# ABSTRACTS


# 49th Brazilian Congress of Pharmacology and Experimental Therapeutics

**Ribeirão Preto Convention Center**
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## 15. Pharmacology: Other

15.001 Nanocarrier-mediated cutaneous co-localization of chemotherapeutic agents and efficacy in 3D **models of skin câncer**. Dartora VFC[1], Lemos DP[1], Zanoni TB[2], Maria-Engler SS[2], Costa-Lotufo LV[1], Lopes LB[1] [1]ICB-USP – Farmacologia, [2]FCF-USP – Análises Clínicas e Toxicológicas

**Introduction:** In spite of the high incidence of skin cancer, safe and self-administered, pharmacological strategies capable of localizing drugs in cutaneous tumors while avoiding systemic exposure and adverse effects are lacking. In this study, multifunctional nanoemulsions for topical use were developed, and their ability to co-localize paclitaxel and C6 ceramide in viable skin layers and improve efficacy in 3D models of skin cancer were studied. **Methods:** Nanoemulsions (NE) were prepared by sonicating the surfactant (Tween 80), oil (tributyrin:oleic acid:miglyol) and aqueous phases, and the formulation was subsequently characterized for droplet size and zeta potential. To evaluate whether drugs co-encapsulation in the nanocarrier improved cytotoxicity, the viability of human melanoma cells (SK MEL 19) plated at 10.000 cell/well in 96 well plates was assessed using MTT after treatment with the unloaded NE, or NE containing ceramide, paclitaxel or both drugs for 24h. Co-localization of the drugs in the skin layers was assessed *in vitro* in porcine skin using fluorescence microscopy after topical treatment for 8h. To verify whether the nanoemulsion with the drugs would be efficacious in the treatment of skin tumors, its cytotoxic effects were studied in 3D models of melanoma. Tissues were topically treated or untreated for 48h with the loaded nanocarrier, and histological analysis was performed. **Results:** The NE displayed size of 55 □ 0,8 nm and slightly negative zeta potential (-6,12 □ 0,24 mV); these characteristics were not affected by drug incorporation. The concentrations of paclitaxel and C6 ceramide necessary to reduce cell viability to 50% decreased approximately 6- and 15-fold, respectively, after incorporation of each drug individually in the NE compared to solutions in DMSO. A further lowering of these concentrations by 4-fold was observed when the drugs were co-encapsulated in the nanoemulsion compared to their use separately, suggesting the potential benefit of the nanocarrier as well as drug combination to improve efficacy. Calculation of “combination index” indicated a synergistic effect between the drugs. An increased localization of the drugs in the viable layers of epidermis was observed after topical administration of the NE compared to drug solutions. Topical administration of the nanoemulsion on 3D bioengineered models of melanoma promoted marked epidermis destruction, with only few cells remaining in this layer. **Conclusions:** Our results support the benefit of the nanocarrier to improve the cytotoxicity of each drug individually against cancer cells compared to their solutions, while co-encapsulation of the drugs in the nanocarrier synergistically improved formulation cytotoxicity. Topical delivery of the nanocarrier promoted skin co-localization, and a marked epidermal destruction was observed in 3D models of melanoma, which demonstrates not only the efficacy of the formulation, but also the importance of localizing the drugs within cutaneous lesions to avoid adverse effects. **Acknowledgements:** This study was supported by FAPESP (grants 2013/16617-7 and 2014/24400-0). V. Carvalho received a CAPES Fellowship.

**15.002 Systemic injection of biperiden reduces ethanol consumption in rats**. Zaniboni CR[1], Moreira JF[1], Santos MG[1], Leão RM[2], Galduróz JCF[3], Cruz FC[1] [1]Unifesp-EPM – Farmacologia, [2]UFBA – Farmacologia, [3]Unifesp-EPM – Psicobiologia

