

3º SINPOSPq - Simpósio Internacional de Pós-graduação e Pesquisa

Pós-Graduando: O que esperar do futuro?

O SINPOSPq, Simpósio Internacional de Pós-graduação e Pesquisa, foi fundado como parte integrante do Projeto de Internacionalização dos Programas de Pós-Graduação (Ciências Farmacêuticas, Biociências aplicadas à Farmácia e Toxicologia) da Faculdade de Ciências Farmacêuticas de Ribeirão Preto da Universidade de São Paulo (FCFRP-USP), criado em 2001, para estimular o intercâmbio científico com pesquisadores estrangeiros. O evento é organizado pelos pós-graduandos da faculdade, com apoio dos docentes.

Os principais objetivos do Simpósio são proporcionar a divulgação das pesquisas desenvolvidas nos três programas de Pós-Graduação da FCFRP-USP, bem como das novas tecnologias e metodologias empregadas relevantes às Ciências Farmacêuticas, promover discussões sobre publicações científica em periódicos nacionais e internacionais e, principalmente, proporcionar maior integração entre os alunos de graduação, pós-graduação e renomados pesquisadores do Brasil e do exterior. Para tanto, são realizadas palestras, conferências, mesas redondas e workshops, além da apresentação dos trabalhos científicos desenvolvidos pelos participantes.

O primeiro SINPOSPq foi realizado em 2004 e, desde então, acontece bienalmente. A edição do ano de 2008 comemora o aniversário de 20 anos da pós-graduação da FCFRP-USP, dando ênfase a seus três programas: Biociências aplicadas à farmácia, Ciências Farmacêuticas (Física Biológica, Medicamentos e Cosméticos, Produtos Naturais) e Toxicologia. As áreas de concentração abordadas pelo evento são Farmacologia e Fisiologia (FF); Parasitologia, Microbiologia, Imunologia e Hematologia (AC); Bioquímica, Genética, Biologia Celular e Molecular (BM); Produtos Naturais (PN); Bioinformática, Física e Química BiológicaS (FQ); Assistência Farmacêutica (AF); Ciências dos Alimentos e Nutrição (NU); Química Medicinal (QM); Análise e Tecnologia Farmacêuticas (TF); Toxicologia (TX).

Comissão Organizadora

AC 01 - ANTI-PHAGOCYtic MECHANISMS IN ATYPICAL ENTEROPATHOGENIC *Escherichia coli* (aEPEC)

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Introduction: EPEC has been associated with diarrhea in several countries. At present it is the most common bacterial agent in children endemic diarrhea in Brazil. The atypical EPEC (aEPEC) differs from the typical (tEPEC) one by not presenting the EAF plasmidium and is considered an emergent pathogen. The increase in diarrhea cases caused by aEPEC, as tEPEC diarrhea cases drop, evokes the capacity of adaptation and pathogenicity that this EPEC subtype is developing. Several virulent bacteria have evolved mechanisms to prevent phagocytosis. The interaction of tEPEC with phagocytic cells inhibits its uptake. This requires a functional type III secretion system and occurs via PI 3 kinase inhibition required for the re-assembly of the cytoskeleton and actin polymerization, both important in phagocytosis. Developing an anti-phagocytic mechanism seems to improve the colonization of the epithelial cells by enteric bacteria, by delaying the activation of the immune response. **Objective:** Investigation of the existence of an anti-phagocytic mechanism in aEPEC. **Methodology:** Macrophages interaction assays were performed with aEPEC isolates: 7(O55:H7); 32O(O55:H7) and tEPEC control (E2348/69) for 10, 30, 60 min infection pulses with different bacteria concentrations. The macrophages were treated with gentamicin and the number of intracellular bacteria was determined. **Results:** Isolate 7 (OD 0,6) was less phagocytosed after 10 and 30 min pulses with 20,3% and 49,9% of infection, respectively, while isolate 32O infected 76,3% and 81,9% and isolate E2348/69 infected 91,2% and 94,2% at the same times. Furthermore, in isolate 7, the number of bacteria adhered or phagocytosed per macrophage is significantly smaller than with isolates 32O and E2348/69. The kinetics of interaction with different bacteria concentrations (OD 1,2; 0,6; 0,06 and 0,006) showed that the anti-phagocytic effect of isolate 7 still occurs at much smaller concentrations, such as OD 0,006. In the 10 min infection pulse, both the bacterial interaction and the number of bacteria/cell were smaller than observed with the control isolate. In the 30 min pulse, only the number of bacteria/cell was smaller than with the control. Isolate E2348/69, with no anti-phagocytic effect under these conditions, showed less invasive after pre-incubation with isolate 7 supernatant in the 10 min infection pulse. **Discussion:** Isolate 7 may present a different anti-phagocytosis mechanism from tEPEC, which triggers the anti-phagocytosis effect after 2h of interaction, and not presented by the other aEPEC isolate studied. These results suggest that aEPEC isolate 7 seems to induce a different anti-phagocytic effect than that presented by the tEPEC, which depends on the adhesion of the bacterium to the macrophage. The existence of an anti-phagocytic mechanism may be of great advantage to the pathogen, since prevention or delay of the immune response may contribute to intestinal colonization.

Keywords: Atypical EPEC; Macrophages; Antiphagocytosis.

Financial support: FAPESP; CNPq.

AC 02 - ANTIBACTERIAL ACTIVITY OF JASMONIC ACID PRODUCED BY *Botryosphaeria rhodina*

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In the present time, practically all the species of bacteria present acquired resistance becoming bacteria problems in the anti-infectious therapeutical, increasing each time the search for new antimicrobial substances. Ahead of this fact it was objectified with this work to test crude extracts contain jasmonic acid (plant growth regulator, which comes being attributed diverse biological activities, amongst them the capacity to inhibit the development of human cancerous cells, inducing death in cells of breast carcinoma and prostate, melanoma, linfoma and leucemic cells) proceeding from the fermentation by *Botryosphaeria rhodina* from its antibacterial activity of *Staphylococcus aureus* ATCC 6538 and physician isolated; strains of *Escherichia coli* ATCC 25922 and physician isolated; and *Pseudomonas aeruginosa* ATCC and physician isolated. The effect of the antimicrobial activity was evaluated by the method of microdilution in medium BHI in plates contains 96 wells, NCCLS M7-A2 (1990) with small modifications, to adapt the protocol in order to evaluate the concentrations of the extract of *B. rhodina* that varied of 5mg the 312mg.L⁻¹. The concentration of inoculum was adjusted in specter using wave length of 550nm, in a band of absorbance of 0,10 the 0,15. After that, 100 µL of inoculum was used by well what it is equivalent 1x10⁴ CFU.mL⁻¹ of each bacterium assayed. The extract was diluted by serial dilutions in medium BHI. The MIC (Minimum Inhibitory Concentration) had been: 187,5 µg.mL⁻¹ for *E. coli* ATCC 25922 and physician isolated, 93,75 µg.mL⁻¹ for *P. aeruginosa* ATCC and physician isolated, 375 µg.mL⁻¹ for *S. aureus* ATCC 6538 and 187,5 µg.mL⁻¹ for *S. aureus* physician isolated. With these results concludes that the crude extract contain jasmonic acid presents high potential antimicrobials for tested bacterias except for the *S. aureus* ATCC 6538 for which the extract presented intermediate potential antimicrobials, evidencing that this extract can be used in the production of new antibacterial drugs.

Keywords: Jasmonic acid; Antimicrobial; *Botryosphaeria rhodina*.

Financial support: CAPES; UNAERP.

AC 03 - BIOFILM FORMATION BY NON PATHOGENIC *Escherichia coli* AND IN DIVERSE PATOTYPES OF DIARRHEAGENIC *E. COLI* STRAINS

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The infectious diarrhea is considered a major cause of infant mortality. Microorganisms belonging to the family Enterobacteriaceae are the main cause of intestinal infection, mainly *Escherichia coli*. Biofilms have been described as sessile bacterial communities that live attached to each other and to surfaces. It plays mechanisms of colonization, conferring bacterial resistance to the action of antibiotics and is also recognized as the source of persistent infection. The biofilm formation has been described in the *E. coli* strains that show the pattern of aggregative adherence (EAEC), as well as in strains of Shiga-toxin-producing *E. coli* (STEC), Enterotoxigenic *E. coli* (ETEC), Diffuse-adhering *E. coli* (DAEC), Enteropathogenic *E. coli* (EPEC), and in commensal *E. coli* K-12. The understanding of biofilm formation is essential to know their role in pathogenesis, enabling the development of alternative therapies. The aim of this study was to verify the capacity of biofilm formation by non pathogenic *Escherichia coli* and diarrheagenic *Escherichia coli* strains (Enteraggregative *E. coli*, typical and atypical Enteropathogenic *E. coli*, Enterotoxigenic *E. coli*, Shiga-toxin-producing *E. coli*, Enterohemorrhagic *E. coli* and Diffuse-adhering *E. coli*), in abiotic surfaces (polystyrene) and cellular surfaces. We analyzed the biofilm formation in 95 *E. coli*: 20 non pathogenic *Escherichia coli* strains and 75 diarrheagenic *E. coli* strains (ETEC, EAEC, aEPEC, tEPEC, DAEC, STEC and EHEC), isolated from children living in Salvador, Bahia. The strains were screened for biofilm production using colorimetric assay of violet crystal using polystyrene 24-well culture dishes and was quantified using enzyme immunosorbent assay plate reader in length of wave of 595 nm. The strains had been capable to form biofilm in polystyrene, regardless of their pathotypes. While 56.8% of the strains showed low biofilm formation (OD = 0 - 0.043), 36.9% of the strains showed intermediate formation (OD = 0.044 - 1.281) and only 6.3% had high capacity for biofilm formation (OD > 1.282). The DAEC, tEPEC, aEPEC, ETEC and EHEC strains showed low to intermediary capacity of biofilm formation, in comparison with the EAEC strains. All *E. coli* pathotypes and non pathogenic *E. coli* presented strains capable of produce biofilms, as described in the literature. The colorimetric assay of violet crystal, a presumptive test used to select the samples better-producing biofilms, showed the high potentiality of the EAEC strains to produce biofilm in regard with other pathotypes and non pathogenic *E. coli*. This capacity can be explained by its aggregative adherence pattern, which is associated with fimbrial adhesins and it is known that the adhesion is the initial process to biofilm development. The samples that showed a higher rate of biofilm formation will be submitted to the methodology of direct counting of CFU/cm² attached to the biofilm after breaking with Triton X-100, and to the interaction with HeLa cells assays.

Keywords: Enterobacteriaceae; *Escherichia coli*; Biofilms.

Financial support: PIBIC; CNPq.

AC 04 - CHALLENGE TEST FOR LIQUID SOAP WITH CHLORHEXIDINE

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Introduction: Nonsterile products might be contaminated with microorganisms, specially those with higher amount of water. Antimicrobial preservatives are added to nonsterile products to protect them from microbiological growth or from microorganisms that are introduced during manufacturing process or using by consumer. The challenge test allows analyzing the efficacy of preservative system in specific formulations. In this test, the formulation is inoculated with a well-known concentration of microorganisms to verify a presence of microorganisms through of colony-forming units (CFU). Chlorhexidine is an antiseptic with large spectrum, active against Gram-positive and Gram-negative bacteria, any fungus and virus used in many medical areas. **Objective:** It was to analyze a formulation of liquid soap with chlorhexidine and the same formulation without chlorhexidine using the method of fast test (value D) and the conventional test (analyzing for 28 days), to verify the speed necessary to kill the microorganisms used. **Method:** The formulations were contaminated with concentration of test microorganisms of 10⁸ CFU/mL of the product. *S. aureus*, *E. coli*, *P. aeruginosa*, *C. albicans* and *A. niger* were used as test microorganisms. The test was carried out during 0 hour, 2nd, 6th, 12th, 24th, 48th hour, 7th day, 14th, 21st and 28th day. The value D (time necessary to degree 1 log, in other word, to kill 90% of microbial population) was determined using the negative reciprocal of the slopes of the regression line, using the linear portions of the survivor curves (log10 CFU/mL versus time of exposure to the antimicrobial preservative). **Results:** Both formulations were satisfactory for challenge test for all microorganisms used because the microorganisms killed in less 48 h and during the time of the test did not have developing of cultures. The value D for *P. aeruginosa* for formulation with chlorhexidine was 3.9 h and for formulation without chlorhexidine, was 8.0 h; for *E. coli*, the value D for formulation with chlorhexidine was 1.9 h and for formulation without chlorhexidine, was 3.9 hours; for *A. niger*, the value D of formulation with chlorhexidine was 3.9 h and for formulation without chlorhexidine, was 8.0 h; for *S. aureus* and *C. albicans* did not was possible to calculate the value D because the time necessary to kill completely these microorganisms was very short, less of 2 h. **Conclusion:** The two formulations were satisfactory for antimicrobial preservative and both were effectiveness to meet criteria specified for challenge test by BP (2003) and USP (2006). The value D agree with conventional method for all microorganisms used in this test. It was observed the among microorganism analyzed, *P. aeruginosa* was the more resistant.

Keywords: Liquid soap; Challenge test; Chlorhexidine; Antimicrobial preservative.

Financial support: CAPES; CNPq; PAD; FAPESP.

AC 05 - CLASSIFICATION OF AVIAN PATHOGENIC *ESCHERICHIA COLI* (APEC) AND *E. COLI* ISOLATED FROM COMMERCIAL CHICKEN OF THE NORTH REGION OF PARANA INTO PHYLOGENETIC GROUPS A, B1, B2 AND D

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Escherichia coli can be classified into three main groups: commensal, intestinal pathogenic and extraintestinal pathogenic strains (ExPEC). The last group involves APEC (avian pathogenic *E. coli*) and UPEC (uropathogenic *E. coli*), and for having diverse virulence factors both of them are capable of living in almost all anatomic sites. There are similarities between these two groups, although not well documented yet. Among the diseases *E. coli* can cause, there are septicemia, diarrhea, urinary tract infections and neonatal meningitis. The phylogenetic analysis shows that they can be divided into 4 phylogenetic groups: A, B1, B2 and D. The virulent extra-intestinal *E. coli* belong mainly to group B2 and D and the commensal strains, to group A. In the present work, 246 *E. coli* samples (180 were APEC isolates and 66 were isolated from commercial chicken) were classified into the phylogenetic groups A, B1, B2 and D, by the polymerase chain reaction, using the short cycle. This methodology consists on the amplification of three primers: *ChuA*, *Yja* and *Tsp*. Of all 246 samples, 69 belonged to group A (28,05%); 80 belonged to group B1 (32,52%); 50 belonged to group B2 (20,33%) and 47, to group D (19,10%). Of the 180 APEC samples, 52 (28,89%) belonged to group A; 62 (34,44%), to group B1; 37 (20,56%), to group B2 and 29 (16,11%), to group D. However, among the 66 commercial chicken samples, 17 (25,76%) belonged to group A; 18 (27,27%), to group B1; 13 (19,70%), to group B2 and 18 (27,27%), to group D. Among APECs, the most prevalent groups were B2 and D, which confirms APEC's pathogenicity, since B2 and D are the two most pathogenic groups. The major percentage of the commercial chicken isolates belong to groups A and B1 (nonpathogenic), however a significant part of these samples are compatible to groups B2 and D. The phylogenetic analysis can help us to understand the similarities between the virulence factor and the colonization standard of APEC and UPEC.

Keywords: ExPEC; APEC; Phylogenetic groups; Virulence factors.

AC 06 - CLONING AND EXPRESSION OF A SINGLE-CHAIN VARIABLE FRAGMENT AGAINST THE HEAT-STABLE TOXIN (ST) OF ENTEROTOXIGENIC *ESCHERICHIA COLI* (ETEC)

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Introduction: Enterotoxigenic *Escherichia coli* (ETEC) is responsible for at least 400 million acute diarrhea episodes and 700,000 childhood deaths per year. Human ETEC pathogenic strains produce heat-labile toxin (LT) and/or heat-stable toxin (ST) that differ in their structure and function while both are used as markers for detection of infections. When compared to other methods, immunoserological assays present some advantages including high specificity and sensitivity with convenient procedures for sample preparation and assay execution. The advances on antibody biotechnology provide alternatives to obtain low cost antibodies with desirable affinities and specificities by manipulating immunoglobulin domains. One approach consists in cloning immunoglobulin's heavy and light variable domains (HV and LV) as a single-chain fusion interspaced by a flexible linker, therefore allowing the correct interaction between the domains and preserving the antigen-binding site. **Objectives:** Construction of a ScFv upon hybridoma cells that produce an anti-ST monoclonal antibody following its bacterial production. **Methods:** After RNA extraction from hybridoma cells and reverse transcription, coding regions of heavy and light chain variable domains (VH and VL) were PCR-amplified and fused to a linker corresponding to (Gly₄Ser)₃ giving rise to the ScFv-ST coding region. The DNA construct was cloned into p-GEMT Easy vector. The recombinant vector was used as template for sequencing and amplification by specific primers harboring restriction sites (*Bam* HI and *Xho* I) intended to permit the subcloning into a pET101D/TOPO vector variant. Aiming to increase the expression and solubility of the target ScFv, this vector was specially designed for its production as a MBP (Maltose Binding Protein) C-terminal fusion. The new recombinant plasmid was used to transform competent *E. coli* BL21(DE3) cells. Transformed cells were cultured (LB-Amp, 37°C) until reach 0,6 O.D._{600nm}. After induction of T7 promoter-associated transcription by IPTG (1mM, 3 hours), the cells were harvested, their periplasmic proteins were isolated by osmotic shock and submitted to metal affinity chromatography using Ni-NTA resin and step-wise elution. Fractions were analyzed by SDS-PAGE. The recognition of ST by purified fractions was tested by western blot. **Results:** The amplification of VH, VL and ScFv-ST showed fragments containing 375 bp, 352 bp and 851 bp, respectively. Alternative forms of the target protein were identified as two major bands with apparent molecular weight 72 and 45 kDa with no biochemical activity. **Discussion:** The design of specific primers was necessary once previously performed PCR reactions exhibited low yield. The expression of the transcript as two electrophoretic protein bands could be explained by truncated translation due to the occurrence of rare codons. MBP removal and, consequently, the release of ScFv-ST from its fusion, could probably allow the recombinant protein to manifest its biochemical activities related to ST recognition.

Keywords: ETEC; ST; ScFv.

Financial support: FAPESP.

AC 07 - CLONING AND EXPRESSION OF C-PHYCOCYANIN β SUBUNIT OF *Spirulina platensis* IN *Escherichia coli*

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Introduction: C-phycoyanin (C-PC) is a pigment soluble in water. It's found in some blue-green microalgae such as *Spirulina platensis*, which are used in many countries as dietary supplements. It is consisted of two subunits, α and β , with molecular masses of 16 and 17kDa, respectively. This protein has shown hepato-protective, anti-oxidant, radical scavenger, anti-arthritic, anti-mutagenic, anti-viral, and anti-tumoral properties. One recent study demonstrated that β subunit of Anabaena C-PC (β C-PC) has anti-tumoral activity, since the β C-PC inhibits cell proliferation and promotes apoptosis on cancer cells. **Objective:** The aim of this study was cloning and expression the β subunit of *S. platensis* C-PC in *Escherichia coli*. **Methods:** The β subunit was cloned in pGEMT-easy plasmid vector originating pTMP-O1 plasmid and subcloned in pET28a plasmid vector originating pTMP-O2 plasmid. When the cells transformed with the plasmid pTMP-O2 reached growth D.O.₆₀₀ between 0.5 to 0.6, it was induced with 1mM of IPTG during 4 h. **Results:** The β subunit was cloned and expressed in *E. coli* BL21. The presence of the β C-PC gene in the pTMP-O1 plasmid was confirmed by sequencing. The gene expression was confirmed by RT-PCR and immunoblotting. Since the construction has one Histag, we use anti-his antibody to detect the recombinant protein. **Discussion:** The production of recombinant proteins in *E. coli* has been very useful in search of new biotechnology products. In this study was obtained a genetically modified microorganism capable of expressing a recombinant protein (β C-PC), which according to the literature, is capable of causing apoptosis in tumor cells. Experiments will be performed to determine the effects of this recombinant protein on cancer cells *in vitro*. Because the number of cases of cancer increase each year and the treatments existing for this disease, are not specific, it is essential to search for new therapies against cancer.

