Press, p555, 1988). The main substances found in genus *Acalypha* are tannins, flavonoids (AMAKURA, Phytochemistry, v.50, p.667, 1999), terpenes, mainly diterpens and alkaloids (SIEMS, Phytochemistry, v.41, p.851, 1996). Many *Acalypha* species have showed antimicrobial (ALADI, Journal of Ethnopharmacology, v.39, p.171, 1993), cytotoxic (AZIZ ULLAH, Bangladesh Pharm. Journal, v.12, p.29, 2002), antitumour and antispasmodic activity (ASTUDILLO, Phytoteraphy Research, v.18, p.102, 2004). Since the secondary metabolites found in species of *Acalypha* are reported in the literature by presenting spasmolytic activity. Based on chimiotaxonomic criterium we chosed *Acalypha multicaulis* Müll. Arg for this research, because it is a new species from the viewpoint of pharmacological studies. Thus, we decided investigate a possible spasmolytic activitie of the crude ethanolic extract from *Acalypha multicaulis* (AM-EtOH) on guinea-pig ileum. **Methods:** The guinea-pig ileum were suspended in organ bath containing modified Krebs solution (pH = 7.4) at 37 ºC, gassed with 95% O_2 and 5% CO_2 carbogen mixture and 1 g resting tension. Isometric contractions were registered through of force transducer coupled to an amplifier, which was connected to a microcomputer. Isotonic contractions were recorded on a smoked drums through levers coupled to kymographs. All the experimental protocols were approved by Ethical Committee in Animal Research of LTF/UFPB (Protocol 0506/05). **Results:** On guinea pig ileum, AM-EtOH antagonized carbachol-induced phasic contractions (E_{max} = 19.83 ± 4.7 %, n = 3) only the concentration of 500 µg/mL. However, AM-EtOH relaxed the organ pre-contracted by KCl (EC_{50} = 255.9 ± 8.7 µg/mL, n = 4) or carbachol (EC_{50} = 25.6 ± 9.5 µg/mL, n= 3) in a significant and concentration-dependent manner, being approximately 10 folds more potent to carbachol. The responsiveness of the ileum was recovered 30 min after withdrawal of the AM-EtOH from the bath. **Discussion:** The extract AM-EtOH shows secondary metabolites with potential spasmolytic action on guinea-pig ileum. Interestingly, AM-ETOH was able to inhibit the tonic component of the contraction, but not its phasic component. Further studies are necessary to elucidate the mode of action of AM-EtOH extract as spasmolytic agent on this tissue. The most important finding this work is the demonstration for the first time that *Acalypha multicaulis* Müll. Arg. shows spasmolytic action on guinea-pig ileum. **Financial Support:** CNPq, CAPES, LTF/UFPB.

09.101

Evaluation of the cytotoxic and spasmolytic activities of green fruits and aerial parts from *Solanum agrarium sendtner* (Solanaceae): comparative study. Correia ACC[1], Santos RF[2], Monteiro FS[2], Pessôa LFP[3], Silva TMS[4], Agra MF[2], Silva BA[2] [1]UFPB - Tecnologia Farmacêutica, [2]LTF-UFPB - Ciências Farmacêuticas, [3]UFPB - Biologia Molecular, [4]UFRPE - Químicaas

Introduction: *Solanum agrarium* Sendtner (Solanaceae) known popularly as "gogóia", and "melancia da praia", is herbaceous at subshrub, having a wide geographical distribution, occurring in Colombia, Venezuela, Caribbean Islands and Brazil (Bahia, Paraíba, Pernambuco, Piauí and Rio de Janeiro) (AGRA, M. F. Dissertação (mestrado), 1991). In folk medicine, the decoction of its roots is used as abortive. Many *Solanum* species have showed spasmolytic activity and, among them some also showed toxic activities. Phamacological studies carried out with the ethanolic extract of aerial parts from *S. agrarium* (SA_{PA}-EtOH) have showed spasmolytic activity on rat uterus and guinea-pig ileum (SANTOS R. F., Iniciados, p. 98, 2003). So, we decided to investigate possible hemolytic and spasmolytic activities of the crude ethanolic extract of green fruits from *S. agrarium* (SA_{FV}-EtOH) on rat erythrocytes and smooth muscles (rat uterus and guinea-pig ileum) and compared with those obtained with SA_{PA}-EtOH. **Methods:** Erythrocytes were isolated from blood of Wistar male rat according to the method described by Rangel et al. (1997). Total hemolysis was obtained with 1% Triton X-100 detergent and the percentage of hemolysis of the SA_{FV}-EtOH and SA_{PA}-EtOH (81, 243, 500, 750 and 1000 µg/mL) was calculated relative to this value. The tissues (rat uterus and guinea-pig ileum) were suspended in organ bath chambers containing appropriate temperature and solutions (pH 7.4) and bubbled with 95 % O_2 and 5 % CO_2 carbogen mixture. Isotonic contractions were monitored. All the experimental protocols were approved by Ethical Committee in Animal Research of LTF/UFPB (Protocol 0506/05). **Results**: SA_{FV}-EtOH presented high hemolytic activity (EC_{50} = 228.7 ± 10.16 µg/mL, n = 3), however SA_{PA}-EtOH showed moderate hemolytic activity (E_{max} = 42.9 ± 3.4% n = 3) only in the concentration of 1000 µg/mL (p < 0.05). On rat uterus, SA_{FV}-EtOH (until 500 µg/mL, n = 3) showed no significant tocolytic activity, in oxytocine- and carbachol-induced phasic contractions, presented E_{max} = 2.1 ± 1.6 and 10.1 ± 2.2%, respectively. Morever, on guinea-pig ileum, SA_{FV}-EtOH antagonized in a significant, equipotent and concentration-dependent manner the carbachol-(IC_{50} = 309.3 ± 45.80, n = 5) and histamine-(IC_{50} = 256.4 ± 29.2 µg/mL, n = 5) induced phasic contractions. The responsiveness of the uterus and ileum was recovered 15 min and 1 h, respectively, after withdrawal of the SA_{FV}-EtOH from the bath. **Discussion**: The secondary metabolites of extract SA_{FV}-EtOH are more toxic than the SA_{PA}-EtOH, since SA_{FV}-EtOH showed high damage to the membrane of rat erythrocytes. On the other hand, extract SA_{FV}-EtOH has secondary metabolites with spasmolytic activity, being most potent on guinea-pig ileum. However, the results observed by Santos (2003) showed that extract SA_{PA}-EtOH presented spasmolytic activity in a significant manner on both organs. So, we can suppose that the secondary(ies) metabolite(s) with spasmolytic activity in green fruits from *S. agrarium* are less concentrated in relation to aerial parts or are different metabolites. **Financial support**: CNPq, CAPES, LTF/UFPB.

09.102

Estudo da atividade cicatrizante de duas espécies do gênero *Lychnophora* (arnicas). Barbosa LCO, Rascado MR, Silva-Barcellos NM, Saúde-Guimarães DA, Grabe-Guimarães A UFOP - Farmácia

Introdução: As espécies do gênero *Lychnophora*, pertencentes à família Asteraceae, são muito conhecidas na medicina popular brasileira como “arnicas”, sendo utilizadas pela população como anti-inflamatórias, no tratamento de contusões e nos reumatismos [Saúde *et al*., *Fitoterapia*, v. **LXIX**, n.1, p.90, 1998]. A atividade analgésica e anti-inflamatória de espécies de *Lychnophora* foi anteriormente demonstrada em nosso laboratório [Guzzo *et al*., *J. Ethnopharmacology,* v. 116, p. 120, 2008], indicando sua potencial atividade em processos com componentes inflamatórios como a cicatrização. O objetivo do presente trabalho foi avaliar a atividade cicatrizante *in vivo* dos extratos etanólicos de *L. trychocarpha* e *L. pinaster* em modelo de feridas induzidas por queimadura. **Métodos:** Foram utilizadas 24 ratos Wistar fêmeas (180-200 g), divididas em três grupos (n=8 cada): ST=sem tratamento (controle), LT= *L. trychocarpha e* LP= *L. pinaster.* Os animais foram submetidos ao procedimento da queimadura para o estudo de cicatrização (aprovado pelo comitê de ética da UFOP nº 2007/98) e receberam tratamento diário com os extratos solubilizados em capriol a 65 %. Nos tempos 0, 3, 7, 11, 14, 21, 28, 35 e 42 dias as áreas das feridas foram mensuradas, com auxílio de um filme de transparência e do programa AutoCad 2008, para posterior análise comparativa dos porcentuais de contração da área queimada (planimetria). **Resultados:** O grupo tratado com *L. pinaster* apresentou melhor perfil de cicatrização em comparação ao grupo tratado com *L. trychocarpha* e ao grupo controle ST. Foram identificadas diferenças estatisticamente significativas (ANOVA seguida do pós-teste de Tukey) nos dias 11 (32,8±3,1% x 56,3±4,5%), 14 (46,1±2,2% x 74,1±4,5%) e 21 (63,8±3,7% x 85,8±4,3%) entre o grupo *L. pinaster* e ST, respectivamente. **Discussão:** Os resultados obtidos sugerem que as arnicas avaliadas apresentam boa atividade cicatrizante no modelo de queimadura, principalmente a *L. pinaster.* Estudos futuros avaliarão a melhor forma farmacêutica a ser utilizada, contribuindo, assim para o uso racional e direcionado destas espécies pela população brasileira. **Agradecimentos**: UFOP e FAPEMIG.

09.103

Preincubation effects of prebiotic oligosaccharides on the oxygen uptake of rat liver mitochondria. Silva GP, Schneedorf, JM UNIFAL - Ciências Exatas

Introduction: Nondigestible prebiotic foods are known as dietary components that may cause physiological effects on the consumer, leading to justifiable claims of health benefits. Although there are a large body of literature concerning to the effects of prebiotic oligosaccharides, little is known about its cellular mechanisms of action[1]. Here we describe the investigation of the potential activity of mananoligosaccharides (MOS), fructoligosaccharides (FOS), inulin (IN) and kefir growth factor (KGF) on the respiratory activity of isolated mitochondria preincubated or not with the oligosaccharides in question. **Methods:** Mitochondria were isolated from Wistar rat liver as described by Lassing and Gnaiger (2006). Organelle viability was monitored at 609nm by a methylene blue test. Samples (1200mg protein/mL) were preincubated with the prebiotic oligosaccharides for 60min before experiments to a 2mL final volume, using too samples not preincubated. The home-made Clark-type electrode containing a PTFE membrane was constructed and connected to a PG39MCSV potentiostat (Omnimetra, RJ), followed by a -600mV applied potential, and the signals were filtered using a INPF and RSF filters. The acquired data were further processed after the calibration of system. Oligosaccharide samples were added at different concentrations after medium equilibration with the mitochondrial suspensions at 50rpm stirring in in 20mM phosphate buffer pH 7.3 containing 70 mM sucrose, 1mM EDTA, 5mM MgCl2. Oxygen consuption was monitored after sequential additions of buffer, mitochondrial samples, 100mM succinate, 100µL of oligosaccharides and 100mM malonic acid during 90min. Inhibition values were determined by difference of linear gradients obtained from minimum least squares applied to data after each compound addition. Data were obtained in triplicate and analyzed by ANOVA ($p<0.05$). **Results and Discussion:** The oxygen electrode was able to identify minor differences in oxygen uptake from mitochondrial suspensions. Calibration parameters prompted a time constant of $10min^{-1}$ with a response time of 68s. Moreover, all the prebiotic oligosaccharides, preincubated or not, was able to inhibit the oxygen consumption of rat liver mitochondria. It was noted mean changes in the inhibitory capability for the oligosaccharides whenever preincubated or not with mitochondrial samples. The results for preincubated suspensions presented mean inhibitions of 94.31%, 48.24% and 45.14% for MOS (0.04%), IN (16%) and FOS (8%), respectively. On the other hand, the results for non preincubated suspensions presented mean inhibitions of 86.64%, 17.65% and 3.32% for MOS, FOS and IN respectively. The overall results suggested an impairment of mitochondrial respiratory activity in the presence of prebiotic oligosaccharides due to metabolic modulations from the outer membrane of the organelle. Acknowledgements: UNIFAL-MG and FAPEMIG. **References:** [1]BRACHT, A.; ISHII-IWAMOTO, E. Métodos de Laboratório em Bioquímica. 1 ed. São Paulo: Manole, 2003. [2]LASSNIG, B.; GNAIGER, E. Laboratory Protocol: Isolation of Rat Liver Mitochondria**.** *Mitochondrial Physiology* Network, 8.13: 1-2, 2006.

09.104

Hemolytic and spasmolytic activity of crude ethanolic extract from *Sapium obovatum* Klotzsch Ex Müll. Arg. (Euphorbiaceae). Oliveira GA[1], Alves AKA[2], Carreiro JN[2], Silva ACL[2], Santos RF[2], Correia ACC[3], Pessôa HLF[4], Tavares JF[2], Silva BA[2] [1]UFPB-LTF-CCS, [2]UFPB - Ciências Farmacêuticas, [3]UFPB - Tecnologia Farmacêutica, [4]UFPB-DBM

Introduction: Euphorbiaceae family is the sixth largest family in the world and is represented by 300 genera and about 7500 species (CRONQUIST, Columbia University Press, v. 55, 1981). *Sapium* genus has 247 species (CRONQUIST, Botanical Garden Press, p. 555, 1988). Many substances are found in genus *Sapium,* as flavonoids, coumarins, phenylpropanoids (HSU, et al., Journal of Natural Products, v. 57, n. 2, p. 308, 1994), terpenoids and mainly diterpenes of trachylobane type (OHIGASHI, et al., Agricultural and Biological Chemistry, v. 47, p. 1617, 1983). *Sapium* species have showed antimicrobial (CHUMKAEW, P. et al., Journal of Natural Products**,** v. 66, n. 4, p. 540, 2003), anti-inflammatory, analgesic and antipyretic activity (PANTHONG, et al., Planta Medica, v. 64, n. 6, p. 530, 1998). Since the secondary metabolites found in *Sapium* species are reported in the literature by present spasmolytic activity and there are no reports in the literature for investigations of this activity in *Sapium obovatum* Klotzsch Ex Müll. Arg*.,* we decided to investigate if the crude ethanolic extract from aerial parts of *S. obovatum* (SO-EtOH) shows spasmolytic activity on guinea-pig ileum. Moreover, we decided to investigate a possible hemolytic effect on rat erythrocytes, since many species of Euphorbiaceae have toxic properties. **Methods**: Erythrocytes were isolated from blood of Wistar male rat according to the method described by Rangel et al. (1997). Total hemolysis was obtained with 1% Triton X-100 detergent and the percentage of hemolysis of the SO-EtOH (81, 243 and 500 µg/mL) was calculated relative to this value. The guinea-pig ileum was suspended in organ bath containing modified Krebs solution (pH 7.4) at 37° C, gassed with 95 % O_2 and 5 % CO_2 carbogen mixture and resting tension of 1g. Isotonic and isometric contractions were monitored. All the experimental protocols were approved by Ethical Committee in Animal Research of LTF/UFPB (Protocol 0506/05). **Results:** In evaluation of cytotoxicity on rat erythrocytes, the SO-EtOH did not induce hemolysis in a significant manner. On guinea pig ileum, SO-EtOH antagonized, in a significant and concentration-dependent manner, the carbachol-(IC_{50} = 10.8 ± 1.5 µg/mL, n = 5) and histamine-(IC_{50} = 3.1 ± 0.2 µg/mL, n = 5) induced phasic contractions, being approximately 3.2 folds more potent to histamine. Morever, SO-EtOH relaxed the guinea-pig ileum pre-contracted by KCl (EC_{50} = 1.8 ± 0.5 µg/mL n = 4) or carbachol (EC_{50} = 0.6 ± 0.2 µg/mL n = 5), in a significant and concentration-dependent manner, being approximately 3 folds more potent to carbachol. **Conclusion:** As the erythrocyte is very susceptible to hemolysis, the absence of hemolytic activity of SO-EtOH suggests that the extract probably does not present chemical constituents able to lyse others cells. However, SO-EtOH shows secondary metabolites with potential spasmolytic action on guinea-pig ileum. These results are inedited, contributing for the pharmacological study of *S. obovatum* Klotzsch Ex Müll. Arg. **Supported by:** CNPq, CAPES, LTF/UFPB.

09.105

Role of proteolytic activity and cell proliferation on gastric healing activity of latex fraction from *Carica candamarcensis.* Silva ACA[1], Lemos FO[1], Viana CTR[1], Figueiredo C[1], Souza CM[2], Cassali GD[2], Salas CE[3], Lopes MTP[1] [1]UFMG - Farmacologia, [2]UFMG - Patologia Geral, [3]UFMG - Bioquímica e Imunologia

Introduction: Previous results from our group demonstrated that a fraction from *C candamarcensis* latex, containing cysteine proteinases (P1G10), displays gastric protective and healing activities, evaluated in acute and chronic gastric lesions in rodent models (Mello et al. *Phytomedicine*, 15(4):237-244, 2008). In this study, we investigated the importance of the proteolytic activity of P1G10 on its gastric healing activity and on other events in this process, such as, cell proliferation and angiogenesis. **Methods:** Gastric lesions were induced on female Wistar rats (180-200g) with acetic acid (Takagi et al. *Jap J Pharmacol*, 19(3):418-426, 1969). After 24 h, animals were divided in groups (n=5) and the treatment (v.o.) was initiated with distilled water (negative control), 10 mg/kg P1G10, 10 mg/kg P1G10 inhibited by iodoacetamide (P1G10-IAA) or, 10 mg/kg trypsin. After 8-days, animals were sacrificed and their stomachs removed to measure the ulcer area followed by fixation of each sample with 10% formalin for histological analysis by HE staining and immunohistochemistry. In this study, PCNA (dilution 1:200) and PECAM/CD31 (dilution 1:20) were used as markers of cell proliferation and angiogenesis, respectively. Results were expressed as mean ± SEM of the ulcer area (mm²), percentage of cells in proliferation and number of vessels/field. Statistical Analysis: ANOVA, Student-Newman-Keuls post-test. Protocol was approved by Local Ethics Committee: CETEA 215/07. **Results and discussion:** We observed that P1G10 displays gastric healing activity, demonstrated by 58% reduction the ulcer area (2.50 ± 0.79 mm^2 – $p < 0.05$) compared to the control group (5.98 ± 0.85 mm^2). The treatments with P1G10 IAA (5.90 ± 1.09 mm^2 – $p > 0.05$) and trypsin (6.95 ± 0.60 mm^2 – $p > 0.05$) showed no ulcer healing activity, evidencing the requirement of the proteolytic activity for the healing effect. The healing property of P1G10 was supported by histological analysis showing a significantly thicker regenerative mucosa and a substantial difference in the organization of granulation tissue in animals treated with P1G10, in relation to the control or P1G10-IAA groups. The immunohistochemical analysis revealed that cell proliferation (22.62 ± 3.89% - $p <0.05$) was strongly stimulated by the treatment with P1G10 when compared to the control group (6.22 ± 1.10 %). With regard to angiogenesis, no significant difference was observed between P1G10 (5.26 ± 0.45 vessels/field – $p > 0.05$) and the control group (3.91 ± 0.86 vessels/field). No significant differences in cell proliferation or angiogenesis were observed between P1G10 (22.62 ± 3.89%; 5.26 ± 0.45 vessels/field) and P1G10-IAA (24.20 ± 5.62%; 5.04 ± 0.38 vessels/field – $p > 0.05$), suggesting that for these effects the proteolytic activity is not relevant. **Conclusion:** The results suggest that the proteolytic activity of P1G10 is important to its gastric healing effect. The cell proliferation stimulus, but not an angiogenic action, seem to be involved in the healing action of P1G10; however, is not influenced by its proteolytic activity. **Financial Support:** CNPq and FAPEMIG.

09.106

Inhibitory effects of *Garcinia gardineriana* and GB2A on tyrosinase activity. Prudente AS[1], Delle Monache F[2], Cechinel-Filho V[3], Cabrini DA[1], Otuki MF[4] [1]UFPR - Farmacologia, [2]UIN - Farmacologia, [3]NIQFar-UNIVALI , [4]UEPG - Ciências Farmacêuticas

Introduction: The major rate limiting step in melanin biosynthesis involves the enzyme tyrosinase (Nesterov et al., 2008). The tyrosinase, a copper containing binuclear enzyme catalyzes three steps of melanin biosynthesis: the hydroxylation of tyrosine to 3,4-dihydroxyphenylalanine (DOPA), oxidation of DOPA to DOPAquinone, and oxidation of 5,6-dihydroxyindole to indolequinone. Because of its key role in melanogenesis, tyrosinase is an attractive target in the search for various kinds of depigmenting agents (Briganti et al., 2003; Solano et al., 2006). The *Garcinia gardneriana* (GG) tree is native from Amazonnic region, though it grows all over Brazil. In a population from southern Brazil, this tree is one option for treating inflammation problems, especially those of the skin, as well as pain and urinary tract and other infections (Guimarães et al., 2004 Castardo et al., 2008). Was isolated from this plant several biflavonoids, one known as GB2a which was also used in our study (Cechinel Filho et al.,2000). Works using the same plant family indicate a possible action the plant under depigmenting study (Masuda et al., 2005; Okunji et al., 2007), justifying this work. **Methods:** We used ten microliters of hydroalcoholic extract of GG and GB-2a in different concentration (0.03-2.1mg/ml) and 20µl of mushroom tyrosinase (500 U/ml) in a 50mM phosphate buffer (pH 6.5), were added to 170µl of an assay mixture containing a 10:10:9 ratio of 1mM L-tyrosine or 1mM L-DOPA solution, or in different concentrations (0,25-2mM), 50mM potassium phosphate buffer (pH 6.5), and distilled water in a 96-well microplate. The samples dissolved in EtOH were subsequent diluted with H2O prior to the experiments. After incubation of the reaction mixture at 37 °C for 40 min or 40 s substrate dependent, the absorbance of the mixture was measured at 490nm using a TECAN Genius Pro plate reader equipped with automatic injectors. The extent of inhibition from the samples was expressed as the concentration necessary for 50% inhibition (IC50) and maxima inhibition (Imax). The results are presented as mean ± S.E.M. The statistical significance between the groups was assessed by means of one-way analysis of variance (ANOVA) followed by post-hoc Newman–Keuls or Bonferroni's test. The accepted level of significance for the tests was $P<0.05$. **Results:** The extract of GG was able to obtained a Imax of 31,33% using as substrate L-tyrosine at a dose of 1200 µg/ml with a IC50 of 2388µg/ml and for GB2a the Imax was 29,87% at a dose of 300µg/ml and IC50 of 546,26µg/ml. Using L-DOPA as substrate was obtained the following results, for GG an Imax of 14,99% and of 42,83% for GB2a and IC50 of 514,15 µg/ml. Already in enzyme kinetics can be observed both by a large inhibition of GG and GB-2a in manner dependent dose. These compounds also inhibited the activity of the enzyme tyrosinase in B16 melanoma cells. **Discussions:** The present study demonstrated that GG and its component GB-2a, have tyrosinase inhibitory effects. The results suggest that GG and GB-2a have potential roles as novel skin-whitening agents for ultraviolet-sensitive skin. However, in order to clarify their beneficial/harmful effects *in vivo*, this aspect should be investigated further.

09.107

Vasodilator effect induced by essential oil of *Lippia microphylla* Cham. in rats. Araujo IGA[1], Silva DF[2], Albuquerque JGF[1], Gomes MAS[1], Nóbrega JV[1], Dias KLG[2], Cavalcante KVM[1], Veras RC[1], Tavares JF[1], Silva MS[1], Correia NA[2], Medeiros IA[1] [1]LTF-UFPB, [2]LTF-UFPB Fisiologia e Patologia, [3]LTF-UFPB/Fisiologia e Patologia, [4]UFPB - Fisiologia e Patologia

Introduction: The genus Lippia (Verbenaceae) has yielded a great number of medicinal and economically important species that are frequently used in folk medicine for treatment of several diseases, such as: coughs, bronchitis, indigestion, liver disorders and hypertension. Generally, essential oil or phenolic compounds obtained from these plant extracts are assumed to contain bioactive molecules. *L. microphylla* Cham. is a plant of the genus Lippia found in the northeast of Brazil, and little is known about the cardiovascular action of *L. microphylla* Cham. Therefore, we studied the effects of essential oil of *L. microphylla* Cham. (EOLM) in rat superior mesenteric artery rings, emphasizing the participation of Ca^{2+} influx in the responses observed. **Methods:** Isolated superior mesenteric rings were mounted in organ baths and the isometric tension changes were measured. Calcium current was recorded using the whole-cell configuration of the patch-clamp technique in freshly dissociated vascular myocytes isolated from rat superior mesenteric artery. All procedures were in compliance with Animal Research Ethics Committee. **Results:** Isometric tension recording in isolated superior mesenteric rings revealed that EOLM (1–300 µg/mL) caused concentration-dependent relaxation in mesenteric rings, without functional endothelium, pre-contracted with 10 µM phenylephrine [EC_{50}=23.5 (21.5-25.7 CI) µg/mL, n=6] or KCl 80 mM [EC_{50}=23.3 (17.9-30.2 CI) µg/mL, n=6]. EOLM (10, 30, 100 µg/mL) also attenuated Ca^{2+} -induced vasoconstriction in a concentration-dependent manner in Ca^{2+} -depleted/high K+ -depolarized mesenteric segments. Furthermore, EOLM antagonized the contractions elicited by the L-type Ca^{2+} channel activator, S(-)-Bay K 8644 [EC_{50}=36.7(31.6-42.5 CI) µg/mL, n=6], indicating that the vasodilatation is related to the inhibition of Ca^{2+} influx through L-type voltage-dependent calcium channels. To confirm this hypothesis, whole-cell L-type Ca^{2+} currents were recorded in freshly dispersed rat mesenteric artery myocytes and characterized using 20 mM Ba^{2+} ions as charge carrier. OELM (1-30 µg/mL) significantly inhibited L-type Ca^{2+} currents in a concentration-dependent manner [EC_{50}=11.9 (9.4-15.0 CI) µg/mL, n=4 for each concentration]. **Conclusion:** These results suggest that OELM induce vasorelaxant effect in isolated rat mesenteric artery due to the inhibition of the Ca^{2+} influx via L-type Ca^{2+} channels. **Financial support**: CNPq/CAPES/ FAPEMIG. The electrophysiological experiments were carried out in the Cardiovascular Biology Laboratory at Universidade Federal de Minas Gerais.

09.108

Avaliação da atividade antitumoral da fração proteolítica do látex de *Carica candamarcensis* Hook F. 1875 e a ação de leucócitos em resposta ao LPS em animais portadores do carcinoma de Ehrlich sólido. Stehling LFO[1], Viana CTR[1], Braga AD[1], Miranda JP[1], Klein A[1], Lopes MTP[1], Salas CE[2] [1]ICB-UFMG - Farmacologia, [2]ICB-UFMG - Bioquímica e Imunologia

Introdução: O tumor de Ehrlich é uma neoplasia que ocasiona ao hospedeiro uma imunossupressão devido à associação deste com o sistema hematopoiético. Nosso grupo vem demonstrando que a fração proteolítica (P1G10) proveniente do látex de *Carica candamarcensis*, apresenta atividade antitumoral sobre tumor ascítico do carcinoma de Ehrlich e de melanomas murinos. Neste trabalho visou-se avaliar o efeito antitumoral da P1G10 e a implicação na migração de leucócitos frente ao estímulo de LPS em animais implantados com tumor sólido de Ehrlich. **Métodos:** Camundongos *Swiss* fêmeas (8 semanas, n=40) foram inoculados *s.c.* no flanco com células tumorais de Ehrlich ($5x10^6$ células/animal) e divididos em 4 grupos para a avaliação da atividade antitumoral. Após 8 dias do inoculo os animais foram tratados *s.c.* durante 30 dias com PBS ou P1G10 (1, 3 ou 5 mg/kg), sacrificados em câmara de CO_2 e então os tumores foram retirados para avaliação da massa e do volume tumoral. Para avaliar a migração leucocitária, camundongos *Swiss* fêmeas (8 semanas, n=48) foram divididos em 2 grupos recebendo inóculo *s.c.* de células tumorais de Ehrlich ($5x10^6$ cél./animal) ou 100 µL *s.c.* de PBS, 15 dias após a inoculação os animais foram pré-tratados *s.c.* com PBS ou P1G10 (1 mg/kg) 1 hora antes da administração intrapleural de lipopolissacarídeo A (LPS, 10 µg/cavidade) e quatro horas após, sacrificados para coleta do sangue periférico e avaliação das contagens total e diferencial dos leucócitos presentes. (Protocolo CETEA/UFMG n.º 090/09) **Resultados e conclusão:** O tratamento com P1G10 (3 mg/kg) reduziu de forma significativa a massa e o volume tumoral em relação ao controle (massa: PBS 0,91 ± 0,26g; P1G10 - 3 mg/kg 0,29 ± 0,32g, $p<0,05$ ANOVA/Dunnet; volume: PBS 1,73 ± 0,40mL; P1G10 - 3 mg/kg 0,56 ± 0,58mL, $p<0,05$ ANOVA/Dunnet). P1G10 (1 mg/kg) reduziu a quantidade total de leucócitos presentes no sangue periférico nos animais portadores do tumor (PBS+PBS 10,2 ± 14,0 x $10^6/mm^3$; P1G10+LPS 7,3 ± 1,6 x $10^6/mm^3$; PBS+LPS 13,7 ± 6,8 x $10^6/mm^3$, inibição de 46 %) e em animais sem tumor (PBS+PBS 8,0 ± 1,7 x $10^6/mm^3$; P1G10+LPS 4,5 ± 2,4 x $10^6/mm^3$; PBS+LPS 12,8 ± 0,7 x $10^6/mm^3$, inibição de 65 %). Estes resultados demonstram a atividade antitumoral de P1G10 e parecem consistentes em demonstrar a relação entre leucócitos periféricos de animais tratados ou não com P1G10, em resposta ao LPS. **Apoio Financeiro:** CNPq, FAPEMIG e CAPES.

09.109

Evaluation of the vasorelaxant effect induced by *Erythroxylum pungens* in rat superior mesenteric arteries. Oliveira AC, Mendes-Junio L, Anjos RM, Furtado FF, Medeiros AAN, Sena Filho JG, Barbosa Filho JM, Medeiros IA UFPB - Tecnologia Farmacêutica

Introduction: *Erythroxylum pungens* (Erythroxylaceae) species unique to northeast Brazil, found in the states of Bahia, Ceará, Maranhão, Pernambuco and Piauí. Various species of the genus *Erythroxylum* have been used in the popular medicine as aphrodisiac, central nervous system stimulant, antihelmintic, among others. The aim of this study was to investigate the vasorelaxant effect induced by EEEP (ethanolic extract from the leaves of *Erythroxylum pungens*) in rat superior mesenteric rings. **Methods:** Rat superior mesenteric rings (1-2 mm) were suspended by cotton threads for isometric tension recordings in Tyrode´s solution, 37ºC, gassed with 95% O2 and 5% CO2, resting tension 0.75 g. **Results and Discussion**: In intact rings pre-contracted with 10 µM Phe, EEEP (0.001-500 µg/ml) induced a marked relaxant effect in a concentration manner (EC50=13,78 ± 5,48 mg/ml, Emax.= 91,94 ± 2,63%). After endothelium removal this effect was not changed (EC50=27.25 ± 4.75 mg/ml, Emax.= 88.28 ± 3.33 %). In endothelium denuded rings, pre-contracted with KCl80 mM, EEEP elicited concentration-dependent relaxation (EC50= 128,18 ± 11,2 µg/ml, Emax.: 76,80± 3,49%). Moreover, in a depolarized medium, EEEP (30-500 µg/ml) inhibited the contractions induced by $CaCl_2$, inducing a rightward shift of the concentration-response curves. The contraction induced by the L-type Ca_{2+} channel agonist S(-)-Bay K 8644 was antagonized, in a concentration dependent manner, by EEEP (CE50= 80,24 ±17,13mg/ml, Emax.= 86,36 ± 13,64%), suggesting that the relaxant activity induced by EEEP involves the inhibition of Ca_{2+} influx through voltage-operated Ca_{2+} channels. Furthermore, in calcium-free media, EEEP (100, 300, 500 µg/ml) inhibited transient contractions induced by Phe (10µM), showing that the inhibition of IP3 sensitive intracellular calcium stores, probably contributes to the vasorelaxant effect induced by EEEP. These results together, suggests that EEEP induces vasorelaxation which may be related to a reduction in [Ca2+]i in vascular smooth muscle cells. CEPA: 0305/07. Financial Support: CAPES

09.110

Effect of the essential oil of *Lippia alba* and the methanolic extract of *Condalia buxifolia* in the transport of silver catfish, *Rhamdia quelen*, juveniles. Becker AG[1], Parodi TV[1], Baldisserotto B[1], Heinzmann BM[2], Morel, A. F.[3], Maldaner G[3], Cunha MA[1], Gomes DP[1]
[1]UFSM - Fisiologia e Farmacologia, [2]UFSM - Farmácia Industrial, [3]UFSM - Química

Introduction: The use of anesthetics during juvenile fish transportation is widely used to reduce stress and mortality. Therefore, the objective of this study was to verify the effect of the essential oil of *Lippia alba* (Mill.) N.E. Brown and the methanolic extract of *Condalia buxifolia* Reissek in the water of transport of silver catfish juveniles. **Methods:** Fish (420.1±8.8g; 21.2±2.3cm) were transported at a load density of 100.6 g/L for 6h in fifteen plastic bags (20 L), divided in five treatments (three replicates by treatment): control, exposed to 30 or 40 µg/L essential oil of *L. alba*, and exposed to 5 and 10 µg/L methanolic extract of *C. buxifolia.* Before transportation fish were exposed to the essential oil of *L. alba* (200µg/L for three minutes) or the methanolic extract of *C. buxifolia* (10µg/L for five minutes). Water samples were collected before and after transport to verify water quality parameters and net Na^+, K^+, and Cl^- flux rates. After transport blood was collected with heparinized syringes and arterial pH (pH*a*), oxygen and carbon dioxide partial pressure (*Pa*O_2 and *Pa*CO_2, respectively) were measured with Roche OMNIC. **Results and Discussion:** There was no significant difference on alkalinity, hardness, temperature, pH, dissolved oxygen, and unionized ammonia between treatments after transporting silver catfish. However, carbon dioxide and total ammonia levels were significantly higher in the control treatment (84.3±1.2 and 3.9±0.2 mg/L, respectively) compared to the other treatments (30µg/L *L. alba*: 66.5±2.2 and 3.3±0.1 mg/L, 40µg/L *L. alba:* 77.4±2.2 and 3.0±0.1 mg/L, 5µg/L *C. buxifolia*: 73.8±2.0 and 2.3±0.2 mg/L, and 10µg/L *C. buxifolia*: 70.5±3.8 and 2.8±0.2 mg/L). In addition, there were significantly higher Na^+, K^+ and Cl^- efflux rates in the fish of the control treatment compared to the other groups. Moreover, blood analyses showed a significant increase of *Pa*O_2 in the treatment with *C. buxifolia* (5µg/L = 39.1±3.4 mmHg), a significantly lower *Pa*CO_2 at both *L. alba* concentrations (30µg/L = 21.5±2.1mmHg and 40µg/L = 22.1±2.7 mmHg) compared to other treatments. Blood pH was similar among all the treatments. Consequently, the essential oil of *L. alba* and the methanolic extract of *C. buxifolia* probably reduced the stress of the fish transport because they reduced carbon dioxide and ammonia excretion, as well as ion loss. The results allow concluding that the use of these substances in the fish transport improves animal welfare. **Acknowledgments and Financial Support:** CNPq, CAPES.

09.111

Neutralization of hemorrhagic activity induced by *Bothrops pauloensis* snake venom by *Schizolobium parahyba* extract and antivenins. Vieira SAPB[1], Lucena MN de[2], Hamaguchi A[1], Rodrigues V M[1], Mendes MM[1], Homsi-Brandeburgo MI[1] [1]UFU Genética e Bioquímica, [2]UFU - Biologia

Local tissue damage is the mainly problem of *Bothrops* snake venoms accidents.Antivenins are used to treat the snake bites, however they are limited to neutralization of local tissue damage. Nowadays, many medicinal plants have been recommended for the treatment of snakebites. The present study compares the efficacy of aqueous extract from *Schizolobium parahyba* (S.p) and antivenins to neutralize hemorrhagic activity induced by *Bothrops pauloensis* (B.p) venom. The neutralization the hemorrhage activity was evaluated by inoculation to S.p and Antivenins by different routes after 15 or 30 min of B. p injection. All procedures were in accordance with the rules of the Ethics Committee on Use of Animals under the number 030/08. Swiss male mice were distributed in 10 groups: *G1*- B.p; *G2*- S.p; *G3*- PBS; *G4*- antivenins; *G5*- B.p + antivenins (1:1.8, w/w, after 15 min), *G6*- B.p + antivenins (1:1.8, w/w, after 30 min); *G7* - B.p + antivenins + S.p (1:1.8:50, w/w/w, 15 min), *G8*- B.p + antivenins + S.p (1:1.8:50, w/w/w, after 30 min), *G9*- B.p + antivenins + S.p (1:1.8:100 w/w/w, after 15 min); *G10*- B.p + antivenins + S.p (1:1.8:100 inhibited by S.p when it was associated with antivenins at ratio 1:1.8:100 (w/w/w, after 30min). The inhibition of hemorrhagic activity, swiss male mice were injected intradermically in the back with dose the 16 µg of venom. After 3 h, the mice were killed and skin of the back was removed and the halo was measured. Hemorrhagic activity was expressed by the mean (in mm) of the hemorrhagic halos induced by venoms in the absence and presence of the plants. Aqueous extract from *Schizolobium parahyba* inhibition significantly the hemorrhagic activity in the ratios 1:1,8:100(w/w/w) after 15 minutes. However, only the treatment with serum is not able to reduce hemorrhagic halo. Ours results indicate that the aqueous extract of the leaves from *Schizolobium parahyba* and serum therapy, contains compounds capable to neutralize activities induced by bothrops venoms. Supported by: CAPES, CNPq and FAPEMIG

09.112

Efeito de uma fração rica em proantocianidinas (FRP) obtida a partir das cascas da *Croton celtidifolius* sobre a aterosclerose *in vivo*. Netto PM[1], Schulz T[1], Hort MA[1], Horst H[2], Pizzolatti MG[2], Ribeiro-do-Valle RM[1] [1]UFSC - Farmacologia, [2]UFSC - Química

Introdução: Aterosclerose é uma doença crônica progressiva caracterizada pela formação de placas fibro-gordurosas arteriais, que podem levar a uma lesão isquêmica. A *Croton celtidifolius* é uma planta nativa das regiões de Mata Atlântica e possui como principais constituintes as proantocianidinas. Estudos anteriores utilizando uma fração (FRP) obtida a partir das cascas dessa planta, demonstraram atividades antiedematogênica, anti-inflamatória, antioxidante, antinociceptiva e vasorrelaxante. Este trabalho teve como objetivo avaliar o potencial antiaterogênico da FRP obtida das cascas da *Croton celtidifolius* em camundongos knockout para o receptor de lipoproteínas de baixa densidade (LDLr -/-) submetidos à uma dieta hipercolesterolêmica (DH). **Métodos**: Camundongos machos C57BL/6 (LDLr -/-) com 12 semanas de idade foram divididos em 5 grupos (n=7-9): C (Controle com Dieta Normal + veículo); CH (Controle com DH + veículo); FRP3 (DH + FRP 3 mg/kg); FRP10 (DH + FRP 10 mg/kg); e FRP30 (DH + FRP 30 mg/kg). Os animais foram tratados por via oral, uma vez ao dia, durante 30 dias. Ao final do tratamento foram avaliados: peso, ingestão alimentar, lipídeos plasmáticos (colesterol total, LDL + VLDL, HDL e triglicerídeos) e reatividade vascular em anéis de aorta torácica isolada. A reatividade vascular foi avaliada através da realização de curvas concentração resposta cumulativas à fenilefrina (agonista □-adrenérgico) e acetilcolina (agonista colinérgico). Os protocolos experimentais foram aprovados pelo Comissão de Ética no Uso de Animais da Universidade Federal de Santa Catarina (nº PP00225). Os resultados foram expressos como média ± erro padrão da média. **Resultados**: A dieta hipercolesterolêmica foi capaz de aumentar significativamente os níveis de colesterol total nos animais LDLr -/- (C,231,60±18,60; CH, 973,96±29,18 mg/dL), entretanto o tratamento com FRP nas 3 doses utilizadas não alterou estes níveis. Da mesma forma os níveis de lipoproteínas plasmáticas (LDL + VLDL e HDL) e triglicerídeos não foram alterados pelo tratamento com a FRP em relação ao grupo CH. Foi possível verificar que a dieta rica em lipídeos promoveu um prejuízo da contração induzida pela fenilefrina (C,0,09±0,017%; CH, 0,03±0,01%) e do relaxamento por acetilcolina em anéis de aorta torácica isolada (C, 81,54±4,83%; CH, 53,88±5,56%), em relação ao grupo C. O tratamento com a FRP, não promoveu alterações na contração induzida pela fenilefrina mas foi capaz de aumentar significativamente o relaxamento máximo induzido pela Ach na dose de 30 mg/kg (C, 81,54±4,83%; CH, 53,88±5,56%; FRP3, 52,9±5,89%, FRP10, 69,50± 5,255%, FRP30, 83,37±3,82%). **Discussão**: Os resultados demonstram que a FRP obtida a partir da *Croton celtidifolius* não foi capaz de alterar os níveis de lipídeos plasmáticos nos animais que receberam uma dieta rica em lipídeos. Entretanto, o tratamento com esta fração na maior dose promoveu um aumento na vasodilatação dependente do endotélio. Estas propriedades moduladoras da função endotelial podem contribuir para a prevenção do processo aterosclerótico em camundongos LDLr -/-. **Apoio Financeiro**: CNPq, Finep, Fapesc

09.113

Cytotoxic activity of caatinga plants: a random approach. Melo JG[1], Rodrigues MD[2], Amorim ELC[3], Nascimento SC[2], Albuquerque UP[1] [1]UFRPE - Botânica, [2]UFPE - Antibióticos, [3]UFPE - Farmácia

Introduction. Plants have been an important source of molecules with pharmacological effects. In the case of cancer, many molecules that directly or indirectly come from plant species are being used on official medicine (Cragg & Newman. Journal of Ethnopharmacology, v. 100, p. 72, 2005). As an example, the species *Catharanthus roseus* (Apocynaceae) has numerous alkaloids like vincristine and vimblastine, which have attested activity against malignant neoplasms. Although superior plants are an important source of bioactive compounds, few surveys have been carried out with caatinga plants in the search of new molecules with antitumoral action. This research aimed to perform *in vitro* essays in order to evidence caatinga plant species with antitumoral potential for further studies. **Methods**. Based on a floristic inventory on anthropogenic zones (with the plot technique; 300 plots of 1m² each) 20 species were randomly selected in an area of caatinga in the country side of Pernambuco. After drying, ten grams of leaves (trees and shrubs) or aerial parts (herbs) of plants were milled and taken in contact to 100mL of methanol for 24 hours for three consecutive times. After that the material was filtered with a filter paper and the solvent removed by reduced pressure. Lineages used were NCI-H (lung cancer) and HEP-2 (laryngeal cancer). After 72h of cell contact with 50µg/ml of the trial product, 25µl of MTT bromide (3-[4,5-dimethylthiazol-2-il]-2,5- diphenyltetrazolium) was added to each case. He trays were left in the hothouse for two hours (37°C). To assess the inhibition rate, optical reading was performed in an automatic microstrip reader in 595nm. Average optical density (OD) of the cases was compared with the means of control cases. **Results and Discussion.** From the twenty species, those which had the best cytotoxic activity (mean in %) were *Mentzelia aspera* L. (Loasaceae) 53.27±5.88; *Delilia biflora* (L.) Kuntze (Asteraceae) 40.19±6.39; and *Ocimum campechianum* Mill. (Lamiaceae) 37.85±5.32, all against HEP-2. Although the above cited results are still preliminary, we believe that plant diversity in caatinga can contribute with interesting molecules for further studies. Therefore, the next steps of the research are: proceed with the preparation of extracts with different solvents; perform the screening against other cancer cell lineages and select more caatinga species with other approaches, like the ethnopharmacological, chemosystematic and chemical ecology. In the end of the research we hope to offer a list of caatinga species which are candidates for more detailed studies.

Support: CAPES; CNPq.