Alcohol addiction is considered a complex behavioral phenomenon and a huge world health problem (WHO, 2014). Studies demonstrate that ethanol reward effects involve the activation of the mesocorticolimbic system by the interaction with gabaergic, glutamatergic, dopaminergic and cholinergic system. Because of its complexity, there is not known an effective pharmacological treatment for ethanol addiction. Recent studies suggest that nicotinic and muscarinic cholinergic receptor affects dopamine levels on mesolimbic system and, consequently change the reinforcing value of the drug (MARK G.P. et al., 2011). Accordingly, it was demonstrated that systemic treatment with biperiden which is a cholinergic antagonist, M1 muscarinic, was capable to block the conditioned place preference induced by cocaine in mice (RAMOS, A.C. et al., 2012; ZACARIAS, M.S. et al., 2012). According to this, we examined whether systemic biperiden (1, 5 and 10mg/Kg; i.p.) was able to reduce ethanol self-administration in Long Evans rats. Animals were first trained to self-administrate in operant conditioning chambers (MARCHANT, N.J. et al., 2013) and the test consisted on a 6-h ethanol binge. On the test day, the animals received biperiden or saline intraperitonially and 30 minutes later, they were put in the self-administrated chambers and ethanol 10% consumption was registered for 6 hours. Results demonstrate that systemic biperiden (1 and 5mg/Kg) decreased ethanol consumption: Saline: 18.65±0,5; Bip 1mg: 7.82±1,5; Bip 5mg: 4.71±0,97; Bip 10mg: 8.79±1,47 ($p<0.05$). Data were presented as Mean ± SEM of ethanol consumption in g/Kg and analyzed by one-way Anova. In conclusion, our data suggest that biperiden may be a new potential drug to ethanol addiction treatment. **CEUA/UNIFESP nº:** 8583220517, **Financial support:** FAPESP 2013/24986-2 and FAPESP 2016/25894-2

**15.003 Effect of fluoxetine on autonomic responses of chemoreflex and baroreflex in rats submitted to chronic stress**. Firmino EMS, Kuntze LB, Lagatta DC, Resstel LBM FMRP-USP – Farmacologia

Stress is capable of altering autonomic regulation and respiratory reflexes in animals, such as baroreflex and chemoreflex activity. Thus, the present study aimed to evaluate if different types of chronic stress modulate the cardiovascular and respiratory responses activated by the baroreflex and chemoreflex and if the treatment with fluoxetine (FLX) is able to prevent the changes caused by chronic stress. For this, adult Wistar rats were used, which were submitted to repeated restraint stress (RRS) or varied chronic stress (CVS) for 14 consecutive days. The animals received chronic treatment with fluoxetine (FLX- 10mg/kg) for 21 days. The RRS increased the tachycardic components (control: -1.59 ± 0.18 e RRS = -2.45 ± 0.34) and bradycardic of the baroreflex (control: -2.07 ± 0.25 e RRS: -2.99 ± 0.35) however, the chronic treatment with FLX prevented the increase of sympathetic components (control: -2.33 ± 0.23; RRS: -3.39 ± 0.32; CVS: -2.56 ± 0.14) and parasympathetic (control: -1.98 ± 0.30; RRS: -2.57 ± 0.24 and CVS: -2.15 ± 0.37). The RRS and CVS also attenuated the magnitude of the pressor response to chemoreflex activation (control: 76.75 ± 2.250; RRS: 56.78 ± 2.373; CVS: 54.22 ± 1.714), which was prevented with treatment with FLX (control: 63.30 ± 2.872; RRS: 66.18 ± 2.910; CVS: 72.30 ± 1.521). Adicionaly, the stress protocols decreased baseline minute ventilation ($V_E$) (control: 1070 ± 73.48; RRS: 806.2 ± 44.67; CVS: 809.5 ± 51.69) tidal volume ($V_T$) (control: 8,914 ± 0.6505; RRS: 6.390 ± 0.2111; CVS: 6.709 ± 0.4243) in addition to increasing the magnitude of the respiratory rate ($\Delta f_R$) front the activation of the chemoreflex (control: 149.1 ± 12.5; RRS: 192.2 ± 15.90; CVS: 192.2 ± 9.816). The chronic treatment with FLX prevented alterations in the basal respiratory parameters of $V_T$ (control: 7.028 ± 0.2628; RRS: 7.270 ± 0.2284; CVS: 7.609 ± 0.4916). The findings of the present study demonstrate that chronic stress causes behavior of the depressive type, besides altering the autonomic baroreflex and chemoreflex responses, which can trigger pathologies in the cardiovascular and respiratory systems. **Financial Support**: FAEPA, CNPQ, CAPES, FAPESP This work was approved by the ethics committee with protocol number 175/2014