Keywords: C-phycoyanin; *Spirulina platensis*; Apoptosis; Recombinant protein.

Financial support: FAPESP.

AC 08 - COMPARISON BETWEEN TWO METHODOLOGIES TO VERIFY BIOFILM FORMATION BY ATYPICAL ENTEROPATHOGENIC *Escherichia coli* (aEPEC) STRAINS

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Atypical Enteropathogenic *Escherichia coli* (aEPEC) has become the most frequent bacterial pathogen in children with diarrhea in Brazil. Microorganisms can live and proliferate in the environment and can possess the capacity to adhere forming biofilms. This type of formation represents colonization mechanisms, conferring resistance to the action of antibiotics in the associated bacteria, related with bacterial persistence. The gene *shf*, regularly present in Enteroaggregative *E. coli* strains, encodes a protein possibly required for the firm biofilm formation of EAEC O42. The aim of this study was to verify the capacity of biofilm formation by atypical EPEC strains in abiotic surface and compare the method of staining with crystal violet (indirect method) with colony forming units (CFU) count (direct method). We analyzed 76 atypical EPEC strains isolated of children with acute diarrhea from Salvador, Bahia. These strains were screened for biofilm production through colorimetric assay of violet crystal using polystyrene 24-well culture dishes and was quantified using enzyme immunoassay plate reader in length of wave of 595 nm. Of these, the 13 highest biofilm formation and 7 who had little biofilm formation were tested through the counting of CFU/cm² attached to the biofilm after disrupting with Triton X-100 to examine the efficiency of the violet crystal assay. The *shf* gene was researched through PCR. The aEPEC strains had been capable to form biofilm in polystyrene. 82.9% of the strains had presented a similar biofilm production comparing with the typical EPEC strain (E2348/69), whereas 17.1% had presented a superior potential (OD>0,100 at 595 nm), reaching maximum OD of 1,026. With regard to the method of CFU counting the largest producer strain obtained a value equals to 8,53x10⁷ CFU while the lower obtained value equals to 1,22x10⁴. The comparison of 20 samples through the direct and indirect methods showed that the majority of them did not show the same order of magnitude in both methodologies. The *shf* gene was found in 2 samples. The violet crystal assay does not seem to be as reproducible as the methodology by CFUs count, but the first can be employed as a presumptive test to select the samples better-producing biofilm, using as a confirmation of these potential to test of CFU adhered to the substrate. The *shf* gene appears not to be as important for the formation of biofilm in atypical *E. coli* strains, because this gene was detected only in 2 of the 20 samples analyzed, and one of them did not present a good biofilm formation. These results raise the possibility of that others structures and/or mechanisms can be involved in pathogenesis of these strains. Adesins not yet described can be involved in the multifactorial process biofilm formation.

Keywords: EPEC Atípica; Biofilme.

Financial support: FAPESP.

AC 09 - DERIVED OF DIBENZILBUTIROLACTONAS, (-) - CUBEFINA: THEIR ACTIVITY IN ANIMALS IN THE CHRONIC PHASE OF CHAGAS DISEASE

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Introduction: Even after almost 100 years of the discovery of Chagas disease, this is still considered in Central and South America a public health problem, which currently reaches between 16 to 18 million individuals. The infection with *T. cruzi* can occur as acute or chronic. In the chronic phase, cardiac and digestive changes can be developed. In view of the need for new substances with biological activity on *T. cruzi*, the interest in research is growing in order to obtain compounds able to act on the parasite. **Methods:** This study was assessed a derivative of dibenzilbutirolactonas, (-)-cubefina in animals cronicados with this strain that contains the gene of β -galactosidase. Albino mice were used, male, strain BALB / C, with control divided into negative, positive control (Benzonidazol) and group treatment of cubefina 20mg/Kg, which saw the analysis of the activity of each substance through the quantitative determination of *T. cruzi* in the tissues of infected animals treated for 20 days, through the strength of the enzyme β -galactosidase expressed by the strain CL Brener. The results for verification of the effectiveness of treatment were obtained, whereas the negative control group (FN) have the maximum number of parasites in tissue, given the lack of treatment. **Results:** We found that for the substance (-)-cubefina the effectiveness of treatment by the intraperitoneal route was statistically similar to the group treated with Bz, the daily dosage of 20mg/Kg weight of animal, or promoting a reduction of 17.4 % of the number of parasites, Bz20 and 18.1% for Cb20, these values are not significant when compared to the group CN. For Bz50 this reduction was statistically significant in relation to the CN, since for that group there was a decrease of 50.7% in the number of parasites. When evaluating the results concerning the treatment by the oral route, we observed a better effectiveness of the response to Bz20 and less activity to Cb, is expected to Bz fact, since this is the appropriate method of treatment of individuals chagasic. **Conclusion:** The results for the differences found for Cb indicate a significantly lower absorption of this class of compounds by digestório system, a fact that this should be further investigated to determine the best formulations for possible employment of such structures in the treatment of diseases.

Keywords: *Trypanosoma cruzi*; Cubefina; Chronic phase.

AC 10 - DETECTION OF VIRULENCE GENES IN ATYPICAL EPEC STRAINS

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Introduction: Diarrhea continues to be one of the most common causes of morbidity and mortality among infants and children, especially in developing countries. Among the bacterial pathogens, diarrheagenic *Escherichia coli* (DEC) is an important agent of endemic and epidemic diarrhea worldwide. The diarrheagenic *E. coli* strains can be classified in six main pathotypes, based upon specific virulence properties, clinical features, association with serotypes O:H, epidemiological aspects, and patterns of interaction with host cells. Enteropathogenic *Escherichia coli* (EPEC) cause a histopathological lesion known as "attaching and effacing" (A/E). Typical EPEC differs from atypical EPEC by the presence of a plasmid called EPEC adherence factor (EAF) that encodes the bundle-forming pilus (BFP). Atypical EPEC comprises a very heterogeneous group. We developed multiplex PCR reactions in order to identify virulence genes present in other DEC pathotypes in a collection of 120 Atypical EPEC strains isolated from children with diarrhea in Salvador, Bahia, Brazil. **Objective:** Search virulence genes of DEC in atypical EPEC. **Methods:** DNA templates for PCR were obtained from overnight *E. coli* cultures that were pelleted, resuspended in 500µl of sterile deionized water and boiled for 10 min. The PCR was developed by combining specific primer pairs. The genes analyzed in this study were *efa*, *pic*, *sat*, *IdaH*, *toxB* and *saa*. Each multiplex reaction was performed in a 50 µl final volume containing 1 µl of template DNA, 0.2mM DNTPs, 10mM Tris-HCl (pH8.8), 1.5 mM MgCl₂, 50mM KCl, 2 U Taq DNA polymerase (Invitrogen) and 10 pmol of each primer (BiosynthesisAmplified). Amplified samples were detected by 1% agarose gel electrophoresis in Tris-borate-EDTA buffer and ethidium bromide staining. **Results:** Of the 120 samples studied, 7 tested positive for the gene *efa1/lifA*; 3 for the gene *toxB*; 13 for gene *sat*; 8, for the gene *IdaH* and 0 for the gene *saa*. The results obtained by the reaction of PCR were confirmed by sequencing. **Discussion:** All samples submitted to the sequencing showed at least 90 % similarity with the genes described in the literature. **Conclusion:** As we expected, atypical EPEC strains carries virulences genes common to other DEC.

Keywords: Gene virulence; PCR; aEPEC.

Financial support: FAPESP; CNPQ.

AC 11 - DISINFECTION IN FLEXIBLE GASTROINTESTINAL ENDOSCOPY USING GLUTARALDEHYDE 2,0%: SEARCH OF SCIENTIFIC EVIDENCE

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Introduction: The endoscope is a medical procedure used for diagnosis and treatment of gastrointestinal diseases which requires a proper cleaning and disinfection/sterilization to prevent the spread of microorganisms associated with the reuse of endoscopes in patients. Nearly 300 cases of complications of infections involve bacteria, viruses and fungi, highlighting the bacteria *Helicobacter pylori* that is a risk factor for gastric and duodenal ulcer, which may lead to cancer of the stomach. **Objective:** to search and evaluate scientific evidence on the disinfection of gastrointestinal endoscopes using glutaraldehyde (GTA) to 2.0%. As a criterion inclusion was set up all articles with language in english, spanish and portuguese. **Methods:** This is an integrative review in databases Medline/PubMed and ISI Web of Knowledge in the last five years. **Results:** From sixteen publications, only eight was related to criteria above. As the results analyzed, showed reduction of 99.9% of *Tropheryma whipplei* in disinfection of gastrointestinal endoscopes with glutaraldehyde to 2.0% which was found in only one publication. *T. whipplei* the bacterium causes a rare and chronic disease that affects the intestines, central nervous system and heart with symptoms of fever, diarrhea, weight loss and abdominal pain. Two publications were comparative studies between GTA to 2.0%, chlorhexidine gluconate to 4.0% and electrolyzed acid water. The five analyzed publication on the effectiveness of glutaraldehyde to 2.0% in the decontamination of flexible endoscopes. **Conclusions:** The publications analyzed, level II, III and VI, they explained on the risk of infections by *Staphylococcus aureus*, *Pseudomonas aeruginosas* and biofilms in gastrointestinal endoscopes and the widespread use is made of various health services difficult making of decision for their replacement.

Keywords: Gutaraldehyde; Endoscopes; Gastrointestinal; Disinfection.

AC 12 - EFFECTS OF DEHYDROEPIANDROSTERONE AND MELATONIN ON TRYPOMASTIGOTE FORMS OF THE Y STRAIN *Trypanosoma cruzi*, AND *in vivo* NITRIC OXIDE PRODUCTION IN J774 MACROPHAGES

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Introduction: In this year of 2008 - closer to the 100th anniversary celebration of the discovery of Chagas' disease, the non-existence of an efficient drug against blood and tissue protozoaries still remain one of the first leading causes of morbidity and mortality by this disease in more than 18 million infected people. All the drugs prescribed nowadays cause several side effects which lead us to the searching of a new drug capable to potencialize and modulate the immune system for host control of parasitic level, and decreased side effects. **Objectives:** To evaluate the *in vivo* actions of Dehydroepiandrosterone (DHEA) and Melatonin (MEL) hormones in trypomastigote form of *T. cruzi* Y strain, and the quantification of nitric oxide in J774 macrophages infected by *T. cruzi* Y strain. **Methods:** trypomastigote form of Y strain (2.5X10⁶) were placed in contact with different hormones concentrations 0.5, 8.0, 20, 32, 40, and 128µM being evaluated by colorimetric procedures where the color intensity was propoportional to the number of viable parasites, and the results expressed according to the inhibitory concentration of 50% of parasite growth (IC50). The J774 macrophages were plated with 5 x 10⁶/well, and infected with trypomastigote forms (2.5 x 10⁶)/well by direct contact with the concentrations 0.5, 8.0, 20, 32, 40, and 128µM. After 24h the NO was measured by quantifying. Concentration was determined by a wave length of 570nm with a microplaque reader. The issues were accomplished in triplicate. **Results:** Parasitic lises after treatment with DHEA/MEL demonstrated IC50 of 4.1.42. Nitric oxide production expressed in µM in J774 infected macrophages treated with DHEA/MEL during 24h showed a decrease when compared to the control infected with P<0.05. **Conclusion:** A tripanomicidal action by the hormones on trypomastigote forms of Y strain by macrophages simultaneously infected and treated with hormones was observed. The necessary concentrations used for rich a tripanocidal action, and an inhibitor of oxidant effects on this infected cells are high in relation to the physiologic concentrations secreted by pineal and adrenal glands. The endogenous productions of DHEA and MEL may be in enough concentrations in the iNO induction process. However our study using concomitant DHEA/MEL may offer a new antioxidant mechanism for individuals carrying acute cases of Chagas' disease.

Keywords: Chaga' diseases; *Trypanosoma cruzi*.

AC 13 - EFFECTS OF ORCHIECTOMY AND MELATONIN THERAPY DURING THE EXPERIMENTAL CHAGAS' DISEASE

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Chagas' disease is due to a long-term complex relationship between parasitic persistence and maladapted host homeostatic mechanisms leading to a severe pathology in chronically infected individuals. Melatonin (MLT) acts as a T-cell immunoregulator promoting a T-helper 1 (Th1) response by increasing interferon gamma (IFN-γ) and TNF alpha (TNF-α) production. Sex-hormones, like testosterone and estradiol, influence the function of all immune cells types

and thus susceptibility for diseases, especially those caused by protozoan parasites. To evaluate the effects of orchiectomy and Melatonin therapy on the course of *T. cruzi* infection in male rats, this study compared blood parasitism, IFN- γ and TNF- α levels. Male *Wistar* rats weighing 100-120g were divided into the following groups: Infected (I), Infected Sham surgery (SH), Infected Orchiectomized (OR), Infected Melatonin treated (M), Infected Melatonin treated Sham surgery (MSH), and Infected Melatonin treated Orchiectomized (MOR). After 4 weeks post orchiectomy, all rats were i.p. infected with 1×10^5 blood trypomastigotes (Y strain) of *T. cruzi*. Studies were performed 7, 14 and 21 days after infection. Animals from all treated groups received, daily and orally, Melatonin at a dose of 5 mg/kg/body weight, dissolved in distilled water, once a day at the same time and during the course of the experiment. Parasites counts were evaluated by Brener's Method and concentrations of TNF- α and IFN- γ were measured by specific two-site enzyme-linked immunosorbent assay (ELISA). Orchiectomized and treated rats displayed reduced levels of blood parasites as compared to their control and sham counterparts. On 7, 14 and 21 days post infection, untreated orchiectomized animals (OR) displayed enhanced concentrations of IFN- γ and TNF- α when compared to control (I) and sham (SH) counterparts. With Melatonin treatment, orchiectomized animals (MOR) displayed the highest concentrations of IFN- γ and TNF- α when compared to untreated orchiectomized counterparts (OR) and also compared to treated non-orchiectomized counterparts (M and MSH). The results of this work point in the direction of a synergic role exerted by orchiectomy and melatonin therapy in immune response against the parasite.

Keywords: Chagas' disease; Melatonin; Interferon gamma; Tumor necrosis factor- α .

Financial support: CAPES; FAPESP.

AC 14 - EFFERVESCENT TABLETS AND ULTRASONIC DEVICES AGAINST *Candida sp* AND MUTANS STREPTOCOCCI IN DENTURE BIOFILM

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A well-established denture hygiene protocol can prevent and treat oral infection in edentulous patients. Such patients are usually elderly and have difficulty brushing their teeth properly. To evaluate the antimicrobial action of effervescent tablets and ultrasound against *Candida sp.* and mutans streptococci from denture biofilm. Seventy-seven complete denture wearers were randomly assigned into four groups, according to the denture hygiene method: (A) Brushing with water (Control); (B) Effervescent tablet; (C) Ultrasonic device; (D) Association between effervescent tablets and ultrasonic device. Samples of denture biofilm were collected at baseline after 21 days. The samples were collected by brushing the dentures with saline and detached microbial cells were quantified by plating. Counts (log (CFU+1) mL⁻¹) for total aerobes, *Candida sp.* and mutans streptococci were compared by means of one-way ANOVA or Kruskal-Wallis test ($\alpha=0.05$). Comparison among methods did not find any significant difference against *C. albicans* (P=.76), *C. tropicalis* (P=.94) e *C. glabrata* (P=.80). Significant lower counts were found for methods B and D when compared with the others for mutans streptococci (P=<.001). Method B provided lower total aerobic counts than for A, whereas C and D showed intermediate results (P=.011). Effervescent tablets showed more pronounced antimicrobial effect than by ultrasonic denture cleaning method. Their association did not provide any further detectable benefit.

Keywords: Complete Denture; *Streptococcus mutans*; *Candida albicans*; Denture cleansers; Ultrasonic.

Financial support: FAPESP.

AC 15 - EVALUATION OF ANTIFUNGAL ACTIVITY OF NOPLAK®, REACH®, ORAL-B® WITHOUT ALCOHOL, LISTERINE® AND PLAX® MOUTHRINSES AGAINST *Candida albicans*

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Oral candidiasis is a common opportunistic human fungal infection. Despite the availability of a number of effective antifungal drugs for the treatment of oral candidiasis, failure of therapy is not uncommon. For these and other reasons mouthrinses are widely indicated in dentistry both as an antiseptic mouthrinse and as a denture disinfectant in order to supplement other antifungals. Our goal was to evaluate the activity of mouthrinses against *C. albicans*. We selected 20 isolates of *C. albicans* kept in the collection of the Mycology Laboratory of FCFRP-USP. The mouthrinses Noplak® (Chlorexidina Gluconate 0,12%; Daudt), Listerine® (Timol 0,064%; Pfizer), Plax® (Triclosan 0,03%; Colgate), Oral-B® (Monohydrated Cetilpiridinium Chloride 0,050%; Gillette do Brasil), Reach® (Monohydrated Cetilpiridinium Chloride 0,050% and Ethilic Alcohol; Johnson Johnson) were obtained from the market in the form of mouthrinse solution. The methodology used with modifications was the broth microdilution published in the document M27-A2 by the Clinical and Laboratory Standards Institute (CLSI). The final concentrations of the mouthrinses for the test were 1:5, 1:10, 1:20, 1:40 and 1:80, they were also tested pure. The growth on the positive control well was compared visually to the growth on the different dilution wells. The highest dilution capable of inhibiting the growth was considered the Minimal Inhibitory Dilution (MID). The mouthrinses Noplak®, Oral B® and Reach® inhibited the growth of 100% of the isolates on the 1:80 dilution; the mouthrinse Plax® inhibited the growth of 90% of the isolates on the 1:80 dilution, 5% on the 1:40 and 5% on the 1:20 dilution. The mouthrinse Listerine® inhibited the growth of 35% of the isolates on the 1:80 dilution, 5% on the 1:40 dilution, 5% on the 1:20 and 55% on the 1:10 dilution. When the mouthrinses Noplak®, Oral-B®, Reach®, Plax® and Listerine® were tested pure, they inhibited the *in vitro* growth of all *Candida albicans* isolates. Noplak®, Reach® and Oral-B® were capable of inhibiting the growth of all the samples in the highest dilution evaluated (1:80).

Keywords: Antifungal activity; Mouthrinses; *Candida*.

AC 16 - HIGH PREVALENCE OF QUINOLONE RESISTANT-ENTEROBACTERIA ISOLATED IN UNIVERSITY HOSPITAL

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Introduction: Quinolones are important alternative of treatment in infections due to extended-spectrum beta-lactams-resistant enterobacteria. However, the prevalence of these bacteria harboring other resistance mechanism is increasing. The overuse of quinolones to treat urinary tract infection contributes to select and to spread multiresistant enterobacteria. **Objective:** The aim of this study was to evaluate the susceptibility profile to quinolones in extended-spectrum beta-lactams-resistant enterobacteria isolated of urine from patients in a university hospital in Brazil. **Material and Methods:** One hundred and twenty one extended-spectrum beta-lactams-resistant enterobacteria were isolated of urine, during April to August of 2007, from patients hospitalized at the Hospital das Clínicas da Faculdade de Medicina de Ribeirão Preto - USP (HC-FMRP-USP). The identification and susceptibility profile was determined by Vitek systems. **Results:** One hundred and five (86.77%) demonstrated resistance to, at least, one quinolone and 16 (13.22%) showed susceptibility to all quinolones evaluated. Enterobacteria demonstrated the most resistance against nalidix acid (83.43%), followed by norfloxacin and levofloxacin (77.68%) and ciprofloxacin (76.03%). *K. pneumoniae* was the most resistant enterobacteria isolated of urine from patients and showed also the most resistance against nalidix acid (96.42%), followed by norfloxacin and levofloxacin (91.68%) and ciprofloxacin (91.07%). **Conclusion:** The overuse of quinolones to treatment of urinary tract infections probably is cause of selection of multiresistant bacteria. There is high prevalence of resistance to quinolones among enterobacteria isolated at the HC-FMRP-USP, mainly *K. pneumoniae*. In contrast with the majority of reports, this specie was the most resistant pathogen isolated of urinary tract infection.

