05. Pain and Nociception Pharmacology

05.001 Curcumin targets different signaling pathways to reduce superoxide anion-induced hyperalgesia. Fattori V[1], Pinho-Ribeiro FA[1], Borghi SM[1], Alves-Filho JC[2], Cunha TM[2], Cunha FQ[2], Casagrande R[3], Verri Jr WA[1] [1]UEL – Ciências Patólogicas, [2]FMRP-USP – Farmacologia, [3]UEL – Ciências Farmacêuticas

Introduction: Pain is one of the most common clinical sign of inflammation and main cause of medical attendance. Reactive oxygen species contribute to inflammatory hyperalgesia, especially superoxide anion ($O_2^{\bullet-}$). Peripheral mechanisms triggered by $O_2^{\bullet-}$ are related to the release of pro-hyperalgesic cytokines, and central mechanisms depends the reaction of $O_2^{\bullet-}$ with nitric oxide generating peroxynitrite that nitrates endogenous MnSOD inactivating this enzyme and maintaining $O_2^{\bullet-}$ levels. Curcumin, a natural compound, presents antioxidant, anti-inflammatory, analgesic, antimicrobial activities and has been used in clinical trials as analgesic and to cancer treatment. **Aim**: Evaluated efficacy of pretreatment with curcumin in a model of $O_2^{\bullet-}$-induced hyperalgesia. **Methods**: Potassium superoxide (KO_2) was used as a superoxide anion donor. Overt pain-like behaviours were countabilized by the number of abdominal writhings or paw flinches and time spent licking the paw. Mechanical and thermal hyperalgesia were determined using an electronic anesthesiometer and hot plate, respectively. Cytokine levels and NF-κB activity were determined by ELISA, and antioxidant effect by nitrobluetretrazolium assay and ABTS radical scavenging ability. Myeloperoxidase activity was measured by colorimetric assay. The Nrf2, heme oxygenase-1 (HO-1) and $gp91^{phox}$ mRNA expression was determined by quantitative PCR. Data were analysed by ANOVA followed by Tukey's *post-hoc* and considered significant when $P<0.05$. **Results**: Curcumin at 10 mg/kg reduced mechanical and thermal KO_2-induced hyperalgesia at all-time points (0.5-7 h after stimulus) in 50% and 85%, respectively. Curcumin reduced in 78% abdominal writhing and 29 and 45% number of flinches and time spent licking, respectively. Neutrophils play major role in maintenance and development of hyperalgesic state and curcumin reduced myeloperoxidase activity in 45%. Corroborating with these, curcumin increased 30% anti-inflammatory cytokine IL-10 and decreased in 45% NF-κB activity, and consequently, reduced KO_2-induced IL-1β and TNF-α production in 37 and 38%, respectively. Curcumin restored antioxidant activity by abolishing $O_2^{\bullet-}$ production and decreasing $gp91^{phox}$ mRNA expression. In line with this, curcumin increased in 45% Nrf2 mRNA expression and 350% HO-1 mRNA expression. **Conclusion**: Curcumin reduced different nociceptives responses targeting diverse pathways and this feature may be beneficial to inflammatory pain. Besides, we provided evidences that support $O_2^{\bullet-}$ as important component of hyperalgesia and advances in the knowledge of curcumin mechanisms of action, and support the use of curcumin as an analgesic. **Financial support**: Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Ministério da Ciência Tecnologia e Inovação (MCTI), Secretaria da Ciência, Tecnologia e Ensino Superior (SETI), Fundação Araucária and Parana State Government. Ethical Comittee approval: Process number 71.2012.68

05.002 Pre-clinical evidence on the benefits of docosahexanoic acid on adverse and anti-tumoral effects of cyclophosphamide. Freitas RDS[1,2], Costa KM[2,1], Nicoletti NF[2,1], Campos MM[3,2,1] [1]PUCRS – Toxicologia e Farmacologia, [2]PUCRS – Medicina e Ciências da Saúde, [3]PUCRS – Odontologia

Introduction: Hemorrhagic cystitis (HC) is an inflammatory and painful side effect of the chemotherapy agent cyclophosphamide (CYP) (Korkmaz, *J Expl Integ Med* 2(2), p.93, 2012.). Omega-3 fatty acids supplementation is often prescribed for cancer patients on chemotherapy (Vaughan VC, *et al*. *Br J Cancer*, 108, p.486, 2013). Previous evidence (unpublished data) demonstrated that supplementation with enriched-fish oil diet for 21 days was able to produce analgesic effects in the mouse model of HC induced by CYP, without any effect on bladder inflammation. **Aims**: This study analyzed the mechanisms underlying the analgesic effects of omega-3-derived docosahexaenoic acid (DHA), in the mouse model of CYP-evoked HC, in addition to the effects of DHA over CYP cytotoxicity, in cultured breast cancer cells. **Methods**: Male Swiss mice were used (N=8/group). The Animal Ethics Committee (12/00303) approved all the experimental protocols. Mice were divided into three experimental groups: Saline + Saline; CYP 300 mg/kg i.p. + Saline, CYP 300 mg/kg i.p. + DHA 1 mol/kg. (1 h prior CYP). The behavioral tests were performed 30 min after CYP injection; each animal was observed for 2 min, every 1/2 h, for 4 h. On the 5th hour, Von Frey (VF) test was performed using a 0.4 g-filament, for abdominal hypersensitivity analysis. After euthanasia, the spinal cords were removed for evaluating the astrocyte activation (GFAP immunolabelling) and GPR40/FFAR1 expression. For *in vitro* experiments, the effects of CYP (0.1 to 50 mM) or DHA (25 to 100 µM) were assessed alone, on the viability of MDA-MB-231 breast cancer cells. Separately, the effects of DHA (75 µM and 100 µM; 30 min before) and CYP (CYP 1 mM and 10 mM) were tested in combination. **Results**: The systemic treatment with DHA significantly inhibited the spontaneous nociceptive behavior caused by CYP (29 ± 8%; $P<0.01$). The DHA i.p. treatment also reduced the abdominal mechanical allodynia on the fifth h after CYP injection (57 ± 15 %; $P<0.05$). CYP administration led to significant astrocyte activation at the right dorsal horn of the spinal cord ($P<0.05$), whereas the i.p. treatment with DHA caused a slight reduction in the number GFAP-positive astrocytes, although this effect was not significant ($P>0.05$). Spinal GPR40/FFAR1 immunolabelling was significantly diminished when CYP was injected (57 ± 15 %; $P<0.05$), while DHA i.p. treatment led its expression to the saline-treated control levels. The treatment with CYP (10 mM and 50 mM) or DHA (100 µM) was able to reduce the viability of MDA-MB-231 human breast cancer cells in a significant manner ($P<0.01$), with inhibitions of 32 ± 9 %, 66 ± 7% and 42 ± 18 %, respectively. The pre-incubation of DHA (100 µM) likely increased the cytotoxic effects of CYP (10 mM) (67 ± 7 %; $P<0.01$), according to the evaluation of MDA-MB-231 cell viability. **Conclusion**: DHA treatment might be useful for patients under chemotherapy with cyclophosphamide, by preventing bladder pain and increasing the anti-tumor effect. Further studies are still required to verify the *in vivo* anti-tumor effects of DHA. **Financial Support**: FINEP/PUCRSINFRA #01.11.0014-00, CAPES and CNPq.

05.003 The role of pattern recognition receptors like toll-like receptors 4 in herpetic and post-herpetic neuralgia. Silva CR[1], Berlink J[1], Raymondi J[1], Cunha FQ[1], Cunha TM[1] [1]FMRP-USP – Farmacologia

Introduction: Herpetic-neuralgia (HN) is a painful vesicular rash resulting from Varicella-Zoster virus reactivation in the dorsal root ganglia (DRGs) or cranial nerves. However, even after the rash resolution, pain may persist for months or even years, defining the Post-herpetic neuralgia (PHN). It is believed that pain during HN and PHN is due to the release of inflammatory mediators on the DRGs, as a result from glial cells activation, cells from the immune response which infiltrate the DRGs or sensory neurons sensitization. Toll-like receptors 4 (TLR4) are involved in hyperalgesia induction during some neuropathic pain models, and are implicated in triggering pro-inflammatory immune system signaling events which may be a target for pain during the PHN. **Aim**: The aim of this study is to verify the possible role of TLR4 activation on DRGs during herpetic and post-herpetic neuralgia. **Methods**: Adult male Wild-type (WT), C57BL/6, TLR4KO and MRP14KO mice (25-35g) were anesthetized and the right midflank depilated for inoculation with HSV-1 ($1x10^6$ plaque-forming units/20µl) for HN and PHN induction. Pain (mechanical allodynia) and weight gain were monitored from 1-42 days post-infection (DPI), and compared with non-infected animals. Levels of TLR4 and MRP14 gene expression were determined by RT-PCR and MRP14 protein expression was analyzed by Western blot in DRGs and spinal cord (L4-L6) from infected animals. **Results**: TLR4KO- and MRP14KO-infected animals had significative less pain (from 5-30 and 5-18 DPI, respectively) if compared with WT HSV-1 infected animals. The TLR4 ablation in infected animals resulted in increased weight gain when compared with MRP14 ablation and WT infected animals. There was an increase in MRP14 and TLR4 RNA levels on DRGs 7-21 DPI. Additionally, 3-7 and 21 DPI MRP14 protein is increased on DRGs of infected animals. Spinal cord MRP14 and TLR4 RNA levels were not altered. **Conclusions**: TLR4 activation seems to be involved in herpetic and post-herpetic neuralgia and the MRP14 protein seems to be, at least one of the triggers for this activation. **Research support**: This study is supported by Fundação de Amparo a Pesquisa do Estado de São Paulo (FAPESP) and Conselho Nacional de Desenvolvimento Científico (CNPq). Animal Ethical Committee: 0208/2014

05.004 Effects of simvastatin on diabetic neuropathic pain in rats. Corso CR, Werner MFP UFPR – Farmacologia

Introduction: Statins, such as simvastatin, in addition to reduce cholesterol levels through inhibition of HMG-CoA reductase, present pleiotropic effects, including anti-inflammatory, immunomodulatory, neuroprotective and antinociceptive effects on diabetic neuropathic pain (Bhalla, S. et al. J. Pain. v. 15, p. 1, 2014). Diabetic neuropathic pain is a common complication of diabetes, involving pain in distal extremities, induced by toxic effects of hyperglycemia. However, it has been related that high dose of simvastatin (80 mg) can induce peripheral neuropathy, but it seems not be evident at lower doses (2 mg) (Fernandez, G. et al. Cleveland C. J. Medicine. v.78, p. 393, 2011). **Aims**: This study aimed to evaluate the effects of treatment with a low (2 mg) and high dose (80 mg) of simvastatin in a model of streptozotocin-induced diabetic neuropathic pain. **Methods**: Diabetes was induced by a single intraperitoneally streptozotocin injection (50 mg/kg) in female Wistar rats (200 – 220 g). Treatments were initiated 2 weeks after confirmation of hyperglycemia (>250 mg/dL) with vehicle (saline, 1 ml/kg, p.o.) or simvastatin (2 and 80 mg/kg, p.o.), repeatedly during 4 weeks. Cold allodynia, induced by acetone instillation on the right paw, started to be measured on week 2, once a week, until week 6. Nociception induced by mevalonate injection on the right paw (1 µmol/50 µl, i.pl.) was performed on the last day (day 42). Following the last behavior test, animals were euthanized and the left portion of sciatic nerve was collected for measure GSH and ROS levels, as well as for histological analysis. Statistical analysis was performed by one or two way ANOVA followed by pos hoc Newman Keuls test. **Results**: Neither simvastatin treatment 2 nor 80 mg/kg attenuated cold allodynia. However, the higher dose of simvastatin (80 mg/kg) diminished the nociception induced by mevalonate in 86 ± 1% compared to the control group (saline p.o + mevalonate i.pl. = 15 ± 2 flinches). Regarding antioxidant effects, both simvastatin 2 and 80 mg/kg decrease ROS generation in 35 ± 1% and 49 ± 8%, respectively (control: 28 ± 3 fluorescence), without modify GSH levels. Finally, morphological analysis of sciatic nerve revealed no differences between normoglycemic and diabetic rats. **Conclusion**: Together, our findings suggest that the antinociceptive effect of higher dose of simvastatin is probably mevalonate-independent pathway and could be related to its antioxidant effects. However, more studies are necessary to elucidate the mechanism underlying these effects in diabetic rats. **Financial support**: Fundação Araucária **Ethics Committee**: All experimental procedures were approved by the Ethics Committee of Federal University of Parana under n° 661.

