

## 05. Pain and Nociception Pharmacology

### 05.001 The transient receptor potential ankyrin 1 antagonism reduces the nociception and inflammation in an ultraviolet B radiation-induced burn model in mice. Fialho MFP, Brum ES, Pegoraro NS, Oliveira SM UFSM

**Introduction:** Burn injuries cause impact psychological and economically since are characterized as debilitating and lifelong injuries cause dramatic clinical effects in humans (Walker, Thermal Burns, 1, 1, 2018; Kaddoura, Ann. Burns Fire Disasters, 20, 95, 2017). These injuries can be caused by several factors, including ultraviolet (UV) radiation. Ultraviolet B (UVB) radiation exposure promotes sunburn and thus acute and chronic inflammatory processes contributing to pain development and maintenance (Church, Clin. Microbiol. Rev, 19, 403, 2006; Lopes, CNS Neurosci. Ther, 22, 118, 2016). Since the typical treatments can cause adverse effects, new therapeutic alternatives are necessary. An alternative potential would be the transient receptor potential ankyrin 1 (TRPA1), which is involved in a variety of inflammatory pain models. TRPA1 channel is activated or potentialized by exogenous irritants compounds, extracellular  $\text{Ca}^{2+}$  influx and several endogenous oxidant molecules [hydrogen peroxide ( $\text{H}_2\text{O}_2$ )] that are produced during tissue damage and inflammation, as by UV radiation (Andrade, Pharmacol. Ther, 133, 189-204, 2012; Julius, Annu. Rev. Cell Dev. Biol., 29, 355-385, 2013; Lopes, CNS Neurosci. Ther, v. 22, p. 118, 2016). We evaluated the peripheral participation of TRPA1 using a topical treatment containing HC030031 (a selective TRPA1 antagonist) on nociception and inflammation caused by a UVB radiation-induced burn model in male mice (25-30g). Experimental protocols were approved by CEUA-UFSM (protocol number 2479201217/2018). **Methods:** The mice were anesthetized and just the right hind paw was exposed to UVB radiation ( $0.75 \text{ J/cm}^2$ ). The topical treatments with different gels formulations were performed consecutively once a day for 8 days. Nociceptive and inflammatory parameters were evaluated at 24h after topical treatment each day.  $\text{H}_2\text{O}_2$  levels (a TRPA1 agonist) in the irradiated paw tissue and  $\text{Ca}^{2+}$  influx in mice spinal cord synaptosomes were determined to evaluate a possible mechanism of activation TRPA1 channel by UVB radiation. **Results:** HC030031 gel presented suitable pH and spreadability factor ensuring its quality and the therapeutic effect. The HC030031 0.05% reversed the mechanical and cold allodynia UVB-induced with maximum inhibition ( $I_{\text{max}}$ ) of  $69 \pm 13\%$  and  $100\%$  (4<sup>th</sup> day), respectively. HC030031 0.05% also reduced the paw edema and MPO activity with  $I_{\text{max}}$  of  $77 \pm 6\%$  on the 5<sup>th</sup> day and  $69 \pm 28\%$ , respectively. Likewise, the UVB radiation increased the  $\text{H}_2\text{O}_2$  levels (a TRPA1 agonist) and the  $\text{Ca}^{2+}$  influx in mice spinal cord synaptosomes. The UVB radiation-induced  $\text{Ca}^{2+}$  influx was reduced by HC030031. **Conclusion:** These findings confirm the activation of the TRPA1 channel by UVB radiation, suggesting that topical TRPA1 antagonists can be a new strategy for the adjuvant treatment of the sunburn-associated pain and inflammation. **Acknowledgments:** Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), and Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul (FAPERGS). We thank CNPq and CAPES for their fellowship support.

### 05.002 Role of kinin receptors in the reserpine-induced pain/depression dyad.

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**Introduction:** The fibromyalgia is a disease characterized by generalized chronic primary pain and comorbidities as depression, which causes functional disability and reduction of patients' quality of life since it does not have specific pathophysiology, diagnostic or appropriate treatment (Macfarlane, G.J. [Ann Rheum Dis](#) 76, 318, 2017; Arnold, L.M. [Int J Clin Pract](#) 70, 99, 2016). Thus, it is important to elucidate the mechanisms involved in this disease. Evidence has shown the contribution of kinins and their B<sub>1</sub> and B<sub>2</sub> receptors in acute and chronic pain conditions (Brusco, I. *Mol Neurobiol*, 54, 7824, 2017). So, the aim of the study was investigating the involvement of the kinins and its B<sub>1</sub> and B<sub>2</sub> receptors in a reserpine-induced pain/depression dyad model in mice. **Methods:** The fibromyalgia model was induced by subcutaneous administration of reserpine (1 mg/kg) once a day for 3 consecutive days (Nagakura, Y. *Pain* 146, 26, 2009). Nociceptive parameters (mechanical [von Frey filaments] and cold allodynia [acetone drop method] and spontaneous nociception) and behaviors of burrowing and forced swimming were evaluated after reserpine administration in male Swiss mice n=6-8/group from CEUA/UFMS: 2770030516/2016. The role of kinin B<sub>1</sub> and B<sub>2</sub> receptors was investigated on these parameters using pharmacological antagonism. Moreover, the mechanical allodynia also was evaluated in wild type C57BL/6 mice and kinin B<sub>1</sub> and B<sub>2</sub> receptor knockout mice n=5/group (USP: 208/2014). Moreover, monoamines levels were measured in the sciatic nerve, spinal cord, and cerebral cortex of the animals. Data were analyzed by Student's t test and one-way or two-way ANOVA-Bonferroni *post hoc* test. **Results:** The B<sub>1</sub> (DALBk) and B<sub>2</sub> (Icatibant) receptor antagonists reduced the reserpine-induced mechanical allodynia from 0.5 up to 2 h, with maximum inhibition (I<sub>max</sub>) of 46±7% (*p*<0.01) at 1 h or from 0.5 up to 1 h with I<sub>max</sub> of 51±8% (*p*<0.001) at 1 h after its administrations, respectively. Moreover, DALBk or Icatibant reduced the reserpine-induced cold allodynia at 1 h after its administration with I<sub>max</sub> of 57±20% (*p*<0.01) and 50±18%, (*p*<0.05), respectively. Low doses of kinin B<sub>1</sub> and B<sub>2</sub> receptor agonists caused spontaneous nociception in animals previously treated with reserpine which was prevented by DALBk (I<sub>max</sub>=59±9%; *p*<0.01) or Icatibant (I<sub>max</sub>=64±8%; *p*<0.05). The kinin B<sub>1</sub> and B<sub>2</sub> receptor gene deletion also reduced the reserpine-induced mechanical allodynia (I<sub>max</sub>=94±6%; *p*<0.001) and (I<sub>max</sub>=88±7%; *p*<0.001) at 1 day or 3 days in kinin B<sub>1</sub> and B<sub>2</sub> receptor knockout, respectively. Only DALBk reversed the decreased burrowing behavior at 2 h after its administration with I<sub>max</sub> of 68±16%; *p*<0.05. Reserpine increased the immobility time (218.7±24 seconds; *p*<0.05) of animals in the forced swimming test when compared to the vehicle group (167.9±14 seconds). Moreover, reserpine also decreased the monoamines levels, such as dopamine and serotonin in the spinal cord and cerebral cortex of the mice (*p*<0.05). **Conclusion:** Kinins B<sub>1</sub> and B<sub>2</sub> receptors are involved in fibromyalgia-associated pain. Our results suggested that the B<sub>1</sub> or B<sub>2</sub> receptors might represent a potential target for the relief of fibromyalgia-associated pain symptoms. **Acknowledgment:** CAPES; CNPq.

**05.003 HUF-101, a cannabidiol analog, prevents mechanical and thermal allodynia in a chemotherapy-induced peripheral neuropathic pain model.** Silva N<sup>1</sup>, Gomes FI<sup>1</sup>, Lopes A<sup>1</sup>, Mechoulam R<sup>2</sup>, Gomes F<sup>1</sup>, Cunha TM<sup>1</sup>, Guimaraes FS<sup>1</sup> <sup>1</sup>FMRP-USP, <sup>2</sup>Hebrew University

**Introduction:** Cannabidiol (CBD) is a phytocannabinoid with multiple pharmacological effects and several potential therapeutic properties. Its low oral bioavailability, however, could limit its clinical use. Preliminary results indicate that the fluorination of the CBD molecule increases its pharmacological potency. Here we investigate antiallodynic effects of HUF-101, a fluorinated synthetic CBD analog, in a chemotherapy-induced peripheral neuropathic pain model and its possible mechanisms of the action. **Methods:** C57BL/6 mice (20-25-g) were treated with HUF-101 (HUF; 1, 3, 10, or 30 mg/kg; i.p.) or vehicle for 7 days. For the chemotherapy-induced peripheral neuropathy (CIPN), mice were treated with paclitaxel (PCX; 8mg/kg; i.p.) or saline on days 0, 2, 4, and 6, 30 min after HUF. In a second experiment, animals received PCX (8mg/kg) or saline in days 0, 2, 4, and 6, and were then treated with HUF or vehicle from the 7th to the 20th day. To evaluate the mechanical and thermal allodynia induced by PCX, Von Frey filaments and acetone test were applied to the right plantar surface of the animal's paw. We also investigated if HUF could attenuate changes in the dorsal root ganglia mRNA expression of inflammatory markers and the peripheral nerve damage induced by PCX. In addition, to evaluate the involvement of PPAR $\gamma$  receptors in HUF effects, mice received GW9662 (a PPAR $\gamma$  receptor antagonist, 2mg/kg) 30 min before HUF. The effects of HUF (0.1, 0.3, 1.0, and 3 $\mu$ M) on the cell viability of breast cancer cells culture (4T1) treated with PCX (3.0 and 10 $\mu$ M) and its potential to produce morphine-like rewarding effects were also investigated. **Results:** The treatment with PCX (8 mg/kg) induced mechanical and thermal allodynia in all protocols. This effect was prevented by the treatment with HUF (1, 3, 10, and 30 mg/kg) for 7 days. However, these same doses were not effective to reverse the already installed allodynia induced by PCX. The antiallodynic effects of HUF were blocked by pretreatment of PPAR $\gamma$  antagonist. Moreover, HUF attenuated the increased Iba-1, Tnf- $\alpha$ , and Il-6 mRNA relative expression in dorsal root ganglia induced by PCX. Preliminary data indicates that HUF attenuates PCX-induced peripheral nerve damage in Nav1.8-Cre-tdTomato mice. Furthermore, HUF did not interfere with the antineoplastic effect of paclitaxel. Unlike morphine, HUF did not show rewarding effects in the conditioned place preference test. **Conclusions:** These results show that the HUF prevents the mechanical and thermal allodynia induced by paclitaxel even at a low dose of 1 mg/kg, without interfering with the chemotherapeutic effect of PCX and producing morphine-like rewarding effects. The antiallodynic effects of HUF seem to be mediated by the activation of PPAR $\gamma$  receptors and the decrease of the increased expression of inflammatory markers in the dorsal root ganglia induced by PCX. Thus, this new compound could be a therapeutic alternative for the treatment of chemotherapy-induced peripheral neuropathic pain. **Financial support:** CNPq, CAPES, CRID, FAPESP, and FAEPA. **Ethical Committee:** 100/2016.