Keywords: Quinolone resistance; Enterobacteria; Urinary tract infection.

AC 17 - HYPOCHLOROUS ACID (HOCL) PRODUCTION IS IMPAIRED IN ELDELY PATIENTS WITH UNEXPLAINED ANEMIA

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Introduction: Epidemiological studies report that a third of the cases of anemia in elderly is unexplained. Reactive oxygen species (ROS) are produced by neutrophils during the oxidative burst, such as HOCl, which is the most ROS involved in antimicrobial defense of mammalian. The purpose of this study was to determine the HOCl production by purified neutrophils in the elderly with unexplained anemia. **Methods:** The study included twenty women patients aged over 60 recruited from geriatric department of healthy public system of Ribeirão Preto city. The ethics committee of the University of São Paulo approved the study, and all selected patients provided their written informed consent. Patients with impaired kidney function, hemoglobinopathy, vitamin deficiency, decompensated diabetes or infectious/malignant diseases were excluded. We had access to their medical history through of their physician. Peripheral blood samples were drawn, hematology and biochemical parameters were performed: complete hemogram (automated Micros 45-ABX®), blood film and leukocyte differential count (Leishman's stain morphology), hemoglobin's electrophoresis, iron status, serum transferrin receptor (sTfR, R&D systems®, USA), creatinine, glucose, albumin, total protein and gamma-glutamyltransferase activity (Labtest®, Brazil). The high sensitivity C-Reactive Protein (hs-CRP) was inflammatory marker measured by immunoturbidimetric assay (BioTécnica®, Brazil). The granulocytes isolation was made by difference of gradient density Ficoll-Hypaque. 1×10^6 cells/mL contained more than 95% neutrophils and less than 5% eosinophils, analyzed by Leishman's stain morphology. The production of HOCl was measured by TMB (tetramethylbenzidine) assay. The absorbance was read at 655 nm with BioSpec-mini UV-Visible Spectrophotometer Shimadzu®. Statistical analysis: Mann Whitney test ($p < 0.05$). Data are reported as median values. **Results:** All patients had normal biochemical tests, hs-CRP levels, hemoglobin's electrophoresis and red blood cell morphology. The control group had no signs of anemia. The hemoglobin levels were significantly different ($p < 0.05$) between control and anemia groups (12.50 and 11.30 g/dL, respectively). Leukocyte counts, iron status, sTfR, platelet and hematimetric indexes did not show significant difference between both groups. However, there was a significant difference ($p < 0.05$) in HOCl production between control and unexplained anemia groups (7.00 and 3.90, respectively). **Conclusion:** Understanding the pathophysiology of unexplained anemia is important because in older persons, it is associated with poor physical functioning, impaired quality of life and reduced survival. These results suggested that lower HOCl production in the elderly with unexplained anemia showed an innate immune system impaired.

Keywords: Hypochlorous Acid; Neutrophils; Elderly; Anemia; Immune system.

AC 18 - IDENTIFICATION OF *Rhodococcus equi* BY CAMP-LIKE REACTION, AND PCR ASSAY TARGETING THE *choE* GENE

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Introduction: *Rhodococcus equi* in recent years has emerged as an important opportunistic pathogen, in human immunodeficiency virus-infected patients, causing a lung disease reminiscent of pulmonary tuberculosis. Identification of *R. equi* by classical bacteriological techniques is sometimes difficult, and misclassification of an isolate is not uncommon. Accurate identification of *Rhodococcus equi* isolates to the species level is possible on the basis of chemotaxonomic properties. However, these techniques are excessively laborious, time-consuming, and expensive for routine use in clinical microbiology laboratories. Navas et al. (J. Bacteriol. 183:4796, 2001) identified the *R. equi choE* gene, a chromosomal locus encoding cholesterol oxidase, an enzyme believed to be a major virulence factor of *R. equi*. Mutational analysis indicated that *choE* is the membrane-damaging factor responsible for the typically shovel-shaped synergistic hemolysis (CAMP-like) reaction elicited by *R. equi* in the presence of sphingomyelinase C-producing bacteria, such as *Listeria ivanovii*. **Objectives:** We aimed to evaluate the use of CAMP-like reaction as a phenotypic marker for the rapid presumptive identification of *R. equi*, and the PCR method for its specific identification, based on the detection of *choE* gene. **Methods:** A total of 84 Gram-positive and partially acid-fast rods isolated from sputum of tuberculosis-suspected patients, and the *R. equi* reference strain, ATCC 6939 were included in the study. The bacteria were routinely cultured at 37°C for 48 h, and the CAMP-like synergistic hemolysis tests were performed on sheep blood agar plates with Mueller Hinton agar base medium, and *L. ivanovii* ATCC 19119 as the indicator strain. All isolates were screened by PCR in accord to Ladrón et al. (J Clin Microbiol. 41(7):3241, 2003). **Results:** The expected presence of *choE* was observed in 50 isolates with positive CAMP-like reaction, and in *R. equi* ATCC 6939. Twenty seven isolates with negative CAMP-like reaction displayed negative *choE* result. Five isolates originally identified as presumptive *R. equi* based on positive CAMP-like reaction displayed negative *choE* gene result, and probably belonged to other bacterial species. **Conclusion:** Our results illustrate the validity of the molecular method as a tool to identify *R. equi*. These assays accurately identify *R. equi* correctly on the basis of phenotypic characteristics.

Keywords: *Rhodococcus equi*; Tuberculosis; CAMP-like; *ChoE*; Sphingomyelinase.

AC 19 - *in vivo* TRYPANOCIDAL ACTIVITY OF TRITERPEN ACIDS AGAINST Y STRAIN OF *Trypanosoma cruzi* - EVALUATION IN THE ACUTE PHASE OF CHAGAS' DISEASE

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Introduction: Chagas' disease is a chronic illness caused by the protozoan *Trypanosoma cruzi* affecting over 18 million people in the three Americas, from Southern Argentina to the Southern United States, with a further 100 million at risk. Vector transmission is under control in Brazil, while infection via blood transfusion is becoming predominant and is estimated responsible for about 20000 new cases annually. Unfortunately, only two drugs are currently available to treat chagasic patients: nifurtimox and benznidazole. Besides presenting severe side effects and requiring long treatment, they are only effective in the acute phase of the disease. Therapeutic agents of several pharmacological classes have been assayed aiming to identify more active and less toxic trypanocidal drugs but none could substitute gentian violet. Several natural products of different structural classes have proven active against *T. cruzi* and screening of plant extracts is a valid strategy being exploited to discover trypanocidal natural products. **Objectives:** The aim of the present work was to evaluate the *in vivo* trypanocidal activity of triterpene acids, ursolic acid, oleanolic acid and potassium salt derivative of ursolic acid, against Y strain of *T. cruzi*. **Methodology:** For the *in vivo* trypanocidal activity, male Balb/c albino mice were used, in which experimental animals were infected with 2×10^4 trypomastigotes and oral treatment was started 48 h post-infection. All compounds of the treatment group were administered at the concentrations of 20 and 50 mg/kg daily for 20 days. **Results:** The obtained results show that animals treated with ursolic acid and potassium salt derivative of ursolic acid (20mg) demonstrated reduction of parasites blood forms (30% and 29%, respectively). On the other hand, oleanolic acid was not effective at the concentration tested. In contrast, ursolic acid, oleanolic acid and potassium salt derivative of ursolic acid, at the concentration of 50mg, reduced the parasites number in about 79%, 76% and 68%, respectively. **Conclusion:** We conclude with obtained results that triterpene acids exhibited trypanocidal activity *in vivo* against Y strain of *Trypanosoma cruzi* at the concentrations evaluated.

Keywords: Chagas' disease; Trypanocidal activity; Ursolic acid; Oleanolic acid.

AC 20 - INFLAMMATORY PHASE OF WOUND HEALING INDUCED BY BIOMATERIALS

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Introduction: Natural latex biomembrane (NLB) of *Hevea brasiliensis* rubber tree seems to enhance wound healing. Studies have described its activity in the repair defects in dogs' esophagus, its biocompatibility, induction of neo-angiogenesis in rabbits' cornea [Materials Research. 7: 277, 2004], effectiveness in healing diabetic, venous and pressure ulcers in humans [Med. Cutan. Ibero. Lat. Am. 34: 133, 2006]. However, its wound healing mechanisms are still unknown. **Objectives:** It was evaluated the activity of NLB on healing and compared it to other biomaterials like: denatured latex (glove) and expanded PolyTetraFluorEthylene (ePTFE). **Methods:** Different biomaterials were subcutaneously implanted on the dorsum of C57BL/6 mice, which were divided into 4 groups: NLB, glove, ePTFE and without implant (sham). Five animals from each group were killed on the 2nd, 7th and 14th days post-implanting (n=5) and histological (inflammatory infiltrate, blood vessels, fibroblast, collagenesis), immunohistochemical (IL-1 β , iNOS, VEGF, TGF- β 1) and myeloperoxidase (MPO) assays were performed (approved by the Brazilian College of Animal Experimentation (COBEA) - process 055/2005). **Results:** NLB group showed more pronounced inflammatory reaction (18.05 \pm 2.21) than glove (5.58 \pm 4.32), ePTFE (4.31 \pm 1.85) and sham (4.96 \pm 7.61) with intense neutrophilic inflammatory infiltrate, confirmed by MPO assay on the 2nd day. Moreover, NLB and ePTFE groups showed lowest expression to immunostaining for iNOS. IL-1 β was highest in NLB and sham groups on the 2nd day. Vascular neo-formation on the 14th day was statistically superior in the NLB (1.73 \pm 0.85) and glove (1.47 \pm 0.32) than ePTFE (0.50 \pm 0.36) groups. Regarding VEGF on the 7th day, all groups showed similar staining which decreasing only in the glove group on the 14th day. Fibroplasia on the 7th day was more stimulated by ePTFE (45.90 \pm 17.39) than NLB (22.30 \pm 4.20) and, on the 14th day NLB (55.67 \pm 13.85) was superior to ePTFE (29.43 \pm 8.21). Collagenesis on the 14th day was higher on the ePTFE (63.81 \pm 11.19) than NLB (42.72 \pm 6.95) and glove (40.72 \pm 4.10). Concerning to TGF- β 1, NLB showed higher staining on the 7th day compared to the other groups, decreasing on the 14th day and increasing to the groups ePTFE and sham. **Conclusion:** NLB stimulated wound healing especially the inflammatory phase, and this seems to enhance the next phases of healing independent of VEGF (angiogenesis) and TGF β 1 (fibroplasia), different from glove which is denatured latex. The ePTFE group was important on proliferation phase of wound healing (Acknowledgements: immuno-histology technician Marilena Heredia).

Keywords: Biomaterials; *Hevea brasiliensis*; Latex; Wound healing; Inflammation.

Financial support: CAPES; CNPq; FAEPA.

AC 21 - INVESTIGATION OF THE MECHANISMS INVOLVED IN HUMAN NEUTROPHIL OXIDATIVE METABOLISM MODULATION BY COUMARINS

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Introduction: Neutrophils are essential cells for the host defense against invading pathogens. They have highly developed mechanisms for killing and intracellular digestion of particles, which involves generation of oxidant molecules and release of the granule components (e.g. enzymes, proteins, peptides). However, increased neutrophil activation and oxidative stress have been implicated in many inflammatory illnesses, such as rheumatoid arthritis, atherosclerosis and Chron's disease. The neutrophil NADPH oxidase complex and the granule enzyme myeloperoxidase have been pointed as important mediators of such oxidative damage, via generation of superoxide anion, HOCl and other reactive species. In this context, modulation of their activities by phenolic compounds has been investigated as a possible therapeutic strategy to treat neutrophil-mediated diseases. **Objectives:** The aims of the present work were to evaluate the modulatory effect of two hydroxylated coumarins (7-hydroxycoumarin, 4-methyl-7-hydroxycoumarin) and their acetylated analogues (7-acetoxycoumarin, 4-methyl-7-acetoxycoumarin) in the human neutrophil oxidative metabolism, as well as to investigate the mechanisms underlying the pharmacological effects observed. **Methods:** Venous blood was collected from healthy volunteers and neutrophils were isolated by using the gelatin method. The neutrophil oxidative metabolism was triggered by normal human serum-opsonized zymosan, in the presence of the reaction medium, or DMSO (0.11%) or the coumarins (1-100 μ mol/L), and the cellular response was evaluated by the lucigenin (CLuc)- or luminol (CLum)-amplified chemiluminescence assays. Toxicity of coumarins (200 μ mol/L) to the neutrophils was evaluated by trypan blue exclusion and measurement of lactate dehydrogenase release. Moreover, the modulatory effect of coumarins (1-200 μ mol/L) was evaluated in a cell-free experimental model (horseradish peroxidase-H₂O₂-luminol) and their oxidation potentials were determined by cyclic voltammetry. **Results:** It was observed that the four coumarins inhibited the CLuc but increased the CLum in a concentration-dependent manner, without being toxic to the cells, under the experimental conditions assessed. In addition, the hydroxylated compounds increased the luminol chemiluminescence generated by the cell-free peroxidase-catalyzed reaction. The oxidation potential of the two hydroxylated compounds were similar (around +0.7 V), but no oxidation peak was detected for the acetylated coumarins in the range of potentials from -1.0 to 1.0 V. **Conclusion:** The tested compounds showed antagonistic effects in the neutrophil oxidative metabolism, depending on the kind of reactive oxygen species measured. Scavenging and/or inhibition of superoxide generation by the coumarins were probably involved in the CLuc decrease. On the other hand, the CLum enhancement seemed to be mediated by interference of the hydroxylated compounds in the neutrophil myeloperoxidase-catalyzed oxidation of luminol, since these coumarins had similar effects in the horseradish peroxidase-H₂O₂-luminol chemiluminescence production. Together, our results provide preliminary information about the mechanisms involved in modulation of the human neutrophil oxidative metabolism by the coumarin derivatives tested.

Keywords: Coumarins; Neutrophils; Chemiluminescence; Reactive oxygen species; Peroxidases.

Financial support: FAPESP; CNPq; Instituto do Milênio Inovação e Desenvolvimento em Fármacos e Medicamentos.

AC 22 - LEISHMANICIDAL ACTIVITY OF EXTRACTS AND NATURAL MOLECULES FROM BRAZILIAN PLANT

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Introduction: *Leishmaniasis* is a group of tropical diseases caused by a number of species of protozoan parasites of the genus *Leishmania*. It affects around 12 million people in 80 countries and it is estimated that are reported about two to three million new cases annually. The drugs currently available for treatment of leishmaniasis are unsatisfactory because of their limited efficacy, frequent side effects, and increasing drug resistance. Research on antiprotozoal drugs of medicinal plant origin is a multidisciplinary task which involves researchers and students in the fields of botanics, phytochemistry, parasitology, pharmacology and medicine. In order to find new drugs against leishmaniasis, we have studied extracts and natural molecules of Brazilian plants such as *Garcinia brasiliensis* (Clusiaceae), commonly known as "bacupari", a native tree in Brazilian forests, rich on metabolites as xanthenes, flavonoids, phenolic acids and benzophenones. Which biological activities as apoptosis induction, antiulcer, anti-oxidant, trypanocidal, cytotoxic, antimicrobial, antifungal and antitumoral pro-perties. **Objective:** To obtain extracts from *G. brasiliensis* fruit: hexane, ethyl-acetate and ethanolic, and to evaluate them to the leishmanicidal activity against promastigote forms of *Leishmania amazonensis*, using bioguide fractionation. **Methods:** The promastigote forms of *L. amazonensis* in log phase of growth (10⁶ parasites/mL) were grown on a 24-well plate in Schneider's medium supplemented with 10% heat-inactivated FBS and 1.0% de penicillin/streptomycin 100000U/mL in the absence or in the presence of different concentrations of the extracts, or isolated compound at 25°C, to evaluate parasite survival. In all tests, 0.5% dimethyl sulfoxide (DMSO) was the concentration used to dissolve the extracts and isolated substances. The controls and reference drug used were the DMSO and Amphotericin B, respectively. After 72h, promastigote forms were counted in a hemocytometer and the percentage of inhibition was determined. Each experiment was

performed in triplicate, and the results were expressed as percentage of inhibition in relation to the control. The 50.0% inhibitory concentrations (IC₅₀) were determined by regression analysis (polynomial and exponential) of the data obtained. **Results:** As much the hexane, ethyl-acetate and ethanol extracts as aromatic compounds (LFQM1, LFQM2 e LFQM3) obtained from hexane extract inhibited growth of the promastigote forms, with IC₅₀ of 1.88, 35.11, 24.30, 3.37, 4.78 and 18.12µg/mL, respectively, after 72h of incubation. The activity is correlated to the non-polarity having been the LFQM1 the most apolar molecule and LFQM3 the most polar. In addition, Amphotericin B showed IC₅₀ of 4.32µg/mL, after 72h of incubation. **Conclusion:** In the present study, we report for the first time a novel pharmacological activity of the extracts and isolated compounds from the fruits of *G. brasilienses*, which showed important activity against promastigote forms of *L. amazonensis*.

Keywords: Leishmanicidal; Natural Products; Benzophenones.

AC 23 - OPTIMIZATION OF EXPERIMENTAL CONDITIONS TO EVALUATE THE ACTIVITY OF HUMAN AND RABBIT NEUTROPHIL NADPH OXIDASE

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Introduction: Neutrophils are professional phagocytes from the innate immune system that play an essential role in the organism's defense against invading microorganisms through generation of reactive oxygen species (ROS). This function begins with formation of the NADPH oxidase complex (NADPHox), located in the plasma and phagosomal membranes of activated neutrophils. This enzymatic complex reduces molecular oxygen to superoxide anion radicals by using NADPH as electron donor, both in the outside of the cells and within the phagosome containing ingested microorganisms. Despite its beneficial effects, excessive ROS production has also been involved in the pathogenesis of many inflammatory diseases. So, modulation of the ROS generation can be relevant to the maintenance of the body's homeostasis. **Objective:** Optimization of experimental conditions to evaluate the NADPHox activity of human and rabbit neutrophils stimulated by immune complexes (IC) and serum-opsonized zymosan (OZ). **Methods:** Neutrophil oxygen consumption was measured using an oxygraph with Clark's electrode type. Different concentrations of cells were incubated at 37°C in Hanks solution (pH 7.2) supplemented with gelatin (0,1%), and the oxygen consumption was determined. Subsequently, different concentrations of IC or OZ were added to the cell suspension and the oxygen consumption by the stimulated cells was measured. The difference between oxygen consumptions before and after neutrophil stimulation refers to NADPHox activation. Oxygen consumption was also measured in the presence of DPI (diphenyleneiodonium), which is a specific inhibitor of NADPH oxidase activity. **Results:** The experimental conditions established for evaluation of both human and rabbit neutrophil NADPH oxidase activities were 4 x 10⁶ cells/mL and 2 mg/mL of OZ. The optimal IC concentration for human neutrophil stimulation was 240µg/mL. It was not possible to establish a viable IC concentration for stimulation of rabbit cells in this assay. **Conclusion:** The experimental conditions established for measurement of the neutrophil NADPHox activity allows evaluation of the pharmacological effect of new natural products, extracts or isolated compounds, and can also be a useful tool for investigating their mechanism of action in the neutrophil ROS generation process.

Keywords: NADPH oxidase, neutrophils; Opsonized zymosan; Immune complexes; Method.

Financial support: FAPESP; CNPq.