05.005 4-HNE levels and TRPA1 expression vary with the severity of temporomandibular joint dysfunction. Klug RJ[1], Mendes SJF[2], Ferro TAF[3], Paiva IC[3], Lamha APSF[4], Almeida LSB[5], Silva MA[1], Monteiro Neto V[6], Muscará MN[7], Calixto JB[8], Grisotto MAG[2], Fernandes ES[1,6] [1]Uniceuma – Odontologia, [2]Uniceuma – Biologia Parasitária, [3]Uniceuma – BIONORTE, [4]Unieuro – Odontologia, [5]UFMA – Odontologia, [6]Uniceuma – Biologia Parasitária, [7]USP – Farmacologia, [8]CIENP

Introduction: The transient receptor potential Ankyrin 1 channel (TRPA1) mediates joint pain in rheumatoid arthritis and osteoarthritis. It is also implicated in orofacial pain and this has been linked to its expression on trigeminal ganglion neurons. Whilst most of the data obtained on this channel are from animal models, little is known of TRPA1 role in human disease. **Aim**: Herein, we investigated the expression levels of TRPA1 on peripheral blood leukocytes as well as the levels of its endogenous agonist 4-HNE in saliva and plasma samples obtained from patients with diagnosed temporomandibular joint (TMJ) dysfunction with different levels of disease severity (n=26), by using commercial enzyme-linked immunosrbent assay kits obtained from Cloud-Clone Corp (TX, USA) and Cell Biolabs (CA, USA); respectively. Samples obtained from healthy subjects were used as controls (n=11). Changes in peripheral blood leukocyte subpopulations were evaluated by flow cytometry on a BD Accuri C6 (BD Biosciences-Immunocytometry Systems) and analyzed using FlowJo software (Tree Star Inc.). **Results**: Increased levels of 4-HNE were detected in saliva samples from patients with moderate/severe TMJ dysfucntion whilst TRPA1 expression levels on peripheral blood leukocytes was augmented in patients with mild TMD ($p<0.05$). These changes were accompanied by increased activation of $CD14^{+}$ circulating cells in mild TMJ dysfunction patients ($p<0.05$) and decrease on the number of circulating T regulatory cells ($CD4^{+}CD25^{+}CD127^{low}$) in patients with moderate/severe TMJ dysfunction ($p<0.05$). **Discussion**: Overall, we show for the first time that TRPA1 expression on peripheral blood leukocytes and the saliva levels of its endogenous agonist 4-HNE vary with the severity of TMJ dysfunction. These changes may reflect on treatment responsiveness at different stages of disease and implicate TRPA1 as a target to treat TMJ dysfunction. **Financial Support**: This work was supported by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Fundação de Amparo à Pesquisa e ao Desenvolvimento Científico e Tecnológico do Maranhão (FAPEMA). S.J.F.M. is an MSc student receiving a grant from FAPEMA. This research was reviewed and approved by the local Research Ethics Comitee (protocol number 264.629); and it was performed in accordance with the Declaration of Helsinki 1975, as revised in 2008.

05.006 Involvement of microglial cells in chemical or sustained isometric contraction-induced muscle hyperalgesia. Melo B, Pelizari M, Oliveira-Fusaro MCG
FCA-Unicamp – Saúde

It is well know the involvement of microglial cells in pain conditions. However, the role microglial cells of spinal cord dorsal horn in muscle pain is still poorly explored. Recently, we developed a model to study the muscle pain induced by sustained isometric contraction in rats. Considering the clinical relevance of muscle pain, the aim of this study was to analyze the involvement of microglial cells of spinal cord dorsal horn in the isometric contraction induced inflammatory muscle pain and to compare it´s efficiency in a classical model of chemically induced inflammatory muscle pain. **Methods**: To investigate the role of microglial cells in muscle pain, minocycline, an inhibitor of the function of microglial cells, was intrathecally administered three days before the hyperalgesic stimuli. The sustained isometric contraction-induced inflammatory muscle pain was performed by an electrical stimulation. To this end, two needle electrodes were inserted into the gastrocnemius muscle and an electrical stimulation (stimulator Grass, S88X) of 1,6V and 19 ms of pulse-width for 1 hour was used. The chemically induced inflammatory muscle pain was performed by administration of carrageenan into the belly of the gastrocnemius muscle of rats. The mechanical muscle hyperalgesia was quantified by the pressure analgesimeter Randal Sellito, applied to the belly of the gastrocnemius muscle. Male Wistar rats from the CEMIB-UNICAMP were used and all experimental procedures were previously approved by the Ethics Committee in Animal Research of the State University of Campinas (3534-1). **Results**: Pre-treatment with minocycline (45 and 100µg/10uL, i.t., 3 days before) prevented the mechanical muscle hyperalgesia induced by carrageenan ($p>0.05$, Tukey, $n=5$). Similarly, the pre-treatment with minocycline (100µg/10uL, i.t., 3 days before) prevented the mechanical muscle hyperalgesia induced by the sustained isometric contraction. Conclusions: These data demonstrated the involvement of microglial cells of spinal cord dorsal horn in mechanical muscle hyperalgesia induced by sustained isometric contraction. Also, it was demonstrated a similar efficiency of minocycline in both inflammatory muscle pain models. Therefore, it is plausible to suggest the microglial cells of spinal cord dorsal horn as a target to control inflammatory muscle pain. Financial Support: FAPESP (2011/11064-4).

05.007 Anti-allodynic effect of nicotinamide in experimental model of rheumatoid arthritis. Dutra MMGB[1,2], Nascimento Jr EB[3], Araújo DP[4], Fátima A[4], Machado RR[2], Coelho MM[2] [1]Centro Universitário Newton Paiva – Farmacologia, [2]UFMG – Farmacologia, [3]UFPI – Farmacologia, [4]UFMG – Química

Introduction: Rheumatoid arthritis (RA) is a painful chronic inflammatory condition that limits the quality of life of patients and affects about 1% of the adult population. The clinical and therapeutic management of the disease still has limitations, in which pain is frequently reported as the most important factor in the disease. Experimental models of RA allow to investigate the pathogenesis of the disease and establish molecular targets for action of potencial new anti-arthritic drugs. Nicotinamide is a vitamin B complex, whose anti-inflammatory and antinociceptive effects were previously described in acute models of pain and inflammation - paw edema and mechanical allodynia induced by carrageenan, formalin and pleurisy. **Aims**: We investigated effects induced by nicotinamide in experimental model of rheumatoid arthritis in rats. The adjuvant induced arthritis (AIA) models are widely used for preclinical testing of new anti-arthritic agents, since they are reproducible and allow studying the inflammatory process involved in the pathogenesis of RA. **Methods**: Female Holtzman rats (140-170 g; food and water ad libitum; 12h light-dark cicle) were provided by Faculdade de Farmácia/UFMG. Arthritis was induced by intradermic administration of 100 uL of Complete Freund Adjuvant (CFA, 1 mg/ml). CFA was injected into the plantar surface and the tail base (0.1 ml in each site, day 1). To enhance systemic effects, an additional injection (100 uL) into the tail base was given the following day (day 2; control = sterile saline). The animals were evaluated during 21 days after the first administration of CFA. The paw edema (by plethysmometer) and mechanical allodynia (by digital analgesymeter) were evaluated every three days or in the 12th day of the experimental protocol. Nicotinamide (75, 125, 250, 500 or 1000 mg/kg, p.o.) was given twice daily or in a single dose in the day 12. In order to investigate possible mechanisms, TNF-α levels in the paw tissue were quantify by ELISA commercial kits (R&D Systems) two weeks after the CFA injections. **Results**: Chronic administration of nicotinamide has anti-allodynic effect in all doses evaluated, markedly after day 6 of treatment, for all the doses evaluated (250, 500, 750 and 100 mg/Kg). This effect occurs when the animals were treated twice daily during all the experimental protocol, and with single dose on day 12, 5 h after oral administration. Nicotinamide has no effect in reduce CFA paw edema. The anty-allodinic activity of nicotinamide could be explained by TNF-α level in the paw tissue, since the rats given nicotinamide 1000 mg/Kg have markedly reduced TNF-α concentration. **Conclusions**: This is the first demonstration of antinociceptive effect of nicotinamide in a chronic experimental model of arthritis. This activity is associated with reduced levels of TNF-α in the arthritic paw. Further mechanisms involved are still under investigation. These results may contribute to raise the interest in evaluating the potential benefits of the nicotinamide, considered the safety profile of this drug, in the treatment of patients with chronic inflammatory disorders. Financial support and acknowledgments: CAPES, CNPq and FAPEMIG. All experiments were approved by the ethical committee of UFMG (CEUA 179/2013).

05.008 Involvement of NO/cGMP/PKG/ATP-sensitive K^+ channels pathway on local antinociceptive effect of dipyrone and its metabolite 4-MAA. Assis DCR[1], Vaz ALL[2], Melo MCC[3], Rae GA[4], Clososki GC[2], Souza GEP[3] [1]FMRP-USP – Farmacologia, [2]FCFRP-USP – Produtos Naturais e Sintéticos, [3]FCFRP-USP – Física e Química , [4]UFSC – Farmacologia

Introduction: and **Aim**: Dipyrone is a pro-drug with potent analgesic and antipyretic effects. After administration, dipyrone is rapidly hydrolysed to 4-methylaminoantipiryne (4-MAA), which is further metabolized to 4-formylaminoantipiryne (4-FAA), 4-aminoantpiryne (4-AA) and 4-acetylaminoantipiryne (4-AAA). Differently from non steroidal anti-inflammatory drugs, the antinociceptive effect of dipyrone is not related only to the inhibition of prostaglandin E_2 (PGE_2) synthesis, but also involves other incompletely understood mechanisms. We working hypothesis is that, like morphine, dipyrone and/or its metabolites induce local antinociception by stimulating the arginine/NO/cGMP/K^+_{ATP} pathway. **Methods**: Male Wistar rats weighing 180 to 200 g were habituated to the test room for at least 1 h prior to experiments. Mechanical hyperalgesia was assessed by electronic von Frey apparatus. Two hours after intraplantar (i.pl.) injection of PGE_2 in rigth hind paws, rats received local injection of dipyrone, 4-MAA (30, 60, 120 µg/paw) or vehicle in a volume of 100µl. As a control of the local effect, rats received injection of PGE_2 in both hind paws but only right paw was treated with dipyrone or 4-MAA (120 µg/paw, both). The hyperalgesic response was evaluated 3h after stimuli injection. The specific PKG inhibitor KT5823 (1.5 µg/paw) or the specific blocker of ATP-sensitive K+ channels glibenclamide (160 µg/paw) were administered 10 min before dipyrone or 4-MAA, respectively. The inhibitor of soluble guanylyl cyclase ODQ (8 µg/paw) and selective neuronal nitric oxide synthase inhibitor, L-NPA (24 µg/paw) were given 30 min before treatment with dipyrone and 4-MAA. Data are expressed as mean ± S.E.M. and were statistically evaluated using ANOVA followed by Tukey test, $p < 0.05$. This study was approved by the Ethic Committee of FMRP/USP (process n° 019/2012). **Results**: Dipyrone (DIP) and it metabolite 4-MAA, showed local antinociceptive effect against PGE_2-induced hyperalgesia and this effect was blocked by L-NPA, ODQ, KT-5823 and Glibenclamide. The intensities of hyperalgesia (Δ withdrawal threshold, in g (g)) were: saline + saline: 0.82 ± 0.13; saline + PGE_2: 6.25 ± 2.05; DIP + PGE_2: (30µg/paw: 6.70 ± 1.68), (60 µg/paw: 5.60± 0.87), (120 µg/paw: 3.85 ± 1.07); 4-MAA + PGE_2: (30 µg/paw: 5.83 ± 0.76); 60 µg/paw: 5.21 ± 1.41; 120 µg/paw: 3.25 ± 1.09); DIP + PGE_2 + L-NPA: (8.24±0.57); DIP + PGE_2 + ODQ: (8.54 ± 0.85); DIP + PGE_2 + KT5823: (9.65±1.53); DIP + PGE_2 + Gli: (8.79±0.64); 4-MAA + PGE_2 + L-NPA: (5.12 ± 1.17); 4-MAA + PGE_2 + ODQ: (7.23±1.02); 4-MAA + PGE_2 + KT5823: (6.23±0.67); 4-MAA + PGE_2 + Glibenclamide: (7.40±1.17); Mor + PGE_2: (2.10±0.74); Mor + PGE_2 + L-NPA: (9.68 ± 1.10; Mor + PGE_2 + ODQ: (10.77±1.04); Mor + PGE_2 + KT5823: (8.36±2.03); Mor + PGE_2 + Gli: (10.64±1.25). **Discussion**: According to the literature, dipyrone showed local antinociceptive effect on PGE_2-induced hypernociception. For the first time it was evidenced that 4-MAA is the metabolite responsible for such effect. Moreover, the mechanisms unrelated to prostaglandin synthesis inhibition seem to rely on NO/cGMP/PKG/KATP pathway. **Financial support**: CNPq.