**05.004 Involvement of spinal CAV2.3 in the secondary hyperalgesia induced by capsaicin.** Ferreira MA<sup>1</sup>, Lückmeyer DD<sup>1</sup>, Macedo SJ<sup>1</sup>, Prudente AS<sup>2</sup>, Ferreira J<sup>1</sup>  
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**Introduction:** The treatment of neuropathic pain is a clinical challenge, since patients have increased sensitivity of nociceptive neurons in the spinal cord (central sensitization). Despite being expressed in nociceptive neurons, the role of Cav2.3 (the main subunit of the R-type VGCC) in central sensitization is poorly understood. Thus, the purpose of the present study was to verify whether the blockade or the knockdown of spinal Ca<sub>v</sub>2.3 reduces capsaicin-induced secondary hyperalgesia in mice, a central sensitization model predictive for effective drugs in the treatment of neuropathic pain.

**Methods:** Male and female C57BL/6-UFSC mice (N=8-10, 20-25 g) were used. To inhibit Cav2.3, animals were treated with its selective blocker SNX-482 (30-300 pmol/site) intrathecally, 10 minutes before or 60 minutes after the capsaicin injection).  $\omega$ -Conotoxin MVIIA, (1-30 nmol/site), a Ca<sub>v</sub>2.2 blocker, used as a positive control. To knockdown Cav2.3, animals were treated with an oligonucleotide antisense (ASO) against Ca<sub>v</sub>2.3 (2.5 nmol/site, intrathecally, twice a day for 3 days) or with a mismatch oligonucleotide (MM). Adverse effects were assessed by neurological tests after these treatments. Mice were evaluated by von Frey's tests to detect paw mechanical thresholds before (baseline) and after capsaicin injection. After baseline detection, mice were injected with capsaicin (20 nmol/site, subcutaneous into the proximal part of the right hind paw). The time spending licking the injected paw (spontaneous nociception) was evaluated in the first 5 minutes after capsaicin injection. Significant reduction at mechanical thresholds (in the distal part of right hind paw, i.e. secondary mechanical hyperalgesia) were detected from 0.5 to 5 hours after capsaicin treatment. **Results:** Capsaicin injection caused spontaneous nociception in the first 5 minutes and secondary hyperalgesia (evaluated by von Frey filaments) from 1 to 5 hours post-injection, and similarly in males and females. Post-treatment, but not pretreatment, with SNX-482 partially (53±7% of inhibition) prevented capsaicin-induced secondary hyperalgesia at a high dose (300 pmol/site, which did not cause adverse effects) and only in female mice. On the other hand, pretreatment or post-treatment with MVIIA largely (34±13 and 82±11% of inhibition) reduced capsaicin-induced secondary hyperalgesia, however only at a high dose (30 pmol/site) that caused adverse effects. Finally, the knockdown of Ca<sub>v</sub>2.3 expression in the DRG (52±6% of inhibition) and spinal cord (51±4% of inhibition) caused by intrathecal pretreatment with an ASO against Ca<sub>v</sub>2.3 (2.5 nmol/site, 12/12 hours for 3 days) did not produced adverse effects and was able to markedly prevent (72±16% of inhibition) the secondary hyperalgesia induced by capsaicin. **Conclusion:** Our results demonstrate that Ca<sub>v</sub>2.3 has a critical role on the maintenance of the central sensitization process in female mice. Since neuropathic pain is more common in woman, Cav2.3 is a potential target for the development of newer drugs for the treatment of neuropathic pain. **Acknowledgments: Financial support** by CNPq, CAPES and INCT-Inovamed. The project was approved by the CEUA of UFSC (PP00872).

**05.005 Effect of lipidic transfer protein isolated from *Morinda citrifolia* (Noni) seeds in sensitive neuropathy peripheral induced by oxaliplatin in mice.** Cesario FRAS, Vale ML, Pereira AF, França JC, Oliveira AR, Dias DBS, Silva CMP UFC

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Oxaliplatin (OXL) is a third generation of platinum agents with spectrum of antitumor activity, which exerts a potential cytotoxic activity on human cancer cells such as colorectal, ovarian and pulmonary cancer. Its toxicity differs from other compounds, presenting a prominent neurotoxic effect, such as peripheral sensory neuropathy, which at one time compromises antineoplastic therapy as a result of experience sensory abnormalities including symptoms of neuropathic pain, such as paraesthesia and dysesthesia (tingling, numbness and pins and needles), allodynia and hyperalgesia KAGIAVA, et al., 2015). lipid transfer protein from *Morindacitrifolia*(noni) seeds (MCLTP1) exhibit potential applications as antioxidant, antinociceptive/analgesics, immunomodulators in the treatment of neurological disorders (CAMPOS; et al., 2016). Data from the literature suggest that the neurotoxic effect of OXL occurs through oxidative stress, neuromodulation of nociceptors and inflammatory processes in peripheral neuronal tissues. Based on this, the objective was to investigate the effect of pre-treatment of MCLTP1 on the peripheral neuropathy model induced by chronic treatment with OXL in mice. **Methods and Results:** The study was approved by the UFC Animal Research Ethics Committee (protocol nº 36/17). Male Swiss mice (20-40g) were treated with Saline (0,1ml/10g, n=6), OXL (2 mg/kg i.v., n=8) for 4.5 weeks (2X/week) concomitantly with the administration of MCLTP1 (4, 2 and 1 mg/kg p.o., n=8) and parallel to the tests nociceptive: tests of Von Frey (mechanical plantar hypernociception) and Acetone (thermal allodynia) to evaluate the development of sensory neuropathy and behavioral tests: Open Field experiment and Rota-Rod tests to verify some motor impairment. Calculations performed from GraphPad Prism 6.0 Statistical Software. The results show that administration of OXL reduce both the nociception time ( $p<0,05$ ) and the mechanical nociception threshold ( $p<0,05$ ), starting on the 14th day for the thermal stimulus ( $p<0,05$ ) and the 1st day for the mechanical stimulus ( $p<0,05$ ) compared to the initial day and the control group, hanging for all 56 days of experiment. Treatment with MCLTP1 at doses of 4, 2 and 1 mg/kg prevented this effect of OXL by increasing the time of thermal nociception and/or delaying the onset of neuropathy from day 14 to day 28. The same occurred for the mechanical test, where MCLTP1 increased the mechanical nociception threshold, with a maximum dose of 4 mg/kg in both tests. As for the Rota-Rod test, there was no increase in the number of falls of the animals in all groups, and in the open field changes occurred only in the number of crosses and rearing in the groups that received OXL. **Conclusion:** Although still preliminary, the data presented suggest that MCLTP1 has a neuroprotective effect, reducing, delaying or preventing the development of peripheral sensory neuropathy by OXL. In addition, the absence of motor impairment suggests a predominantly sensitive neuropathy, with possible cognitive alterations caused by oxaliplatin. **Financial support:** PRONEX/CNPq. CAMPOS, D. C. O.; et al. First isolation and antinociceptive activity of a lipid transfer protein from noni (*Morindacitrifolia*) seeds. *Internat. J. Biolog. Macromol.* v. 86, p. 71–79, 2016. KAGIAVA, A. A.; et al. Oxaliplatin induced neurotoxicity is mediated through gap junction channels and hemichannels and can be prevented by octanol. *Neuropharmacol.* v. 97, p. 289-305, 2015.



**05.006 Classical analgesic substances induce thermal antinociceptive effects in *Drosophila melanogaster* larvae.** Silva TS<sup>1</sup>, Lopes C<sup>2</sup>, Guimarães JDS<sup>3</sup>, Kuhn GCS<sup>3</sup>, Romero T<sup>3</sup>, Naves LA<sup>3</sup>, Duarte IDG<sup>3</sup> <sup>1</sup>FMRP-USP, <sup>2</sup>USP, <sup>3</sup>UFMG

**Introduction** Attempting to minimize the use of mammalian vertebrates in pharmacological pain research, non-mammalian vertebrates, as zebrafish, have been adopted for partial replacement. Some invertebrates, as the fruit fly *Drosophila melanogaster*, can detect and respond to noxious stimuli. *D. melanogaster* larvae exposed to thermal nociceptive stimuli elicit a stereotypical rolling escape behavior, characterized by 360° rolling along body axis. Since this behavior is well defined, *D. melanogaster* larvae represent a potential model to evaluate putative analgesics. We assessed the thermal antinociceptive effects of morphine, anandamide (AEA), dipyrrone, acetylsalicylic acid (ASA) and dexamethasone (DXM) in *D. melanogaster* larvae, in order to validate such nociception model. **Methods** *D. melanogaster* larvae, placed in a 20 µl water droplet on a Petri dish, were positioned on a glass surface of the Hargreaves apparatus. Infrared radiation source was perpendicularly positioned under the glass, towards the water droplet. 95 infrared (IR) intensity units was used as the thermal noxious stimulus. **Rolling Behavior Latency**, the time from infrared incidence until the first rolling behavior, was defined as the nociceptive measurement and visualized by binocular loupe. The substances tested were injected into larvae hemocoel using a microinjection apparatus. Rolling behavior latencies were evaluated in specific time points after hemocoel injections. To induce infrared nociceptive sensitization, each larva was stimulated for 32 s on 97 IR units. **Results** Rolling behavior latency was increased by morphine (2, 4, 8, 16 ng) in a dose-dependent manner. 4 ng of naloxone, a non-selective opioid antagonist, fully reversed the maximum effect of morphine. AEA (8, 16, 32 pg) induced dose-dependent antinociception. This effect was not reversed by CB<sub>1</sub> (AM251, 80 ng) or CB<sub>2</sub> (AM630, 100 ng) receptor antagonists. Dipyrrone (32, 64, 128 ng) also elicited dose-dependent antinociceptive effects. DXM 8 and 16 ng produced antinociceptive effects. Exposing larvae to 97 IR units induced nociceptive sensitization, i.e., reduction of rolling behavior latency. In the sensitization peak (180 min), the rolling behavior latency reduced from 12.1 to 7.5 s. ASA and DXM were administrated 150 min after infrared-induced nociceptive sensitization. ASA (25, 50, 100 ng) displayed a dose-dependent reversal of sensitization. DXM (4, 8, 16 ng) reversed sensitization in a rapid onset manner (30 min after injection). DXM (16ng), injected prior to nociceptive sensitization, displayed a late onset of action (150 min after injection). **Conclusion:** Classical analgesics are capable to elicit antinociceptive effects on thermal nociception in *D. melanogaster* larvae. Infrared-induced nociceptive sensitization was achievable in the proposed model and was reversed by ASA and DXM. Together, our findings open perspectives for evaluation and discovery of antinociceptive drugs using a *D. melanogaster* larvae model. **Financial support:** CNPq, CAPES, FAPEMIG.