AC 24 - ORCHIECTOMY AND DEHYDROEPIANDROSTERONE THERAPY IN *Trypanosoma cruzi* INFECTED RATS

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Introduction: *Trypanosoma cruzi*, the causative agent of Chagas' disease is an intracellular protozoan parasite. The control of parasitism during the acute phase of infection is associated with synthesis of IFN-γ by cells from lymphocytic lineage, activation of macrophages and the production of nitric oxide for the destruction of intracellular amastigotes. Male sex steroids play fundamental role in determining the outcome of disease, through modulation of the activity of immune response. DHEA is secreted by human adrenal cortex and it is considered potent immune-activator. **Objective:** The effects of orchietomy on the course of *T. cruzi* infection in rats treated with DHEA were examined, by comparing blood parasitism, nitric oxide production and IFN-γ levels. **Methodology:** Male *Wistar* rats weighing 100g were divided into the following groups: Infected Control (IC), Infected Sham surgery (ISH), Infected Orchietomized (IOR), Infected DHEA treated (ID), Infected DHEA treated Sham surgery (IDSH), and Infected DHEA treated Orchietomized (IDOR). Four weeks post orchietomy, all rats were i.p. infected with 1 x 10⁸ blood trypomastigotes (Y strain) of *T. cruzi*. Studies were performed 7 and 14 days after infection. Animals from all treated groups received s.c. 0.1 mL of DHEA, at a dose of 40 mg/kg body weight. Parasites counts were evaluated by Brener's Method. Cells were harvested from the peritoneal cavity, adjusted to 5x10⁶ cells/ml and dispensed into 96-well flat-bottom plates with or without LPS (1µg/ml). Cultures were incubated at 37°C, 5%CO₂ for 48h. NO was measured in the supernatants by using the Griess reaction. The absorbance was determined at 540nm. Serum levels of IFN-γ were quantified using BD OptEIA™ SET Rat IFN-γ (BD Biosciences, U.S.A.). **Results:** Orchietomized and treated rats displayed reduced levels of blood parasites as compared to their control and sham counterparts. DHEA administration triggered enhanced number of macrophages and IFN-γ concentrations in all groups when compared to untreated animals. But, for orchietomized group, these values were greatest, triggering a rise in the levels of NO and IFN-γ. **Conclusion:** DHEA improves the immune response against the parasite and orchietomy contributes to this up modulation, reducing the immunosuppressive effects of male androgens.

Keywords: *Trypanosoma cruzi*; Dehydroepiandrosterone (DHEA); Interferon gamma (IFN-γ); Nitric oxide (NO).

Financial support: CAPES.

AC 25 - PARASITES WITH ZOONOTIC POTENTIAL IN DOG FAECES COLLECTED FROM PUBLIC PARKS OF THE CITY OF FERNANDÓPOLIS, SP, BRAZIL

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Introduction: The rule of the dog as a definitive host of some parasites with zoonotic potential has been studied and recognized as an important problem of public health. Dogs that harbor adult worms pass the parasite eggs into the environmental in their faeces, some of which can also infect humans. Children are most commonly infected when they eat soil contaminated with eggs or put objects contaminated with eggs into their mouths. Dog's ownership and its widening in urban agglomerations creates a continuing increasing trend. A growing danger of contamination of human environmental with infectious stages of zoonoses originating from dogs is related to this phenomenon. **Objective:** The objective of this study was to investigate the occurrence of zoonotic parasites in dog's faeces collected from public parks, from March to June, 2007, in Fernandópolis city, São Paulo state, Brazil. **Methods:** We collected 428 samples faeces individually, from four Public Parks in the Fernandópolis city. The faeces were examined by using a spontaneous-sedimentation method and fluctuation in saturated sodium chloride solution. **Results:** Parasites with zoonotic potential were present in 46% (196) of the 428 samples studied. Among them the most frequent agent was *Ancylostoma spp.* (48.3%). *Toxocara canis* eggs were found on 32.3% samples. *Trichuris vulpis* eggs (12.1%) were also found followed by *Giardia spp* cysts (8.2%). Multiple infections with two or more parasite were less common than infections with a single parasite type. The most frequently multiple infection was of *Ancylostoma spp* with other parasites in 20

samples, *Toxocara canis* 45% (9), *Trichuris vulpis* 30% (6) and *Giardia spp.* 25% (5). **Conclusions:** The present results showed a high frequency of parasites with zoonotic potential, among them *Ancylostoma spp* and *Toxocara canis*, parasites that cause cutaneous and visceral larva migrans respectively. The ascarid worm *Toxocara canis* is considered to be one of the most frequent canine parasites that represents a considerable health risk, especially for children. Others parasites found to exhibit only minor pathological potential for the human. The findings suggest the importance about the implantation of an active zoonoses control center and adoption of sanitary preservation programs in these sites is necessary in order to prevent environmental contamination by parasites potentially pathogenic to human.

Keywords: Parasites; Dog faeces; Zoonotic potential; *Ancylostoma spp*; *Toxocara canis*.

AC 26 - PHENOTYPICAL VERSUS MOLECULAR DETECTION OF VAGINAL *Candida albicans* FROM HEALTHY AND INFECTED BRAZILIAN WOMEN

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Introduction: Vulvovaginal candidiasis (VVC) is a significant problem affecting 75% of women at least once during lifetime. The condition is mainly implicated with *Candida albicans*, a commensal dimorphic fungal organism of the lower female reproductive tract. Vaginal discharge, irritation, vulvar burning, dyspareunia, and external dysuria are the main complaints associated with VVC. Culture is believed to be the gold standard method for the diagnosis of VVC. However, Polymerase Chain Reaction (PCR) represents a faster alternative method for the detection of *Candida spp.* **Objectives:** To determine the prevalence of *C. albicans* in vaginal samples obtained from healthy women and those diagnosed with VVC, by comparison of culture-dependent (biochemical tests) and culture-independent (PCR-based) methodologies. **Materials and Methods:** Sixty four healthy women [mean age 31.2 ± 8.4 years (ranging from 18 to 47)] and sixty eight subjects diagnosed with VVC by clinical and laboratory criteria [mean age 28.9 ± 8.7 years (ranging from 16 to 51)] were recruited in the city of Ribeirão Preto (São Paulo, Brazil) and participated in this study. Each subject voluntarily signed an informed consent and answered a questionnaire using a format approved by the Ethics Review Board of the Centro de Saúde Escola da Faculdade de Medicina de Ribeirão Preto - Universidade de São Paulo (Protocol # 0146). From each patient, two samples were obtained with two sterilized swabs from the lateral vaginal wall. One of the swabs was transferred to a tube containing a 0.85 (w/v) sodium chloride solution and the suspension was seeded onto plates containing a selective and differential culture medium [CHROMagar® *Candida* (Probac - São Paulo)] for isolation and presumptive identification of yeasts belonging to the genus *Candida*. One or two typical colonies were selected and the isolates were further confirmed by biochemical tests. The other swab was used for DNA extraction and detection of *C. albicans* by PCR by amplification of an HSP 90 gene fragment according to Crampin and Matthews (J. Med. Microbiol., v. 39, p. 233-8, 1993). **Results:** In the group of healthy women, according to biochemical identification, 11 out of 64 women (17.2%) were positive for vaginal *C. albicans*, compared to 14 out of 64 subjects (21.9%) assessed by PCR method (excellent agreement: Kappa coefficient = 0.852). On the other hand, in the VVC group, 50 out of 68 women (89.2%) exhibited positive vaginal samples for *C. albicans*, whereas 51 out of 68 subjects (75.0%) showed positive vaginal samples for the microorganism by the use of PCR method (moderate agreement: Kappa coefficient = 0.547). **Conclusions:** The highest agreement rate between phenotypical and PCR methods for detection of *C. albicans* was obtained in the vaginal samples from healthy women. PCR method presents potential application as a diagnostic test, but further improvements are needed.

Keywords: *C. albicans*; VVC; PCR; Biochemical identification.

Financial support: FAPESP; CAPES; NSERC.

AC 27 - POTENTIAL ROLE OF MESENCHYMAL STEM CELLS THERAPY IN WOUND HEALING: PRELIMINARY RESULTS

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Introduction: Despite better understanding of the pathophysiology of cutaneous wound healing, more studies need to be taken to allow more efficient treatments. The potential use of bone marrow-derived mesenchymal stem cells (MSCs) has been discussed, and since their immunobiological properties have been established, it is important to assess them in healing process according to their differentiation and histocompatibility. **Objectives:** The aims of this work were to assess the MSCs xenografting from mice to cutaneous wounds in rabbits, topically and systemically treated, according to their efficacy in accelerating the tissue repair, the quality of the healed tissue and the differences between two applied treatments during the follow-up. **Methods:** MSCs were isolated from bone marrow of BALB/c positive-GFP (Green Fluorescent Protein) mice. Three full thickness excisional wounds were produced with biopsy punch on the dorsal ear surface of three rabbits. The rabbit one was topically treated with 1.2 x 10⁶ MSCs-GFP+ per wound on the left ear and with saline solution on the right ear. The rabbit two was intravenously treated (auricular artery) with 1.0 x 10⁷ MSCs-GFP+. The rabbit three received no treatment. All wounds were covered with a dressing, gauze and adhesive tape. Wounds were assessed clinical-photographically, wound areas were analyzed (ImageJ software) and biopsies were taken: on the 3rd, 7th and 14th days post-wounding, in order to evaluate the properties of MSCs in wound healing by fluorescence. **Results:** The xenografting presented no immune rejection. The presence of the MSCs-GFP+ was verified in the biopsies. On the 3rd day following the wounding, granulation tissue was more evident in the topically treated wounds than in the controls or the systemically treated wounds; on the 7th day, the repair was similar in treated wounds and different from the controls; on the 14th day, the scars from treated wounds showed faster reepithelialization and less fibrotic scarring than the controls (showing scabs). Topically treated wounds had prominent decreasing of area (61.6%) compared to systemically treated (23.0%) and the control (5.4%) from days 3 to 7. On the 14th day both treatments were satisfactory (100% reduction for treated wounds and 55.2% for non-treated). **Conclusions:** These preliminary results showed the effectiveness of MSCs in cutaneous wound healing with better tissue formation and without histocompatibility problems, encouraging us to clarify the properties and clinical applications of MSCs in regenerative medicine for non-healed acute and chronic wounds, as well for severe burns.

Keywords: Mesenchymal stem cells; Xenografting; Wound healing; Rabbit ear model.

Financial support: CAPES.

AC 28 - PREVALENCE OF VARIANT S (HBS) AND C (HBC) HEMOGLOBINS IN CHILDREN FROM RIBEIRÃO PRETO-SP PUBLIC SCHOOLS

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Hemoglobinopathies are a group of hereditary diseases that are classified based on presence of structurally abnormal hemoglobin, such as S, C, D and E hemoglobins, and/or one or more globin chains deficiency such as thalassemias. A punctual mutation occurs in most of the abnormal hemoglobins, in other words, substitution of a single amino acid, involving α , β , δ and γ chains, having already been described more than 1,100 variant hemoglobins. The production of variant hemoglobins and A hemoglobin (HbA) occurs without serious clinical manifestations, in heterozygous state. HbA

is totally absent in the homozygote state and anemia is a consequence of this state. Interactions of two variants hemoglobins or combination of variants hemoglobin and thalassemia have been described. Studies in several regions show that the most variant hemoglobins in Brazil are HbS and HbC. These are from Africa. However, after Brazilian miscegenation, those hemoglobins were found in other ethnic groups. The objective of this study was to evaluate the prevalence of S and C hemoglobins, in children from Ribeirão Preto-SP public schools, Brazil. We examined 253 children aged 6-9 years. The variant Hb were identified by hemoglobin eletroforesis in cellulose acetate in Tris-EDTA-Borate buffer pH 8,6 and by High Performance Liquid Chromatography - HPLC (β -thalassemia Kit; Variant II - Bio-Rad equipment). It was observed 11 (4.35%) children with AS phenotype and 1 (0.4%) with AC phenotype. This study confirms the prevalence described in the literature, which emphasizes the importance of genetic counseling and early diagnosis seeking to increase of quality of life hemoglobinopathies bearers.

Keywords: Hemoglobinopathies; Hemoglobin eletroforesis.

Financial support: CNPq.

AC 29 - PROFILE OF ANTIBIOTIC SUSCEPTIBILITY FROM ISOLATED STRAINS OF THE ORAL CAVITY OF BOTHROPS JARARACA SNAKES, *Bothrops jararacussu* AND OTHER SNAKES

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Introduction: Besides the normal microbiota of the skin of patients, the bacteria present in the oral cavity and prey of snakes may penetrate the skin and subcutaneous tissue, finding favorable conditions for your proliferation. The snakes have in its oral cavity, a variety of bacteria that may be potential pathogens for a secondary infection on bite site. The environmental conditions and form of the collect of the snakes could be a variable in determining these bacteria in oral cavity of snakes. The identification and characterization of microbiota of these snakes have fundamental importance in both the specific guidance of antimicrobials, as the mapping of the agents present in the environment and its reservoirs. **Objective:** In this study, we evaluated the profile of antibiotic susceptibility of bacteria isolated from the oral cavity of 76 *Bothrops jararaca* snakes (caught in the metropolitan area of São Paulo), 17 strains from *Bothrops jararacussu*, and the oral cavity of 19 snakes from other genus (Colubrids). **Methods:** The bacteria were tested by the Kirby-Bauer method for antimicrobial susceptibility, utilizing commercially available sensitivity discs. **Results:** The bacteria tested were Gram-negative bacilli, mainly Enterobacteriaceae, and the isolated strains from snakes *Bothrops jararaca* were sensitive to antibiotics: amikacin, amoxicillin, ciprofloxacin, chloramphenicol, cotrimoxazole and gentamicin. Resistance was seen to cephalothin (50%) and ampicillin (32%). There were observed samples with intermediate sensitivity (22%) and samples resistant (15%) to the antibiotic tetracycline. The bacterial strains isolated from *Bothrops jararacussu* were sensitive to antibiotics: amikacin, ciprofloxacin, chloramphenicol, cotrimoxazole, gentamicin and tetracycline, presenting resistance to cephalothin (76%), ampicillin (65%), amoxicillin/clavulanic acid (24%) and cotrimoxazole (24%). The isolated bacterial strains from the oral cavity of other genus presented sensitivity to antibiotics: amikacin, ciprofloxacin, chloramphenicol, cotrimoxazole, gentamicin and tetracycline, as well as resistance to ampicillin (25%) and cephalothin (56%). There were not found multiresistant strains. **Discussion:** These results showed the variable antimicrobial susceptibility of aerobic gram-negative bacillus. Isolated strains from other genus of snakes have a high index of antimicrobial resistance to ampicillin and cephalothin, as well as those of *B. jararaca*. In contrast, strains isolated from oral cavity of *B. jararacussu* showed high resistance to cephalothin and ampicillin, and lowest level of resistance to amoxicillin/clavulanic acid and cotrimoxazole. Strains of other genus and *B. jararacussu* will be collected to compare with isolated strains from *B. jararaca*. In the cases of secondary infection after snakebite accidents, it is important to know the predominant bacteria commonly found in the oral cavity of snakes and the antimicrobial susceptibility profile of bacteria strains, as well as in the case of others captivity animals unlike from *Bothrops ssp*, because these bacteria may act how potential pathogens to these animals.

Keywords: *Bothrops jararaca*; *Bothrops jararacussu*; Snakes.

Apoio: FAPESP; PIBIC-CNPq.

AC 30 - RECOMBINANT HUMAN ANTIBODY FRAGMENTS PRODUCED BY PHAGE DISPLAY TECHNOLOGY AGAINST THYMOCYTES RECOGNIZE PHERIPHERAL T CELL SURFACE ANTIGEN

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Introduction: The main treatment for the acute rejection of grafts consists of immunosuppressant drugs which inhibit or lyse T lymphocytes. However, such treatment is followed by the immune susceptibility to infections and proneness to other diseases. Polyclonal anti-thymocytes antibodies (ATG) are also used; however, this procedure results in an increase of the susceptibility to linfoproliferative and infectious diseases and could lead to the development of the Serum Sickness Disease, due to heterologous antibodies. In an attempt to avoid the inconvenience of using heterologous antibodies, humanized chimerical antibodies have been developed. These antibodies, nonetheless, stimulate the activation of the complement system and the opsonization of cells by fagocytosis. **Objective:** The aim of this work is to produce scFv portions of human anti-thymocytes immunoglobulins, capable to recognize ppheripheral T cells, for a subsequent test in human lymphocytes proliferation *in vitro* assay and use as suppression therapy after allotransplants. **Methodology:** To produce human monoclonal antibodies, we used the phage display technology and the Griffin.1 library. This consists of the scFv phagemid library constructed by recloning the heavy and the light chain variable regions into the phagemid vector pHEN2. The thymocytes used in the selection were obtained from thymus of children submitted to cardiovascular surgery at the University Hospital of Faculty of Medicine, USP at Ribeirão Preto (HCFMRP-USP). To test whether the phage-antibodies produced against thymocytes were capable of recognizing peripheral T lymphocytes, the ELISA technique was used. The peripheral T lymphocytes were separated by using Ficoll and later isolated in columns of CD4 and CD8. A flow cytometry was performed to evaluate the separation. **Results:** After 3 rounds of selection, the polyclonal ELISA was performed, and at rounds 2 and 3 thymocytes and peripheral T lymphocytes were recognized. Next, a monoclonal ELISA for each round was performed and a total of 162 positive clones to peripheral T lymphocytes were identified. **Conclusion:** The phage-antibodies produced recognize the peripheral T lymphocytes. New experiments are in progress to assess whether the fragments scFv produced by these phages are able to inhibit the proliferation of T lymphocytes becoming in the future a suppression therapy for allotransplants.

Keywords: scFv; Phage display; Transplant rejection; T cell.

Financial support: FAPESP.

AC 31 - SCREENING OF METALLO-ÂETA-LACTAMASE-PRODUCING PSEUDOMONAS AERUGINOSA IN UNIVERSITY HOSPITAL IN RIBEIRÃO PRETO - SP

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Introduction: The metallo-beta-lactamase (MBL) production is an important mechanism of carbapenem resistance in *Pseudomonas aeruginosa*. These enzymes are part of Ambler class B beta-lactamases and they hydrolyze all beta-lactams antibiotics, except aztreonam. This fact severely restricts treatment options against many Gram-negative rods, including *P. aeruginosa*. MBL are not inhibited by commercially available beta-lactamase inhibitors.

As these enzymes require two ions divalentes, commonly zinc, as cofactor for your activity, they have sensitivity to ethylene diamine tetracetic acid (EDTA) and mercaptopropionic acid (MPA). Actually, six subclasses of MBL are known: IMP, VIM, SPM, SIM, GIM, AIM. **Objective:** The aim of the present study was to screen and detect MBL-producing *P. aeruginosa* isolated from patients hospitalized at the Hospital das Clínicas de Ribeirão Preto - USP in the period of April to August, 2007. **Material and Methods:** All imipenem, meropenem or ceftazidime-resistant *P. aeruginosa* isolates were recovered from hospitalized patients admitted in this institution. The isolates were submitted to screening for MBL production with ceftazidime and imipenem in the presence of MPA and EDTA using double-disk synergy test (DDS). Isolates that presented ghost zone in the DDS was considered as presumptive MBL-producers, and these will be submitted to polymerase chain reaction (PCR) for detection of resistance genes. **Results:** *P. aeruginosa* isolates were obtained from urine (31 isolates, 41,33%), sputum (16 isolates, 21,33%), catheter (6 isolates, 8%) and blood (5 isolates, 6,66%). Seventeen (22,67%) isolates were obtained from others sites. Seventy-five isolates were resistant to ceftazidime and/or imipenem/meropenem. Thirty-five out of 75 isolates, corresponding to 46,7%, were presumptive MBL-producers according to DDS. **Conclusion:** The founded results about MBL-producers indicates the necessity of measures to controlling the special of resistance to carbapenems.

Keywords: Metallo-beta-lactamase; *Pseudomonas aeruginosa*; Carbapenem.