**15.004 Raltitrexate**: **A promising alternative for the treatment of toxoplasmosis in immunosuppressed patients**, Reis MP[1], Pauli KB[1], Lima DA[1], Moraes ALS[1], Lívero FAR[1], Lourenço ELB[1] [1]Unipar – Preclinical Investigation of Natural Products
**Introduction:** Toxoplasmosis is a worldwide infection caused by *Toxoplasma gondii*. The disease has great importance in public health since about 500 million people worldwide have positive serological reaction to the parasite, making it one of the most successful parasitic infections. In immunocompetent individuals, the parasite replication is controlled and a mild infection occurs. However, in patients with Acquired Immunodeficiency Syndrome (AIDS), the multiplication of tachyzoites becomes rapid causing a serious infection that if untreated can lead to death. Currently, pyrimethamine is used as reference drug in the treatment of toxoplasmosis. This drug inhibits the dihydrofolate reductase (DHFR), key enzyme to folic acid formation, that is used by *T. gondii* to replication. However, the growing development of parasite resistance mechanism in AIDS patients coupled with the lack of adequate sanitary conditions make the search for new prevention, diagnosis, treatment and management strategies extremely necessary. Thus, after the use of molecular modeling by homology and virtual doping, we identified that raltitrexate (RTX), an antineoplastic drug, has affinity for DHFR and could be a promising compound for the eradication of acute infections by *T. gondii*. **Methods:** Female Swiss mice were exposed to *T. gondii* RH strain. After 48 hours, mice were weight and divided into five groups ($n$ = 5-6) for the treatment with vehicle (negative control; C-); pyrimethamine (12.5 mg/kg; C+) or raltitrexate (0.15, 0.75 and 1.5 mg/kg), by oral route (gavage). Then, 48 hours after the treatment, the animals were submitted to euthanasia, by cervical dislocation. To count the number of tachyzoites in abdominal cavity, 10 ml of 0.9% NaCl solution was injected in peritoneum of mice and aspired with a syringe. After centrifugation (1000 rpm), the pellet was resuspended in 1 ml of 0.9% NaCl and tachyzoites were counted in Neubauer chamber. Liver, kidneys and spleen were collected to relative weight determination. One liver sample was collected to count the number of tachyzoites in the organ and other was collected to hematoxylin-eosin histological analyzes. **Results:** Treatment with RTX 1.5 mg/kg reduced the number of tachyzoites in peritoneal lavage and in liver imprint by 70% and 97%, respectively, when compared with C- (9.10 ± 1.06 tachyzoites/ml and 300.3 ± 3.43 tachyzoites/mm$^2$, respectively). In the same way, a reduction in the number of tachyzoites was found in C+ group. No differences were found in body or organ relative weights for all experimental groups. Regardless histopathological evaluation, no changes were observed in the tissue for all groups, except in the RTX 1.5 mg/kg group, in which cellular hypertrophy of hepatocyte was observed. **Conclusion:** The antineoplastic drug raltitrexate presented a significant antiparasitic effect, highlighting this drug as a possible therapeutic agent against *T. gondii* in HIV patients with pyrimethamine resistance. **Financial support**: CAPES, DEGPP-UNIPAR. **CEUA-UNIPAR:** The institutional committee for the animal care # 25452/2014 approved all the procedures.

**15.005 *Hibiscus acetosella* (Welw) hydroalcoholic extract cytotoxicity to epithelial cell**. Fontes VC, Castelo Branco SJS, Lima Neto LG, Zago PMW Universidade Ceuma

**Introduction:** *Hibiscus acetosella,* (Welw) is an African plant popularly known as purple vinegar, sage-okra, purple okra, gooseberry and garden guaxima. Preliminary studies showed the *Hibiscus* genus has high anti-inflammatory, anthelmintic and cardioprotective potentials, however, little is known about *H. acetosella* species pharmacological properties. Therefore, this study aimed to evaluate, *in vitro,* the toxicity of the crude hydroalcoholic *H. acetosella* extract in rat fibroblasts (3t3). **Methods:** Cells were inoculated into 96-well plates ($4x10^4$ cells / mL; DMEM/ 10% Fetal Bovine Serum, incubation at humid atmosphere, 5% $CO_2$). After a 24 h cell adhesion period, the culture medium containing *H. acetosella* at different concentrations (500; 50; 5; 0.5 µg / mL) was added to the wells. Cells were incubated for 48 h and submitted to MTT viability evaluation, subsequently. The experiments were performed in triplicate and with the appropriate controls. Data were submitted to analysis of variation with significance level of 5%. Results and conclusion: A viability of 98.8% (± 11.40%), 112.70% (± 4.60%), 107.19% (± 5.40%) and 81.70% (± 15, 60%) was obtained with the *H. Acetosella* concentrations of 0.5, 5, 50, 500 µg / mL, respectively. No statistical difference was found between the test groups and the control group. These preliminary data suggest that the *H. acetosella* hydroalcoholicis not cytotoxic to epithelial cells, however, further studies are required to confirm the obtained results.

**15.006 Cytotoxic effect of *Chenopodium ambrosioides* in epithelial cells** Castelo Branco SJS, Fontes VC, Lima Neto LG, Gonçalves LM, Zago PMW Universidade Ceuma

**Introduction:** *Chenopodium ambrosioides* (mastruz) is a native Central and South America plant, with antifungal, anti-inflammatory and wound healing effects previously described. Recently, the hydroalcoholic plant extract showed antimicrobial activity against *Candida albicans* biofilms developed on poly (methyl methacrylate) dental prostheses's surface. That antimicrobial effect induced the present study that aimed to evaluate, *in vitro*, the cytotoxic effect of the *Chenopodium ambrosioides*'s hydroalcoholic extract. **Methods:** Rat fibroblasts (3t3) were inoculated into 96-well plates ($4x10^4$ cells / mL; DMEM (Dulbecco's Modified Eagle Medium) / 10% Fetal Bovine Serum; incubation at humid atmosphere, 5% $CO_2$). After cell adhesion period, *C. ambrosioides* extract was added to cell medium at concentrations of 2500; 1250; 250; 50 and 25 µg / mL. Plates were incubated for 24 hours and subsequently submitted to MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) viability evaluation. The experiments were performed in triplicate and with the appropriate controls. Data were submitted to the analysis of variance with significance level of 5%. **Results and Conclusion:** A viability of 97.45% (± 12.84%), 124.56% (± 10.54%), 98.80% (± 6.60%), 110.36% (± 22.51%) and 109.77% (± 23.04%) was obtained with *C. ambrosioides* extract concentrations of 1250; 250 50; 25 and 10 µg / mL, respectively; not differing from control group viability ($p>0,05$). The highest concentration (2500 µg / mL) was cytotoxic to the cells (viability 17.3% ± 1.49%, $p <0.05$). These preliminary outcomes suggest that concentrations lower than 2500 µg / mL of the *C. ambrosioides* hydroalcoholic extract were not cytotoxic to epithelial cells; however, further studies are needed to confirm the present data.

**1515.007 Bioresponsive nanostructured systems for sustained drug release and treatment of alcoholism**. Santos R[1], Lopes LB[1], Rae M[1], Camarini R[1], Stainer A[2]
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**Purpose:** Development of delivery systems for treatment of drug addiction has attracted considerable attention due to the possibility of reducing the frequency of administration and increasing patient compliance. However, simple and safe formulations that can be self-administered are still needed. In this study, we have developed a fluid nanocarrier (microemulsion) capable of transforming into nanostructured gels *in vivo* upon uptake of biological fluids for naltrexone release, and evaluated (i) the *in vivo* kinetics of gel formation after subcutaneous administration, (ii) its ability to prolong drug release, and (iii) its efficacy to reduce the conditioned place preference (CPP) to ethanol in a behavioral model to study the rewarding effects of drugs. **Methods:** Microemulsions were prepared using monoolein as structure-forming surfactant in combination with water, tricaprylin and propylene glycol. *In vivo* phase transformation was assessed 2, 15 and 34 days after subcutaneous injection of 100 µL of the microemulsion in mice. To assess the formulation ability to provide sustained release, a fluorescent compound (Alexa fluor 647, 0.05% w/w) was encapsulated in the nanocarrier, and its *in vivo* release was evaluated using a bioimagining system for 34 days. Biological effects of the formulation were assessed using the CPP paradigm. Mice were distributed in four groups and received one of the following treatments: subcutaneous saline injection (control), daily subcutaneous naltrexone injection (1 mg/kg, eight days) or subcutaneous administration of the microemulsion containing naltrexone (100 µL, single administration) at 5 or 10% (w/w); ethanol (2g /kg) or saline were administered intraperitoneally before CPP testing. **Results:** The microemulsion displayed nanometric size (30.2 nm), and transformed into hexagonal nanostructured gels *in vivo* approximately 48 h after subcutaneous administration. The gel persisted locally for 34 days without further phase transformation, and release of the encapsulated fluorescent compound was sustained during this period. For comparison, release of the fluorescent compound from a propylene glycol solution lasted only 10 days, demonstrating the ability of the gel to prolong drug release. Subcutaneous daily administration of the naltrexone solution decreased the CPP to ethanol, but a more robust effect was observed following administration of a single dose of the microemulsion containing 10% of naltrexone. **Conclusions:** A single subcutaneous administration of the microemulsion resulted in a hexagonal phase gel capable of prolonging the release of a fluorescent compound for over 30 days and decreased ethanol-induced CPP, supporting its use as a platform for sustained release of drugs useful in the treatment of alcoholism. Acknowledgements**:** The authors are grateful to CNPq (grant#443549-2014-1) and FAPESP (2013-16617-7) for **Financial Support**. Protocol for animal use approved by the Institute of Biomedical Sciences Institutional Animal Care and Use Committee (protocol number 72, p.20, book 3) **Key words:** nanocarrier, sustained release, naltrexone, alcoholism**.**

**15.008 Dietary fatty acids content modifies memory parameters in rats**: **Comparative study between Mediterranean- versus western-based diet**. Haygert PF, Roversi K, Milanesi LH, Vey LT, Duarte MMF, Burguer ME UFSM – Farmacologia

**Introduction:** The balanced intake of fatty acids (FA) of both omega-6 (n-6) and omega-3 (n-3) series exerts an important rule on cognitive development [1]. Studies have shown beneficial influences of the Mediterranean diet (MD), which is rich in polyunsaturated fatty acids (PUFA) n-3 and a low n-6/n-3 PUFA ratio [2,3]. On the other hand, Western diet (WD) contains saturated fats such as hydrogenated vegetable fat (HVF, rich in *trans* -TFA) and interesterified fat (IF, rich in interesterified FA), making high the n-6/n-3 PUFA ratio. Due to the health damages caused by TFA, *trans* fat have been replaced by IF in the processing of foods, which has low levels of TFA [4]. Here we compared a Mediterranean-like diet (MD- n-6/n-3 PUFA ratio about 1) with two different Western-like diets (WD1 and WD2- n-6/n-3 PUFA ratio above 10), on learning process *per se* and following scopolamine (SCO) administration, which is a inductor of cognitive impairment in animals. **Methods:** Male Wistar rats (21 days old) were fed with one of three different diets: balanced diet (MD, fish oil plus soybean oil 1:1 20% in XXX ) or two Western diets (WD1- HVF-20% or WD2- IF 20% in XXX). On post-natal day (PND) 90, half of the animals were injected with scopolamine (SCO- 1.0mg /kg i.p.) or saline (0.9% NaCl). After 30 min of this administration, animals were evaluated in the Novel Object Recognition Task (NORT) and Water Maze (WM). All animals were anesthetized and euthanized 24h after behavioral evaluations for quantification of pro and anti-inflammatory factors in plasma and hippocampus. **Results:** WD1- andWD2-fed rats showed decline *per se* in the NORT recognition index in comparison to MD diet, whose impairment was intensified after SCO exposure. In addition, while WD1 and WD2 showed higher latency time *per se* to find the platform in the WM task, after SCO administration no difference among the experimental groups was observed. Both Western diets showed increased plasma levels of IL 1β and IL6 together with decreased IL-10 in relation to MD group. SCO administration increased IL1β in MD group, however this proinflammation marker stayed lower than those observed in WD1 and WD2. **Conclusion:** Our findings allow us to suggest that the type of dietary FA exerts pivotal on influence on the susceptibility to neurological disorders development such as cognitive impairment. Here, we demonstrating that the current replacement of HVF by IF in the Western diet is not beneficial to health, since both exert deleterious effects on memory , thus increasing proinflammatory cytokines, in opposition to FA from Mediterranean diet. **Financial support:** Authors are grateful to CNPq, CAPES and PRPGP/UFSM (PROAP) for the fellowships and financial support. The authors are grateful to Tiaraju® for their donation of fish oil capsules. The experimental protocol was approved by the CEUA/UFSM committee (nº 9373231116/2016). [1] HARDMAN,et al. **Front Nutr**., v.22, p.3-22,2016. [2] SIMOPOULOS, A.P. **BiomPharm.**,v. 60, p. 502-5072006. [3] MORRIS, etal.**Alzh e Dem**.,v.11, p.1007-1014,2015. [4]MAGRI, et al. **ClinNutr.**, v.14, p.242-248,2015.

**15.009 Determination of manganese in blood using graphite furnace atomic absorption spectroscopy**. Carrara MYW, Jacobucci SR, Trape AZ, Garlippi CR, Rosa PCP Unicamp – Farmacologia

**Introduction:** Manganese is an essential metal that participates in several metabolic functions, cellular, being able to be a cofactor of enzymatic reactions or even to be part of metalloenzymes such as: arginase, glutamine synthetase, manganese superoxide dismutase and enolpyruvate decarboxylase. The main sources of manganese intake are vegetables, grains, fruits, nuts and teas. The excess of this in the body, however, can be harmful to health. This occurs mainly in occupational exposures such as in battery industries, metallurgies that occur in smelting and welding processes, and even in plantations where organometallic pesticides are used. [1] Manganese in the body is mainly concentrated in the pancreas, liver, kidneys, intestines And has high ability to cross the blood-brain barrier. The brain is considered the most sensitive organ to prolonged exposure to metal and results in neurotoxicity. The first clinical manifestations are headaches, insomnia, memory loss, emotional instability, dystonia.[2] There are studies that demonstrate results from workers exposed to manganese in mining companies, in welding works, but little is said about exposure to agrotoxics in Brazil . For that, a method of analysis for the determination of manganese in blood and its validation was developed, according to the parameters of RDC 27/2012 of Anvisa, which deals with bioanalytical tests. To date, no studies on the determination of Mn in blood validated according to this standard have been found in the literature. Method: For determination of Mn, a graphite furnace atomic absorption spectrometer equipped with an automatic sampler and Zeeman background corrector.350µL of whole blood were diluted in a solution containing a mixture of 0.01% Triton-X 100 (V / V) and 0.2% nitric acid (V / V) until the final volume reached 10mL. From this preparation were injected 10µL in the graphite furnace under the following conditions: wavelength: 279,5nm, pyrolysis temperature: 900 oC, atomization temperature: 1800 oC and the flow rate of nitrogen gas at 0,2L / min. Through the experimental planning, the best analytical conditions will be evaluated, involving the following factors: pyrolysis temperature, atomization temperature, heating time and use of modifiers. And then, for the validation will be evaluated the parameters of selectivity, accuracy, residual effect, calibration curve, precision and stability. **Results:** Initial data indicate that there is no possibility of using a substitute matrix to the whole blood to make the calibration curve. The most appropriate working range was 1 to 7 ppb, with repeatability and proportional response to blood sample concentrations, and adequate recovery for the range studied. The next steps will confirm the best conditions for the quantification of the serum level of manganese, validation and analysis of the samples. **Conclusion:** The analytical conditions defined for the method were the best found so far because of the greater sensitivity and analytical efficiency.References: 1 Casarett,Toxic.Bas.Scie.of Poisons,955,2007. 2 ATSDR,2012 Research approval by the Human or Animal Research Ethical Committee: 56715216.9.0000.5404 **Financial Support**: Faepex Unicamp, Syngenta and Ihara

**15.010 Structure-activity relationship between cardenolides and bufadienolides for the inhibition of porcine kidney $Na^+K^+$-ATPase and the antagonistic effect of $K^+$.** Azalim PN[1], Rendeiro MM[1], Leitão SG[2], Quintas LEM[1], Noël F[1] [1]UFRJ – Farmacologia Bioquímica e Molecular, [2]UFRJ – Farmácia

**Introduction:** The $Na^+$,$K^+$-ATPase (NKA) is an integral membrane protein present in all eukaryotic cells and a member of the P-type ATPases. It is classically described as responsible for maintaining the cell's electrochemical gradient. The main inhibitors of NKA are cardiotonic steroids (CTS), characterized by a steroidal nucleus in configuration Cis-Trans-Cis, a lactone ring of five carbons (cardenolides) or six carbons (bufadienolides) at C17 and, for some of them, an osidic portion at C3. Laursen et al. (Proc. Natl. Acad. Sci. USA, 112:1755, 2015) showed that bufalin (a bufadienolide) was insensitive to the antagonistic effect exerted by potassium ($K^+$), differently from the cardenolide digitoxigenin. The objective of present work was to evaluate 13 CTS, among cardenolides and bufadienolides, to establish a structure-activity relationship (SAR) regarding the inhibitory effect on porcine kidney NKA and its sensitivity to the antagonistic effect exerted by $K^+$, aiming mainly to verify if there is a class difference between cardenolides and bufadienolides. **Methods:** The ATPase reaction was initiated by adding 50 µg protein of a porcine kidney preparation to the medium containing (in mM) NaCl 87, $MgCl_2$ 3, $ATPNa_2$ 3, EGTA 1, $NaN_3$ 10, Maleate-Tris 20 (pH 7.4 at 37 °C) and different concentrations of the CTS. After 2 hours, the reaction was stopped and the inorganic phosphate liberated from ATP hydrolysis was measured by the Fiske and Subbarow colorimetric assay. The antagonistic effect exerted by $K^+$ was studied by comparing the $IC_{50}$ values of the CTS at two different concentrations of KCl (1 and 50 mM). The experiments were performed in triplicates and repeated three times. **Results:** SAR at low $K^+$ concentration: 1. Osidic portion: For the cardenolide genins (without osidic portion) the inhibitory potency was inversely proportional to the number of hydroxyls present in the steroidal nucleus, as indicated by the $IC_{50}$´s measured at 1 mM $K^+$ (ouabagenin: 319 nM; digoxigenin: 134 nM; digitoxigenin: 88 nM) whereas the opposite was observed in the presence of the osidic portion (ouabain: 38 nM; digoxin: 129 nM, digitoxin: 190 nM). 2. Cyclization at C14: the presence of the epoxy differently affected the potency of bufadienolides, with emphasis for a dramatic increase of the $IC_{50}$ of marinobufagin (824 nM) compared to telocinobufagin (74 nM). 3. Lactone ring: at least for digitoxigenin and bufalin, the number of carbons in the lactone ring had no effect on the $IC_{50}$ (88 and 75 nM, respectively) at 1 mM $K^+$. Antagonistic effect of $K^+$**:** As expected, all 7 cardenolides were sensitive to the antagonist effect of $K^+$, since their $IC_{50}$´s were increased 7.2-11.5 times when incubated in the presence of 50 mM KCl (only 3.5 times for digitoxin). By contrast, the bufadienolides were poorly sensitive to the $K^+$ effect, since their $IC_{50}$´s increased only 1.7-2.9 times (4.0 times for resibufogenin). **Conclusion:** The insensitivity to the antagonistic effect exerted by $K^+$ initially reported for bufalin appears to be a class characteristic of the bufadienolides as a whole. **Financial Support:** FAPERJ, CNPq, CAPES. **Animal Research Ethical Committee**: no needed (kidneys obtained at slaughterhouse).

**15.011 Development and evaluation of the cytotoxic potential of topical nanocarriers co-encapsulating endoxifen and metformin for breast cancer chemoprevention** Lemos DP[1], Giacone DV[1], Lotufo LV[1,2], Lopes LB[1,3] [1]ICB-USP – Farmacologia, [2]UFC, [3]Albany College of Pharmacy and Health Sciences

**Introduction:** Even though breast cancer is responsible for the death of a large number of women worldwide, there are no widely accepted chemoprevention strategies. Considering the location of the breast tissue and the need for therapeutic drug concentrations only locally in the breast in high-risk groups, we developed topical nanocarriers co-encapsulating metformin and endoxifen for local delivery to the mammary tissue and chemoprevention of cancer. After nanocarrier development and selection, its cytotoxic potential against cancer cells was assessed. **Methods:** For development of oil-in-water nanoemulsions, polysorbate 80 and a poloxamer solution were employed as surfactant and aqueous phase, respectively, while various combinations of monocaprylin, tricaprylin, oleic acid, tributyrin were tested as oil phase. After selection of a mixture of oleic acid and tricaprylin as oil phase, nanoemulsions were obtained by probe sonication, and characterized for size and zeta potential. Metformin and endoxifen were co-encapsulated at 1% (w/w). The cytotoxic potential was assessed in MCF-7 cells plated at 10.000 cell/well in 96 well plates using MTT after treatment with the unloaded nanocarrier (0.016-2 mg/mL), or the nanocarrier containing endoxifen, metformin or both drugs for 48h. **Results:** The selected nanocarrier displayed a small size (33.52 ± 2.51 nm) and slightly negative zeta potential (-6.55 ± 1.57 mV). While drug incorporation did not affect the nanocarrier size, a zeta potential inversion (to +7.89 ± 0.45 mV) was observed. Compared to the endoxifen solution in DMSO, its nanoencapsulation shifted the cell viability curve to the left, and the drug concentration necessary to reduce cell viability to 50% decreased approximately 6-fold. Encapsulation of metformin had a smaller impact on cell viability, and the effect of the nanoemulsion containing metformin was very similar to the unloaded nanocarrier. Compared to the nanoemulsion containing only endoxifen, a further reduction on cell viability was observed when this drug was co-encapsulated with metformin (2.5-fold). **Conclusion:** Stable nanoemulsions capable of co-encapsulating endoxifen and metformin were obtained. Nanoencapsulation of endoxifen individually, but not of metformin, decreased the drug concentration necessary to reduce cell viability to 50% compared to its solution. Co-encapsulation of both drugs in the nanocarrier further improved cytotoxicity, demonstrating the benefit of drug combination. **Acknowledgements:** This study was supported by FAPESP (2013/16617-7) and CNPq (443549-2014-1). D. de Lemos received a CAPES fellowship, and D. Giacone, a FAPESP fellowship (2016/04913-9).

**15.012 Development and validation of a stability indicating method to determination of Cloxazolam and its degradation products by HPLC-DAD**. Resck RR, Rosa PCP FCM-Unicamp – Farmacologia

For the determination of the shelf life of a drug product, a stability study under controlled conditions of temperature and humidity is required, where it is verified whether the physical, chemical, biological and microbiological characteristics of a pharmaceutical product remain within defined specifications during the period of use.[1] For this, it is necessary to have methods that are able to detect and quantify possible changes in the formulation. These methods are described as stability indicating **Methods:**[2] Cloxazolam is a benzodiazepine used in the treatment of anxiety and commercially available as tablets at dosages of 1, 2 and 4 mg [3], where in the last 5 years the shelf life has been reduced by Anvisa. The objective of the present work was to develop and validate a stability indicating method by HPLC-DAD for the quantification of Cloxazolam along with its degradation products in Cloxazolam tablets, which is compatible with LC-MS. The final chromatographic method was achieved using Luna Phenyl-Hexyl column (150 x 4.6 mm, 3 µm), eluted through a gradient composed of 20 mM ammonium formate solution pH 8.6 and acetonitrile at a flow 1.0 mL/min. The wavelength used was 244 nm and the oven temperature of the column employed was 35 °C. The stress test of the drug substance, drug product and placebo was evaluated for the selectivity of the method. The conditions evaluated were acid and basic hydrolysis, oxidation by peroxide and metal ions, dry and moist termal stress, and photolysis. The major degradation products obtained under the degradation conditions employed were analyzed by LC-MS to have their structures identified. Validation was conducted according to ICH Q2 Guide, using the work range of 0.0004 mg/mL to 0.00096 mg/mL for the evaluation of impurities and of 0.32 mg/mL to 0.48 mg/mL for the evaluation of the main peak. The method presented suitable chromatographic resolution between the most relevant peaks and satisfactory results of peak purity in all tested stress conditions. In linearity, the results obtained presented $R > 0.99$ and $R^2 > 0.98$ in both working ranges. The accuracy showed recovery results between 95.0 to 105.0% for the impurities and between 98.0 to 102.0% for the main peak. In precision, the RSD results obtained were lower than 2.0%. The method was robust in all the modifications employed. Therefore, it is concluded that the proposed method is adequate, safe, effective and indicative of stability for the quantification of Cloxazolam and its degradation products in Cloxazolam tablets. Support CNPq.

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**15.013 Evaluation of the antimicrobial activity of phenylacrylates series** (**AL**)
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**Introduction:** Phenyl acrylate esters are derivatives formed by the Knoevenagel condensation reaction, reacting an aromatic aldehyde and ethyl 2-cyanoacetate in the presence of organic bases as catalysts. Although important intermediates in synthesis, they are still little known as to their biological properties. **Methods:** In this work, the ethyl-2-cyano-3-phenylacrylates (**AL**) series: AL01, AL02, AL09, AL10, AL11 e AL12 were tested against Gram-positive (bacteria *Staphylococcus aureus* ATCC 25923 and *Staphylococcus epidermidis* ATCC 12228) and Gram-negative bacteria (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *Salmonella Enteritidis* INCQS 500258). The antimicrobial activity was evaluated quantitatively by the broth microdilution technique (CLSI, 2016) in 96-well plates, and the Minimum Inhibitory Concentration (MIC) was determined using the CTT solution (2,3,5-triphenyl-tetrazolium chloride) to 0.5% as a developer of bacterial growth. The tests were performed in triplicate, by serial dilution. The negative control used was aqueous solution of Tween 80 at 5% and the positive control (gentamicin) was performed in serial dilutions. The Minimum Bactericidal Concentration (MBM) was determined by sowing the contents of the wells in BHI agar, in order to observe the lowest concentrations that promoted total inhibition of bacterial growth. **Results**: For *S. aureus*, none of the samples exerted antibacterial activity. For *S. epidermidis*, samples **AL09** and **AL12** presented bactericidal effect, while the other samples were bacteriostatic. For *E. coli*, bacteriostatic action was observed for **AL02**, **AL10** and **AL11** samples. On the other hand, the other samples did not inhibit the growth of this bacterium. For *P. aeruginosa*, a bactericidal action occurred for **AL01** and **AL02**, while in the other samples, bacteriostatic action was observed. For *S. Enteritidis*, **AL02** presented bactericidal action and **AL12** showed to be bacteriostatic, unlike the other samples, which did not exert any antibacterial effect. **Conclusion:** The antibacterial activity of the phenylacrylate derivatives of the **AL** series, against the tested bacterias, is a promising result due to the demonstrated antibacterial potential. These results also corroborate new assays about the cytotoxicity and elucidation of possible molecular targets of the antibacterial action, as well as the advance in structure-activity relations (SAR) of these molecules, aiming at the availability of candidate molecules to antimicrobial drugs that aid in the treatment of infectious diseases. **Keywords:** Etil-2-ciano-3-fenilacrilatos; microdilution in broth; CIM; bactericidal activity.