**05.007 Cisplatin-induced neurotoxicity in dorsal root ganglia: The rosiglitazone neuroprotective effects.** Oliveira HR, Neves FAR, Duarte DB UnB

**Introduction:** Neurotoxicity is the major dose-limiting side effect of antitumoral therapies, such as cisplatin and paclitaxel. When the Peripheral Nervous System is affected is called Chemotherapeutic-Induced Peripheral Neuropathy (CIPN). Clinically, patients with CIPN present sensory loss, paresthesia and neuropathic pain (acute or chronic). Currently, there is no treatment to prevent and/or revert this toxicity and one of the prominent strategies is neuroprotection. There are many pathways that could induce neuroprotection, and we believed that this can be reached by activating the Peroxisome Proliferator-Activated Receptor  $\alpha$  (PPAR $\alpha$ ), which already demonstrated to be neuroprotective in many neurodegenerative diseases, including Alzheimer's disease. Our hypothesis is that the activation of PPAR $\alpha$  could be also neuroprotective on CIPN.

**Methods:** To investigate the PPAR $\alpha$  role on CIPN, we used primary cultures from Dorsal Root Ganglia (DRG) from adult naïve Wistar rats weighing 200–350 g as a model. The cells were cultured in HAM F12 culture media with nerve growth factor (NGF -250  $\mu$ g/mL) for 9 days. On day 8, cells were treated for 24 hours with cisplatin (3, 10 or 30  $\mu$ M), rosiglitazone (PPAR $\alpha$  agonist- 1, 3 or 9  $\mu$ M) and/or T0070907 (PPAR $\alpha$  antagonist- 10  $\mu$ M). To evaluate gene expression, the mRNA was extracted with Trizol and quantified in triplicate by RT-PCR. The mRNA expression level was normalized to expression of  $\beta$ -actin mRNA levels. The TNF $\alpha$  and CGRP released levels was evaluated by ELISA.

**Results:** Cisplatin treatment with 30  $\mu$ M decreased the PPAR $\alpha$  and PPAR- $\alpha$ / $\beta$  gene expression in 72.4% and 74.4%, respectively ( $p < 0.05$ ). We next investigate whether PPAR $\alpha$  activation could modulate Cisplatin effects, and we observed that the co-treatment with rosiglitazone recovered the mRNA levels of these 2 receptors decreased by the cisplatin challenge ( $p < 0.05$ ). Further, we investigated whether these observed effects were due to PPAR $\alpha$  activation or to rosiglitazone off-target effects. Thus, the cells were treated with T0070907 and we observed that the rosiglitazone effects also occurs in an independent PPAR $\alpha$  activation pathway (the T0070907 10  $\mu$ M did not altered the mRNA levels of PPAR- $\alpha$  and  $\beta$ / $\alpha$  in co-treatment with cisplatin 30  $\mu$ M and Rosiglitazone 9  $\mu$ M -  $p < 0.05$ ). To investigate whether PPAR $\alpha$  activation modulate the cisplatin effects on cytokine expression, we treated DRGs cells with cisplatin 30  $\mu$ M and Rosiglitazone. We observed that cisplatin increased TNF $\alpha$  gene and protein release, while the co-treatment with rosiglitazone did not modulate the observed effects ( $p < 0.05$ ). Also, we investigated the neuronal function using the release of CGRP and we observed that cisplatin induced decrease in the high potassium-evoked release of CGRP, which is not modulated by PPAR $\alpha$  activation by rosiglitazone treatments ( $p < 0.05$ ). **Conclusions:** We observed rosiglitazone dependent and independent PPAR $\alpha$  activation effects on cisplatin neurotoxicity. Thus, we concluded that rosiglitazone treatment could be beneficial on Cisplatin's neurotoxic effects. **Financial support:** This project was financed by IASP EC 2013 Grant to DBD and FAPDF 1930006682015 to DBD.

**05.008 Alfa-phellandrene reduces cancer hypernociception in an experimental model in mice.** Reis Filho AC, Pinheiro Neto FP, Nogueira MRSN, Lopes EML, Almeida FRC, França ARSF, Lima MPD, Gomes LS, Acha BT, Ferreira PMP UFPI

**Introduction:** Oncologic pain is a type of pain that has an enigmatic and multifactorial neurobiology. Because of the complex nature and etiology of cancer pain, several pharmaceutical agents are used as part of the to treat it, such as tricyclic antidepressants and anticonvulsants. In this context, pharmacological research for the development of new therapeutic agents has become increasingly necessary. Essential oils, especially the secondary metabolites, such as monoterpenes, are extracted from plants that exhibit the most varied biological characteristics, among them we highlighted  $\alpha$ -phellandrene ( $\alpha$ -Phel) which displayed important biological activities such as; antitumor, anti-inflammatory and antinociceptive. The purpose of this study was to investigate a possible antinociceptive effect of  $\alpha$ -Phel in animal model of cancer pain. **Methods:** Female Swiss mice weighing between 25 and 35 g were used, and the experiments were previously approved by the Ethics Committee on the Use of Animals (CEUA/UFPI N<sup>o</sup> 146/16). For induction of hypernociception, 180 sarcoma cells were inoculated into the subaxillary region of the mice, and the mechanical allodynia was evaluated with von Frey filaments, using two treatments: (16 days): sham (normal animals), vehicle (0.9% NaCl with 2% Tween 80), antinociceptive positive control (Pregabalin 10 mg/kg po), antitumoral positive control (5-Fluoracil- 5 FU, 25 mg/kg, ip) and 3 doses of  $\alpha$ -Phel (12.5, 25 and 50 mg/kg po). Subacute treatment (24 days): sham, vehicle, antinociceptive positive control, 4 doses of  $\alpha$ -Phel (6.25, 12.5, 25 and 50 mg/kg po). The relative weight of the animal organs as well as the gross tumor weight were evaluated to investigate possible antitumor activity. Antioxidant (GSH, SOD and MDA) and cytokines (TNF- $\alpha$ , IL-1 $\beta$ , IL-4 and IL-6) analysis were performed. **Results:** In the direct evaluation when assessing the mechanical sensitivity score the reduction of hypernociception was evidenced by the comparison between the groups means on the 12th day of evaluation (sham =  $0.67 \pm 0.49$ , vehicle =  $8.00 \pm 0.32$ , Pregabalina =  $3,833 \pm 0,601$ , 5-FU =  $4,5 \pm 1,333$ , and  $\alpha$ -Phel 12.5 =  $4,833 \pm 0,703$ , 25 =  $3,5 \pm 0,563$  e 50 =  $1,167 \pm 0,401$ ) (\*p < 0.05) In the reproducible in filaments 0.07g, 0.16g and 1.0g and In the indirect evaluation evaluating the mechanical nociceptive threshold (sham =  $9,333 \pm 0,422$ , vehicle =  $2,22 \pm 0,49$ , Pregabalina =  $4,667 \pm 0,989$ , 5-FU =  $3,1 \pm 0,790$ , and  $\alpha$ -Phel 12.5 =  $2,767 \pm 1,071$ , 25 =  $2,667 \pm 0,803$  e 50 =  $8,0 \pm 0,516$ ) during the following days.  $\alpha$ -Phel reduced tumor size by 74.6% up to 82.74% in subacute treatment, reduced TBARS (MDA) levels, increased glutathione reductase (GSH) levels, and ultimately demonstrated ability to modulate inflammatory cytokines (TNF- $\alpha$ , IL-1 $\beta$ , IL-4 and IL-6). **Conclusion:**  $\alpha$ -Phel has a potential antinociceptive effect in a model of cancer hypernociception possibly by antioxidant action and modulation of inflammatory cytokines.

**Financial support:** PPSUS-MS-FAPEPI/UFPI.



**05.009 Antinociceptive properties of Tonantzitlolone B isolated from *Stillingia loranthacea* (Euphorbiaceae).** Villarreal CF<sup>1</sup>, Espírito-Santo RF<sup>1</sup>, Santos DS<sup>1</sup>, Lauria PSS, Abreu LS<sup>2</sup>, Tavares JF<sup>2</sup>, Velozo ES<sup>1</sup> <sup>1</sup>UFBA, <sup>2</sup>UFPB

**Introduction:** The pharmacological treatment of pain remains a clinical challenge because some painful conditions are irresponsive to the currently available painkillers. Natural products are rich sources of analgesic compounds traditionally used in the drug discovery process. Tonantzitlolone B (TZL-B) is a known diterpene recently isolated from the bark roots of *Stillingia loranthacea*. The therapeutic potential of TZL-B and structurally related molecules has been tested on experimental models of cancer and viral infections. However, the pharmacologic actions of these substances are still poorly understood. Once terpenoids frequently exhibit anti-inflammatory and antinociceptive activities, the present study aimed to investigate the antinociceptive properties of TZL-B.

**Methods:** Male Swiss mice (25 – 28g) were treated with TZL-B (1 – 1000 µg/kg) by intraperitoneal route. The antinociceptive properties were investigated on different nociception assays, i.e. formalin test, CFA-induced paw inflammation, von Frey filaments, cold plate and tail flick tests. The involvement of opioid pathways was evaluated in pharmacologic antagonism assay. Motor function was accessed by the rota-rod test. **Results:** TZL-B induced dose-dependent antinociception in the second phase of the formalin test, with a minimum effective dose of 10µg/kg ( $p < 0.5$ ) and a maximum effective dose of 100 µg/kg ( $p < 0.01$ ). The first phase of the formalin was also inhibited by TZL-B, but with a minimum effective dose of 100µg/kg. Despite the formalin test results, TZL-B did not prevent the CFA-induced edema at any of the tested doses. TZL-B at 100 ( $p < 0.1$ ) and 1000 µg/kg ( $p < 0.1$ ), reduced the CFA-induced mechanical allodynia for up to 6 h following the treatment. At the same dose range, TZL-B induced antinociceptive effect in the cold plate ( $p < 0.01$ ) and tail flick ( $p < 0.1$ ) tests, without affecting the motor function of the mice. The TZL-B-induced antinociception was abolished by the pretreated with naloxone (5 mg/kg). **Conclusion:** TZL-B induced consistent antinociceptive effects at a low range of doses. The mechanisms of the antinociception promoted by TZL-B involve the activation of the opioid system, but the subtype of receptor responsible for the action need to be further investigated. It is unlikely that the antinociceptive effects of TZL-B are associated with an anti-inflammatory mechanism considering that it did not prevent paw edema formation. These results, along with the good safety profile previously reported, point out TZL-B as a promising candidate for the development of new analgesic drugs. **Financial support:** CNPq and CAPES.

**05.010 Morphine exposure and maternal deprivation during the early postnatal period alter neuromotor development and nerve growth factor levels.** Torres ILS<sup>1</sup>, Oliveira C<sup>1</sup>, Scarabelot VLS<sup>1</sup>, Vercelino RV<sup>2</sup>, Silveira NPS<sup>1</sup>, Adachi LNA<sup>1</sup>, Regner GG<sup>1</sup>, Santos LS<sup>3</sup>, Macedo ICM<sup>4</sup>, Souza AD<sup>5</sup>, Caumo WC<sup>1</sup> <sup>1</sup>UFRGS, <sup>2</sup>UFCSPA, <sup>3</sup>Unilasalle, <sup>4</sup>Unipampa, <sup>5</sup>Unime

**Introduction:** The objective of this study was to verify whether repeated morphine administration and maternal deprivation in early life alter neurobehavioral development and central nerve growth factor (NGF) levels. **Methods:** A total of 58 male Wistar rat pups were used in our study. From postnatal day 1 (P1), litters were daily deprived of their mother for 3 hours; this was continued for the first 10 days of life. Animals were divided into 5 groups: total control (C), did not receive any intervention; saline (S), received saline solution; morphine (M), received morphine; deprived-saline group (DS), were subjected to maternal deprivation and received saline solution; and deprived-morphine (DM), were subjected to maternal deprivation and received morphine. From P8, newborns received subcutaneous (s.c.) injections of morphine or saline (5 µg) once a day, for 7 days. Righting reflex, negative geotaxis and gait were chosen as postural parameters to evaluate neuromotor reflexes. All experiments and procedures were approved by the Institutional Committee for Animal Care and Use (GPPG/HCPA protocol No. 150614), and followed the recommendations of the Brazilian Law #11794 (2008).

**Results:** In the righting reflex test, a delay in the development of animals was evidenced in the M group (Wald  $\chi^2= 15.09$ ; 4,  $p<0.05$ ). Performance of negative geotaxis was slower in the M and DM groups (Wald  $\chi^2= 122.09$ ; 20,  $p<0.05$ ). In the gait test, all groups showed a daily improvement in performance in terms of locomotion frequency (Wald  $\chi^2= 112.52$ ; 5,  $p<0.05$ ). An increased frequency of rearing was observed in the M, DS, and DM groups from P16 to P20 (Wald  $\chi^2= 49.46$ ; 20,  $p<0.05$ ). The DM group presented an increase in NGF levels in the brainstem (one-way ANOVA/SNK,  $F(4,36)=4.88$ ;  $p<0.05$ ). An increase in cerebral cortex NGF levels in the M, DS, and DM groups was observed as well (one-way ANOVA/SNK,  $F(4,38)=40.77$ ;  $p<0.05$ ).

**Conclusion:** Our results suggest that changes in environmental conditions and the disruption of mother–infant interactions during the neonatal period can produce changes in the neurobiology, physiology, and emotional behavior of rats. This finding has important implications for the maternal-neonate interaction needed for normal brain development in newborns. **Acknowledgements and Financial support:** CNPq; CAPES; Graduate Research Group of Hospital de Clínicas de Porto Alegre – GPPG (ILS Torres – Grant 150614) BIC/PROPESQ-UFRGS.

**05.011 NAD + modulation in neuropathic pain induced by partial ligation of the sciatic nerve in mice.** Miranda ALP, Silva VDCS, Santos BLR, Lima CKFL, Oliveira JT, Camacho-Pereira J UFRJ

**Introduction:** Nicotinamide adenine dinucleotide (NAD) is a coenzyme that is present in the oxidized (NAD<sup>+</sup>) and reduced (NADH) state. The decline of NAD is implicated in the development of diseases related to aging, being an important target for new therapies. Expression and activity of CD38, an ectoenzyme, increase with aging and are responsible for NAD decline and mitochondrial dysfunction through an SIRT3-dependent mechanism (Camacho-Pereira et al, Cell Metabolism 23, 1127, 2016). The aim of this study was to investigate the participation and modulation of NAD<sup>+</sup> in neuropathic pain (NP) in mice. **Methods:** NP was induced by partial sciatic nerve ligation (PSNL) in 3-month-old mice: *knockout* for CD38 (CD38 KO), *double knockout* for CD38 and SIRT3 (CD38/SIRT3 KO) and wild type (WT; C57Bl/6). The locomotion capacity and the short-term memory were evaluated by the open-field and object recognition tests, to assess the cognitive deficit associated with aging. Mechanical sensitivity (allodynia) was assessed by von Frey filaments and hypersensitivity to heat and cold by the Hargreaves and acetone test, respectively, during two weeks after surgery. The mitochondrial function and O<sub>2</sub> consumption will be evaluated by High Resolution Respirometry (HRR) (Oroboros Instruments). **Results:** Firstly, the effect of deletion of CD38 or CD38/SIRT3 was evaluated on the locomotor activity of 7-month-old adult mice to verify the absence of locomotor alterations that could influence the interpretation of the memory test. There were no significant differences in the number of quadrants crossed by WT, CD38 KO and CD38/SIRT3 KO animals, evidencing a full locomotor capacity of the groups. The WT and CD38/SIRT3 KO groups showed a loss of recent memory building capacity by exploring the same objects for a similar period of time without significant differences. In the CD38 KO group we observed a longer exploration time of the new object, showing maintenance of the recent memory and learning capacity even at animals in aging process. CD38 KO animals showed reduced mechanical allodynia compared to WT and CD38/SIRT3 KO animals and electrophysiology studies revealed a better nervous conduction velocity. No significant differences between the animal's groups were observed for heat and cold hypersensitivity. **Conclusion:** The results point to CD38 as a target involved in the development of NP suggesting an important role of NAD<sup>+</sup> in the regenerative processes. Studies on mitochondrial function and neuromodulation involved in the painful response of these animals are underway as well as the evaluation in older animals.

**05.012 Dopamine D1 and D2 receptors mediate the neuropeptide s-induced antinociception in the mouse formalin test.** Oliveira MC<sup>1</sup>, Holanda VA<sup>1</sup>, Souza LS<sup>1</sup>, Soares BL<sup>1</sup>, André E<sup>2</sup>, Silva Júnior ED<sup>1</sup>, Guerrini R<sup>3</sup>, Calo G<sup>3</sup>, Ruzza C<sup>3</sup>, Gavioli EC<sup>1</sup>  
<sup>1</sup>UFRN, <sup>2</sup>UFPR, <sup>3</sup>Universidade de Ferrara

**Introduction:** Neuropeptide S (NPS) is the endogenous ligand of the NPSR receptor. NPS/NPSR system controls a myriad of biological actions, including anxiolysis, wakefulness, food intake, and analgesia [1]. A growing body of evidence support a facilitatory effect for NPS on dopaminergic neurotransmission [2]. The present study is aimed to investigate the role of dopamine receptors signaling in the antinociceptive effects of NPS in the formalin test in mice. **Methods:** the following dopamine receptor antagonists were employed: SCH 23390 (selective D1 antagonist, 0.05 mg/kg, ip), haloperidol (non-selective D2 antagonist; 0.03 mg/kg, ip), and sulpiride (selective D2 antagonist; 25 mg/kg, ip). Mice were pretreated with dopamine antagonists before the central NPS administration (0.1 nmol, icv). The positive controls morphine (5 mg/kg, sc) and indomethacin (10 mg/kg, ip) were used to set up the experimental conditions. Morphine-induced antinociceptive effects were observed during phases 1 and 2 of the test, while indomethacin was active only at the later nociceptive phase. Animals were randomly assigned to 5-8 animals / group and statistical significance was tested by one-way ANOVA followed by Tukey's test. All the experiments were approved by ethic committee from UFRN (n. 012/2011). **Results:** Central NPS significantly reduced formalin-induced nociception during both phases. The systemic administration of SCH 23390 slightly blocked the effects of NPS only during phase 2. Haloperidol prevented NPS-induced antinociceptive effects. Similar to haloperidol, sulpiride also counteracted the antinociceptive effects of NPS in the phases 1 and 2 of the formalin test. **Conclusion:** In conclusion, the present findings suggest that the analgesic effects of NPS are dependent on dopaminergic neurotransmission, mainly through D2 receptor signaling. By contrast, a discrete involvement of D1 receptors in the antinociceptive effects of NPS was observed only during the phase 2 of the formalin test. **References:** 1. Guerrini et al. Med Res Rev. 30(5): 751-77, 2010. 2. Taylor et al. Pain. 157(6): 1194-1198, 2016 **Acknowledgments:** CNPq (CNPq grant no. 507331/2010-9 and no. 302302/2015-8 to ECG) and CAPES (PNPD to VADH).

**05.013 Investigation of antinociceptive potential of synthetic 4-Aminoquinoline derivatives.** Moura IG, Silva SMA, Viana MDM, Moreira MSA, Meneghetti MR, Campesatto EA UFAL

**Introduction:** There are several pharmacological alternatives for pain treatment. However, the development of new analgesic drugs is of great interest because the drugs currently available on the market have limited efficacy and/ or several side effects that restrict their use. Chloroquine is already widely used in the treatment of diseases that cause pain and inflammation such as: rheumatoid arthritis, systemic lupus erythematosus, sarcoidosis and malaria. **Objective:** To investigate the antinociceptive potential of three synthetic derivatives of 4-aminoquinoline (DS4AMQs):  $C_{12}H_9ClN_2$ ,  $C_{11}H_9ClN_2O_2$ ,  $C_{17}H_{20}ClN_3$ . **Materials and Methods:** DS4AMQs were obtained from the structure of the drug Chloroquine. *In vivo* assays were performed using Swiss mice (25-35g, n = 6), of both genders, through following tests: nociception induced by acetic acid (0,6%, i.p.) and glutamate (30  $\mu$ mol/paw, i.p.), in which the number of writhes and paw licking time were evaluated after administration of the respective phlogistic agents. Nociception in hot plate test was measured as the time of removal of the paw from the plate at 54°C. As standard drugs were used: dipyron (40 mg/kg, p.o.) morphine (5,4 mg/kg, i.p.) and as negative control, vehicle (10 mL/kg, p.o.). In all tests, the dose of DSAAMQs used was 50 mg/kg. All experimental models were approved by the Research Ethics Committee of the Federal University of Alagoas (protocol n° 04/2018). **Results:** In the abdominal writhing test, all DS4AMQ were able to statistically reduce ( $p < 0.001$ ) the number of writhes. Similar to that observed in glutamate-induced nociception, in which all DS4AMQs were able to statistically reduce ( $p < 0.001$ ) paw licking time. In the hot plate assay, the latency time at temperature rise was statistically reduced ( $p < 0.001$ ) by all DS4AMQs. **Conclusion:** The results suggest that the DS4AMQs induce a central and peripheral antinociceptive action, corroborating the discovery of new pharmacological therapies, in the analgesic treatment. **Acknowledgment:** CAPES; FAPEAL; UFAL.



#### 05.014 Mitochondrial dysfunction in a fibromyalgia-like symptoms model in mice.

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**Introduction:** Fibromyalgia (FM) is a musculoskeletal pain condition (Choy, *Nat Rev Rheumatol*, 11, 513, 2015), with mitochondrial dysfunction and reactive oxygen species (ROS) production (Meeus, *Expert Opin Ther Targets*, 17, 1081, 2013). Until now, the reserpine-induced FM-like model enables the evaluation of pain and depressive-like behaviours (pain-depression dyad model) (Taguchi, *Pain*, 156, 415, 2015). However, few studies address whether this model could alter the mitochondrial function in mice muscles and spinal cord. **Methods:** Reserpine (1 mg/kg; s.c.) or vehicle (0.1% acetic acid in saline 0.9%; 10 ml/kg; s.c.) was injected once daily for 3 consecutive days in male Swiss mice (30g; n=5-10/group) (CEUA-UFSM: 3525100119/2019). Analyses mitochondrial (high-resolution respirometry in O<sub>2</sub>k-system oxygraphy), oxidative status [ROS production, H<sub>2</sub>O<sub>2</sub> levels, catalase (CAT) activity, and lipid peroxidation (LP)], monoamine levels (HPLC), depressive-like behaviour [forced swimming test (FST) and thigmotaxis], and pain {mechanical [paw withdrawal threshold (PWT) by von Frey filaments] and cold allodynia [behavioural scores by acetone drop method], and muscle strength [grams]} were carried out. Another set of reserpinized mice received a mitochondrial insult using oligomycin [ATP synthase inhibitor (1 mg/kg, i.p.)]. Others received 5-day supplementation with a mitochondrial function enhancer coenzyme Q10 (150 mg/kg, p.o.). Data were analyzed by Student's t-test or two-way ANOVA-Bonferroni *post hoc* test. **Results:** Reserpine induced peripheral [reduced O<sub>2</sub> flux in gastrocnemius and soleus mitochondria when stimulated by their substrates ( $p=0.09$ ), in ATP synthesis ( $p<0.05$ ), as well as, in the electron transfer system (ETS;  $p<0.01$ )] and central [reduced O<sub>2</sub> flux related to ATP synthesis ( $p<0.05$ ) and to ETS ( $p<0.05$ ) in the spinal cord] mitochondrial dysfunction. Reserpine induced peripheral and central ROS production ( $p<0.05$ ). Reserpine altered oxidative parameters in soleus muscle [H<sub>2</sub>O<sub>2</sub> levels, CAT activity, and LP ( $p<0.05$ )] more severely than in gastrocnemius. Instead, reserpine reduced the central H<sub>2</sub>O<sub>2</sub> levels and LP, and increased CAT activity ( $p<0.05$ ). Reserpine caused behavioural changes in mice consistent with FM symptoms [monoamine levels decrease ( $p<0.01$ ); mechanical (PWT=0.22g; 83±6%;  $p<0.001$ ) and cold allodynia (score=5; 2.5x;  $p<0.05$ ); reduced muscle strength (139.7g; 39±6%;  $p<0.01$ ); thigmotaxis behaviour (46±7%;  $p<0.01$ ); immobility in the FST (204s; 28±4%;  $p<0.05$ )], without inducing adverse effects. Oligomycin enhanced the reserpine-induced mechanical (PWT=0.04g; 81±3%;  $p<0.001$ ) and cold allodynia (score=7; 54±3%;  $p<0.01$ ). Otherwise, coenzyme Q10 prevented the reserpine-induced mechanical (PWT=2.05g; 100%;  $p<0.001$ ) and cold allodynia (score=2; 91±6%;  $p<0.01$ ), limbs strength loss (227g; 86±26%;  $p<0.05$ ), and immobility time in the FST (145.6s; 100%;  $p<0.05$ ). **Conclusion:** We characterized the pain-depression dyad model using reserpine contributing to advance the mechanisms in the pathophysiology of FM. **Acknowledgments:** CAPES; CNPq

**05.015 Correlation between metabolic syndrome and migraine: the role of adipokines and omega-3.** Barbosa IR, Dagnino APA, Cunha GD, Campos MM PUC-RS

**Introduction:** Metabolic Syndrome (MS) is an exacerbating factor for migraine, and both conditions have been correlated with anxiety symptoms (Andreeva et al., *Neuroepidemiology* 51: 25, 2018; Klenofsky et al., *Curr Pain Headache Rep*, 23: 1, 2019). Omega-3 fatty acids (n-3) display anti-inflammatory and analgesic properties, but their benefits on migraine and MS remain unraveled (Sanders et al., *Prostaglandins Leukot and Essent Fatty Acids*, 135: 47, 2018). This study evaluated anxiety parameters after induction of migraine in a rat model of MS, investigating the effects of n-3 and the role of hypothalamic adipokines in this context. **Methods:** The local Animal Ethics Committee approved the protocols (9088/18). MS was induced in male Wistar rats (180-200 g; 8-weeks old) by supplementation with a 10-% fructose solution, during 8 weeks (Tavares et al., *J Endod* 45: 174, 2019). At four weeks, the animals were randomized to receive n-3 (1g/kg; by gavage) or saline solution. At the last two weeks, chronic migraine was induced by nitroglycerin administration (NTG; 10 mg/kg; i.p), every 2 days, totaling five injections (Harris et al., *J Neurosci Methods*, 284: 63, 2017). To assess photophobia and anxiety, the animals were evaluated in the light-dark box and in the elevated plus maze, respectively. ELISA was used to determine the hypothalamic leptin levels. **Results:** The induction of migraine by NTG led to a significant decrease in the time spent in the bright compartment of the light/dark box, as an indicator of photophobia ( $P < 0.05$ ). The treatment with n-3 failed to significantly improve this aspect ( $P > 0.05$ ). MS migraineur rats displayed a reduction in the time spent in the open arms ( $3.5 \pm 2.3$ ), according to the evaluation in the plus-maze apparatus, in comparison with negative control animals ( $12.9 \pm 7.8$ ), MS rats ( $23.7 \pm 6.1$ ) or migraineur rats ( $10.1 \pm 4.1$ ). Of note, the treatment with n-3 led to a recovery of this parameter ( $20.0 \pm 11$ ). Moreover, n-3 administration was able to reduce the hypothalamic leptin levels of MS migraineur rats ( $P < 0.05$ ). **Conclusions:** NTG led to photophobia, a hallmark of migraine, in either MS or control rats, without any benefits for n-3 supplementation. Alternatively, the induction of MS potentiated the anxiety-like behavior in migraineur rats, an effect that was clearly improved by long-term administration of n-3, likely involving the modulation of leptin levels in the hypothalamus. **Financial support:** FINEP, CNPq, CAPES, PUCRS.

**05.016 Cannabidiol is a promising treatment for chronic pain: anxiolytic-like and analgesic effects in animal model of chronic constriction injury (CCI), modulation via CB1 and TRPV1 receptors.** Cardoso GKRS<sup>1</sup>, Zuardi AW<sup>2</sup>, Crippa JA<sup>2</sup>, Hallak J, Leite-Panissi CRA<sup>3</sup> <sup>1</sup>USP, <sup>2</sup>FMRP-USP, <sup>3</sup>FFCLRP-USP

In the general population, the incidence of chronic pain is 6% to 8%, and its impact on the quality of life, mood, and sleep exceed the burden of its causal pathology. In this perspective, cannabidiol (CBD) is considered a promising strategy for the treatment of neuropathic pain. Our objective was to evaluate the possible modulation of the effect of cannabidiol via CB1 and TRPV1 receptors using a systemic CBD treatment (3 days) in rats submitted to sciatic nerve constriction (CCI) and evaluated in the open field test (OP) and nociceptive tests (NT). For this study, we used 80 male Wistar rats (220 g) (CEUA/USP/2018.1.103.58.5). The rats underwent a surgical procedure (CCI or false operated/SHAM) on day zero, and the development of neuropathy was followed for 3 weeks by nociceptive tests (I: von Frey, II: Hot plate and III: Acetone). On the 23rd day, the rats were submitted to the open field test (% of time center and periphery and number of crossings). Nociceptive tests were performed on the 24th day after 3 days of CBD treatment. Immunofluorescence was performed to evaluate receptor expression in the insular cortex (IC) and anterior cingulate cortex (ACC). Three sections of each animal were analyzed (n = 8), and images from three different regions were acquired in each section. Binders without binders were used to control tissue autofluorescence. Fluorescence intensity was quantified using ImageJ® software. The two-way ANOVA test was used, followed by the Tukey test (p < 0.05). Results showed that treatment with CBD at different doses (0.3, 3, 10 and 30 mg/kg i.p.) had an effect antiallodynic (I: F (4,24) = 237.2; P < 0.0001; II: F (4,24) = 172.3, P < 0.0001) and antihyperalgesic effects (III: F (4,24) = 375.4 P < 0.0001) in CCI rats. The open-field test, CBD showed an anxiolytic-like effect (F (4,25) = 6.7, P = 0.0008) in the injured animals. The immunofluorescence showed statistical significance in the condition factor (F (1,32) = 17.4, P = 0.0002) and in the treatment factor (F (1,32) = 99.1, P < 0.0001) in the ACC. In the IC region, statistical significance was found in the condition factor (F (1,32) = 125, P < 0.0001), in the treatment factor (F (1,32) = 491.5, P < 0.0001). In both analyses, the 3 mg/kg (Baseline: I: 56g, II: 0 score and III: 25s; 18th day I: 21g, II: 8 score, III: 12s and CBD 3 mg/kg I: 54g, II: 0 score and III: 24s) dose showed an increase in receptor expression in the studied regions, when compared to the SHAM group. These results demonstrated that CBD has antinociceptive and anxiolytic effects in the neuropathic pain model. Our results suggest a synergistic effect, CBD would be modulating the sensory-discriminatory and affective-motivational aspects of chronic pain. This synergistic effect offers favorable perspectives for new pharmacological approaches in the treatment of neuropathic pain, and we can suggest that this effect can involve CB1 and TRPV1 receptors. **Financial support:** FAPESP (2018/06877-5); INCT - Translational in Medicine (FAPESPn<sup>o</sup> 465458/2014-9), CAPES.

**05.017 Synergy and Additivity Between Cannabinoidergic, Opioidergic and Adrenergic Systems in the modulation of peripheral nociception in mice.** Lopes C<sup>1</sup>, Santos-Silva T<sup>2</sup>, Fonseca F<sup>1</sup>, Castro-Júnior C<sup>2</sup>, Romero T<sup>2</sup> <sup>1</sup>USP, <sup>2</sup>UFMG

**Introduction:** Pain is a relevant clinical condition ubiquitously present among individuals, representing a challenge for healthcare providers due to devastating consequences upon life quality of patients. The therapeutic management of pain relies on a diverse repertoire of pharmaceuticals, but most of them are not enough effective or lead to diverse side effects, justifying the need for new treatments. Despite the efforts to develop new substances to treat pain, diverse approaches seek benefits on the co-administration of already existing analgesics, an attempt to maximize therapeutic actions and to concurrently reduce side effects. In fact, such perspective relies on pharmacological synergism, which can be defined as supra-additive effects associated to a certain biological response. The aim of this work was to evaluate, using Isobolographic Analysis, the synergistic effects of three antinociceptive substances, administered in binary doses, on the mechanical algometric test of paw withdrawal. The method herein developed is extensible to any binary combination of substances for which dose-response relationships are known and is statistically validated, providing a good standard to properly evaluate synergy, additivity or sub-additivity of pharmacological effects. **Methods:** Swiss male mice (ethics committee approval CEUA/ICB-UFMG nº69/2018) were acutely co-treated with prostaglandin E<sub>2</sub> (PGE<sub>2</sub>, 2µg) and ascending doses of either anandamide (AEA, 12.5, 25 and 50ng), xylazine (XYL, 25, 50 and 100µg) or DAMGO (0.25, 0.5, 1 and 2µg) and a four-parameter sigmoidal non-linear regression model was used to interpolate data and derive pharmacodynamical values. Extensive modelling based on Loewe's principles for drug synergy assessment and Isobolographic Analysis was performed and three paired doses of AEA+XYL (7.5ng+7.5µg, 12ng+12µg and 16ng+16µg), AEA+DAMGO (6ng+0.1µg, 9ng+0.2µg and 14ng+0.3µg) and DAMGO+XYL (6µg+0.2µg, 11µg+0.4µg and 17µg+16µg) were tested in hindpaws previously sensitized with 2µg of PGE<sub>2</sub> for synergy assessment. The nociceptive thresholds were evaluated, and the results obtained were compared with the additive predicted effects (10, 30 and 50 Maximum Possible Effect - %MPE) of the given doses, considering an ideal additivity. Two metrics were evaluated: combination indexes (CI), to compare theoretical and experimental doses; and effect levels for the tested doses, revealing deviations from an ideal additive effect. **Results:** It was observed a profound pharmacological synergism for the antinociception of AEA+XYL and AEA+DAMGO, with CI lower than 1. For DAMGO+XYL combination, the doses were additive for this metric (CI ~ 1). Statistical validation is feasible on the scope of effect levels analysis, and indeed such effects were significantly higher than those predicted considering additivity, for all the levels tested, for the pairs AEA+XYL (theoretical effect/%MPE VS. experimental effect/%MPE: 11 VS. 37, 33 VS. 63, 49 VS. 65) and AEA+DAMGO (theoretical effect/%MPE VS. experimental effect/%MPE: 11 VS. 34, 30 VS. 55, 50 VS. 66). For the pair DAMGO+XYL, experimental effects were significantly higher only for 10 and 50% MPE (theoretical effect/%MPE VS. experimental effect/%MPE: 6 VS. 15, 28 VS. 29, 47 VS. 62). Isobolograms were also constructed, and their overall depiction of data agrees with the observations. **Conclusion:** According to the proposed model of synergy assessment - Isobolographic Analysis - AEA+XYL and AEA+DAMGO acts as synergistic analgesics in the referred nociception model in mice, in contrast with DAMGO+XYL, which appear to display a more additive antinociceptive effect. Thus, the method proposed appears to be a good standard and a feasible technique to evaluate synergy in pain models using rodents, capable to distinct between diverse combined substances synergism, additivity or even sub-additivity. **Financial support:** CAPES, FAPEMIG and CNPq.

**05.018 Antioxidants antinociceptive effect in animal model of oxaliplatin-induced peripheral neuropathy is associated with decreasing oxidative damage and inflammation in the spinal cord.** Agnes JP, Gonçalves RM, Delgobo M, Macedo SJ, Ferreira J, Zanotto-Filho A UFSC

**Introduction:** Cytotoxic chemotherapeutics display low selectivity to cancer cells, and attention is increasingly being paid to the side effects of anticancer drugs aiming to improve the quality of life of individuals undergoing chemotherapy. Chemotherapy-induced peripheral neuropathy (CIPN) is the major neurological condition reported in protocols containing platin derivatives, taxanes and bortezomib. It has been reported that Reactive Oxygen Species (ROS) are increased by chemotherapeutics, causing oxidative damage to several tissues, including peripheral nerves and spinal cord thereby contributing to CIPN pathobiology. In this study, we evaluated the antinociceptive effect of antioxidants, namely N-acetylcysteine (NAC), lipoic acid (LA) and vitamin E (VE), in animal model of peripheral neuropathy induced by OXA well as their impact in oxidative damage and inflammation on spinal cord. on tumor growth., to evaluate the involvement of inflammation in nociceptive changes of CIPN we evaluated the effects of OXA in animal knockouts for TLR4 and caspase 1/11 that are involved in neuroinflammation.

**Methods:** The CIPN model was induced in both Swiss (wild-type) and C57BL/6 wild-type, TLR4<sup>-/-</sup> and caspase 1/11<sup>-/-</sup> backgrounds. Treatment with OXA (5mg/kg I.P) was administered every 48h for a total 14 days; NAC, LA and VE administered daily at the dose 50mg/kg orally by gavage. In some experiments, subcutaneous Ehrlich tumors were developed in Swiss mice, and treatments started after the presence of palpable tumors, and tumor volume was measured over the 14 days treatment. Von Frey (mechanical nociception) and hot and cold plate (thermal nociception) were used to evaluate nociceptive behaviors associated with CIPN. At the end of treatments, the tumors and spinal cord were collected, and lipoperoxidation (TBARS assay) and cytokines content (by ELISA) were quantified. Statistical analysis for differences between two groups at the same time were analyzed by Test T-student, and between groups by 1-way ANOVA and Tukey test as post-hoc. Differences between three or more groups in two different conditions were analyzed by two-way repeated measures ANOVA and Bonferroni's test as post-hoc. All experimental procedures were approved by CEUA-UFSC (nº 3722260417). Results: We observed that antioxidant treatments significantly reduced nociceptive changes resulting of CIPN whereas they did not alter the antitumor efficacy of OXA. Antioxidants decreased OXA-induced oxidative damage (TBARS), as well as promoted anti-inflammatory modulation in OXA-treated mice as inferred by means of reduced IL-1 $\beta$  and TNF- $\alpha$  and increased IL-10 contents in the spinal cord lysates. In TLR4<sup>-/-</sup> and caspase 1/11<sup>-/-</sup> mice, we observed that the animals did not develop mechanical allodynia, as well as presented lower levels of IL-1 $\beta$  and TNF- $\alpha$  in the spinal cord in comparison to the wild animals treated with OXA, indicating that nociceptive changes may be related to neuroinflammation.

**Conclusion:** Our data suggest that antioxidants may exert antinociceptive effects in OXA-induced CIPN through mechanisms involving decreased oxidative damage and inflammation at the spinal cord level. These data corroborate to previous studies which reported the involvement of spinal cord/dorsal horn inflammation in CIPN. Thanks to LAMEB-UFSC for technical assistance and CAPES for **Financial support**.



**05.019 The activation of cannabinoid receptors inhibits the development of oxaliplatin-associated neurotoxicity in mice.** Pereira AF, Lisboa MRP, Alves BWF, Silva CMP, Dias DBS, Menezes KLS, Cesario FRAS, França JC, Oliveira AR, Alencar NMN, Lima-Júnior RCP, Vale ML UFC

**Introduction:** Oxaliplatin is a third-generation platinum compound used as first line treatment for metastatic colorectal cancer. However, it has an important dose limiting side effect: a neurotoxicity leading to a peripheral sensory neuropathy (PSN). The purpose of the study was to evaluate the role of the endocannabinoid system in the development of oxaliplatin-induced PSN. **Methods:** This work was approved by the Ethics Committee on the Use of Animals of the Federal University of Ceará (protocol number 41/2016). For the induction of PSN, Swiss male mice received oxaliplatin injections (2 mg/kg, i.v.), twice a week, totaling nine administrations. Before each oxaliplatin administration, cannabidiol (10 mg/kg, p.o.), a non-selective cannabinoid agonist (WIN 55, 212-2; 0.5 mg/kg, s.c.) or cannabinoid receptors selective antagonists, AM251 (CB1 antagonist) and AM630 (CB2 antagonist) (3 mg/kg, i.p.) were administered. For 56 days, mechanical and thermal nociceptive tests were performed once a week. In addition, other behavioral tests, such as rota rod, catalepsy and hot plate tests were performed to evaluate possible cannabimimetic effects. On the 14<sup>th</sup>, 28<sup>th</sup> or 56<sup>th</sup> experimental days, the mice were euthanized and the dorsal root ganglia, the trigeminal ganglia, the spinal cord, the trigeminal spinal tract and the periaqueductal gray were harvested. Immunofluorescence assay was performed to evaluate CB1, CB2, c-Fos and ATF3 immunoections in nervous tissues. **Results:** The results showed that oxaliplatin increased ( $P < 0.05$ ) the mechanical (from the 14<sup>th</sup> day to 56<sup>th</sup> day) and thermal (from the 21<sup>st</sup> day to 56<sup>th</sup> day) nociceptive responses, leading to increased c-Fos and ATF3 neuronal expressions. In addition, oxaliplatin increased CB1 and CB2 expressions in different areas of peripheral and central nervous systems ( $P < 0.05$ ). Moreover, cannabidiol and WIN 55, 212-2 attenuated the oxaliplatin-related nociceptive response ( $P < 0.05$ ). The CB1 antagonist, AM251, anticipated the thermal nociceptive response from the 21<sup>st</sup> day to the 14<sup>th</sup> day ( $P < 0.05$ ). Alterations were not observed with the injection of AM630 in the development of PNS. **Conclusion:** In conclusion, cannabinoid receptors are involved in the development of oxaliplatin-induced PSN, promoting an inhibitory effect. When activated, it prevents PSN and, when inhibited, it could anticipate the nociceptive response. This suggests a protective role exerted by the endocannabinoid system in the pathophysiology of oxaliplatin-associated neurotoxicity and it should be considered as a therapeutic target. **Financial support:** National Council for Scientific and Technological Development (CNPq) and the Foundation for Support in Scientific and Technological Development of Ceará (FUNCAP) (PRONEX - Process PR2-0101-00054.01.00/15).

**05.020 Increase of kynurenine 3-monooxygenase in the spinal cord astrocytes mediates the maintenance of neuropathic pain.** Maganin A, Souza GS, Lopes AHP, Silva RL, Gomes FIF, Alves-Filho JCF, Cunha FQ, Cunha TM FMRP-USP

**Introduction:** The previous study from our group has identified that after peripheral nerve injury there is an increase of kynurenine in the plasma, which seems to be involved in the maintenance of neuropathic pain. However, the mechanisms which peripheral kynurenine (Kyn) mediates neuropathic pain is unknown. **Objectives:** The aim of the present study was to test the hypothesis that peripheral Kyn reaches the spinal cord and maintain neuropathic pain by KMO metabolism, leading to downstream nociceptive metabolites. **Methods:** Spared Nerve Injury (SNI) model of neuropathic pain was induced in C57BL/6 mice and the following test and methods were used: von Frey filaments nociceptive test, Real-time PCR and western blotting, inhibition of KMO activity and astrocytes primary cultures. This study was approved by the Local Ethical Commission in Animal Research (045/2013). **Results:** SNI-induced mechanical allodynia was associated with an increase in the expression of KMO in the spinal cord, mainly at day 10 and 14 after injury. KMO expression was restricted to spinal cord astrocytes. Functionally, pharmacological inhibitor against KMO injected intrathecally after SNI, reduced mechanical allodynia. Also, kyn injected systemically (i.v) promoted mechanical allodynia, which was reduced when KMO was pharmacologically inhibited. Primary cultured astrocytes stimulated with TNF increased the expression of activation cell makers including GFAP and also of KMO, also activated microglia is capable to activate astrocytes. Also in culture of human astrocytes stimulated with TNF increase the KMO expression. **Conclusions:** In summary, these results indicated that after peripheral nerve injury spinal astrocytes-expressing KMO plays a critical role in the development of neuropathic pain. In conclusion, these data reveal a previously unappreciated role for the kynurenine metabolic pathway as a critical link between peripheral nerve injury, spinal cord glial cells (astrocytes) and the maintenance of neuropathic pain.

#### **05.021 Photobiomodulation in the treatment of inflammation and muscular pain.**

Oliveira CGDO, Chacur M, Giorgi R USP, IBu

Muscular injury often occurs in sports, falls and work, including the youth, adult and elderly population. This kind of injury causes impaired pain and muscle function, triggering important functional limitation. In recent years, the application of photobiomodulation therapy (PBM) has been shown to be an interesting strategy to accelerate the process of tissue regeneration and reduce the release of inflammatory mediators. Several articles in the literature demonstrate that PBM has therapeutic properties in various musculoskeletal disorders. This proposal aims to understand the effect of photobiomodulation, using low level laser therapy (LLLT) and light emitting diode (LED), in the chronic muscle injury model (myositis). For this, we performed behavioral tests, measuring nociceptive and edematogenic responses and histology of muscle fibers. Male Wistar rats weighing 200-220 grams were divided into four groups: naive (control), CFA (injured), CFA + LLLT and CFA + LED, with three different energy intensities for both types of photobiomodulation, being LLLT 1.08J, 1.8J and 3J and for LEDs - 0.29J, 0.71J and 3J per session. PBM treatment was initiated six days after CFA injury, and had duration of 5 sessions on consecutive days. After the behavioral analysis, animals were euthanized, and muscle were collected for histology analysis. Statistical analysis was performed using GraphPad Software version 6, two-way ANOVA and Bonferroni post-test, the assumed significance level was  $p \leq 0.05$ . Our results demonstrated an improvement of mechanical, tactile and edematogenic response using for both LLLT and LED. However, in relation to the response to the thermal stimulus, no improvement of the nociceptive picture of the animals was observed. In addition, we observed a greater amount of inflammatory infiltrate in the injured group (CFA) compared to the control group (naive) in the histological analyzes. We suggest a beneficial effect of the treatment with photobiomodulation, and may also propose as an adjuvant in the treatment of patients with muscle pain. **Acknowledgement:** BrightPhotomediceand FAPESP

**05.022 Local effects of natural alkylamides from *Acmella Oleracea* and synthetic isobutylalkyl amide on neuropathic and postoperative pain models in mice.** Werner MFP, Souza LMD, Maria-Ferreira D, Luz BB, Nascimento AM, Cipriani TR, Dallazen JL UFPR

**Introduction:** *Acmella oleracea*(L.) R.K.Jansen (“jambu”), (family Compositae) is a native plant from Amazon region. We have previously demonstrated that the intraplantar (i.pl.) administration of an hexanic fraction (HF) rich in alkylamides from jambu flowers, and the synthetic isobutylalkyl amide (IBA) displayed anesthetic, antinociceptive and antiinflammatory properties at 0.1 µg/20 µL (Dallazen, JL et al. *Fitoterapia*, v. 131, p. 225, 2018; Dallazen, JL et al. *Inflammopharmacology*, p. 1, 2019). This study aimed to evaluate the local effect of HF and IBA on neuropathic (partial sciatic nerve ligation, PSNL) and postoperative pain (plantar incision surgery, PIS) models. **Methods:** The experiments were conducted in male Swiss mice (~30 g, CEUA/BIO-UFPR 1107). Previous (basal values: B) and seven days after (d7) to PSNL surgery, the mechanical (von Frey test) and cold (acetone-evoked evaporative cooling) allodynia, and changes in digital gait parameters (CatWalk® apparatus) were analyzed. Then, the animals were treated intraplantarly with vehicle (V: 0.002% tween 80 or 0.02% DMSO, 20 µL), HF or IBA (0.1 µg/20 µL) or intraperitoneally with gabapentin (GABA: 30 mg/kg) and evaluated for 6 h. Likewise, prior to PIS (basal values: B) and one day after surgery (Post: 24 h), the mechanical allodynia, heat hyperalgesia (hot plate, 52 ± 0.1 °C), and spontaneous nociception scores were evaluated. These parameters were analyzed for 3 h after topical treatment with vehicle (V: acetone, 20 µL), HF or IBA (0.1 µg/20 µL), or subcutaneously with morphine (MOR: 1 mg/kg). **Results:** PSNL produced an intense mechanical and cold allodynia by decreasing the paw withdrawal threshold (PWT) from 1.6 ± 0.3 g, to 0.05 ± 0.0 g and increase the total scores in acetone-evoked evaporative cooling from 0.0 ± 0.0 to 7.7 ± 0.6 on the d7 in vehicle group. The sham procedure did not induce mechanical and cold allodynia in mice. The systemic treatment with GABA completely abolished the mechanical allodynia for 5 h, and the local treatment with HF and IBA for 3 h, when compared to vehicle group. GABA and HF treatments reverted the cold allodynia for 3 h, and IBA for 2 h, comparing to vehicle group. The PSNL induced several changes in mice footprint and gait parameters: reducing the max contact, print area, stand duration and swing speed of paws, as well as increasing the swing duration at d7, comparing to its basal value (B). Sham-operated mice did not show changes on gait parameters. The treatment with GABA, HF and IBA, increased the max contact area, paw print, and stand duration after 0.5 h of treatments, when compared to the vehicle group. On the day after PIS (Post), mice developed mechanical allodynia and thermal hyperalgesia to heat revealed by decreasing of PWT from 1.4 ± 0.1 g (B) to 0.04 ± 0.0 g (Post), and 12.1 ± 0.4 s (B) to 4.5 ± 0.3s (Post), respectively, in vehicle group. The sham procedure did not modify mechanical threshold and heat latency. Systemic and topical administration of MOR and HF, respectively, reverted the mechanical allodynia and thermal hyperalgesia for 2 h, whereas IBA partially reverted the mechanical allodynia for 1 h. PIS also induced alteration on position and borne weight upon incised paw 24h post-incision increasing the spontaneous nociception scores in vehicle treated group from 0.0 ± 0.0 (B) to 17.0 ± 1.6 scores (post). Only MOR and HF treatments reduced the total scores for 2 h after their administration. **Conclusion:** Collectively, the local treatment with natural alkylamides from jambu flowers (HF) was effective on both neuropathic and postoperative pain model, instead of IBA, which only demonstrated effect on neuropathic pain. Thus, our findings showed that alkylamides from jambu and IBA are suitable target for drug development and treatment of pain. **Financial support:** CAPES: Finance Code 001. **Keywords:** Jambu; Isobutylalkyl Amide; Partial Sciatic Nerve Ligation; Plantar Incisional Surgery; Allodynia; Digital Gait Analysis;

**05.023 Antinociceptive activity of Priprioca (*Cyperus articulatus* var. *nodosus* L.).** Moraes WP<sup>1</sup>, Pereira AMNP<sup>1</sup>, Pires TM<sup>1</sup>, Moraes JC<sup>1</sup>, Almeida Júnior JS, Lopes JMC, Barata LESB, Saroratto AS<sup>2</sup> Ufopa, Unicamp

**Introduction:** Medicinal plants are one of the best sources of bioactive compounds that are candidates for new drugs. Among them, stands out *Cyperus articulatus* L., native of the Amazon, in which its essential oil is widely used in traditional medicine. Considering ethnopharmacological applications, this study aims to investigate the chemical composition and to evaluate the antinociceptive activity of essential oil of *Cyperus articulatus* L (OECA) in experimental models of nociception in mice. **Methods:** The botanical material was collected in the Tabocal region in the municipality of Santarém-PA. The botanical identification was performed by Dr. Antônio Elielson Sousa da Rocha. Analysis of the chemical composition of OECA was performed on an Agilent gas chromatograph, HP-6890 model equipped with an Agilent mass sorting detector, model HP-5975 using an HP-5MS capillary column. Acute oral toxicity was assessed according to OECD protocol 423/2001. The animals were orally treated with OECA at three different doses (10, 100 and 400 mg/kg), defined according to the acute oral toxicity test. These doses corresponded to 1/200, 1/20 and 1/5 of the maximum dose (2000 mg/kg) used in the acute toxicity test. Morphine (10 mg/kg) was used for comparative antinociceptive effect. The negative control was treated vehicle (Tween 80 and 0.9% SF). The volume administered in all tests was 1 ml/100 g of animal weight. The antinociceptive activity followed the protocols of hot plate, abdominal and formalin contortion tests. This project was submitted to the Ethics Committee on the use of animals of UFOPA - CEUA / UFOPA was approved under protocol No. 07004/2013. **Results:** GC-MS analysis identified 40 chemical compounds corresponding to 88.43% of the constituents present in OECA. Mustacona (10.65%) is the major component followed by  $\beta$ -selinene (8.45%), cyclochloroethylene (6.99%) and  $\alpha$ -copaene (6.57%). There was no death and no animals showed signs of toxicity at the dose of 2,000 mg/kg, which makes it possible to classify OECA as a low acute toxicity product with a toxic dose higher than 2000 mg/kg. OECA showed significant antinociceptive activity, increasing the pain threshold induced by chemical substances (acetic acid and formalin) and thermal stimulation. In the hot plate test, OECA significantly prolonged the latency time at 60, 90 and 120 minutes. The abdominal writhing test, the treatment with OECA at 10, 100 and 400 mg/kg significantly reduced the number of abdominal writhing in 24%, 39% and 50% respectively when compared to the control group. In formalin test, OECA at doses of 100 and 400 mg/kg significantly inhibited the paw licking time in the first phase in 42% and 53% respectively, when compared to the control group. In the second phase of the test OECA at doses of 100 and 400 mg/kg significantly inhibited the paw licking time by 42% and 52% respectively, when compared to the control group. Naloxone at the dose of 5 mg/kg inhibited the antinociceptive effect of morphine and OECA (400 mg/kg) on formalin-induced nociception by decreasing the paw-licking time in mice at both phases of the test. **Conclusion:** In conclusion, the results obtained in the present study evidenced that the OECE consists mainly of sesquiterpenes and monoterpenes. The safe dose is less than 2.000 mg/kg administered orally. OECA has antinociceptive activity increasing the pain threshold induced by chemical substances and thermal stimulation. **Keywords:** Priprioca, *Cyperus articulatus*, medicinal plant, pain. **Financial support:** Ufopa



**05.024 Activities of neurons and glial cells are increased after hyperalgesia induced by platelet releasate by mechanisms dependent on P2X7 purinergic receptors.** Giorgi R<sup>1</sup>, Bom AOP<sup>1</sup>, Francisco KM<sup>1</sup>, Campos ACP<sup>2</sup>, Santoro ML<sup>1</sup>, Pagano RDLP<sup>2</sup> <sup>1</sup>IBu, <sup>2</sup>Hospital Sírio Libanês

**Introduction:** Prior studies developed by our group demonstrated that both whole platelets and platelet releasate (PR) evoked mechanical hyperalgesia in rats evaluated by paw pressure test, suggesting that platelets play a primordial role in the genesis of inflammatory pain (YAMASHITA KM. J Thromb Haemost, 9, 2057, 2011). Recently, we have demonstrated that prostanoids, sympathomimetic amines, TNF $\alpha$  and IL-1 $\beta$  cytokines and B1 and B2 bradykinin receptors, NK1 and NK2 tachykinin receptors, and P2X7, P2X3 and P2X2/3 purinergic receptors participate of the peripheral mechanisms involved in the mediation of PR-induced hyperalgesia. However, the central mechanisms involved in hyperalgesia evoked by PR are until unknown. In the present study, we investigate the effects on the central nervous system during hyperalgesia induced by PR, evaluating the activity of neurons, astrocytes and microglial cells in the dorsal horn of the spinal cord. Considering that purinergic receptors interfere with glial cells activity and the peripheral blockade of P2X7 receptor reverses the hyperalgesia induced by PR, the involvement of this receptor in mediating the central effects observed will also be investigated after i.p.l administration of PR. **Methods:** To evaluate hyperalgesia, male Wistar rats were submitted to the paw pressure test before (initial measure) and two hours after (final measure) injection of PR (100 $\mu$ l/paw; 200x10<sup>9</sup>platelets/L). Also, animals injected with 50 $\mu$ L P2X7 antagonist (300 $\mu$ g/paw, A438079), followed by 50 $\mu$ L PR, were equally evaluated. Control animals received sterile saline or Tyrode under the same conditions. After the test, animals were submitted to euthanasia, spinal cord segments (L4-L6) were obtained and prepared for immunohistochemical staining of neurons (EGR-1, 1: 1000), astrocytes (GFAP, 1: 1000) and microglia (Iba-1, 1: 1000). Ethical Committee Protocol: 1108/13;1270060519. **Results:** The results showed that during the peak of hyperalgesia induced by PR an increase in the activity of neurons, astrocytes and microglia was observed on both sides of the dorsal horn of the spinal cord, when compared to the saline or Tyrode group. Moreover, in the three cell types evaluated, the ipsilateral side of the injection demonstrated a significantly higher marking pattern when compared to the contralateral side of the dorsal horn of the spinal cord. Peripheral P2X7 purinergic receptor blockade totally reversed the PR-induced hyperalgesia and the bilateral increase in activation of neurons and glial cells. **Conclusions:** Taken together, the data obtained previously with the results presented here suggest that both peripheral and central mechanisms are involved in the genesis of hyperalgesia evoked by platelet releasate. In addition, demonstrate that greater activity observed on the CNS during hyperalgesia is mediate by mechanisms that depend, at least in part, on the peripheral P2X7 purinergic receptors. **Acknowledgement:** Fundação Butantan, FAPESP (2012/24621-1)

### 05.025 Therapeutic action of bergenin in an animal model of diabetic neuropathy.

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**Introduction:** Diabetes is a metabolic syndrome affecting millions of people worldwide<sup>1</sup>. Peripheral neuropathy is a frequent complication of diabetes, leading to sensorial alterations such as paresthesia, anesthesia and neuropathic pain<sup>2,3</sup>. Bergenin is a natural bioactive compound that presented antioxidant as well as antinociceptive properties in pre-clinical trials<sup>4</sup>. For this reason, this study aimed to evaluate the therapeutic effects of bergenin from *Cenostigma gardnerianum* on experimental diabetic neuropathy. **Methods:** Diabetic neuropathy was induced in C57Bl/6 mice (CEUA-FIOCRUZ/IGM 022/2015) by daily injections of streptozotocin (STZ, 80 mg/kg, i.p.) for three days. Four weeks after STZ injections, mice were once or daily (14 days) treated with bergenin (3.125 - 50 mg/kg) or vehicle (control). Glycemia, body weight and mechanical nociceptive threshold (von Frey filaments) were evaluated throughout the experimental period. Cytokine levels, iNOS mRNA and antioxidant factors expression were measured by ELISA and RT-PCR. Cytotoxicity and nitrite levels were evaluated in stimulated J774 macrophages by the Alamar Blue and Griess assays, respectively. All data are presented as means  $\pm$  standard error of the mean (S.E.M) of measurements made on six animals in each group. Statistical analysis of the weight was performed using one sample Student t-test. Behavioral data were analyzed using two-way ANOVA (group and time) followed by Bonferroni's multiple comparisons. Remaining data were analyzed using one-way ANOVA followed by Tukey's post-test. Differences were considered statistically significant for p values <0.05. **Results:** STZ-induced neuropathy was characterized by the reduction of nociceptive thresholds, which was reversed in mice treated with a single dose of bergenin (3.125 - 50 mg/kg) for up to 8 h. Daily treatment with bergenin at 25 mg/kg reversed the mechanical allodynia throughout the experimental period with consistent efficacy, suggesting the absence of tolerance development. Diabetic mice developed hyperglycemia (>250 mg/dL), although their body weight was not altered. Bergenin had no effect on glycemic levels, indicating that its antinociceptive effect is not due to glycemic control. The *in vitro* assay corroborated the antioxidant action, considering that bergenin (12.5 - 100  $\mu$ M) reduced nitrite production by stimulated J774 macrophages, with no cytotoxic effect. In both spinal cord and sciatic nerve of diabetic mice, the levels of TNF- $\alpha$  and IL-1 $\beta$  were reduced while those of TGF- $\beta$  were increased by daily treatment with bergenin (25 mg/kg), as shown by ELISA. The expression of iNOS was reduced, while Nrf2 and glutathione peroxidase mRNA were enhanced, in the dorsal root ganglion and spinal cord of neuropathic mice daily treated with bergenin. **Conclusion:** Daily treatment with bergenin was able to reverse the behavioral signal of sensory diabetic neuropathy, probably due to its immunomodulatory and antioxidant properties. **Financial support:** CNPq and CAPES. **References:** 1 Saklayen, M. G. *Curr. Hypertens. Rep.* v.20, 2018. 2 Schreiber, A. K. *World J. Diabetes.* v. 6, 432. 2015. 3 Evangelista, A. F. J. *Neuroinf.* v. 15, 189. 2018. 4 Oliveira, C. M. de. *J. Nat. Prod.* v. 74, 2062. 2011.