05.009 The resveratrol peripheral antinociceptive effect is mediate by μ-opioid receptor activation. Oliveira CC, Costa AF, Duarte IDG, Perez AC, Santos SHS, Romero TRL UFMG – Farmacologia e Fisiologia

Introduction: Substances derived from plants play an important role in the development of new analgesic drugs. The resveratrol (3,5,4'-trihidroxistilbene) is a polyphenol, not flavonoid type, found in approximately 72 vegetal species**.** The analgesic properties of this natural substance have been demonstrated**,** although the base of these mechanisms is not completely elucidated yet. **Aims**: Assess the opioid system involvement in the peripheral antinociceptive effect induced by resveratrol. **Methods**: The mouse paw pressure test was used and hyperalgesia was induced by intraplantar injection of carrageenan (200 μg/paw). All drugs were administered by intraplantar injection in Swiss male mice (n=4). **Results**: Resveratrol (100 μg/paw) administered into the right hind paw induced a local antinociceptive effect that was antagonized by naloxone, a non-selective antagonist to opioid receptors. Clocinnamox, a selective antagonist to μ opioid receptors, reverted the resveratrol peripheral antinociception significantly. However, naltrindole and nor-binaltorphimine, selective antagonists to δ and κ receptors, respectively, were not able to revert the peripheral antinociception induced by resveratrol. Furthermore, the bestatine, an inhibitor of the endogenous opioids degrading enzyme, aminopeptidase, has shown to intensify the resveratrol antinociceptive effect, low dose (50 μg/paw)**. Conclusions**: The experimental data suggest that resveratrol is capable to inducing peripheral antinociceptive effect by endogenous opioids peptides release and μ opioid receptor activation. **Financial support**: CNPq, CAPES and FAPEMIG**. Ethics Committee License Number**: 51/2014**.**

05.010 Mechanical muscle hyperalgesia induced by sustained isometric contraction is mediated by P2X3, AMPA E NMDA receptors. Jorge CO, Marques ACS, Melo B, Santos DFS, Azambuja G, Oliveira-Fusaro MCG FCA-UNICAMP – Saúde

Introduction: Musculoskeletal pain is an important health issue in the world. Among the kinds of muscle pain, the one induced by sustained isometric contraction is associated with body movements of the daily life and has a high socio-economic impact. Despite its clinical relevance, the molecular mechanisms involved in the development of muscle pain induced by sustained isometric contraction are poorly understood. Recently, our research group developed a model of muscle pain induced by sustained isometric contraction of gastrocnemius muscle of rats and we demonstrated the involvement of peripheral inflammatory mechanisms. **Aim**: Considering that the biochemical and neuroplastic alterations of central nervous system are crucial to the transition from acute to chronic pain the aim of this study was to evaluate the involvement of P2X3, AMPA and NMDA receptors present in the central nervous system in response to sustained isometric contraction. **Methods**: Male Wistar rats (200 - 250g), from CEMIB-UNICAMP were used and all experimental procedures were previously approved by the Ethics Committee in animal research of the State University of Campinas. The sustained isometric contraction was performed by an electrical stimulation. To this end, two needle electrodes were inserted into the gastrocnemius muscle and an electrical stimulation (stimulator Grass, S88X) of 1,6V and 19 ms of pulse-width for 1 hour was used. The mechanical muscle hyperalgesia was quantified by the pressure analgesimeter (Randal Sellito) applied to the muscle belly. To investigate whether the mechanical muscle hyperalgesia was mediated by P2X3, AMPA or NMDA receptors expressed on spinal cord dorsal horn, the selective antagonists A317491, AP5 and CNQX, respectively, was administered via intrathecal 5 minutes before sustained isometric contraction. **Results**: A317491 (180µg/10µL), AP5 (20 and 60µg/10µL) or CNQX (1µg/10µL) significantly reduced ($p<0,05$, Tukey test) the mechanical muscle hyperalgesia induced by sustained isometric contraction. **Conclusion**: This study demonstrated that the mechanical muscle hyperalgesia induced by sustained isometric contraction is modulated by P2X3, AMPA and NMDA receptors expressed on spinal cord dorsal horn. It also suggests these receptors as target to control inflammatory muscle pain. **Financial support**: CNPq, 473790-2013-0. CEUA: 3083-1.

05.011 Antinociceptive effect of decoction extract *H. crenata* Pohl and possible mechanism involved. Donald GR[1], Giorno TBS[1], Carvalho PR[1], Fernandes PD[1] – [1]LaFDI-UFRJ – Farmacologia da Dor e da Inflamação

Introduction: *Hyptis crenata* Pohl (HC) is a medicinal plant mainly used in the Northeast and Central part of Brazil. It has been particularly used as a treatment for pain symptoms such as stomach-aches and headaches. Research testing HC decoction extract, which is the main method of HC preparation in folk medicine, has shown antinociceptive properties in animal models of pain (acetic acid and Hargreaves tests). However, there is still no clarification regarding the possible mechanism behind this effect. **Aim**: The aim of this study was to investigate the antinociceptive effect of *H.crenata* decoction extract through acute pain models of the hot plate and glutamate tests, in addition to carrying out assessments of the possible mechanism involved. **Methods**: The glutamate nociception is mediated by N-methyl-D-aspartate (NMDA) and non-NMDA receptors and by the release of nitric oxide. The test consists of injecting the hind paw with 20 µl of glutamate (20 µmol) and counting the amount of time the animal spent licking its paw. The hot plate test (HP) mainly involves central antinociception where this effect is measured through behavioural components (jumping, withdrawing or licking the hind paw) on a plate heated at a constant temperature (55 Celsius). The measurements were taken at different time points after the treatments (30 min, 60 min, 90 min, 120 min, 150 min and 180 min). Swiss mice were used for this study. Receptor antagonists naloxone, atropine, yombine and ondansentron were used to investigate the possibility of the antinociceptive effect involving opioid, muscarinic, α-adrenergic and serotoninergic (5-HT) actions. The animal protocol was approved by DFBCICB015-04/16. The HC decoction extract used in this study had its compound identified through liquid chromatography–mass spectrometry (LC-MS). Statistical analyses were performed by ANOVA with Dunnett's post-test (* $p < 0.05$). **Results**: The HC extract at doses of 30 mg and 100 mg/kg were able to reduce the glutamate nociception by 45 and 39% respectively. An antinociceptive effect was also observed through the HP for 10, 30 and 100 mg/kg doses tested when compared to vehicle (percentage increase compared to baseline of 85 ±24SD, 87 ±10SD, 75±43 SD for the respective HC doses) and all of them presented no difference when compared to morphine. The 30 mg/kg dose was the treatment chosen to carry out the test involving the antagonist, because this dose presented a more constant effect during the different times measured after the treatment on the HP. According to the results the antinociceptive effect observed on the hot plate involves intervention on opioid, muscarinic and α-adrenergic receptors, since their antagonists were able to reduce the effect of HC extract. However, 5-HT antagonist was unable to reduce that effect. The LC-MS analysis showed 91 compounds present in the HC extract. **Conclusion**: The results suggest that 5-HT receptors do not play a role for HC antinociception but the other receptors (opioid, muscarinic and α-adrenergic) seem to be part of the key inducing this effect. In addition, HC extract has action on the glutamatergic pathway. **Keywords**: Pain, medicinal plant, opioid receptor, hot plate **Financial support**: CAPES, CNPq, FAPERJ

05.012 Muscle pain induced by chemical stimulus or sustained isometric contraction is modulated by PPAR-γ receptors in Wistar rats. Santos DFS, Oliveira-Fusaro MCG Unicamp

Introduction: It is well know that, during an inflammatory process, the organism has anti-inflammatory mechanisms to modulate it. It has been described that 15d-PGJ_2, a derivative of aracdonic acid metabolism, via activation of PPAR-γ receptors has potent anti-inflammatory and anti-hyperalgesic effects. However, it is unknown whether activation of PPAR-γ receptors expressed in peripheral tissue modulates muscle inflammatory pain. **Aim**: to evaluate whether activation of PPAR-γ receptors of muscle tissue by 15d-PGJ_2 modulates muscle inflammatory pain performed by two different models. **Methods**: To investigate the role of PPAR-γ receptors in muscle pain, 15d-PGJ_2, an endogenous agonist of PPAR-γ receptors, was administered into the gastrocnemius muscle 30 minutes before the hyperalgesic stimuli. To confirm the involvement of PPAR-γ receptors, the GW9662, a selective PPAR-γ receptors antagonist, was used. The sustained isometric contraction was performed by an electrical stimulation. To this end, two needle electrodes were inserted into the gastrocnemius muscle and an electrical stimulation (stimulator Grass, S88X) of 1,6V and 19 ms of pulse-width for 1 hour was used. The chemically induced inflammatory muscle pain was performed by administration of carrageenan into the belly of the gastrocnemius muscle of rats. The mechanical muscle hyperalgesia was quantified by the pressure analgesimeter Randal Sellito, applied to the belly of the gastrocnemius muscle. Male Wistar rats from the CEMIB-UNICAMP were used and all experimental procedures were previously approved by the Ethics Committee in Animal Research of the State University of Campinas (n° 3919-10). **Results**: Pre-treatment with 15d-PGJ_2 (10 and 100ng/muscle, 30 minutes) prevented the mechanical muscle hyperalgesia induced by carrageenan or sustained isometric contraction when administered in the ipsilateral ($p<0,05$, Tukey test, $n=5$) but not in the contralateral gastrocnemius muscle. Pre-treatment with GW9662 (9ng/muscle, 30 minutes) reversed the muscle hypoalgesia induced by 15d-PGJ_2 in both models ($p<0,05$, Tukey test). **Conclusion**: These data demonstrated that the activation of PPAR-γ receptors reduce the mechanical muscle hyperalgesia in two different models of inflammatory pain. This study suggests the PPAR-γ receptors of muscle tissue as important pharmacological targets to control muscle hyperalgesia. **Financial support**: CNPq, 473790-2013-0.

05.013 Effect of the selective TRPV4 channel antagonist on the scratching behavior in mice. Matias DO[1], Alves VS[1], Fabiana DC[1], Miranda ALP[2], Costa R[2]
[1]UFRJ – Acadêmico, [2]UFRJ – Ciências Farmacêuticas

Introduction: Chronic itching is a debilitating condition commonly associated with skin diseases such as dermatitis, psoriasis e dry skin. However, little is known about the cellular and molecular mechanisms that mediate itch. Due to the poor efficacy of available drugs, emerged the necessity of finding new molecular targets for development of more efficacious anti-pruritic drugs. Among the molecules recently associated with itch transmission, we can highlight the transient receptor potential (TRP) channels family. TRP vanilloid-1 (TRPV1) and ankyrin-1 (TRPA1) members are classic transducers of noxious stimuli, such as noxious heat or cold; however, on the past few years, these channels have also been implicated in acute and chronic itching transduction. Nonetheless, the involvement of TRPV4 channel in itch has not been explored and, considering its involvement in sensorial transduction, it is possible that this member modulates itch signalling. **Aim**: To investigate the participation of TRPV4 channel on the scratching behaviour in mice by the use of its selective antagonist, HC067047. **Methodology**: HC067047 effect was evaluated on two modes of acute itching in mice: compound 48/80 (C48/80)- and chloroquine (CQ)-induced scratching behaviour. Female Swiss mice (8 weeks old) were treated by intraperitoneal (i.p.) injection with HC067047 (10 mg/kg) 30 min. before pruriginous agent. Control animals were treated with vehicle (8% DMSO and 2% Tween 80 in saline). After treatments, mice received an intradermal (i.d.) injection (on the back of the neck) of C48/80 (10 □g/site) or CQ (100 □g/site) in 50 □l of saline. The number of scratches bouts to the injected site was quantified for 30 min. Experimental procedures were approved by the local ethics committee of the UFRJ (protocol number: 054/14). **Results**: I.d. administration of C48/80 or CQ elicited significant scratching behaviour in mice when compared to the injection of saline ($p<0.05$). Importantly, pre-treatment with the selective TRPV4 antagonist HC067047 significantly reduced both C48/80- and CQ-induced scratching behaviour by 33% ($p<0.05$) and 57% ($p<0.05$), respectively. The pre-treatment with vehicle solution did not interfere on this response. **Conclusion**: These initial results suggest that TRPV4 channel activation mediates the scratching behaviour in mice. Additional studies are in course to clarify the mechanisms involved in this process. **Keywords**: Pruritus, chronic itching, TRPV4. **Financial support**: FAPERJ, CNPq and CAPES.

05.014 Pharmacological characterization of fish oil concentrate treatment on experimental model of neuropathic pain. Silva RV, Lima CKF, Lobo BW, Miranda ALP UFRJ – Medicamentos

Introduction: Neuropathic pain (NP) is a multifactorial condition arising from injury or malfunction of peripheral or central nervous system. The pathophysiology is characterized by strong neuro-immune interaction. Omega 3 polyunsaturated fatty acids such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are well known for their immunomodulatory activity displayed by their endogenous conversion to resolvins and protectins as lipid mediators (Serhan, *J Exp Med*, p1025, 2002). **Aim**: To evaluate therapeutic efficacy of fish oil concentrate (FOC), rich in EPA and DHA, for the treatment of partial sciatic nerve ligation- induced (PSNL) NP in mice. **Methodology**: NP was induced by PSNL on the left paw (Seltzer, *Pain*, p245, 1990). Two treatment protocols were employed: daily oral treatment initiated at 5th day after surgery (therapeutic protocol) and daily oral treatment initiated prior to surgery (preventive protocol). Animals were treated with vehicle, FOC (4.6 g/kg and 2.3 g/kg) or Gabapentin (100 mg/kg). Thermal hypernociception and mechanical allodynia were assed up to 24 hours following first administration on 5^{th} day and also at 7th and 9th days after surgery. **Results**: The highest dose of FOC increased mechanical and thermal withdrawal threshold in animals receiving daily oral administration in a therapeutic protocol initiated on 5^{th} day after NP induction. In the first 24 h after FOC administration it was observed a decrease in mechanical allodynia, but not in thermal hypernociception. Additionally, a statistically significant reduction in mechanical allodynia was observed at 7^{th} and 9^{th} days post-surgery whereas thermal hypernociception was totally reversed at 9^{th} day. In a preventive treatment protocol, beginning at the day of the surgery, the highest dose of FOC prevented mechanical allodynia behavior induced by PSNL. **Conclusion**: Our results indicate that FOC oral treatment reverses mechanical and thermal hypersensitivity after peripheral nerve injury in mice in preventive and therapeutic treatment protocol. Therefore, it might arise as an alternative treatment for neuropathic pain. **Financial Support**: CNPq, CAPES. Ethics committee protocol number FARMACIA04

05.015 Investigation of antinociceptive and anti-inflammatory potential of naringenin in mice. Dallazen JL, Silva CF[1], Baggio CH, Werner MF UFPR – Farmacologia