AC 32 - SEROTYPE DISTRIBUTION AND SENSITIVITY TO PENICILLIN OF STREPTOCOCCUS PNEUMONIAE ISOLATES FROM 1998 TO 2007 IN THE REGION OF RIBEIRAO PRETO, STATE OF SAO PAULO, BRAZIL

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Introduction: Streptococcus pneumoniae (pneumococci) is usually found in the nasopharyngeal passages of healthy individuals, 20-40% children and 5-10% adults but it could be disseminated through the blood stream causing bacteremia, meningitis, pneumonia or other infections. Pneumococci are the most common agents of bacterial meningitis and the main causes of pneumonia in the elderly and children. In the 1960s, most pneumococci strains were sensitive to penicillin and the treatment of choice was the use of β -actam antibiotics. However, resistance to this class of drugs has been increasing through the years. **Objective:** To evaluate serotype distribution and the susceptibility pattern of pneumococci in the region of Ribeirão Preto. **Material and Methods:** Five hundred and three pneumococcal isolates were processed at the Adolfo Lutz Institute during the period of 1998 to 2007. The samples, isolated from Cerebrospinal Fluid, blood, pleural effusion and other clinical samples were from municipal and regional patients. Isolate identification was by conventional methods. Oxacillin discs, 1 mg, were used to detect resistance to penicillin. Antibiotic Minimal Inhibitory Concentrations (MIC)s, determined as indicated by the Clinical and Laboratory Standards Institute (CLSI) and typing utilizing the Quellung reaction were conducted at the Regional Laboratory São Paulo, Adolfo Lutz Institute. **Results:** During the last 10 years, the origins of the 503 pneumococcal isolates were as follows: 221 (43.94%) from meningitis patients; 217 (43.14%) from pneumonia; 15 (2.985) from bacteremia and 50 (9.94%) from other clinical sources. Serotype 14 prevailed in 17.49% of the cases, followed by types 3, 1, 9V, 19F, 23F, 6A, 6B, 19A and 4. Most isolates were from patients of 16 to 60 years of age. After the general survey with the oxacillin discs, MICs were determined in 132 samples (26.24%) of which 108 (21.47%) showed intermediate resistance, 42 (8.35%) were resistant and 72 (14.31%) were sensitive. **Conclusion:** Decisions on vaccine formulations depend on regional and temporal information about which serotypes may cause disease, as epidemiological aspects of this condition vary from country to country and in the course of time. Thus, establishing control strategies needs local periodic evaluations. In addition, rational choice of the initial empirical treatment of pneumococcal diseases is based on the clinically important monitoring of resistance to antimicrobials.

Keywords: Streptococcus pneumoniae; Serotypes; Resistance; Penicillin; Meningitis.

AC 33 - STANDARDIZATION AND EVALUATION OF IMMUNOLOGICAL DETECTION METHOD FOR SHIGA TOXIN EXPRESSING-*Escherichia coli* ISOLATES

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Introduction: STEC infection is an important food borne pathogens that may cause a wide range of disease symptoms, since an asymptomatic event until hemolytic-uremic syndrome and hemorrhagic colitis. The main virulence factors for STEC, the Shiga toxins (Stx) are responsible for protein synthesis inhibition. Numerous assays for its diagnosis have been developed and some of the evaluated kits showed variability in sensitivity and specificity when tested by different reference laboratories. The commercially available immunological kits offered by different companies are economically unaffordable in developing countries. **Objectives:** The aim of this study was the standardization and evaluation of a specific, sensitive and low cost diagnostic method for Shiga toxins detection. **Methods:** The definition of a growth media that induce better expression and production of Stx was determined: STEC strains were grown at 37°C for 24 h in eight different media and toxin production was measured by enzyme-linked immunosorbent assay (ELISA) in the pellet and/or supernatants of cultures in the presence or absence of ciprofloxacin or novobiocin. Besides, selected monoclonal antibodies supernatants were purified by affinity chromatography, isotyped and characterized. Then a capture ELISA using polyclonal and monoclonal antibodies anti-Stx1 and anti-Stx2 were developed. For assay standardization, different concentrations of polyclonal and monoclonal antibodies were tested. Also, different dilutions of mouse anti-goat peroxidase conjugated and plates were tested. **Results:** The results showed that the most suitable medium for Stx production was *E. coli* broth when the bacterial isolates were grown for 4 h in the presence of ciprofloxacin and the production is detected in the supernatant. The monoclonal anti-Stx1 and anti-Stx2 antibody was subcloned several times and both were classified as IgG1. Anti-Stx1 antibody was able to recognize the A subunit of both toxins, besides showing higher affinity constant than anti-Stx2 antibody. Thus, the capture ELISA was standardized using 250 μ g/ml of the IgG enriched fraction of rabbit anti-Stx1 and anti-Stx2 sera for coating and 2.5 μ g/ml of monoclonal anti-Stx1 antibody for the capture of the toxin. After development of the assay with 149 isolated (between positive and negative strains) the capture ELISA showed 80.7% of sensitivity, 100% of specificity and 93% of efficiency. **Conclusion:** These results indicated the capture ELISA had the sensitivity similar to those in the literature, but isolates expressing-Stx2 were not recognized, probably due anti-Stx1 specificity, as it was able to recognize the A subunit of both toxins only in denaturation conditions, which lead us to the improvement of this method in order to increase the accuracy of the immunoassay.

Keywords: STEC; Immunodiagnostic; Shiga toxin; Capture ELISA.

Financial support: FAPESP.

AC 34 - STANDARDIZATION OF A ONE-STEP REAL-TIME RT-PCR FOR RAPID DIAGNOSIS OF YELLOW FEVER VIRUS INFECTION

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Yellow fever, the prototype viral haemorrhagic fever, is an acute infection characterized by hepatitis, renal failure, cardiovascular collapse and bleeding. The causative agent, a mosquito-borne RNA virus belonging to the family Flaviviridae, as dengue and Japanese encephalitis viruses. Yellow fever was a severe public health problem until the early 20th century, and today it remains endemic and epidemic in tropical regions of South America and Africa. In Brazil, from December of 2007 until July of 2008, 75 suspicious cases of jungle yellow fever were notified. Among 45 yellow fever confirmed

cases, 25 evolved to death (55.6% of case fatality rate). A rapid and reliable detection method is crucial for the patient's appropriate treatment and to monitor and to control the virus dissemination. We have standardized a Real-Time RT-PCR technique, using Yellow Fever Virus (YFV) specific designed primers. The YFV strain 17D (vaccine) was propagated in C6/36 *Aedes albopictus* cell culture. Viral RNA was extracted using QIAamp Viral RNA Mini Kit (QIAGEN, Inc.). Real-Time RT-PCR was performed by using SuperScript III Platinum SYBR Green One-Step qRT-PCR Kit (Invitrogen Corp.). The test took 2 hours to be performed. The temperature of melting (T_m), 85.7°C, was determined increasing the temperature from 60°C to 95°C at 0,2 degrees per second. The method showed a high sensitivity.

Keywords: Yellow fever; Real-Time RT-PCR; Diagnosis.

Financial support: CNPq; FAPESP.

AC 35 - THE EFFECTS OF ZINC SUPPLEMENTATION DURING *Trypanosoma cruzi* EXPERIMENTAL INFECTION

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Introduction: Chagas' disease, caused by the protozoan *Trypanosoma cruzi*, is considered the sixth most important neglected tropical disease worldwide. During the first week of infection, NK cell-derived gamma interferon (IFN- γ) is involved in controlling intracellular parasite replication, mainly through the induction of NO biosynthesis by activated macrophages. Nitric oxide (NO) is considered the major effective molecule of intracellular amastigotes' killing. Zinc is essential for growth and development of all organisms. Several studies have demonstrated the benefits of zinc supplementation on infectious diseases in human populations. **Objective:** Evaluate the effects of zinc supplementation on cytokines and NO production and the resistance to *T. cruzi* during the acute phase of infection. **Methodology:** Male *Wistar* rats weighing 100g were divided into the following groups: non-infected males-without-zinc supplementation (MWZNI), non-infected males-zinc supplemented (MZNI), infected males-without-zinc supplementation (MWZI) and infected males-zinc supplemented (MZI). Rats were i.p. infected with 1 x 10⁵ blood trypomastigotes (Y strain) of *T. cruzi*. Studies were performed 7 and 14 days after infection. Parasites counts were evaluated by Brener's Method. Animals from all treated groups received 0.1 mL of zinc sulfate (gavage), at a dose of 20 mg/kg body weight, once a day over the course of the experiment. Cells were harvested from the peritoneal cavity, adjusted to 5 x 10⁶ cells/ml and dispensed into 96-well flat-bottom plates with or without LPS (1 μ g/ml). Cultures were incubated at 37°C, 5%CO₂ for 48h. NO was measured in the supernatants by using the Griess reaction. The absorbance was determined at 540nm. Serum levels of IFN- γ were quantified using BD OptEIA™ SET Rat IFN- γ (BD Biosciences, U.S.A.). **Results:** Zinc supplemented groups displayed reduced levels of blood parasites when compared to infected and unsupplied animals. Zinc supplementation triggered enhanced IFN- γ concentrations as well as a higher production of NO by peritoneal cavity cells in all infected and treated groups when compared to untreated and infected animals. **Conclusion:** zinc supplementation led animals to a more efficient immune response reducing the pathogenic effects of the experimental disease.

Keywords: *Trypanosoma cruzi*; Zinc; Interferon gamma (IFN- γ); nitric oxide (NO).

Financial support: CAPES.

C 36 - TOXICITY EVALUATION OF THE SOLVENT DIMETHYL SULPHOXIDE (DMSO) ON CULTURES OF *Leishmania amazonensis*

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Introdução: The dimethyl sulphoxide (DMSO) was synthesized in Germany between 1866 and 1867, when was describe a notable solvent capacity. It is a molecule with a polar domain and two apolar groups, making it the most common solvent experimentally used to dissolve hydrophobic substances for *in vivo* and *in vitro* purposes. The characteristic amphipathic of the molecule became it soluble in both aqueous and organic media. This explicate your capacity of penetrate rapidly through biological membranes and cellular barriers, probably by altering lipid packing and producing structural defects in the bilayer, what her award a potential effect cytotoxic. A wide range of pharmacological effects exerted by DMSO has been documented in experimental animal models, where can be observed permeability increase of the molecules and transport through biological membranes. **Objective:** In function from this application for DMSO in tests *in vitro*, justify the determination of concentration in what him raisin to exert toxicity for cells. In this sense, the present study assessed the toxic potential from DMSO on promastigotes cultures of *Leishmania amazonensis*. **Methods:** Promastigote forms of *L. amazonensis* in log phase of growth (10⁶ parasites/mL) were grown on a 24-well plate in Schneider's medium supplemented with 10% heat-inactivated FBS and 1% of penicillin/streptomycin 10000UI/mL in the presence of different concentrations of DMSO (0.1 to 15.0% v/v) at 25°C, to evaluate parasite survival. The medium alone were used as controls. After 72h, promastigote forms were counted in a hemocytometer and the percentage of inhibition was determined. Each experiment was performed in triplicate, and the results were expressed as percentage of inhibition in relation to the control. The 50.0% inhibitory concentration (IC50) was determined by non linear regression model and the variance analysis of the data obtained was performed to evaluate the differences among the concentrations. **Results:** There were differences significant in the effect of DMSO on the growth of *Leishmania* promastigotes (p<0,05), except above of 7.5%, where the resulted were equals. It inhibited growth of the promastigote forms, with IC50 of 1,06%, after 72h of incubation. This information show which the use of higher concentrations de DMSO how solvent in *in vitro* tests can influence the results obtained. **Conclusion:** The DMSO is a tool necessary and widely used in dissolution of substances. However, this use must be discerning, because the same in short concentrations can show citotoxicit. Nevertheless, this effect is private for each type of experiment, due your higher capacity for to cross and disorganizer biologics membranes. This interference can be minimized by use of the DMSO in same concentration in whole experiment and a control only with the solvent.

Keywords: Toxicity; DMSO; *Leishmania*.

AC 37 - BACTERIAL BIOFILM FORMATION ON BIOMATERIAL ABIOTIC SURFACES

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The surfaces that allow biofilm formation can range from abiotic surfaces to biotic surfaces. Bacterial biofilms are formed from individual planktonic cells in a complex developmental process and can survives on abiotic surfaces in hospital environments and colonizes different medical devices. The objective of this research was to assess the capacity of staphylococcal survival on abiotic surfaces. Polymethylmetacrylate (PMMA) discs and 1-centimeter-long segments of siliconized latex and polyurethane catheters were cut and served as the biomaterial substratum in this study. Each biomaterial sample was tested against each of the bacterial isolates *Staphylococcus aureus* ATCC 25923 and *Staphylococcus epidermidis* ATCC 12228 in duplicate. Samples were separately and aseptically introduced in a test tube containing 5ml of Brain Heart Infusion (BHI) broth inoculated with staphylococcal suspension of 10⁸ CFU/mL each one. The samples were incubated under aerobic conditions for 24 and 48 hours at 37°C. Afterward the coupons were removed and maintained in a dry test tube under sterile conditions for 7 days at room temperature. Then, three dry coupons were inserted, one by one, into separated tubes containing BHI broth (5mL) and incubated for 24 and 48 hours at 37°C. Also, tubes containing three of the samples in 1.0mL of phosphate buffer saline were sonicated for 8 minutes in a water bath sonicator to dislodge adherent bacteria. The number of viable staphylococci cells were assessed by quantitative culture of serial 10-fold dilutions of the sonicate. The sonicated coupons were cultured in BHI for 24

h at 37°C. SEM was used to observe bacterial adherence on abiotic surfaces. The staphylococcal growth was evaluated at 24 and 48h observing the turbidity of BHI broth. The results shown that before and after the 7 day sterile preservation of the tested biomaterials, the BHI broths containing samples of each materials were visually cloudy before and after 7 day sterile preservation and subsequent incubation. The number of bacteria surviving on the three abiotic surfaces was below the level of inoculation of the assay. The average cfu count for *S. aureus* after 24 h was about 28.3×10^2 and after 48 h was 6.6×10^2 , and for *S. epidermidis* after 24 h was about $10^9.6 \times 10^2$ after 48 h was 7.0×10^2 . SEM shown that abiotic surfaces allowed bacterial adherence. In conclusion bacteria were able to colonize and proliferate on these abiotic surfaces, then, these results suggest that the abiotic surfaces studied are not resistant to biofilm formation.

Keywords: Bacterial adherence; Biomaterials; Polyurethane; PMMA; Biofilm.

AC 38 - EVALUATION OF POSSIBLE SINERGISM BETWEEN MELATONIN AND MELOXICAM IN THE ACUTE PHASE OF EXPERIMENTAL CHAGAS DISEASE

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Introduction: Modulation of immune responses by administration of pharmacological active substances in models experimentally infected with *Trypanosoma cruzi* were hardly contributed in the research of new therapies against Chagas' disease. Due immunosuppressor characteristics of the acute phase of the disease, the pharmacological interventions on *in vivo* models capable of modulate the immune system through Th-1 cytokine production are exhaustively studied, been also relevant the effects of E2 prostaglandin synthesis blocker in the acute phase of Chagas' disease. **Objectives:** To evaluate a possible synergic and immunomodulatory effects subsequent to the administration of Meloxicam and Melatonin through stimulation of Th-1 cytokines production, increase of the nitric oxide production, and E2 Prostaglandin synthesis block, verifying the impact of treatment on parasitemic blood of male *Wistar* rats infected by *Trypanosoma cruzi* Y strain. **Methods:** Five male *Wistar* rats by group weighting 90-100g being divided into: (IWT) - Infected without treatment; IT (Mel) - Infected Treated with Melatonin; IT (Mx) - Infected Treated with Meloxicam, IT (Mel + Mx) - Infected Treated with Melatonin and Meloxicam. After infection with 1×10^5 Y strain blood forms of *T. cruzi* groups were treated only in the morning with an oral solution of the substance diluted in 400 Polietilenoglycol, and distilled water (1:1 proportion). Experiments were accomplished in the 7th, 14th, and 21st days after infection being the parasitemy peak determined by Brener method. Animals were killed by decapitation post anesthesia. Nitric oxide was quantified by means after the peritoneal washed cells recovering, and cytokines were measured after serum extract by specific Immunological kits (R&D Systems). **Results:** We could observe the increase of I FN- γ and IL-2 levels also a parasitemic decrease in experimental infected groups. Animals presented an increase of NO production which could be explained by the probable modulation of the immune systems of experimental groups treated mainly with the drugs combination in relation to TH1 cytokines production. **Conclusion:** The results suggested a synergic effect of the studied substances which could contribute to establish alternative treatment mechanisms by a better understanding of the immune responses against *T. cruzi* acute infection.

Keywords: *Trypanosoma cruzi*; Meloxicam; Melatonin.
Financial support: FAPESP.

AC 39 - CROSSTALK AMONG COMMENSAL *E. coli* AND ENTEROPATHOGENIC *Escherichia coli* (EPEC)

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Introduction: Quorum sensing is used to designate a mechanism of gene regulation depending on cellular density. The bacteria produce substances that accumulate in growth media, and when these substances reached a threshold concentration, a variety of responses can occur. These molecules are called autoinducers and the phenomena called quorum sensing. Quorum sensing is widespread in gram negative and gram positive bacteria and is involved in various cellular mechanisms, including virulence regulation. At least, three autoinducers were described in literature, and were designated AI-1 (autoinducer 1), AI-2 (autoinducer 2) and AI-3 (autoinducer 3). **Objectives:** This study was developed to verify the possibility of crosstalk among EPEC and other enterobacteria, in intestinal infections. **Methods:** Pre conditioned medium: Bacterial strains were grown to 37°C with agitation of 250 rpm in LB 0.4M NaCl until OD_{600} of 1.0. The growth was centrifuged, and supernatant were filtered in 0.22 μ m membrane. β -galactosidase assay: A reporter TEV5232 strain containing the *LEE1:lacZ* fusion was grown in pre conditioned medium until reach an $OD_{600} \leq 0.2$, and β galactosidase activity was measured in Miller units. Adherence assays: Bacterial strains were grown for 18h in LB medium at 37°C. From the uninduced overnight cultures, 10^5 CFU was added to HEp-2 cells, incubated for 3h at 37°C with 5% CO_2 , washed with PBS, fixed with methanol and stained with Giemsa stain. For quantification of adherence, after 3h incubation, the nonadherent bacteria were removed by PBS washing and HEp-2 cells were lysed with 1% Triton. Serial dilutions of the bacterial cells were plated in LB agar, and CFU were counted. **Results:** It was possible to verify the production of autoinducer AI-3 in the strains tested, and also quantify their ability of induction. Apparently, the inoculation of cell cultures with enterobacteria influences the cellular responses to EPEC strains. Discussion and conclusion: Pre conditioned media of this strains were capable to activate the transcription of *LEE1:lacZ* fusion, indicating that the molecule of autoinducer can be produced by a strain and recognized by another, highlighting the possibility of interspecific crosstalk. Using strains of enterobacteria in adherence assays, we observed a different interaction of EPEC with HEp-2 cells.

Keywords: Quorum sensing; Enteropathogenic *Escherichia coli* (EPEC).
Financial support: FAPESP.

AF 01 - REDUCTION OF CARDIOVASCULAR RISK IN PATIENTS WITH METABOLIC SYNDROME AFTER PARTICIPATION OF A PHARMACEUTICAL CARE PROGRAM IN A PUBLIC HEALTH CENTER

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Introduction: the Metabolic Syndrome (MS) is a set of chronic diseases with metabolic origin and related with hypertension, obesity, dislipidemia and fasting hyperglycemia. These combination of factors put patients in a treatment that needs the use of a set of medicines which could determine a high degree of non-adherence to the treatment as well as the development of drug-related problems (DRP). **Objective:** the aim of this study was to verify whether the institution of a Pharmaceutical Care (PC) program in a Public Health Center (PHC) to MS patients would result in positive influence on their clinical profile with reduction of the cardiovascular risk. **Methods:** it was performed a case control study with 73 patients participants of *HiperDia* program from a PHC (Vila Velha-ES, Brazil) previously identified with MS. They were randomized into two groups: Control (C; n=37) and Intervention (I; n=36). The presence of MS was confirmed by clinical and laboratory evaluation according to the National Cholesterol Education Program's Adult Treatment Panel III (NCEP-ATP III) and the cardiovascular risk was analyzed by the updated Score of Framingham (SF). These evaluations were performed in both groups by a pharmacist blind to the experiment before and after the follow-up program. The only intervention of the pharmacist team to the C group was an initial interview and pharmacotherapy history analysis. A team of four pharmacists developed a PC program according to *Dáder* methodology. DRP were identified according to the Second Consensus of *Granada* and categorized in DRP related to: necessity, effectiveness and safety. In I group these problems were analyzed and the best solution was proposed in accordance with the patient. The pharmacotherapy follow-up program consisted of a monthly meeting at the PHC during six month from April to September of 2007. In each meeting arterial blood pressure and capillary blood glucose,

cholesterol and triglycerides were measured. **Results:** there were no significant differences on the initial socio-economic and clinical profile data between the groups. DRP were identified in both groups (C=123; I=129) with predominance of the DRP related to effectiveness (C=47.3%; I=45.8%). The improvement (81.5-100%) or resolution (50-90%) of the DRP in I group resulted in a improvement in almost all parameters analyzed (PAS=131±3; women waist circumference=94±2; glucose: 130±12; cholesterol=182±5; men HDL-c=47±2; women HDL-c=49±1; LDL-c=111±5; p<0.05 or 0.01) and a reduction of the SF (14.3±1.5%; p<0.01) compared with C group (PAS=141±4; women waist circumference=105±2; glucose: 173±13; cholesterol=210±4; men HDL-c=40±3; women HDL-c=41±3; LDL-c=132±4; p<0.05 or 0.01. SF=26.4±2.7%). **Conclusion:** the institution of a PC program in a PHC determines the reduction of the cardiovascular risk of the patients' adherent to the program as a result of the improvement or resolution of the majority of the DRP after the pharmacist interventions (UVV).