09.114

Isobrucein B diminish the hypernociception and neutrophil tissue infiltration induced by carrageenan. Talbot J[1], Vieira SM[2], Pinto LG[1], Cunha TM[1], Lemos HP[1], Amorim RCN[3], Silva ECC[3], Pohlit AM[3], Cunha FQ[1] [1]FMRP-USP - Farmacologia, [2]FMRP-USP/INPA, [3]CPPN-INPA

Introduction. The isolation of quassinoid compounds isobrucein B from *Picrolemma sprucei* has been described previously by Moretti in 1982. Our laboratory had demonstrated the anti-inflammatory and gastro-protective activities of isobrucein B (IsoB) in mice. In the present study, we investigated the antinociceptive effect of IsoB associated with the reduction of neutrophil migration. **Methods.** C57Bl/6 mice weighing 18-23 g were used. The animals were treated with IsoB (0,5, 1, 5 mg/kg) via intraperitoneal (i.p.) 30 minutes before the challenged. Paw inflammation was induced by intraplantar injection of carrageenan (carrageenan 100ug/25ul saline) and hypernociception was evaluated using an electronic version of the von Frey test. Granulocyte infiltration in paw was determined by myeloperoxidase. The experimental arthritis model in mice was induced by immunizing the animals with methylated bovine serum albumin (mBSA) and complete Freud´s adjuvant through subcutaneous (s.c.) injection. Twenty-one days after the initial injection, arthritis was induced in the immunized mice by intra-articular (i.a.) injection of mBSA dissolved in PBS. Recruitment was assessed directly in knee joint exsudate. Neutrophil chemotaxis to IL-8 or fMLP was made for cells incubated or not with IsoB. This study was approved by Animal Ethics Committee of FMRP/USP (nº. 127/2008) **Results.** IsoB induced a dose-dependent decrease of paw hypernociception (1,067$\pm$0,1764 g; $p<0,0001$; $F=76,65$) that was associated with diminution of neutrophil infiltration in tissue (2104$\pm$749,9 neutrophil/mg tissue; $p<0,0001$). In addition, articular cavity of mBSA challenged immunized mice treated with IsoB demonstrated a dose-dependent reduction in neutrophil recruitment when compared with non-treated mice (0,8925$\pm$0,229 neutrophil x 10^6/cavity; $p<0,0001$). IsoB modulate neutrophil chemotaxis response to IL-8 (13,25$\pm$0,2363 cells/field; $p<0,0001$) but not to fMLP. **Discussion.** These results suggest that IsoB can reduce the intensity of hypernociception, interfering in the neutrophil migration to endogenous stimulus like IL-8, and reducing the neutrophil infiltration. This natural substance has potential as new medication for treatment of inflammatory diseases. Moretti C. Tetra. Lett 23:647. (1982); Vieira, S. M. Nature Chemistry. *submitted* (2009). FAPEAM, CNPq/PNOPG&PPG-7, CAPES and FAPESP

09.115

Triagem farmacológica comportamental de uma fração de alcalóides de *odontocarya Acuparata* miers em camundongos. Nora DE[1], Hofmann Junior AE[2] [1]URI - Farmácia, [2]URI - Ciências da Saúde

Introdução: A família Menispermaceae apresenta grande relevância científica devido às atividades farmacológicas identificadas e as potencialidades que apresenta, estes efeitos ocorrem pela presença de alcalóides em seus exemplares. No ocidente, os estudos iniciaram com a descoberta do uso do preparado “curare” pelos índios de Bacia Amazônica do qual posteriormente foi utilizada a tubocurarina em processos cirúrgicos. Exemplares da família demonstraram efeitos sobre o SNC e seguindo esta análise o vegetal *Odontocarya acuparata* Miers (Menispermaceae) foi estudado. **Métodos**: O vegetal foi coletado, macerado e por extração, pH dependente, com solventes orgânicos obtida a fração enriquecida em alcalóide denominada CH_2Cl_2-B. Para a triagem desta fração sobre o comportamento foram utilizados camundongos divididos em 3 grupos de 5 animais cada, grupo controle (solução fisiológica), grupo 1 (extrato a 15 mg/kg) e grupo 2 (extrato a 45 mg/kg). A administração foi realizada por via intraperitoneal e após os animais ficaram sob observação de 4 horas em caixas de contenção diferentes das que estavam habituados. O estudo foi aprovado pelo comitê de ética da URI- Campus de Erechim (016/TCC/09). Resultados e Discussão: Verificou-se no grupo 2 diminuição na atividade de ambulação, diminuição de elevações dos membros anteriores, ptose palpebral e maior período de imobilidade. Os resultados apontam para a presença de atividade depressora na dose 45 mg/kg. Este estudo representa a primeira avaliação das potencialidades farmacológicas de *Odontocarya acuparata*, sugere avanços em modelos animais para depressão do SNC e o isolamento/ identificação de alcalóides responsáveis pelas ações verificadas. Apoio financeiro URI - Campus de Erechim.

09.116

The flavonoid quercetin diminishes inflammatory hypernociception in mice by preventing oxidative stress and cytokine production. Valério DA[1], Georgetti SR[2], Magro DA[3], Casagrande R[4], Cunha TM[5], Moura-de-Carvalho FT[6], Vieira SM[7], Fonseca MJ[2], Ferreira SH[5], Cunha FQ[5], Verri WA, Jr[8] [1]UFTM/FMTM, [2]FCFRP-USP - Ciências Farmacêuticas, [3]USP - Farmacologia, [4]UEL - Ciências Farmacêuticas, [5]FMRP-USP - Farmacologia, [6]FORP-USP - MEF, [7]COPE-INPA, [8]UEL - Ciências Patológicas

Introduction: Flavonoids are polyphenolic compounds of which quercetin (3,5,7,3´,4´-pentahydroxyflavone) is the major representing member because it has all active structures of this group. Its biological activities include antioxidant and antinociceptive effects. However, the mechanism involved in its antinociceptive effect is not fully elucidated. Cytokines and reactive oxygen species have been involved in the cascade of events implicated in the genesis of inflammatory pain. Therefore, in the present study we evaluated the antinociceptive mechanism of quercetin focusing on the role of cytokines and oxidative stress. **Methods:** Mechanical hypernociception was evaluated in carrageenan, cytokines, PGE_2 and dopamine injected hindpaws (subcutaneous route) using an electronic version of von Frey test. Overt pain-like behavior was quantified by the number of abdominal contortions (acetic acid or phenyl-p-benzoquinone tests, intraperitoneal route) or paw flinches (formalin test, subcutaneous plantar route). Cytokines, neutrophil migration and reduced gluthatione (GSH) were evaluated by ELISA, MPO activity and fluorescence, respectively in cutaneous skin paw tissue samples. Experiments were performed in male Swiss mice (n=5 per group) of 25-30g. The Ethics Committee on Animal Research of the Faculty of Pharmaceutical Sciences of Ribeirão Preto-USP approved this study (no. 04.1.950.53.5). **Results and discussion:** The intraperitoneal pretreatment (30 min) with quercetin dose-dependently inhibited inflammatory nociception induced by acetic acid, phenyl-p-benzoquinone, the second phase of formalin and also carrageenin hypernociception (up to 88, 82, 90 and 58%, respectively at 100 mg/kg). Quercetin (100 mg/kg) also inhibited the hypernociception induced by cytokines (TNFalpha, IL-1beta and CXCL1; 43%, 45% and 38%, respectively), but not of inflammatory mediators that directly sensitize the nociceptor (PGE_2 and dopamine; not altered). Quercetin did not affect carrageenin- or cytokine (TNFalpha and CXCL1)-induced leukocyte recruitment (not altered). On the other hand, quercetin reduced carrageenin-induced IL-1beta production (36%) as well as abolished carrageenin-induced decrease of GSH levels. Conclusion: Quercetin exerts its antinociceptive effect by inhibiting pro-nociceptive cytokine (IL-1beta) production- and the oxidative imbalance mediation of inflammatory pain. Financial support: CNPq, FAPESP, CAPES and Fundação Araucária.

09.117

Efeito do tratamento com *Lithothamnion calcareum* na resposta inflamatória associada ao GVHD em camundongos. Rezende B[1], Castor MGM[2], Bernardes, PTT[3], Silva AFC[3], Resende BC[4], Vieira AT[4], Arantes RME[5], Teixeira MM[4], Pinho V[4] [1]ICB-UFMG - Bioquímica e Imunologia / Morfologia, [2]UFMG - Fisiologia e Farmacologia, [3]ICB-UFMG - Morfologia, [4]ICB-UFMG - Bioquímica e Imunologia, [5]UFMG - Patologia Geral

Introdução: A Doença do Enxerto-Versus Hospedeiro (GVHD) é a principal limitação para o sucesso do transplante alogênico de medula óssea. A GVHD se inicia quando linfócitos T enxertados reconhecem aloantígenos do hospedeiro, ocasionando grave inflamação sistêmica, que pode levar à morte. Em modelos animais, o transplante de esplenócitos do C57BL/6J para o B6D2F1 resulta na GVHD aguda. *Lithothamnion calcareum* (LTC) é uma espécie de alga vermelha calcária do filo Rhodopyta. Estudos relacionados aos polissacarídeos desta demonstraram suas propriedades anti-inflamatórias, antitumoral e imunomodulatória. Assim, objetivamos investigar possíveis propriedades terapêuticas de LTC em relação à GVHD. **Métodos**: Para indução da GVHD aguda os camundongos receptores, B6D2F1, receberam intravenosamente um pool de $3x10^7$ células de camundongos C57Bl/6 (grupo GVHD). Camundongos do grupo controle receberam células isogênicas (B6D2F1) (grupo controle). Após a transferência, houve avaliação dos parâmetros clínicos e da resposta inflamatória associada à indução do GVHD. Para testar o efeito da alga *Lithothamnion calcareum* na GVHD, inicialmente fez-se uma extração bruta da alga. Os extratos foram obtidos por percolação exaustiva do material seco e pulverizados utilizando etanol em diferentes proporções (96%, 70% e 50%) como solvente extrator. Os solventes foram então removidos dando origem ao produto final. Após este processamento, os animais foram tratados com dieta específica onde foram acrescentadas 1g da alga em estudo para cada 100g de ração (grupo GVHD tratado com LTC). Após tempos determinados da evolução da doença, o intestino dos animais dos diferentes grupos foram retirados e processados para detecção de citocinas e quimiocinas por ELISA, quantificação indireta de neutrófilos (MPO) e macrófagos teciduais (NAG) por ensaios enzimáticos. Amostras de intestino também foram processadas para análises histopatológicas. A translocação bacteriana foi avaliada em cultura de lavado peritoneal. Todos os experimentos foram realizados com grupos de 5 animais. A análise estatística foi determinada por one-way ANOVA e teste Student-Newman-Keuls. A diferença entre os grupos foi considerada estatisticamente significativa quando $p < 0,05$. **Resultados e Discussão**: Os animais transplantados com células de C57Bl/6 e tratados com *Lithothaminion calcareum* apresentaram menor taxa de mortalidade e diminuição da ocorrência e intensidade dos sinais clínicos da GVHD quando comparados aos camundongos do grupo GVHD. Estes resultados correlacionaram com uma menor translocação bacteriana para o peritôneo, menor grau de lesão e recrutamento de leucócitos para o intestino, além de níveis diminuídos de citocinas e quimicionas pró-inflamatórias tais como CCL-3 (pg/mL; controle: 72,6±18,69; GVHD: 354±62,96; GVHD+LTC: 145±18,53), IFN-γ (pg/mL; controle: 0,02±0,01; GVHD: 0,124±0,04; GVHD+LTC: 0,01±0,01), TNF-α (pg/mL; controle: 2±0,5; GVHD: 136±51; GVHD+LTC: 2±0,5);MCP-1(pg/mL; controle: 6,6±4,9; GVHD: 45,8±9,1; GVHD+LTC: 2,8±2,8) e CCL-5 (pg/mL; controle: 813±432; GVHD: 4561±1415; GVHD+LTC: 102,7±23). **Conclusão**: A alga *Lithothaminion calcareum* reduziu de forma significativa sinais clínicos e a reação inflamatória ocasionada pela GVHD, podendo ser utilizada como futura estratégia terapêutica para tratamento da doença. **Apoio financeiro**: CNPq,CAPES e FAPEMIG. Comitê Ético de Experimentação Animal (CETEA/UFMG) protocolo No. 024-09.

09.118

Investigation of the gastroprotective action mechanism and healing action of the ethanolic extract of *Maytenus obtusifolia* Mart. Mota KSL[1], Dias GEN[1], Montenegro CA[1], Lima GRM[1], Medeiros VM[1], Tavares JF[1], Silva MS[1], Pellizzon CH[2], Hiruma-Lima CA[2], Batista LM[1] [1]LTF-UFPB, [2]UNESP - Botucatu

Introdution: *M. obtusifolia* (Celastraceae) is distributed in many states of the Northeast and Southeast of Brazil. It is popularly known as “bom-nome”, “carne-de-anta” or “carrancudo” and it is used in the folk medicine for the treatment of ulcers, general inflammations and cancer. Despite of the ethnopharmacologic importance of this species there are few studies about the toxic and pharmacological activities. Previous studies in our laboratory revealed the antiulcerogenic activity of the ethanolic extract obtained from the leaves of *M. obtusifolia* (EEtOH). Therefore, the aim of this study was evaluate the gastroprotective action mechanism and the healing activity of the EEtOH. **Methods:** Male Wistar rats (180-250 g, n=6-10) were used, which were treated orally with vehicle (saline), carbenoxolone (100 mg/kg), cimetidine (100 mg/kg) or EEtOH. In the evaluation of the gastroprotective action mechanism were investigated the participation of the nitric oxide (NO) (SIKIRIC, Eur. J. Pharmacol., 332, 23, 1997) and sulphydryl compounds (MATSUDA, Life Sci., 65, 27, 1999). In the evaluation of the healing activity the gastric ulcers were induced by acetic acid 30 % (TAKAGI, JPN J. Pharmacol., 19, 418, 1969). At the end of the treatment period the ulcerative area (UA) and toxic parameters (body and organs weight, water and food consumption and biochemical and hematological parameters) were determined. Then, the slides were observed after haematoxylin and eosin (HE) and Periodic Acid Schiff (PAS) staining. The results are expressed in mean ± S.D. and were compared using ANOVA followed by Dennett’s or Tukey’s test, $p<0.05$. Number of Ethical in Animal Research license is 0205/07. **Results and Discussion:** In the models that investigate the involvement of the NO the EEtOH (250 mg/kg) did not promote significant changes in the ulcerogenic index (UI) among the groups treated in the absence (155.4 ± 19.3) and presence of L-NAME (169.0 ± 42.6), a non-selective inhibitor of NO synthase. In the models that evaluate the participation of the sulphydryl compounds the EEtOH (250 mg/kg) showed UI of 153 ± 17.1 for the groups pretreated with saline, however, when the groups were pretreated with NEM, an inhibitor of the sulphydryl compounds, the UI was significantly increased for 281.7 ± 52.2, with consequent reduction of the gastroprotection. In the acetic acid-induced ulcer model, the treatment during 14 days with EEtOH (250 mg/kg) (21.4 ± 3.7 mm^2) decreased the gastric lesions, when compared to vehicle (44.3±12 mm^2), with healing of 52 %. The histological analysis using HE staining showed better degree of organization of the stomach glands in the group treated with EEtOH in relation to vehicle. The results using PAS staining the EEtOH showed an increase on mucus production when compared to vehicle. In this model, during the 14 days of treatment, it was observed that the extract showed the reduction of water intake and increase in serum urea of the animals tested. **Conclusion**: The data demonstrate that EEtOH shows low toxicity, healing activity and the gastroprotective action mechanism of EEtOH is independent of NO and dependent on the participation of sulfhydryl compounds, supporting the popular use of this species. **Financial Support:** CNPq/LTF/UFPB

09.119

Effect of P1G10 pretreatment in tumor growth and metastasis. Freitas KM[1], Viana CTR[1], Stehling LFO[1], Gomes MT[4], Salas CE[2], Lopes MTP[1] [1]ICB-UFMG - Farmacologia, [2]UFMG - Bioquímica e Imunologia

Introduction: Our group previously showed that P1G10, a papain like cysteine proteinase containing fraction from *Carica candamarcensis*, promoted a reduction in tumor size of melanoma B16F1 and Ehrlich models and the number of lung metastasis in melanoma B16F10. Furthermore, other studies showed that long-term rectal administration of an enzyme mixture containing papain displayed antitumoral effect in C57Bl6 inbred mice inoculated with Bl6 melanoma cells [1]. In addition, when mice were immunized with papain, the growth rate, invasiveness and metastasis of both the B16 melanoma and Lewis lung carcinoma were inhibited [2]. Based on these observations, we now evaluate the role of pretreatment with P1G10 in tumor growth and metastasis. **Methods:** Two experimental models were performed to access the role of PIG10 when administrated previously to tumor progression. *i* P1G10 fraction (1-5 mg/kg) was daily administrated (*s.c.*) to Swiss mice (n=20) during 10-days. Then, 1 x 10^7 Erlich ascites viable tumor cells were administered intraperitoneally and 10-days later, the tumor size was evaluated by counting the cells in the ascite fluid. *ii* C57Bl6 mice (n=35) were non-immunized or immunized with 50 µg of P1G10 diluted in aluminium hydroxide solution by weekly intraperitoneal administration during one month. Then, 1 x 10^6 B16F10 viable cells were inoculated *s.c*. into the ear. After 15-days, tumor was extirpated and the total tumor volume determined by measuring the tumor sizes with a caliper. Subsequently, on the 21st the presence of lung metastasis was evaluated in these animals. (Protocol # 090/09, CETEA UFMG). **Results and Discussion:** *i* The pretreatment of Swiss mice with 1 and 5 mg/kg of P1G10 reduced the number of tumorigenic cells in ascite fluid compared with untreated control mice (11.14 ± 1.6 and 10.6 ± 1.0 x 10^7 cell/ml respectively; control 14.3 ± 1.6 x 10^7 cell/ml, p< 0,05, ANOVA, Bonferroni test). *ii* On the other hand, C57Bl6 immunized with P1G10 attained a total tumor volume of 5.13 ± 1.14 cm^3, while the non-immunized control had 1.27 ± 0.32 cm^3 (p< 0,05, ANOVA, Bonferroni test). In addition, 40% of non-immunized animals display lung metastasis, while this frequency increased to 60% in immunized mice. Thus, based on this and prior results, pretreatment or treatment with P1G10 was efficient to reduce Ehrlich ascite carcinoma. However, the growth rate and metastasis of B16F10 melanoma were enhanced in mice immunized with P1G10, different from what was described previously for papain immunized mice. Although these discrepant results suggest an opposite effect of P1G10 in these two models, a more conclusive interpretation must be avoided given the intrinsic physiopathological differences of each tumor cell line. **References**: 1- Wald, M. Life Sci. v62,p 43, 1998. 2- Bellelli, A. Invas. Metast. v10, p142, 1990. **Financial Support:** CNPq, FAPEMIG and CAPES.

09.120

Combretum leprosum fruit extract inhibits homeostasis alterations induced by *Bothrops* snake venom. Fernandes FFA[1], El-Kik CZ[1], Facundo VA[2], Melo PA[1] [1]UFRJ - Farmacologia Básica e Clínica, [2]UNIR - Química

Introduction: In this work we evaluated the ability of *Combretum leprosum* ethanolic fruit extract in inhibiting hemostasis alterations induced by *Bothrops jararacussu* and *Bothrops jararaca* snake venoms. The *Combretum* genus (Combretaceae) is distributed in Asia, Africa and Americas, including about 250 species, with cosmopolita distribution. It presents about 10% of its species with known ethnopharmacology use, mainly in the treatment of snakebites, cancer, leprosy, abdominal pain, tropical fevers, as cicatrizant agent and others. The protocols were submitted and approved by the Committee of Animal Care from Health Science Center, Biomedical Sciences Institute, UFRJ, and received the number DFBCICB 023. **Material and Methods**: We evaluated the inhibition of *Bothrops jararacussu* crude venom lethality. Also we tested the anti-hemorrhagic activity of the extract (Kondo & col., Japanese Journal of Medical Science and Biology, v.13, p.43, 1960) and the clotting time evaluated by Lee-White modified method (Raphael, Med. Lab. Tecnol., 4ed, p.742, 1983) against *B. jararaca* (1 mg/kg) crude venom. We evaluated the inhibition of *Bothrops jararacussu* crude venom azocaseinolytic activity according with the method described by Garcia & col (Archives of Biochemistry and Biophysics, v.188, p.315, 1978) and the collagenase activity of the enzyme collagenase and *B. jararacussu* venom according Chavira e col (Anal. Biochem, v.136, p.446, 1984) modified method. **Results**: The lethality induced by *B. jararacussu* crude venom (5 mg/kg) was reduced in a dose dependent way (30 – 300 mg/kg, i.p.), where the last protect 100% of the animals. The proteolytic and collagenase activities of *B. jararacussu* crude venom (10 mg/mL and 50 mg/mL, respectively), and the collagenase activity of collagenase enzyme (50 mg/mL) were inhibited by the extract in a concentration-dependent way (10-300 mg/mL), where 300 mg/mL abolished both activities of *B. jararacussu* venom and 50% of collagenase enzymatic activity. The hemorrhage caused by *B. jararaca* (1 mg/kg) crude venom was also completely inhibited by the extract (100 mg/kg). **Conclusion**: These results suggest that the *C. leprosum* crude extract is able to inhibit some important activities from *Bothrops* venoms. **Supported by:** CAPES, CNPQ-PIBIC, FAPERJ, PRONEX, MIRT-FOGARTY

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Involvement of K^+ channels on spasmolytic effect and investigation of hemolytic activity of trachylobane-360. Martins IRR[1], Santos RF[2], Correia ACC[3], Silva ACL[2], Pessôa HLF[4], Tavares JF[2], Silva MS[3], Silva BA[3] [1]LTF-CCS-UFPB, [2]LTF-UFPB - Ciências Farmacêuticas, [3]UFPB - Tecnologia Farmacêutica, [4]DBM-UFPB

Introduction: The species *Xylopia langsdorfiana* A. St.-Hil. & Tul. (Annonaceae) 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 diterpene ent-7a-acetoxytrachyloban-18-oic acid (trachylobane-360), isolated from hexanic phase of the crude ethanolic extract of the stem bark of *X. langsdorfiana* showed spasmolytic effect on guinea-pig ileum partially due to blockage of the voltage-gated calcium channel (Janebro D. I., SBFTE 2007), but there is not studies that investigate the involvement of the potassium channel in this effect. Furthermore, this trachylobane presents antitumoral effect in leukaemia cells (SILVA, M. V. B., Rev. Bras. Cienc. Farm., 41, p. 481, 2005). Thus, we aim to verify a hemolytic activity in rat erythrocytes to guarantee that it is not toxic on non-cancer cells and to deepen the mechanism of spasmolytic action of trachylobane-360 on guinea-pig ileum, investigating a possible participation of the potassium channel in this effect. **Methods:** Erythrocytes were isolated from blood of Wistar male rat according to the method described by Rangel (1997). Total hemolysis was obtained with 1 % Triton X-100 detergent and the percentage of hemolysis of the trachylobane-360 (10^{-5}, $3x10^{-5}$ and 10^{-4} $_M$) was calculated relative to this value. To investigate the mechanism of action spasmolytic, the guinea-pig ileum was suspended in organ bath containing modified Krebs solution (pH 7.4) at 37° C, gassed with 95 % O_2 and 5 % CO_2 mixture and resting tension of 1 g. Isometric contractions were registered. All the experimental protocols were approved by Ethical Committee in Animal Research of LTF/UFPB (Protocol 0101/08). **Results:** In the evaluation of hemolytic activity on rat erythrocytes, trachylobane-360 did not induce a significant hemolysis at concentrations of 10^{-5}, $3x10^{-5}$ and 10^{-4} M (n = 3). About the spasmolytic action, trachylobane-360 relaxant effect (EC_{50} = 1.5 ± 0.3 x 10^{-5} M, n = 5) was attenuated significantly in the presence of TEA^+ 5 mM, a non-selective blocker of K^+ channels (EC_{50} = 5.0 ± 0.4 x 10^{-5} M, n = 5). We decided to investigate what subtype of K^+ channels participate in this trachylobane-360 effect. The relaxation promoted to diterpene was not reduced significantly by 1 mM TEA^+ (BK_{Ca} blocker) and 100 nM apamin (SK_{Ca} blocker). **Discussion:** As the trachylobane-360 showed no damage to the erythrocyte membrane of rats on the concentrations used on guinea-pig ileum assays, *in vitro*, it is an indicator of safety to continue the studies and, probably, it would have low or no toxicity when tested *in vivo*, as well as others diterpenes as labdano-302 (AMORIM, S. S. et al., Anais de II Simponature, 2007). The relaxant effect of trachylobane-360 appears to be due to activation of K^+ channels, and apparently the subtypes BK_{Ca} and SK_{Ca} are not involved. However, others subtypes of K^+ channels, have not been investigated, as K_{ATP} and K_V, could be involved in spasmolytic effect of this diterpene. **Supported by:** CAPES, CNPq, LTF/UFPB

09.122

Investigation of the endothelium-independent vasorelaxant effect induced by *Aspidosperma tomentosum* (Apocynaceae) in rat mesenteric rings. Furtado FF[1], Menezes[1], Anjos RM[1], Costa CDF[2], Ferreira AKB[2], Herculano EA[2], Aquino PGV[3], Araújo-Júnior JX[3], Sant'ana AEG[3], Ribeiro EAN[2], Medeiros IA[1] [1]LTF-UFPB, [2]ESENFAR-UFAL, [3]IQB-UFAL

Introduction: *Aspidosperma tomentosum* Mart. is a plant popularly known as "peroba-do-campo", and any study on its effects on the cardiovascular system was found in the literature. In a preliminary study we demonstrated that, the ethanol extract of the stem of *Aspidosperma tomentosum* (ATEE) produced hypotensive and bradycardic effects in normotensive non-anaesthetized rats, and in superior mesenteric rings, produced vasorelaxant responses, endothelium-independent, in part due to the blockade of Ca^{2+} influx. The aim of this study was to further investigate the mechanisms involved in the vasorelaxant effect induced by ATEE in rat mesenteric rings, with emphasis to the participation of the K^+ channels and intracellular calcium stores. **Methods:** Male Wistar rats (250-300 g) were used for all experiments. Rat superior mesenteric rings (1-2 mm) were suspended by cotton threads for isometric tension recordings in tyrode's solution, 37°C, gassed with 95 % O_2 and 5% CO_2, resting tension 0.75 g. Statistical analysis were performed by Student "t" test**.** Protocols were approved by the Ethics Committee in Animal Research (CEPA/LTF 0106/08)**. Results and Discussion:** In isolated rat mesenteric rings with intact endothelium ATEE (0.03-300 µg/mL) induced concentration-dependent relaxation of the contractions induced by PHE (10 µM) (EC_{50} = 37.07 ± 4.72; E_{max} = 97 ± 4.0 %, n=6). After endothelium removal the vasorelaxant response elicited by ATEE was not significantly attenuated (EC_{50} = 32.93 ± 3.16; E_{max} = 99 ± 3.6 %, n=6). The vasorelaxant effect induced by increasing concentrations of ATEE was not significantly altered in the presence of KCl 20 mM (EC_{50} = 37.42 ± 8.4 µg/mL; E_{max} = 100 ± 2.7 %, n=6). In rings pre-contracted with $PGF_{2\alpha}$, ATEE induced relaxant effect (EC_{50} = 44.88 ± 11.9 µg/mL; E_{max} = 95.1 ± 4.47 %, n=6), which was not significantly different from those obtained in the presence of Phe 10 µM. In endothelium-denuded mesenteric rings, Phe (10^{-9} -10^{-5} M)-induced contractions were reduced in presence of ATEE (10; 30; 100 and 300 µg/mL). In addition, ATEE (10; 30; 100 and 300 µg/mL, n=6) inhibited the transient contractions induced by Phe (10 µM) in Ca^{2+}-free solution containing EGTA. Nevertheless, in these same conditions, ATEE (300 µg/mL) was ineffective to inhibit caffeine (20 mM)-induced contractions. **Conclusion:** These results demonstrate that ATEE induces endothelium-independent vasorelaxation, which is not related to the opening of K^+ channels. Vasorelaxation is sems to be rather due to the inhibition of the Ca^{2+} release from IP_3-sensivite Ca^{2+} stores. **Financial Support:** CNPq.

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Ca^{2+} and K^+ channels contribute to trachylobane-318-induced spasmolytic effect on guinea-pig ileum. Santos RF, Martins IRR, Carreiro JN, Travassos RA, Oliveira GA, Tavares JF, Silva MS, Silva BA LTF-UFPB - Ciências Farmacêuticas

Introduction: The ent-7a-acetoxytrachyloban-18-oic acid (trachylobane-360) is a diterpene isolated from hexanic phase of the crude ethanolic extract of the stem bark of X*ylopia langsdorfiana* A. St.-Hil. & Tul. (Annonaceae). From the trachylobane-360 was obtained a hydroxylated derivative, due a structural modification, which was identified as ent-7a-hidroxitrachyloban-18-oic acid (trachylobane-318), and this diterpene shows spasmolytic activity on guinea-pig ileum and trachea (Santos, R.F. et al., SBFTE 2008; Martins, I.R.R., et al., SBFTE 2008). How spasmolytic effect was most potent in guinea pig ileum, we decided to investigate the spasmolytic mechanism of trachylobane-318 in this organ, assessing the contribution of the calcium and potassium channels. **Methods:** Guinea-pig ileum was suspended in organ bath containing modified Krebs solution (pH 7.4) at 37° C, gassed with 95 % O_2 and 5 % CO_2 mixture. Isometric contractions were recorded. All the experimental protocols were approved by Ethical Committee in Animal Research of LTF/UFPB (Protocol 0101/08). **Results:** Trachylobane-318 spasmolytic effect on guinea-pig ileum was evaluated on cumulative $CaCl_2$ curves in depolarizing medium nominally without calcium**.** Trachylobane-318 antagonized the contractions induced by $CaCl_2$ (n = 5) in a significant and concentration-dependent manner. The concentration-response curve to $CaCl_2$ in the presence of trachylobane-318 (3 x 10^{-5}; 10^{-4} and 3 x 10^{-4} M) was shifted rightward in a non-parallel manner, with reduction of maximum effect (E_{max}) to 87.3 ± 4.0, 17.7 ± 3.7 and 3.1 ± 0.5 %, respectively. Furthermore, trachylobane-318 relaxed (EC_{50} = 5.5 ± 0.3 x 10^{-5} M, n = 3) the guinea-pig ileum pre-contracted with S-(-)-Bay K8644, an agonist of L-type voltage-gated calcium channels (Ca_V-L). Trachylobane-318 spasmolytic effect (EC_{50} = 0.1 ± 0.01 x 10^{-5} M, n=5) was attenuated significantly in the presence of TEA^+ 5mM (EC_{50} = 0.6 ± 0.2 x 10^{-5} M), a non-selective blocker of K^+ channels. We decided to investigate what subtype of K^+ channels participate in this trachylobane-318 response. Interestingly, the diterpene relaxation effect was reduced significantly by glibenclamide (EC_{50} = 1.1 ± 0.3 x 10^{-5} M, n = 5), blocker of K_{ATP}; 4-aminopyridine (EC_{50} = 0.7 ± 0.2 x 10^{-5} M, n = 5), a blocker of K_V and apamin (EC_{50} = 0.7 ± 0.2 x 10^{-5} M, n = 5), blocker of SK_{Ca}. The curve of relaxation of trachylobane-318 has been shifted to right on 11, 7 and 7 times in the presence of the K^+ channels selectives blocker (glibenclamide, 4-AP and apamin, respectively). **Discussion:** According to obtained results, we can suggest that in functional level, the spasmolytic effect of trachylobane-318 on guinea-pig ileum seems to involve non-competitive antagonism of the Ca_V-L and a non-selective activation of K^+ channels (K_{ATP}, K_v and SK_{Ca}), and probably trachylobane-318 is indirectly blocking the calcium channels due to a positive modulation of the potassium channels, thus leading to relaxation of smooth muscle. However forward studies are necessaries to elucidate the mechanism spasmolytic fully. **Supported by:** CAPES, CNPq, LTF/UFPB

09.124

Evaluation of the cytoprotective action mechanism and healing effect induced by phase ethyl acetate of *Maytenus obtusifolia* Mart. Mota KSL[1], Dias GEN[1], Lima GRM[1], Montenegro CA[1], Medeiros VM[1], Tavares JF[1], Silva MS[1], Pellizzon CH[2], Hiruma-Lima CA[2], Batista LM[1] [1]LTF-UFPB, [2]UNESP - Botucatu

Introdution: *M. obtusifolia* is popularly known as "bom-nome", "carne-de-anta" or "carrancudo". It is used in the folk medicine for the treatment of ulcers, general inflammations and cancer. Despite of the ethnopharmacologic importance of this species there are few studies about the toxic and pharmacological activities. Previous studies in our laboratory revealed the antiulcerogenic activity of the phase ethyl acetate obtained from the leaves of *M. obtusifolia* (FAcOEt). Therefore, the aim of this study was to evaluate the cytoprotective action mechanism and the healing activity of FAcOEt. **Methods:** Male Wistar rats (180-250g, n=5-10) were used, which were treated orally with vehicle (saline), carbenoxolone (100 mg/kg), cimetidine (100 mg/kg) or FAcOEt. In the cytoprotective action mechanism were used following protocols: quantification of prostaglandin E_2 (PGE_2) in the gastric mucosa (CURTIS, Can. J. Physiol. Pharmacol, 73, 130, 1995), determination of the nitric oxide (NO) (SIKIRIC, Eur. J. Pharmacol, 332, 23, 1997) and sulphydryl compounds (MATSUDA, Life Sci., 65, 27, 1999). In the evaluation of the healing activity, the gastric ulcers were induced by acetic acid 30% (TAKAGI, JPN J. Pharmacol, 19, 418, 1969). At the end of the treatment period the ulcerative area (UA) and toxic parameters (body and organs weight, water and food consumption and biochemical and hematological parameters) were determined. Then, the slides were observed after haematoxylin and eosin (HE) and Periodic Acid Schiff (PAS) staining. The results are expressed in mean ± S.D. and they were compared using ANOVA followed by Dennett's or Tukey's test, $p<0.05$. The protocols were approved by the Ethics Committee in Animal Research (CEPA/LTF 0205/07). **Results and Discussion:** The FAcOEt (125 and 250 mg/kg) did not increase the levels of PGE_2 in both groups treated in absence and presence of indomethacin, a non-selective inhibitor of cyclooxygenase, when compared to vehicle. The FAcOEt (250 mg/kg) did not promote significant changes in the ulcerogenic index (UI) among the groups treated in the absence (70.1±21.8) and presence of L-NAME (62±23), a non-selective inhibitor of NO synthase. However the FAcOEt (250 mg/kg) showed significant changes in the UI among the groups treated in the absence (53.8±18.9) and presence of NEM (224.8±46.3), an inhibitor of the sulphydryl compounds, showing significant reduction of the gastroprotection in the group treated with NEM. In the acetic acid-induced ulcer model, the chronic treatment with FAcOEt (250 mg/kg) decreased the gastric lesions for (19±4.5mm^2), when compared to vehicle (44.3±12mm^2), with cure rate of 57%. The histological analysis using HE staining showed higher degree of organization of the stomach glands in the group treated with FAcOEt in relation to vehicle. The results using PAS staining the FAcOEt showed an increase on mucus production when compared to vehicle. During the 14 days of treatment the FAcOEt did not change the toxic parameters. **Conclusion**: These results indicate that FAcOEt shows low toxicity, healing activity and the cytoprotective action mechanism which may be related to the participation of sulfhydryl groups. **Financial Support:** CNPq/LTF/UFPB

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The essential oil of fresh leaves of *Rollinia leptopetala* R. E. Fr presents spasmolytic activity on guinea pig ileum. Carreiro JN[1], Travassos RA[1], Silva ACL[1], Oliveira, G. A.[1], Monteiro FS[1], Martins IRR[1], Santos RF[1], Agra MF[2], Costa VCO[3], Silva MS[2], Silva BA[2] - [1]LTF-UFPB - Ciências Farmacêuticas

Introduction: genus *Rollinia* (Annonaceae) is constituted of about 65 species (LEBOEUF et al., Phytochemistry, v.21, p. 2783, 1982). Many these species present cytotoxic, antitumor, pesticide, vermifuge, abortive, antimicrobial, immunosuppressive, antiemetic, antimalarial activity and inhibiting appetite (RUPRECHT et al., J. Nat. Prod., v. 53, p. 237, 1990; CAVÉ et al., Progress in the Chemistry of Organic Natural Products, 1997). *Rollinia leptopetala* species is popularly know as “pinha brava”, “araticum”, “bananinha” and “pereiro” (MAAS et al., Organization for Flora Neotropica, v. 57, p. 121, 1992). In folk medicine is used in the Cariri Paraibano as digestive (AGRA et al., J. Ethopharmacol, v. 111, p. 383, 2007). Recently, Costa et al. (2008) demonstrated that the essential oil obtained from fresh leaves of the *Rollinia leptopetala* (RE-OL) showed modulator activity of bacterial resistance of *Staphylococcus aureus* to the antibiotic norfloxacin. Since there is no study relate with spasmolytic activity to *R. leptopetala* we aim to investigate a possible spasmolytic activity on guinea-pig ileum. **Methods:** guinea-pig ileum was suspended in organ bath containing modified Krebs solution (pH 7.4) at 37 °C, gassed with 95 % O_2 and 5 % CO_2 carbogen mixture and resting tension of 1g. Isotonic contractions were recorded on a smoked drums through levers coupled to kymographs and isometric contractions were recorded through force transducer coupled to amplifier, which was connected to a microcomputer. All the experimental protocols were approved by Ethical Committee in Animal Research of LTF/UFPB (Protocol 0506/05). **Results:** RL-OE (0.1-243 µg/mL) antagonized the carbachol and histamine- induced contractions on guinea-pig ileum in a significant and concentration-dependent manner (IC_{50} = 49.8 ± 6.5 µg/mL and 2.4 ± 0.3 µg/mL, respectively; n = 5), being about 25 times most potent when the ileum was contracted by histamine. The concentration-response curves to histamine in the presence of RL-OE (3-81 µg/mL) was shifted rightward in a non-parallel manner with reduction of the maximum effect (E_{max}) to 91.2 ± 1.3; 57.3 ± 9.4 and 21.8 ± 2.2%. In addition, RL-OE relaxed (0.1-243 µg/mL) the guinea pig ileum pre-contracted by 40 mM KCl, 10^{-6} M carbachol or histamine (EC_{50} = 13.6 ± 2.5; 8.3 ± 2.0; 6.8 ± 0.1 µg/mL, respectively), in a significant, equipotent and concentration-dependent manner. **Discussion:** RL-OE shows non-selective spasmolytic activity for agonists tested. So, we can suggest that RL-OE is not directly interacting with histamine receptors, characterizing an antagonism not competitive in functional level. As RL-OE relaxed the guinea-pig ileum pre-contracted by both KCl, carbachol and histamine equipotent manner. The inhibition of tonic contractions on guinea-pig ileum it is suggestive the blockade of calcium influx through voltage-operated calcium channels, once these channels are responsible by maintenance for this contractile response. **Financial Support**: CNPq, CAPES, LTF/UFPB

09.126

Ability of fucosylated chondroitin sulfate to inhibit *Bothrops jararacussu* snake venom activities. Machado MM[1], Tomaz MA[1], Cons, BL[1], Strauch MA[1], Ricardo HD[1], Borges PA[1], El-Kik CZ[1], Dip EC[2], Mourao PAS[3], Melo PA[1] [1]UFRJ - Farmacologia Básica e Clínica, [2]UFF - Odontologia, [3]HUCFF-UFRJ - Tecido Conjuntivo

Introduction: Snakebite by *Bothrops jararacussu* snake induces an intense local tissue damage. The venom contains a complex mixture of enzymes and small peptides. Phospholipases A_2 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). We assessed some venom activities, as well as some antagonists that could help to neutralize these effects. Polyanions have been shown to present antivenom properties (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 some activities of *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 and phospholipase activities were measured using the azocasein and the chicken egg yolk as substrate, respectively. The coagulant effect was evaluated by the modified Lee-White method. 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 (50 µg/mL). It was observed that FucCS inhibits 75% of proteolytic venom activity and 80% of phospholipase venom, in concentration-dependent manner. The coagulant effect of *B. jararacussu* (0,1 µg/mL) is abrograted with 0,8 µg/mL of FucCS. Incubation of FucCS with the venom eliminates the increase of plasma CK, *in vivo*, but pre and posttreatment were ineffective. The oedema was reduced by 1 and 10 mg/kg of FucCS. **Discussion:** FucCS was capable to inhibit all venom activities evaluated. Although the plasma CK levels did not reduce in the pre- and posttreatment (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. **Financial Support:** CNPq, CAPES, FAPERJ and PRONEX.

09.127

Antimicrobial activity of *Urtica dioica* and *Vaccinium macrocarpon* extracts. Ribeiro ZEA[1], Lodi KB[1], Back-Brito GN[3], Teodoro GR[2], Rocha RF da[3], Koga-Ito CY[1] [1]FOSJC-UNESP - Biociências e Diagnostico Bucal, [2]UNESP - Microbiologia, [3]UNESP - São José dos Campos

Introduction There are evidences that *Vaccinium macrocarpon* (cranberry) and *Urtica dioica* (nettle) may be effective for the treatment of recalcitrant infections. There is an increasing use of phytotherapic agents in medical field. Few studies on these plants are available in the literature. The purpose of this study was to assess the antibimicrobial activity of these extracts on *Escherichia coli*, *Pseudomonas aeruginosa, Enterococcus faecalis, Salmonella typhimurium*, *S. pyogenes, S. epidermidis, Candida albicans* and *Staphylococcus aureus*. **Methods** Screening of antibacterial activity was performed with the following microorganisms using standard strains: *E. coli* (ATCC 23922), *P. aeruginosa* (ATCC9027)*, E. faecalis* (ATCC19433)*, S. typhimurium* (ATCC14028), *S. pyogenes* (ATCC1500)*, S. epidermidis* (ATCC1228)*, C. albicans* (ATCC18804) and *S. aureus* (ATCC 6538). Susceptibility testing was performed by microdilution method (CLSI). For these tests, 10 oral isolates and a *C. albicans* sample-standard (ATCC 18804), a sample *S. aureus* (ATCC 6538) and 9 oral isolates were included. The bacterial strains were grown in Brain Heart Infusion agar and *C. albicans* on Sabouraud agar. Suspensions standardized were prepared (0.5 Mc Farland). Solutions (100 mg/mL) of each extract were prepared within propyleneglycol in distilled water (50:50). Two-fold *serial dilutions* were obtained in *Mueller-Hinton broth (for S. aureus)* or RPMI + MOPS, pH 6.9 (for *C. albicans*) in microtiter plates. Then it was inoculated into plates and incubated for 24h. After this period, aliquots of the final suspension were plated on agar plates containing mannitol or Sabouraud agar to verify the minimum microbicidal concentration (MMC). This study was approved by the Local Ethics Committee (061/06-PH/CEP). **Results** Nettle was fungicide to 100% of *C. albicans* isolates at concentrations from 0.025 mg/mL. For *S. aureus*, 5 isolates and the standard sample were susceptible to nettle (0.025 mg/mL). The remaining samples were resistant to concentrations of 50 mg/mL (n = 1), 3.125 mg/mL (n = 1), 1.563 mg/mL (n = 1), 0.781 mg/mL (n = 1) and 0.391 mg/mL (n = 1). Regarding cranberry, all clinical isolates and standard sample of *C. albicans* were susceptible to concentrations from 0.025 mg/mL. For *S. aureus*, cranberry was bactericidal for 9 samples at concentrations from 0.025 mg/mL. Only one isolate was resistant to all the concentrations of *V.macrocarpon*. **Discussion** The extracts were fungicide for 100% of *C. albicans* in a considerably low concentration. Also, low concentrations of nettle and cranberry extracts *(50% and 90%,* respectively) were effective against *S. aureus isolates. C*ranberry*,* in particular, has some important components that can inhibit the adhesion of uropathogens. Also, considering that similar adhesion occurs to the tooth surface, this plant can inhibit bacterial adhesion and can thus slow development of dental biofilm. *T*hus suggesting that both extracts may contain phytochemical compounds with antibacterial and fungicidal properties and they can be considered promising alternatives for the treatment of oral and systemic diseases. Supported by FAPESP/CNPq.

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Evaluation of the cytotoxic and spasmolytic activities of ethyl acetate extract of aerial parts from *Solanum stramonifolium* Jacq (Solanaceae). Macedo CL[1], Correia ACC[1], Monteiro FS[1], Cavalcante FA[2], Pessôa HLF[3], Silva TMS[4], Agra MF[5], Silva BA[5] - [1]LTF-UFPB, [2]ICBS-UFAL, [3]DBM-UFPB, [4]DQ-UFRPE, [5]DCF-LTF-UFPB

Introduction: *Solanum* L. genus is most representative of the Solanaceae family, with about 1400 species (BOHS, L., Missouri Botanical Garden Press, p. 27, 2005), habiting tropical and subtropical regions of the world (AGRA, M. F., Royal Botanic Gardens, p. 197, 1999). Many *Solanum* species have showed spasmolytic activity, among them some also showed toxic activities. These activities, in general, have been attributed to presence of a great variety of steroidal saponins and glycoalkaloids (FRIEDMAN, M., *et al*. Food Chem. Toxicol. p. 537, 1991). *Solanum stramonifolium* Jacq. is a shrub with 3 m tall. It's popularly known by "jurubeba-branca-doce". Its distribution extends from the Northern Amazon, Colombia and Peru to the Guianas and Northern Brazil (MARTINS, F. C, (Monografia), 1998). In folk medicine, the fruit from *S. stramonifolium* are used as food for humans (MURAKAMI, A. et al. Cancer Lett, p. 137, 1995) its roots used to treat constipation in the form of juice (BHANDARY, M. J. et al. J. Ethnopharmacol, p. 149, 1995) and its MeOH extract shows antitumor activity (MURAKAMI, A et al. Cancer Lett, p. 137, 1995). So, we decided to investigate if crude ethyl acetate extract of aerial parts from *S. stramonifolium* (SS-AcOEt) presents hemolysis on rat erythrocytes and spasmolytic activity on smooth muscle (rat aorta and guinea-pig ileum). **Methods:** Erythrocytes were isolated from blood of Wistar male rat according to the method described by Rangel et al. (1997). Total hemolysis was obtained with 1 % Triton X-100 detergent and the percentage of hemolysis of the SS-AcOEt (81, 243 and 500 µg/mL) was calculated relative to this value. While the tissues (rat aorta and guinea-pig ileum) were suspended in organ bath chambers containing appropriate temperature and solutions (pH 7.4) and bubbled with 95 % O_2 and 5 % CO_2 carbogen mixture. Isotonic and isometric contractions were monitored. All the experimental protocols were approved by Ethical Committee in Animal Research of LTF/UFPB (Protocol 0506/05). **Results**: SS-AcOEt caused a weak hemolytic activity (7.2 ± 1.4 %, n = 3, $p < 0.05$) only at concentration of 500 mg/mL. On rat aorta, SS-AcOEt no show effect relaxant (until 500 mg/mL, n = 3). However, on guinea-pig ileum, SS-AcOEt inhibited both the carbachol-(IC_{50} = 119.4 ± 8.1 µg/mL, n = 3) and histamine-induced phasic contractions (IC_{50} = 93.1 ± 16.4 µg/mL, n = 3) in a significant, equipotent and concentration-dependent manner. **Discussion**: Based on the fact that the membrane of rat erythrocytes is highly susceptible to hemolysis, whereas SS-AcOEt only had effect in high concentrations, we suggest that this extract has low or no toxicity when tested in other cells. Moreover, SS-AcOEt presented selective effect for guinea-pig ileum, when was compared to rat aorta. **Financial support**: CNPq, CAPES, LTF/UFPB.

09.129

Atividade gastroprotetora do extrato bruto hidroalcoólico da *Achillea millefolium* L.: envolvimento de sistemas antioxidantes. Potrich BP[1], Allemand A[1], Mota L[1], Freitas CS[1], Baggio CH[1], Andre E[2], Werner MFP[3], Marques MCA[1] [1]UFPR - Farmacologia, [2]University of Ferrara - Experimental and Clinical Medicine, [3]UFSC - Farmacologia

Introdução: A planta medicinal *Achillea millefolium* L. é conhecida popularmente como mil-folhas, sendo utilizada por sua propriedade gastroprotetora. O objetivo deste trabalho foi investigar o efeito gastroprotetor do extrato bruto hidroalcoólico (EBH) e o envolvimento de sistemas antioxidantes na gastroproteção. **Métodos:** Ratos Wistar fêmeas (250 g) foram submetidos à indução de úlcera aguda por etanol P.A. e úlcera crônica induzida por ácido acético 80% (Wallace et al., *Am J Physical Gastrointest Liver Physiol*, 279, 2000). No modelo de lesão aguda por etanol os animais foram pré-tratados com veículo, omeprazol (40 mg/kg, vo), e EBH nas doses 30, 100 e 300 mg/kg vo 1h antes da administração oral de 0,5 ml de etanol. Após 1 h, os animais foram sacrificados, os estômagos retirados e abertos para a mensuração da área da úlcera através do programa *Image tool* 3.0. No modelo de úlcera crônica induzida por ácido acético, o tratamento (duas vezes ao dia) com veículo, omeprazol (40 mg/kg, vo) e EBH nas doses 0,1, 1 e 10 mg/kg vo iniciou-se no 2º dia após a indução da úlcera e foi mantido durante 7 dias. Após o tratamento os animais foram sacrificados e a área das lesões mensuradas com auxílio de régua (altura x comprimento x profundidade). Amostras dos estômagos com úlceras induzidas por etanol e por ácido acético foram retiradas para posterior avaliação dos grupos sulfidrílicos não-protéicos (GSH), catalase (CAT) e superóxido dismutase (SOD). A atividade da mieloperoxidase (MPO) foi avaliada apenas nas amostras de lesão induzida por ácido acético. Foi avaliada também a atividade do EBH em seqüestrar radicais livres no modelo de DPPH. Todos os protocolos experimentais foram aprovados pelo CEEA da UFPR sob número 161. **Resultados:** O EBH (100 e 300 mg/kg, vo) foi capaz de reduzir a área da lesão induzida por etanol em 45,1% e 79,4%, respectivamente. Semelhantemente, o EBH (1 e 10 mg/kg, vo) também protegeu a lesão gástrica induzida por ácido acético em 50% e 85%, respectivamente, quando comparado ao grupo controle. O EBH nas doses de 30, 100 e 300 mg/kg foi capaz de restabelecer os níveis de GSH e a atividade da SOD, em lesões induzidas por etanol. Em lesões induzidas por ácido acético, o EBH nas doses de 1 e 10 mg/kg foi capaz de restabelecer a atividade da SOD, e na maior dose, restabeleceu a atividade da CAT. Além disso, o EBH nas doses de 0,03; 0,01 e 0,3 mg/ml mostrou atividade sequestradora de radicais livres (26,3%, 57,6% e 71,8%, respectivamente) no modelo de DPPH. O aumento da atividade da MPO causada pela lesão gástrica induzida por ácido acético foi completamente inibido pelo tratamento dos animais com o EBH (1 e 10 mg/kg, vo). **Conclusão:** O EBH da *Achillea millefolium* L. foi capaz de reduzir a lesão gástrica induzida por etanol e ácido acético. Essa atividade gastroprotetora pode estar relacionada com a capacidade do EBH em (i) sequestrar radicais livres, (ii) restituir os sistemas antioxidantes estudados a níveis basais e (iii) reduzir a migração de neutrófilos, diminuindo o processo inflamatório gástrico. **Apoio Financeiro:** CAPES, FUNPAR

09.130

Lack of effect of mangiferin on dexamethasone-induced insulin resistance in rats. Vieira AB, Carvalho VF, Silva PMR, Cordeiro RSB, Martins MA [1]IOC-FIOCRUZ - Fisiologia e Farmacodinâmica

Introduction: Dexamethasone (DEX) is an anti-inflammatory steroidal drug known by its ability to induce insulin resistance and widely used to treat allergic diseases. In allergic individuals with co-morbidities like diabetes or insulin resistance glucocorticoid treatment could increase morbidity and mortality rates. Mangiferin *(Mangifera indica L.)* have been showed to possess antidiabetic and anti-inflammatory properties in different experimental models and so represent a possible new adjuvant drug for this disease combination. The aim of this study was to investigate the antidiabetic activity of mangiferin in a model of insulin resistance induced by dexamethasone. **Methods:** Experiments were performed on male Wistar rats and approved by the Institutional Committee for Ethics and Animal Experimentation (0085/1). Rats (n=20) were divided in 4 groups. Control group (CON) received saline (i.p.) while the other 3 groups received dexamethasone phosphate (1.0 mg/kg/i.p.) in saline for 5 consecutive days. Rats that received DEX were also treated orally 1 hour before with saline alone (DEX group) or mangiferin (MF) dissolved in saline (30 mg/kg or 60 mg/kg). On the day 6, fasted rats (6 h) were weighed and submitted to measurement of blood glucose with a glucometer. Animals were anesthetized for blood collection and then killed in a CO_2 chamber. Serum was isolated and assayed for triglycerides, cholesterol and insulin levels (IL). Liver, pancreas and epididimal fat were weighed. HOMA-IR index was calculated based on fasted glucose and insulin levels. Results were expressed as mean± s.e.m. **Results:** While before treatment no difference was seen in body weight of rats, after experimental period MF30 (196.4g ± 2.6) and MF60 (201.0g ± 4.6) groups had the same significantly ($p<0.001$) weight loss that DEX group had (200.8g ± 4.4) in comparison with control group (255.6g ± 4.3). Although there were no difference in fasted glucose levels between groups, the injection of DEX significantly ($p<0.05$) increased insulin levels (46.36 µUI/ml ± 4.66) and HOMA-IR index (12.22 µUI/ml ± 1.72) in DEX group compared with control group (IL: 19.87 µUI/ml ± 2.30 ; HOMA: 5.60 ± 0.74), but treatment with mangiferin did not reversed these changes (MF30 IL: 57.14 µUI/ml ± 3.55; MF30 HOMA: 13.75 ± 1.27; MF60 IL: 56.28 µUI/ml ± 6.65; MF60 HOMA: 15.61 ± 1.80). Mangiferin also did not show protective effect on liver mass increase induced by DEX administration (CON: 0.038g ± 0.00989; DEX: 0.052g ± 0.00255; MF30: 0.047g ± 0.0006; MF60: 0.049g ± 0.00160). No difference was seen after treatment on pancreas and epididimal fat mass between groups. Colesterol levels did not changed after experimental period, while triglycerides had great increase ($p<0.01$) induced by DEX (CON: 26.40mg/dL ± 2.58; DEX: 117.80mg/dL ± 29.40) and no decrease with 30 mg/kg treatment (146.8mg/dL ± 13.35). Interestingly, triglycerides had a significant ($p<0.001$) increased on MF60 group (235.00mg/dL ± 17.37) compared with DEX group. **Discussion:** Our findings indicate that mangiferin did not alter dexamethasone-evoked insulin resistance in rats. Furthermore, co-administration of dexamethasone and mangiferin, at the highest dose, unexpectedly led to increased triglycerides levels, suggesting that the combination of these treatments may be associated to undesired side effects. **Supported by:** CNPq and Faperj

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Avaliação da atividade inibitória do óleo essencial da sálvia (*Salvia officinalis* L.) sobre a sobre a quimiotaxia de leucócitos *in vivo.* Fonseca JP, Farinha TO, Anteguera AAC, Nogueira de Melo GA, Miranda CR, Caparroz-Assef SM, Bersani-Amado CA, Cuman RKN
UEM - Farmácia e Farmacologia

Introdução: A espécie vegetal *Salvia officinalis* L. (*Lamiaceae*) é conhecida popularmente como Sálvia. O extrato bruto e o óleo essencial da sálvia (OES) obtido das folhas desta planta têm sido utilizados na medicina popular para o tratamento de diversas enfermidades, desde o desconforto gastrintestinal até processos infecciosos. **Objetivo:** Avaliar a atividade anti-inflamatória do óleo essencial da sálvia (OES) sobre a quimiotaxia de leucócitos *in vitro*. **Métodos:** Os ensaios de quimiotaxia foram realizados em câmara de Boyden, utilizando-se filtros de nitrocelulose (poros de 8mm). Os leucócitos foram obtidos do exsudato peritoneal de ratos machos da linhagem Wistar 4h após a injeção intraperitoneal de carragenina (200µg). Após avaliação da viabilidade celular, as células foram incubadas com OES em diferentes concentrações (10^{-4}mL/mL, 10^{-3}mL/mL e10^{-2}mL/mL) durante 30 min. A dexametasona (Dexa10 $^{-5}$mol/L) foi utilizada como droga anti-inflamatória padrão. No compartimento superior da câmara foi colocada suspensão de células ($1x10^6$) e no inferior, a caseína (5%) como agente quimiotáxico. Após incubação em estufa de CO_2 durante uma hora, os filtros foram retirados da câmara, fixados em etanol absoluto e corados com hematoxilina-eosina. O comportamento celular (quimiotaxia) foi avaliado com o auxílio da microscopia óptica por meio da contagem da distância percorrida através do filtro (µm) e o número de células migradas. Os procedimentos experimentais foram aprovados pelo Comitê de ética em Experimentação Animal / UEM (CEAE) e registrados sob n° 016/08. **Resultados:** A incubação de leucócitos com OES, nas diferentes concentrações testadas, reduziu significativamente a distância percorrida por estas células no filtro ($p<0,05$): **Controle:** 78,20 ± 1,36mm; **$Dexa_{10}^{-5}$**mol/L: 53,40 ± 1,39*mm; **OES_{10}^{-4}**mL/mL**:** 65,60 ± 1,72* mm; **OES_{10}^{-3}**mL/mL**:** 52,93 ± 1,22* mm; **OES_{10}^{-2}**mL/mL**:** 48,20 ± 1,48* µL/mL. O tratamento com OES não promoveu redução no número de células migradas quando comparado ao número de células não tratadas ($p<0,05$): **Controle:** 27,67 ± 1, 49 cél.; **$Dexa_{10}^{-5}$**mol/L**:** 27,73 ± 1,45* cel.; **OES_{10}^{-4}**mL/mL**:** 30,67 ± 1,6 cél.; **OES_{10}^{-3}**mL/mL**:** 22,2 ± 0,54 cél.; **OES_{10}^{-2}**mL/mL**:** 24,13 ± 1,34 cél. **Discussão:** Os resultados preliminares indicam que o OES interfere na movimentação de leucócitos no filtro (distância percorrida), porém não modifica o número de células migradas. **Apoio Financeiro:** CAPES/CNPq/FADEC

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Ability of heparin to antagonize the cardiotoxic effect of the *Bothrops asper* venom. Ricardo HD[1], Machado MM[2], Martins V[1], Cons, BL[1], Strauch MA[1], Gutiérrez JM[3], Lomonte B[3], Melo PA[1] [1]UFRJ - Farmacologia Básica e Clínica, [2]FMC/UFRJ - Farmácia / Farmacologia Básica e Clínica, [3]ICP-UCR

We investigated the *in vitro* cardiotoxic activity of *Bothrops asper* crude venom and the antivenom effect of a heparin on isolated rat hearts. Cardiotoxicity was evaluated in a Langendorff preparation with adult Wistar rat heart bathed and continuously perfused (2-5 mL/min) with Ringer solution at 37°C. Heart tension was recorded continuously with a transducer coupled in a 7D Grass Polygraph, as well as the electrocardiogram (EKG). In the heart preparation, *B. Asper* venom at concentrations (1 -10 µg/mL) induced a progressive negative inotropic effect, time and concentration-dependent. The crude venom (10 µg/mL) decreased to 0% the heart tension after 15 min, increasing perfusion press, PR interval, decreasing QRS amplitude, with changes on the EKG waves. The addition of heparin 30; 100; 300 µg/ml decreased in concentration-dependent way the venom cardiotoxic effect in the heart tension reaching 100% of the inhibition with 300 µg/ml, perfusion press and EKG waves changes. The heart was then removed from the Langendorff apparatus and the ventricles were sliced and incubated in 1% triphenyl tetrazolium chloride (TTC) at 37°C (pH 7.4) for 4 min. At the end of the incubation period, the heart slice was placed in formaldehyde solution, which not only fixes the tissue but also enhances the color contrast. The normal myocardium was stained. Heparin was able to antagonize completely the cardiac arrest, the changes in EKG and the damaged induced by *B. Asper crude venom* in this isolated preparation. Financial Support by: CAPES, CNPq, PRONEX e FAPERJ

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Evaluation of effects induced by *Pradosia huberi* ethanolic extract on blood pressure and heart rate in rats. Medeiros AAN[1], Medeiros FA[1], Queiroz TM[2], Oliveira AC[2], Medeiros IA[2]
[1]DF-IEPA, [2]LTF-UFPB

Introduction: *Pradosia huberi* Ducke (SAPOTACEAE), popularly known as “casca-doce”, is a species of the Amazonian forest that is used against gastritis. The flavonoids 2,3-dihydromyricetin 3-a-L-rhamnoside, astilbin, engelitin, and 2,3-dihydromyricetin were identified in the steam bark (JACQUEMIN, Ann. Pharm. Fr., 43, 521, 1985), and these present anti-inflammatory (KANBARA, Jpn. A2, JP, n. 06256194, 1994) and anti-oxidative effects (HARAGUOHI, Biosci. Biotechnol. Biochem., 60, 513, 1996). The aim of this study was to investigate the effect induced by *Pradosia huberi* ethanol extract (EPH) on blood pressure and heart rate in rats. **Methods:** Male Wistar rats (250-300 g) were anesthetized and the abdominal aorta and inferior vena cava were cannulated for pressure recordings and administration of drugs. All protocols were approved by the Ethics Committee in Animal Research of LTF/UFPB (n. 0603/07). **Results:** In non-anaesthetized rats, EPH (5, 10, 20 $mg.kg^{-1}$ i.v.) injections produced hypotension (-5.6±0.5; -8.8±1.3 and -32.6±6.6%, respectively) and bradycardia (-0.3±0.9; -4.4±2.2 and -45.3±6.0%, respectively) (n=6). After acute treatment with a muscarinic agonist (atropine, 2 $mg.kg^{-1}$, i.v.), hypotension (-5.0 ± 0.8, -6.4 ± 0.9 and -11.6 ± 1.8%, respectively) and bradycardia (-1.2 ± 0.4, -2.9 ± 0.8 and -8.1 ± 2.2%) were significantly attenuated. Hexamethonium (20 $mg.kg^{-1}$ i.v.), a ganglionic blocker, also attenuated the effect of EPH. After L-NAME (20 $mg.kg^{-1}$, i.v.) both responses were not modified. **Conclusion:** In conclusion, the results suggested that EPH produces hypotension and bradycardia in non-anesthetized normotensive rats. Hypotension appears to be caused by a transient decrease in cardiac output as a function of intense bradycardia, that seems to be partly due to an indirect activation of muscarinic receptors. However the EPH effect is not influenced by endogenous production of nitric oxide. **Financial Support:** CNPq/LTF/UFPB/IEPA.

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A proteolytic fraction from the latex of *Carica candamarcensis* exhibits thrombolitic activity and effects on haemostatic patterns *in vitro*. Bilheiro RP[1], Gomes MT[2], Rodrigues KCL[2], Salas CE[2], Carvalho, MG[3], Sanchez E[4], Lopes MTP[1] [1]UFMG - Farmacologia, [2]UFMG - Bioquímica e Imunologia, [3]UFMG - Análises Clínicas e Toxicológicas, [4]FUNED

Introduction. Our previous results show that P1G10, a fraction with proteolytic activity from the latex of *C. candamarcensis,* sequentially activates proteolytic enzymes that generate a clot in a way similar to blood coagulation in mammals. **Objective**. We evaluated the action of CMS2MS2, a cysteine protease composing P1G10, as a fibrinolytic/fibrinogenolytic agent and its inhibition by serum α2-macroblobulin (α2-M). We also evaluated the haemostatic activity of P1G10 by the activated partial thromboplastin time (APTT) and the prothrombine time (PT). **Methods.** Fibrinogen was clotted by addition of thrombin on a Petri dish. CMS2MS2 (0.13–1 µg) or papain were placed and incubated for 2 h at room temperature and the lysed circles were measured. Fibrinogenolytic activity was measured after incubating a 6.25 mM fibrinogen solution with CMS2MS2 (0.086 mM) (0–120 min, 37°C) by denaturing 10% SDS-PAGE. The effect of incubating CMS2MS2 (50 ng) with increasing amounts of α2 -M (5, 25 and 50 µg) for 5 min at 37°C was analyzed on reduced 7.5% SDS-PAGE. The APTT (Actin®) and PT (Thromborel®) assays were performed in a coagulometer (*Dade-Behring* BFT *II*) with increasing amounts (0.01, 0.1, 1 and 10 µg) of P1G10 mixed with 100 µL of human plasma (COEP-UFMG ETIC439/06). **Results and Discussion.** The fibrinolytic activity of CMS2MS2 was 3-fold higher than papain and 1.5 lower than that of plasmin. Fibrinogenolysis by CMS2MS2 at different intervals showed Aα>Bβ>g chain degradation. When incubated with CMS2MS2, α2-M is hydrolyzed, showing that CMS2MS2 is not inactivated by α2-M. The APTT evaluation showed that P1G10 had procoagulant activity at 10 µg and anticoagulant activity at lower concentrations, while PT of P1G10 was procoagulant at 10 µg and had no significant effect at lower concentrations. These preliminary results obtained with CMS2MS2 suggest a thrombolytic action, not inhibited by α2-M and, in the lower concentration range P1G10 demonstrated an anticoagulant by APTT and no activity by PT. **Supported by CNPq and FAPEMIG.**

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Genotoxic effects of *Bothrops alternatus* and *Bothrops neuwiedi* venom in mice by micronucleus test. Zobiole NN[1], Pereira CAS[1], Okubo, BM[2], Ricci-Azevedo R[3], Schiaveto de Souza A[4], Moreno SE[5] [1]UCDB - Biotecnologia, [2]UCDB - Biologia, [3]UCDB - Biologia, [4]FMRP-USP - Fisiologia, [5]FMRP-USP - Farmacologia

Introduction: In Brazil, snakes are the most important cause of accidents occasioned by venomous animals. The *Bothrops* genus is responsible for 90% of these cases [1]. The main characteristic of these envenomations is the localized inflammatory reaction, with liberation of local and systemic chemical mediators. The cytokines have central role in this process, since they induce the release of other inflammatory mediators, including nitric oxide. Recent studies demonstrated that many of these proinflammatory mediators, especially NO, are able to induce DNA damage. However, the studies about genotoxic effects induce by snake venoms are not enough. Therefore, the present study aimed at the evaluation of genotoxic effect of the *Bothrops neuwiedi* (VBN) and *Bothrops alternatus* venom (VBA) in peripheral red blood cells from mice. **Methods**: The genotoxicity was determined using the Micronucleus Test in peripheral blood erythrocytes of Balb-c mice, weighing between 18-22g. The animals were treated with VBA and VBN, in doses of 10, 30 and 80 µg/animal. Negative control (CN) group was injected with the saline, used for dilution of the venom. In the positive control (CP) group, the mice were injected with cyclophosphamide (50 mg/kg, i.p.). For all the treated groups, the micronucleus frequency in peripheral blood erythrocytes was evaluated 24 hours after the treatments. All experiments were approved by UCDB's Ethics Committee for research on animals, under the protocol 010/2008. **Results:** VBN and VBA (80 µg/animal) caused similar significant ($P<0.01$) increases in the frequency of micronucleus as cyclophosphamide (CP). On the other hand, the micronucleus frequency in mice treated with 10 and 30 µg of VBN was lower than with CP, but still higher than in CN. Similarly, the results obtained with 10 and 30 µg of VBA, showed a decrease in the number of micronucleus in red blood cells when compared with CP. However, both doses are not able to induce increase in micronucleus, when compared with saline group. **Discussion:** The data suggests that this increment in the number of micronucleus in the high dose can be possibly explained by the increased toxicity and consequently incapacity the reparation in the DNA damage induced by VBA and VBN. Research Grants from: *UCDB, CAPES, FUNDECT.* **References**: 1. Barraviera B. Acidentes ofídicos. In: Focaccia, Roberto (org.). *Veronesi*: tratado de infectologia. 3.ed. São Paulo:Atheneu, 2005, 2: 1929-1947.

09.136

Evaluation of the antiophidic activity of the extract of an Amazon plant named *Humirianthera ampla* and some isolated compounds (lupeol and sitosterol). Strauch MA[1], Azevedo MS[2], Ricardo HD[1], Cons, BL[1], Fernandes FFA[1], Tomaz MA[1], El-Kik CZ[1], Machado MM[3], Martins VV[1], Melo PA[1] [1]UFRJ - Farmacologia Básica e Clínica, [2]UNIR - Química, [3]FMC / UFRJ - Farmácia / Departamento de Farmacologia Básica e Clínica

Introduction: In spite of being the only therapy officially recommended against snakebites, polyvalent antivenoms are not always available wherever needed, being frequently replaced by folk medicine based on plant, mainly in the Amazon area of Brazil. One of these plants is named *Humirianthera ampla* (HA), which has been investigated for its antiophydic activities in different experimental protocols against some *Bothrops* snake venoms. **Methods**: We investigated the effects of the crude extract of HA, as well as lupeol and sitosterol, on the phospholipase, proteolytic, pro-coagulant, hemorrhagic and myotoxic activities of some venoms. The myotoxic activity *in vitro* was accessed by measuring the rate of creatine kinase (CK) release from mice *extensor digitorum longus* to the bathing solution, and *in vivo* by measuring CK activity in plasma 2 hours after i.m. injection of venom, as described by Melo and Suarez-Kurtz (1988). The haemorrhagic activity was evaluated by intradermal injection of the venoms alone or pre-incubated with the extract of HA and compounds in mice (Melo et al., 1994). The antiproteolytic activity was by using azocasein as substrate in according to Garcia et al., (1988). The phospholipase activity was determined by adjusting in our laboratory the turbidimetric method of Marinette (1965) using as substrate a suspension of chicken egg yolk. The timing of blood clotting was evaluated by the modified method of Lee-White (Raphael, 1983). Animals were used according to the rules of the Committee for Animal Manipulation of Federal University of Rio de Janeiro (Protocol CEUA: DFBCICB 024). **Results:** The extract of HA (300 mg/ml) inhibited 83.40%, 87,48% and 65,04% of the proteolytic activities of the venoms of *B. atrox*, *B. jararacussu* and *B. jararaca*, respectively, and 92,20%, 48,50% and 41,75% of the phospholipase activity the venoms of *B. jararaca*, *B. atrox* and *B. jararacussu*, respectively. *B. jararacussu* venom proteolytic activity was inhibited by sitosterol and lupeol (74,84% and 86,61% of inhibition, respectively). *B. jararacussu* venom phospholipase activity was also inhibited by sitosterol and lupeol (82,80% and 60% of inhibition, respectively). In the study of the hemorrhagic activity *in vivo*, the venom of *B. atrox* was completely abolished by the extract at a dose of 300 mg/kg (100% of inhibition). The extract showed anti-myotoxic *in vitro* and *in vivo* activities against the venom of *B. atrox* (83,83% and 44,85% of inhibition, respectively), as well as lupeol (49,17% and 33,17% of inhibition, respectively). HA also decreased pro-coagulant effects of the venoms of *B. jararacussu, B. atrox* and *B. jararaca* (38,5%, 94,6% and 95,8% of inhibition, respectively). *B. atrox* haemorrhagic activity was 100% inhibited by HA extract. **Discussion:** Our studies indicate that the extract of HA and compounds present relevant antiophidic activity, demonstrating that some information about popular culture plants should be investigated. **Financial support:** FAPERJ; FUJB-UFRJ; CAPES; CNPQ; PRONEX

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Atividade do extrato bruto de *Arrabidaea chica* verlot em modelos experimentais de inflamação. Jorge MP[1], Souza IMO[2], Jankowsky L[1], Marchetti GM[1], Ruiz ALTG[1], Tinti SV[1], Magalhães PM[3], Rodrigues RAF[2], Foglio M[2], Carvalho JE[1] [1]CPQBA-UNICAMP - Farmacologia, [2]CPQBA-UNICAMP - Fitoquímica, [3]CPQBA-UNICAMP - Agrotecnologia

Introdução: *Arrabidaea chica* VERLOT, popularmente conhecida como pariri é encontrada em todo território nacional e utilizada popularmente no tratamento de processos inflamatórios e também para cicatrização de ulcerações externas. Como a atividade cicatrizante já foi comprovada experimentalmente[1], este trabalho teve como objetivo avaliar a atividade anti-inflamatória em modelo de edema de pata de ratos e de orelha de camundongos. Além disso, os extratos dessa espécie apresentaram atividade antioxidante *in vitro* que estimulam a sua comprovação *in vivo.* **Metodologia**: As folhas secas de *A. chica* foram submetidas ao processo de maceração dinâmica com metanol/ácido cítrico 0,3% originando o extrato bruto metanólico (EB). O EB foi administrado por via tópica para avaliar a capacidade de reduzir o edema de orelha induzido por óleo de cróton (5%) em camundongos (Swiss macho, n=8)[2] e por via oral (1000 mg/kg) para avaliar a capacidade de reduzir o edema de pata induzido por carragenina (2%) em ratos (Wistar machos, n=5)[3]. A dexametasona e a indometacina foram utilizadas como controles positivos, respectivamente. O protocolo experimental número 1346-1 foi aprovado pelo CEEA/Unicamp. **Resultados:** O EB não apresentou atividade anti-inflamatória nas duas vias de administração avaliadas. Administrado por via tópica não foi capaz de reduzir o edema de orelha produzido pelo óleo de cróton. Já a dexametasona reduziu o edema em 60% após 240 minutos (salina 8,21mg/dexametasona 3,36mg). Administrado por via oral não foi capaz de reduzir o edema de pata induzido por carragenina, enquanto a indometacina reduziu em 40% o edema após 240 minutos (acetona 1mL/indometacina 0,6 mL). **Discussão**: A moderada atividade antioxidante de *A. chica* se deve à presença de polifenóis glicosilados e livres em sua composição. Esses polifenóis (antocianinas e flavonóides) em virtude de sua elevada polaridade geralmente apresentam baixa biodisponibilidade quando administrados pela via oral o que pode explicar a ausência de atividade anti-inflamatória do EB no modelo de edema de pata. No entanto a ausência de atividade anti-inflamatória está condizente com os efeitos cicatrizantes, pois a cicatrização é a derradeira etapa do processo inflamatório[4]. Apoio Financeiro: Capes, Fapesp, CNPq. 1. Jorge, M. P., *et al*. J. Ethno., 118: 361-366.2008; 2. Tubazo, A., *et al*. Inflam. Rese. 17: 347-349.1985; 3. Winter C.A., *et al*. Proc Soc Exp Biol Med. 111:544-547.1962; 4. Balbino, C. A., *et a*l. Brazi. J. Pharma. Sci., 41:27-51.2005

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Avaliação das atividades gastroprotetora e antioxidante do extrato etanólico de *Encholirium spectabile* Mart. em modelos de úlceras por etanol/HCL e etanol. Carvalho KIM[1], Machado FDF[1], Fernandes HB[1], Passos FFB[1], Silva FV[1], Oliveira RCM[2], Lima JT[3], Almeida JRGS[3] [1]UFPI - Pesquisa em Plantas Medicinais/CCS, [2]NPPM-CCS-UFPI - Biofísica e Fisiologia, [3]UNIVASF - Medicina

Introdução: *Encholirium spectabile* Mart. (Bromeliaceae), conhecida popularmente por macambira-de-flexa, é uma bromélia terrestre restrita aos afloramentos rochosos sob as condições semi-áridas do Nordeste. Algumas espécies dessa família são utilizadas na medicina popular para o tratamento de doenças do aparelho digestivo, entretanto, não há dados farmacológicos que comprovem seus efeitos no sistema gastrintestinal. Em estudos anteriores o extrato etanólico bruto de *Encholirium spectabile* (ES-EtOH) apresentou atividade gastroprotetora em modelos de úlcera induzida por etanol e por ibuprofeno. O presente trabalho tem por objetivo avaliar a atividade antiulcerogênica do extrato etanólico em modelo de úlcera gástrica induzida por Etanol/HCl, bem como avaliar a atividade antioxidante do mesmo. **Métodos:** Licença de Autorização do C.E.P. /UFPI 007/08. Camundongos Swiss albinos (25-30 g, n = 10), ambos os sexos, em jejum, foram tratados v.o. com salina, ES-EtOH (50, 100 e 200 mg/kg) e Carbenoxolona (100 mg/kg) no modelo de úlcera por etanol/HCl. 1 h após receberam 0,2 ml de etanol/HCl (0,3M HCl/etanol 60%). 1 h após a indução das úlceras, os animais foram sacrificados, os estômagos retirados e abertos pela curvatura maior e a área de lesão calculada (mm^2). Na avaliação da atividade antioxidante do ES-EtOH para mensurar a atividade enzimática da catalase (CAT) utilizou-se o método de Beers e Sinzer (1952). Os animais foram pré-tratados v.o. com salina (10ml/kg), carbenoxolona (100 mg/kg) e ES-EtOH (100 mg/kg), uma hora após a administração, receberam $etanol_{abs}$ (0,2/animal). Após 30 min os animais foram sacrificados, os estômagos abertos pela grande curvatura, a porção glandular foi retirada, os estômagos foram homogeneizados em solução tampão fosfato de potássio (pH 7,4) e a absorbância lida a 240nm dentro de 6 minutos após a adição de uma solução reagente de peróxido de hidrogênio. O valor da absorbância foi medida para uma curva padrão de CAT e expressa em mmol/minuto/100mg de tecido. **Resultados e Discussão**: De acordo com os resultados obtidos, o ES-EtOH apresenta efeito gastroprotetor no modelo de úlcera induzida por etanol/HCl nas doses 100 (3,10±0,38***) e 200 mg/kg (3,62±0,63***) quando comparado ao controle salina (11,57±0,98) e carbenoxolona (1,46±0,29). No ensaio da atividade da catalase, os grupos tratados com carbenoxolona (151,78±10,70*) e com ES-EtOH (192,74± 18,10***), foram capazes de aumentar de forma significativa ($p<0,05$* e $p<0,001$***) os níveis de CAT quando comparados ao controle salina (83,55±7,76) e ao grupo SHAM (154,93±12,01). O estresse oxidativo pode ser prevenido tanto por ação enzimática quanto por defesas antioxidantes químicas. A CAT é uma enzima que promove a primeira linha de defesa contra o H_2O_2 do ambiente celular pela conversão em oxigênio e água. O aumento do dano é acompanhado por uma diminuição nos níveis desses compostos na mucosa gástrica, os resultados indicam um forte envolvimento da catalase no efeito gastroprotetor do extrato. Evidenciado pelo aumento da atividade da enzima no grupo tratado com o ES-EtOH. **Apoio:** UFPI/UNIVASF/CAPES/CNPq

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Avaliação da participação do óxido nítrico na atividade gastroprotetora de *Neoglaziovia variegata* Mez. em modelo animal. Machado FDF[1], Carvalho KIM[1], Fernandes HB[1], Passos FFB[1], Alves AAR[1], Oliveira RCM[2], Almeida JRGS[3], Lima JT[3] [1]NPPM-CCS-UFPI, [2]NPPM-CCS-UFPI - Biofísica e Fisiologia, [3]UNIVASF - Medicina

Introdução: Estudos anteriores demonstram que o extrato etanólico de *Neoglaziovia variegata* Mez (NV-EtOH), conhecida popularmente por "caroá" apresenta atividade gastroprotetora em modelos animais de úlcera por etanol, etanol/HCl e ibuprofeno. O presente estudo tem por objetivo avaliar a participação do óxido nítrico (NO) na atividade evidenciada pelo extrato NV-EtOH. **Métodos:** Licença de Autorização do C.E.P. /UFPI 007/08. Camundongos Swiss albinos (25-30g, n = 8), ambos os sexos, em jejum, foram divididos em grupos, no qual o primeiro grupo de animais recebeu injeção de solução salina via i.p. (controle), o segundo grupo recebeu somente uma injeção de L-arginina (L-ARG), o terceiro recebeu extrato NV-EtOH (400 mg/kg), no quarto, após 30 min da injeção de L-ARG, os animais receberam injeção de L-NG-nitro arginina (L-NOARG) e o quinto recebeu injeção de L-NOARG (70 mg/kg, i.p.) Decorridos 30 min, o tratamento foi feito via oral com água (no grupo salina) e com NV-EtOH na dose de 400 mg/kg (no quinto grupo L-NOARG). Uma hora após a administração (v.o.) do grupo salina e extrato, a úlcera gástrica foi induzida com $etanol_{abs}$ (0,2 ml/animal), enquanto nos grupos L-ARG e L-ARG+L-NOARG, a indução ocorreu após 30 min da injeção i.p. Em seguida, os animais foram sacrificados, os estômagos retirados, abertos pela curvatura maior e a área de lesão calculada por planimetria (mm^2). Os dados foram analisados por ANOVA seguida do teste de Tukey, com significância para valores de p< 0,01** e p<0,001***. **Resultados e Discussão:** O pré-tratamento com L-NOARG e NV-EtOH (400 mg/kg), aumentou de forma significativa a área de lesão ulcerativa em mm^2 (NV-EtOH + L-NOARG 20,61 ± 3,21***) e (L-ARG +L-NOARG 19,53 ± 2,39**) em relação à salina (10,26 ± 2,15), ao grupo L-ARG (3,18 ± 0,58) e ao NV-EtOH (2,40±0,45***). O NO está envolvido na proteção da mucosa por promover vasodilatação, fato observado no grupo L-ARG (substrato da NOS). Os resultados com o inibidor da NOS (L-NOARG) mostram que o efeito gastroprotetor de NV-EtOH (400 mg/kg) foi revertido com a prévia administração deste inibidor, sugerindo uma provável participação do NO endógeno na atividade evidenciada. **Apoio:** UFPI/UNIVASF/CAPES/CNPq

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Histological and *in vivo* effect of capsaicin and local anesthetic agent on the tongue edema induced by *Dieffenbachia picta* Schott in mice. Dip EC[1], Pereira NA[2], Borges PA[2], Fernandes FFA[2], Ricardo HD[2], Strauch MA[2], Machado MM[2], Tomaz MA[2], Martinez AMB[3], Melo PA[2] [1]FOUFF - Ciências Básicas, [2]ICB-UFRJ - Farmacologia Básica e Clínica, ICB, [3]UFRJ - Embriologia e Histologia

Introduction: Acute inflammation caused by *Dieffenbachia picta* Schott, a tropical poisonous and ornamental plant, can induce angioedema, glottis obstruction, respiratory compromise and death in children and pets. Neurogenic inflammation results when substance P (SP) and calcitonin gene-related peptide (CGRP) are released from peripheral terminals of capsaicin-sensitive sensory neurons activated by nociceptive input or activated antidromically by dorsal root reflexes. We examined, the effect of chronic administration of capsaicin (1%) or the local anesthetic agents, lidocaine (2%), bupivacaine (0,5%), ropivacaine (1%) and benzocaine (20%), on tongue edema caused by the crude *D. picta* juice in mice (DIP et al.; Toxicon, 43(6):729, 2004). The protocols were submitted and approved by the committee of animal care from Health Science Center, Biomedical Science Institute, UFRJ and received the number 0512/2001. **Methods and Results:** Tongue edema was induced by topical application of 100 mL of *D. picta* juice and it was evaluated with a digital tachymeter during 2 hours. The tongue edema reached the maximum at 60 min. in the control mice, and at this time it was completely inhibited by topical application of benzocaine or tongue tissue injection of ropivacaine. Tongue previous treatment injection (5 min.) of the animals with lidocaine (2%) decrease in a volume dependent way the tongue edema expressed as percent of *D.picta* effect in 51,25 % ± 2,63 (10 µl) to 76,63 ± 1,01 (50 µl). Isobaric bupivacaine also reduced significantly the tongue edema (80,11% ± 1,54) (n=10). Animals received intradermal injection of capsaicin (50 mg/kg) for four days and after 15 days were submitted to tongue edema. Chemical denervation using capsaicin partially reduced tongue edema in 55,34% ± 3,23 from control groups (n=8). For histological examination, the tongue was removed 1 h after the edema induction, and the dorsal area was immersed in fixative solution and processed for light microscopy. Our results showed that local anesthetic agents and pre treatment with capsaicin can decrease the acute inflammatory response and tissue damage induced by *D. picta* juice in mice. **Conclusions:** Our results suggest that the angioedema caused by *D.picta* juice depends on the stimulation of primary sensitive neurons leading to neurogenic plasma protein extravasations due to the release of neurokinins (SP and CGRP) resulting in the triple inflammatory response. **Financial Support**: CAPES, CNPq, FAPERJ, PRONEX, FUJB-UFRJ

09.141

Avaliação anti-inflamatória tópica de compostos pró-cicatrizantes. Batista SD, Salomão ACS, Riveros BS, Otuki MF, Cabrini DA UFPR - Farmacologia

Introdução: A cicatrização é um processo altamente dinâmico e complexo que tem objetivo de reparar o tecido lesionado trazendo novamente a integridade e a homeostase. Neste processo de reparação ocorre a interação entre moléculas da matriz extracelular, mediadores solúveis, células residentes e leucócitos, organizados em etapas que se superpõem e são didaticamente denominadas de inflamação, fibroplasia e remodelagem. Apesar dos grandes avanços na compreensão desse fenômeno, a incidência e prevalência de úlceras crônicas na clínica é ainda alta repercutindo em custos elevados e conseqüências sociais. Além disso, poucos são os estudos que certificam a segurança e eficácia de um tratamento cicatrizante na prática, principalmente dos produtos já utilizados clinicamente para este fim. Assim, o objetivo desse estudo foi avaliar a atividade anti-inflamatória dos compostos utilizados clinicamente como pro-cicatrizantes, tais como calamina (CA), alantoína (AL) e sulfadiazina de prata (SDP) no modelo de edema de orelha induzido por diferentes agentes flogísticos. **Métodos**: Camundongos Swiss fêmeas (20-30 g; N=5-6) foram utilizados para a realização dos experimentos. A atividade tópica dos compostos foi avaliada nos modelos de edema de orelha induzido por óleo de cróton (OC), ácido araquidônico (AA) e fenol. Para tanto, o aumento da espessura (µm) da orelha foi medida com o auxílio de um micrômetro digital, antes e 6 h, 1 h ou 2 h após a aplicação de OC (0,4 mg/orelha), AA (2 mg/orelha) ou fenol (10%), respectivamente. Em seguida foram aplicados os compostos AL, CA, SPD (1,0 mg/orelha), indometacina (1,0 mg/orelha) ou dexametasona (DX) (0,1 mg/orelha). Os agentes flogísticos, assim como os compostos foram dissolvidos em 20 µL de acetona e aplicados na face interna da orelha direita. Após 24 h da indução da inflamação os animais foram mortos e amostras dos tecidos das orelhas foram coletadas para avaliação dos níveis da enzima mieloperoxidase (MPO), sendo que a atividade desta indica a migração de neutrófilos. **Resultados**: A aplicação de AL, SDP e DX inibiu em 53,6 ± 11,85 %, a 56,9 ± 8,8 % e 81 ± 5,17 %, respectivamente, o edema induzido de orelha por OC. Na MPO a AL reduziu a infiltração de PMN em 25,0 ± 9,64 %, a SPD em 19,2 ± 10,52 % e a DX (controle positivo) em 90,5 ± 2,14 %. Já a CA não foi capaz de reduzir o edema por OC e a MPO. No edema induzido por AA, todos os compostos inibiram o edema na dose testada, com valores de 40,7 ± 1,60 % para a AL, 68,1 ± 8,66 % para a CA e de 51,7 ± 13,23 % para a SPD, enquanto a indometacina (controle positivo) inibiu 90,0 ± 8,96 %). Já no modelo de edema de orelha induzido por fenol, nenhum dos compostos foi capaz de causar alteração significativa. **Discussão**: Este estudo mostra que as aplicações tópicas principalmente de AL e SDP apresentaram eficácia sobre eventos inflamatórios como edema e migração leucocitária, sugerindo uma atividade anti-inflamatória que pode se somar a sua possível ação pró-cicatrizante pelo tratamento tópico com estes compostos. **Apoio Finaceiro**: CNPq

09.142

Evaluation of toxicity of DPT vaccines and its correlation with the bacterial endotoxin levels. Fingola FF, Albertino SRG, Domingos R, Zamith HPS INCQS/FIOCRUZ - Farmacologia e Toxicologia

Introduction: The administration of triple bacterial vaccine against diphtheria, pertussis and tetanus (DPT) to children almost invariably leads to adverse post-vaccination events such as flush, heat, fever, irritability, anorexia, and, at a lower frequency, hypotonic-hyporesponsive episodes, among others. The National Institute of Health Quality Control (INCQS) of FIOCRUZ performs toxicologic control of the DPT vaccine by performing *in vivo* nonspecific toxicity (NT) test and specific body weight gain test in mice (BWGT). Although it is not recommended in Pharmacopoeias for the evaluation of toxicity of the DPT vaccine, the *in vitro* test with *Limulus* amebocyte lysate (LAL) is a valuable test for the determination of endotoxin (LPS) concentrations present in the DPT vaccine since LPS is the main component of the DPT vaccine responsible for the production of fever. The objective of the present study, in addition to the assessment of the toxicity of DPT vaccine samples analyzed at the INCQS from 2000 to 2008, was to determine whether there is a correlation between the results of the BWGT and LAL tests. **Methods**: Thirty samples of DPT vaccines from a Brazilian producer were analyzed by the NT, BWGT and LAL tests. The *in vivo* tests were performed in accordance with the Committee for Ethics in Animal Use of the FIOCRUZ (CEUA PO137-02). Albino Swiss mice (18-22 g) and short hair guinea pigs (250-350 g) were used for the NT test and NIH mice (14-16 g) supplied by the Center of Laboratory Animal Breeding of FIOCRUZ were used for the BWGT. The LAL test was performed in the bacterial endotoxin laboratory of the INCQS using kits provided by Cambrex Bio Science, Walkersville Inc., MD, USA. Fifteen of the 30 samples analyzed were obtained from 2000 to 2002 and the remaining 15 were obtained from 2006 to 2008. **Results and Discussion**: Only one of the 30 samples analyzed was unsatisfactory for the NT test during the period from 2000 to 2002. In the BWGT, 14 samples were unsatisfactory, 12 of them from the samples from 2000 to 2002 and only 2 from the samples from 2006 to 2008. In the LAL test, LPS concentrations ranged from 1250 EU/mL to 80,000 EU/mL, with the maximum LPS concentration being always below 20,000 EU/mL during the period from 2006 to 2008. Animal weight loss determined by the BWGT in 9 samples analyzed was always associated with LPS concentrations above 20,000 EU/mL. We conclude that the quality of DPT vaccines regarding toxicity improved significantly from 2000 to 2008. We suggest that the weight loss of the animals determined by the BWGT may be associated with high LPS concentrations starting from 20,000 EU/mL. Finally, no clear relation was observed between the occurrence and frequency of deaths in the BWGT and the concentrations of LPS in the LAL test. Financiamento: INCQS/FIOCRUZ.

09.143

Development of *in vitro* methodology for the assessment of sensibilization phenomenon to histamine induced by pertussis toxin and pertussis vaccine *in vivo*. Miller RA[1], Domingos, R[1], Corrado AP[2], Zamith HPS[1] [1]INCQS/FIOCRUZ - Farmacologia e Toxicologia, [2]FMRP-USP - Farmacologia

Introduction: Among the effects induced by the pertussis toxin (PT) in mammalian species the sensitization to the biological and lethal effects of the histamine provided the establishment of an *in vivo* quality control assay to evaluate the safety of the pertussis vaccine (PV) against whooping cough and of the bacterial triple vaccine, (DPT) against the diphtheria, whooping cough and tetanus. The histamine sensitization assay (HSA) performed with NIH female mice (20 to 24 g) is highly sensitive to pertussis toxin (PT) detecting levels as low as 20 ng of administered PT/dose, PT levels of 84 and 147 ng/ml in DPT vaccines causing 50% lethality. Although the HSA has been conclusive in relation to the high specificity to PT, the high number of mice employed leading to high cost and the suffering of animals are limiting factors that make difficult its routine use as quality control assay of DPT vaccine. **Methods**: The aim of our study was to develop an *in vitro* methodology in ileum segments from female Short Hair guinea pigs (250-300 g) from the animal facilities of the FIOCRUZ to evaluate the sensitization to the histamine by PT. All experiments were approved in accordance with the guidelines of the Committee for Ethics in Animal Use of the FIOCRUZ (CEUA PO137-02). Concentration-effects curves to histamine in guinea pig ileum were studied and the parameters of mean effective concentration (EC_{50}), maximum effective concentration (EC_{max}) and dissociation constant of drug-receptor complex (K_d) were determined. **Results and Discussion**: It was not detected any increase of ileum contractile response to histamine in relation to control PBS, 4 days after intraperitoneal treatment of guinea pigs with doses and dilutions corresponding to mean histamine sensitization dose (HSD_{50}) obtained in NIH female mice of PT (40 ng), PV and of 5 DPT vaccines (0.26 IU). In all of ten assays performed by experimental group, the data followed a normal distribution, the variances were homogeneous and there weren't significant differences among assays. With doses 10 times higher than the HSD_{50} of PT (400 ng) and of PV (2.6 IU) the data showed the same behavior above. Contrary to anticipated results, histamine EC_{50} and K_d values in ileum of guinea pigs treated *in vivo* with PT were significantly higher than the control and PV ($p< 0.05$) with no alteration in EC_{max} ($p= 0.3672$). *In vitro* 15 min treatment of guinea pig ileum with 30 ng/ml of PT reduced about half the EC_{max} in relation to control ($p= 0.0028$) without significant reduction in the mean values of histamine EC_{50} and K_d ($p= 0,09$). Differently, *in vitro* 15 min treatment of ileum with 40 ng/ml of PT significantly reduced histamine EC_{max} ($p< 0.0069$), EC_{50} ($p= 0.0261$) and K_d ($p= 0.0479$) in relation to control ileum. *In vitro* 15 min treatment with PBS did not alter significantly the mean values of histamine EC_{50} ($p> 0.1$), EC_{max} ($p> 0.2$) and of K_D ($p> 0.1$) in relation to control without treatment, demonstrating no effect of solvent control (PBS) on the ileum contractile response by histamine. In conclusion, we demonstrated an increase of the histamine sensitization in female guinea pig ileum after *in vitro* treatment of 30 and 40 ng/ml of PT**.** **Financial support**: INCQS/FIOCRUZ

09.144

Estudo da miotoxicidade do veneno de *Bothrops jararacusu* em camundongos diabéticos: tratamento com heparina. Borges PA[1], Machado MM[2], Fonseca TF[2], Gaban GA[2], Tomaz MA[2], Calil-Elias S[1], Martinez AMB[4], Melo PA[2] [1]FF-UFF - Farmácia e Administração Farmacêutica, [2]ICB-UFRJ - Farmacologia Básica e Clínica [3]UFRJ - Farmacologia Básica e Clínica, [4]UFRJ - Embriologia e Histologia

Introdução: O Diabetes mellitus (DM) é desordem metabólica irreversível, cuja prevalência global continua a aumentar devido ao envelhecimento populacional e alterações no estilo de vida. Para se estudar alterações induzidas pelo DM existem vários modelos em animais. Dentre estes, a indução com substâncias como aloxana, um análogo de glicose que se acumula nas células beta pancreáticas através do transportador de glicose GLUT 2. A diabetogenicidade da aloxana é devida à formação de espécies reativas de oxigênio e consequente necrose (Lenzen S., 2008). Os venenos de serpentes podem ser utilizados como modelo de lesão muscular aguda e crônica para se estudar a regeneração deste tecido em condições favoráveis e patológicas (Calil-Elias et al., 2002). O veneno de *B. jararacussu* (BJU) induz miotoxicidade, seguida de regeneração frequentemente incompleta. Demonstrou-e que a heparina previne e até favorece a regeneração muscular após a lesão por este veneno.(Calil-Elias et al., 2002). **Métodos:** Induzimos diabetes administrando em dose única i.v. de aloxana (70 mg/kg) em camundongos suíços divididos em cinco grupos: controles não diabéticos (CND), controle diabéticos (CD), diabéticos + BJU, diabético + BJU + pós-tratamento com heparina de alto peso molecular (HMWH) e diabético + BJU + pós-tratamento com heparina de baixo peso molecular (LMWH). O veneno foi injetado perimuscular, na dose de 1,0 µg/g (50 µL), próximo ao músculo EDL da pata direita (Calil-Elias et al., 2002). Os grupos controle receberam injeção similar de solução salina fisiológica. O tratamento com heparina (10 µg/g i.v.) foi feito 15 e 240 min. após a injeção do veneno. Após 24 horas de injeção do veneno, os animais foram sacrificados. Os músculos EDL foram retirados, liberados do tecido conjuntivo, secos, pesados e homogeneizados segundo Melo e Ownby (1996). Após a homogeneização observou-se, o conteúdo total de creatinoquinase (CK) analisado em espectrofotômetro. Os valores são expressos em unidades de CK por grama de tecido muscular (U/g). **Resultados e Discussão:** Vinte a quarenta horas após injeção, o tratamento com aloxana reproduziu o aparecimento de hiperglicemia de acordo com o já descrito na literatura, (4-5 vezes superior ao CND), observou-se perda de peso (aprox. 20-30%) e perfil comportamental debilitado do DM. A injeção perimuscular do veneno induziu aumento da atividade de CK no plasma sem diferença significativa entre os grupos CDN, e DM, 2 horas após injeção do veneno. A análise do homogeneizado 24 h após, mostrou redução em mais de 50% do conteúdo de CK, este efeito do veneno não foi antagonizado pela heparina. Os resultados nos levam inicialmente a concluir que: 1-O veneno de *B. jararacussu* apresenta atividade miotóxica de mesma intensidade em camundongos DM comparados aos CDN; 2- O pós-tratamento com heparina não interfere nesta etapa de lesão pelo veneno em camundongos DM, sendo necessárias avaliações em período mais prolongado, no qual ocorre a regeneração (cerca de 28 dias). Calil-Elias et al., Histol Histopathol. 217: 463(2002). Melo e Ownby, 34: 653 (1996). **Suporte Financeiro:** CAPES, CNPq, FAPERJ, PRONEX e FUJB-UFRJ

09.145

Potassium channel activation is involved in the negative inotropic effect of aqueous fraction of the *Plectranthus amboinicus* (Lour.) spreng in guinea pig left atrium. Rodrigues de Oliveira V[1], Gondim ANS[2], Brandão WB[3], Silva BA[3], Conde-Garcia, EA[1] - [1]UFS - Fisiologia, [2]UNEB - Educação, [3]LTF-UFPB - Ciências Farmacêuticas

Introduction: *Plectranthus amboinicus* (Lour.) Spreng. (Lamiaceae) has been largely used in folk medicine. Usually known as “hortelã-graúda”, "alfavaca grossa" or "malvarisco" (in Brazil) it has been used as antiseptic, antithermic, and to treat bronchial asthma. Their leaves are rich in triterpenes, apigenin, and quercetin among other compounds. In spite of its biological activities, the scientific literature is scarce concerned to its action on the mammalian myocardium. The objective of the present study was to investigate the contractile effect produced by *P. amboinicus* leaf aqueous fraction (FAq) and the existence of inhibitory contractile mechanism such as the activation of the membrane potassium channels, on the guinea pig left atrium. **Methods**: The hydroalcoholic crude extract (EBH) was obtained by macerating dry leaves in water:ethanol (1:1, v/v, 8 days). The FAq was prepared by dissolving EBH in deionized water. Insolubles residues were discarded by filtration. This work was submitted and approved by Ethical Committee of Animal Research / Federal University of Sergipe (Process # 31/05). The experiments were carried out on the guinea pig (*Cavia porcellus*) left atrium. The preparation, was maintained in an organ chamber (5mL, stretched to 1gf, stimulation: 2 Hz; 400 V; 0.5 ms), bathed by Tyrode solution, aerated by carbogen mixture (95 % O_2 / 5 % CO_2, 27 ± 0.1 °C). Atrial contraction force was measured isometrically (HP FTA10 force transducer) coupled to a HP 8805B amplifier. Electrical signals were amplified, digitalized (DATAQ DI400) and stored in computer. Concentration-effect curves concerned to the inotropic effect of FAq were obtained before and after adding tetraethylammonium (TEA, 20 mM) to the bath. **Results and Discussion:** FAq reduced the atrial force (CE_{50} = 430 ± 110 µg/mL), in a concentration-dependent fashion. This effect disappeared during washout. TEA (20 mM), a non-selective potassium channel blocker, reduced the FAq inotropic effect, shifting rightward the concentration-effect curve of FAq and increasing CE_{50} from 430 ± 110 µg/mL to 1600 ± 138 µg/mL (n = 3 atria, $p < 0,05$). The negative inotropic effect can be explained by the activation of sarcolemmal potassium channels. **Financial Support:** ELETROBRÁS, FAPITEC/SE, CNPq, UFS.

09.146

Avaliação da atividade antimicrobiana de folhas de pata de vaca adquiridas no mercado Municipal de Campo Grande, MS. Maldonado KS[1], Garcia DCB[1], Gimenes AHG[1], Schwab L[1], Tomazoni E[1], OLIVEIRA EJT[1], Mariano YY[1], Araújo BS[1], Negrete CL[1], Oliveira RF[2], Yano M[2] [1]UCDB - Farmácia, [2]UCDB - Biotecnologia

Introdução: A *Bauhinia forticata,* conhecida popularmente como pata de vaca, é pouco usada em arborização urbana devido a seu tronco espinhoso, no entanto portadora de uma das mais belas flores e folhagem entre as *Bauhinias,* como também é bastante difundida na medicina popular, frequentemente encontrada na composição de fitoterápicos industrializados, além de ser comercializada em feiras livres. As folhas de pata de vaca são empregadas popularmente como diurético e especialmente no tratamento de diabetes, pois é cientificamente comprovada a presença de insulina em sua composição. Esse trabalho teve como objetivo avaliar a atividade antimicrobiana do extrato de folhas de pata de vaca obtidas no Mercado Municipal de Campo Grande, MS. **Métodos:** As folhas de pata de vaca foram adquiridas no Mercado Municipal de Campo Grande, MS, moídas e preparado o extrato bruto etanólico das folhas por maceração estática. O extrato foi filtrado e seco no rotaevaporador e, em seguida, preparadas alíquotas de 0,25g do extrato. Após seco, o extrato foi ressuspendido em 2,5 mL de solução salina a 0,9% e preparadas as seguintes concentrações: 100%, 50% e 25%. A avaliação da atividade antimicrobiana foi realizada *in vitro* utilizando-se os seguintes microrganismos: *Staphylococcus aureus* ATCC 6538, *Pseudomonas aeruginosa* ATCC 9027, *Klebsiella pneumoniae* ATCC 700603 e *Candida albicans* ATCC 10231. Para os testes, foram utilizados discos estéreis de papel de 6 mm de diâmetro, impregnados com 20 µL de cada concentração (100%, 50% e 25%). Após secagem, os discos foram colocados em placas de Petri com meio Ágar Mueller-Hinton, para as bactérias e Ágar Batata Dextrose, para o fungo, onde foram inoculados os microrganismos em solução padronizada. Numa placa de Petri, além dos discos de extrato, foram colocados também um controle negativo (solução salina a 0,9%) e um controle positivo (penicilina para *S. aureus*, gentamicina para *P. aeruginosa*, tetraciclina para *K. pneumoniae* e itraconazol para *C. albicans*), sendo os testes realizados em triplicata. As placas foram incubadas em estufa a 37°C por 24 horas. Após o período de incubação, os resultados foram lidos para a verificação da presença ou não de halos de inibição (mm). **Resultado e Discussão:** O extrato bruto etanólico de folhas de pata de vaca não demonstrou atividade antimicrobiana frente aos microrganismos *S. aureu*, *P. aeruginosa, K. pneumoniae* e *C. albicans,* e nas concentrações testadas, sendo essas cepas não sensíveis ao extrato. Especialmente nas últimas décadas, inúmeros esforços têm sido dirigidos para conferir às plantas seu real papel e valor na terapia. O consumidor tem se tornado cada vez mais exigente e mais criterioso com a qualidade do produto que consome, sendo crescente a sua preocupação em fazer uso de produtos menos agressivos de origem natural ou o mais próximo possível desta origem, portanto, a não inclusão de matérias primas sintéticas para, por exemplo, conservação do produto final. Por tal motivo remete-se à pesquisa de produtos extraídos de fitoterápicos e produtos naturais, como a busca de atividade antimicrobiana no extrato de folhas de pata de vaca aqui praticado **(PIBIC/UCDB e CNPq)**.

09.147

Influence of chronic intake of passion fruit bark flour in biochemical parameters in type II diabetic patients. Braga A[1], Araujo, BV[2] [1]URI - Acadêmica, [2]URI - Ciências da Saúde

Introduction: Research about the potential use of soluble fibers in diabetic patients, showed the positive effect of this food supplement in glycemic control and cholesterol levels (Wheeler, ML, J Am Diet Assoc, v.108, p.S34, 2008). The dried bark of *Passiflora edulis* shows a source of soluble fiber, considering that 19% of its constitution is pectin (Yapo, BM, J. Agric. Food Chem, v.54, p.2738, 2006), which makes it plausible to use as an adjuvant in the treatment of diabetic patients. **Methods:** This study evaluated the efficacy of soluble fiber present in passion fruit in the maintenance of glucose and lipidic levels in type II diabetic patients. The patients were allocated into two groups: treated group (n=22) and control group (n=22). The treated group received 24g of passion fruit bark flour daily, divided in three doses, which had been administered with water before main meals for 60 days. Blood samples were collected at 0, 30 and 60 days after treatment from treated and control groups began. The samples underwent assess biochemical parameters such as glucose, total cholesterol, HDL cholesterol, triglycerides and frutosamine plasma and compared through ANOVA in two ways considering time and treatment (Sigma Stat® v.3.5.1). All the experiments were realized in the Clinical Biochemistry Laboratory at University and previously approved by Ethics Committee of URI (#042-04TCH-08). **Results:** After treatment, no statistic reduction ($p < 0.05$) was observed for fasting glucose in the treated group at the baseline (210.42 ± 70.0 mg/dL) at 30 days (172.5 ± 52.73 mg/dL) and at 60 days (178.75 ± 47.11 mg/dL). Reduction of total cholesterol ($p < 0.05$) in hypercholesterolemic patients of the treated group were observed (n=5), at baseline (255.25 ± 27.60 mg/dL) at 30 days (204.31 ± 43.82 mg/dL) and at 60 days (220.79 ± 41.35 mg/dL). No significant differences were observed in the control group and the other parameters tested in the treated group. The side effects frequently experienced by patients in the treated group included diarrhea, nausea and sleepiness. **Discussion:** The results show the effect of passion fruit bark flour in reducing levels of fasting glucose and total cholesterol in hypercholesterolemic subjects in the first 30 days of treatment, lasting to the end of the study. It is postulated that the antihyperglycemic effect is associated with soluble fiber fermentation by intestinal bacteria and the formation of a highly bioadhesive viscous mucous in the intestines, which diminishes the surface of contact during meal intake with the gastrointestinal mucous, delaying the glucose absorption (Sartorelli, Arq Bras Endocrinol Metab, v.50, p.415, 2006). Regarding the reduction of total cholesterol in hypercholesterolemic patients, it can be associated with the capacity of soluble fiber to reduce the amount of bile reabsorption in the intestines, and therefore the cholesterol. The results point out a positive effect of regular use of this food supplement in type II diabetic patients.

09.148

Studies on antidiarrhoeal activity of the roots from *Solanum paludosum* Moric. in mice.

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Introduction: *Solanum paludosum* Moric. (Solanaceae) is herbaceous species, known popularly as "jurubeba-roxa" in the Northeast of Brazil. Pharmacological studies of the root bark of this species showed spasmolytic activity on rat jejunum and hypotension on rats. Recently, total alkaloids fraction from root bark *of Solanum paludosum* showed spasmolytic effect on guinea-pig ileum. In addition, some *Solanum* species also showed antidiarrhoeal activity. Therefore, we decided to investigate a possible antidiarrhoeal activity of the methanol extract obtained from roots of *Solanum paludosum* (SP-$MeOH_R$) in mice. **Methods:** *Castor oil-induced diarrhoea***:** mice were weighted and divided into control (saline), positive control (loperamide 10 mg/kg) and test groups (SP-$MeOH_R$ 250, 500 or 750 mg/kg), containing four mice in each group. Each animal was placed in an individual cage which had the floor was lined with blotting paper and it was changed every hour. Diarrhoea was induced by oral administration of 0.4 mL castor oil/mice 30 min after the above treatments. During an observation period for 3h, the total number of faecal output and number of wet faeces excreted by the animals were recorded. *Normal intestinal transit***:** animals were divided into 3 groups of 6 animals each. Group 1 received saline 10 mL/kg, p.o. (negative control); group 2 were administered atropine 2 mg/kg p.o. (positive control); and group 3 were administered SP-$MeOH_R$ 125, 250 or 500 mg/kg p.o. (test groups). After 30 min, standard charcoal meal (0,4 mL/mice) were given to mice orally. Animals were sacrificed 30 min after administration of charcoal meal and the small intestine immediately isolated. All the experimental protocols were approved by Ethical Committee in Research of UFAL (Protocol 027241/2008-11). **Results**: the SP-$MeOH_R$ (250, 500 and 750 mg/kg) produced antidiarrhoeal activity in the study, when inhibiting significantly ($P < 0.001$), both the frequency of defaecation (100, 70.7 and 93.3 %, respectively) as well as the wetness of the faecal droppings (100, 96.7 and 100 %) on mice. This effect of the extract was similar to that of the standard drug, loperamide (10 mg/kg), which produced a maximum inhibition of 96,7 % of the total number and 100 % of the wet faeces. However, this effect of the extract not may be related to an inhibition of muscle contractility and motility, since SP-$MeOH_R$ was unable to inhibit the intestinal transit by charcoal meal, unlike from atropine (2 mg/kg) that inhibited 54,5 ± 4,4 %. **Discussion**: diarrhoea is the frequent passage of wet faeces and it involves both an increase in the motility of the gastrointestinal tract, along with increased secretion and decreased mucosal absorption of fluid, and thus a loss of electrolytes and water. Hence 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 obtained in this study suggest that the SP-$MeOH_R$ possesses significant antidiarrhoeal activity, however other studies must be carried out to elucidate the mechanisms involved in these activity. **Financial support**: PIBIC/UFAL/FAPEAL.

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Study of the therapeutic effects of copaíba oils (*Copaifera sp*) in patients with knee inflammation disorders. Oliveira ED de, Moura TO, Almeida GKM, Souza VTC, Cruz KML
UFS - Fisioterapia

Aim: The aim of this work is to investigate the effect copaíba oil the knee inflammation disorders (gonoarthrosis). **Methods and results:** Fourteen patients (11 women and 03 men) with gonoarthrosis and with range age of 45 – 65 years. The patients were randomly in test and control groups. The control group receiving pulsation ultrasound wave (model: Sonacel Dual, sing: Bioset) of 1 MHz frequency and 0,8 watt/cm^2 power were applied for 8 minutes followed of massage mineral oil for 5 min. to the knee joint for a total treatment period of 10 sessions. The test group receiving pulsation ultrasound wave (model: Sonacel Dual, sing: Bioset) of 1 MHz and 0,8 watt/cm^2 power were applied for 8 min. followed of massage copaíba oil for 5 minutes to the knee joint for a total treatment period of 10 sessions. After the application of massage the groups to performed exercise in order to complement of the treatment through passive muscle stretching. In both the groups the time of each session for 30 minutes. The evolution of flogistics signals were evaluated in the first and ten session, through of the goniometry, perimetry, muscle power scale and visual analogue scala (VAS). In the assessments of the VAS were observed that the test group reduced the 72,6 ± 1,2% pain while the control group reduced the 61,3 ± 3,4 % pain. The movement range were increase on the control at 9,8 ± 0,9% and the test at 20 ± 0,8%. After the assessment of the 10 sessions were observed that control group didn't present to increase of the power scale and the test group presented to increase of the 7,2 ± 0,8%. **Conclusion:** In accord with results achieved, the group that receiving copaíba oil treatment in the knee inflammation disorders (test group) obtained the better therapeutic efficacy in the reduced of disease symptom.CEP/UFS: CAAE: 0004.0.107.000-09.
Support: Paird/UFS

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Involvement of K^+ channels on tocolytic effect and investigation of hemolytic activity of labdane-302. Travassos RA[1], Macedo CL[1], Correia ACC[1], Pessôa HLF[2], Tavares JF[1] Silva MS[1], Silva BA[1] [1]LTF-DCF-CCS-UFPB, [2]DBM-UFPB

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 tocolytic effect on rat uterus through modulation of K^+ channels that, indirectly, can block the Ca_V-channels leading to that effect. Furthermore, this diterpene presents cytotoxic effect in lung fibroblasts of Chinese hamster strain V79 (TAVARES, Journal of Natural Products, v.69, p.960, 2006). Thus, we aim to verify a hemolytic activity in human erythrocytes to guarantee that it is not toxic and to deepen the mechanism of tocolytic action of labdane-302 on rat uterus, investigating a possible participation of the potassium channel in this effect. **Methods:** Erythrocytes were isolated from blood of human according to the method described by Rangel (1997). Total hemolysis was obtained with 1 % Triton X-100 detergent and the percentage of hemolysis of the labdane-302 ($3x10^{-5}$, 10^{-4} and $3x10^{-4}$ M) was calculated relative to this value. To investigate the mechanism of tocolytic action, the uterus was suspended in organ bath containing Locke Ringer solution (pH 7.4) at 32 °C, gassed with 95 % O_2 and 5% CO_2 mixture and resting tension of 1 g. Isometric contractions were registered. All the experimental protocols were approved by Ethical Committee in Animal Research of LTF/UFPB (Protocol 0506/05). **Results:** In the evaluation of hemolytic activity on human erythrocytes, labdane-302 did not induce a significant hemolysis at concentrations of $3x10^{-5}$, 10^{-4} and $3x10^{-4}$ M (n = 3). About the tocolytic action, labdane-302 relaxant effect (EC_{50} = 4.9 ± 0.6 x 10^{-5} M, n = 5) was decreased on 7 folds (EC_{50}= 3.5 ± 1.1 x 10^{-4} M) in the presence of CsCl ($p < 0.001$), a non-selective K^+ channels blocker (TRAVASSOS, R. A., SBFTE, 2008). We decided to investigate what subtype of K^+ channels participate in this labdane-302 effect. The relaxation promoted to diterpene was not significantly reduced (n = 5) by 3 mM 4-AP (K_V blocker), however in the presence of 1 mM TEA^+ (BK_{Ca} blocker) the relaxation action was decreased on 3 folds (EC_{50}= 1.4 ± 0.05 x 10^{-4} M, n = 5) in a significant and concentration-dependent manner. **Conclusion:** As the labdane-302 showed no damage to the erythrocyte membrane of humans on the concentrations used on uterus rat assays, *in vitro*, it is an indicator of safety to continue the studies and, probably, it would have low or no toxicity when tested *in vivo.* The relaxant effect of labdane-302 appears to be due to activation of K^+ channels, and the subtype BK_{Ca} can be involved in tocolytic effect of this diterpene, in functional level. However, others subtypes of K^+ channels, have not been investigated, could be involved in tocolytic effect of this diterpene. **Supported:** CAPES, CNPq, LTF/UFPB

09.151

Efeito do extrato etanólico da *Chamomilla recutita* (L) Rauschert sobre a contração atrial de cobaia. Oliveira ED de, Oliveira, LR, Ubirajara WM, Souza JB UFS - Fisioterapia

Objetivo: Este trabalho visou avaliar os efeitos inotrópicos do extrato etanólico da *C. recutita* (camomila) na contração do átrio esquerdo de cobaia. **Métodos e Resultados**: Os estudos foram realizados em átrio esquerdo de cobaia (AE) montado em cuba (Tyrode, 27±0-1^{0}C, 95%O_2, 5% CO_2), estirado (1gf) e estimulado (400 V, 0.5 ms). A execução deste trabalho foi aprovada pelo CEPA/UFS (Comitê de Ética em Pesquisa Animal da Universidade Federal de Sergipe) com protocolo número 34/209. O extrato foi adicionado cumulativamente à cuba e a força isométrica (HP FTA-10, HP 8805B) foi registrada (HP 7754ª, HP 7754B), digitalizada (DATAQ DI400) e gravada em computador. O efeito do extrato foi testado no controle (n = 4) onde a curva concentração efetiva (CE_{50}) foi obtida através da equação de Hill-Langmuir e a excitabilidade atrial investigada pelos períodos interpicos. A força isométrica da contração atrial foi reduzida em 80% apresentando uma CE_{50} de 350 ug/mL. O efeito foi totalmente revertido a lavagem. Os tempos de contração (Tc) e relaxamento (Tr) medidos a 80, 50 e 20% da amplitude da força diminuíram em 15% e aumentaram entre 25, 31 e 38% respectivamente de acordo com as respectivas concentrações: 100 ug/mL, 300ug/mL e 1000 ug/ml. O período interpicos no controle e no teste foi de 500±1ms e 500±4ms respectivamente (p>0,05). **Conclusões**: No átrio esquerdo de cobaia a camomila deprimiu a força de contração com CE_{50} de 350 um/mL e não alterou a excitabilidade tissular. Apoio: CNPq/UFS

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Possíveis efeitos ansiolítico e antidepressivo do extrato bruto de *Achillea millefolium* L.

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Introdução: A *Achillea millefolium* L (Compositae) é popularmente conhecida como "Mil-folhas" apresenta relevância etnofarmacologica por ser uma planta útil para o tratamento de distúrbio do sistema nervoso central (Vafaei et al. (2006) , embora poucos dados científicos foram publicados. **Objetivo**: avaliar a atividade de ansiolítica e antidepressiva do extrato bruto hidroalcoolico das partes aéreas de *Achillea milefollium* (EB-Am) em camundongos. A participação gabaérgica foi estudada por administração prévia de picrotoxin e flumazenil. **Métodos**: Foram utilizados camundongos Suíços adultos machos (40 – 50g; n=6 por grupo), para a verificação de um possível efeito no sistema nervoso central. Os animais foram tratados por gavagem com EB-Am nas doses de: 0, 100, 300 e 600 mg/kg; imipramina (10 mg/kg) e diazepam (0,75 mg/kg). A dose utilizada para os tratamentos agudo e crônico (25 dias) foi de 300 mg/kg. Todas as drogas foram administradas em volume final de 5ml/kg de peso corporal. Picrotoxina (0,5 mg/kg) e flumazenil (1,0 mg/kg) foram utilizados para avaliar a participação GABAérgica no efeito ansiolítico do EB-Am. Para avaliar o comportamento geral, após a administração do EB-Am, os animais foram observados nos tempos de 30 minutos, 1h, 2h até 96h. Para avaliar a performance motora e a atividade exploratória foram realizados os testes de abdução de patas posteriores e placa perfurada. A possível ação ansiolítica foi avaliada pelo teste do labirinto em cruz elevado. A ação antidepressiva foi avaliada nos testes de natação forçada e suspensão pela cauda. Todos os experimentos foram aprovados pelo Comitê de Ética em Experimentação Animal da Universidade Paranaense (UNIPAR). **Resultados**: Na avaliação comportamental, após 30 min, a dose de 600 mg/kg promoveu sedação que perdurou até a terceira hora. Na dose de 300 mg/kg foi observado um aumento da atividade exploratória (30 min até 24h; C=19,5±10; DZP=71,0±10**; EB-Am300=49,75±10**). Foi observado um aumento da percentagem de tempo gasto nos braços abertos após a administração aguda (C=8,2± 5; DZP=53±20*; EB-Am300= 65,2±21*) e crônica (C=36± 6; DZP=75±3*; EB-Am300= 56±3*) do EB-Am (ANOVA seguido de Newman-Keuls, p <0,05). Na natação forçada, a administração crônica do EB-Am diminui o tempo de imobilidade (C=23± 15; EB-Am300=5±3*; DZP=54±3,0*; IMI= 16±9), um efeito semelhante a drogas antidepressiva. Por outro lado, nenhum efeito foi observado no teste de suspensão pela cauda. Os resultados preliminares indicaram que o EB-Am tem um potencial efeito antidepressivo e ansiolítico. Embora este resultado corroborou com o efeito anticonflito encontrado por Molina-Hernadez *et al*. (2004) o efeito antidepressivo foi observado pela primeira vez. Além disso, o efeito ansiolítico parece ser independente da neurotransmissão GABAérgica, uma vez que não foi bloqueado pelo tratamento prévio com flumazenil. As doses mais elevadas de EB-Am utilizadas no presente estudo não induzem sinais clínicos de toxicidade após tratamento de longo prazo em ratos (Dalsenter *et al*., 2004). Estes resultados demonstram que o EB-Am apresenta uma possível ação ansiolítica e antidepressiva. **Apoio Financeiro**: Universidade Paranaense – Umuarama / PR

09.153

Investigação da inviabilidade de cepas patogênicas humanas pelo extrato etanólico de galhos de *Mamica* obtidos no mercado municipal de Campo Grande-MS. Sarate SO[1], Gimenes AHG[2], Schwab L[2], Negrete CL[2], Tomazoni E[2], Yano M[3], Oliveira RF[4] [1]UCDB - Ciências Biológicas e Saúde, [2]UCDB - Farmácia, [3]UCDB, [4]UCDB - Biotecnologia

Introdução: A mamica é uma espécie vegetal pertencente à família Rutaceae. É comumente encontrada na região sul-americana, utilizada popularmente para o tratamento de quadros inflamatórios, infecciosos e cancerosos (SILVA et al, 2007; MOURA et al, 1997). Estudos recentes confirmam a capacidade exercida pelo β-cariofileno, extraído do óleo essencial de *Zanthoxylum rhoifolium* Lam. frente à células tumorais ascíticas de camundongos suíços *in vitro* e *ex vivo*. A validação da sua eficácia na inviabilidade do *Plasmidium falciparum* também tem sido reportada (JULLIAN et al, 2006). Objetiva-se avaliar o potencial antimicrobiano dessa espécie, adquirida no Mercado Municipal de Campo Grande -MS, frente a microorganismos promotores de elevado grau patogênico em humanos. **Materiais e Métodos:** Amostras de 0,25g do extrato etanólico seco de cada espécie foram ressuspendidos em 2,5 mL de solução salina 0,9% (concentração de 100%) e preparada outra diluição de 50%. Posteriormente, foram micropipetados alíquotas de 20µL de cada extrato diluído em discos de papel filtro com diâmetro de 6mm. Em placas de vidro contendo ágar Müller-Hinton, cultivou-se cepas de *Staphylococcus aureus* ATCC *25923*, *Pseudomonas aeruginosa* ATCC *9027* e *Klebsiella pneumaniae* ATCC *13833* e em ágar Batata Dextrose, a levedura *Candida albicans* ATCC *10231* sendo os discos contendo os extratos, aplicados sobre as placas semeadas como sugere o método de difusão em disco proposto por Bauer *et al.* 1966. Para o grupo controle, utilizou-se o antibiótico penicilina para *S. aureus*, gentamicina para *P. aeruginosa*, tetraciclina para *K. pneumoniae* e itraconazol para *C. albicans*. **Resultados e Discussão:** Das concentrações testadas, o EBEG de mamica demostrou inviabilidade apenas para a cepa de *S. aureus*, com halo de inibição correspondente a 9mm na maior concentração (100%) e 7mm para a menor (50%). Não houveram diâmetros inibitórios para as *P. aeruginosa, K. pneumoniae* e *C. albicans*, como sugere a Tabela abaixo, que representa a média de resultados obtidos em triplicata para cada microrganismo:

Tabela 01. Média de halos de inibição do EBEG de mamica frente aos microrganismos: *S. aureus, P. aeruginosa, K. pneumoniae* e *C. albicans.*

Microrganismos	**Halo de inibição (mm)** Concentrações		
	C. posit.	100% (2,5 mg/mL)	50% (1,25 mg/mL)
S. aureus ATCC 25923	21 PEN	9	7
P. aeruginosa ATCC 54919	20 GEN	-	-
K. pneumoniae ATCC 13883	20 TET	-	-
C. albicans ATCC 10231	28 ITR	-	-

Legenda: (-) Resultados negativos; (PEN) Penicilina; (GEN) Gentamicina; (TET) Tetraciclina; (ITR) Itraconazol.

Conclusão: Apesar dos halos mínimos, a atividade antimicrobiana para *S. aureus* é presente para o EBEG de mamica, sendo interessante, estudos posteriores de prospecção e isolamento químico da(s) substância(s) promotoras de tal atividade. (PIBIC/UCDB).

09.154

Verificação do efeito antimicrobiano do extrato etanólico de galhos de *Suma* comercializados no Mercado Municipal de Campo Grande, MS (Mercadão). Sarate SO[1], Gimenes AHG[2], Negrete CL[3], Schwab L[2], Yano M[4] [1]UCDB - Ciências Biológicas e Saúde, [2]UCDB - Farmácia, [3]Curso de Farmácia, UCDB - Saúde, [4]UCDB

Introdução: A suma é uma planta da família Malpighiaceae, geralmente encontrada nos estados de Minas Gerais, São Paulo, Goiás, Mato Grosso, e Espírito Santo. Por sua propriedade tóxica, tem se tornado constante problema para empreendedores rurais. De fácil adaptação a terrenos não muito férteis e por causa da boa palatabilidade, a espécie *Mascangia coriacea* é ingerida por animais bovinos junto com a forragem, causando morte por intoxicação (MARTINS, 1998). Este trabalho tem por finalidade, avaliar a eventual citotoxicidade do extrato etanólico de galhos de suma adquiridos no Mercado Municipal de Campo Grande em quatro cepas de microrganismos de potencial patogenicidade em humanos. **Materiais e Métodos:** Amostras de 0,25g do extrato do extrato seco de cada espécie foram ressuspendidos em 2,5 mL de solução salina 0,9% (concentração de 100%) e preparadas outras duas diluições a 50 e 25%. Posteriormente, foram micropipetadas alíquotas de 20µL de cada extrato diluído em discos de papel de filtro com diâmetro de 6mm. Em placas de Petri contendo ágar Müller-Hinton, cultivou-se cepas de *Staphylococcus aureus* ATCC *25923*, *Pseudomonas aeruginosa* ATCC *9027* e *Klebsiella pneumoniae* ATCC *13833* e em ágar Batata Dextrose, a levedura *Candida albicans* ATCC *10231* sendo os discos contendo os extratos, aplicados sobre as placas semeadas segundo o método de difusão em disco proposto por Bauer *et al.* 1966. Para o grupo controle, utilizou-se o antibiótico penicilina para *S. aureus*, gentamicina para *P. aeruginosa*, tetraciclina para *K. pneumoniae* e itraconazol para *C. albicans*. **Resultados e Discussão:** Foram demonstrados resultados positivos apenas para a cepa de *S. aureus,* com diâmetro de inibição correspondente a 8 mm na maior concentração (100%) e 7 mm para a concentração intermediária (50%). Para a concentração de 25%, todos os resultados mostraram-se negativos. O halo exercido pelo grupo controle, penicilina, gentamicina, tetraciclina e itraconazol foram de 21, 20, 20 e 28 mm, respectivamente. **Conclusão:** Os valores obtidos podem sugerir uma possível aplicação do vegetal como adjuvante no tratamento de infecções causados pelo gram-positivo *S. aureus*. Estudos podem ser realizados, verificando demais propriedades tóxicas do extrato, para sua posterior utilização no preparo de formulações tópicas. (PIBIC/UCDB).

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Avaliação do efeito agudo do extrato hexânico de *Siparuna guianensis* (negramina) sobre o comportamento de ratos da linhagem Wistar. Barros WM[1], Vanzeler MLA[2], Molina CV[3], Da Silva LE[1], Valentini CMA[4] [1]UFMT - Química, [2]UFMT, [3]UNIVAG - Farmácia, [4]FAMEV-UFMT - Agricultura Tropical

Introdução: As plantas contém metabólitos secundários que promovem efeitos farmacológicos características de cada classe. *Siparuna guianensis* é nativa principalmente da Amazônia, e conhecida popularmente como negramina, muito utilizada como anti-inflamatório e antireumático. O objetivo foi avaliar a atividade aguda do extrato de *Siparuna guianensis* sobre o comportamento de ratos da linhagem – Wistar*,* de acordo com protocolo no. 123 do CEEA/FCMSC-SP. **Método:** Para a preparação dos extratos, amostras das cascas dos frutos foram secas, trituradas e submetidas à extração em aparelho Soxhlet inicialmente com hexano e em seguida com etanol, por três repetições e o solvente removido completamente por evaporação a vácuo, obtendo-se o extrato etanólico das cascas (EMCSG) e o extrato bruto hexânico (EBHSG). Foram administradas EBHSG (00, 100 e 500 mg/kg) em *Rattus novergicus* da linhagem Wistar, 1 horas antes para a observação da atividade comportamental em campo aberto e labirinto em cruz elevado por 5 min. **Resultados**: O teste de ANOVA não demonstrou diferenças estatísticas $p > 0,05$ nos parâmetros analisados: a) locomoção total nas doses de 100 mg/kg (82,2± 10,5) e 500 mg/kg (63,7±10,9) quando comparados ao grupo controle (68,5± 10,5); b) locomoção central nas doses de 100 (1,1± 1,5) e 500 mg/kg (1,3± 0,5) e o controle (2,6± 1,0); c) *rearing* em número de unidades, para as doses de 100 (5,7±0,7) e 500 mg/kg (7,0± 1,8) e o controle (4,7± 1,4); d) cropólitos para as doses de 100 (0,87±0,6) e 500 mg/kg (0,87± 0,5) e o controle (3,1± 1,2); e) *grooming* para as doses de 100 (21,9±7,6) e 500 mg/kg (15,5± 6,6) e o controle (3,4±1,7); f) entradas no braço aberto nas doses de 100 (2,1± 0,7) e 500 mg/kg (1,5 ± 0,4) e o controle (2,5± 0,5); g) entradas no braço fechado para as doses de 100 (3,6± 1) e 500 mg/kg (1,9± 0,5) e controle (2,9 ± 0,4); h) *head-dips* em número de unidades, para as doses de 100 (0,0 ± 0,0) e 500 mg/kg (0,5± 0,19) e controle (0,12 ± 0,12). **Discussão**: O presente resultado sugere que o EBHSG não alterou a atividade motora nos parâmetros analisados, porém outros trabalhos serão necessários para esclarecer estes dados. **Apoio financeiro:** IFMT - CAMPUS BELA VISTA,UNIVAG - CENTRO UNIVERSIÁRIO

09.156

Effect of treatment with a proteolytic fraction from the latex of *Carica candamarcensis* Hook. F. 1875 in the immunohematopoietic response of mice with or without Ehrlich ascites carcinoma. Viana CTR[1], Stehling LFO[1], Dittz D[1], Silva ACA[1], Figueiredo C[1], Villalba MIC[1], Salas CE[2], Lopes MTP[1] - [1]UFMG - Farmacologia, [2]UFMG - Bioquímica e Imunologia

Introduction: The Ehrlich carcinoma produces severe changes in the hematopoietic system of the host, which displays key roles in the development of the tumor. Previous results from studies involving P1G10, a fraction rich in cysteine proteases obtained from the latex of *Carica candamarcensis* by chromatography in Sephadex G10, showed a significant reduction in cellularity in the ascites fluid of mice bearing this tumor. Considering this, we investigated the effect of P1G10 on the immunohematopoietic response of mice bearing Ehrlich ascites carcinoma. **Methods**: Swiss mice (n = 40) were divided into 4 groups: 2 of which were inoculated, *i.p.* with 1 x 10^7 Erlich tumor cells and the others received 100 µL of PBS (phosphate-buffered saline), *i.p*. The treatment was carried out for 10 days with PBS or P1G10 (1 mg/kg), *s.c*. In another test, Swiss female mice (n = 20) were pre-treated for 10 days and then inoculated with Ehrlich tumor cells (1 x 10^7 cells). On the 11^{th} day after inoculation, in both trials, the animals were sacrificed and blood samples taken for determining leukocytes count; the femurs were removed for determining the total number of cells in the bone marrow. (Protocol # 090/09, CETEA UFMG). **Results and Discussion**: In animals bearing Ehrlich tumor, P1G10 (1 mg/kg) significantly reduced the number of cells in the bone marrow (*2.55 ± 1 x 10^7 cells / ml; Control: 4.26 ± 1.5 x 10^7 cells / ml). There was no increase in the number of leukocytes in peripheral blood (19.19 ± 6x 10^7 cells / ml, control 16.04 ± 3.3 x 10^7 cells / ml). In animals without tumor, the administration of P1G10 did not increase the number of cells in the bone marrow (6.6 ± 1.7 x 10^7 cells / ml, Control: 5.2 ± 0.1 cells / ml). When the animals were pre-treated with the fraction, however, there was a significant increase of the cellularity (*1.7 ± 0.2 x 10^7 cells / ml; Control 1.2 ± 0.03 x 10^7 cells / ml). The number of circulating leukocytes did not differ in any of the trials. These results show that the treatment with the fraction P1G10 was able to reduce the number of cells in the bone marrow of mice with the tumor and increase it in mice without the tumor. On the other hand, when animals are pre-treated with P1G10 there is no change in the number of cells, suggesting that when the tumor is present the fraction acts on the hematopoietic system and on the release of cells from bone marrow to peripheral blood. The results seem promising and further trials are in progress. * $p < 0.05$, Student´s *t* test. **Financial Support**: CNPq, FAPEMIG and CAPES.

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Estudo do mecanismo da ação antinociceptiva dos extratos de *Eugenia brasiliensis* E *Eugenia beaurepaireana* no modelo da formalina em camundongos. Beirith A[1], Cabrini DA[2], Otuki MF[2], Pizollatti MG[4], Brighente IMC[3], Arruda F[4], Magina MDA[4] [1]FURB - Ciências Naturais, [2]UFPR - Farmacologia, [3]UFSC - Química, [4]FURB - Ciências Farmacêuticas

Introdução: A *Eugenia brasiliensis* Lamarck, é conhecida popularmente como "grumixama", "grumixameira", "grumixaba", "itapoiroti" e "cumbixaba" e a *Eugenia beaurepaireana* (Kiaerskou) Legrand, é conhecida como "ingabaú" e "guamirim-ferro". São encontradas desde a região Nordeste do Brasil e se distribuem uniformemente, em toda a mata atlântica, desde o extremo norte ao extremo sul de Santa Catarina. São utilizadas na medicina popular para o tratamento de inflamações, dores, infecções urinárias e outros tipos de infecções. O presente estudo investiga o mecanismo da ação antinociceptiva dos extratos hidroalcoólicos das folhas e galhos da *E. brasiliensis e E. beaurepaireana,* no modelo de nocicepção induzida pela formalina em camundongos. **Métodos:** Foram utilizados camundongos suíços machos (30-40 g, seis por grupo) e os resultados expressos como média ± e.p.m. Quatro diferentes grupos de animais foram pré-tratados naloxona (1 mg/kg, via intraperitoneal) e, após 30 min, com os extratos de *E. brasiliensi*, *E. beaurepaireana* (600 mg/kg, via oral), morfina (1 mg/kg, via subcutânea) ou o veículo utilizado para diluir as drogas (PBS). Após 30 min para os grupos tratados com morfina e naloxona e 60 min para os grupos tratados com os extratos, todos os animais foram avaliados em relação à primeira e segunda fase da nocicepção induzidas pela formalina (2,5%) em camundongos. **Resultados:** O pré-tratamento dos animais com naloxona (antagonista não seletivo de receptores opióides, 1 mg/kg, i.p.) causou reversão do efeito antinociceptivo da morfina (agonista não seletivo de receptores opióides, 1 mg/kg, s.c.). No entanto, o pré-tratamento com naloxona não alterou a antinocicepção causada pelos extratos de *E. beuarepaireana* ou *E. brasiliensis* (600 mg/kg, v.o.), quando analisada em relação à dor induzida pela formalina. **Discussão:** Estes resultados demonstram que a ação antinociceptiva dos extratos não envolve a participação dos receptores opióides. Novos experimentos serão realizados para investigar o mecanismo de ação dos extratos de *Eugenia*. **Apoio financeiro:** FURB e FAPESC.

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Antitumor activity of a sulfated polysaccharide rich fraction from *Passiflora edulis*. Lacerda KOA[1], Figueiredo IST[1], Cavalcante IJM[1], da Silva DC[2], Freitas ALP[2], Alencar NMN de[1], Moraes MO[1], Pessoa C[1], Costa-Lotufo LV[1] [1]UFC - Fisiologia e Farmacologia, [2], [3]UFC - Bioquímica

Introduction: In the last years, much attention has focused on polysaccharides isolated from natural products such as mushrooms, alga and plants. Their wide range of biological properties with a relatively low toxicity are the mainly reasons for the increase of the researches (Paulsen, *Cur. Org. Chem*., 5:939, 2001). *Passiflora edulis* is a plant from the *Passifloreaceae* family. This plant is economically important and very common in Brazil. The aim of this study was to investigate the effects of a fraction of sulfated polysaccharides from *P. edulis* in experimental models. **Methods:** The cytotoxicity of the fraction from *P. edulis* was tested against HL-60, MDA-MB-435, SF-295, and HCT-8 cell lines by MTT assay. For the *in vivo* test were used 48 Swiss mice (female, 25 - 30 g), obtained from the central animal house of Universidade Federal do Ceará, Brazil. All procedures were accepted by the animal Committee of Universidade Federal do Ceará (protocol n° 44). One day after inoculation of the Sarcoma 180 tumor, the fraction of polysaccharides (10 or 25 mg/kg/day^{-1}) were dissolved in distilled water and administered intraperitoneally for 7 days in healthy mice or transplanted with the tumor. 5-FU (25 mg/kg/day^{-1}) was used as a positive control. The negative control was injected with 0.9% NaCl. On day 8, the mice were sacrificed. Tumors, livers, spleens and kidneys were extirpated, weighed and fixed in 10% formaldehyde. The inhibition ratio (%) was calculated by the following formula: inhibition ratio (%) = [(A-B)/A] x 100, where A is the tumor weight average of the negative control, and B is that of the treated group. Biochemical analyses, as well as histopathological and morphological analyses of the tumor and the organs, including liver, spleen and kidney, were performed in order to evaluate the toxicological aspects of the polysaccharide treatment. **Results and Discussion:** The results *in vitro* showed that the fraction of polysaccharide had IC_{50} values greater than 25 µg/ mL^{-1} for all tumor cells tested, therefore it was considered to be non-toxic. The results *in vivo* showed that the inhibition ratios of the tumor growth were 78.11% and 82.62% for the polysaccharide treatment (10 and 25 mg/kg/day), respectively. 5-FU reduced tumor weight by 80.61%. There was statistically significant difference between the compounds in relation to the control group. The histopathological analyses suggest that both the kidney and liver could be considered as a weak potential target of polysaccharide toxicity. The findings in the present study suggest that the fraction of polysaccharides has *in vivo* antitumor effects, with low toxicity in the liver and kidney. Therefore, others studies must be done to clarify the mechanisms by which this fraction of polysaccharides has its anticancer potential. **Financial Support:** CNPq, CAPES, BNB, FUNCAP, FINEP, Claude Bernard Institute.