Introduction: Naringenin is a flavonoid abundant in grapefruit and other citrus fruits, that presents several pharmacological effects, as antitumor, antimutagenic, antioxidant, anti-inflammatory, analgesic among others. In the present study, we investigated some of the mechanisms of action underlying the antinociceptive effects of naringenin. **Methods**: Experiments were performed in female Swiss mice (~30 g) (CEUA/BIO-UFPR; approval number 705). Naringenin (1-100 mg/kg, i.p.) was administered to mice 30 min before the following the overt pain induce by acetic acid (writhing test, 0.6%, 450 µl i.p.) or intraplantar injection (20 µL) of formalin (2,5%), glutamate (20 µmol), capsaicin (5,2 nmol), cinnamaldehyde (10 nmol), prostaglandin E_2 (30 nmol) and bradykinin (3 nmol). Paw edema (micrometer) and mechanical allodynia (0.6 g, von Frey hair) was studied through carrageenan model (300 µg/20 µL). Finally, mice were submitted to open-field and rotarod tests. Statistical significance was determined by one-way ANOVA followed by pos hoc Newman Keul's test and by two-way ANOVA followed by Bonferroni's post hoc test for the difference between groups in mechanical allodynia ($P < 0.05$). **Results**: Acetic acid-induced abdominal writhing was reduced in 36, 45, 56 and 91%, by naringenin 1, 3, 10 and 30 mg/kg, respectively (C: 35 ± 3). Naringenin attenuated only the second phase of formalin- and also the glutamate-induced nociceptive response behaviors (DE_{50}= 47.8 mg/kg, DE_{50}= 66.0 mg/kg, respectively), but did not reduce the nociception induced by TRP agonists (capsaicin and cinnamaldehyde). Nociceptive response produced by prostaglandin E_2 was reduced in 98% (C: 92 ± 10 s) at 100 mg/kg and that of bradykinin was reduced in 49 and 79% (C: 52 ± 5 s) at 10 and 30 mg/kg, respectively. Carrageenan-induced paw edema was not modified by naringenin, but mechanical allodynia was reduced by naringenin 10, 30 and 100 mg/kg until the 2^{nd} h (At 2 h, 30 mg/kg: 42% reduction compared to control 78.3 ± 3.1%). Interestingly, at higher dose (100 mg/kg) naringenin reduced crossings in the open-field apparatus in 59% (C: 168 ± 10), but the performance of mice in the rotarod apparatus was not modified by naringenin. **Conclusion**: Our findings demonstrated that intraperitoneal treatment with naringenin exerts an antinociceptive effect in several chemical and inflammatory models of nociception, probably through inhibition of inflammatory mediator's release. However, more studies are warranted to better clarify the mechanism of action of naringenin, since at higher dose it appears to decreased locomotor activity of animals. **Financial support**: (PIBIC/CNPq 2012011550)

05.016 Gedunin induces anti-nociceptive effect in Swiss mice. Chaves AS, Brito TM, Rodrigues SA, Amendoeira FC, Ferraris FK Fiocruz – Farmacologia e Toxicologia

Introduction: Carapa guianensis Aublet (Meliaceae), known as Andiroba is a tree that grows up to 30 meters tall occurring in tropical rainforest of Americas' and found in Amazonas State of Brazil. Gedunin is a limonoid also known as tetranortriterpenoid (TNTP) isolated from the seeds of the Meliaceae family exhibiting potential anticancer activity, antimalarial, insecticidal, anti-allergic effects, inhibit neutrophil migration and stress-induced chaperone protein Hsp90, and down regulates the anti-apoptotic proteins Hsp70 and Hsp27. **Aim**: The aim of this study is to identify whether gedunin promotes anti-nociception effect in vivo models. **Methods**: Male Swiss mice (20 – 25g) obtained from the Oswaldo Cruz Foundation breeding colony were used in this study and caged with free access to food and water and kept at 22°C (± 2) and 12h light/dark cycle. All experimental procedures were performed according to The Committee on Ethical Use of Laboratory Animals of Oswaldo Cruz Foundation (FIOCRUZ; license P17/13-5). The writhing test was carried out according to the method previously used by Carvalho and collaborators (2013). The animals were selected randomly per dose and divided into six groups each. They received an intraperitoneal (i.p.) injection of saline (vehicle), diclofenac, gedunin (0.05, 0.5, 5 and 50 mg/kg) diluted in sterile phosphate buffered saline (PBS) with 20µl 1 hour before stimulation with acetic acid 0.8%. Five minutes after the i.p. injection of acetic acid, the number of writhes exhibited by each mice was counted in 10 min. The results were reported as the mean ± S.E.M. and statistically analyzed by Student's t-test and by the one-way analysis of variance (ANOVA) followed by the Turkey's multiple comparisons test. Values of $p \leq 0.05$ were regarded as significant. **Results**: The pre-treatment with gedunin (from 0.5 to 50 mg/kg i.p.) was able to induce analgesia in mice treated one hour before acetic acid 0.8% injection (0.5 mg/kg = 17.29 ± 1.136; 5 mg/kg = 20.00 ± 1.271; 50 mg/kg = 13.58 ± 1.357, respectively), compared to saline group (24.92 ± 1.174). Among them, the lower dose of gedunin (0.05 mg/kg) was not able to inhibit the number of times each animal writhes (25.15 ± 1.181). **Conclusions**: Thus, our result shows that gedunin, a natural tetranortriterpenoid, in different doses from 0.5 to 50 mg/kg induces anti-nociceptive activity supporting other findings of its pharmacological use. Moreover, the analgesic ability of gedunin might be explained by the induction of anti-inflammatory pathways. CARVALHO et al. Marine Drugs. v.11, p. 1221 - 1234, 2013. We would like to thank Ministério da Saúde (MS), CAPES, CNPq and FIOCRUZ for the financial support.

05.017 Nitroxyl reduces chronic constriction injury-induced neuropathic pain in mice. Longhi Balbinot DT[1], Rossaneis AC[1], Pinho-Ribeiro FA[1], Bertozzi MM[1], Casagrande R[2], Katrina MM[3], Verri Jr WA[1] – [1]UEL – Ciências Patológicas, [2]UEL – Ciências Farmacêuticas, [3]University of Arizona – Química

Introduction: Chronic pain is a major health problem worldwide. We have recently demonstrated the analgesic effect of the nitroxyl donor, Angeli's salt (AS) in models of inflammatory pain. **Aim**: In the present study, the experimental model of chronic constriction of the sciatic nerve (CCI) in mice was used to evaluate the acute and chronic analgesic effects of AS in neuropathic pain. **Methods**: Male Swiss mice were used (20-25g; n=6) and the experiments were approved by the Institutional Ethics Committee under the protocol 26292.2012.91. Seven days after CCI surgery mice were treated subcutaneously with AS (s.c.; 0.3-3mg/kg) and the mechanical and thermal hyperalgesia were evaluated (0, 1, 3, 5, 7 and 24 h after AS treatment). Furthermore, chronic (7-14th after CCI) antinociceptive effect of AS was evaluated. In addition, pharmacological treatments targeting guanylate cyclase (ODQ; 0.3mg/kg, i.p.), PKG (KT5923; 0.5µg/mouse, i.p.) and ATP-sensitive potassium channels (glibenclamide; 0.3mg/kg, i.p.) were used. At 5 h after the acute or chronic treatment with AS (3mg/kg, s.c.) treatment, spinal cord samples of mice were collected to verify the mRNA expression, by qPCR, of GFAP, Iba-1, pro-IL-1β, TNF-α, IL-33 and ST2, Nav 1.8 and TRPV1. **Results**: AS treatment (1 and 3mg/kg; s.c.) reduced CCI-induced mechanical, but not thermal hyperalgesia. The antinociceptive effect of AS was maximal at 5 h after treatment (CCI: 5.73□0.25; AS 1: 3.81□0.2; AS 3: 3.05□0.3). Chronic (7-14th after CCI) treatment with AS (3mg/kg, s.c.) promoted a sustained reduction of CCI-induced mechanical (CCI: 6.59□0.22; AS: 2.7□0.23) and thermal (CCI: 7□0.71; AS 3: 13.5□0.68) hyperalgesia. The antinociceptive effect of AS was prevented by ODQ, KT5823 and Glib (5 h: CCI: 6.4□0.32; AS 3: 3.03□0.49; AS+ODQ: 5.16□0.44; AS+KT: 5.04□0.69; AS+Glib: 5.01□0.61). Furthermore, AS chronic treatment significantly reduced the rises in GFAP (CCI: 2.41□0.17; AS: 0.96□0.03), TNF-α (CCI: 2.06□0.13; AS: 1.36□0.07), IL-33 (CCI: 3.62□0.28; AS: 1.81□0.16), Nav 1.8 (CCI: 2.47□0.16; AS: 1.26□0.09) and TRPV1 (CCI: 3.17□0.32; AS: 1.43□0.16) mRNA expression induced by CCI. On the other hand, either AS acute (A) or chronic treatment (C) reduced the pro-IL-1β (CCI: 2.08□0.08; A: 1.56□0.19; C: 1.08□0.10), Iba-1(CCI: 2.83□0.38; A: 1.56□0.19; C: 1.08□0.10) and ST2 (CCI: 1.81□0.16; A: 1.2□0.17; C: 0.81□0.09) mRNA expression induced by CCI. **Conclusions**: Together, these results suggest that AS diminishes CCI-induced mechanical and thermal hyperalgesia by reducing the activation of microglia and astrocytes, decreased TNF-α, IL-1β and IL-33 cytokines as well as reduces TRPV1 and Nav 1.8 channel expression. Hence, the analgesic effect of AS depends on activating the cGMP/PKG/K^+ATP signaling pathway. Thus, donating nitroxyl is a conceivable approach to limit neuropathic pain states that merits further investigation. **Financial Support**: CAPES, CNPq, MCTI/SETI/Fundação Araucária and Parana State Government.

05.018 Antihyperalgesic synergistic effect of diclofenac associated with terpinolene in inflammatory pain in rats Macedo EMA[1], Santos WC[1], Piauilino CA[1], Reis Filho AC[1], Sousa DP[2], Oliveira FA[1], Almeida FRC[1] [1]NPPM-UFPI, [2]UFPB – Ciências Farmacêuticas

Introduction: Pharmacological treatment of inflammatory pain is usually done with non-steroidal anti-inflammatory agents (NSAIDs) to produce good efficacy, but cause side effects, especially gastrointestinal lesions. Then pharmacological alternatives may be used, such as the combination of NSAIDs with natural products to induce synergistic analgesic effect with reduced side effects. The monoterpene terpinolene (TPL) is a chemical constituent of the essential oil of many plant species that exhibit various pharmacological activities, among them, analgesic and anti-inflammatory. The aim of this study was to investigate the anti-hyperalgesic effect of diclofenac associated with TPL in inflammatory pain models. **Methodology**: Female Wistar rats (170-230 g/n = 6-9) (Animal Ethics Committee/UFPI, no. 82/2014) received 50 uL of Complete Freund's Adjuvant (CFA) in the intraplantar region. After 24 hours, the rats were treated orally with TPL (3.125; 6.25; 12.5 and 25 mg/kg); sodium diclofenac (1.25; 2.5 and 5 mg/kg); combination of diclofenac (1.25 mg/kg) with TPL (3.125 mg/kg) or vehicle (2% Tween 80 in saline 0.9%), followed by mechanical compression test (Randall Selitto) at the times (0, 1, 2, 3, 4, 5 and 6 hours), and during the subchronic phase (10 days), the rats were treated and assessed their pain threshold daily. To investigate the involvement of serotonergic receptors in the association anti-hyperalgesic effect, 24 h after CFA injection the animals were pretreated (30 min before) with ketanserin (3 mg/kg, sc, 5- HT2A antagonist) or vehicle, and then the association and the isolated substances were administered. The animals were submitted to Randall Selitto test every hour after this administration until the sixth hour. Statistical analyzes were performed using ANOVA (two way) followed by Bonferroni Test, $p<0.05$. **Results**: Animals that received the lowest doses of diclofenac and TPL did not alter the mechanical threshold; but the 2.5 and 5 mg/kg diclofenac groups increased threshold (44.44±2.31 and 48.04±2.83g, respectively) in the third hour of the acute phase, when compared to control (22.44±1.02g). TPL (6.25; 12.5 and 25 mg/kg) increased the threshold (42.50±1.32g, 4th hour); 48.33±1.86g (3rd hour) and 46.79±2.42g (3rd hour), respectively. The combination of ineffective doses of diclofenac and TPL showed anti-hyperalgesic effect (42.86±1.84g in the 3rd hour) similar to the positive control, diclofenac 5 mg/kg (48.04±2.83g), and this effect was kept in sub-chronic phase. The pre-treatment with ketanserin completely reversed the anti-hyperalgesic effect of TPL. This reversal was not identified to diclofenac or association. The ketanserin not completely reversed the effect of association, but reduced the pain threshold **Conclusion**: Diclofenac associated with TPL showed synergistic anti-hyperalgesic effect in acute and subchronic inflammatory nociception and one of the possible mechanisms of action involves 5-HT2A serotonergic receptor. **Financial support**: UFPI/CAPES

05.019 Role of Transient Receptor Potential Vanilloid-4 (TRPV4) channel in diabetic peripheral neuropathy in mice. Dias FC, Alves VA, Matias DO, Silva RV, Santos BLR, Lima CKF, Miranda ALP, Costa R UFRJ – Biotecnologia Farmacêutica