Keywords: Drug-related problem; Metabolic Syndrome; Pharmaceutical Care.

AF 02 - STUDY OF THE GESTACIONAL PROFILE IN BRAZIL, COMPARED TO THE STATE OF SÃO PAULO AND THE CITY OF RIBEIRÃO PRETO-SP

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Introduction: The pregnancy causes biological, family, emotional, social and economic changes that affect the woman herself and the society as a whole. However it is important that these changes happen in a structured way, the social and governmental actions with these users are essential. **Objectives:** Evaluating the profile of the Brazilian pregnancy compared with the State of São Paulo and the city of Ribeirão Preto. **Methods:** Data collection was conducted between May and July of 2008 with the database of the Information Department of the Brazilian Public Health System (DATASUS), the Ministry of Health. The variables of age pregnancy woman, educational level, civil status, delivery routes and number of prenatal visits in Brazil in São Paulo state and the city of Ribeirão Preto, during the years 2001 to 2005, were collected. **Results:** The analysis of the data has shown that from 2001 to 2005 the average age of women has increased in places studied, and the level of schooling that in 2001 the percentage of women who had 8 to 11 years the education was 34.5%, in 2005 this group accounted for 42.9% of pregnant women. It was also observed that the status of women changed as the number of single mothers increased to the detriment of women married or living in a consented union. Regarding delivery routes an average increase in the number of caesarians was found, 48% in 2001 to 51.4% in 2005, reducing the number of normal childbirth. The number of prenatal visits increased during the period of study, because in 2001, 59% of women have held seven or more consultations before the completion of delivery, and this representation in 2005 reached an average of 68% of pregnant women. **Conclusion:** The profile of Brazilian gestational, compared to the State of São Paulo, and the city of Ribeirão Preto take a similar pattern, showing uniformity in the evaluated data.

Keywords: Pregnant; Brazilian Public Health System (SUS).

AF 03 - USER RIGHTS WITH DIABETES ON PHARMACEUTICAL ASSISTANCE

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Introduction: On September 29, 2007, entered into force the Federal Law 11.347/06, which has over the free distribution of medicines and materials needed for their implementation and monitoring of capillary blood glucose for diabetics enrolled in programmes of education for diabetes. **Objective:** This study aims to describe what users with diabetes know about this law and what they have enjoyed the same. **Methods:** Data were collected through interviews audiogravada and submitted to the analysis of thematic content, the Center for Nursing Education for Adults and elderly, in July 2008. **Results:** The themes that emerged from the analysis were: difficulty in access to health services, to delay purchases of medicines and supplies, impersonal care by the team of health and ignorance of the existence of the law 11.347/06. These issues relate to the existence of a health system still fragile in its practicality, where existing laws are not charged or by their own benefit, users of health. Based on the results, it is observed that for the rights of the user specified in diabetic Law are implemented there is a need to disseminate the same, so as to make them known to the public and health professionals themselves. **Conclusion:** Since the nursing as a link between users and the current models of existing health, it is clear that, increasingly, there is the need for the nursing staff know and educate users in order to provide information about their disease, its treatments and now their rights as belonging to health systems. Thus, it is clear the responsibility of the health professional, while being pro-active, to incorporate a reflexive stance and ethics that addresses the adoption of new knowledge and new skills necessary for effective communication with the health of users, configuring an environment in which the humane aspect is indispensable.

Keywords: Diabetes; Nursing; Rights; Users.

BM 01 - ALTERED EXPRESSION OF THE MITOTIC CHECKPOINT GENES *BUB1*, *BUBR1*, *BUB3*, *MAD1*, *MAD2* AND *AURKA* IN CELL LINES OF CENTRAL NERVOUS SYSTEM TUMORS (CNST)

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Most solid malignant neoplasm show chromosomal aberrations and aneuploidy. The spindle checkpoint is the primary mechanism to ensure that two daughter cells get the same amount of DNA, and its dysfunction has been suggested to contribute to aneuploidy and carcinogenesis. Several proteins, including Mad1, Mad2, Bub1, BubR1 and Bub3, which are specifically recruited to unbound kinetochores upon checkpoint activation, are required for the spindle checkpoint. Significantly, spindle checkpoint defects are frequently observed in human cancer including breast, colon, lung, ovarian, and hepatocellular carcinomas. AURKA plays a critical role in centrosome maturation and bipolar spindle assembly, and its overexpression has been shown to induce centrosome amplification and aneuploidy in human cell lines. In this study, we investigated the expression of the genes *BUB1*, *BUBR1*, *BUB3*, *MAD1*, *mad2* and *AURKA* in seven cell lines of CNST in relation to the not-neoplastic tissue (white brain substance) by real time-polymerase chain reaction (RQ-PCR). We used the cell lines of the pilocytic astrocytoma (R286), anaplastic astrocytoma (UWV467), pediatric glioblastoma (SF188), adult glioblastoma (U343), medulloblastoma (UWV3), oligodendoglioma (R280) and ependymoma (R253). The expression of the genes *BUB1*, *BUBR1* and *AURK* was significantly higher in relation to the not-neoplastic tissue in all the cell lines. On the other hand, all the cell lines showed underexpression of the genes *MAD1*, *mad2* and *BUB3*. Similar results were observed in others works studying cell lines with chromosomal instability. Our findings suggest that a defective mitotic checkpoint characterized by reduced expression of *MAD1*, *mad2* and *BUB3* contribute to chromosomal instability in CNST. High levels of the particular transcripts of the *AURKA*, *BUB1* and *BUBR1* could represent a cellular compensation for defects in molecular components of the mitotic spindle damage checkpoint. These results suggest that these genes may play a relevant role in CNST, and may represent putative therapeutic targets for CNST. The white substance of the brain does not is the normal counterparty of all tumors of this study, so these findings should be validated with functional tests and with studies in primary tissue samples from CNST patients.

Keywords: Mitotic checkpoint genes; Central nervous system tumors.

Financial support: CAPES; FAEPA; CNPQ.

BM 02 - ANTIGENOTOXICITY OF ROSMARINIC ACID IN V79 CELLS EVALUATED BY THE COMET ASSAY

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Rosmarinic acid (RA), a natural phenolic compound, is mainly found in species of the family Boraginaceae and in the subfamily Nepetoideae (family Lamiaceae). A number of bioactivities have been assigned to RA, such as antidepressive, hepatoprotective, anti-inflammatory, antiangiogenic, antitumor, and HIV-1-inhibiting properties. RA is also known to possess marked antioxidant properties as a reactive species scavenger and lipid peroxidation inhibitor. RA has a broad range of applications, including products ranging from food preservatives and cosmetics to medications. Therefore, the evaluation of a possible genotoxicity activity of RA is important to guarantee its safe use in humans. Thus, the objective of the present study was to evaluate the genotoxic and/or antigenotoxic potential of RA on Chinese hamster lung fibroblasts (V79 cells) using the Comet assay. Three doses of RA (100, 200 and 400 µg/mL) were used for the evaluation of its genotoxic potential. In the antigenotoxicity assays, the different concentrations of RA were combined with the chemotherapeutic agent doxorubicin (DXR, 0.5 µg/mL). Cell viability was measured by the trypan blue dye-exclusion test, all measurements conducted with V79 indicated viability of >90% for all experimental groups. The Comet assay revealed that the different concentrations of RA tested presented no genotoxic activity. Treatment with different concentrations of RA combined with DXR showed a significant reduction in the frequency of DNA damage compared to cultures treated with DXR only. Although the mechanisms underlying the antimutagenicity of RA are not completely understood, the putative antioxidant activity of RA might explain its effect on DXR genotoxicity.

Keywords: Rosmarinic acid; Comet assay; V79.

Financial support: FAPESP; Universidade de Franca.

BM 03 - ANTISERUM AGAINST RECOMBINANT NCTRAP-2 DECREASES THE *in vitro* INVASION PROCESS OF *Neospora caninum*

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The protozoan *Neospora caninum* is responsible for infecting a wide range of animals and in bovines it can induce abortions, which represents huge economical losses. *N. caninum* is an Apicomplexan parasite, phylum of intracellular parasites where the invasion step is crucial for survival. One important group of proteins secreted prior to invasion is the TRAP (Thrombospondin Related Anonymus Protein) family. Upon contact with host cells the TRAPs undergo exocytosis onto the apical surface of the parasite where they initiate tight binding. The adhesive complexes are translocated towards the posterior pole of the parasite via actin-myosin-based motility machinery, resulting in invasion of the host cell. In *N. caninum* one member, NcTRAP1 was previously described. A second possible member called NcTRAP2 was detected by non-annotated EST clustering. The aim of the present work was the cloning of the NcTRAP-2 full-length sequence, production of recombinant antisera, localization of native form in 2D western blot and *in vitro* invasion inhibition assay. The full-length gene was obtained with a predicted protein sequence with 39% of identity and 53% of similarity with its homologues of *Toxoplasma gondii* (TgMIC-2); 39% and 53% with *Neospora caninum* (NcTRAP-1). The TRAP homologues have a signal peptide, two adhesive domains (an integrin-like domain and one or more thrombospondin type I repeats) and a transmembrane domain. Two recombinant fragments (fragments 1 and 2, both without signal peptide and transmembrane region) of NcTRAP-2 were generated (pET28 vector), MW of 50 and 78 kDa (fragment 2 is 163 aa longer towards the C-terminal end). Antisera against the recombinant forms were obtained and monitored by ELISA. The antiserum against recombinant fragment 1 recognized the native NcTRAP-2 (80 kDa, acidic PI) and putatively two isoforms (50 kDa, neutral PI) in 2D western blot. *In vitro* assays were performed to test inhibition of the invasion with the sera against recombinants 1 and 2 which were estimated both by: microscopic counting (10 fields/well in Giemsa stain) on 8 well plates and Real Time PCR on 24 well plates. The sera against recombinants forms of NcTRAP-2 (fragments 1 and 2) decreased the *in vitro* invasion process in 46% and 27% respectively.

Keywords: *Neospora caninum*; Apicomplexa; Thrombospondin related anonymous protein.

BM 04 - CHROMOSOMAL IMBALANCES OF OSTEOSARCOMA DETECTED BY COMPARATIVE GENOME HYBRIDIZATION (CGH)

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Osteosarcoma (OS) is the most frequent aggressive bone malignancy affecting children and young adults with an event-free survival of 50-70% after 3 years. The incidence peak occurs during the second decade of life, suggesting a relationship between rapid bone growth and the development of this tumor. The knowledge of the genetic basis behind tumor progression is still limited. Conventional cytogenetic studies have demonstrated that OS exhibits high karyotypic heterogeneity, with different degrees of aneuploidy and complex structural rearrangements. The CGH is an important tool for studying the genomic profiles of solid tumors, and has confirmed the complexity of karyotypic alterations in OS. However, previous studies have shown divergent results and few have correlated them with tumor progression. The objective of present study was to identify chromosome imbalances in nine samples of OS by CGH. 3 biopsies, 5 resections before chemotherapy and 1 metastasis were analyzed. The experiments were performed accordingly with Kallioniemi et al. (1994). Briefly, test and control genomic DNAs were labeled by nick-translation using the commercial Biotin-Nick translation Mix and Dig-Nick translation Mix kits (Roche, Mannheim, Germany), respectively, according to the manufacturer's instructions, to obtain DNA fragments ranging from 300 to 1000 pb. Hybridization was performed at 37°C for 72 h. 15 metaphase images were analyzed by using an epifluorescence microscope (Olympus BX-40) equipped with a cooled charge-coupled device camera. Ratio profiles were calculated using the CGHView™/EXPO software (Applied Spectral Imaging®, Carlsbad, CA, USA). The thresholds for identification of imbalances were established through control experiments. Chromosome or chromosomal regions outside the calculated interval were considered to be over- or underrepresented. Telomeric and heterochromatic regions were excluded from the analysis, according to Kallioniemi et al. (1994). CGH detected chromosomal imbalances in all samples. Gains were more frequent than losses. Many chromosomal alterations were observed, especially gains at 1q, 2, 3p, 4, 5p, 6, 7, 8, 11p, 14q, 16, 21q and X; and losses at 1p, 2q, 3q, 5q, 9q, 11q and 17q. The minimal regions of superposition were gains of 2p13-p14, 2q36-q37, 4q21 and 8p22, and losses of 1p43.2, 3q22-q23 and 3q24. Three patients had consecutive samples, and the chromosomal alterations varied, reflecting the chromosomal heterogeneity for each case. The highest clonal divergence among the consecutive samples was observed between resection and the corresponding metastatic sample, showing the chromosomal complexity acquired during the progression and dissemination in this case. Additional investigations for the characterization of genes at these regions are necessary. Reference: Genes Chromosomes Cancer. 1994 Aug;10(4):231-43.

Keywords: Osteosarcoma; CGH; Cytogenetics; Imbalances.

Financial support: CAPES; FAPESP; FAEPA.

BM 05 - EVALUATION OF CITOTOXICITY, CELLULAR PROLIFERATION AND APOPTOSIS IN HTC CELLS TREATED WITH B-GLUCAN EXTRACTED FROM BARLEY

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Introduction: The β -glucans (β G) are polysaccharides produced by several plants (such as oats, barley and algae) and constitute the cell wall of pathogenic bacteria and fungi. Some of its benefits are: the stimulation of the immune system, of haematopoiesis, the prevention of mutagenic effects, antigenotoxic capacity against DNA damage inducing agents, such as hydrogen peroxide, reduction of growth of the mammary carcinoma and melanoma, anticlastogenic potential, and others. **Objectives:** evaluate citotoxicity of five concentrations of β G extracted from barley (Sigma) through the cytotoxicity assay (MTT); observe whether β G changes the cellular cycle, accelerating or delaying; and whether occurs cellular death, through Cellular Proliferation Kinetics Analysis; evaluate apoptosis inductor effects on cells culture exposed to β G through Apoptosis detection *in situ* assay with Acridine Orange. All of these assays were made in hepatic lineage of *Rattus norvegicus* (HTC)- cellular cycle of 24 hours. **Materials and Methods:** The cells were cultivated in DMEM-F12 medium. The cytotoxicity analysis in five concentrations (10, 50, 100, 200 e 400 μ g/mL) was made through MTT assay in the times 24, 48, 72h. The Cellular Proliferation Kinetics Analysis was made in eight treatments: Mitomicina-C: 0,3 μ g/mL, control (medium + 1% of DMSO), three concentrations of β G (10, 50 e 100 μ g/mL) and these same concentrations of β G associated to chemotherapeutic agent Mitomicine C. The analysis was made in Neubauer plate after 24, 48, 72 and 96 hours of the treatment to form a growth curve. In these treatments, the cell viability was also evaluated through Trypan Blue method. The Apoptosis detection *in situ* assay analyzed the apoptosis rate of the HTC cells exposed to the same three concentrations of β G. Cisplatin was used as damage induction control for apoptosis and medium with 1% of DMSO as control. The analysis was made in fluorescent microscope after 24 hours of treatment. **Results:** The concentrations 10, 50 and 100 μ g/mL of β G did not show cytotoxicity. In the Cellular Proliferation Kinetics Analysis, the cycle was amended only in the cells exposed to Mitomicine C, though the treatments with β G didn't significantly changes the cellular cycle ($p < 0,05$). The results of Apoptosis detection *in situ* assay with Acridine Orange were analyzed by Chi-square test ($p < 0,05$) and there wasn't significant difference between treatments. **Conclusion:** The data obtained in the experimental tested conditions, didn't show that β -glucan of the barley induces apoptosis or changes the cellular cycle.

Keywords: β -glucan, cytotoxicity; Cellular Proliferation Kinetics; Apoptosis.
Financial support: CNPq; CAPES; FUNDAÇÃO ARAUCÁRIA; UEL.

BM 06 - EXPRESSION, PURIFICATION AND PRELIMINARY CRISTALLOGRAPHIC STUDIES OF CHLOROCATECHOL 1,2-DIOXYGENASE FROM *Pseudomonas putida*

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The increasing demand of industrialized products and decades of modern agriculture practices are responsible for the increase of organic pollutants discarded in the environment. Aromatic compounds, such as catechol, chlorocatechol and their derivatives, are among the most hazardous ones due to their carcinogenesis potential and recalcitrant properties to degradation. A modern and efficient biotechnological strategy for the elimination of these compounds is called bioremediation and it is based on the use of living microorganisms, or their enzymes, to clean up contaminated soil or water. An example, it is the use of dioxygenases, which are bacterial nonheme iron enzymes responsible for the aerobic biodegradation of aromatic compounds. In this work, we present the overexpression, purification and preliminary crystallographic studies of chlorocatechol 1,2-dioxygenase from *Pseudomonas putida* (Pp 1,2-CCD). This enzyme is a dioxygenase belonging to the intradiol class which shows high specificity to catechol, chlorocatechol and halogenated substrates derived from substituted catechol. Recombinant Pp 1,2-CCD was produced in *Escherichia coli* BL21(DE3) as a intein-tag fusion protein. Protein expression was induced with 0,5 mM IPTG, for 5 h at 22°C. Pp 1,2-CCD was purified by affinity chromatography using chitin beads resin. The protein is eluted with 30 mM of DTT. Following, the enzyme was successfully crystallized using both hanging and sitting drop vapor diffusion methods. Crystals of protein have been obtained using polyethylene glycol 8000 and 10000 as a precipitant agent plus magnesium acetate tetrahydrate as an additive. The brown crystals, with dimensions of 450x80x80 μ m, appeared after three days and display a rectangular shape. In order to solve the crystal structure, the crystals will be used for X-ray diffraction experiments at MX-1 beam line located at Laboratório Nacional de Luz Sincrotron. Our studies, corroborated with previous biochemical and biophysical analysis, will be used to fully characterize the enzyme. Our results can be further explored to potentialize the biotechnological use of Pp 1,2-CCD as a bioremediator.

Keywords: Chlorocatechol 1,2-dioxygenase; Bioremediation; Crystallization, Tertiary structure.

BM 07 - FREQUENCY OF CHROMOSOMAL ABERRATIONS IN PERIPHERAL LYMPHOCYTES OF TANNERY WORKERS IN BRAZIL

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The mutation defined as any qualitative and quantitative alteration to the genetic material, may occur in spontaneous or induced forms. It raises a great interest due to being directly related to a diversity of degenerative disease development, specially cancer. It has been estimated that millions of workers are exposed to different chemical products in their work routine. The region of Franca is characterized by its concentration of shoes factories and tanneries where a great variety of chemical products have been used. The aim of this paper was analyzing the frequency of chromosome aberrations (CA) in lymphocyte from periferic blood of individuals occupationally exposed to different chemical agents used in tannery industries from the municipality of Franca, besides control individuals. Twenty individuals voluntarily joined this work (10 tannery workers and 10 control) all of them were male, age group from 25 to 46 years old. The participants answered a pattern questionnaire with the aim to collect important characterized data such as age, exposition time to chemical agents, smoking habit and coffee and; or alcohol ingestion. A total of 100 metaphases individual were analysed, and for the calculus for mitotic index (MI) and the frequency of metaphasic cells was observed in an amount of 1000 cells division-individual. The results didn't present difference between the MI calculated for two different groups. For the frequency of CA the values observed were 69 and 35 considering the "gaps", and 34 and 18 excluding the "gaps" for the exposed and control groups respectively. This data was analysed over a non-parametric test of Mann-Whitney with no meaningful difference. Despite these statistics results, a bigger frequency of CA in a workers' group was related showing the importance and necessity of protection for professionals occupationally exposed.

Keywords: Chromosomal aberrations; Tannery workers; Human lymphocytes; occupational exposure.

BM 08 - GENE EXPRESSION OF HSP70 IN HUMAN OSTEOBLASTIC CELLS CULTURED ON TITANIUM

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The heat shock protein of 70 kD (HSP70) plays a role in the maintenance of cellular homeostasis and prevention of apoptosis induced by stress. HSP70 presents three isoforms, one constitutive and two induced, mainly for temperature increase. The aim of this study was to evaluate the effect of

culturing human osteoblastic cells in presence of titanium (Ti) on expression of HSP70. Cells enzymatically released from bone alveolar fragments were cultured in osteogenic medium on Ti discs and tissue culture polystyrene (TCP) for 10 days. After that, total RNA was obtained for gene expression evaluation of constitutive HSP70 (HSP1L) and induced ones (HSPA1A and HSPA1B) by real-time PCR. Cells grown on TCP were exposed to 37, 43 or 47°C for 120 min as a positive control. The results of experiments carried out in triplicate were compared by Kruskal - Wallis test or Mann-Whitney test. The increase in temperature has not changed the gene expression of constitutive hsp70, but increased the expression of both induced HSP70. For cells grown on Ti, gene expression of constitutive and induced HSP70 were reduced in comparison to TCP. The results suggest that Ti does not induce stress in human osteoblastic cells.

Keywords: Bone; Titanium; Gene expression.

Financial support: FAPESP.

BM 09 - HNRNP K PROTEIN IS A PROGNOSTIC MARKER IN HEAD AND NECK CANCER

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Introduction: hnRNP K protein has been found in the nucleus and cytoplasm and has been implicated in a variety of cellular functions such as regulation of transcription and translation, RNA splicing, mRNA stability, chromatin remodeling, signal transduction and cell adhesion. The identification of specific protein expression profiles in tumors may lead to the discovery of new markers that can distinguish normal and neoplastic cells, and may also provide new molecular targets in cancer chemoprevention and treatment. hnRNP K is an important regulator of transcription and one of its putative targets is c-Myc gene. Recently, we identified hnRNP K as one protein differentially expressed in tumor samples using proteomic tools. **Objectives:** To validate hnRNP K expression in cancer we used 93 tumor samples spotted in duplicate on one tissue microarray and analyzed by immunohistochemistry (IHC). We also evaluated its expression in 30 tumors paired with normal counterpart by using immunoblotting technique. Material and **Methods:** Sixty micrograms of total protein from the tumor and surgical margin samples were separated on 12 % SDS-polyacrylamide gels for immunoblotting analysis. The tissue microarray slide containing 93 samples was prepared by GENCAPO pathologists and gently yielded to us. The antibodies (anti-hnRNP K, anti-B-actina, and anti-rabbit) were purchased from Santa Cruz Biotechnology. The staining system used was Detection System Peroxidase DAB DAKO for IHC and the Super Signal West Pico Chemiluminescent Substrate System (Pierce) or ECL Western Blotting System (GE) for immunoblotting assays. **Results:** Tissue microarray analyses showed 100% positive tumors for hnRNP K expression, and 68 % of the tumors with hnRNP K nuclear and score 1 or 2 by IHC staining are classified as moderately and well-differentiated. The western blotting confirmed up regulation of hnRNP K in head and neck tumors, and interestingly it was associated with non-survival. **Conclusion:** Our results showed hnRNP K protein is overexpressed in head and neck cancer and suggest its application as a prognostic marker. These findings appoint it as a potential and new therapeutic target in HNSCC. New studies have already been performed to understand how this protein is acting on HNSCC tumorigenesis.

Keywords: Signaling; Therapeutic target; Head and neck cancer; Molecular marker.

Financial support: FAPESP; CNPq; GENCAPO RESEARCH GROUP.

BM 10 - PREVALENCE OF FIMBRIAL ADHESINS-ENCODING GENES OF DIARRHEAGENIC *Escherichia coli* IN ATYPICAL ENTEROPATHOGENIC *E. coli* STRAINS

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Diarrheagenic *E. coli* is classified into six main pathotypes: enteropathogenic *E. coli* (EPEC), enterohemorrhagic *E. coli*, enterotoxigenic *E. coli* (ETEC), enteroaggregative *E. coli* (EAEC), diffusely adherent *E. coli* and enteroinvasive *E. coli*. Among these pathotypes, EPEC and the EAEC are subdivided in typical and atypical. EPEC is identified on the presence of *eae* and *bfpA* genes that encode the adhesin intimin and the type IV pili BFP, respectively. Both genes are used for classification of EPEC in two groups: typical (*eae+*/*bfpA+*) and atypical (*eae+*/*bfpA-*). Current data demonstrate that atypical EPEC (aEPEC) are more prevalent than typical EPEC, either in developed or developing countries. Adhesion, an essential first step in bacterial pathogenesis, is mediated by adhesins, and is critical for successful *E. coli* colonization of host's mucosa. The distinct surface structures can be divided into fimbrial and non-fimbrial adhesins. The objective was to investigate the prevalence of genes that encode fimbrial components described in some pathotypes of *E. coli* among 76 isolates of atypical EPEC. The PCR technique was employed to search for the presence of the gene sequences in 76 strains of atypical EPEC isolated from cases of acute diarrhea: *fimA*, *fimH*, *lpfA0113*, *cs3*, *cs4* and *cs6*. These gene sequences correspond to the type I fimbriae of Enterobacteriaceae (*fimA* and *fimH*), long polar fimbriae of Shiga toxin-producing *E. coli* (STEC) of serogroup O113 (*lpfA0113*) and the CS3, CS4 and CS6 colonization factors of ETEC. The PCR reactions were developed employing specific primers based on published sequences to amplify the amplicons: *fimA* - 161 bp, *fimH* - 508 bp, *lpfA0113* - 573 bp, *cs3* - 264 bp, *cs4* - 250 bp, *cs6* - 261 bp. The genes *fimA* and *fimH* were found in 75 (94.7%) and 77 (97.4%) isolates, respectively. Regarding the *lpfA0113* gene sequence, 16 (21%) of the isolates harbored that sequence. None of the isolates presented the *cs3*, *cs4* and *cs6* genes. The absence of genes encoding colonization factors of ETEC among atypical EPEC suggests that these structures are not spread among other pathotypes of *E. coli* and that their encoding genes do not suffer horizontal transfer. The detection of *lpfA0113* gene, originally described in isolates of STEC belonging to the serogroup O113, in atypical EPEC strains corroborates the proposal phylogenetic relationship between atypical EPEC and STEC. The high prevalence of *fimA* and *fimH* genes were expected since they encode the type I fimbriae, a common structure in *E. coli*. The role of these fimbrial structures in atypical EPEC pathogenesis is unclear.

Keywords: *Escherichia coli*; EPEC; ETEC; EAEC.

BM 11 - PROTECTIVE EFFECT OF SIMULTANEOUS AND POST-TREATMENT WITH *Baccharis dracunculifolia* EXTRACT ON GENOTOXICITY OF METHYL METHANESULFONATE

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Baccharis dracunculifolia D. C. (Asteraceae), a native plant from the Brazilian 'cerrado', is widely used in folk medicine as an anti-inflammatory agent and for the treatment of gastrointestinal diseases. *B. dracunculifolia* has been described as the most important plant source of propolis in southeastern Brazil, which is called green propolis due to its color. The aim of the present study was to evaluate the genotoxic and antigenotoxic effects of the ethyl acetate extract of *B. dracunculifolia* leaves (Bd-EAE) on Chinese hamster lung fibroblasts (V79 cells) by the comet assay. Methyl methanesulfonate (MMS; 200µM) was used as an inducer of DNA damage. For the evaluation of the genotoxicity, four different concentrations of EAE-Bd were used: 12.5; 25.0; 50.0 and 100.0 µg/mL. The antigenotoxicity protocols used were before, simultaneously, and after the treatment with the mutagen. Cell viability was measured by the trypan blue dye-exclusion test, all measurements conducted with V79 indicated viability of >90% for all experimental groups. The results showed a significant increase in the frequencies of DNA damage at the cultures treated with 50.0 and 100.0 µg/mL of Bd-EAE. In relation antigenotoxicity, the lowest Bd-EAE concentration tested (12.5 µg/mL) showed protective activity in simultaneous and post-treatment when compare to MMS. The present

results indicate that Bd-EAE has the characteristics of a so-called "Janus" compound, i.e., Bd-EAE is genotoxic at higher concentrations, whereas it displays a chemopreventive effect on MMS-induced genotoxicity at lower concentrations. The constituents of *B. dracunculifolia* responsible for its genotoxic and antigenotoxic effects are probably flavonoids and phenylpropanoids, since these compounds can act either as pro-oxidants or as free radical scavengers depending on their concentration.

Keywords: *Baccharis dracunculifolia*; Propolis; Comet assay; V79 cells.
Financial support: FAPESP; Universidade de Franca.

BM 12 - PROTECTOR EFFECT OF THE ANNATTO ON THE CISPLATIN-INDUCED MUTAGENICITY IN PC12 CELLS

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Cisplatin is one of the most potent and highly effective chemotherapeutic agents in treatment of several types of cancers. A major dose limiting side effect of this drug is neurotoxicity. About 20% of patients are unable to complete a full course of cisplatin therapy due to sensory neuropathy. Previous reports have demonstrated that this event can be mediated by oxidative stress as a result of generation of reactive oxygen species, and that the administration of antioxidants could be able to reduce the damage and protect the tissues. The annatto contains carotenoids with efficient antioxidant activity, and the present study was designed to investigate whether annatto has a protective effect against cisplatin-induced mutagenicity in PC12 cells. Thus, cultures were divided in groups for treatment: 1) a negative control, 2) cisplatin (0.1 µg/mL), 3) annatto pre-treatment (concentrations ranging from 0.2 to 1.0 µg/mL) with cisplatin (0.1 µg/mL). The analyzed parameters were cytotoxicity assessment by MTT method and mutagenicity by micronucleus test (MN) in PC12 binucleated cells. Cisplatin was not cytotoxic in the tested concentrations and annatto did not demonstrate expressive cytotoxicity, maintaining cell viability above 80% in all concentrations. We could identify that treatment with cisplatin 0.1 µg/ml induced more than 80 MN by 1000 BN cells, and that the pre-treatment with annatto was able to reduce MN induction more than 55%. Under the tested conditions we can say that cisplatin, in the concentration of 0.1 µg/mL was not cytotoxic, but was mutagenic in PC12 cells and that the annatto presented protective activity against this mutagenicity.

Keywords: Cisplatin; Annatto; Mutagenicity; Antioxidant.

BM 13 - RELATIONSHIP BETWEEN CLASS I HDACS GENE EXPRESSION AND MULTIDRUG RESISTANCE GENES IN CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA

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Introduction: One of the most important causes of failure in the treatment of acute lymphoblastic leukemia (ALL) is cellular multidrug resistance (MDR). The altered expression of genes related with MDR as *MDR1*, *MRP1*, *MRP3*, *BCRP* and *LRP*, is a common feature observed in cancer and recently, some studies have shown that epigenetic drugs known as histone deacetylase inhibitors (HDACi) promote apoptosis in drug-resistant leukemia cells although the molecular mechanisms involved in this process remain unclear. Histone deacetylase (HDACs) enzymes, which are associated with chromatin condensation and transcriptional repression, virtually can control transcriptional activity of many genes including MDR genes. **Objectives:** To investigate the relationship between gene expression of class I HDACs (*HDAC1*, *HDAC2*, *HDAC3* and *HDAC8*) and multidrug resistance genes (*MDR1*, *MRP1*, *MRP3*, *BCRP* and *LRP*) in ALL. **Methods:** Expression of class I HDACs and MDR genes were analyzed in 89 consecutive bone marrow samples obtained from pediatric patients with ALL using real-time polymerase chain reaction. The Spearman correlation test was the statistical method utilized. **Results:** We observed a negative correlation between *HDAC3* and *MRP1* ($p=0.002$); *HDAC8* and *MRP1* ($p=0.039$); *HDAC8* and *BCRP* ($p=0.094$). It was observed correlation in gene expression of *HDAC1*, *HDAC2*, *HDAC3* and *HDAC8*. MDR genes also showed correlation, except *BCRP* that was associated only with *MRP1* ($p=0.007$). We also found correlation between *HDAC3* and *MDR1* ($p=0.042$); *HDAC3* and *MRP3* ($p=0.046$); *HDAC2* and *MRP3* ($p=0.047$). **Conclusion:** These results suggest that class I HDACs gene expression can be related with MDR genes and this association is important for understanding the molecular mechanisms involved in MDR in childhood ALL, however it is necessary to perform functional investigation to confirm these results.

Keywords: HDACs; Multidrug Resistance; Acute Lymphoblastic Leukemia.

BM 14 - SERIAL ANALYSIS OF GENE EXPRESSION IN CD8+ T CELLS ISOLATED FROM HUMAN T-CELL LYMPHOTROPIC VIRUS TYPE 1 INFECTED PATIENTS

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Human T cell lymphotropic virus type 1 (HTLV-1) infection is associated with two distinct clinical pathologies. About 2-3% of infected people develop an aggressive T-cell tumor, adult T cell lymphoma/leukaemia (ATLL) and another 2-3% develop chronic inflammatory diseases, of which the best known is HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP). Ninety-five percent of infected people remain life-long asymptomatic carriers of the virus. The mechanisms involved in HTLV-1 related diseases are not well elucidated, but host factors are an essential part in HAM/TSP pathogenesis. CD8+ cytotoxic T lymphocytes (CTL) have an important function in the immune response against the HTLV-1 and so in the risk of HAM/TSP. In the present study, we compared gene expression profile of CD8+ T cells among non-infected individuals, asymptomatic (HAC) and HAM/TSP patients using Serial Analysis of Gene Expression (SAGE). HAC group was composed by pooled samples (n=4) with low proviral load (CPV), while HAM/TSP group showed high CPV (n=4). SAGE analysis of 51,017, 62,432 and 60,620 tags from control, HAC and HAM/TSP groups respectively, allowed identification of approximately 12,000 different transcripts in each library. We identified around 900 genes differentially expressed between control group and HAC or HAM/TSP. However, only 300 genes were identified between HAC and HAM/TSP groups. The expression profile revealed the presence of highly frequent transcripts related to regulation of transcription, surface antigen and adhesion molecules, apoptosis and antigen processing and presentation. This study represents the first extensive serial analysis of gene expression of CD8+ T cells in this infection and will contribute to the identification of novel genes involved in cellular and molecular events associated to HTLV-1 infection and related diseases.

Keywords: Gene; HTLV-1; CD8+ cytotoxic T lymphocytes.

Financial support: FAPESP; CTC/FUNDHERP.

BM 15 - TARGETS FOR HUMAN ENCODED MICRORNAS IN HUMAN T-CELL LYMPHOTROPIC VIRUS TYPE 1 (HTLV-1) *tax* REGION

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HTLV-1 is a retrovirus associated with two distinct diseases: HTLV-1 associated myelopathy/tropical spastic paraparesis (HAM/TSP) and adult T-cell leukemia/lymphoma (ATLL). Only 5% of HTLV-1 infected individuals develop clinical manifestations and the risk or prognosis of diseases development is still unknown. MicroRNAs (miRNAs) are non-coding RNAs that mediate post-transcriptional gene repression by inhibiting protein translation or by destabilizing target transcripts. Retroviruses depend on the host machinery, so they are susceptible to the host gene-regulatory mechanism. For this reason, human miRNAs (hsa-miRNAs) could be responsible for host-virus interaction and be associated with diseases. In this work, bioinformatics tools and experimental approaches have been used to find hsa-miRNAs targets in regulatory gene (*tax*) of HTLV-1. Firstly, we predicted hsa-miRNAs which target *tax* of the HTLV-1 genome using two softwares - miRanda and RNAhybrid. The algorithms were set up with personal parameters (Score 120; Mfe -20 kCal/Mol). To validate these findings, mature miRNAs expression was quantified by qRT-PCR employing stem-loop primers strategy. For this purpose, total RNA from PBMC cells was obtained from HTLV-1 patients classified according to the clinical status: asymptomatic (HAC) (n=6) and HAM/TSP patients (n=9). Proviral load (CPV) was measured for all HTLV-1 positive samples. A control group (negative samples, n=6) was also included. Bioinformatics analysis demonstrated that several miRNAs presented good results for established parameters, but hsa-mir-221 (Score, 159 Mfe -21,4 kCal/Mol); hsa-miR222 (Score 168, Mfe -30,5kCal/Mol) and hsa-miR-125b (Score 138, Mfe -25,6kCal/Mol) were used for initial investigation. For validation approaches, control group was used as calibrator. We obtained high levels of hsa-mir- 222 expressions in HAM/TSP group (more than 2.5 times) in contrast to the other groups. Hsa-miR-125b presented higher levels in patients than controls (more than 10 times). Additionally, statistical analysis showed a correlation between this hsa-miR-125b and CPV (p=0,0428). Until now, these miRNAs (hsa-mir-221, hsa-miR222 and hsa-miR-125b) were associated to cancer. This is the first study attempting to the differential expression of miRNAs in HTLV-1 related diseases.

Keywords: MicroRNAs; HTLV-1;

Financial support: FAPESP; CNPq; CTC/FUNDHERP.

FF 01 - ANATOMOPATHOLOGICAL FEATURES OF HYPERTENSIVE PATIENTS OF A HEALTH PUBLIC UNITY OF RIBEIRÃO PRETO-SP

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The mortality analysis by hypertensive diseases (HD) from 1991 to 2002 in Brazil (BR), state of São Paulo (SSP) and city of Ribeirão Preto (RP) identified that the proportional mortality and the mortality coefficient by HD in RP was greater than those of SSP and BR during all the studied period. The objective of this work was to analyze the anatomopathological features of hypertensive patients of a health public unity of RP. The casuistic was formed by a sample of adult individuals, from 20 years old, of both sexes, excluding pregnant with HD, into the 1601 patients seen during 1999 in the Ambulatory of Cardiology and Arterial Hypertension of CSE-FMRP-USP. 155 patients (9,68%) end up dead, of which 41 (26, 45%) were submitted to necropsy, being 25 (61%) men. Their age varied between 33 and 90 years old (X=68,41 ± 0,08); 30 (73, 1%) were white, with height from 1,41 to 1,78m (X= 1,60± 0,08), weight between 30 and 117 Kg (X= 66,9± 15,92); brain weight from 1070 to 1520g (X= 12,75±10,37); heart weight from 240 to 850g (X= 513,25±150,53); kidney weight from 40 to 300g (X=139,35± 54,72). The main *causa mortis* was acute myocardial infarction in 11 patients (26,8%). With exception of HD, the main disease was systemic atherosclerosis in 9 (21,95%). The main finding in the heart was coronary atherosclerosis in 27 (65,85%), followed by left ventricle concentric hypertrophy in 22 (53,65%). In the brain, the main finding was brain edema in 14 (34,14%). The ischemic brain vascular accident only happened in 7 (17,07%). In the kidneys, benign nephrosclerosis was found in 16 (39,02%). In the circulatory system, the most frequent finding was moderated systemic atherosclerosis in 20 (48,78%). In other organs, the main finding was chronic passive congestion of liver in 22 (53,65%). We concluded that the coronary atherosclerosis and systemic atherosclerosis were, possibly, the main determinants of the death of hypertensive people in RP, based on findings of necropsy, being that brain vascular accident did not happen in a significant number of cases.

Keywords: Hypertension arterial; Mortality; Hipertensive diseases; Atherosclerosis; Anatomopathological.

Financial support: CAPES.

FF 02 - BLOCKING NNOS DOES NOT AFFECT SURVIVAL RATE BUT MODIFIES THE HYDROELECTROLYTIC BALANCE IN EXPERIMENTAL SEPSIS

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Introduction: Sepsis, the systemic response to severe infection, and its complication, the septic shock, show several physiological alterations that include changes in vasopressin secretion, hematocrit, refractory hypotension and hormone secretion. Experimental sepsis induced by cecal ligation and puncture (CLP) is the model that better mimics the clinical course of the sepsis because presents a polymicrobial infectious focus and shows a large complexity of the inflammatory response. The nitric oxide (NO) is a not-conventional neuromodulator, that is reported to be involved in neuroendocrine and hemodynamic mechanisms. During sepsis, the high production of NO contributes to the deleterious effects of this syndrome, causing alterations such as the increase of vascular permeability, loss of plasma protein, hypovolemia and persistent hypotension. Recently a peripheral injection of 7-nitroindazole, a blocker of neuronal nitric oxide synthase (nNOS), was shown to reduce the NOS activity in the hypothalamus and the vasopressin secretion without modifying the hydroelectrolytic changes caused by salt loading. **Objective:** To evaluate the effect of the intraperitoneal injection of the 7-nitroindazole in the survival rate, the peripheral NO production and the hydroelectrolytic balance during experimental sepsis in rats. **Methods:** Male *Wistar* rats (250-300 g) were submitted to CLP or sham operation. Thirty minutes before the surgery, they were intraperitoneally injected with 7-nitroindazole (50mg/kg) or with vehicle (DMSO 10%/sesame oil, 1:9) as control. In one group the survival rate was analyzed for five days. In another group the animals were decapitated 0, 4, 6 and 18 h after surgery and blood was collected to analyse hematocrit, plasma osmolality, serum sodium, plasma protein and nitrate levels. **Results:** The drug did not change significantly the survival rate in rats submitted to the sepsis (p = 0,1403) The plasma osmolality and sodium did not show temporal alterations in both groups, vehicle or drug. The hematocrit increased 4 and 6h after CLP and the pretreatment with 7-nitroindazole recovered the volemia (p<0,05). The plasma protein levels show a decrease only 6h after the CLP and the 7-nitroindazole pretreatment anticipated this decrease at 4h (p<0,05). The nitrate production increased only after 18h of CLP and the drug administration anticipated this increase to 6h (p<0,05). **Conclusion:** Blocking the nNOS does not change the survival rate, osmolality and serum sodium but worsens the hypovolemia and the loss of protein and anticipate the production of nitric oxide in experimental sepsis. (Technical support: Nadir Martins Fernandes).

Keywords: Polymicrobial sepsis; CLP; Hydroelectrolytic balance; nNOS; Nitric Oxide.

Financial support: CNPq; FAPESP.

FF 03 - cAMP REDUCES THE ATROPHY-RELATED UBIQUITIN LIGASE ATROGIN-1 IN SKELETAL MUSCLES FROM NORMAL RATS

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Aim: This study was undertaken to investigate the role of beta-2 adrenoceptors and cAMP in regulating the Ubiquitin-proteasome system (UPS) in skeletal muscles from normal rats. **Methods and results:** The activity of UPS and the Akt/FoxO signaling pathway were measured in skeletal muscle from rats treated with clenbuterol (3 mg/kg wt; sc), a selective beta-2 adrenergic agonist, for 3 days. In extensor digitorum longus (EDL) muscle, clenbuterol increased by 30% muscle weight, reduced by 45% the UPS activity and increased by 30% the phosphorylation of Akt and FoxO3. The addition of isobutylmethylxanthine (IBMX; 10⁻³M), a cAMP phosphodiesterase inhibitor, to the incubation medium increased cAMP levels (4-fold) and decreased by 50% the UPS activity in isolated soleus and EDL muscles from normal rats. IBMX *in vitro* also reduced the levels of ubiquitin-protein conjugated and the mRNA levels of the atrogin-1/MAFbx (50%) and the E2-14kDa ubiquitin conjugating enzyme (30%) transcripts in muscles from normal rats. Ubiquitin and MuRF1 mRNA were not altered by IBMX *in vitro*. **Conclusions:** These data suggest that stimulation of beta-2 adrenoceptors, through the activation of cAMP and Akt signaling pathways, inhibit ubiquitin-proteasome proteolysis by increasing FoxO3 phosphorylation and suppressing atrogin-1 mRNA expression in skeletal muscle from normal rats.

Keywords: cAMP; Beta-2 adrenoceptors; Ubiquitin-proteasome system; Skeletal muscles.

Financial support: CNPq; FAPESP.

FF 04 - DECREASED NUMBER OF CAVEOLAE IN ENDOTHELIAL CELLS IMPAIRS THE RELAXATION INDUCED BY ACETYLCHOLINE IN HYPERTENSIVE RAT AORTAS

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Endothelium-dependent relaxation induced by acetylcholine is impaired in renal hypertensive two kidney-one clip (2K-1C) rat aortas. It has been proposed that endothelial caveolae are important to the synthesis of nitric oxide (NO). The present study aimed to investigate the contribution of the endothelial cells caveolae to the endothelium dependent relaxation and their integrity in endothelial cells from 2K-1C rat aortas. The maximal relaxant effect (ME) of acetylcholine were greater in 2K than in 2K-1C aortas. The caveolae disassembler methyl- β -cyclodextrin (CD) reduced the potency and ME to acetylcholine in 2K. However, CD did not modify the potency but it reduced ME of acetylcholine in aortas from 2K-1C. NO production stimulated with acetylcholine was greater in 2K than in 2K-1C endothelial cells. CD impaired the NO production in endothelial cells from 2K-1C and 2K. We verified that the increase of cytosolic Ca²⁺ concentration ([Ca²⁺]_i) induced by acetylcholine in endothelial cells was higher in 2K than in 2K-1C, which was impaired by CD only in 2K. Acetylcholine decreased [Ca²⁺]_i in the vascular smooth muscle cells, which response was higher in 2K than in 2K-1C. CD impaired this response only in 2K. We have quantified a larger number of caveolae in the endothelial cells from 2K than in 2K-1C rats. Moreover, CD reduced the number of caveolae in both 2K and 2K-1C endothelial cells. Our results support the hypothesis that the integrity of endothelial cells caveolae plays an important role in the NO production induced by acetylcholine in 2K-1C endothelial cells.

Keywords: Caveolae; Endothelial cells; Renal hypertension; Nitric oxide; Cytosolic calcium.

Financial support: FAPESP; CNPq.

FF 05 - EFFECT OF PURMORPHAMINE ON GENE EXPRESSION OF HUMAN OSTEOBLASTS DERIVED FROM BONE MARROW MESENCHYMAL CELLS

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Introduction: Purmorphamine promotes osteogenesis in mesenchymal progenitor cells as showed by the upregulation of the bone transcription factor RUNX2 (Run-related transcription factor 2) and the bone marker ALP (alkaline phosphatase). **Objectives:** This study investigated the immunophenotype profile of human bone marrow mesenchymal cells (hBMMCs) exposed to purmorphamine by fluorescence-activated cell sorting (FACS). Furthermore, it was evaluated the effect of purmorphamine on osteogenesis of osteoblasts differentiated from hBMMCs. **Methods:** Experiments were carried out using cells from two healthy bone marrow donors obtained under approval of Committee of Ethics in Research. For immunophenotype profiling primary and first-passaged cells were cultured in non-osteogenic medium and FACS of representative cell surface makers for mesenchymal, hematopoietic and endothelial cells was conducted. First-passaged cells were exposed or not to purmorphamine (2 μ M) for 7 days after what the same cell surface markers were evaluated by FACS. For gene expression assay, subconfluent cells in primary culture were enzymatically harvested and first-passaged cells were subcultured in 75 cm² tissue culture flasks (1.4 x 10⁵ cells/flask) for 7 days in osteogenic medium to allow osteoblastic differentiation. Cells were exposed to purmorphamine (2 μ M) or vehicle (dimethyl sulphoxide) for 7, and for the last 4, 3, 2 and 1 days of subculture. Gene expression of ALP, RUNX2, osteopontin (OP) and osteocalcin (OC) were evaluated by quantitative real-time PCR. All experiments were done in triplicate and submitted to Mann-Whitney test. **Results:** FACS of primary culture revealed at least two distinct cell populations, the predominant hBMMCs (CD90⁺, CD73⁺, CD105⁺, CD166⁺, CD29⁺, CD13⁺, HLA class I⁺, CD146⁺, CD44⁺, CD14⁺, CD106⁺, CD31⁺, CD45⁻) and the fewer hematopoietic cells (CD34⁺). After 7 days, FACS demonstrated that purmorphamine treatment reduced hBMMCs surface markers expression (excluding CD44, HLA class I and HLA-DR) while untreated culture did not change their immunophenotype profile being comparable to primary culture. Exposition of osteoblastic differentiated cells to purmorphamine resulted in upregulation of all evaluated genes. RUNX2 was upregulated at all periods (7 days: 1.54-fold; 4 days: 1.27-fold; 3 days: 1.38-fold; 2 days: 1.40-fold; 1 day: 1.13-fold). OC and ALP were upregulated at 7 (1.10-fold for OC; 1.12-fold for ALP) and 4 days (1.54-fold for OC; 1.13-fold for ALP). OP was upregulated at 4 (1.07-fold), 3 (1.23-fold) and 2 days (2.83-fold). **Conclusion:** FACS analysis suggests that purmorphamine treatment reduces mesenchymal cell population probably by inducing osteoblastic differentiation as revealed by increased gene expression of osteoblastic markers.

Keywords: Purmorphamine; Factor RUNX2; Bone marker ALP; Mesenchymal cells.

Financial support: FAPESP.

FF 06 - LERCANIDIPINE-FLUVASTATIN INTERACTION: PHARMACOKINETICS STEREOSELECTIVITY IN EXPERIMENTAL STUDY

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Hypertension and dyslipidemia are independent risk factors for cardiovascular mortality and are frequently present in the same patient. Fluvastatin (FV), used to reduce cholesterol levels, and lercanidipine (LER), used to control blood pressure, are marketed as racemic mixtures. Therapeutic activities are 30-fold higher for (+)-3R,5S-FV and 100- to 200-fold higher for S-LER compared to their respective antipodes. The present study describes the enantioselective

pharmacokinetic interaction between LER and FV in *Wistar* rats. Male *Wistar* rats (n = 6 per group) received racemic LER (3mg/kg) or FV (5mg/kg) or LER plus FV. Serial blood samples were collected from 0-24 h. Plasma concentrations of the LER and FV enantiomers were determined by LC-MS/MS and pharmacokinetic parameters were evaluated using the WinNonlin software. The Wilcoxon and Mann-Whitney tests ($P < 0.05$) were used to analyze enantiomer ratios and the pharmacokinetic drug interaction. Data are expressed as medians. In monotherapy, the kinetic disposition of both FV and LER was enantioselective. AUC values were significantly higher for (-)-3S,5R-FV than for (+)-3R,5S-FV (281.6 vs 274.6 ng.h/mL) and for R-LER compared to S-LER (67.9 vs 18.9 ng.h/mL). The pharmacokinetic parameters of FV were not enantioselective when combined with LER (AUC: (-)-3S,5R-FV: 545.1; (+)-3R,5S-FV: 504.8 ng.h/mL). There was a significant reduction in S-LER (18.9 vs 13.3 ng.h/mL) AUC values when FV was co-administered. In conclusion, the interaction between FV-LER might be relevant since AUC values of (+)-3R,5S-FV were increased when LER was co-administered and AUC values of the S-LER, the eutomer, was reduced when FV was co-administered.

Keywords: Cardiovascular mortality; Fluvastatin; Lercanidipine; Racemic mixtures.

Financial support: FAPESP.

FF 07 - PATHWAYS INVOLVED IN THE VASODILATATION INDUCED BY THE NEW NO DONOR

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Introduction: Nitric oxide (NO) produced in the endothelial cells or released by NO donor migrates to vascular smooth muscle cells where it activates the soluble guanylyl-cyclase (sGC) that produces cGMP and activation of cGMP-dependent protein kinase (PKG). This enzyme can phosphorylate several proteins that could be involved in the vascular relaxation. Aim: This study aimed to investigate the pathways involved in the vasodilatation induced by the new NO donor *cis*-[Ru(bpy)₂(py)(NO)](PF₆)₃ (Py) synthesized in our laboratory. **Methods:** Rats (180-200g) were killed and the thoracic aorta was removed. Vascular reactivity to this new NO donor was studied in denuded rat aortic rings pre-contracted with phenylephrine (Phe, 100nM) or 60mM KCl. Cumulative concentration-effect curves for the NO donor were constructed in the presence or in the absence of the selective guanylate cyclase (sGC) inhibitor (ODQ, 1µM), non-selective K⁺ channel blocker (TEA, 1mM), Cl⁻ channel blocker (NPPB, 10µM) and sarcoplasmic reticulum Ca²⁺-ATPase (SERCA) inhibitor (Tapsigargin, 1µM). Cumulative concentration-effect curves for CaCl₂ stimulated with 100nM Phe or 60mM KCl were constructed in the presence or in the absence of the NO donor (Py). We analysed the values of maximum effect (Emax) and the potency (pD₂) of the NO donor. **Results:** Py induced concentration-dependent relaxation in rat aorta pre-contracted with Phe (Emax: 105±1.08%; pD₂: 6.54±0.1; n=5) or KCl (Emax: 67.5±4.6%; pD₂: 5.79±0.11; n=5). However, the maximum effect (Emax) and potency (pD₂) values were lower KCl-contracted aorta. ODQ reduced the Emax (75±4.5%) and pD₂ (75±4.5%) in aortas pre-contracted with Phe. On the other hand, in KCl-contracted aortas, ODQ reduced the Emax (60.3±5.7%), but did not change pD₂ (5.29±0.09; n=8). TEA and NPPB did not alter the Emax (TEA: 98.6±2.1%; n=6; NPPB: 100.7±2.2%; n=7), but both reduced the potency (pD₂) of Py (TEA 5.85±0.1; n=6 and NPPB: 5.96±0.07, n=7). Interestingly, Tapsigargin reduced the Emax (82.1±4.6; n=5) and the potency of Py (5.82±0.21; n=5). The cumulative concentration-effect curves for CaCl₂ stimulated with 100nM Phe were similar to those stimulated with 60mM KCl (Phe Emax: 1.4±0.16%; pD₂: 0.47±0.19, n=9 and KCl Emax: 1.6±0.21%; pD₂: 0.51±0.12, n=7). Incubation with the NO donor reduced the Emax of both curves (Phe: 0.1±0.06% and KCl: 0.7±0.11%). **Conclusion:** Taking together, our results show that the relaxation induced by the new NO donor involves the activation of sGC, K⁺ channels sensitive to TEA, Cl⁻ channels sensitive to NPPB and SERCA. Moreover, the NO donor in study almost abolished Ca²⁺ influx.

Keywords: Nitric oxide (NO); NO donor; Vasodilation; Ion channel.

Financial support: FAPESP; CNPq.

FF 08 - BLOCKING PERIPHERAL LEUKOTRIENES DOES NOT DECREASE THE PERIPHERAL NITRIC OXIDE PRODUCTION AND DOES NOT AFFECT HYDROELECTROLITY BALANCE AND SURVIVAL RATE DURING EXPERIMENTAL SEPSIS

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Introduction: Recently in our laboratory it was seen that the central or peripheral administration of MK-886 (a LTs biosynthesis inhibitor) abolished or reduced the secretion of AVP in the initial phase of the sepsis. Furthermore, was observed that in this phase occurs an increase of the enzyme LTC₄ that is found usually in the hypothalamic neurons that synthesize vasopressin. Additionally we saw that the central administration of MK-886, but not the peripheral, reduced the increase of the enzyme LTC₄ and of serum nitrate that usually occurs during the sepsis. Those data suggest that the leukotrienes have a role in the vasopressin secretion that could occur direct or indirectly through the production of nitric oxide. Since vasopressin secretion is also stimulated by hydroelectrolytic balance our Objective was to analyze the effect of blocking peripheral leukotrienes in the osmolality and hematocrit during experimental sepsis. **Materials and Methods:** Male *Wistar* rats received i.p. injection of MK-886 (2.0 or 4.0 mg/kg) or vehicle (DMSO 5%) 1h before cecal ligation and puncture (CLP) or sham operation. In one group of animals, the survival rate was monitored for 3 days. Another group, the animals was decapitated at 0, 4, and 24h after CLP or sham operation, and blood was collected for hematocrit, plasma osmolality and serum nitrate levels measurement. **Results:** The serum NO production increased 24 h after CLP and the peripheral administration of the LTs blocker did not decrease this production ($p < 0.001$). The hematocrit increased 4h after CLP and the pretreatment with MK-886 did not modify this effect ($p < 0.001$). The plasma osmolality showed temporal alterations during the analyzed times and the MK-886 also did not modify these parameters ($p < 0.05$). The mortality rate also was not affected by the drug ($p < 0.15$). **Conclusion:** The results suggest that the peripheral LTs does not affect the hydroelectrolytic parameters neither the survival rate during experimental sepsis. (Technical Support: Nadir Martins Fernandez).

Keywords: Polymicrobial sepsis; Leukotrienes; CLP; Hydroelectrolytic balance.

Financial support: FAPESP; CAPES.

FQ 01 - A CLASSIC EVOLUTIONARY ALGORITHM TO PROTEIN STRUCTURE PREDICTION

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Introduction: The Protein Structure Prediction (PSP) can be an optimization problem and it aims to determine the protein tertiary structure from its amino acids sequence. This paper presents a classic Evolutionary Algorithm (EA) to PSP problem using an ab initio approach. **Objective:** Development a classic evolutionary algorithm to PSP problem with hydrophobic interactions. **Methodology:** The algorithm starts initializing a random conformation. The torsion angles (Φ, Ψ, χ) are generated at random from the constrained regions. Afterwards, the energy of the conformation is evaluated. First, the protein's structure in internal coordinates (backbone and side-chain torsion angles) is transformed into Cartesian coordinates. We proposed three kinds of crossover

operators: one based on BLX- α operator; one using uniform crossover and the last is a two-point crossover. Three kinds of mutation operators were proposed: the first acts on the peptide chain; the second and the third apply a uniform mutation, modifying all the values of the backbone and side-chain torsion angles. **Results:** this classic EA used the population size is 200 chromosomes and the maximum number of generations is 100. The cost function has dielectric constant equal to 4.0. The 1A11 protein presented lowest energy (Fitness = -171.959772) and your Distance Matrix Error (DME (Å) = 9.987). **Conclusion:** This approach can explore any region of the search space to find an adequate structure conformation even though there is no similar protein structure previously known. Because of this it is a pure ab initio algorithm that does not use any heuristics in the prediction process. Today, we have worked in other features for this approach.

Keywords: Protein Structure Prediction; Classic Evolutionary Algorithm.

FQ 02 - MOLECULAR DYNAMICS AND MONTE CARLO METHOD APPLIED TO *in silico* PROTEIN FOLDING

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With the aid of computational simulation, it is possible to assess the structures and processes concerning polymers, like their stabilities, folding pathways and techniques of developing de novo molecular designs. Nowadays, among several known biopolymer classes, the proteins are particularly the most studied through *in silico* modeling. Among numerous available computational methods, basically two of them are widely employed to the task: the molecular dynamics and Monte Carlo. The first one is substantiated in classic Newtonian dynamics, through movement equations. With given initial conditions and the proper force field, it is possible to follow the temporal evolution from all atoms in the system. The second one, the Monte Carlo Method, is capable to drive this temporal evolution by stochastic means, for that, it hands over resources as random generator of numbers along with the Metropolis criteria. The minimalist models are a useful analytical tool to focus specific aspects of the protein folding problem. These models are based on the application of a methodological reductionism, whose minimizes the number of possible variables of the system. We adopt those approach because it's produces a model as simple as possible, but not simpler. Moreover, the minimalist models preserve at last an analogy with particular aspects of original problem. