05. Pain and Nociception

05.001

Local administration of mangiferin prevents hyperalgesia in models of inflammatory pain. Rocha LW, Parada CA Unicamp – Biologia Estrutural e Funcional

Introduction: Mangiferin is a natural xanthone C-glucoside [2-C-β-Dgluco-pyranosyl-1,3,6,7-tetrahydroxyxanthone], which the molecular formula is $C_{19}H_{18}O_{11}$ (MW, 422.35). Mangiferin is the main bioactive compound of Vimang®, a Cuban pharmaceutical formulation which has been used to control stress. Several studies have demonstrated the anti-hyperalgesic effect of mangiferin (Dar *et al.*, 2005, Izquierdo *et al*, 2013; Lopes *et al*, 2013) in models of acute pain. The aim of this study is to verify whether local peripheral administration of mangiferin reduces the inflammatory hyperalgesia induced by inflammatory stimuli, as well as, to verify the mechanisms underlying this anti-hyperalgesic effect. **Methods:** Male Wistar rats were used in this study (180-200g, N = 6-8). The animals were pre-treated with Mangiferin (150, 300, 600 and 1200 µg/paw) or vehicle (20% DMSO in saline, 50 µL/paw) and after 30 minutes they were subjected to an intraplantar injection of ʎ- carrageenan (100 µg/paw), PGE_2 (100 ng/paw), IL-1β (0.5 pg/paw) or CINC-1 (1pg/paw). The mechanical hyperalgesia was assessed three hours after flogistic agent injection, using handheld force transducer (electronic anesthesiometer, IITC Life Science, Woodland Hills, CA, USA) fitted with a 0.5 mm^2 polypropylene tip. The tip is applied to the central area of the plantar hind paw with a Gradual Increase in pressure. After paw withdrawal, the intensity of the pressure was automatically recorded. All experiments were approved by the Ethics Committee on Animal Research at Unicamp (protocol number 2813-1). **Results:** Mangiferin locally administrated in subcutaneous tissue of rat hindpaw prevented the hyperalgesia induced by carrageenan administration (100 µg/paw) in a dose-response manner. The 150 µg/paw dose was not significant, but mangiferin presented inhibition values of 26.83 ± 7.87%, 77.58 ± 7.81% and 91.66 ± 10.21%, respectively with the administrated dose (300, 600 and 1200 µg/paw). The ID_{50} was 399.4 µg/paw (259.1-615.6). Local administration of mangiferin reduced the IL-1β- or CINC-1-, but not by PGE_2-induced hyperalgesia three hours after their intraplantar injections, with inhibition of 43.43 ± 9.98% and 37.70 ± 6.86%, respectively. **Discussion:** Mangiferin demonstrated anti-hyperalgesic activity in inflammatory pain models tested and our results suggest that the anti-hyperalgesic action of mangiferin is possibly related to the inhibition of the enzyme cyclooxygenase 2 (COX-2) and also with a possible action on sympathomimetic amines. More experiments are being conducted to better understand these effects. **Financial support:** Fundação de Amparo à Pesquisa do Estado de São Paulo (Fapesp). Dar, A., Biol. Pharm. Bull., v. 28, p. 596, 2005. Izquierdo, T., *Eur J Pharmacol*, v. 718, p. 393, 2013. Lopes, S.C., *Pharm Biochem Beh*, v. 110, p. 19, 2013.

05.002

Beneficial effects of docosahexaenoic acid supplementation on cyclophosphamide-induced alterations in mice. Freitas RDS[1,2], Costa KM[1], Campos MM[1,2,3] [1]PUCRS – Medicina e Ciências da Saúde, [2]INTOX-PUCRS, [3]PUCRS – Faculdade de Odontologia

Introduction: Hemorrhagic cystitis (HC) is an inflammatory and painful alteration commonly associated to the use of the chemotherapy agent cyclophosphamide (CYP) (de Jonge *et al.*, Clin Pharmacokinet 44(11), p.1135, 2005; Korkmaz *et al.*, *J Expl Integ Med* 2(2), p.93, 2012.). The supplementation with omega-3 fatty acids has been clinically used for the management of cancer patients (Calder, Am J Clin Nutr 83(6), p.1505S, 2006; Carmo *et al.*, Braz J Oncology 55(3), p.279, 2009). The beneficial effects of omega-3 are likely related to the production of lipid pro-resolution mediators, such as resolvins and protectins. These mediators are derived from the omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), both exclusively found in fish oils (Serhan, Am J Pathol 177(4), p.1576, 2010; Ariel, Front Immunol 3(4), p.1, 2012). Previous pilot studies from our laboratory demonstrated a decrease of CYP-induced nociceptive parameters after fish oil supplementation for 21 days. Furthermore, literature evidence demonstrated the effectiveness of DHA in reducing nociception in several pre-clinical inflammatory pain models (Lu *et al.*, Neuroscience 241, p. 22, 2013; Nakamoto *et al.*, *Eur J Pharmacol*, 666 (1), p. 100, 2011). The present study was aimed to evaluate the effects of acute DHA treatment in a mouse model of CYP-induced HC. **Methods and Results:** Male Swiss mice were used (N = 8-12/group). The experimental protocols were approved by the Animal Ethics Committee (CEUA-PUCRS, 12/00303). Mice were divided into three groups: Saline + Saline, CYP 300 mg/kg i.p. + Saline, and CYP 300 mg/kg i.p. + DHA 1 µmol/kg i.p. (DHA was injected 1 h before CYP administration). The behavioral tests were performed 30 min after CYP injection, and each animal was observed for 2 min, every 1/2 h, for 4 h, in order to assess nociception scores. Following the behavioral assessments, Von Frey test was performed using a 0.4 g-filament, for mechanical threshold determination. Six hours after, the animals were euthanized, total blood was collected for differential hematological analysis, and the urinary bladders were removed and scored for inflammatory changes. DHA supplementation was not able to significantly affect the inflammatory bladder changes elicited by CYP, including hemorrhage and edema scores. Nevertheless, it was possible to observe a significant reduction of nociceptive behavior and frequency of response to mechanical stimulation, with inhibition percentages of 41 ± 8% and 58 ± 14%, respectively. Differential hematological analysis showed that DHA administration partially reduced the neutrophil contents (14 ± 6%) in CYP-treated mice, whereas it restored the lymphocyte levels toward the normality (44 ± 14%). **Discussion:** These results suggest that pretreatment with DHA might be useful as adjuvant for patients under chemotherapy with CYP, by preventing painful alterations related to HC. Further studies are being conducted to identify the mechanisms of action of DHA in the present model. **Financial Support:** FINEP/PUCRSINFRA #01.11.0014-00, Capes and CNPq.

05.003

Melatonin treatment entrains the rest-activity circadian rhythm in rats with chronic inflammation. Torres ILS[1], Laste G[1], Vidor L[1], de Macedo IC[3], Rozisky JR[1], Rozisky JR[1], Medeiros LF[1], Souza A[1], Souza A[1], Meurer L[2], Meurer L[2], Souza ICC[3], Caumo W[1] [1]UFRGS – Farmacologia, [2]HCPA, [3]UFPel

Introduction: Melatonin (N-acetyl-5-methoxytryptamine) is a hormone synthesized and secreted by the pineal gland. Among the broad range of effects attributed to melatonin, its potential antinociceptive and anti-inflammatory actions have been studied in an animal model of acute pain. This study aim to evaluate the therapeutic effect of exogenous melatonin, dexamethasone, and a combination of both on nociceptive response induced by chronic inflammation and on the rest-activity circadian rhythm in rats. **Methods:** Sixty-four adult male Wistar rats were randomly and divided into 8 groups (n=8): control group and 7 groups with complete Freund's adjuvant-inflamed animals (CFA; injection into the footpad). Control CFA-inflamed group not receive any treatment, the other 6 groups were treated with melatonin (MEL), dexamethasone (DEXA), melatonin + dexamethasone (MELDEXA), and respective vehicles. Fifteen days after CFA injection, animals were treated with intraperitoneal injection of MEL (50 mg/kg) or vehicle (8% ethanol in saline), DEXA (0.25 mg/kg) or vehicle (saline), and MEL + DEXA or vehicle, for 8 days. Von Frey test was performed 24 hours after the treatment and the hind paw thickness was measured using a pachymeter. Swelling and histological findings were analyzed. This study was approved by the Animal Care and Ethics Committee-GPPG-HCPA (protocol no. 10.0013). **Financial support**: CNPq, Capes, GPPG-HCPA, FAPERGS/PRONEM. For statistical analysis was used two-way ANOVA or ANOVA repeat measures when necessary, followed by Bonferroni's, with $P<0.05$. **Results:** All treated groups significantly reduced the severity of inflammation when compared with their vehicles (ANOVA repeat measures, $P<0.05$ for all analyses). Inflamed animals treated with dexamethasone alone or associated with melatonin showed marked inhibition of histological findings. On the other hand, the group treated with melatonin remained with moderate inflammation. The CFA group showed a decrease in the mean rest-activity circadian rhythm, determined by the number of touch-detections per hour during water intake in comparison with the control group; only the group treated with melatonin showed a synchronized rest-activity rhythm. At the end of treatment, a significant increase was observed in hind paw withdrawal threshold on the von Frey test in the treated groups (one-way ANOVA, $P<0.05$ for all). **Discussion:** Our findings showed that melatonin (50 mg/kg) has strong chronobiotic and antinociceptive effects, but only mild anti-inflammatory effects. This evidence supports the hypothesis that melatonin can induce phase advance and circadian rhythm synchronization in rats with chronic inflammation. **Acknowledgment**s: CNPq, Capes, GPPG-HCPA, FAPERGS/PRONEM (grant no. 11/2050).

05.004

Antinociceptive and anti-inflammatory activities of LQFM008 molecule. Silva DPB[1], Florentino IF[1], Oliveira LP[1], Menegatti R[2], Costa EA[1] [1]DCiF-ICB-UFG – Natural Products, [2]FF-UFG – Medicinal Pharmaceutical Chemistry

Introduction: As well as anti-inflammatory, many other medications used to treat pain have side effects, requiring the development of specific, effective and safe new drugs. The piperazine derivatives have being reported to possess a potent and effective analgesic activity. Thus, the objective of this study was to evaluate the antinociceptive and anti-inflammatory activity of LQFM008, a new piperazine derivative, as well as its mechanisms of action. **Methods:** The animals used were adult male *Swiss* mice weighing 35 – 40g (n= 9). The antinociceptive and anti-inflammatory effects of LQFM008 were evaluated using the methods of formalin induced pain, tail flick test, hot plate test, paw edema induced by carrageenan and pleurisy induced by carrageenan. All the experimental protocols were approved by the Research Ethics Committee of the UFG (Protocol Nº. 182/10). **Results and Discussion:** In the first phase of formalin-induced pain test (0-5 min.), the treatment with LQFM008 30 mg/kg reduced the time of reactivity to pain by 30.4% as compared to the control group treated with vehicle 10 mL/kg (± 76.2 seconds). In the second phase (15-30 min.), the treatments with LQFM008 15 or 30 mg/kg reduced the time of reactivity to pain by 25.8 and 39.7%, respectively, as compared to the control group (± 211.1 seconds). In the tail flick test, the treatment with LQFM008 15 mg/kg (p.o.) increased the latency to thermal stimulus by 77.3 and 93.8%, of at 60 and 90 minutes, respectively, as compared to the control group (± 4.4 and 4.8 seconds, respectively). In the hot plate test, treatment with LQFM008 at a dose of 15 mg/kg (p.o.) did not increase the latency to thermal stimulus. In the test of paw edema induced by carrageenan, treatments with LQFM008 7.5, 15 or 30 mg/kg (p.o.) reduced the edema by 14.2, 29.2 or 25.6%, respectively in the first hour, as compared to the control group (Difference between the paws ± 158.6 µL). The treatments with LQFM008 15 or 30 mg/kg (p.o.) reduced the edema by 28 or 26.1% (second hour), 35.3 or 29.3% (third hour) and finally in the fourth hour by 31.4 or 27.9% as compared to the control group (Difference between the paws ± 152.9; 151.4 and 140 µL, respectively). In the test of pleurisy induced by carrageenan, treatments with LQFM008 30 mg/kg reduced cell migration by 26.2% compared to the control group (±7.79 Leukocytes x 10^6/mL). The treatment with LQFM008 also reduced the proteic exudation by 24.3% as compared to the control group (Blue Evan's concentration ± 3.417 µg/mL). The positive result of LQFM008 in the tail flick test shows that this molecule has a central-spinal analgesic effect, thus justifying the effect seen in the first phase of the formalin test, where antinociceptive effect independent of anti-inflammatory activity can be observed. The effects of LQFM008 in the paw edema and pleurisy induced by carrageenan show that this molecule in addition to presenting nociceptive effect has anti-inflammatory effect. **Financial Support**: Capes, CNPq, and FUNAPE/UFG

05.005

Involvement of kinins on pain induced by treatment with paclitaxel. Oliveira SM[1], Brusco I[2], Bastos C[2], Dalla Pozza CC[2], Rigo F[3], Trevisan G[4], Tonello R[2], Ferreira J[5] [1]UFSM – Bioquímica e Biologia Molecular, [2]UFSM, [3]UFMG, [4]UNESC, [5]UFSC

Introduction: Paclitaxel, a chemotherapy agent widely used in the treatment of solid tumors cause adverse effects that limits its use, such as peripheral sensory neuropathy, compromising the quality of life of patients. Among the mechanisms involved in neuropathic pain are the kinins receptors; so the aim of this study was to investigate the involvement of kinins in painful hypersensitivity caused by paclitaxel in mice. **Methods**: Adult male Swiss mice (weighing 30-35 g) were treated with single intraperitoneal injection (1 mg/kg) or four injections of paclitaxel (1+1+1+1 mg/kg in alternate days) and the development of mechanical hyperalgesia or the ongoing-like pain behavior (induced by B1 or B2 receptor agonists) was evaluated after single (24 h) or repeated (14 days after the last injection) administration of paclitaxel (Rigo *et al.*, 2013). To prevent the development of ongoing-like pain induced by B1 or B2 receptor agonists or to reverse mechanical hyperalgesia induced by paclitaxel, the animals were treated with the B1 (des-Arg^{9}-Leu^{8}-Bradykinin) or B2 (Hoe140) receptor antagonists. The effect of enalapril maleate on the mechanical hyperalgesia paclitaxel-induced and the bradykinin-related peptides levels in the paw tissue of animals treated with paclitaxel also were evaluated. All experiments were approved by Committee on the Use and Care of Laboratory Animals of the UFSM (106/2011). **Results**: The animals present mechanical hyperalgesia at 12 and 24 h (86 ± 19% and 91 ± 10% of hyperalgesia, respectively) after single injection of paclitaxel or at 14 and 21 (91 ± 12% and 92 ± 6% of hyperalgesia, respectively) but not at 7 days after the last paclitaxel injection. The selective B1 or B2 receptor agonist des-Arg-Bradykinin (1 nmol/paw) or Bradykinin (1 nmol/paw), respectively, administered intraplantar route induced ongoing pain-like behavior at 24 h after single injection or 14 days after the last paclitaxel injection; this ongoing pain-like behavior was prevented by previous intraperitoneal administration (30 min) of B1 or B2 receptor antagonists Des-Arg-Leu^{8}-Bradykinin (DALBk; 150 nmol/kg) or Hoe140 (100 nmol/kg) with inhibitions of 76 ± 8% and 68 ± 12% or 71 ± 22% and 100% after single or repeated paclitaxel administration, respectively. Moreover, DALBk (150 nmol/kg) or Hoe140 (100 nmol/kg) reversed the mechanical hyperalgesia induced by single or repeated paclitaxel administration with inhibitions of 98 ± 8% and 93 ± 7% at 30 min or 90 ± 10% and 95 ± 4% at 60 min after treatment, respectively. Enalapril maleate (30 mg/kg, oral route) administered after single or repeated paclitaxel injection, increased the mechanical hyperalgesia paclitaxel-induced. Therefore, animals that received paclitaxel presented bradykinin-related peptides tissue levels higher than animals that received vehicle. **Discussion:** Our data evidence the involvement of kinins and their receptors in painful hypersensitivity induced by paclitaxel and point out the potential of kinins receptors antagonists to treat the pain related to paclitaxel. **Acknowledgements**: Capes, CNPq, FAPERGS. **References:** Rigo FK, *et al. Pharmacol Biochem Behav* 115:16-22;2013.

05.006

Effects of simvastatin on acute and neuropathic pain models in rats. Corso CR[1], Martins DF[2], Werner MFP[1] [1]UFPR – Farmacologia, [2]UNESC – Ciências da Saúde

Introduction: Several studies have been shown that statins, such as simvastatin, in addition to reduce cholesterol levels due inhibition of HMG-CoA reductase, present pleiotropic effects, including anti-inflammatory, immunomodulatory and neuroprotective [1]. It is well know that injury to the peripheral nerves induces neuropathic pain, which is related to a lesion or disease of the somatosensory nervous system. Furthermore, the reduction in cholesterol levels can be associated to the decrease in the nerve myelination. Thus, this study evaluate conflicting evidence with regard to simvastatin accelerates or hinders neural regeneration after sciatic nerve crush in rat. **Methods:** All procedures used in the present study were approved by the Ethics Committee of the UFPAna (n° 661). Male Wistar rats (~200 g, N= 5-8) were orally treated with vehicle (saline, 1 ml/kg), simvastatin (2 and 80 mg/kg) or gabapentin (30 mg/kg), 1 h before intraplantar injection of 50 µl of a 2.5% formalin. Furthermore, paw edema was measured 1 and 3 h after the formalin injection. Neuropathic pain was induced by crush injury using a non-serrated clamp [at 10 mm of the sciatic nerve trifurcation (no ties in sham-operated rats) for 30 s [2]. Mechanical (von Frey up-down method) and cold allodynia (acetone test), as well as nerve function (measurement of sciatic functional index and sciatic static index) were evaluated at 3, 6, 12 and 18 days after nerve crush. Animals were treated as reported above, 1 day after surgery repeatedly during 18 days. **Results and discussion:** Simvastatin treatment (2 and 80 mg/kg) attenuated the second phase of formalin-induced nociceptive behaviors in 48 ± 9 and 59 ± 9%, respectively, but not decrease the paw edema. At the 3rd day after nerve injury, mechanical allodynia was reduced for up to 6 h by simvastatin 2 mg/kg and only for up 3 h by simvastatin 80 mg/kg (at 3 h: 52 ± 14 and 66 ± 20%, respectively and gabapentin 109 ± 17%). Again, at 6th day, only treatment with simvastatin 2 mg/kg reduced for up to 6 h mechanical allodynia (at 3 h: 89 ± 18% and gabapentin 144 ± 21%). However, no antiallodynic effects were observed after simvastatin treatments in the 12th or 18th days after sciatic crush and treatments did not attenuate cold allodynia (3rd to 18th day). Moreover, both gabapentin and simvastatin treatments (2 and 80 mg/kg) were ineffective in ameliorating nerve function. Confirming previous studies, simvastatin showed antinociceptive effects on formalin-induced inflammatory pain. The lowest dose of simvastatin reduced partially the mechanical allodynia in the initial phase of neuropathic pain, but the antiallodynic effect was not maintained throughout the days following the nerve injury. In the opposite way, simvastatin treatment did not prevent cold allodynia and did not ameliorate the nerve function. Therefore, future additional histological studies will evaluate whether simvastatin could affects nerve myelination to better correlate with the antiallodynic effect. [1]Shi XQ *et al. Pain*, p. 1033, 2011. [2]Martins DF *et al. Pain*, p. 2653, 2011.

05.007

The involvement of opioid system on analgesia induced by environmental enrichment on injured rats. Kimura LF[1], Mattaraia VGM[2], Picolo G[1] [1]IBu – Dor e Sinalização, [2]IBu – Biotério Central

Introduction: Chronic pain is a public health serious problem, since many types of pains are still intractable. It has been found that environmental enrichment (EE) can alter the perception of nociceptive stimuli as well as the analgesic response induced by opioids, suggesting a relationship between well-being and analgesia. The aim of this work was to evaluate the role of animal welfare in pain sensitivity of rats against chronic noxious stimuli and the response of these animals to opioid drugs. **Methods:** Male Wistar rats (IBu) were used. All procedures were approved by the Institutional Animal Care Committee of the Butantan Institute (CEUAIB, protocol number 1050/2013). Animals were assigned to these conditions: enriched rats were housed in groups of five in large cages (60 x 50 x 22 cm) and given different novel objects (Ping-Pong balls, tunnels, huts, retreats, and surgical cap), every week on a regular basis, since birth. The control group remained in standard cages and did not receive objects. After 7 weeks under these conditions, the effects of EE were analyzed on anxiety, using Plus Maze test, and on the sciatic chronic constriction-induced pain sensitivity (CCI), using mechanical stimuli (the paw pressure test). **Results:** The results showed that the EE decreased the anxiety of rats, since enriched animals entered in opened arms (number of entries = 6.03) and spent more time on them (79.83 seconds) when compared to control group (number of entries = 3.8; 53.3 seconds). Concerning mechanical pain sensitivity, after 14 days of CCI, hypernociception was detected on non-enriched animals (threshold = 42.27 g), whereas enriched animals did not present any pain threshold alteration (similar preoperative – 68.08 g – and postoperative – 65.38 g – thresholds). Moreover, when animals were treated with naloxone (1 mg/kg, s.c.), a non-selective antagonist of opioid receptors, the analgesic effect of EE on enriched animals was completely abolished (threshold = 41 g). In addition, the treatment with a subanalgesic dose of morphine (2 mg/kg, s.c.) on injured rats after 14 days of CCI, had no outcome in control group (threshold = 50 g) whereas increased enriched animals pain threshold (75 g). **Discussion:** The results presented herein show that EE protocol used in this work is effective, since it was able to diminish the anxiety of experimental animals, as described in the Literature. Enriched rats exhibited decreased pain sensitivity, which involves the endogenous opioid pathway activation, when compared to non-enriched animals. Taken together, the data presented here bring up the importance of EE on both limbic and analgesic system, and demonstrate for the first time the modulation of opioid system by EE on chronic conditions. **Financial Support:** Supported by CNPq and São Paulo Research Foundation (Fapesp).

05.008

Ovariectomy as a pre-clinical model of spontaneous scratching behaviour in mice.

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Introduction: Women typically reach their menopausal period between the fourth and fifth decades of life, when a marked decrease in estrogen levels is observed, leading to various immunological and behavioral changes (Studd & Zamblera, *Gynecol Endocrinol*, 8, 191, 1994). Among the various changes, we highlight the progressive reduction of the skin collagen directly related to the deficiency of estradiol (Delattre *et al.*, *Exp Dermatol* 21, 205, 2012). In fact, hormone replacement therapy leads to increased collagen contents, improving skin elasticity and dryness (Calleja-Agius *et al.*, Menopause Int 13, 60, 2007). Pruritus is an unpleasant cutaneous sensation that elicits itching reflex (Steinhoff *et al.*, *J Invest Dermatol* 126, 1705, 2006). It is associated with self-protective function in acute forms, but chronic states seriously compromise patients' quality of life. Therefore, it would be useful to evaluate the pruritogenic transmission under menopause. The present study evaluated the scratching behavior associated with dry skin model in ovariectomized mice. **Methods:** The experimental protocols were approved by the Local Animal Ethics Committee (12/00293). Female Swiss mice (8/group, 25-30g) were divided into 2 surgical protocols: (a) ovariectomy (Rocha *et al.*, *Psychopharmacology Berl* 179, 637, 2012) and (b) sham-operated. After 14 days of surgery, the animals were submitted to the dry skin model (a five-day-treatment based on application of diethylether and acetone solution, 1:1, AEW, on the shaved back of the neck). One group of animals received the same treatment, but only with the application of distilled water for 45 s (W). After the 5th day, the animals were placed individually and acclimatized for 30 min in glass cylinders. The scratching behavior was measured for 60 min, as the number of scratches with forepaws/hindpaws close to or behind the ears. Finally, the animals were euthanized and their uteri were weighed, and the uterine atrophy was considered as a signal of hypoestrogenism. **Results:** The dry skin AEW model increased the scratch bouts in the sham-animals in comparison with the W groups (69 ± 7 and 23 ± 3 scratch bouts, respectively). Ovariectomized mice had a scratching behavior superior to the sham animals (68 ± 4 and 45 ± 4 scratch bouts, respectively), regardless the protocol they had been submitted (AEW or W groups). No statistical significant differences were observed between ovariectomized animals in both groups ($p>0.05$). The uterus weight of the ovariectomized mice was 36 ± 2 mg in comparison to 212 ± 15 mg in the sham group, indicating uterine atrophy. **Conclusion:** The ovariectomy protocol, simulating the menopause status, seems to be an appropriate model of chronic pruritus and might provide additional insights on the dermatological changes associated to menopause. These results suggest an important relationship between estrogen levels and pruritus. Additional studies are in progress to determine the effects of estradiol replacement on ovariectomy-induced itching, as well as to determine the effectiveness of anti-histamine agents and topical corticoids on this kind of pruritus. **Financial support:** CNPq, PROBIC/FAPERGS 2013-2014.

05.009

Tingenone, a pentacyclic triterpene, induces peripheral antinociception due to NO/cGMP pathway activation in mice. Veloso CC[1], Rodrigues VG[2], Ferreira RCM[1], Duarte LP[2], Klein A[1], Duarte ID[1], Romero TRL[1], Perez AC[1] [1]UFMG – Farmacologia, [2]UFMG – Química

Introduction: Natural substances derived from plants play an important role in the development of new analgesic drugs, among them, triterpenoids. The participation of L-arginine/NO/cGMP pathway has been established on the peripheral antinociception induced by various drugs. The present study assessed the involvement of L-arginine/NO/cGMP pathway in the antinociceptive effect induced by tingenone, a natural triterpene, from *Maytenus imbricata* Mart. ex. Reissek, against the hyperalgesia evoked by prostaglandin E_2 (PGE_2) in peripheral pathway. **Methods:** The mice paw pressure test was used to induce hyperalgesia by intraplantar injection of prostaglandin E_2 (2 µg). All testing procedures were approved by Ethics Committee in Animal Experimentation at the UFMG (protocol 115/2012). **Results and discussion:** Tingenone (200 µg/paw) administered into the right hind paw induced a local antinociceptive effect (7.00 ± 1.41), that was antagonized by L-NOArg 24 µg/paw (60.00 ± 10.29), nonselective NO synthase (NOS) inhibitor and by L-NPA 24 µg/paw (76.33 ± 2.18), selective neuronal NOS (nNOS) inhibitor. The L-NIO, selective inhibitor of endothelial (eNOS), and the L-NIL, selective inhibitor of inducible (iNOS), did not alter the peripheral antinociceptive effect of tingenone. The ODQ 100 µg/paw (60.50 ± 3.37), selective soluble guanylate cyclase inhibitor, reversed the antinociceptive effect of tingenone, and zaprinast 50 µg/paw, inhibitor of the phosphodiesterase that is cGMP specific, intensified (11.75 ± 8.52) the peripheral antinociceptive effect of the smaller dose of tingenone 50 µg/paw (43.50 ± 1.04). The results suggest evidence that tingenone induced a peripheral antinociceptive effect by L-arginine/NO/cGMP pathway activation, with potential for a new analgesic drug. Financial agencies and acknowledgments: Capes, FAPEMIG and CNPq.

05.010

Antinociceptive effect of monoterpene geraniol not involves opioid system. La Rocca V[1], Fonseca DV[1], Nóbrega FFF[2], Almeida RN[1] [1]UFPB – Psicofarmacologia, [2]CDSA-UFCG

Introduction: Essential oils are complex mixtures of volatile natural compounds extracted from different aromatic plant species. Chemical analysis of these products has revealed the predominant presence of monoterpenes, sesquiterpenes and arylpropanoids. Several works, using different animal models, report activity in the Central Nervous System (CNS) of various essential oils, where there has been a majority presence of monoterpenes. The geraniol (GER) is a monoterpenic alcohol found in essential oils of some aromatic plants, many of which are used for their analgesic, anti-inflammatory and anxiolytic action. We evaluated the possible involvement of opioidergic mechanism in the antinociceptive effect of geraniol in mice. **Materials and Methods:** We studied the effect of the opioid receptor antagonist naloxone on geraniol induced analgesia in the acetic acid-induced writhing test in mice. Male Swiss mice were divided into five groups (N=6). A group was treated intraperitoneally (i.p) with GER 25 mg/kg, and compared with control groups: vehicle (distillation water + Tween80) and positive control (Morphine 6 mg/kg, i.p.). Another group was pretreated with Naloxone [5 mg/kg, subcutaneally (s.c.)], fifteen minutes before to receive GER 25 mg/kg i.p, and compared with control group (Naloxone 5 mg/kg s.c. + Morphine 6 mg/kg, ip). Thirty minutes after the specific treatment, the animals were injected with a solution of 1% acetic acid (0.1 ml/10g) i.p. and placed in individual polyethylene boxes to recorded the latency time for the first abdominal contortion; after five minutes was counted the number of abdominal constrictions presented by each animal over a fifteen minutes period of observation. The results were analyzed by analysis of variance ANOVA, supplemented by Dunnett's test ($p<0.05$). Ethics Committee on Animal Use of the Biotechnology Center of Federal University of Paraíba analyzed and approved the procedure, whit license number 0106/12. **Results:** GER (25 mg/kg i.p) decreased significantly ($p<0,05$) by 68,1% the number of contortions (7,5 ± 2,6) induced by intraperitoneal injection of acetic acid in mice, compared to the control animals (23,5 ± 2,3). The same dose of GER into animals pretreated with naloxone decreased ($p<0,05$) the abdominals contortions (8,8 ± 5,6) by 62,1%, similarly to the group tested in the absence of opioid antagonist and increased significantly ($p<0,05$) the latency time for the first contortion (562,8 ± 119,3) compared to the vehicle animals (231,7 ± 13,2). **Conclusions and discussion:** The administration of geraniol resulted in an analgesic effect into pain model tested. However, as the naloxone, opioid receptor antagonist, not reverted the antinociceptive action of geraniol, an involvement of opioidergic system is improbable. The mechanism remains to be elucidated.

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05.011

Melatonin analgesia is associated with improvement of the descending endogenous pain-modulating system in fibromyalgia: A Phase II, randomized, double-dummy, controlled trial. Deitos A, Zanette SA, Vercelino R, Laste G, Rozisky JR, Schwertner A, Machado CB, Xavier F, Souza ICC, Torres ILS, Caumo W HCPA-UFRGS

Introduction: Central disinhibition is a mechanism involved in the physiopathology of fibromyalgia. Melatonin can improve sleep quality, pain and pain threshold. The objective of this study was evaluate if the treatment with melatonin alone or in combination with amitriptyline (AMIT) would be superior to AMIT alone in modifying the endogenous pain-modulating system (PMS) as quantified by conditional pain modulation (CPM). **Methods:** Sixty-three females, aged 18 to 65, were randomized to receive bedtime AMIT (25 mg) (n=21), melatonin (10 mg) (n=21) or melatonin (10 mg) + AMIT (25 mg) (n=20) for a period of six weeks. The descending PMS was assessed with the CPM-TASK. It was assessed the pain score on the Visual Analog Scale (VAS 0-100 mm), the score on Fibromyalgia Impact Questionnaire (FIQ), pressure pain threshold (PPT), and BDNF serum. The outcomes variables were collected before and six weeks after initiating treatment. Linear mixed models were used to compare outcomes within and between subjects in which the independent variable was the treatment, with Bonferroni's Multiple Comparison Test. Delta values (post- minus pre-treatment) were used to compare the treatment effect. $P<0.05$ was considerate significant. The statistical analysis was performed in SPSS 18.0. This study was approved by the Research Ethics Committee at the HCPA (Institutional Review Board IRB 0000921). **Results:** Melatonin alone or in combination with AMIT reduced significantly pain on the VAS compared with AMIT alone ($P<0.01$). The delta values on the VAS scores were -12.85 (19.93), -17.37 (18.69) and -20.93 (12.23) in the AMIT, melatonin and melatonin + AMIT groups, respectively. Melatonin alone and in combination increased the inhibitory PMS as assessed by the Numerical Pain Scale [$NPS_{(0-10)}$] reduction during the CPM-TASK: -2.4 (2.04) melatonin AMIT, -2.65 (1.68) melatonin, and -1.04 (2.06) AMIT ($P<0.05$). Melatonin + AMIT treated displayed better results than melatonin and AMIT alone in terms of FIQ and PPT improvement ($P<0.05$, fort both). From the baseline, the mean of serum BDNF decreased 22.57% in the AMIT group, whereas the melatonin group and the melatonin + AMIT group presented a mean reduction of 36.6% and 34.49%, respectively. **Discussion:** Melatonin increased the inhibitory endogenous pain-modulating system as assessed by the reduction on NPS during the CPM-TASK. Melatonin alone or associated with AMIT was better than AMIT alone in improving pain on the VAS, whereas its association with AMIT produced only marginal additional clinical effects on FIQ and PPT. **Financial agencies and acknowledgements:** Capes – PNPD/Capes; CNPq; Postgraduate Program in Medical Sciences at the School of Medicine – UFRGS; Postgraduate Research Group at the HCPA; FAPERGS.

05.012

Antinociceptive and anti-inflammatory activity of *Eugenia brasiliensis* in mice. Simões RR[1], Dal-Secco D[2], Frederico MJ[2], Costa AF[2], Syracuse S[2], Siebert DA[3], Alberton MD[3], Santos ARS[2] [1]UFSM, [2]UFSC, [3]FURB

Introduction: The plants of the genus *Eugenia Lam* (Myrtaceae) are used in folk medicine for the treatment of various diseases such as arthritis, rheumatism and diabetes. *Eugenia brasiliensis* is known by the popular names of grumixama, grumichameira and ibaporoiti. However, to date no pharmacological study has been performed concerning the anti-inflammatory and analgesic action for the extract of this specie. Therefore, this study evaluates the possible antinociceptive and anti-inflammatory effect of the hydroalcoholic extract of *E. brasiliensis* (HEEb). **Methods**: Female Swiss mice were used (26-30g). Leaves of *E. brasiliensis* were collected in Florianópolis/SC (27°36'13.65"S, 48°31'14.75"O), and a voucher specimen (FLOR 34.675) was deposited in the Herbarium of the Department of Botany, UFSC. The air-dried leaves of *E. brasiliensis* (1.813 g) were extracted with ethanol 70% at room temperature for 15 days. After the plant material was filtered and the residue rejected, being the solvent evaporated under reduced pressure, to yield the HEEb. In the formalin test were evaluated the nociceptive response (licking/biting) in the injected paw, and paw oedema e temperature. In the acetic acid (0.6%) model was evaluated the nociceptive response (number of writhes), leukocyte migration and protein extravasation. In addition, the effect of the HEEb (100 mg/kg, i.g.) was also analyzed in the nociception and paw oedema induced by prostaglandin (PGE_2) and compound 48/80. Mice were habituated to the laboratory conditions for at least one hour before testing, and treated orally with the HEEb (30-300 mg/kg, diluted in saline 0.9%) or vehicle (saline, 10 mL/kg). The statistical significance of differences between groups was detected by One-way Anova followed by post hoc test of Newman-Keuls, and all protocols used were approved by CEUA-UFSC (protocol number PP00745). **Results**: In the formalin test, the HEEb reduced the inflammatory phase (100-300 mg/kg, i.g.) and paw temperature with inhibitions of 43 ± 7% and 58 ± 9% at dose of 300 and 100 mg/kg, respectively, when compared to control. In addition, the HEEb (30-300 mg/kg, i.g.) decreased the number of writhing and leukocyte migration caused by acetic acid with inhibitions of 66 ± 4% and 71 ± 7% at dose of 100 and 300 mg/kg when compared to control, respectively. Furthermore, the HEEb (30-100 mg/kg, i.g.) was able to inhibit only the neutrophil migration (inhibition of 100% at 300 mg/kg), but not did not alter the concentration of protein induced by acetic acid when compared to control. The nociceptive response and paw oedema induced by PGE_2 was not changed with HEEb (100 mg/kg, i.g), but it was able to reduce only the nociceptive response (41 ± 5%) induced by compound 48/80. **Conclusions**: These data show for the first time that HEEb presents significant antinociceptive and anti-inflammatory effects in mice, which appear to be mediated by an inhibition of synthesis or release of inflammatory mediators, and by leukocyte migration, especially neutrophils. Such findings are of interest because they support, at least, the use of *E. brasiliensis* in popular medicine. **Financial support**: CNPq, FAPESC, Capes.

05.013

4-methyl–(4')–methyl-naphthalimide, a cyclic imide with important effects against inflammatory and cancer pain in mice. Silva GF, Buzzi FC, Correa R, Cechinel Filho V, Quintão NLM Univali – Ciências da Saúde

Introduction: Preliminary studies have shown that cyclic imides have important anti-hypersensitivity activity in mice experimental model of pain. Despite the great interest by the Pharmaceutical Industries in search of new drugs for chronic pain treatment, a completely effective and safe analgesic has not been found. This study aims to investigate the anti-hypersensitive effect of 4-methyl–(4')–methyl-naphthalimide (4M4MN) by the use of different models of inflammatory and cancer pain in mice. **Methodology:** Female Swiss and C57BL/6 mice (25-35g, N=6-8) were used throughout this study (ethics committee nr: 033-13). Mice were pre-treated with the compound (10 mg/kg, i.p.) and 30 min later they were submitted to the carrageenan-induced mechanical hypersensitivity (300 µg/paw) to determine the ID50% and the time-course of the compound. After that other set of experiment was carried out to analyze the mechanism involved on the compound effect. Mice were pre-treated with 4M4MN (10 mg/kg, i.p.) or vehicle (10 mL/kg, i.p.) and 30 min later they received an intraplantar injection of prostaglandin E2 (PGE2; 0.1 nmol/paw), bradykinin (BK; 500 ng/paw), TNF-α (40 pg/paw), KC (20 pg/paw) or IL-1β (20 pg/paw). Then the animals were evaluated 3 h after the i.pl. injection. The anti-hypersensitivity effect of the compound was also evaluated using a model of mechanical hypersensitivity induced by bone cancer. Mice were previously anesthetized with ketamine (77 mg/kg, i.p.) and xylasine (7 mg/kg, i.p.) and then a small incision was performed at the knee joint for the inoculation of 4T1 breast carcinoma murine cells (3x10 ^{4}cells per site/50 µL/tybia) using an insulin syringe with 28G needle. Fifteen days after the cells inoculation mice were submitted to X-ray evaluation to detect bone tumor and then initiate the treatment with the compound (10 mg/kg, i.p.), saline (10 mL/kg, i.p.) or zoledronic acid (150 µg/kg, i.p.) once a day for 60 days. In all experiments the mechanical withdrawal threshold was evaluated using the von Frey monofilament 0.6g. **Results:** The compound 4M4MN, when administered i.p. 30 min before carrageenan i.pl. injection was able to significantly reduce the mechanical hypersensitivity for up to 6 h, with inhibition of 68 ± 4%. The compound was also capable of reducing the hypersensitivity induced by PGE2 (% of inhibition of 62 ± 3%) and BK (% of inhibition of 73 ± 2%). The mechanical hypersensitivity induced by, KC and IL-1β was also significantly affected, with inhibitions of 63 ± 2% and 75 ± 2%, respectively. However no effect was observed with the TNFα i.pl. injection. The hypersensitivity caused by bone cancer induced by 4T1 cells inoculation was significantly reduced by the compound 4M4MN and by the positive control drug zoledronic acid, with inhibitions of 49 ± 3% and 53 ± 2%, respectively. **Discussion:** This preliminary data suggests that the 4M4MN presented a persistent anti-hypersensitivity effect, probably interfering with inflammatory chemical mediators such as the cytokine IL-1β, which are directly involved with release of other subsequent inflammatory mediators such as PGE2, BK and KC. The compound was also effective against the hypersensitivity induced by bone cancer. Further studies should be performed to verify its exact mechanism of action, pharmacokinetic characteristics and possible adverse effects. **Financial Support:** CNPq, Capes, FAPESC, ProPPEC/UNIVALI.

05.014

Essential role played by tumor necrosis factor alpha in urate crystal-induced inflammation and hypernociception in mice. Bastos LFS[1], Oliveira THC[1], Amaral FA[1], Dias ACF[2], Oliveira VLS[1], Tavares LD[2], Costa VV[1], Galvão I[1], Soriani FM[3], Sachs D[4], Ryffel B[5], Souza DG[2], Teixeira MM[1] [1]UFMG – Biochemistry and Immunology, [2]UFMG – Microbiology, [3]UFMG – General Biology, [4]UNIFEI – Pharmacology, [5]CNRS – Molecular Immunology and Embryology

Introduction Gout is a disease that most commonly manifests as recurrent episodes of acute joint inflammation and pain secondary to the deposition monosodium urate (MSU) crystals within the synovial fluid and lining. The precise mechanisms of gouty inflammation remain unclear because several mediators participate in this arthritic disease. Among them, interleukin (IL)-1b seems to be the most widely studied. Tumor necrosis factor alpha (TNF-a) plays a role in gouty arthritis as well. A polymorphism in the gene for TNF-a was shown to contribute to gout pathogenesis and the efficacy of anti-TNF-a therapies for patients with gout has been demonstrated in a few studies. **Methods** Eight-to-10 week old wild-type (WT) C57BL/6J and TNF-$a^{-/-}$ or TNFR1/$2^{-/-}$ male mice were used. All procedures were approved by the animal care and use ethics committee of UFMG (protocol # 192/12). MSU crystals (100 μg; 10 μl) or phosphate-buffered saline (PBS; vehicle) were injected intra-articularly (i.a.) in the mouse tibiofemoral joint using an ultrafine needle (30 gauge) after shaving under isoflurane anesthesia. Mechanical hypernociception was measured by using an electronic pressure-meter, colloquially known as electronic von Frey. The test consisted of evoking a hindpaw dorsiflexion reflex with a hand-held force transducer adapted to a blunt polypropylene tip. Mice were euthanized and the articular cavity was washed with PBS containing bovine serum albumin (3% m/v; 2 x 5 μl) for total or differential cell counting. Periarticular tissue was collected and homogenized in PBS containing antiproteases for determinations of cytokine (IL-1b and TNF-a) and chemokine (CXCL1) production by ELISA. **Results and discussion** TNF-a production was markedly increased 15 h after i.a. MSU injection, but not after PBS injection. Both TNF-$a^{-/-}$ and TNFR1/$R2^{-/-}$ mice exhibited lower number of neutrophils in the articular cavity, concentrations of IL-1b and CXCL1 in periarticular tissue and mechanical hypernociception than WT mice. Either intraperitoneal (i.p.) etanercept or infliximab (both 10 mg/kg) – a TNF-a receptor 2-Fc conjugate and a monoclonal antibody against this cytokine, respectively – reduced articular neutrophils, IL-1b, CXCL1 and mechanical hypernociception. Pentoxyfilline (0.6 and 1.8 mg/kg, i.p.), a clinically available drug, reduced periarticular TNF-a concentration. Moreover, pentoxyfilline also reduced periarticular neutrophils, IL-1b and CXCL1 concentrations, and hypernociception. Using different genetic and pharmacological tools, the present study contributes to a better understanding of the role played by TNF-a in the pathophysiology of gout. This knowledge may be useful for the development of new therapeutic tools, especially for gout refractory to conventional management. **Acknowledgements** CNPq, Capes and FAPEMIG and PRPq-UFMG.

05.015

Antinociceptive activity of *Citrus limon* (L.) Burm. f. essential oil in mice. Monteiro-Silva JF[1], Oliveira LMS[1], Almeida EKC[1], Silva NKGT[1], Viana MDM[1], Silva-Neto GJ[1], Vieira ACS[1], Sant'Ana AEG[2,3], Alexandre-Moreira MS[1], Campesatto EA[1] [1]UFAL – Pharmacology and Immunity, [2]IQB-UFAL – Natural Resources

Introduction: A variety of species of the genus *Citrus* has been used in folk medicine to the treatment of chronic pain and inflammatory conditions. *Citrus limon* species, popularly known as lemon, has been studied. Aiming at the interest of potentially analgesic drugs of natural source, this study evaluated the antinociceptive activity of the essential oil from the peel of the fruit of *Citrus limon* (CLEO) in murine models. **Methods:** The experimental procedures were performed with CLEO (Ferquima, São Paulo). The Gas Chromatography-Mass Spectrometry (GC-MS) analysis revealed the quality and purity of CLEO and identified the presence of (-)-D-limonene (65.0%) and β-pinene (15.0%). *Swiss* mice (25-35g, n = 6), of both genres, were used for tests of abdominal writhing induced by acetic acid, formalin and hot plate. In all tests, positive control group was treated with saline 0.9% (10 mL/kg, p.o.). In writhing and formalin assays, standards drugs used were dipyrone (40 mg/kg, p.o.) and indomethacin (35.7 mg/kg, p.o.), respectively; besides the groups with CLEO in the doses 30, 100 and 300 mg/kg, orally. In the hot plate test, morphine (4.3 mg/kg, sc) was used as standard drug and only one dose of CLEO was tested (100 mg/kg). This project was submitted to Ethics Committee for Animal Research of UFAL and approved at the protocol number 02/2014. **Results and discussion:** In the writhing test, all doses of CLEO were able to reduce significantly ($p<0.001$) the number of writhes. In the formalin test, no dose of CLEO significantly inhibited the response in the neurogenic phase, suggesting no involvement in the central pain modulation. However, in the inflammatory phase, CLEO was able to reduce the licking time when compared to control group ($p<0.05$) at doses of 100 and 300 mg/kg. In the hot plate test, tested dose (100 mg/kg) was unable to significantly increase the response time of the animal to stimulation. Thus, these results suggest that CLEO does not show central antinociceptive action in preclinical trials, but show significant peripheral antinociceptive activity, corroborating the popular use of lemon and contributing to its ethnopharmacological study. **Financial Agencies**: Capes, FAPEAL and INCT-INOFAR. **Acknowledgments:** Prof. Dr. Antônio Euzébio Goulart Sant'Ana for technical assistance.

05.016

Mechanisms involved in the antinociceptive effect of 5-(1-(3-fluorophenyl)-1H-pyrazol-4-YL)-2H-tetrazole (LQFM-021)-new pyrazole derivative. Florentino IF[1], Oliveira LP[1], Silva DPB[1], Menegatti R[2], Costa EA[1] [1]ICB-UFG, [2]FF-UFG

Introduction: Pain is one of the most prevalent unpleasant feeling that limits productivity and decreases quality of life. The development of compounds that can treat pain with little or no side-effects remains a major challenge in research. Pyrazol compounds are known to possess antipyretic, analgesic and anti-inflammatory activities. The aim of this work was to evaluate the antinociceptive effect of a new pyrazole compound 5-(1-(3-Fluorophenyl)-1*H*-pyrazol-4-yl)-2*H*-tetrazole (LQFM-021) and investigate the involvement of the opioid receptors and NO/cGMP/K_{ATP} pathway in this effect. **Methods:** The LQFM-021, pyrazole derivative, was synthesized at the Faculty of Pharmacy/UFG. Male Swiss albino mice weighing approximately 30 g (n=8) were used in this study. The experimental protocol was approved by the Research Ethics Committee of Federal University of Goiás (number 017/13). The pharmacological tests like acetic acid-induced abdominal writhing, formalin-induced pain, tail-flick, hot plate, chimney, open field and pentobarbital-induced sleep test were performed. **Results and Discussion:** The oral treatments of mice with LQFM-021 (17, 75 and 300 mg/kg) decreased the number of writhing by 47.6, 51.8 and 64.1%, respectively. In the neurogenic phase of formalin test, the treatments with LQFM-021 (15, 30 and 60 mg/kg) reduced the licking time (s) by 33.8, 35.3 and 43.0%, respectively. In the inflammatory phase of this test, the same doses of LQFM-021 reduced the licking time by 43.9, 58.4 and 52.4%, respectively. LQFM-021 (30 mg/kg) did not elicit antinociceptive effect in the tail-flick and hot plate tests. Furthermore, pre-treatments with naloxone (3 mg/kg i.p.), L-name (10 mg/kg i.p.), ODQ (10 mg/kg i.p.) or glibenclamide (3 mg/kg i.p.) antagonized the antinociceptive effect of LQFM-021 in both phases of the formalin test. In addition, it was observed that the treatments of mice with LQFM-021(15, 30 and 60 mg/kg) did not compromise motor activity of the animals in the chimney test. In the open field and pentobarbital-induced sleep tests, treatments of the animals at the doses that induced antinociceptive effect did not alter animal's behavior. This result rules out possible false positive antinociceptive effect. In conclusion, our data showed that LQFM-021 produced antinociceptive effect in the acetic acid-induced writhing and formalin test. The thermal pain tests indicate that antinociceptive action of LQFM-021 only involved peripheral mediators. In addition, our data suggest the participation of peripheral opioid receptors and NO/cGMP/$K^{+}_{(ATP)}$ pathway in the antinociceptive action of LQFM-021. **Sources of research support:** CNPq and Capes.

05.017

The antipsychotic aripiprazole induces antinociceptive effects in animal models through partial agonism at dopamine D2 receptors. Almeida-Santos AF, Ferreira RCM, Aguiar DC, Romero TR, Moreira FA UFMG – Pharmacology

Introduction: Several neurotransmitters are known to modulate pain perception, including dopamine. Direct and indirect dopamine agonists, such as D-amphetamine and apomorphine, promote antinociception in experimental animals. In this sense, Aripiprazole (Ari) is an antipsychotic proposed to acts as a partial agonist at dopamine D2 receptors, leading to a favourable pharmacological profile. Thus, we tested the hypothesis that systemic or peripheral administration of Ari could induce the antinociceptive responses. **Methods:** Male Swiss mice (n=3-4/group) were submitted to different models of nociception. Exp. 1: The animals received administration of Ari (0.1, 1 or 10 mg/kg, *i.p*), morphine (5 mg/kg, *i.p*) or indomethacin (10 mg/kg, *i.p*) 20 min before formalin (2%, into the right plantar surface). The time licking the paws was measured from 0 to 5 and from 15 to 30 min after the inflammatory stimulus. The next experiments were conducted using the Randall-Sellito test, in which the force required to induce paw withdrawal was measured from 185 to 240 min after prostaglandin E_2 administration (PGE_2; 2 µg, intraplantar). Exp. 2: Ari (0.1, 1 or 10 mg/kg, *i.p*) was administered 180 min after PGE_2. Exp. 3: Ari (12, 25, 50 or 100 µg, intraplantar) was administered 180 min after PGE_2. Exp. 4: Haloperidol (dopamine D2 receptor antagonist; 0.1, 1 or 10 µg, intraplantar) was administered 150 min and Ari (100 µg) 170 min after PGE_2. Exp. 5: Quinpirole (dopamine D2 receptor full agonist; 25, 50 or 100 µg/intraplantar) was administered 180 min after PGE_2. Exp. 6: Ari (25 µg/intraplantar) was administered 150 min and Quinpirole (25 µg/intraplantar) 170 min after PGE_2. **Results:** Systemic administration of Ari induced antinociceptive effects, demonstrated by increase in the time licking the paw in the first [$F_{(5,31)}$=5.871; p<0.0001] and second phases [$F_{(5,31)}$=7.342; p<0.0001] of formalin test and by increases in nociceptive thresholds in Randall-Sellito test [$F_{(4,16)}$=1002; p<0.0001]. Intraplantar administration of Ari (25, 50 and 100 µg) mimicked the systemic effects [$F_{(5,18)}$=368.1; p<0.0001]. The peripheral effect of Ari (100 µg) was prevented by Haloperidol [$F_{(6,21)}$=154.2; p<0.0001] and mimicked by Quinpirole [$F_{(4,15)}$=326.6; p<0.0001]. Finally, low dose of Ari prevented the effect of Quinpirole (both 25 µg) [$F_{(5,18)=}$ 137.8; p<0.0001]. **Discussion:** The antipsychotic Ari induced systemic and peripheral antinociceptive effects in different animal models. The D2 antagonist, Haloperidol, prevented the effect of Ari which, in turn, prevented the effect of the full agonist, Quinpirole. These data provide evidence that the mechanism involve partial agonism at D2 receptor. This substance should be further investigated as a possible alternative for the treatment of certain types of pain. Approval by Animal Ethics Committee: 109/2011. **Financial support:** Capes.

05.018

Role of spinal cord PI3K, MAP kinases, and glial cells in superoxide anion-induced pain in mice. Carvalho TT[1], Calixto-Campos C[1], Mizokami SS[1], Pinho-Ribeiro FA[1], Manchope MF[1], Casagrande R[2], Verri WA[1] [1]UEL – Ciências Patológicas, [2]UEL – Ciências Farmacêuticas

Introduction: Reactive oxygen species (ROS), which are abundantly produced by activated phagocytes, may promote different responses during inflammation. The ROS superoxide anion radical ($O_2^{\cdot-}$) is responsible for triggering and maintaining the inflammatory process and generating, among other signs, pain. In this sense, we have recently developed an animal model of inflammatory pain (writhing response and mechanical hyperalgesia) and oxidative stress induced by potassium superoxide (KO_2 – a superoxide anion donor) injection in mice. Spinal cord activation of PI_3K, MAP kinases, and glial cells mediate the nociceptive process during inflammatory and neuropathic pain conditions, in which superoxide anion has a role. In the present study, the spinal participation of PI_3K, MAP kinases, and glial cells in the KO_2-induced pain model was investigated. **Methods:** Male Swiss mice (n= 5/group) were used in this study with the approval of the Ethics Committee for Animal Use from UEL, process nº 71.2012.68. KO_2 (30 µg/i.pl. or 1000 µg/i.p.) or vehicle (saline) was injected and the mechanical hyperalgesia was measured at 30 min, 1, 3, 5, and 7 hours after stimulation, abdominal contortions was evaluated during 20 min after stimulation, and mRNA expression was evaluated by qPCR 30 min, 1, 3, 5 and 7 hours after KO_2 injection. The groups that received KO_2 were also pretreated (30 minutes before) intrathecally with Wortmannin (PI_3K inhibitor – 0.3, 1 or 3 µg), PD98059 (MEK 1/2 inhibitor – 1, 3 or 10 µg), SP600125 (JNK inhibitor – 1, 3 and 10 µg), SB202190 (p38 inhibitor – 1, 3 or 10 µg), Fluorocitrate (astrocyte inhibitor – 0.05, 0.15 or 0.45 µg), Minocycline (microglia inhibitor – 15, 50 or 150 µg) or vehicle (2 or 20% DMSO in saline, 5 µL). **Results:** KO_2 induced significant mechanical hyperalgesia and abdominal contortions in mice, which was reduced by treatment with Wortmannin (up to 89.8% and 79.2%, respectively), PD98059 (up to 63.8% and 66.7%, respectively), SP600125 (up to 70.9% and 85.4%, respectively), SB202190 (up to 72.8% and 46.5%, respectively), Fluorocitrate (up to 49.0% and 67.2%, respectively), and Minocycline (up to 41.8% and 65.5%, respectively). Additionally, co-treatment with PI_3K and MAP kinase inhibitors, at doses that were ineffective as single treatment, reduced in 36.5% and 78.6% the mechanical hyperalgesia and abdominal contortions, respectively, induced by KO_2. Furthermore, KO_2 induced a 14.3 and 12.1 fold increase in mRNA expression of Iba-1 (microglia marker) at 7 h and GFAP (astrocyte marker) at 1 h, respectively. **Discussion:** These results demonstrate that superoxide anion-induced mechanical hyperalgesia and writhing responses are dependent on spinal PI_3K, MAP kinases, microglia and astrocyte activation in mice. **Financial support:** Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenadoria de Aperfeiçoamento de Pessoal de Nível Superior (Capes), MCTI, SETI/Fundação Araucária and Paraná State Government, Brazil.

05.019

Transient receptor potential ankyrin 1 blockage reduced hyperalgesia in a model of trigeminal neuralgia. Trevisan G[1], Nassini R[2], Di Siena G[2], Materazzi S[2], Fusi C[2], Rossato MF[3], Ferreira J[4], Geppetti P[2] [1]UNESC – Ciências da Saúde, [2]UniFi – Ciências da Saúde, [3]UFSM – Química, [4]UFSC – Farmacologia

Introduction: Trigeminal neuralgia (TN) causes intractable pain and reduced quality of life of patients. Then, novel agents for the management of this painful condition are urgently needed. Interestingly, the defunctionalization of transient receptor potential vanilloid 1 (TRPV1) and TRP ankyrin 1 (TRPA1) positive sensory fibers by capsaicin (a TRPV1 agonist) provide pain relief in TN. The TRPA1 is multimodal sensor for noxious stimulus, and it was highlighted as a novel target for analgesics development. Thus, the goal of this study was to explore the TRPA1 role in a model of TN in mice. **Methods**: Animal experiments were carried out according to the European Communities Council (ECC) guidelines for animal care procedures and the Italian legislation (DL 116/92) application of the ECC directive 86/609/EEC. Studies were conducted under the University of Florence research permit ♯204/2012-B. C57BL/6 mice, wild-type (*Trpa1*$^{+/+}$, or TRPA1-deficient mice (*Trpa1*$^{-/-}$) were submitted to the constriction of the infraorbital nerve (CION) or sham procedure. Then, 10 days after animals previously submitted to CION procedure or not were systemically (intragastric, i.g.) or s.c. (into the left upper lip just lateral to the nose) treated with the TRPA1 selective antagonist (HC-030031, 300 mg/kg or 100 µg/site), the antioxidant compound α-lipoic acid (100 mg/kg or 10 µg/site), or the NADPH oxidase inhibitor apocynin (100 mg/kg or 1 µg/site). Orofacial nociception was assessed using von Frey hair filaments (mechanical allodynia), acetone drop test (cold allodynia), and the non-evoked nociceptive behavior as assessed by the spontaneous facial rubbing. The chemical hyperalgesia was observed by the injection of different TRPA1 agonists locally (s.c., into the left upper lip just lateral to the nose). In addition, we have also observed the TRPA1 immunoreactivity, the NADPH oxidase and the superoxide activity, and the production of hydrogen peroxide (H2O2, a TRPA1 agonist) after the CION procedure. **Results**: The systemic (intragastric) or local (subcutaneous in the left upper lip) administration of TRPA1 antagonist (HC-030031) reduced mechanical and cold allodynia, and the non-evoke nociceptive response in CION mice (10 days after procedure). In addition, TRPA1 agonists local injection-induced nociception was enhance after CION. Different from *Trpa1*$^{+/+}$ CION mice, the *Trpa1*$^{-/-}$ CION mice did not showed nociceptive response. The TRPA1 immunoreactivity was not altered in trigeminal ganglia of CION mice, but CION increase the NADPH oxidase and superoxide dismutase activity, and the hydrogen peroxide content in the trigeminal ganglion. Finally, the systemic or local injection of an antioxidant agent (α-lipoic acid) or a NADPH oxidase inhibitor (apocynin) reduced CION-elicited nociception. **Discussion:** Taken together these results showed that TRPA1 activation by oxidative substances might be relevant to nociception development in a TN model. Then, the blockage of the TRPA1 channel could be a novel option for TN pain management. **Financial** Agencies and acknowledgments: The authors thank the fellowships from CNPq and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (Capes). We are grateful to Dr. Delia Preti (University of Ferrara, Italy) for providing HC-030031.

05.020

The jararhagin a snake venom metalloproteinase induces mechanical hyperalgesia, paw edema and neutrophil recruitment in mice. Ferraz CR[1], Calixto-Campos C[1], Manchope MF[1], Casagrande R[2], Moura-da-Silva AM[3], Baldo C[4], Verri WA[1] [1]UEL – Departamento Patologia, [2]UEL – Departamento de Ciências Farmacêuticas, [3]IBu – Laboratório de Imunopatologia, [4]UEL – Departamento Bioquímica e Biotecnologia

Introduction: Jararhagin is a metalloproteinase isolated from *Bothrops jararaca* snake venom. The Snake venom metalloproteinases (SVMPs) are responsible by the tissue damage and inflammatory response in bothrops snakebites, including necrosis, hemorrhage and edema. The pain is a common symptom in victims of these accidents, but there is few information about the mechanisms and the involvement of SVMPs in pain. Herein, it was investigated whether injection of jararhagin induces mechanical hyperalgesia, paw edema and myeloperoxidase activity in mice. **Methods:** Male Swiss mice were used (20-25g; n=6) and the experiments were approved by the Institutional Ethics Committee under the protocol 7786.2014.42. Mechanical hyperalgesia was assessed by an electronic pressure meter, an electronic version of the von Frey filaments. Paw edema was measured using a caliper and myeloperoxidase activity by colorimetric assay. Mice received intraplantar (i.pl.) injection of jararhagin (1, 10, 100 or 1000 ng/paw, diluted in saline) and mechanical hyperalgesia and paw edema were evaluated betweem 0.5-48 h. Myeloperoxidase (MPO) activity was determined 7 h after jararhagin injection. **Results:** The injection of jararhagin induced mechanical hyperalgesia and paw edema in a dose-depend manner. The higher dose tested induced significant mechanical hyperalgesia and edema until 24h. The doses of 100 and 1000 ng/paw of jararhagin were also able to induce significant increase of myeloperoxidase activity. **Discussion:** The jararhagin induced mechanical hyperalgesia, paw edema and neutrophil recruitment (myeloperoxidase activity) in mice. This model can be employed for the study of the hyperalgesic and inflammatory mechanisms of jararhagin, an important metalloproteinase from *Bothrops jararaca* snake venom. **Financial Support:** Capes, CnPQ, MCTI, SETI/Fundação Araucária and Paraná State Government, Brazil.

05.021

Supraspinal kynurenine pathway contributes to the maintenance of neuropathic pain

Santana DAR, Santanna MB, Fonseca MDM, Souza GR, Cunha TM FMRP – Pharmacology

Background: Injury to the somatosensory nervous system due to multiple sclerosis, diabetes mellitus or traumatic injury often causes a debilitating chronic pain syndrome, termed neuropathic pain. One factor that might be involved in the genesis of neuropathic pain is the down modulation of the endogenous descending pain pathway by increased degradation of tryptophan (Trp). In this context, the activation of the indoleamine 2,3-dioxygenase 1 (IDO1) might play a role. IDO1 converts Trp into kynurenine (KYN) that is further metabolized into other catabolites through the action of enzymes within the kynurenine pathway. Kynurenine 3-monooxygenase (KMO) converts KYN in other biologic active products. The final product of the kynurenine pathway, quinolinic acid (QUIN) acts as a NMDA receptor agonist and the persistent activation of NMDA receptor induced phosphorylation of subunits of this receptor (e.g. phosphorylation NR1 subunit, pNR1) with is relevant for development chronic pain. In this study, we evaluated the relationship between the neuropathic pain process induced by the spared nerve injury model (SNI) and the expression of IDO/KMO into the periaqueductal gray matter (PAG), the nucleus raphe magnus in addition to adjacent structures of the rostral ventromedial medulla (RVM) and hippocampus. **Methods:** The RVM and PAG were removed 3, 7, 14, 21 days after SNI. After that, we evaluated the protein levels of IDO, KMO, phospho-NR1 and NR1 subunit of NMDA receptor using western blotting technique. The contribution of supraspinal IDO1 or KMO to the induction and maintenance of neuropathic pain was evaluated by microinjecting Norharmane (Ido1 Inhibitor) or Ro61-8048 (KMO inhibitor) into the intracerebroventricular (i.c.v.) spaces 7 and 14 days after SNI. Mechanical hypersensitivity was evaluated using von Frey filaments. Next, kynurenine pathway metabolites were injected i.c.v. with or without the pretreatment with NMDA receptor antagonist, MK801, and mechanical allodynia was determined. Finally, to determine the effect of up regulation of kynurenine pathway after SNI was microinjection MK801 into i.c.v. **Results:** The expression of IDO1 significant increased at 7 days in RVM and 3, 7, 14 and 21 days in PAG, but not in hippocampus after SNI. In addition, KMO expression also increases at 7 and 14 days in RVM and 7 days in PAG after SNI. The microinjection of Norharmane, 14 days after SNI, reduced in a dose and time-dependent manner mechanical hypersensitivity. Accordingly, the microinjection of Ro61-8048 into the intracerebroventricular spaces, 7 and 14 days after SNI, also reduced in a dose and time-dependent manner mechanical hypersensitivity. Interestingly, the microinjection of QUIN but not of L-kynurenine or 3-hydroxyanthranilic acid into the i.c.v, produced mechanical hypersensitivity in a dose and time-dependent manner, which it was blocked by the pretreatment with MK801. Finally, 14 days after the SNI the microinjection of MK801 into the i.c.v. reduced in a dose and time-dependent manner mechanical hypersensitivity **Conclusions:** These results suggest that the main enzymes of kynurenine pathway (IDO and KMO) in the supraspinal sites play an important role in the maintenance of neuropathic pain through enhancement of NMDA signaling by QUIN. In conclusion, it is plausible to suggest that kynurenine pathway could be a real target in the development of new drugs to control neuropathic pain. **Financial support**: Fapesp, FAEPA, CNPq Animal Ethics Committees: Protocol nº 045/2013 was approved by Local Committee from Ribeirao Preto Medical School of the University of Sao Paulo 06/24/2013

05.022

Antinociceptive and anti-inflammatory effects of hydroalcoholic extract of *Licania rigida* in mice. Lima MJA, Vasconcelos FL, Silva MGP, Vasconcelos AG, Melo CTV NUBEM-INTA,

Introduction *Licania rigida* is a native plant from Northeast region of Brasil. It is found mainly in Ceará, Paraíba, Piauí and Rio Grande do Norte states. Prior studies showed that the extract of that plant is used in folk medicine for inflammation and diabetic pathologies. Therefore, this work aim to investigate if the hydroalcoholic extract of *L. rigida* (EELR) presents antinociceptive and/or anti-inflammatory effects in animal models such as acetic acid-induced writhings and formalin test in mice. **Methods** Female mice (20-25g) were divided into 4 groups (6-13 animals per group): controls (vehicle – tween 80 10%), EELR-200 (EELR 200 mg/kg), EELR-400 (EELR 400 mg/kg) and MOR-7.5 (morphine 7.5 mg/kg). Morphine was used as standard for both tests. Experimental groups and vehicle were administered by gavage and morphine groups received intraperitoneal (i.p.) injections. After 30 minutes of i.p. injections and 60 minutes after gavage administration, mice received the stimulus of acetic acid i.p. or formalin in the paw. Only after 10 minutes of acetic acid injection, the writhings were accounted for 20 minutes. In the formalin test, the nociceptive response was peacked 5 minutes (min) after formalin injection (early phase), and 20-25 min after formalin injection (late phase), representing the tonic and inflammatory pain response, respectively. Data were analyzed by One way ANOVA followed by Student Newman Keuls as the *post hoc* test. Data are here presented by mean ± S.E.M (number of animals). Ethical approval was obtained from the Ethics Committee on Animal Research (CEUA) of Instituto Superior de Teologia Aplicada – INTA with the protocol number 2013.07.003-P. **Results** In the acetic acid-induced writhings test, EELR 400 decreased writhings at 76% (7.12 ± 1.38(8)) as compared to control (30.68 ± 5.67(13)). In addition, in the first phase of formalin test, both doses, EELR 200 (43.00 ± 10.1 (7)) and EELR 400 (34.00 ± 5.1 (13)) reduced the paẃs licking time at 32.7% and 46.7%, respectively, comparing to control group (63.9 ± 7.96 (11)). On the other hand, in the late phase of formalin test, only EELR 400 (6.75 ± 2,86 (13)) decreased the paẃs licking time at 80% as compared to control (33.88 ± 6.07 (11)). MOR-7.5, as expected, decreased all parameters analyzed in both tests comparing to respective controls. **Discussion:** The results showed that EELR presents antinociceptive effects better then anti-inflammatory effects once the lower dose was able to decrease pain only in the first phase of formalin test. On the other hand, when the dose of EELR is increased it can be observed that the extract presents both antinociceptive and anti-inflammatory effects once EELR 400 decreased both phases of formalin test and the number of writhings in the acetic acid-induced writhings, concluding that better effects occur with higher doses of EELR. **Acknowledgments:** Grateful to INTA for the Financial support

05.023

Quercetin reduces ehrlich cells-induced cancer pain in mice: Inhibition of neutrophil recruitment, oxidative stress, cytokines production and activating an opioid receptor-dependent analgesic pathway. Calixto-Campos C[1], Carvalho TT[1], Zarpelon AC[1], Hohmann MSN[1], Corrêa M[1], Casagrande R[2], Verri WA[1] [1]UEL – Ciências Patológicas, [2]UEL – Ciências Farmacêuticas

Introduction: Cancer pain directly affects the quality of life of patients. Several studies have shown that the flavonoid quercetin presents important biological effects, including anti-inflammatory, antioxidant, antinociceptive, and antitumor activity. Therefore, the analgesic effect and mechanisms of quercetin were evaluated in Ehrlich cells-induced cancer pain in mice. **Methods:** Male Swiss mice were used (20-25g; n6) and the experiments were approved by the Institutional Ethics Committee under the protocol 12107.2012.10. For the assessment of overt-pain like behavior, mice received intraplantar (i.pl.) injection of Ehrlich tumor cells ($1x10^7$ cells). For mechanical (electronic version of the von Frey filaments) and thermal (hot plate) hyperalgesia and paw volume (caliper), mice received ($1x10^6$ cells, i.pl.) and, after 30 min, were treated daily with quercetin (100 mg/kg; 2% DMSO diluted in saline). Measurements were performed on alternated days during 12 days. On the 12^{th} day, mice were terminally euthanized and the cutaneous plantar tissue was collected for the determination of myeloperoxidase (MPO) activity, oxidative stress, and cytokine production. The participation of opioid receptor-dependent analgesic pathway (naloxone 1 mg/kg i.p) in the effect of quercetin (100 mg/kg i.p during 8 days) over mechanical and thermal hyperalgesia and paw volume (10^6 cells i.pl.) or in overt-pain (10^7 i.pl.) was investigated in the 8^{th} day after injection the cells. The effect of co-treatment with opioid (morphine 1 mg/kg i.p) plus quercetin (10 mg/kg i.p during 8 days) in pain-evoked were similarly accessed. **Results:** Treatment with quercetin reduced Ehrlich tumor-induced overt pain (64%), mechanical (44%) and thermal hyperalgesia (46%) (average of measurements on 0-12 days), but not the increase in paw volume. Additionally, quercetin reduced neutrophil recruitment (67%), oxidative stress, as assessed by ABTS test (100%) and FRAP test (39%), GSH (100%), and the production of IL-1β (25%) and TNF-α (72%). The analgesic effect of quercetin was amenable by naloxone (opioid receptor antagonist) treatment in overt pain, mechanical and thermal hyperalgesia. Importantly, co-treatment with morphine and quercetin at doses that were ineffective when administered alone reduced overt-pain and mechanical and thermal hyperalgesia. There was no effect on paw edema. **Discussion:** Quercetin reduces Ehrlich cells-induced cancer pain by reducing the production of hyperalgesic cytokines, neutrophil recruitment, oxidative stress and cytokine production, as well as by activating an opioid receptor-dependent analgesic pathway and potentiating morphine analgesia. Thus, quercetin treatment seems to be a suitable therapeutic approach for cancer pain that merits further investigation. **Financial Support:** Capes, CnPQ, MCTI, SETI/Fundação Araucária and Paraná State Government, Brazil.

05.024

Effect of infrared light emitting diodes infrared on neuropathic pain in rats. Pigatto GR[1], Agne JE[2], Bauermann LF[1], Ferreira J[3], Santos GT[3], Freitas RB[1] [1]UFSM – Departamento de Fisiologia e Farmacologia, [2]UFSM – Fisioterapia e Reabilitação, [3]UFSM – Química

Introduction: The peripheral nerve injury can cause sensory and motor functional changes, promoting important damages, especially neuropathic pain. In nervous tissue damage, an innocuous stimulus begins to be perceived as painful as a result of increased sensitivity to a painful stimulus. Various treatments which promote non-pharmacological and noninvasive analgesia are used, such as low-intensity monochromatic light, for example the light emitting diode (LED). The LED is among different treatments options because that is recognized as an effective adjuvant in scarring and neurophysiological Results: **Objective:** This study proposes an evaluation of analgesic effect of LED in the infrared spectrum in experimental model of neuropathic pain induced by constriction of the sciatic nerve in rats. **Methods:** This study was approved by the Ethics Committee on Animal Experimentation and Research under protocol 0802011 (2) of UFSM. Twenty-four Wistar rats were randomized into 4 groups (n = 6). Group I: control group (neuropathic animals-NA); group II: NA and LED treated (LEDt); group III: Sham and LEDt; group IV: Sham untreated. Neuropathy was performed by crushing the sciatic nerve in the region next to its trifurcation. For this purpose the nerve was pressed for 30 seconds using a traumatic hemostat adapted tweezers Halsted. For LED applications the Anodyne ® device (LED infrared light calibrated at high speed 890 nm with a power of 780 mW) was used. The animals received a power of 390 mW energy density of 124.8 J/cm². The nociception parameters performed were: static and mechanical allodynia, thermal allodynia (cold), thermal hyperalgesia (heat) and spontaneous nociception in order to evaluate treatment efficacy. The statistic analysis was carried by two-way ANOVA followed by Bonferroni. $p<0,05$ were considered as significant. **Results and discussion:** The animals of groups I and II developed neuropathy compared to basal measurement ($p<0,05$). They showed allodynia to mechanical and thermal cold stimulation, hyperalgesia in thermal heat test, and behavior of spontaneous nociception in the 7th day post-injury (time 0). In LEDt animals was observed that LED accelerated the recovery in all nociceptive parameters with an increase in the threshold. These effects were observed from the 7th day with regarding to hyperalgesia in thermal heat (19.70 ± 2.07; $p<0,001$; control group- 10.33 ± 0.77) and spontaneous nociception (0.75 ± 0.25; $p<0,05$; control group- 1.16 ± 0.16), and from the 14th with respect to allodynia static (29.80 ± 4.54; $p<0,001$; control group- 8.71 ± 1.27) and dynamic (10 ± 1.78; $p<0,05$; control group- 5.83 ± 0.60), and allodynia thermal cold (0.25 ± 0.25; $p<0,001$; control group- 0.83 ± 0.16). **Conclusion:** The continuous LED treatment with infrared spectrum promotes analgesic effect in experimental model of neuropathic pain in rats. The results of this study suggest that this alternative treatment may be used to treat patients with neuropathic pain. **Acknowledgment:** UFSM and Capes.

05.025

NOD1 and NOD2 contribute to the genesis of neuropathic pain and are involved in glial cells activation. Santa-Cecília FV, Ferreira DW, Fonseca MD, Cunha FQ, Zamboni DS, Cunha TM FMRP-USP – Pharmacology

Introduction: Among pattern recognition receptors (PRRs), the TOLL-like receptors and NOD-like receptors(NLRs) are the most important in recognizing the pathogen-associated molecular patterns(PAMPs). NOD2 are responsible by intracellular detection of muramyl dipeptide(MDP), PAMP found in the peptidoglycan from bacteria. Upon recognition, NLRs recruit directly RIPK2, an adaptor protein, important in NLRs-mediated NFκB activation. PRRs play a crucial role in the activation of spinal cord glial cells and in the induction and maintenance of neuropathic pain. In the present study, we aimed to evaluate the role of receptors NOD1 and NOD2 in the genesis of neuropathic chronic pain, focusing on their signaling pathways (RIPK2), release of pronociceptive cytokines and activation of glial cells. **Methods:** The experiments were carried out on male C57BL/6(WT), male NOD1, NOD2, RIPK2, as NOD1/2 double (DKO), TNFR1, CCR2, TLR4, MyD88 and TRIF deficient (-/-) mice. All animal care and experimental procedures were conducted according to the guidelines of the Ethics Committee (106/2011) of FMRP-USP. Mice were submitted to the Spared Nerve Injury neuropathy model. The mechanical threshold was determined by application of von Frey filaments. Spinal activation of NOD2 was done by intrathecal administration of MDP. Mice were treated with propentofylline, SB 203580, IL-1ra, fluorocitrate and minocycline before the injection of MDP. The levels of gene expression were determined by RT-PCR, ELISA and Western blot. Brains of newborn C57BL/6 mice were used to prepare primary microglial cell cultures. After stimulation, the supernatants were used to evaluate the levels of cytokines by ELISA. **Results:** The results demonstrate that NOD1-/-, NOD2-/-, RIPK2-/-, as well as DKO-/- showed a significant reduction for the days 5-21, with peak at 14 day (50-60%), in mechanical hypersensitivity after peripheral nerve injury when compared to WT. Interestingly, CFA-induced chronic inflammatory hyperalgesia was not reduced in these mice. The reduction in neuropathic pain in NOD1-/-, NOD2-/-, RIPK2-/- and DKO-/- mice was associated with a reduction in the expression of Iba-1(50-63%), GFAP(55%-73%), IL-1β(43-71%) and TNF-α(40-73%) in spinal cord when compared with WT. WT mice treated with an intrathecal injection of MDP(NOD2 ligand), but not with I-Dap(NOD1 ligand), showed a decrease in mechanical nociceptive threshold (peak 3 to 5 hs,50%) compared with the control group. The MDP pronociceptive effect was not observed in NOD2-/-, RIPK2-/-, TNFR1/2-/- and in mice treated with IL-1ra, propentofylline, minocycline, fluorocitrate and SB203580. On the other hand, TNFR1-/-, CCR2-/-, TLR4-/-, MyD88-/- and TRIF-/- mice present similar mechanical hypersensitivity after MDP challenge compared with WT. Corroborating, intrathecal injection of MDP, increase the mRNA expression of IL-1β (63%), IL-6(50%) and TNF-α(50%) in the spinal cord. *in vitro*, it was observed that primary cultures of microglia did not produce IL-1β, TNF-α, IL-6 in response to MDP(10 μg/mL). However, MDP, together with an ineffective concentration of LPS(0.1 ng/mL), showed a robust production of these cytokines. **Discussion:** The results suggest that NOD1 and NOD2, via RIPK2, contribute to the genesis of neuropathic pain, possibly by mediating the release of pronociceptive cytokines and increased glial cells activation. Moreover, it seems that NOD2 activation seems to synergy with TLR4 in attempt to stimulate glial cells activation. These mechanisms represent a novel approach for elucidating the pathophysiology of chronic pain, and a target for the development of drugs for the treatment of neuropathic pain. **Financial Support:** CNPq, Fapesp, FINEP.

05.026

Antinociceptive effect of a new compound derived from pyrazole. Oliveira LP[1], Florentino IF[1], Silva DPB[1], Oliveira TS[2], Ghedini PC[2], Menegatti R[3], Costa EA[1] [1]DCiF-ICB-UFG – Pharmacology of Natural Products, [2]DCiF/ICB/UFG – Biochemistry and Molecular Pharmacology, [3]FF-UFG – Medicinal Pharmaceutical Chemistry

Introduction: Pain has been associated to the mechanism of alerts that triggers appropriate protective responses to real or impending injury (Milano, 2008). However, in the case of chronic pain, pain could outlives its usefulness by interfering with human confort and wellbeing (Julius and Basbaum, 2001). The need for the treatment of pain has led to the search for new analgesic drugs with good efficacy. The pyrazole compounds have been reported to possess antipyretic, analgesic, anti-inflammatory and vasorelaxant effects. The present study sought to investigate analgesic effect of a new pyrazole derivative-LQFM 020 and elucidate your action mechanism. **Material and Methods:** LQFM020 was synthesized in the 'Laboratório de Química Farmacêutica Medicinal/FF/UFG' (LQFM). Experiments were performed using male *Swiss* albino mice (25–30 g) and *Wistar* male rats (200-300 g). The antinociceptive activity of LQFM020 was evaluated by acetic acid-induced writhing test and formalin-induced pain test. The vasorelaxant effect in thoracic aorta of rats of this compound was investigated. The experimental protocols were approved by the Ethic Commission of UFG (number: 017/13 and 020/13). **Results and Discussion:** In the acetic acid-induced writhing test (n=10), the LQFM020 (9, 17.5 and 35 mg/kg, p.o.) and indomethacin reduced the number of writhes by 40, 45, 60 and 43%, respectively, when compared with the control value of 88.9 ± 2.23. LQFM020 (35 mg/kg, p.o.) and morphine reduced the licking time in the neurogenic phase (0–5 min) by 31 and 92%, respectively, in relation to the control value of 76 ± 3.2 s. In the inflammatory phase (15–30 min), treatment with LQFM020 (35 mg/kg), indomethacin and morphine reduced the licking time from control value of 144 ± 6.7 s by 47, 42 and 98%, respectively (n=8). In vascular reactivity tests (n=6), LQFM 020 induced endothelium- dependent (E_{max}= 93 ± 2%) and independent relaxation (E_{max} = 91 ± 2%). Incubation of aorta with L-NAME, ODQ and Glibenclamide significantly inhibited LQFM 020-induced relaxation. In conclusion, LQFM020 demonstrated significant antinociceptive and vasorelaxant activities that suggest participation of-NO/cGMP/K_{ATP} pathway and calcium channels. **Financial Support**: Capes and CNPq. **References:** Julius D., Basbaum A. Molecular mechanisms of nociception. *Nature*, 413 (2001), 203-210. Milano J. *et al.*, Antinociceptive action of 4-methyl-5-trifluoromethyl-5-hydroxy-4, 5-dihydro-1*H*-pyrazole methyl ester in models of inflammatory pain in mice. *Life Sciences*, 83 (2008), 739-746.

05.027

Chemokine decoy receptor D6 did not modulate the induction and maintenance of neuropathic pain. Quadros AU, Violante VD, Alves-Filho JCF, Cunha FQ, Cunha TM FMRP-USP – Pharmacology

Introduction: One of the main challenges in pain therapy is the treatment of chronic pain, represented by neuropathic pain. There are growing bodies of evidence that chemokines (eg. CC, CXC and CX3C) play an important role in the physiopathology of this type of pain, either by their role in the recruitment of immune cells to site of nerve damage, spinal cord and dorsal root ganglion or direct activation of nociceptive fiber. Chemokine decoy D6 is an atypical receptor, which binds with high affinity to CC/β chemokines. It is able to rapidly internalize and degrade its ligands, exerting effective scavenging activity. Although D6 role is already established in several models of inflammatory diseases, its participation in chronic pain has not been investigated. Thus, the aim of this study was to evaluate the role of D6 receptor in the genesis and maintenance of neuropathic pain focusing on its capacity to modulate the pronociceptive chemokines. **Methods**: Animal care and handling procedures were done in accordance with University of Sao Paulo Animal Ethics Committee (003/2013) and IASP guidelines. Peripheral neuropathy was induced in male C57/Bl6 (WT) or D6 deficient ($D6^{-/-}$) mice by three different models: SNI (spared nerve injury), PSNL (partial sciatic nerve ligation) and MPNL (medial plantar nerve ligation). Mechanical threshold was evaluated by using von Frey hair and thermal (heat) threshold was measure in PSNL model by Hargreaves test. The quantification of chemokines CCL2/MCP-1, CCL3/MIP-1α and CCL4/MIP-1β was performed by ELISA in samples from sciatic nerve of WT or $D6^{-/-}$ mice submitted to PSNL model. Evaluation of D6 expression was done in sciatic nerve, dorsal root ganglia (DRG) and spinal by real time RT-PCR. Dates are expressed in mean ± SEM and analyzed by *one way* ANOVA, with *post hoc* Bonferroni. **Results**: It was observed no statistic difference in mechanical allodynia between WT and $D6^{-/-}$ animals submitted to SNI (WT: 2,06 log mg ± 0,22/$D6^{-/-}$: 1,97 log mg ± 0,24), PSNL (2,02 log mg ± 0,18/2,10 log mg ± 0,16) or MPNL model (2,03 log mg ± 0,15/1,92 log mg ± 0,19). In addition, heat hypersensitivity in PSNL model was also similar between $D6^{-/-}$ and WT mice (10,84 seg ± 0,52/10,17 seg ± 0,75). The expression of D6 receptor was not detected in the sciatic nerve, DRGs or spinal cord of naïve mice or even after peripheral nerve injury. Interestingly, the concentration of CCL2 and 3 in the sciatic nerve of $D6^{-/-}$ animals reveled to be reduced 6 hours after surgery (664,6 pg/mL ± 116,7/ 441,4 ± 37,9 respectively) when compared with WT mice (3390 ± 583,4/ 773,4 ± 120,4 respectively), while the amount of CCL4 was reduced 12 hours after surgery in D6 $^{-/-}$ animals (430,8 ± 96,6) when compared to WT in the same time (1543 ± 491,4). **Conclusion**: These results indicate that different of our initial hypothesis, D6 chemokine receptor did not influence the induction or maintenance of the neuropathic pain process. Surprisingly, they also indicate that D6 could be involved in the increase in the expression of chemokines at the site of peripheral nerve injury. **Acknowledgments**: Fapesp

05.028

Nitroxyl inhibits Ehrlich cells-induced cancer pain in mice: Activation of THE cGMP/PKG/ATP-sensitive potassium channel signaling pathway and inhibition of TNF-α and IL-1β production. Longhi-Balbinot DT[1], Calixto-Campos C[1], Medeiros DC[1], Zarpelon AC[1], Corrêa M[1], Miranda KM[2], Verri WA[1] [1]UEL – Patologia, [2]UA – Chemistry and Biochemistry

Introduction: There are evidences that nitric oxide (NO) plays varied roles in the modulation of pain/analgesia. However, the roles of charged and uncharged congeners of NO are less well understood. In the present work, we investigated the antinociceptive effect of the nitroxyl (HNO) donor, Angeli's salt ($Na_2N_2O_3$; AS), in Ehrlich cells-induced cancer pain. Moreover, whether the antinociceptive effect of nitroxyl was dependent on the activation of cGMP (cyclic guanosine monophosphate)/PKG (protein kinase G)/ATP-sensitive potassium channels was addressed. **Methods:** Male Swiss mice were used (20-25g; n=6) and the experiments were approved by the Institutional Ethics Committee under the protocol 12089.2013.40. To induce cancer pain, the animals received an intraplantar (i.pl.) injection of Ehrlich tumor cells ($1x10^6$ cells). At 8 days after tumor injection (peak of hyperalgesia), mice were treated with HNO by intrathecal route (i.t.; dose range: 0.01-1µg/5µl) and the mechanical and thermal hyperalgesia was evaluated (0, 1, 3, 5, 7 and 24 h after HNO treatment). At 3 h after the treatment, spinal cords of mice were collected to verify the levels of TNF-α and IL-1β by ELISA assay. The effect of HNO (0.1µg/5µl, i.t.) on overt-pain like behavior (flinches) induced by Ehrlich tumor cells ($1x10^7$ cells) was also investigated. In both pain models pharmacological treatments targeting guanylate cyclase (ODQ; 0.3 mg/kg, i.p.), PKG (KT5923; 0.5µg/mouse, i.p.) and ATP-sensitive potassium channel (glybenclamide; 0.3 mg/kg, i.p.) were used. **Results:** The results are expressed as mean ± SEM. The injection of Ehrlich tumor cells (ETC) induced a significant mechanical hyperalgesia (g) that was inhibited by HNO (0.01-1 µg/5µl, i.t.) treatment. The antinociceptive effect of HNO was maximal at 5 hours after treatment (Sal: 0.63 ± 0.39; ETC:7.28 ± 0.32; HNO 0.01: 6.60 ± 0.25; HNO 0.1: 4.88 ± 0.39; HNO 1: 4.46 ± 0.35). Thus, the dose of 0.1µg/5µl of HNO was chosen for further experiments. HNO treatment (0.1 µg/5µl, i.t.) also significantly reduced the thermal hyperalgesia induced by ETC (5 h: Sal: 25.45 ± 2.37; ETC: 9.94 ± 2.25; HNO: 25.71 ± 2.90). The i.t. injection of HNO (0.1 µg/5µl) diminished the levels of ETC-induced TNF-α and IL-1β production in the spinal cord (3 h: ETC: 22.73 ± 3.16; HNO: 5.13 ± 1.96 for TNF-α and ETC:561.98 ± 80.00; HNO: 5.13 ± 1.96, respectively). Additionally, the number of flinches induced by ETC was reduced by HNO treatment (0.1 µg/5µl, i.t.) (5 h: Sal: 0.37 ± 0.18; ETC: 30.34 ± 0.55; HNO: 5.60 ± 1.37). The antinociceptive effect of HNO (0.1 µg/5µl) was prevented by ODQ, KT5823, and glibenclamide treatments. **Discussion:** The present study demonstrated the efficacy of a nitroxyl donor and its analgesic mechanisms in overt pain-like behavior, mechanical and thermal hyperalgesia in Ehrlich cells-induced cancer pain in mice. This effect is probably due to the activation of the cGMP/PKG/ATP-sensitive potassium channel (K^+) signaling pathway and reduction of the production of TNF-α and IL-1β cytokines. **Financial Support:** Capes, Fundação Araucária and CNPq.

05.029

Central MU, delta and kappa opioid receptors mediate the protective effect of a sulfated polysaccharide isolated from the red seaweed solieria filiformis on temporomandibular joint nociception in rats. Araújo IWF[1], Santos RS[2], Rivanor RLC[3], Monteiro VS[3], Pachêco JMS[4], Vieira LV[5], Freitas AR[5], Val DR[6], Clemente-Napimonga JT[7], Brito GAC[8], Bezerra MM[4], Chave HV[4], Benevides NMB[3] [1]UFC – Curso de Engenharia de Pesca, [2]UFC – Medicina, [3]UFC – Bioquímica, [4]UFC – Ciências da Saúde, [5]UFC – Odontologia, [6]RENORBIO-UFPE, [7]FOP-Unicamp, [8]UFC – Ciências Morfofuncionais

Introduction: Many clinical studies on peripheral opioid analgesia have been developed in patients suffering from persistent articular pain, a problem of major relevance and prevalence. The aim of the current study was to determine if central opioid receptors play a role in the antinociceptive effect of a sulfated polyssacharide from the red seaweed *Solieria filiformis* (FII) on temporomandibular joint (TMJ) nociception. **Methods:** FII was extracted by enzymatic digestion, followed by ion exchange chromatography (DEAE-cellulose). We used male *Wistar* rats (200-220 g) (CEPA 76/12). The TMJ injection of 1.5% formalin (50 µL) was used as a nociceptive stimulus. The nociceptive behavior was quantified for 45 minutes and measured as flinching the head and rubbing the orofacial region, as a nociceptive assay. Animals (n=5) were treated with FII (0.03, 0.3 or 3 mg/kg; s.c.) or mophine (5 mg/kg; s.c.) 30 min before injection of formalin. The intrathecal injection of mu-opioid receptor antagonists CTOP (0,2 or 10µg/10µl), delta-opioid receptor antagonist Naltrindole (10 or 30µg/10µl), kappa-opioid receptor antagonist nor-binaltorphimine (15 or 45µg/10µl), or vehicle (0.9% NaCl) 15 min before treatment with FII (0.3 mg/kg) was performed. ANOVA, Bonferroni's test. **Results and discussion:** The injection of formalin into the TMJ region of rats increased ($p<0.05$) the behavioral response (205.8 ± 13.36 s) compared to sham group (48.30 ± 2.25 s). Pretreatment with FII (0.03, 0.3 and 3 mg/kg) inhibited ($p<0.05$) the nociceptive response of rats by 27.3%, 75.5% and 57.8%, respectively. Administration of the opioid receptor antagonists: naloxone (15 µg/10 µl), CTOP (0,2 and 10µg/10µl), Naltrindole (10 and 30µg/10µl) and Nor-Binaltorfimina (15 and 45µg/10µl) increased ($p<0.05$) TMJ formalin-induced nociception in the treated groups with FII (0.3 mg/kg). These data suggest that the antinociceptive effect of FII on the TMJ nociception depends on the subsequent activation of µ-, δ- and κ- opioid receptors in the central nervous system. Thus, the understanding of the mechanisms by which the fraction FII of *S. filiformis* inhibits TMJ nociception could potentiate strategies for the treatment of the TMJ pain. **Financial support:** Conselho Nacional de Desenvolvimento Científico e tecnológico (CNPq), Fundação Cearense de Apoio ao Desenvolvimento Científico e Tecnológico (FUNCAP) and INCT-IBISAB.

05.030

IL-1B and HO-1-independent anti-nociceptive action of strontium ranelate in zymosan-induced temporomandibular joint inflammatory hypernociception in rats. Teixeira SC[1], Alves SM[2], Lemos JC[1], Aguiar LMV[2], Pereira KMA[2], Brito GAC[3], Benevides NMB[3], Filho GC[3], Pinto VPT[4], Bezerra MM[4], Chaves HV[2] [1]UFC – Medicina, [2]UFC – Ciências da Saúde, [3]UFC, [4]UFC – Biotecnologia

Introduction: Temporomandibular joint (TMJ) disorders are associated with high levels of inflammatory pain-related disability. Strontium ranelate (Sran) is a dual agent that reduces bone resorption and simultaneously increases bone formation used in the current treatment of osteoporosis. Herein, we investigate strontium ranelate efficacy in the zymosan-induced TMJ inflammatory hypernociception in rats evaluating the possible role of both IL-1β and hemeoxygenase-1 (HO-1). **Methods:** Experiments were approved by the Institutional Animal Care and Use Committee of the Federal University of Ceará, Fortaleza, Brazil (74/2013). Male Wistar rats (180-220g) were pretreated per os with Sran (0.5, 5 or 50 mg/kg) or saline (non-treated group) 60 min before the intra-articular injection of zymosan (2 mg, 40 µL) in the left TMJ. Von Frey test was used to evaluate hypernociception (g) at 4 h after Zy. 6 h after Zy injection it was collected synovial lavage for leukocyte counting and myeloperoxidase (MPO) measurement, and joint tissue and trigeminal ganglion for histopathological analysis (H&E) and IL-1β dosage by the method (ELISA). To perform immunohistochemistry, histological sections of ATM were subjected to IL-1β antibody using method of streptavidin-biotin-peroxidase. In another series of experiments rats were treated with ZnPP-IX (3 mg/kg), a specific HO-1 inhibitor, before Sran (0.5 mg/kg). At another time Ran was administered prior to Zy, and 45min before euthanasia, Evans Blue (5 mg/kg, iv) was administered to assess plasma extravasation. Vascular permeability was evaluated by Evans Blue extravasation measurement. Results: Sran (0.5, 5 or 50 mg/kg) increased ($P>0.05$) the nociceptive threshold (91.8 ± 5.4; 101.5 ± 11.1; or 105.9 ± 3.6, respectively) when compared to non-treated group (52.84 ± 4.1). Nevertheless, Sran (0.5 mg/kg) is not able to reduce leukocyte counting (17142 ± 2334), MPO activity (127 ± 30.5), Evans Blue extravasation measurement (158.2 ± 4.6), and IL-1β levels in joint tissue (7.3 ± 0.78), and trigeminal ganglion (4.3 ± 0.4), when compared to non-treated group (10738 ± 2197; 227.1 ± 32.2; 166.3 ± 6.7; 9.2 ± 1.3; and (5.1 ± 0.9), respectively. ZnPP-IX did not change Sran anti-nociceptive efficacy (91.8 ± 5.4 *versus* 85 ± 4.9). Conclusions: Sran reduced hypernociception in zymosan-induced TMJ independently of IL-1β and HO-1 pathway in rats and it may represent a potential therapeutic to ameliorate the TMJ painful condition. Funding Sources: FUNCAP, CNPq, Capes, and INCT-IBISAB.

05.031

Participation of the NO/cGMP/K^+_{ATP} pathway and muscarinic receptor in the antinociceptive action of quercetin. Lopes EM, Piauilino CA, Araújo JM, Freitas FFBP, Reis Filho AC, Gomes BS, Almeida FRC, Brito SMRC UFPI, Brazil. – Medicinal Plants

Introduction: Pain is an unpleasant feeling caused by damaging stimuli and is often associated with various diseases. There are a variety of treatment options for pain, a growing amount of evidences point out the L-arginine/NO/cGMP/K^+_{ATP} channel pathway, as a relevant factor for antinociceptive effect of many drugs. Natural compounds have been used for treatment of many pathological processes, some studies shows effect antinociceptive the quercetin. The aim of this study was to delineate the antinociceptive effect and to elucidate possible action mechanisms involved. **Methods:** Male Swiss mice (n = 6-8, 20-30 g) were treated systemically with quercetin (5, 10 and 20 mg/kg, ip) 30 min before the intraplantar injection of glutamate (10 µmol). Control animals received vehicle (dimethylsulfoxide – DMSO – 1% in distilled water) or morphine (5 mg/kg, sc). The local effect was evaluated after intraplantar administration of quercetin (20 and 40 ng/paw). Nociception was evaluated by quantifying paw licking time after glutamate (15 min). To determine the mechanism of action in the glutamate test, the animals were pretreated (20 min or 15 min before quercetin) with naloxone (opioid antagonist, 2 mg/kg ip), L-arginine (precursor of nitric oxide, 600 mg/kg i.p.), methylene blue (a non-specific inhibitor of guanylyl cyclase, 1 mg/kg i.p.), glibenclamide (ATP-sensitive K^+ channels inhibitor, 3 mg/kg ip), atropine (an antagonist of muscarinic receptors 1 mg/kg ip) and mecamylamine (an antagonist of nicotinic receptors, 2 mg/kg ip). All experimental protocols were approved by Ethics Committee of Animal Experimentation, CEEA/PI n° 008/2012). Statistical analyzes were performed using ANOVA (one way) followed by Tukey test, $p<0.05$. **Results and discussion:** The systemic administration of quercetin reduced the paw licking time at doses of 20 mg/kg (44,97 ± 4,92) and 10 mg/kg (58,45 ± 2,66) when compared to vehicle (117,20 ± 12,10), the morphine (5 mg/kg) also showed a decrease of the response (17,26 ± 4,02). The intraplantar treatment with quercetin (20 and 40 ng/paw) also presented a significant reduction in nociception (59,18 ± 9,85 and 48,90 ± 7,00 respectively) relative to vehicle (100,13 ± 8,26). The pretreatment of animals with l-arginine (71,75 ± 5,86), glibenclamide (117,71 ± 13,53) and atropine (82,32 ± 8,88) completely reversed the antinociceptive effect of quercetin in the glutamate test. The pretreatment with methylene blue maintained antinociceptive effect of quercetin. However, there was no reversal when the animals that received the quercetin were pretreated with the naloxone and nicotine. In conclusion, antinociceptive activity of quercetin in an acute nociception model involves the participation of the L-arginine/NO/cGMP/$K+_{ATP}$ channel pathway and muscarinic receptor. **Financial support:** UFPI / Capes

05.032

Sulfated polyssacharides from the red seaweed solieria filiformis reduces mechanical hyper-nociception in the rat temporomandibular joint during zymosan-induced arthritis.

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Introduction: Temporomandibular disorders (TDM) involving the temporomandibular joint (TMJ) are often associated with inflammation and secondary hyperalgesia, allodynia, referred pain and arthritis. This study aimed to investigate the effect of sulfated polyssacharides from the red seaweed *Solieria filiformis* (FI and FII) in the model of arthritis induced by zymosan (Zy) in the temporomandibular joint of rats. **Methods:** FI and FII were extracted by enzymatic digestion, followed by ion exchange chromatography (DEAE-cellulose). We used male *Wistar* rats (200-220 g) (CEPA 80/10). Animals (n=6) were treated with FI and FII (0.03, 0.3 or 3 mg/kg; s.c.) 30 min. before induction of arthritis. Rats were anesthetized and received an intra-articular (i.art.) injection of zymosan (2 mg/40 µL) or saline (sham) into the left TMJ. Mechanical hypernociception in the TMJ was evaluated by measuring the threshold of force intensity that needed to be applied to the TMJ region until the occurrence of a reflex response of the animal. The force threshold value was recorded before the i.art. injections of either zymosan or vehicle and after 4 h. After 6 h, the rats were sacrificed under anesthesia and exsanguinated. The superficial tissues were dissected, the TMJ cavity was washed to collect the synovial fluid (total cell counting and myeloperoxidase (MPO) assay and TMJ tissues were excised to perform histological changes. **Results and discussion:** Pretreatment with FI and FII in low doses inhibited ($p<0.05$) the nociceptive response of rats compared to zymosan group. The injection of zymosan resulted in a significant increase in the number of leukocytes (14310 ± 3210) in the synovial fluid compared to sham group (75 ± 21). FI and FII (0.03, 0.3 or 3 mg/kg) did not reduce ($p>0.05$) the neutrophil accumulation, as demonstrated by MPO activity. Intense influx ($p>0.05$) of inflammatory cells was observed in the synovial membrane and in the periarticular tissue, beyond the thickness in synovial membrane in the treated groups with FI and FII. Other studies have reported that sulfated polysaccharides of seaweeds showed antinociceptive effects, but did not significantly reduced inflammatory processes induced by carrageenan or dextran in rats. Thus, these data suggest that these sulfated polysaccharides may be key tool by which to study the mechanisms of their antinociceptive activity.

05.033

Analgesic and anti-inflammatory effects and mechanisms of action of pimaradienoic acid. Zarpelon AC[1], Possebon MI[1], Mizokami SS[1], Hohmann MSN[2], Staurengo-Ferrari L[1], Carvalho TT[1], Ambrosio SR[3], Arakawa NS[2], Casagrande R[2], Verri WA[1] [1]UEL – Patologia, [2]UEL – Ciências da Saúde, [3]Unifran – Ciências Exatas e Tecnológicas

Introduction and aims: Pimaradienoic acid (PA) is a pimarane diterpene (ent-pimara-8 (14),15-dien-19-oic acid) extracted at high amounts from various plants, such as *Vigueira arenaria* Baker (Asteraceae). This compound presents a wide variety of activities, including antispasmodic and relaxant actions on vascular smooth muscle and inhibition of rat carotid contractions, however, carrageenan-induced paw edema and acetic acid-induced abdominal writhing are the only known anti-inflammatory activities demonstrated for it. Therefore, it is important to further investigate the analgesic and anti-inflammatory effects of PA. **Methods:** Firstly, mice received per oral (po), subcutaneous (sc) or intraperitoneal (ip) treatment with PA (1, 3 and 10 mg/kg) or vehicle (saline) 30 min before or 24 h after inflammatory stimulus with acetic acid and PBQ. Afterwards, mice were treated with PA before intraplantar (i.pl.) stimulus with carrageenan or complete freund adjuvant (CFA). The dose 10 mg/kg of PA was chosen for the next experiments. We also evaluated the involvement do NO pathway, cytokine production and nuclear factor-κB (NFκB) activation. Statistical differences were considered significant for $P<0.05$. Experimental procedures were approved by the Ethics Committee of Londrina State University (Of. Circ. 1531.2013.76). **Results and discussion:** Per oral pre-treatment with PA at the doses of 1, 3 and 10 mg/kg inhibited the acetic acid-induced abdominal writhing (up to 28.13, 28.90, 54.91% respectively). This was also observed at 10 mg/kg dose via sc and ip routes. Both phases of the formalin (up to 87.50, 87.09% respectively), and CFA (up to 42,57%)-induced were inhibited by the dose of 10 mg/kg of PA. Compound PA inhibited the carrageenan- and CFA-induced mechanical hyperalgesia (up to 100, 59.58%), edema (up to 34.25, 45,56%), and myeloperoxidase activity (up to 88,42, 77,43%) and the hyperalgesia induced by PGE_2 (up to 62.27%). Pharmacological inhibitors also demonstrated that the analgesic effects of PA depends on activation of the NO-pathway. The treatment prevented the reduction of the antioxidant defenses and the superoxide production in the plantar tissue, and the production of TNF-α, IL-1β, IL-33 and IL-10, and nuclear factor κB activation. The compound did not alter plasma levels of AST and ALT and myeloperoxidase activity in the stomach per se. These results demonstrate that PA shows analgesic effects and the mechanisms involve the inhibition of cytokine production, activation of the NO signaling pathway, inhibition of oxidative stress, and NFκB activation and does not induce liver or stomach damage. **Financial support:** Fundação Araucária, Fapesp, and CNPq (Brazil), 2010 IASP Early Career Research Grants Program funded by Scan/Design by INGER & JENS BRUUN Foundation.

05.034

Vanillic acid inhibits inflammatory pain in mice: Effect on oxidative stress, cytokine production and NFkB. Zarpelon AC[1], Calixto-Campos C[1], Carvalho TT[1], Hohmann MSN[1], Pinho-Ribeiro FA[1], Fattori V[1], Manchope MF[1], Casagrande R[2], Verri WA[1] [1]UEL – Patologia, [2]UEL – Ciências Farmacêuticas

Introduction and aims: Vanillic acid (VA) is a benzoic acid derivative that is used as a flavoring agent. It is an oxidized form of vanillin. Most of these phenolic compounds are antioxidants *in vitro* and have many beneficial biological activities. However, the analgesic effects and mechanisms of VA are incompletely understood. In the present study, it was evaluated the effect and mechanisms of VA in models of inflammatory pain in mice. **Methods:** We evaluated the effect of VA in different models of acute and chronic inflammation. Firstly, mice received intraperitoneal (i.p.) treatment with VA (3-30 mg/kg; diluted in tween 5%) 1 hr before i.p. stimulus with acetic acid and phenyl-p-benzoquinone (PBQ). Afterwards, mice were treated with VA (3-100 mg/kg i.p.) before intraplantar (i.pl.) stimulus with carrageenan or treated with 30 mg/kg of VA (i.p.) before i.pl. administration of complete Freund's adjuvant (CFA). The dose 30 mg/kg of VA was chosen for the next experiments. The model of carrageenan was used to evaluate: cellular recruitment (myeloperoxidase activity [MPO]), oxidative stress, cytokine production and nuclear factor-κB (NFκB) activation. Statistical differences were considered significant for $P<0.05$. Experimental procedures were approved by the Ethics Committee of Londrina State University (Process 10716.2013.53). **Results and discussion:** VA reduced acetic acid-induced writhing over 20 min (up to 66.8%). Using the VA dose of 30 mg/kg, it was observed reduction of PBQ-induced writhing over 20 min (83.9%) and CFA-induced paw flinch over 30 min (58%). VA also inhibited carrageenan-induced mechanical hyperalgesia (up to 69.86%), thermal hyperalgesia (up to 64.05%) and paw edema (up to 11%) 1-5h after stimulus, MPO activity (up to 57.12%) at 5h, and prevented the reduction of antioxidant capacity evaluated by FRAP (ferric reducing ability potential, up to 42.95%) and ABTS (scavenge of the ABTS cationic radical, up to 60.92%) assays at 3h. VA also inhibited CFA-induced mechanical (up to 40.55%) and thermal (up to 100%) hyperalgesia during a 7 days treatment protocol, which did not alter the blood levels of AST and ALT or MPO activity in the stomach. VA also reduced the carrageenan-induced production of TNF-α, IL-1β and IL-33, reduced NFκB activation and increased IL-10 levels at 3 h after stimulus. The present data demonstrated that VA presents analgesic effect in varied models by mechanism related to inhibition of oxidative stress, cytokine production and NFkB activation. Therefore, VA merits further investigation on its analgesic potential. **Financial support:** This work was supported by Brazilian Grants from Coordenadoria de Aperfeiçoamento de Pessoal de Nível Superior (Capes), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), SETI/Fundação Araucária, and Governo do Estado do Paraná.

05.035

Spinal cord BDNF levels increase after dexamethasone treatment in male rats with chronic inflammation. Souza ICC[1], Laste G[2], Rozisky JR[2], Macedo IC[2], Souza VS[2], Caumo W[2], Torres ILS[3] [1]UFPel – Morfologia, [2]UFRGS, [3]UFRGS – Farmacologia

Introduction: Dexamethasone is widely used in the therapy of chronic inflammatory diseases for its pain-modulating effects. **Objective:** to evaluate the effect of dexamethasone on nociception, as well as spinal cord and spinal cord levels of brain-derived neurotrophic factor (BDNF) in male rats with chronic inflammation induced by Complete Freund's Adjuvant (CFA). **Methods:** Sixteen rats with CFA-induced chronic inflammation were randomly assigned to receive dexamethasone (**D**; 0.25 mg/kg) or vehicle (**V**; saline solution) for 8 days. The hot plate and electronic von Frey tests were performed 24 hours after the end of treatment. BDNF levels, in spinal cord were determined by enzyme-linked immunosorbent assay (ELISA). The level of inflammation in the tibiotarsal joint (the ankle region) was evaluated histologically **Results:** A significant increase in paw withdrawal threshold in the hot plate test was noted in the dexamethasone-treated group (Student's *t* test, **V:** 3.38 ± 0.29s; **D:** 4.87 ± 0.45s, $F_{(1,16)}$:0.95, $P<0.05$). Dexamethasone administration increased the hind paw withdrawal threshold to punctuate mechanical stimuli (Student's *t* test, **V**: 24.81 ± 2.6g; **D**:48.31 ± 5.86g, $F_{(1,15)}$: 4.81, $P<0.005$). Rats in the dexamethasone group showed significantly increased spinal horn levels of BDNF as compared with animals in the saline group (Student's *t* test, **V**: 51.63 ± 1.38 pg/mL, **D**: 62.59 ± 3.75 pg/mL; ($F_{(1,15)}$: 3.84, $P<0.05$). Histological analysis showed a local inflammatory response only in animals treated with vehicle demonstrating that the dexamethasone treatment decreased the inflammatory process. **Discussion:** This study corroborates the antinociceptive and anti-inflammatory properties of dexamethasone in this model of inflammatory chronic pain. Also, dexamethasone exerted its pain-modulating effects by increasing BDNF levels in the spinal cord. Taken together, our findings predict a potential influence of corticosteroids on the modulation of spinal cord nociceptive transmission by increases in BDNF levels. This relation is still unclear and contradictory in the literature, and requires further studies for elucidation. The number of ethics committee approval is 100013. **Acknowledgements:** CNPq, Capes, HCPA.

05.036

Differential modulation of nociception after mild dorsal periaqueductal gray stimulation: influence of aversive context. Souza AS, Mujica EMM, Bottamedi M, Tonussi CR UFSC – Farmacologia

Introduction: Pain is influenced by cognition and emotion. The dorsal Periaqueductal Gray (dPAG) can enhance nociception as this is the main source of fear/anxiety. Mild stimulation of the dPAG with glycine produced anxiety-like behavior (1) and hypernociception (2). **Objective:** The aim of the present study was to investigate whether the dPAG, sensitized by glycine, can change the modulation of nociception in an aversive context. **Methods:** Male Wistar rats were implanted with guide cannula aimed at the dPAG. One-week after surgery, subjects were submitted to the experimental procedure. On the conditioning day, rats were placed in a fear conditioning/observation box and after 10 min received the first of 5 foot shocks spaced 30s apart (0.4 mA, 1s duration). Two minutes after the last foot shock, rats were returned to their home cage. Controls did not receive foot shocks, but remained in the conditioning box an equal time. On the testing day, rats were infused into the dPAG with PBS (phosphate buffered saline) or glycine (Gly; 80 nmol/0.3 µL; 60s). Five min later the rats received formalin (2%/50µL) into the hind paw and were immediately placed in the conditioning/observation box. Fear-related behavior (freezing) and nociception-related behaviors (lifting and licking/biting of the injected paw) were registered. All procedures were approved by the Local Ethical Committee for Animal Use (P00723). **Results:** Repeated measures ANOVA followed by Duncańs test showed that fear conditioning was associated with significant increases in the duration of freezing upon re-exposure to the context irrespective of Gly treatment (NoFC-PBS vs. FC-PBS; $F(3,24)= 11.58$, $P<0.001$; NoFC-Gly vs. FC-Gly; $p<0.05$). Re-exposure of rats to the box previously paired with foot shock resulted in a significant reduction of formalin-evoked lifting behavior compared with non-fear-conditioned, PBS-treated counterparts (NoFC-PBS vs. FC-PBS, $p<0.005$), confirming expression of fear-conditioned analgesia (FCA). Intra-dPAG administration of Gly significantly increased lifting (NoFC-PBS vs. NoFC-Gly; $F(3,24)= 15.62$, $p<0.05$) and licking/biting (NoFC-PBS vs. NoFC-Gly, $p<0.01$) behaviors of non-fear-conditioned rats and significantly suppressed lifting-related FCA (FC-Gly vs. FC-PBS, $p<0.001$). Fear conditioning was associated with a significant reduction of licking/biting-related hypernociceptive effect induced by Gly into dPAG (NoFC-Gly vs. FC-Gly; $F(3,24)= 4.67$, $p<0.01$). **Discussion:** This study showed that intra-dPAG Gly increased formalin-evoked paw lifting despite the re-exposure to the aversive context. However, regarding to the licking/biting behavior, fear contextual conditioning prevented the Gly-induced hipernociceptive effect. Keeping in mind that the lifting behavior is predominantly a spinal reflex, so little influenced by the perception and licking/biting behavior is a supraspinally mediated response which leads to the suggestion that depends on an emotional and/or attentional component to be expressed, the results suggest that dPAG sensitized by Gly, appears to play a differential role in modulation of nociception-related behaviors depending on the emotional valence that is assigned to the context. **Financial support**: Capes and CNPq. **References:** (1) Teixeira, KV Behav. *Neurosci* 113, 196 (1999), (2) Martins, MA Behav. *Brain Res* 214, 260 (2010)

05.037

Aldehyde dehydrogenase 2 activation reduces neuropathic pain and 4-hydroxinonenal adducts in spinal cord. Netto BS[1], Ferreira JC[2], Chen CH[3], Mochly-Rosen D[3], Cury Y[1], Zambelli VO[1] [1]IBu – Dor e Sinalização, [2]ICB-USP – Anatomia, [3]Stanford University – Chemistry and Systems Biology

Aim of Investigation: Neuropathic pain control remains a challenge and an unmet clinical need. Aldehyde- dehydrogenase 2 (ALDH2) is a mitochondrial enzyme responsible for the metabolism of reactive aldehydes. Aldehydes accumulation has been recently related to increased pain. Recent data from our group has been shown that activation of ALDH2, using a small molecule called Alda-1, displays a potent antinoceptive effect in a model of carrageenan-induced hyperalgesia (intraplantar, i.pl) in rats. ALDH2 activation induces analgesia by reducing aldehydic load (submitted manuscript). However, the role of ALDH2 in neuropathic pain control is still unknown. Therefore, we propose to investigate the involvement of the mitochondrial aldehyde dehydrogenase 2, ALDH2, in neuropathic pain, using Alda-1, an ALDH2 pharmacological agonist which selectively enhances the activity of ALDH2 (Chen *et al.*, Science, 2008). **Methods:** The experiments were conducted in C57/BL mice following the protocols approved by the IBu Ethical Committee (976/12). Neuropathic pain was induced by sciatic nerve chronic constriction injury (CCI). The nociceptive threshold was determined, before and 14 days after surgery, using the electronic von Frey Method: After the behavior assessment, samples from spinal cord were obtained for detection of 4-hydroxinonenal (4-HNE) aldehydic adducts by Western blot. Fourteen days after surgery, a dose response curve for Alda-1 was performed (5, 10 and 20 mg/kg, s.c. route) and the pain threshold evaluated. Results: CCI decreased the pain threshold when compared to values obtained before surgery (3.3 ± 0.3g vs 9.7 ± 0.5g). Alda-1 (5 mg/kg) increased the pain threshold at 1 and 2 hours after its administration when compared to vehicle-treated mice (6.7 ± 0.4g and 7.7 ± 0.3g, vs 4 ± 0.6g and 3.4 ± 0.6g, respectively). Alda-1 (10 and 20 mg/kg) also increased the pain threshold compared to vehicle-treated mice at 1h (8.6 ± 0.8g and 8.9 ± 0.5g, respectively, vs 4 ± 0.6g) and at 2 h (8.1 ± 1g and 9.3 ± 0.6g, respectively, vs 3.4 ± 0.6g). No differences in pain threshold were detected 3 h after Alda-1 or vehicle control injection at all doses of Alda-1. CCI induces an ipsilateral increase in 4-HNE levels in spinal cord (4-fold). Alda-1 (10 mg/kg) significantly decreased these adducts levels. Conclusions: The results indicate that activation of ALDH2 by Alda-1 reduces CCI-induced nociception by decreasing the aldehydic load in spinal cord. The dose of 10 mg/kg provided maximal reduction of nociception that latest 2h. Our data propose a novel mitochondrial target for neuropathic pain control. Therefore, Alda-1 may be a novel therapeutic drug class to reduce neuropathic pain. **Financial support**: Fapesp (2011/08873-8, 2012/05035-4, 2013/05937-0) and NIAAA1147 to DM-R

05.038

Evaluation of antinociceptive and anti-inflammatory effects of LQFM-023 – New derivative of PDE3 inhibitors. Lino RC[1], Melo GS[2], Pazini F[3], Menegatti R[3], Costa EA[4]
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Introduction: The clinical cases of side effects and poor response to the first line antinociceptive and anti-inflammatory drugs make the development of new analgesic agents a necessity. LQFM-023 was designed from the milrinone and cilostazol prototypes (inhibitors of PDE_3). Though these drugs are mainly cardiotonic, there are reports in the literature showing that they also have anti-inflammatory or analgesic activity. The present study sought to evaluate antinociceptive and anti-inflammatory effects of LQFM-023 that was synthesized in the in the Laboratory of Pharmaceutical Medicinal Chemistry. **Methods:** Female Swiss albino mice weighing approximately 30 g were used in acetic acid-induced abdominal writhing, formalin-induced pain and carrageenan-induced paw edema following prior approval of experimental protocol by the Research Ethics Committee of UFG-number 017/13. Assay of PLA_2 activity was also realized. **Results and discussion:** In this study, treatments with LQFM-023 (35, 70 e 140 µmol/kg p.o.) reduced the number of writhing to 54.4 ± 5.4; 46.9 ± 4.5; 30.2 ± 6.3, respectively when compared to control group (90.35 ± 2.1). The treatment with indomethacin (28 µmol/kg, p.o.) also reduced this parameter to 55.0 ± 3.5. In the first phase of the formalin test, treatments with LQFM-023 (140 µmol/kg p.o.) or morphine (37 µmol/kg s.c.) reduced the licking time (s) to 51.7 ± 5.6 or 3.0 ± 2.5, respectively when compared to control group (70.5 ± 4.2). In the second phase of formalin test, the same doses of LQFM-023, morphine and indomethacin (28 µmol/kg, p.o.) reduced the licking time (s) to 171.8 ± 21.07, 8.04 ± 8.04 and 115.14 ± 23.2, respectively when compared to control group (244.8 ± 25.9). In the paw edema test, treatments with LQFM-023 (140 µmol/kg p.o.) reduced the edema by 34.1% (at 2nd hour), 35.5 (at 3rd hour) and 41.2% (at 4th hour) while indomethacin (28 µmol/kg p.o.) reduced this parameters by 54.9%(at 1st hour), 52.6%(at 2nd hour), 52.6% (at 3nd hour) and 60.7% (at 4th hour). LQFM-023 (5.29 or 10.6 µmoL/mL) reduced PLA_2 activity to 2.9 and 48.00%, respectively. In conclusion LQFM-023 reduced the number of writhing and licking time in both phases of formalin-induced pain test. This compound also reduced the paw edema after 2 hours and inhibited the activities of PLA_2 (an enzyme that participates in inflammatory processes) *in vitro*. These results demonstrate analgesic and anti-inflammatory activities of LQFM-023.

05.039

Antinociceptive activity of the novel compound isolated from Peperomia. Queiroz APS[1], Freitas MCC[2], Lima AB[1], Silva MN[3], Arruda MSP[2], Do Nascimento JLM[4], Bastos GNT[1]
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Introduction: The Peperomia species are frequent in American and Asiatic continents commonly used as a traditional medicine in order to treat pain and inflammation. Their extracts are known by its antinociceptive, antibacterial, antimalarial, antioxidant and anti-inflammatory activities. However there are few studies with the chemical compound isolated. The PNI-23 was isolated from the aerial parts of only species of the Peperomia genus for analyses in this study. **Material and methods:** We evaluated the antinociceptive effect of PNI-23 compound in male Swiss albino mice (20-25 g), used in accordance to the CEPAE-UFPA. We used the writhing test, the formalin test and hot plate test as models of chemical and thermal pain. The PNI-23 were administered at 0.5; 1 and 5 mg/kg i.p. and compared to vehicle and indomethacin (5 mg/kg i.p.) for writhing test as morphine (10 mg/kg; 4 mg/kg s.c) for the hot-plate and formalin test. As chemical models we used the writhing and formalin tests. The PNI-23 was injected 30 min before of test as indomethacin, the morphine (4 mg/kg s.c.) 15 min before in formalin test. For the hot plate test, the PNI-23 (5 mg/kg i.p) and morphine (10 mg/kg s.c) were administered and forthwith we started the test. Results were expressed as mean ± S.E.M. Statistical evaluation were made using ANOVA followed Dunnett's *t*-test and Tukey test and values were considered significantly different when $P \leq 0.05$. **Results and discussion:** As a result the study demonstrated that abdominal writhes provoked by acetic acid injection were reduced 50% in dosed at 1 and 60% at 5 mg/kg by PNI-23 meaning dose-dependency. In the formalin test the PNI-23 at 5 mg/kg reduced significantly the second phase of the test decreasing the time of licks. The animals submitted to the hot plate test did not have had induced alterations in the latency time when compared to the control. These results suggest antinociceptive activity of PNI-23 that could be peripheral. The formalin test resembles the clinical pain more closely, as in comparison with other tests that employ mechanical or thermal stimuli. **Conclusion:** Thereby, according to the tests employed PNI-23 is dose-related to analgesic activity and its action may be related to its interference in the inflammatory process. **Financial Agencies**: CNPq; Fapespa, UFPA. Number of ethics committee- CEPAE-UFPA: 124-13

05.040

Therapeutical efficacy of fish oil extract on experimental model of neuropathic pain. Silva RV, Lima CKF, Moreira CC, Santos EAP, Lobo BW, Miranda ALP FF-UFRJ – Fármacos e Medicamentos

Introduction: Neuropathic pain is a multifactorial condition arising from injury or malfunction of peripheral or central nervous system. Neuroinflammation initially established in peripheral nerve injury-induced neuropathic pain is a crucial process to its physiopathology. Neuro-imune interaction drives the process of peripheral and central sensitization, essential phenomena in neuropathic pain. Omega 3 polyunsaturated fatty acids as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are well known for their anti-inflammatory activity displayed by their endogenous conversion to resolvins and protectins as lipid mediators (Serhan, *J Exp Med* 196(8): 1025, 2002). **Aim**: To evaluate fish oil extract (FOE), rich in EPA and DHA, for the treatment of partial sciatic nerve ligation induced-neuropathic pain in mice. **Methodology**: Swiss mice were anesthetized and submitted to surgery which exposed sciatic nerve at the mid-thigh level on the left paw allowing ligation from 1/3 to 1/2 (Seltzer, *Pain* 43: 243, 1990). Before surgery, animals received thermal (Hargreaves) or mechanical (Von Frey) stimuli on the left hind paw. Daily oral treatment was initiated at 5th day after surgery with vehicle (gum arabic 5%) or FOE (4.65 g/kg) during 5 days. Thermal hypernociception and mechanical allodynia were assed 1, 2, 3, 4, 6, and 24 hours following first administration and also at 5th, 7th, and 9th day after surgery. COX -2 and ATF-3 expression in dorsal root ganglia (DRG) were quantified by western blot analysis. Additionally, TNF-alfa production in the spinal dorsal horn was evaluated. **Results**: FOE reversed mechanical allodynia on the first day of treatment at 6h and 24h following oral administration (n=6 animals, *p<0,05). However, for thermal hypernociception FOE treatment was efficacious only after 2 days of administration. COX-2 and ATF-3 expression diminished in the DRG of animals treated with FOE for 5 days, indicating reduced inflammation and neuronal activation. Similarly, TNF-α production was decreased in the spinal dorsal horn, suggesting reduced microglial activation. **Conclusion:** Results indicate that FOE reduces neuroinflammation and neuronal activation after peripheral nerve injury induced-neuropathic pain in mice. FOE oral treatment reverses mechanical and thermal hypersensitivity. Furthermore, FOE arises as a safe and efficacious therapeutical alternative for the treatment of neuropathic pain. **Finacial Support:** PIBIC-CNPq/UFRJ; Faperj; Capes. CEUA-UFRJ number FARMACIA04.

05.041

Involvement of dorsal and ventral regions of the anterior pretectal nucleus in descending modulation of neuropathic pain. Rossaneis AC[1], Prado WA[2] [1] UEL – Ciências da Saúde, [2]FMRP-USP – Farmacologia

Introduction: The anterior pretectal nucleus (APtN) is known to be involved in descending modulation of nociception. However, there is little evidence of its involvement in chronic pain. Previous works showed that the dorsal (dAPtN) and ventral (vAPtN) regions of APtN may act differently in nociceptive modulation, depending on the duration of pain. Preliminary studies developed by our group showed that the APtN seems to be involved in descending inhibitory control of neuropathic pain only in its early phase. The present study evaluated the participation of the dAPtN and vAPtN as well as the neuronal activation of these regions, through the expression of Fos protein in rats with neuropathic pain. **Methods:** The experiments were conducted in accordance to the Ethical Committee for Animal Experimentation of the Faculty of Medicine of Ribeirao Preto/USP (N°. 199/2009). The Wistar rats were submitted to stereotaxic surgery to implant cannula directed to dAPtN or vAPtN five days before the chronic constriction injury (CCI) of the sciatic nerve. Immediately before, and in the 2nd and 7th days following the CCI, measures of the paw withdrawal threshold to mechanical stimulation (MT) were performed by applying electronic Von Frey. Lesion of dAPtN or vAPtN was performed by injection of NMDA (2.5 µg/0.25 µL) 48 h before (for evaluation of early stage neuropathy) or 5 days after CCI (for evaluation of the maintenance phase). To assess the neuronal activation of the APtN, other animals were submitted to CCI or false ligation and sacrificed 2 hours after receiving intermittent mechanical nociceptive stimulus in the 2nd or 7th day after ligation. The brains were removed, submitted to immunohistochemical analysis, and the number of c-Fos-immunoreactive (Fos-ir) cells was quantified. The MTs (in grams) are reported as mean ± SD and compared by MANOVA with repeated measures and Bonferroni post-hoc test. Comparisons of number of Fos-ir cells were made using ANOVA and Tukey's multiple comparison test ($p<0.05$). Results: The lesion of the vAPtN significantly accentuated the decrease in MT measured on the 2nd day, but did not alter the MT on the 7th day. The results were different regarding treatment ($F_{3, 20} = 922.11$, $p<0.001$) and time ($F_{2, 40} = 1181.51$, $p<0.0001$) and showed a significant treatment x time interaction ($F_{6, 40} = 468.90$, $p<0.0001$). On the other hand, lesion of the dAPtN produced no significant change in the threshold in any of the treatment days. The number of Fos-ir cells, both in vAPtN as dAPtN, on the 2nd day after CCI was significantly higher mechanically stimulated animals than in non-stimulated and control group ($F_{9,20}= 9,24$; $p<0,001$), however, there was no significant difference in the number of Fos-ir cells between the dorsal and ventral regions. **Discussion:** The vAPtN appears to be responsible for the inhibitory descending modulation that APtN exerts on the initial phase of neuropathy. Our results suggest the hypothesis that during the installation of neuropathy, the APtN is activated by mechanical nociceptive stimulus, and its ventral, but not dorsal region, is able to respond to this stimulus by activating inhibitory descending nociceptive pathway. **Financial support**: Fapesp.

05.042

Spinal TLR9 is involved in the genesis of chronic inflammatory and neuropathic pain states. Ferreira DW, Fonseca MDM, Santa-Cecília FV, Cunha FQ, Cunha TM FMRP – Farmacologia

Introduction: Despite advances in the pharmacological treatment of pain, the mechanisms involved in the induction and maintenance of chronic painful process are poorly understood. Previous work has indicated that pattern recognition receptors (PRRs) play crucial roles in the activation of glial cells in the spinal cord contributing to the induction and maintenance of neuropathic pain. Among PRRs receptors, toll-like receptor TLR9 are activated by unmethylated CpG dinucleotides (CpG-DNA) motifs, a PAMP present in bacteria and viruses, as well as mitochondrial DNA, a mitochondrial DAMP released during processes of cellular injury. However, there is no evidence that TLR9 might be involved in the genesis of chronic pain conditions. Thus, in the present study, we aimed to evaluate the role of TLR9 activation in the genesis of chronic inflammatory and neuropathic pain, as well as the mechanisms involved. **Methods:** The experiments were carried out on male C57BL/6 (WT) mice or TLR9, MyD88, IL1R, IL-6 and TNFR1/2 knockout ($^{-/-}$) mice. All animal care and experimental procedures were conducted according to the guidelines of the Ethics Committee (109/2011) of the Ribeirão Preto Medical School (University of São Paulo, São Paulo, Brazil) and according to the IASP guidelines on the use of laboratory animals. Neuropathic pain was induced in isoflurane-anesthetized mice through the Spared Nerve Injury model (SNI). Chronic inflammatory pain was induced through the injection of CFA (10 µL) into the plantar surface of the hindpaw. Spinal activation of TLR9 was performed by intrathecal administration of ODN-CpG. To assess the mechanisms involved in ODN-CpG-induced mechanical hypersensitivity, mice were treated with fluorocitrate (astrocytes inhibitor 0,33 nmol; 30 minutes) and minocycline (microglia inhibitor 0,4 ng; 60 minutes) before the injection of ODN-CpG 10 ng. The mechanical threshold was determined by application of von Frey filaments to the hindpaws. The levels of gene expression were determined by RT-PCR in spinal cord samples (segments L4, L5 and L6). **Results:** Firstly, it was shown that CFA-induced mechanical allodynia was reduced in TLR9$^{-/-}$ mice compared with WT mice. However, TLR9$^{-/-}$ mice had reduced allodynia in the SNI model of neuropathic pain only in the induction phase (3-7 days) but not in the maintenance phase (7-28 days). Underlying the mechanisms involved in the participation of TLR9 in the genesis of chronic pain conditions, WT mice treated with intrathecal injection of ODN-CpG showed a decrease in mechanical nociceptive threshold (peak 1 to 3 hours) compared with the control group (vehicle), returning to the baseline 24 and 48 hours after treatment. Furthermore, ODN-CpG-induced mechanical hypersensitivity in WT mice was not observed in TLR9$^{-/-}$, MyD88$^{-/-}$, IL1R$^{-/-}$, IL-6$^{-/-}$ and TNFR1/2$^{-/-}$ mice. The pretreatment of WT mice with minocycline and fluorocitrate had little effect in inhibiting the mechanical hypersensitivity induced by ODN-CpG. Furthermore, the levels of IL-1β, IL-6 and TNF-α mRNA were increased (peak – 1 hour) in the spinal cord after administration of ODN-CpG. **Conclusions:** These data suggest that spinal activation of TLR9/MyD88 signaling is involved in the cascade of events of chronic pain conditions such as inflammatory and neuropathic pain. Moreover, spinal TLR9/MyD88 signaling mediates chronic pain through a mechanism which depends on the subsequent production of IL-1β, IL-6 and TNFα. These mechanisms might represent a novel approach for the development of new analgesic drugs to control chronic pain. **Financial Support:** CNPq, Fapesp, FINEP

05.043

IFN-γ mediates the induction of indolamine (2,3)-dioxygenase (IDO) in the spinal cord that account for the genesis of neuropathic pain. Fonseca MDM, Santana DAR, Souza GR, Cunha FQ, Cunha TM FMRP-USP – Farmacologia

Introduction The production of spinal interferon-γ (IFN-γ) has been implicated in the genesis of neuropathic pain. There are current data showing that IFN-γ increases the expression and activity of indoleamine (2,3)-dioxygenase 1 (IDO1) in several tissues. The IDO catalyzes the conversion of serotonin (5-HT) or tryptophan into biologically active metabolites. Thus, it can participate in one of the mechanisms responsible for deregulation of descending inhibitory pathway, since it limits the synthesis of serotonin. Unpublished data of our laboratory indicate that IDO participate in the genesis of neuropathic pain through enhancement of NMDA receptor activity due to increase in spinal concentration of quinolinic acid. The present study evaluated whether the increase in the expression of IDO1 in the spinal cord during neuropathic pain is a mechanism dependent on IFN-γ. **Methods:** The experiments were performed in male C57BL/6 (WT), IFN-γ, IFN-γ receptor (IFN-γR) and IDO1 deficient (-/-) mice. All animal care and experimental procedures were performed according to the guidelines of the Faculty of Medicine of Ribeirao Preto (University of Sao Paulo, Sao Paulo, Brazil) Ethics Commission (097/2011). Neuropathic pain was induced by the spared nerve injury model (SNI). The mechanical threshold (in grams) was evaluated by application of von Frey filaments to the right hindpaws. IFN-γ was administered intrathecally (i.t) in different doses. Animals were treated with IDO inhibitor, D, L-1 methyltryptophan (1-MT) via i.t (15 μg/site) or orally (v.o) (3 mg/animal) or with MK801 (NMDA receptor antagonist 10 nmol/site). The gene expression of IDO and IFN-γ were determined by RT-PCR. IDO1 protein expression and activity were performed by western blotting and HPLC, respectively and its localization was assessed in spinal cord by immunofluorescence. All assays were performed on samples from the spinal cord (L3, L4 and L5 segments). **Results:** Firstly, it was shown that the expression of IFN-γ increase in the spinal cord of WT mice during the course of neuropathic pain. Functionally, IFN-γ-/- and IFN-γR-/- mice developed less mechanical allodynia compared with WT mice, which was associated with a reduction in the expression (mRNA and protein) and activity of IDO1 in the spinal cord after SNI. Interestingly, IDO1 is co-expressed in the same cells that express NeuN in the spinal cord after IFN-γ injection. Corroborating, intrathecal injection of IFN-γ induced mechanical allodynia in a dose-and time dependent manner. Spinal pronociceptive effect of IFN-γ was associated with an increase in IDO1 expression (mRNA and protein) and activity that was absent in IDO1-/- mice. Finally, the pronociceptive effect of IFN-γ was also reduced by the systemic and local treatment with IDO1 inhibitor and MK801, suggesting the participation of IDO1 products on the enhancement of NMDA receptor activity. **Discussion:** The present study indicates that a neuro-immune activation in the spinal cord through an IFN-γ dependent mechanism plays critical role in the geneses of neuropathic pain. Moreover, IFN-γ contributes to neuropathic pain through induction of IDO expression and function that in turn enhance NMDA receptor activity. **Financial Support:** Capes and Fapesp.

05.044

Investigation of the antinociceptive activity of riparin – IV using different animal models. Dias ML, Carvalho AMR, Rios ERV, Rocha NFM, Vasconcelos LF, Barbosa Filho JM, Brito GAC, Silva-Alves KS, Costa FL, Leal-Cardoso JH, Sousa FCF UFC

Riparin IV is an alkamide, produced by synthetic process, which has the same group (nucleus) of the Riparins isolated from *Aniba riparia*, and has that name because it is an analogue of Riparins. Previous results of our group demonstrated that Riparin IV presentes an antinociceptive activity in diferent animal models. The aim of this study was to investigate the mechanism of action of Riparin IV. We used male *Swiss* mice, weighing 25-32g (6 to 8 animals in each group) to perform the first part of the experiments. Riparin IV was used at the doses of 25 and 50 mg/kg, by gavage. Data were analyzed using One-Way ANOVA and Student Newman- Keuls test *post hoc*. This study was aproved by Ethics Comitee, with the number of protocol 38/2011. Animals were pre-treated with Rip.IV at both doses 1 hour before Cg (0,1%), PGE2 (100μg/ml) and epinephrine (100ng/ml) application (20μl/paw) and assessed 30, 60 and 180 minutes after intraplantar injection. Pre-treatment with Rip.IV at (25 mg/kg) was able to decrease the intensity of hypernociception observed at 60 and 180 minutes after intraplantar injection of Cg and PGE2, and pre-treatment with Rip.IV (50 mg/kg) decreased the mechanical inflammatory hipernociception at all times observed, when compared to the animals treated with vehicle. After 3 hours of Cg injection, subplantar tissue was collected to quantify the scores of edema and inflammatory infiltrate. Animals pre-treated with Rip.IV at both doses showed no statistical difference when compared to the control group, but animals pre-treated with indomethacin presented a significant reduction at both scores analyzed. The results indicated that Rip.IV presents an antinociceptive activity, probably due to prevention of nociceptors sensibilization, with no anti-inflammatory activity. Then, we decided to investigate the effects of Riparin IV on compound action potential (CAP) of the rat sciatic nerve, in order to verify if the substance was able to evocate any change on nerve excitability. The experiments were carried out on sciatic nerves dissected from Wistar rats. Nerves, mounted in a moist chamber, were stimulated at a frequency of 0.2 Hz, with electric pulses of 100 μs duration at 40 V, and evoked CAP were monitored on an oscilloscope and recorded on a computer. The recorded CAP showed two waves that were denominated 1st and 2nd components. The parameters observed were the peak amplitudes of the components and the conduction velocity. Riparin IV was able to reduce both parameters observed, when compared to control group. These effects developed slowly and were reversible. In conclusion, Riparin IV presents an antinociceptive activity, which seems to be related to the blockade of nerve excitability. **Financial support**: CNPq/Capes/FUNCAP

05.045

α-phellandrene administration reduces inflammatory pain in rats: Preliminar evaluation.

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Introduction: Inflammatory pain is a very common symptom in clinical practice. Patients mainly suffer with spontaneous pain, evoked pain, and hyperalgesia. Among several types of chronic pain, chronic inflammatory pain has been recognized to be most common and difficult to treat. Therapy for inflammatory pain has been composed of symptomatic treatment with nonsteroidal anti-inflammatory drugs, but long-term use of these agents has shown side effects such as gastric lesions. The anti-inflammatory and antinociceptive activity of some essential oils and their isolated components, the monoterpenes, is well reported. In view of the broad range of effects attributed to the α-phellandrene (α-PHE), a monoterpene, this study evaluated acute and chronic antinociceptive property of α-PHE in complete Freund's adjuvant (CFA)-induced inflammatory pain model. **Methods:** The inflammation was induced in Male Wistar rats (180-250 g; n= 6-8/group) by intraplantar CFA injection in the hind paw. They were distributed into groups that received either 25, 50 and 100 mg/kg of α-PHE, vehicle (saline) or dexamethasone (DEXA – 0.5 mg/kg), orally, and subjected to digital von Frey test at different time intervals (1, 2, 4, 6, 8, 10, 12 and 24 h). To investigate the effects of chronic treatment in mechanical hypernociception, animals were treated once daily with an interval of 24 hours on 1st, 2nd, 3nd, 4nd, 5nd, 9nd and 10nd days. Biochemical parameters (blood urea nitrogen – BUN, creatinine and ALT), body and wet organs weight changes (heart, liver, kidneys, lungs and spleen) and macroscopic estimation of gastric lesions were assessed after α-PHE treatment. Swiss mice (20-30g) were utilized in order to evaluate spontaneous locomotion and muscle relaxant activity, through the open field and rota rod tests. All experimental protocols were approved by Ethics Committee of Animal Experimentation, CEEA/PI n° 008/2012). Statistical analyzes were performed using ANOVA (two way) followed by Bonferroni Test, $p<0.05$. **Results and discussion:** The α-PHE demonstrated an antinociceptive effect in the acute phase from 1ª (29.1 ± 3.3) to 24ª (25,8 ± 1.0) hours at a dose of 100 mg/kg, from 1ª (30.0 ± 1.8) to 12ª (32.5 ± 2.3) hours at a dose of 50 mg/kg, and from 2ª (40.3 ± 2.3) to 12ª (28.6 ± 1.3) hours at the dose of 25 mg /kg, and it remained similar to DEXA (0.5 mg/kg po). Prolonged treatment with α-PHE once a day with an interval of 24 hours significantly reduced the CFA induced hypersensitivity with all of the doses (43.0 ± 1.2; 43.6 ± 2.7;39.2 ± 0.9) (***$p<0.001$). α-PHE (100 mg/kg p.o.) did not change the mice spontaneous movement (open-field and rota rod tests), suggesting no central depressant or muscle relaxant effect. Biochemical parameters (blood urea nitrogen – BUN, creatinine and ALT), body and wet organs weight (liver and kidneys) and macroscopic estimation of gastric lesions assessed after treatment for eight days, did not show significant changes. In conclusion, the preliminary results of this study suggest anti-hypernociceptive effect of α-PHE in CFA-induced inflammatory pain model.

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05.046

Antinociceptive effects of essential oil of *Piper rivinoides* Kunth. Costa NF[1], Siqueira AM[1], Nascimento DD[1], Souza SP[2], Valverde SS[2], Castro-Faria-Neto HC[1], Frutuoso VS[1]
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Introduction: The Piperaceae family has about 1,200 species, which 700 correspond to the *piper* genus. These species are used for relevant biological activities. The aim of this study was to investigate the antinociceptive effect of the essential oil from *Piper rivinoides* Kunth (OEPR). **Methods:** Acetic acid writhing test: mice received i.p. injection of 0.8% acetic acid solution, writhing numbers were counted for 10 minutes. Imidazoline and adrenergic antagonists were used in this model. Capsaicin-induced nociceptive: mice were stimulated with capsaicin injection in the right hind paw (1,6 µg/paw). The time licking spent was counted for 5 minutes. Formalin test: mice received formalin injection in the right paw (20 µg/paw). The time licking spent was counted in two phases (1ª -first 5 minutes and 2ª -from 15 min to 30 min). *Tail Flick* and hot plate: The mice tails were exposed to 40 W light and the withdrawal of the tails were evaluated (latency). In the hot plate, animals were put in the apparatus and the time of withdrawal, bite or lick the paw in contact with the plate were observed. *Von Frey* and Paw edema: mice received carrageenan injection in the right paw (300 µg/paw). The paw withdrawal was counted after 10 applications of *Von frey* hair on the paw (0.4g) and the paw edema was measured in the plethysmograph after 1 and 3 hours of the stimulus. Animals were treated (o. p). with OEPR (0.1, 1, 10, 100 and 300 mg/kg) or vehicle 1 hour before each assay. Efaroxan (α_2 adrenergic antagonist and imidazoline I_1 - 1 mg/kg), idazoxan (α_2 adrenergic antagonist and imidazoline I_2 - 3 mg/kg) and yohimbine (α_2 adrenergic antagonist – 0.15 mg/kg) were injected (i.p.) 20 minutes before the administration of OEPR, vehicle or agonist clonidine (α_2 adrenergic agonist 0.01 mg/kg). Morphine (10 mg/kg, i.p.) and diclofenac (50 mg/kg, o.p.) were used as standard drugs. All experiments had been performed in accordance to the Fiocruz Council Animal Care (CEUA), number 033/09. **Results and discussion:** The OEPR effect was dose dependent, which 100 mg/kg (28.27 ± 8,3) reduced 50% the number of writhes in relation to control group (50,6 ± 12,0). The same pattern was observed in the capsaicin assay: the animals treated with 100 mg/kg of OEPR showed good inhibition (36.5 ± 19.7) in relation control (73.3 ± 24.4), so 100 mg/kg was chosen for the next experiments. In the formalin test, OEPR induced a higher inhibition (42.88%, 38.71 ± 18.47) in the 1st phase (neurogenic) than in the 2nd phase (inflammatory, 25.87%, 221.68 ± 80) compared with the vehicle (71.28 ± 16.38 and 1307 ± 54, 1st and 2nd phases respectively). No effect was observed in Tail Flick or Hot Plate, suggesting no interference in reflex response. OEPR showed inhibition only in the 3rd hour in *Von frey* test (53.63 ± 12.0) compared to vehicle (80.0 + 6.3). The paw edema inhibition could be observed in the first hour (0.040 ± 0.0 mL) and the 3rd hour (0.055 ± 0.0 mL) compared to vehicle (0,076 ± 0.0 mL) (0,112 ± 0.0 mL). The effect of OEPR was not altered by Yohimbine and idazoxan. Efaroxan was able to reverse the analgesic effect of OEPR. These results suggest that OEPR inhibits neurogenic pain, mediated by activation of the I_1 receptor. **Financial support**: Capes, CNPq-Fiocruz.)

05.047

Polysaccharide extracts of *Ximenia americana* barks ameliorate abdominal hipernociception in cerulein-induced acute pancreatitis. Silva KES[1], Assreuy AMS[1], Pires AF[1], Girão DKFB[2], França FV[3], Criddle DN[4], Pereira MGP[3], Soares PMG[2] [1]ISCB-UECE, [2]UFC – Morfologia, [3]FECLESC-UECE, [4]University of Liverpool

Acute pancreatitis is a potential lethal disorder involving inflammation, cell death and neuroimmune interactions. Pain management in acute pancreatitis represents a major clinical challenge and influences the disease clinical outcome. Nowadays, the pharmacological treatment of pancreatitis is based in the use of nonspecific drugs, which reflects the poor understanding of the pain signaling pathway involved and makes necessary the search for new compounds. Plant polysaccharides have been highlighted for its effects in several pathologies. However, there are few studies in the pancreatitis pain. Barks of *Ximenia americana* Linne is used to treat gastric pain and the analgesic effect of its aqueous extracts had been experimentally demonstrated. This study aimed characterize the total polysaccharides from *X. americana* barks and to evaluate the toxicity and anti-hipernociceptive effect in the mice model of acute pancreatitis (AP). Barks powder (5 g) of *X. americana* was depigmented in methanol, filtered and the insoluble residue was added to 0.1 M NaOH, filtered and centrifuged. Alkaline supernatants were neutralized, precipitated (ethanol), centrifuged and the final supernatant was lyophilized (TPL) in according to Yoon *et al.* (2002). TPL was analyzed mass (agarose and polyacrylamide gel electrophoresis, stained with Stains-All). AP was induced in male Swiss mice (20-30 g) by 10 intraperitoneal (i.p.) injections of cerulein (50 µg/kg). Animals received TPL (10 mg/kg, i.v.) in a single dose, 30 min before induction, or in two administrations, 30 min before and 30 min after the last injection of cerulein (i.p.) or saline. Plasma levels of amylase and lipase, pancreatic myeloperoxidase-MPO activity and pancreatic histology were analyzed. Abdominal hypernociception was evaluated at the 1st, 24th and 36th h in the von Frey test. Assessment of motor coordination of the animals was conducted by the Rota-rod test and subchronic toxicity assessed after treatment for 14 days with TPL (10 mg/kg, i.v.). Protocols were approved by CEUA/UECE nº 12.783.679-9. Mean ± SEM (n=6-8), ANOVA and Bonferroni's test ($p<0.05$). TPL showed high content of carbohydrates (43%, including 15% of uronic acid) and low protein content (6.5%). Electrophoresis of TPL revealed a polydisperse band. TPL (10 mg/kg) increased the abdominal hypernociception threshold in 44% (single dose) and in 42% (2 doses). TPL (two doses) inhibited the pancreatic MPO activity in 58%, and on histological evaluation markedly improved acinar cell necrosis, edema and neutrophil infiltration, besides, decreased serum levels of amylase (28.4%) and lipase (52%). TPL did not alter animal's motricity, body/organs (kidney, stomach, liver, heart and spleen) mass or hematological markers of renal and hepatic function. The TPL inhibits hypernociception and decreases inflammation in AP induced by cerulean in mice, revealing to be an important tool of interest to treat this condition. **Acknowledgements**: Capes, CNPq and FUNCAP.

05.048

Antinociceptive properties of physalins from *Physalis angulate*. Lima MS[1], Evangelista AF[2], Santos GGL[2], Ribeiro IM[3], Tomassini TCB[3], Soares MBP[2,4], Villarreal CF[1,2] [1]FF-UFBA, [2]CPqGM-Fiocruz, [3]FarManguinhos-Fiocruz, [4]CBTC-HSR

Introduction: Pain is the most common reason a patient sees a physician [1]. Nevertheless, the use of typical painkillers is not completely effective in controlling all pain syndromes, therefore further supporting attempts have been made to develop improved analgesic drugs [2]. In the present study the antinociceptive properties of physalins B (**1**), D (**2**), F (**3**) and G (**4**) from *Physalis angulata* were evaluated. **Methods:** The antinociceptive properties of physalins **1-4** were evaluated in male Swiss mice (25-30g) on the writing, formalin and Freund's adjuvant (CFA)-induced paw inflammation tests. Physalins (25, 50 and 100 mg/kg) or vehicle were administrated intraperitoneally (ip) 40 min before testingThe threshold to mechanical stimulation was measured with von Frey filaments (Stoelting, Chicago, IL, USA) and the volume of each mouse paw was measured with a plesthismometer (Ugo Basile, Comerio, Italy). The paw levels of cytokines were determined by ELISA as previously described [3]. In order to investigate central mechanisms contribution, the antinociceptive properties of physalins were evaluated on the tail flick and hot plate tests. To evaluate possible non-specific muscle-relaxant or sedative effects of physalins (100 mg/kg), mice were submitted to the rotarod test. Animal care and handling procedures were in accordance with International Association for the Study of Pain guidelines for the use of animals in pain research and the Institutional Animal Care and Use Committee Fiocruz (L-IGM-012/09). **Results and discussion:** Physalins **1-4** produced dose-related antinociceptive effects on the writhing and formalin tests. On the other hand, only **3** inhibited inflammatory parameters such as hyperalgesia, edema and local production of TNF-α on the CFA test. Data presented here showed that **3** induce a consistent anti-inflammatory effect, while **1**, **2** and **4** presented no anti-inflammatory effect. These results suggested that the antinociceptive effect of physalins is due, at least in part, to a specific analgesic action. Reforcing this hypothesis, physalins produced antinociceptive effect on the tail flick and hot plate tests, suggesting a centrally-mediated antinociception. Mice treated with physalins did not demonstrate motor performance alterations. The results from this study show that physalins **1-4** present antinociceptive properties associated with central, but not anti-inflammatory events and indicate a new pharmacological property of physalins. **Acknowledgments:** This work was supported by grants from Fundação de Amparo à Pesquisa do Estado da Bahia (FAPESB), Fiocruz and Conselho Nacional de Pesquisa (CNPq). **References:** [1] *J Am Osteopath Assoc* 2013, 113, 620-627. [2] *J Pharmacol. Rep*. 2013, 65, 1601-1610. [3] *Eur J Pharmacol*. 2013, 699, 112-117. [4] *Pharmacol Rev* 2001, 53, 597-651.

05.049

Neryl acetate is effective in atenuate acute pain in animal model in comparason with nerol. Araujo JM[1], Lopes EM[1], Vasconcelos ALM[1], Freitas FFBP[1], Reis Filho AC[1], Reis Filho AC[1], Reis Filho AC[1], Sousa DP[2], Sousa DP[2], Sousa DP[2], Almeida FRC[1], Almeida FRC[1] [1]UFPI – Plantas medicinais, [2]UFPB – Ciências Farmacêuticas

Introduction: Acute pain is a main issue and so difficult to treat. Several medications are used to relieve acute pain, but most have side effects that limit their usefulness. Essential oils is the most souce of monterpenes. Therefore, alternative therapies are still needed. Neryl acetate was obtained from nerol and both are monoterpenes with unknown properties. Until this time there was no research with antinociceptive action of the nerol and neryl acetate. The aim of the present study was to investigate the acute antinociceptive activity of those monoterpenes, the toxicity of neryl acetate (NerylAc), to compare the structures and activity in acute models of pain, to evaluate its anti-hypernociceptive activity and some of the mechanisms involved, besides its action in skeletal muscle activity and central nervous system. **Method:** Male and female Swiss mice (20-30 g) and male Wistar rats (180-240 g) were used (n= 6-9/group). Animals were treated with neryl acetate (3.125, 6.25, 12.5, 25 or 50 mg/kg, p.o. and 40 or 80 ng/paw,i.pl.), Nerol (12,5, 25 and 50 mg/kg,p.o.) mg saline 0.9% p.o. or standard drug such morphine (5mk/kg s.c.), diazepam (4 mg/kg,i.p.), indometacin (10 mg/kg,p.o.), naloxone (2 mg/kg.i.p,), pilocarpine (3 mk/kg, i.p.), atropine (1 mg/kg I.p.), nicotine (1 mg/kg,i.p.), mecamylamine (2 mg/kg,i.p.), L-Noarg (75 mg/kg,i.p.), L-arginine (600 mg/kg, i.p), glibenclamide (3 mg/kg,i.p.). The acute chemical nociception was determined by means of capsaicin, formalin, acetic acid and glutamate. Thermic nociception was evaluated by hot plate and inflammatory hypernociception by Randall and Selitto test. Rota rod and open field test were used to study the monoterpenes effect on locomotor activity and skeletal muscle. Actions mechanisms were searched using glutamate test. All experimental protocols were approved by Ethics Committee of Animal Experimentation, CEEA/PI n° 008/2012). Statistical analyzes were performed using ANOVA (one way) followed by Tukey test, $p<0.05$. **Results and discussion:** Were used 2g/kg, p.o. in unic dose and the animals did not present any signs of acute toxicity for the NerylAc, and the DL50 was not calculated. NerylAc showed antinociceptive action in the glutamate (25 and 50 mg/kg, p.o.) and capsaicin (3,125, 6,25, 12,5 and 25 mg/kg,p.o.) tests, it exerted significant increase in the response latency period in hot plate test at 25 mg/kg), beyond significant reduction on licking time at both of the phases in the formalin test in 25 mg/kg,p.o. NerylAc (3,125, 6,25 e 12 mg/kg,p.o) also reduced the number of acetic acid-induced writhings in mice. Nerol had no effect on these tests. NerylAc (25 mg/kg,p.o) showed an increase in nociceptive mechanical threshold on the fourth hour in the Randall and Selitto test. NerylAc (25 and 50 mg/kg,p.o.) and nerol (25 and 50 mg/kg,p.o) showed no muscle relaxant activity or central depressant effects in open field and rota rod tests at doses used. In conclusion, data showed that NerylAc presents low acute toxicity, antinociceptive property and a possible involvement of muscarinic system and the L-arginine/nitric oxide pathway in this effect. NerylAc demonstrated lower acute toxicity and more potent antinociceptive action than nerol. It seems that the acetylation of the molecule produces an ester responsible for different effects observed. Thus, the continuation of the studies with this molecule looks attractive for future development of a phytochemical for clinical trials. **Financial support**: UFPI / Capes.

05.050

Riparina B, an alkaloid obtained from *Aniba riparia*, acting in the reduction of acute pain. Santiago RF[1], Fontenele AM[1], Braúna IS[1], Brito TV[1], Cruz Júnior JS[1], Batista JA[1], Fernandes HB[1], Dias JM[1], Freitas RM[2], Medeiros JVR[1], Barbosa ALR[1] [1]UFPI – Plantas Medicinais, [2]UFPI – Pharmacy

Introduction: Riparin is the name given to the pharmacological potential derived from amides isolated and synthesized, attributed to extracts of the fruit and the cup of *Aniba riparia* (*Nees*) *Mez Lauraceae* plant typical of the Amazon. Through the Schotten-Baumann reaction of riparin new derivatives were obtained and called, riparin A and B. However, there are few studies in support of the analgesic activity of Riparin B. **Methods and Results:** Mice were pretreated with subcutaneous injection of Riparin B (10 mg.kg-1) Morphine (5 mg.kg-1) or vehicle (3% DMSO or Tween 80). For evaluation of writhings was administered intraperitoneally 30 minutes after acetic acid pretreatment and in 10 minutes it started the counting of abdominal writhes for 20 minutes. In the formalin test, the verification of paw lick was performed 30 minutes after the subplantar injection of formalin (20 µ / paw) and counting was divided into two phases (0-5 minutes and 20-25 minutes); whereas for the hot plate test animals were placed on a hot plate at 50 ± 1˚C immediately after the pre-treatment and after 30, 60, 90 and 120 minutes. Riparin B (10 mg.kg-1) significantly decreased the writhing induced by acetic acid (± 13.4) compared with the group receiving DMSO to 3% (± 42.6). In the formalin test, the group that received Riparina B (10 mg.kg-1) reduced the paw licking time (1st phase: ± 32.205; 2nd phase: ± 3.3575) compared to the control group (1st phase: ± 68.005; 2nd phase: ± 57.7125), both in the first and second phase. Riparin B (10 mg.kg-1) significantly decreased the writhing induced by acetic acid (± 13.4) compared with the group receiving DMSO to 3% (± 42.6). In the formalin test, the group that received Riparina B (10 mg.kg-1) reduced the paw licking time (1st phase: ± 32.205; 2nd phase: ± 3.3575) compared to the control group (1st phase: ± 68.005; 2nd phase: ± 57.7125), both in the first and second phase. In the hot plate test, the group that received Riparin B (10 mg/kg-1) showed more time spent on the board at 30, 60 and 120 minutes (30 min: ± 17,874; 60 min: ± 14,936; 120 min: ± 10,706) compared to the group that received Tween 80 (30 min: ± 4,604; 60 min: ± 6, 028, 120 min: ± 9.22). **Conclusion:** The study confirms that, given Riparina B reduces acute pain, reducing writhing, licking the paw and increasing the residence time of the animal on the hot plate.

05.051

Effect of transplantation of bone marrow mesenchymal stem cells in murine model of diabetic peripheral neuropathy. Evangelista AF[1], Silva DN[2], Soares MBP[1,2], Villarreal CF[1,3]
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Introduction: Diabetes is a highly prevalent disease that often compromises the peripheral nervous system [1-2]. Clinical characteristics of diabetic peripheral neuropathy include spontaneous symptoms, changes in sensitivity, hyperalgesia and allodynia, with patients often suffering from severe pain that can last for many years. Currently there is no gold standard for the treatment of neuropathic pain. Based on the potential of stem cells for functional reestablishment of the damaged nervous system, the cell therapy represents a promissory alternative to the neuropathic pain control.[3,4, 5]. In this study, the potential of mesenchymal stem cells derived from bone marrow in a mice model of diabetic peripheral neuropathy was evaluated. **Methods:** Male C57BL/6 mice were injected intraperitoneally (i.p.) with streptozotocin 80 mg/kg (Sigma) diluted in a citrate buffer. Negative control group received vehicle only. Mice were considered diabetic if glycemia were above 250 mg/dl. After 28 days, hyperglycemic mice were transplanted by orbital plexus injection with 1 x 10^6 cells/mouse in a final volume of 200 µl or saline (200 µl). Paw mechanical and thermal nociceptive thresholds were evaluated by using von Frey filaments and Hargreaves test, respectively. Nociceptive threshold, body weight, blood glucose level and motor function (rotarod test) were evaluated during the experimental period of 90 days. Animal care and handling procedures were in accordance with International Association for the Study of Pain guidelines for the use of animals in pain research and the Institutional Animal Care and Use Committee Fiocruz (L-IGM-025/09). **Results and discussion:** Blood glucose levels and body weight loss were reduced in diabetic cell-treated mice when compared to diabetic saline-treated controls during the follow up period. STZ-induced mechanical allodynia and thermal hypoalgesia was also reduced after the cell therapy. Sixteen days after transplantation, cell-treated diabetic mice exhibited nociceptive thresholds similar to that of non-diabetic mice, an effect maintained throughout the 90-day evaluation period. In addition, the cell therapy improved the motor function in diabetic mice. The present study demonstrates that mesenchymal stem cells produce a powerful and long-lasting antinociceptive effect on diabetic neuropathy. Our results suggest stem cell therapy as an option for the control of diabetes complications such as intractable diabetic neuropathic pain. **Acknowledgments:** This work was supported by grants from Fundação de Amparo à Pesquisa do Estado da Bahia (FAPESB), Fiocruz, Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (Capes) and Conselho Nacional de Pesquisa (CNPq). **References:** [1] IDF. *Intern Diab Fed* 6, 2013. [2] Boulton AJ *Diab. Care* 27: 1458, 2004. [3] Tesfaye S. *Hum. press* 2, 2007. [4] Tesfaye, S. *Curr Opin Support. Palliat Care.* 3: 136, 2009. [5] Csete, M *Anest. Res. Soc.* 104, 2007

05.052

Heme oxygenase-1/carbon monoxide pathway peripherically activated reduces inflammatory hypernociception in the temporomandibular joint dependent from ATP-sensitive K^+ channel but not from NO/cGMP/protein kinase G pathway. Freitas HC[1], Alves SM[2], Maciel GF[3], Val DR[4], Gondim DV[5], Pereira KMA[2], Brito GAC[5], Napimoga JTC[6], Filho GC[2], Pinto VPT[2], Bezerra MM[2], Chaves HV[2] – [1]UFC – Medicina, [2]UFC – Ciências da Saúde, [3]UFC – Odontologia, [4]RENORBIO-UFPE, [5]UFC – Ciências Morfofuncionais, [6]Unicamp – Odontologia

Introduction: The signs and symptoms associated with the temporomandibular joint (TMJ) arthritis include acute or persistent pain. Several lines of evidence indicate the antinociceptive activity of the heme oxygenase-1/carbon monoxide pathway (HO-1/CO). The purpose of this study is to investigate if the antinociceptive role of HO-1/CO pathway peripherically activated depends on the nitric oxide (NO)/cGMP/ protein kinase G (PKG)/ATP-sensitive K^+ channel (K^+_{ATP}) on the TMJ inflammatory hypernociception in rat. **Methods**: We used male Wistar rats (200-220 g) (CEPA 76/12). Inflammatory hypernociception was induced by intra-articular injection of zymosan (Zy) into left TMJ, and sham group received saline. Animals (n=6) were treated with Hemin (0,12 µmol/art., 15µL), DMDC (0,16 µmol/art., 15µL) or its vehicles (15µL) injected (i.art.) 15 min before zymosan. 15 min prior hemin or DMDC, aminoguanidine (0,1 mol/art., 15µL), ODQ (8 µg/art., 15µL), KT5823 (1,5 µg/art., 15µL) or glibenclamide (10 µg/art., 15µL) was administered. Also, naloxone (10 µg/art., 15 µl) was administered 5 min before hemin or DMDC. Mechanical hypernociception in the TMJ was evaluated by measuring the threshold of force intensity that needed to be applied to the TMJ region until the occurrence of a reflex response of the animal. The force threshold value was recorded before the i.art. injections of either zymosan or vehicle and after 4 h. **Results and discussion:** Hemin (110.5 ± 5.4) and DMDC (110.9 ± 4.7) reduced ($p<0.05$) the TMJ inflammatory hypernociception compared to Zy group (74.3 ± 4.3). The HO-1/CO pathway antinociceptive action depends on the K^+_{ATP} since the antinociceptive action of hemin (83.5 ± 2.9) and DMDC (82.3 ± 2.9) was prevented by the pre-treatment with K^+_{ATP} (glibenclamide). Hemin (71.8 ± 6.4) but not DMDC (118.6 ± 2.5) antinociceptive action depends on the TMJ opioid receptors since it was prevented by the pre-treatment with opioid receptors antogonist (naloxone). However, the HO-1/CO pathway antinociceptive action does not depend on the NO/cGMP/PKG pathway since the antinociceptive action of hemin (115.8 ± 3.0, 119.4 ± 3.9, 94.3 ± 5.9) and DMDC (90.4 ± 5.0, 114.5 ± 3.4, 83.3 ± 1.2) was not prevented by the inhibitors of nitric oxide synthase (aminoguanidine), cGMP (ODQ), or PKG (KT5823), respectively. Our data provide evidence that up-regulation of HO-1/CO pathway provides a robust anti-nociceptive effect in zymosan-induced TMJ hypernociception, and that HO-1/CO acts through ATP-sensitive K^+ channel. **Financial support:** Conselho Nacional de Desenvolvimento Científico e tecnológico (CNPq), Fundação Cearense de Apoio ao Desenvolvimento Científico e Tecnológico (FUNCAP) and INCT-IBISAB.

05.053

LASSBio-1141: A neuro-immune modulator effective in a model of neuropathic pain.

Lima CKFL[1], Silva RV[1], Yekkirala AS[2], Lacerda RB[3], Barreiro EJ[4], Fraga CAM[4], Cunha TM[5], Woolf CJ[2], Miranda ALP[1] [1]LEFEx-UFRJ – Farmácia, [2]Harvard Medical School, [3]UFRRJ – Química, [4]LASSBio-UFRJ – Farmácia, [5]FMRP-USP

Introduction: Therapeutic approach of neuropathic pain is a great challenge nowadays and multi-target therapies, focusing on neuro-imune interaction, have arisen as a positive pharmacological approach. LASSBio-1141 was previously described as anti-TNF-α compound effective in models of inflammation (Lacerda, R.B. *Bioorg Med Chem* 17: 74, 2009). Then, the aim of this work was to investigate its efficacy in a model of neuropathic pain as well as its mechanism of action. **Methods:** Culture of glia cells was obtained from newborn (1-2 days) Swiss mice and stimulated with LPS for quantification of TNF-α and IL-10. DRG and TG neurons were obtained from C57 mice and cultivated in 384-wells for Ca-imaging assay. Neuropathic pain was induced in Swiss mice using partial sciatic ligation (PSL), as described by Seltzer (Seltzer, Z. Pain, 43:245, 1990). 5 day after surgery mechanical and thermal hypersensitivity were evaluated. After 5 days of treatment samples of DRG and spinal cord were collected and analyzed by RT-PCR for IL-1β and TNF-α. All the procedures were approved by CEUA-UFRJ, protocol nº FARMACIA04 **Results and discussion:** LASSBio-1141 at 10 μM and 1μM reduced TNF-α production after stimulation of glia cells with LPS for 24 h, these concentrations also increased IL-10 production in the same culture. Regarding neuronal effect, LASSBio-1141 at 10 μM diminished calcium influx in culture of DRG and TG neurons stimulated with capsaicin (1 μM), showing reduction of TRPV1 activity. LASSBio-1141, orally administered at 100μmol/kg, showed efficacy through 10 days of treatment, blocking completely the thermal hypersensitivity and mechanical allodynia (n=10). After 5 days of treatment mice were euthanized and samples of DRG and spinal cord were submitted to RT-PCR for IL-1β and TNF-α and the treatment with LASSBio-1141 was efficacious in reducing mRNA expression of those cytokines in both samples (TNF-α= 92% DRG, 85% Spinal cord; IL-1β= 91% DRG, 88% spinal cord), It was also observed an increase in IL-10 in mice spinal cord. Additionally, LASSBio-1141 (100μmol/kg, v.o.) treatment was inefficacious to reduce mechanical allodynia in IL-10 KO mice submitted to PSL. Also, LASSBio-1141 reduced neuronal activation after PSL, as shown by reduced ATF3 expression in DRG. In order to identify LASSBio-1141 mechanism of action, we investigated the participation of adenosinergic system. The ADORA1 antagonist, DPCPX (10 mg/kg, i.p.), completely reversed LASSBio-1141 effect at the first day of treatment with LASSBio-1141. However, the ADORA2A antagonist, CGS15943 (5 mg/kg, i.p.), reversed mechanical allodynia after 6 h of treatment. LASSBio-1141 reversed thermal hypersensitivity even 5 days after interruption of treatment. **Conclusion**: LASSBio-1141 arises as a novel candidate compound with an innovative mechanism of action, modulating neurons and immune activation. **Financial Support:** Faperj, CNPq, Capes, INCT-INOFAR.