Introduction: Painful diabetic neuropathy is a chronic pain condition resultant from metabolic damage to primary afferent neurons caused by hyperglycemia. Chronic pain can be debilitating and, despite many efforts, there are so far no available pharmacotherapies for its satisfactory relief. Transient receptors potential (TRP) family is formed by cation channels permeable mainly to calcium, and some of its members are involved in pain transmission. TRP vanilloid 4 (TRPV4) is expressed in almost all tissues and can be activated by a variety of stimuli, including osmotic changes, temperature and inflammatory mediators. TRPV4 channels mightplay an important role in pathological pain states caused by osmotic changes, such as in asthma, alcoholism and diabetes. **Aim**: To assess the involvement of TRPV4 channel in painful diabetic neuropathy using an experimental model of diabetes in mice. **Methods**: Male Swiss mice (8 weeks) were subjected to induction of type 1 diabetes model by intraperitoneal (i.p.) injection of streptozotocin (STZ). Painful diabetic neuropathy was assessed by plantar sensitivity of animals to thermal cold (acetone solution) and mechanical (von Frey filaments) stimuli. It was also evaluated the sensitivity of animals to the selective TRPV4 agonist GSK1016790A (GSK 0.3 to 3 nmol/paw), which was administered by intraplantar (i.pl.) route, by assessing overt nociception during 10 min. To assess the involvement of TRPV4 in the maintenance of mechanical allodynia, diabetic animals were treated by subcutaneous (s.c.) injection with the selective TRPV4 antagonist HC-067047 (10 mg/kg) 6 weeks after diabetes induction. **Results**: From one week after STZ injection, mice showed increased glycemia and less weight gain in comparison to control animals. Painful diabetic neuropathy has appeared from the 4^{th} week after induction of diabetes and it was characterized by cold and mechanical allodynia. I.pl. injection of GSK (0.3, 1 and 3 nmol/paw) in healthy animals caused dose-dependent overt nociception. The dose of 0.3 nmol/paw of GSK was tested in diabetic mice every 2 weeks for 12 weeks. Interestingly, diabetic animals responded to GSK similarly to control animals ($p>0.05$). However, treatment with HC-067047reversed mechanical allodynia in diabetic animals when compared to vehicle-treated diabetic animals ($p<0.05$). Conclusions: The present results suggest that the activity of TRPV4 channel expressed in plantar tissue is not altered in diabetic animals. However, TRPV4 channels seem to play an important role in diabetic mechanical allodynia. Additional studies are in progress to elucidate the involvement of TRPV4 in painful diabetic neuropathy. **Financial support**: FAPERJ, CNPq and CAPES. Ethics committee of the UFRJ protocol number: 054/14

05.020 Anti-inflammatory and anti-nociceptive effects of GYY-4137, a slow-releasing hydrogen sulfide (H_2S) donor, on temporomandibular joint synovitis induced by carrageenan in rats. de Lira FBC[1], de Paula MAV[1], Teixeira SA[1], Wood M[2], Whiteman M[2], Costa SKP[1], Muscará MN[1] [1]USP – Farmacologia, [2]University of Exeter Medical School

Introduction: Temporomandibular joint (TMJ) disorders are usually associated with inflammation and pain. We have previously demonstrated that hydrogen sulfide (H_2S) exerts beneficial effects on nociception and inflammation secondary to carrageenan (CGN)-induced knee joint synovitis in rats. GYY-4137 is a slow H_2S-releasing compound that has shown promising results as anti-inflammatory agent, although the mechanisms involved have not been yet completely defined. We thus decided to investigate the effects of GYY-4137 on the nociception and inflammation induced by CGN when injected into the TMJ of rats, and to pharmacologically characterize the mechanisms involved. **Methods**: The protocol was approved by the local Ethics Committee for Animal Experimentation (CEUA-ICB 46, book 2/85, 2010). Under anesthesia with inhalatory isofluorane (3% in O_2), male Wistar rats (7 wk. old) received an intra-articular (i.art.) injection of 500 µg of CGN. Four hours later, mechanical allodynia was evaluated by measuring the force threshold necessary for head withdrawal with the aid of an electronic analgesimeter based on the Von Frey filaments principle. Myeloperoxidase (MPO) activity was measured in the TMJ capsule tissue as a marker of neutrophil infiltration. GYY-4137 (1,25-20 µg/joint), glibenclamide (a KATP channel blocker, at 10 and 30 µg/joint) and ODQ (1H-(1,2,4) oxadiazolo [4,3-a] quinoxalin-1-one, a specific soluble guanylate cyclase inhibitor, at 0,8 and 8 µg/joint) were co-injected with CGN. The results were analysed by unpaired Student t-test or ANOVA followed by the Dunnett's test, when applicable. **Results**: In comparison with saline (control group), the intra-articular injection of CGN into the rat TMJ evoked mechanical allodynia, as evidenced by the significant decrease in the force threshold (-31.6 ± 3.5 vs. 0.3 ± 4.0 g; $P<0.001$) and increased MPO activity (26.8 ± 5.2 vs. 1.3 ± 0.3 U/joint; $P<0.001$). GYY-4137 significantly reduced CGN-induced mechanical allodynia in a dose-dependent manner (between 2.5 and 20 µg/joint, $P<0.01$) as well as MPO activity (12.9 ± 4.5 vs. 26.8 ± 5.2 U/joint, $P<0.05$) at the 2.5 µg/joint dose. Glibenclamide (30 µg/joint) prevented the antinociceptive effect of GYY-4137 (22.3 ± 2.9 vs. 8.8 ± 0.8 g; $P<0.05$) while ODQ did not alter these effects. **Conclusions**: These data provide evidence on the anti-inflammatory and antinociceptive effects of GYY-4137 on the CGN-induced TMJ synovitis in rats. Pathways involving KATP channels but not cGMP seem to be involved in the antinociceptive effects of GYY-4137. **Financial Support**: FAPESP (grant #2014/24518-1), CNPq and CAPES.

05.021 Evaluation of antinociceptive activity of methanolic fractions of sugarcane juice (*Saccharum officinarum* L.). Soares MA, Silva NLC, Gomes AC, Simas NK, Kuster RM, Miranda ALP, Tributino JLM – UFRJ

Introduction: Different sugarcane (Saccharum officinarum L.) segments are popularly used for the treatment of pathological conditions such as anemia, infections, and hypertension. Some components identified in the sugarcane stalk juice are flavonoids derived from apigenin, luteolin and tricine, for which there are important biological actions described, such as anti-inflammatory activity. Furthermore, some flavonoids are described as ligand of opioid receptors, key pharmacological targets for analgesia. The methanol fraction of natural (SJ) and fermented (FSJ) sugarcane juice were obtained from reverse chromatography of the sugarcane stalks juice, variety SP71-1406, which has high concentration of flavonoids, constituting an interesting object of study for evaluating analgesic activity. **Aims**: Considering the actions described for isolated flavonoids and extracts of plants rich in flavonoids, the ease of obtaining the sugar cane juice as a source of flavonoids and the need to develop new approaches for the treatment of painful disorders, this study aims at evaluating the analgesic potential of SJ and FSJ using animal models of nociception and inflammation. **Methods**: SJ and FSJ 100 mg/kg, orally administered, were evaluated in models of writhing induced by acetic acid 0.1 N (CEUA FARMACIA03), nociception induced by formalin 2.5% (Ipl.) (CEUA FARMACIA02), paw edema induced by carrageenan 1% (Ipl.) and modified hot plate assay for evaluation of thermal hyperalgesia (CEUA FARMACIA01). **Results**: SJ shows a 41% reduction in the number of writhes compared to the control group, while FSJ shows 31% reduction, which points to an analgesic effect of these substances. In the formalin test, SJ and FSJ 100 mg/kg (po) reduce the licking time only in neurogenic phase, with inhibition of 44.2% and 56.7%, respectively. This effect was reversed by naloxone, which shows that their analgesic effect is related to modulation of the opioid system. SJ and FSJ not significantly reduced either paw edema or the carrageenan-induced thermal hypersensitivity. **Conclusion**: These results indicate analgesic effect for SJ and FSJ, which is related to modulation of the opioid system without significant activity on inflammatory components. This work contributes to the ethnopharmacological study of Saccharum officinarum, potential source of flavonoids which can be developed for the treatment of pain. We consider prospects of work toconfirm the results so far using other experimental models. **Financial support**: FAPERJ, CAPES, PIBIC / UFRJ

05.022 Antinociceptive activity of bergenin in a mice model of neuropathic diabetic pain. Santos DS[1], Gama KB[2], Nascimento OA[1], Alves CQ[3], David JPL[4], David JM[4], Soares MBP[2], Villarreal CF[1] [1]UFBA – Farmacologia e Terapêutica Experimental, [2]CPqGM-Fiocruz-BA, [3]UFBA – Química, [4]UFBA

Introduction: Diabetic neuropathy is a major complication of diabetes mellitus (Partanen, J. N Eng J Med, v. 333, p. 89, 1995). It is associated with decreased work productivity and quality of life of patients, and is often unresponsive to conventional pharmacological treatments. Berginin is a C-glucoside of 4-O-methyl gallic acid that occurs naturally in several plant genera, with antidiabetic (Kumar, R. Fitoterapia, v. 83, p. 395, 2012) and antinociceptive properties in inflammatory pain models (Oliveira, C. M. J. Nat. Prod, v. 74, p. 2062, 2011). Therefore, the hypothesis of the antinociceptive effect of bergenin for neuropathic pain of diabetic origin was investigated in the present study. **Aim**: Evaluate the antinociceptive activity of bergenin from Cenostigma gardnerianum Tul. in a mice model of diabetic neuropathy induced by streptozotocin (STZ). **Methods**: Male C57BL/6 mice (20-23g) were used. Four weeks after the induction of the model by daily administering of STZ (80 mg/kg, ip; 3 days) the animals received the test treatment by intraperitoneal route. Mice were considered diabetic if glycemia were above 250 mg/dl. Bergenin (3,125 a 50 mg/Kg) was administered only once or according to a repeated dosing schedule (for 7 or 14 days). The treatment with gabapentin (30 mg/kg, orally every 12 hours for six consecutive days) was used as gold standard. The paw mechanical nociceptive threshold was evaluated by von Frey filaments test. Nociceptive threshold, glycemia and body weight were assessed throughout the whole experimental period. All data were analyzed by one-way ANOVA followed by Tukey test. **Results**: Thirty days after STZ treatment, the nociceptive threshold was significantly lower for diabetic groups relative to the control group, indicating the development of diabetic neuropathy. A single treatment with bergenin (3.125 to 50 mg / kg) induced significant and dose- related increase of the mechanical nociceptive threshold when compared to the control group. This effect was statistically significant until 8 hours after bergenin. According to the behavioral data, daily treatment with bergenin (25 mg/kg) for 7 and 14 consecutive days induced antinociceptive effect, leading nociceptive thresholds of neuropathic animals to levels similar to those of non-neuropathic animals. The antinociception effect of bergenin was not diminished by the onset of tolerance, after repeated administrations. Additionally, at 25 mg/kg/12h over 14 days the administration of bergenin produced a continuous increase of the nociceptive threshold throughout the treatment period and even 2 days after discontinuation. Throughout the experimental period, there were no significant changes in body weight and blood glucose levels between groups. **Conclusion**: Bergenin induced a long lasting and dose-related antinociceptive effect in an experimental model of diabetic neuropathy, suggesting their therapeutic potential to the neuropathic pain control. **Support**: CNPq, FAPESB. Animal Research Ethical Committee: L-IGM-025/09

05.023 Inhibition of gastrin-releasing peptide receptor by PD176252 markedly prevents the chronic pruritus in a mouse model of atopic dermatitis. Canevese FF, Machado GDB, Pereira PSJ, Campos MM PUCRS – Farmacologia e Toxicologia

Introduction: It has been described that gastrin-releasing peptide receptor (GRPR) plays a pivotal role in itch transmission, which suggests that neurons expressing GRPR can be a specific component on itch transmission (Davidson and Giesler, *Trends Neurosci*, 33: 550, 2010). Atopic dermatitis is a chronic inflammatory disease without satisfactory treatment, having itch as the main symptom. The participation of GRPR remains to be evaluated in chronic itching models. **Aims**: This study analyzed the involvement of GRPR in the pruriceptive responses observed in the mouse model of oxazolone-induced atopic dermatitis, by testing the selective non-peptide GRPR antagonist PD176252. **Methods**: Male Swiss mice (5/group, 25-30 g) were used. Atopic dermatitis was induced by oxazolone application 0.5 % (10 µl/site) at intervals of 2-3 days, for 16 days (Tsukumo et al, *J Pharmacol Sci.,* 115: 21, 2011). Three different protocols of treatment with PD176252 (5 mg/kg, i.p.) were adopted. Firstly, the antagonist was administered in a single dose, at the 16^{th} of the protocol to induce atopic dermatitis by oxazolone. In a second protocol, the treatment with PD176252 started at 9th day, and was repeated daily until the 16^{th} day of atopic dermatitis induction. Finally, the administration of PD176252 was initiated on the 16^{th}, together with the last application of oxazolone, continuing from days 17 to 19. Control groups received saline solution at the same schedules of treatment. The scratching behavior was measured for 60 min, at the end of the experimental protocols, as the number of scratches with fore- and hind-paws close to or behind the ears. **Results**: The single treatment with PD176252 resulted in a significant decrease of scratching behavior induced by the repeated administration of oxazolone, with an inhibition percentage of 40 ± 9 %. Similar percentages of inhibition were obtained when PD176252 was administered chronically, from the 9^{th} to the 16^{th} day, or from the 16^{th} to the 19^{th} days, after the onset of atopic dermatitis induction. For this experimental set, the inhibition percentages were 48 ± 3 % and 48 ± 15 %, respectively. **Conclusion**: The present data show that selective inhibition of GRPR by PD176252 was effective in reducing the scratching behavior, in the mouse model of atopic dermatitis induced by oxazolone. Of note, PD176252 was effective when tested in either prophylactic or therapeutic protocols of administration. **Financial Support**: CAPES, CNPq, FINEP/PUCRSINFRA #01.11.0014-00.The Local Ethics Committee (13/00338-PUCRS) approved all the experimental protocols.

05.024 Study of the analgesic activity of Solidago chilensis Meyen extract enriched with diterpenes. Brito TM, Chaves AS, Rodrigues SA, Amendoeira FC, Ferraris FK Fiocruz – Farmacologia e Toxicologia

Introduction: *Solidago chilensis* Meyen, native from South America, popularly known as Brazilian-arnica has been used in folk medicine for therapeutic purposes such as antiinflammatory, analgesic, muscle relaxant, in the treatment of wounds, kidney disease and colds. **Objective**: To determine whether treatment with a fraction rich in diterpenes obtained from *S. chilensis*, modulates peripheral pain in a nociceptive experimental model induced by acetic acid in mice. **Methods**: The fraction rich in diterpenes was obtained from inflorescences of *S. chilensis* and was prepared at the Laboratory of Natural Products, Institute of Technology in Drugs, Oswaldo Cruz Foundation, Rio de Janeiro, Brazil. C57BL/6 mice, weighing 20–25 g, were housed under standardized conditions (room at constant temperature (25oC) with alternating 12-h periods of light and darkness), were fed on a standard mouse diet with water ad libitum before use. This study was approved by the Committee for Ethics in Animal Research (CEUA, Fiocruz, license no P17 / 13-5). For induction of peripheral mediation of pain it was used writhing test induced by acetic acid 0.8%. Abdominal constrictions were caused by the intraperitoneal injection of 0.8% acetic acid. The animals were previously treated, intraperitoneal administration (i.p), with different doses of *S. chilensis* diterpenes rich fraction (0.005, 0.05, 0.5, 5 and 50 mg/kg) 1 h before the stimulation with acetic acid. Control animals received the same volume of vehicle (saline). Five minutes after the acetic acid injection, the number of times that each animal presented abdominal constriction was counted for 10 minutes. Sodium diclofenac (25 mg/kg i.p.) was used as standard drugs for comparison. Each experimental group was performed with at least six animals. The results were statistically evaluated by analysis of variance (ANOVA) and the Newman-Keuls-test, considering significant p values 0.05. **Results**: Intraperitoneal administration of S. chilensis diterpenes rich fraction (S.ch) dose-dependently inhibited the abdominal constrictions induced by acetic acid in mice. Dose of 0.5 to 50 mg/kg induced a significant antinociceptive effect (Sal – 23.5 + 6.78; S.ch 0.5 – 16.2 + 4.72 ; S.ch 5 – 14.7 + 3.15 and S.ch 50 – 10.8 + 6.61, n=8). The analgesic effect of S. chilensis fraction 50 mg/kg were similar to those observed with sodium diclofenac, a classical non-steroidal analgesic (Sal - 23.5 + 6.78 ; Diclo - 9.8 + 6.93, n=8). **Conclusion**: We conclude that the Solidago chilensis fraction rich in diterpenes has analgesic activity, but there is a need for further investigation to identify the diterpene compounds related to this activity. Thank you FAPERJ for the financial support.

05.025 Evaluation of central and peripheral changes in different models of tooth pulp inflammation in rats. Filippini HF[1,2], Scalzilli PA[1,3], Costa KM[1,4], Freitas RDS[1,4], Campos MM[1,2,3,4] [1]PUCRS – Toxicologia e Farmacologia, [2]PUCRS – Odontologia, [3]PUCRS – Odontologia, [4]PUCRS – Medicina e Ciências da Saúde

Introduction: The orofacial region has peculiar patterns of pain transmission. The trigeminal ganglion and trigeminal subnucleus caudalis are important anatomical sites related to nociceptive information from orofacial structures. Previous data showed that application of the inflammatory agent mustard oil to the rat tooth pulp induces central sensitization in brainstem nociceptive neurons of trigeminal subnucleus caudalis (Itoh, *Neurosci* 192, p.721, 2011). Additional data demonstrated nociceptive behavior, accompanied by central sensitization of caudalis nociceptive neurons, in a chronic model of dental pain after exposure of the tooth pulp to the oral cavity (Tsuboi, *Europ J Neurosci*, 34, p.292, 2011). **Aim**: This study investigated whether different models of tooth pulp inflammatory pain display distinct patterns of behavioral nociception, hematological changes and glial activation in the trigeminal ganglion. **Methods**: Male Wistar rats were used (N=5-7/group). The Animal Ethics Committee (CEUA 13/00362) approved the experimental protocols. The pulps of the maxillary left first molars were surgically exposed with a 1/2 size drill in low-speed rotation, under irrigation. In the group A, the pulps were left exposed to the oral cavity. For the group B, the pulps were exposed and the teeth were immediately sealed with temporary dental filling. In the group C, the pulps were exposed, and 0.2-□l of Complete Freund's Adjuvant (CFA) were applied with a small piece of paper-point for 1 min, followed by dental sealing. Naïve rats, without surgical pulp exposure were used as a negative control group (D). Body weight (g), water and food consumption, and locomotor activity in the open-field test were evaluated at 1, 2, 3 and 8 days after pulp exposure. At each time point, a separate group of animals was euthanized and total blood was collected for differential hematological analysis using Giemsa staining. In addition, trigeminal ganglions (ipsilateral and contralateral) were removed for immunohistochemistry analysis to determine GFAP-positive satellite glial cells. **Results**: There was no statistical significant difference when comparing body weight gain, or food and water consumption among the groups. A significant reduction of locomotor activity was observed in the group C (treated with CFA), in relation to the group D (naïve), with an inhibition percentage of 33 ± 14 %. Differential hematological analysis showed a reduction of monocyte counts at 8 days in the group B (sealed teeth), when compared to the naïve rats (93 ± 32%). Qualitative immunohistochemical analysis revealed an increase of satellite glial cell activation, as indicated by GFAP-immunostaining, at 8 days in the groups B and C, in both TG sides. **Conclusion**: Based on the present results, it is possible to conclude that different models of tooth pulp inflammation in rats led to distinct central and peripheral changes. Further studies are in progress to assess additional parameters of pain and inflammation in these experimental models. **Financial Support**: CAPES, CNPq, FINEP/PUCRSINFRA #01.11.0014-00. **CEUA** (13/00362).

05.026 Effects of hydrogen sulfide (H2S) donors on pruritus induced by a type-2 protease activated receptor (PAR-2) agonist in mice. Coavoy-Sánchez SA, Rodrigues L, Costa SKP, Muscará MN ICB-USP – Pharmacology

Introduction: Pruritus is the most common symptom of cutaneous diseases and anti-histamines are the usual treatment; however, anti-histamine-resistant pruritus is common in some clinical settings. In this way, the involvement of mediators other than histamine in the context of pruritus requires new therapeutic targets. Considering that the intradermal (i.d.) injection of the PAR-2 agonist peptide SLIGRL-NH_2, evokes a scratching behavior in mice in a dose-dependent manner (Shimada et al., 2006), and that H_2S donors can inhibit histamine-mediated itching (Rodrigues et al., 2013), in the present work we investigated the effects of H_2S donors on PAR-2 mediated acute itching behavior in mice, as well as some of the possible mechanisms involved. **Methods**: The experimental protocol was approved by the local ethics committee (CEUA - ICB/USP; n° 100, fls. 09, livro 03/2013). Male C57BL/6 mice (7-10 wk-old) received an i.d. injection of SLIGRL-NH_2 (40 nmol/site), a proteinase-activated receptor-2 (PAR-2) agonist, into the dorsal neck region. GYY4137 and NaHS (a slow-release and a spontaneous H_2S donor, respectively) were injected either concomitantly with or 30 min before SLIGRL-NH_2 into the same site. The itching response was quantified by the number of scratching bouts during the 40 min following SLIGRL-NH_2 injection. The participation of transient receptor potential ankirin-1 (TRPA1) receptor and KATP channel was evaluated by treating the animals with HC-030031 (a TRPA1 antagonist), allyl isothiocyanate (AITC; a TRPA1 agonist) or glibenclamide (a K_{ATP} channel blocker). **Results**: Intradermal injections of SLIGRL-NH_2 (8–80 nmol) evoked a dose-dependent scratching behavior, which peaked at 10 min and returned to the basal response along the next 30 min. The co-injection of either GYY4137 (1 and 3 nmol) or NaHS (0.3 and 1 nmol) with SLIGRL-NH_2 (40 nmol/site) resulted in significant itching inhibition (45 and 46%, respectively; $P<0.05$), which was abolished by 200 nmol glibenclamide. However, pre-treatment with the H_2S-donors had no significant effects. In addition, HC-030031 (20 µg/site) significantly reduced by 43% the SLIGRL-NH_2-induced itching ($P<0.05$), but AITC-induced pruritus (at 1000 nmol/site) was unaffected by NaHS. **Conclusions**: Our data show that pruritus secondary to PAR-2 activation can be reduced by H_2S acting through K_{ATP} channel opening. On the other hand, TRPA1 receptors can also mediate the SLIGRL-NH_2 itching behavior, but H_2S does not interfere with this pathway. **Financial Support**: CAPES, CNPq, FAPESP. **References**: Shimada SG et al. Eur J Pharmacol. 2006 Jan 20;530(3): 281-3. Rodrigues L et al. Nitric Oxide 2013 31(Suppl 2), S54–S55.

05.027 Blockage of gastrin-releasing peptide receptor by PD176252 ameliorates acute and chronic pruritus in mice. Machado GDB, Danesi GM, Pereira PJS, Campos MM PUCRS

Introduction: Pruritus is an unpleasant cutaneous sensation that leads to the desire to scratch (Steinhoff et al., J Invest Dermatol. 126, 1705, 2006). Acute pruritus serves as a self-protective mechanism, and plays an important role in the “itch-scratch cycle” that leads to chronic pruritus, compromising the general quality of life (Dong et al., Annu Rev Biophys. 43, 331, 2014). Recent evidence has implicated gastrin-related peptide (GRP) in itching sensation (Patel et al., Biochim Biophys Acta. 1766, 23, 2008; Weber et al., Curr Opin Endocrinol Diabetes Obes. 16, 66, 2009). This peptide exerts its effects by binding to its receptor, denoted GRP-R (also known as BB2-R) (Patel et al., Biochim Biophys Acta. 1766, 23, 2008). Despite the countless medical conditions that present pruritus as the main symptom, the arsenal for the treatment of itch remains limited so far. Previous evidence from our research group revealed beneficial effects for the non-peptide GRP-R antagonist PD176252 in the acute scratching behavior caused by trypsin and compound 48/80 in mice (unpublished data). **Aims**: The present study further evaluated the effects of PD176252 in either acute or chronic models of pruritus in mice. **Methods**: Male Swiss mice (8 per group, 25-30 g) were used. The Local Ethics Committee (13/00338-PUCRS) approved all the experimental protocols. The animals were treated with PD176252 (1 mg/kg or 5 mg/kg), dosed by i.p. route, 30 min before the induction of scratching behavior. The control groups received vehicle (saline solution), at the same interval of time. The acute scratching behavior was evoked by a single injection of the MrgprA3 agonist chloroquine (200 □g/site), or the inducer of oxidative stress H_2O_2 (0.3%/site), into the back of the mouse neck. To induce chronic scratching, the dry skin model of was used. For this purpose, the animals received five-daily applications of a solution containing diethylether and acetone 1: 1 (AEW), upon the shaved back of the neck. PD176252 (5 mg/kg, i.p.) was given 30 min before the last AEW application. Scratching behavior was measured for 40 min, as the number of scratches with forepaws and hindpaws close to the injected site and/or behind the ears. **Results**: The treatment with PD176252 (1 and 5 mg/kg) produced a significant and dose-dependent reduction of the scratching behavior induced by chloroquine, with inhibition percentages of 61 ± 5 % and 82 ± 4 %, respectively. A dose-dependent effect for PD176252 was also observed in the model of scratching behavior elicited by H_2O_2, with inhibitions of 68 ± 9 % and 86 ± 4 %, for the doses of 1 and 5 mg/kg, correspondingly. Finally, PD176252 (5 mg/kg) displayed marked inhibitory effects on the chronic scratching behavior in the model of dry skin, with an inhibition of 64 ± 9%. **Discussion**: Data presented herein confirm and extend the evidence about the relevance of GRP-R for itching transmission. It is tempting to suggest that GRP-R antagonists, such as PD176252, might be useful options to treat pruritus, especially in the cases refractory to the currently available therapies. **Financial support**: PIBIC-FAPERGS / CNPQ. CEUA 13/00338-PUCRS.

05.028 Anti-inflammatory and Antinociceptive Properties of the Ethanol Extract of *Trema micrantha* **(Cannabaceae) leaves.** Carvalho MGB[1], Silva RV[1], Carbonezi LH[2], LIma CKF[1], Miranda ALP[1] – [1]FF-LEFEx-UFRJ – Biotecnologia Farmacêutica, [2]IPPN-UFRJ –

Inflammation is given by a series of physiological responses generated by the host in response to a stimulus such as infection or trauma. Inflammation can have rapid onset and short duration (acute inflammation), or persist due to a continuous stimulus or injury (chronic inflammation). The nociception is the process where intense thermal, mechanical or chemical stimuli are detected by a subpopulation of peripheral afferent nerve fibers called nociceptors and interpreted as pain, one of the classical signs of inflammation. *Trema micrantha*, popularly known as *Pau pólvora* (Stick powder) or *Curindyba*, is a native tree species in Brazil that can be found throughout the country (Pio Correia, 1931). In folk medicine the leaves are used to combat skin disorders, rheumatism and syphilis (Lorenzi, 2000). The objective was to evaluate the anti-inflammatory and antinociceptive activities of ethanolic extract from the leaves of Trema micrantha (TMF) through the formalin test (2.5%; 20 µl/paw) and mechanical allodynia (von Frey filaments) induced by Freud's Adjuvant (CFA, 20 µl/paw), both in mice, and quantifying cells and mediators of the inflammatory infiltrate. The extract (10, 30 and 100 mg/kg, *p.o.*) was administered 1 hour before the intra-plantar injection of formalin and stimulated paw licking time recorded in two different times after injection: 0-5 min (neurogenic phase) and 15-30 min (inflammatory phase). The results were expressed in % of inhibition compared to the vehicle control group (Tween 80/ethanol /water) (n = 6-8 animals, * $p < 0.05$, *** $p < 0.001$, ANOVA). The extract significantly inhibited the 1st phase of the formalin test in 40%*, 65.6%* and 35.5%*** at all three doses (10, 30 and 100 mg/kg), respectively. It was able to inhibit significantly the 2nd phase of the test by 84%** only at a dose of 100 mg/kg. There was no significant effect of the extract in mechanical allodynia even at a higher dose of 300 mg/kg. These results suggest an anti-inflammatory and antinociceptive profile for *Trema micrantha* leaves extract, since nociception in the second phase is due to an inflammatory response by the presence of neutrophils and mediators such as TNF and PGs, and in the first phase to the chemical stimulation of nociceptors. TMF extract at 100 mg/kg significantly decreased TNF and MPO levels, inhibiting by 50%* and 55% **, respectively, confirming the anti-inflammatory profile of the extract observed in phase 2 of the formalin test. We can conclude that *Trema micrantha* leaves extract has distinct antinociceptive and anti-inflammatory profile, which may be due to the presence of different components responsible for these actions involving also distinct signaling pathways and modulation. Studies to understand the possible mechanisms of action are underway and consist perspectives of this work. Financial Support: FAPERJ, CNPq, CAPES. CEUA FARMACIA01, FARMACIA02.

05.029 Antinociceptive mechanisms of a lipid transfer protein isolated from noni seeds in mice. Campos DCO[1], Costa AS[1], Rocha AD[1], Carmo LD[2], Alencar NMN[2], Oliveira HD[1] [1]UFC – Bioquímica, [2]UFC – Fisiologia e Farmacologia

Introduction: Noni is the Hawaiian name for the fruit of Morinda citrifolia L. (Rubiaceae). In traditional pharmacopoeia, this plant part is claimed to prevent and cure several diseases and also for pain management and treatment. Previous studies from our research group have reported the isolation and characterization of a lipid transfer protein (McLTP1) from noni seeds exhibiting potent antinociceptive activity in mice. **Aims**: To have greater insights into the mechanisms and sites of action, this study examined the effects of different pain modulators on the antinociceptive action of McLTP1, using three experimental models of pain. **Methods**: McLTP1 was purified from noni seed as previously describe and was administered to male Swiss mice weighing 25 - 30g at a dose of 8 mg/kg by intraperitoneal (i.p.) or oral route (p.o.). Aiming to characterize the antinociception induced by McLTP1 in the acetic acid-induced abdominal writhing test, the following drugs were administered 30 min before the protein treatment: yohimbine (1 mg/kg, i.p.), atropine (2 mg/kg, i.p.), L-nitro arginine methyl ester (L-NAME 20 mg/kg, i.p.) and glibenclamide (2 mg/kg, i.p.). To elucidate the possible mechanism in the antinociceptive effect of McLTP1 in capsaicin test, the effects of capsazepine (5 mg/kg, i.p., administered 15 min before McLTP1 treatment) was analyzed. The antinociceptive effect of McLTP1 was also analyzed in the tail flick test. Groups of mice (n = 6) were treated with McLTP1 or morphine (5 mg/kg, s.c.) and the reaction time was measured before and after 30, 60, 90, and 120 min of drug administrations. **Results and Conclusion**: Pretreatment of mice with yohimbine did not modify significantly ($p>0.05$) the antinociceptive effect of McLTP1 (85.71 i.p and 80.95% p.o. of inhibition of abdominal writhes). However, systemic treatment of mice with L-NAME (61.42% i.p and 54.76% p.o. of inhibition) or glibenclamide (59.52% i.p and 54.28% p.o. of inhibition) reversed significantly the effect of McLTP1. McLTP1 significantly reduced the paw-licking response in capsaicin test after pretreatment of mice by oral (46.55% of inhibition) or intraperitoneal route (56.89% of inhibition). The capsaicin-induced nociception was significantly blocked by capsazepine (43.10% of inhibition of nociception) and the combination of this drug with McLTP1, significantly reversed the antinociceptive effect observed after oral (0.21%) or intraperitoneal (13.70%) treatment with this protein. In tail flick model, McLTP1 failed to demonstrate antinociceptive activity. These findings demonstrate that McLTP1 has the antinociceptive potential to be developed as a therapeutic agent. Based on the results obtained, it was suggested that the antinociceptive action of McLTP1 involves the participation of NO/cGMP, PKG/ATP pathway and TRPV1 receptors. **Keywords**: Morinda citrifolia L., seed proteins, LTP, antinociceptive mechanisms. Supported by: CAPES and CNPq. Research approval by the Animal Care and Use Committee of the Federal University of Ceará - Proc. No. 37/13

05.030 α-Phellandrene presents anti-inflammatory and anti-hyperalgesic effects: **Role of the antioxidant mechanism, inhibition of the neutrophils migration and release of the pro-inflammatory cytokines.** Santos WC[1], Macedo EMA[1], Cunha FVM[1], Sousa DP[2], Santos IMSP[3], Araújo KS[3], Oliveira FA[1], Almeida FRC[1] [1]UFPI – Farmacologia, [2]UFPB – Ciências Farmacêuticas, [3]Facid

Introduction: Aromatic species have been used as an important complementary native source for the pharmacological treatment of inflammatory diseases. Essential oils from these plants have pharmacological properties such as analgesic and anti-inflammatory. They consist of several secondary metabolites, among them are the monoterpenes. The α-phellandrene (α-Fel) is a monoterpene present in the essential oils from aromatic plants, and previous studies have demonstrated anti-inflammatory, antinociceptive and antioxidant activities. **Methods**: The inflammation was induced in female Wistar rats (170-230 g; n=6-8/group) by intraplantar CFA injection (50 µL) in the hind paw (Animal Ethics Committee/UFPI, Nº. 82/2014). Twenty-four hours after CFA injection, we used Randall Selitto and plethysmometer aparatus to evaluate inflammatory hyperalgesia and edema, respectively. The rats were orally treated with α-PHEL (6.25, 12.5, 25, 50 and 100 mg/kg), vehicle (2%Tween 80 in saline 0,9%) or diclofenac (5 mg/kg) and evaluated on the times 1, 2, 3, 4, 5 and 6 h (acute phase) and treated once daily for 10 days (chronic phase). On day 11, all female rats were euthanized. Hind paws were removed for histological examination (n=5). We used the mouse air pouch model of carrageenan-induced inflammation to evaluate the cytokine levels (TNF-α and IL-1β), nitrite concentration, MPO and SOD activity. Statistical analyzes were performed using two-way ANOVA followed by Bonferroni's post test or one-way ANOVA followed by Tukey's post test, $p<0.05$. **Results**: The α-PHEL reduced inflammatory hyperalgesia during both phases of the test (α-Fel 12.5 mg/kg: 45.4±1.8 g, α-Fel 25 mg/kg: 51.2±2.4g, α-Fel 50 mg/kg: 52.1±1.6g and α-Fel 100 mg/kg: 56.3±2.2g) (***$p<0.001$), except at the dose of 6.25 mg/kg. Doses tested did not reduce the edema in the acute phase, whereas in the chronic phase the α-PHEL 100 mg/kg reduced the edema from the 3rd day of the treatment (0.37±0.02 mL) (**$p<0.01$), similar effects were observed with doses of 25 (0.37±0.02, *$p<0.05$) and 50 mg/kg (0.35±0.02 mL,**$p<0.01$) from the 7th day of treatment. The treatment with the α-PHEL promoted an important decrease of the cellular infiltrate to the inflammatory site when evaluated by histology and total leukocyte infiltration in the model of air pouch (**$p<0.01$). The α-PHEL (50 to 100 mg/kg) significantly reduced the levels of TNF-α, IL-1β (***$p<0.001$), nitrite and MPO enzyme activity, in addition, increased the activity of the SOD, an antioxidant enzyme (**$p<0.01$). **Conclusion**: Our results suggest that the anti-inflammatory and antinociceptive action of α-PHEL are related to the inhibition of the production/release of pro-inflammatory mediators, leukocyte migration and possible antioxidant effect. **Financial support**: UFPI/CAPES **Keywords**: α-Phellandrene. Monoterpene. Antinociceptive. Anti-inflammatory.

05.031 Antihyperalgesic and antiallodynic effect of γ-TPN in the model of sciatic nerve partial ligation. Passos FFB, Piauilino CA, Lopes EM, Oliveira AP, Almeida FRC UFPI

Introduction: Neuropathic pain is characterized by a direct result of injury or disease affecting the peripheral or central somatosensory system. Natural compounds have been used for treatment of many pathological processes. The gamma terpinene (γ-TPN) is a monoterpene present in several plant species and studies with monoterpenes that exhibit structural similarity have shown sedative, antinociceptive and antidepressive activities. The aim of this study was to evaluate the analgesic effect of γ-TPN. **Methods**: In this study we used the sciatic nerve partial constriction model (PSNL). Male Wistar rats (n=6-8, 180-240g) received the bandage on the right hind paw and 24 hours after were treated with γ-TPN (100 and 200 mg/kg, po). Control animals received vehicle (Saline + 0.9% Tween 80, po) or pregabalin (10 mg/kg, po). The mechanical allodynia was evaluated by measuring paw withdrawal threshold (g) using digital von Frey. The brisk paw withdraw was considered as a positive response. Hyperalgesia was assessed by the Randall and Selitto method where the nociceptive threshold (g) was measured by applying increasing pressure on the ligated foot. In the cold allodynia test, the animals received acetone (0.05 mL/paw) on the ligated paw during 20 s and the result was quantified by scores. The hyperalgesia and the mechanical and cold allodynia were observed in alternated days (3, 6, 9, 12 and 15). All of the protocols were approved by Ethics Committee of Animal Experimentation (CEEA/UFPI n° 008/2012). Statistical analyzes were performed using one way ANOVA followed by Tukey test, $p<0.05$. **Results**: The administration of γ-TPN increased the mechanical threshold (von Frey test) at doses of 100 mg/kg (38.47±1.76; 41.63±1.61; 39.29±0.71; 38.58±2.03; 41.50±1.39) and 200 mg/kg (43.64±1.11; 40.35±1.02; 45.03±0.96; 42.03±0.92; 40.89±0.78) when compared with control (28.36±1.15; 27.61±0.78; 26.53±1.12; 26.52±0.78; 26.25±1.38). Pregabalin (10 mg/kg) increased the threshold (38.11±1.83; 41.79±1.15; 40.52±1.81; 41.04±0.67; 42.62±1.10). In the test of Randall Selitto γ-TPN at doses of 100 mg/kg (37.88±4.08; 48.06±2.36; 40.56±1.66; 45.56±1.69; 45.56±1.43) and 200 mg/kg (47.05±2.14; 51.44±2.23; 50.39±3.12; 49.83±1.67; 49.33±1.38) increased threshold when compared to control (26.02±1.36; 25.13±1.74; 28.84±1.47; 28.27±1.08; 29.74±1.17), and pregabalin (10 mg/kg) had the expected effect (41.79±3.13; 46.15±2.10; 47.05±2.70; 46.21±1.82; 46.98±1.90). In the cold allodynia test, the γ-TPN treated animals presented higher scores at 100 mg/kg (1,33 ± 0,53; 1,00 ± 0,58; 2,00 ± 0,73; 1,78 ± 0,64; 2,44 ± 0,84) and 200 mg/kg (2,75 ± 1,38; 1,50 ± 0,87; 1,75 ± 1,03; 3,00 ± 0,71; 2,00 ± 0,91) when compared with control (4,50 ± 0,76; 3,75 ± 0,77; 5,00 ± 0,50; 4,88 ± 0,64; 5,75 ± 0,16). Pregabalin (10 mg/kg) had the expected effect (2,50 ± 1,32; 1,50 ± 0,87; 1,75 ± 0,75; 2,25 ± 0,75; 2,75 ± 0,85). **Conclusion**: The γ-TPN has antiallodynic and antihyperalgesic activities in the sciatic nerve ligation neuropathic pain model. **Financial support**: UFPI/FAPEPI-CAPES

05.032 Microneedles enhance antinociceptive effect of topical 15d-PGJ_2 cream in a rat model of temporomandibular joint pain. Macedo CG[1], Jain AK[2], Franz-Montan M[1], Napimoga MH[3], Clemente-Napimoga JT[1], Gill HS[2] [1]FOP-UNICAMP, [2]Texas Tech University – Chemical Engineering, [3]SLMandic

Introduction: Temporomandibular Disorders (TMDs) are related with multifactorial etiology and manifest through varied symptomatology. The pain arising from TMDs is often treated with opioids and agents that inhibit the immune response and are associated with substantial adverse effects and long-term risks. Thus, the development of new therapies that are safer and more effective is of great interest to patients and clinicians. **Objectives**: The objective was to evaluate whether the antinociceptive effect of topically-applied 15d-PGJ_2 cream can be enhanced with microneedles (MNs) in a rat temporomandibular joint (TMJ) model. **Methods**: Male Wistar rats (150g) were used (n=4-6/group). A positive control group received an intra-TMJ injection of 15d-PGJ_2 (100 ng/TMJ) 15 min or 2 hours prior an ipsilateral intra-TMJ injection of 1.5% formalin, a nociceptive stimulant. The negative control group received no15d-PGJ_2 treatment. Efficacy of MNs to increase drug penetration through skin was assessed by applying 100, 200 or 500 ng of 15d-PGJ_2 in 10 mg cream base on TMJ skin surface either after hair removal, or after hair removal and creating micropores in skin by applying stainless steel MN patches. Then 15 min later an ipsilateral intra-TMJ injection of 1.5% formalin was done. To evaluate duration of antinociceptive effect from MN-treatment, two concentrations of 15d-PGJ_2 cream (200 and 500 ng) were applied and an ipsilateral intra-TMJ injection of 1.5% formalin was done 2, 4, 6 or 8 h later. Animal's nociceptive behavior was observed during a 45 minute-period after formalin injection, and then they were euthanized and their periarticular tissue was removed to evaluate the release of TNF-α and IL-1β by ELISA. **Results**: Topical application of 15d-PGJ_2 cream (100, 200, and 500 ng) for 15 min did not induce any significant ($p>0.05$) antinociceptive effect compared to direct TMJ injection of 15d-PGJ_2 (100 ng/TMJ). However, when TMJ skin was first treated with MNs and then cream was applied, a significant ($p<0.05$) reduction in formalin-induced nociceptive behavior was observed compared to rats that received no 15d-PGJ_2, and importantly this antinociceptive effect was comparable to the intra-TMJ injection of 15d-PGJ_2. A concentration-dependent effect was observed, with 200 and 500 ng 15d-PGJ_2 showing better effect compared to the 100 ng dose. Furthermore, the antinociceptive effect of 200 and 500 ng 15d-PGJ_2 with MN treatment persisted up to 8h ($p< 0.05$ compared to negative control); on the other hand direct intra-articular injection of 15d-PGJ_2 was not able to maintain the antinociceptive effect for more than 2 h. The 15d-PGJ_2 (200 and 500 ng) cream associated with MNs also significantly reduced the release of TNF-α and IL-1β, up to 8 h. **Conclusion**: The 15d-PGJ_2 cream associated with MNs provides antinociceptive and anti-inflammatory effect, and can offer a potential patient-friendly therapeutic option for pain control related to inflammatory disorders of TMJ. **Financial Support**: CAPES/PDSE and TTU. TTU/ACUC Approval Number: 14040-05

05.033 Spinal cord mechanisms involved in Ehrlich cells-induced cancer pain. Zarpelon AC, Calixto-Campos C, Verri Jr WA UEL – Ciências Patólogicas

Introduction: **and aims**: The cancer affects a significant number of people actually, and the pain is the major symptom related to poor quality of life on patients. About 62%–85% of patients with advanced cancer described pain, therefore, pain management in cancer patients is a public health issue, and the mechanisms of cancer pain are not completely understood. This complex pain involves inflammatory and/or neurophatic compounds and despite it affect several patients, the pharmacological control is not well defined. An important characteristic of cancer pain is that the hyperalgesia, allodynia and spontaneous pain are usually not controlled by opioids. Our group demonstrated recently a model of mechanical and thermal hyperalgesia induced by administration of Ehrlich tumor cells on paw, the pain is susceptible to treatment with opioids, but not to cyclooxygenase inhibitor or to tricyclic antidepressant. In this model we demonstrated bone degradation, indicating that it is a nociceptive process extremely difficult to be modulated. There are evidences that the inflammatory response against the tumor cells results in the production of cytokines and chemokines that sensitize the nociceptors by receptor-mediated activation of protein kinases and/or activation of mytogen activated protein kinases such as p38. The activation of these intracellular pathways results in activation of complex mechanisms. Despite all of the research performed in an attempt to inhibit cancer pain, it cannot be stated the exact mechanisms involved in the maintenance and chronicity of cancer pain. **Methods**: Therefore, our group was investigating the involvement of PI3K/AKT, MAP quinases (p38, JNK e ERK), cytokines and glial cells on different times after paw tumor inoculation (2, 4, 6, 8, 10 and 12 days). On day 8, mice received a intratecally injection of following inhibitors: wortmannin (PI3K inhibitor, 1, 3, 10 µg/mice) PD98059 (MEK 1/2 inhibitor, prevents ERK 1/2 activation, 1, 3, 10 µg/mice), SP600125 (JNK inhibitor, 1, 3, 10 µg/mice), SB202190 (p38 inhibitor, 1, 3, 10 µg/mice), fluorocitrate (astrocytes inhibitor, 0.48, 1.6, 4.8 µg/ mice) and minocycline (microglia inhibitor, 15, 50, 150 µg/ mice). Furthermore, we are also evaluating if tumor growth can induce glial cells to be reactive and release inflammatory mediators, which may increase the nociceptive signals in the spinal cord. Understanding the underlying mechanisms related to the development of cancer pain is important for effectively pharmacological treatment of the patients. Statistical differences were considered significant for $P<0.05$. Experimental procedures were approved by the Ethics Committee of Londrina State University (Of. Circ. 08982.2014). **Results and Discussion**: Ehrlich tumor cells induced significant mechanical and thermal hyperalgesia and pain thickness starting at day 2 up to day 12, with the maximum response on day 8. The mechanical, thermal hyperalgesia induced by ehrlich tumor cells was reduced by the i.t. treatment with the inhibitors of PI3K (10 µg/mice, 52%, 66,8%), ERK 1/2 (10 µg/mice, 71,2%, 61,2%), JNK (10 µg/mice, 78,1%, 67,8%), p38 (10 µg/mice, 80,4%, 68,3%), astrocytes (1.6 µg/mice, 76,7%, 59,8%) and microglia (50 µg/mice, 53,3%, 55,05%). The tumor growth was not reduced with the treatments. All the inhibitors diminishes the overt pain like behavior tumor induced. There was an increase on glial cells expression detected by PCR method. **Financial support**: Fundação Araucária, CAPES, CNPq. ACZ is fellowship of CAPES/ Fundação Araucária

05.034 Antinociceptive effect of 15-deoxy-^{Delta12,14}-prostaglandin J_2 is mediated by the activation of proliferator-activated receptor- g on macrophage cells in the temporomandibular joint. Abdalla HB[1], Macedo CG[1], Napimoga MH[2], Bonfante R[1], da Rocha LM[1], Clemente-Napimoga JT[1] [1]FOP-UNICAMP, [2]SLMandic

Introduction: We have previously demonstrated that peripheral administration of 15-deoxy-^{12,14}-prostaglandin J_2 (15d-PGJ_2) in the TMJ of rats can prevent nociceptor sensitization, and that this antinociception effect is mediated by proliferator-activated receptor- (PPAR-), and - and - opioid receptors. However, the mechanism that underlies the signaling of PPAR- (upon activation by 15d-PGJ_2) to induce antinociception, and how the opioid receptors are activated via 15d-PGJ_2 are not fully understood. **Objectives**: Therefore, in this study we sought to better understand the role of opioid receptors in the antinociceptive effect of 15d-PGJ_2. **Methods**: Male Wistar rats (± 150 g, n=4-6/group) were pretreated (15 min) with an intra-TMJ injection of PPAR-γ receptor antagonist GW9662 (3 ng/TMJ) followed by an ipsilateral intra-TMJ injection of 15d-PGJ_2 (100 ng/TMJ); or κ-opioid receptor agonist U50,488 (30 µg/TMJ); or δ-opioid receptor E6264 (10 µg/TMJ) 15 min prior an ipsilateral intra-TMJ injection of 1.5% formalin. An additional group of animals was pretreated with the inducer of macrophage (1% Thioglycollate; 30 µl/TMJ/3 days) followed by an ipsilateral intra-TMJ injection of 15d-PGJ_2 (100ng/TMJ) 15 min prior an ipsilateral intra-TMJ injection of 1.5% Formalin. Animals' nociceptive behavior was observed during 45 minutes-period and then they were euthanized by deep anesthesia and their periarticular tissue removed to evaluate the release of Dynorphin, β-Endorphin and Transcription factor of PPAR- by Enzyme-Linked Immunosorbent Assay (ELISA). **Results**: Pretreatment with an intra-TMJ injection of the PPAR- antagonist GW9662 (3 ng/TMJ) blocked the antinociceptive effect of 15d-PGJ_2 in formalin-induced pain behaviour in the TMJ of rats, but did not affect the antinociceptive effect induced by - and -opioid receptor agonists. Because 15d-PGJ_2 requires - and -opioid receptor activation, but is not a ligand for them, this result suggested that PPAR- activation by 15d-PGJ_2 must mediate release of endogenous opioid peptides to activate - and -opioid receptors. Intra-TMJ injection of 15d-PGJ_2 (100 ng/TMJ) significantly increased the release of endogenous opioid receptor peptides dynorphin, β-endorphin and transcription factor of PPAR- in the periarticular tissue of animals pretreated with the macrophage-inductor thioglycollate (1%, 30 µl/TMJ/day for 3 days). **Conclusion**: The data presented supports the hypothesis that peripheral antinociceptive effect of 15d-PGJ_2 is mediated by PPAR expressed in the resident macrophages of TMJ tissues. Once activated by 15d-PGJ_2, PPAR induces the release of β-endorphin and dynorphin, which activates - and -opioid receptors in primary sensory neurons to induce the antinociceptive effect. **Financial Support**: CNPQ. CEUA/UNICAMP: 3414-1

05.035 Microglial Cells: **no role in diabetes-induced hyponociception into rats TMJ.** da Rocha LM, Muzilli A, Freitas FF, Macedo CG, Abdalla HB, Bonfante R, Clemente-Napimoga JT FOP-Unicamp

Introduction: Diabetes in early phase in known to result a variety of painful conditions induced by a modification in the neuronal activation and transmission. Following peripheral nerve/tissue injury, one important contributor to change nociceptive transmission are microglial cells in central nervous system. **Objectives**: The aim of this study was evaluate the role of microglial cells of the *trigeminal subnucleus caudalis* in the diabetes-induced hyponociception in the TMJ of rats. **Methods**: Wistar rats (± 150g, n=4-6/group) were treated with an intraperitoneal injection of vehicle (normoglycemic – NG) or Streptozotocin 75 mg/kg (diabetic – DB). Diabetes-induced hypernociception was assessed by the animals' nociceptive behavior induced by an intra-articular injection of formalin 7, 14, 21, 28, 35 and 42 days after the diabetic induction. After behavioral assays, animals were terminally anesthetized and their trigeminal subnucleus caudalis were removed for analyzed the release of Fraktalkine (FKN) and Catepsin S (CatS) (ELISA); and the expression of the CD11b/c and p38MAPK (Western Blot). **Results**: Early phase of diabetes induced hyponociception into TMJ of rats 7, 14, 21, 28, 35 and 42 days after the diabetic induction ($p<0.05$: Two-way ANOVA, Bonferroni's test). Western Blot analysis demonstrated no statistical difference in the expression of CD11b/c or p38MAPK among DB rats and NG rats ($P>0.05$: Two-way ANOVA, Bonferroni's test). ELISA analysis demonstrated no statistical difference in the release of FKN or CatS among DB rats and NG rats ($P>0.05$: Two-way ANOVA, Bonferroni's test). **Conclusion**: The results suggest that microglial cells seem to have no role in the diabetes-induced hyponociception in the TMJ of rats. **Financial Support**: CNPq. CEUA/UNICAMP: 3415-1

05.036 Evaluation of the involvement of microglial cells in the induction and persistence of inflammatory hyperalgesia induced rheumatoid arthritis in rats ATM. Bonfante R[1], Abdalla HB[1], da Rocha LM[1], Macedo CG[1], Clemente-Napimoga JT[1]
[1]FOP-Unicamp – Ciências Fisiológicas

Introduction: Rheumatoid arthritis is an autoimmune polyarthritis resulting in a systemic clinical condition of chronic inflammation that primarily affects the synovial joints that presents distorted, rigid and with reduced function. The temporomandibular joint is affected in about 65% of patients with rheumatoid arthritis, and the pain is the main complaint, especially during movement and function. The phagocytic cells are microglial cells responsible for innate immunity in the central nervous system and are related as an important factor both the induction and maintenance of sensory disorders followed by neuronal disorders or peripheral tissue injury as those induced by chronic diseases such as arthritis. **Objectives**: This study aims to evaluate the expression and activity of microglial cells of the trigeminal subnucleus caudalis (Vc) in the induction and development of persistent inflammatory hyperalgesia induced by AR in the TMJ of rats. **Methods**: Male Wistar rats were sensitized subcutaneously with an injection of Methylated Bovine Serum Albumin (mBSA, 500µg) in an emulsion containing phosphate buffered saline (PBS) and Freund's Complete Adjuvant. mBSA dissolved in Freund's Incomplete Adjuvant was injected in different sites in the back of the rat 7 and 14 days after the first immunization. Twenty-one days after the initial injection, TMJ-arthritis was induced in the animals immunized by intra-articular injection of an mBSA (10mg / TMJ / week) during 3 weeks. After 24 hours, 7 and 14 days after the last mBSA intra-articular injection, the animals were terminally anesthetized and their Vc was collected to biochemical analysis (Western Blot and ELISA) to measure the release of Fraktalkine (FKN) and Catepsin S (CatS), and expression of the CD11b / c by RA induced hypernociception. **Results**: The release of CatS and FKN showed significantly higher on day 14 when compared to the control group ($p < 0.05$: ANOVA, Bonferroni), the FKN also showed significantly higher on day 7 compared to the control ($p < 0.05$: ANOVA, Bonferroni). The expression of CD11b / c showed no significant difference between the treated and control groups. **Conclusion**: Peripheral injuries in the oral region are able to promote persistent activation of microglial p38 MAPK pathway in the Vc sustained by the persistence of the release of mainly Cathepsin S and Fractalkine up to 14 days after the last challenge. **Financial support**: Fundação de Amparo à Pesquisa do Estado de São Paulo #2014/09975-7 Ethics Committee/UNICAMP: 3413-1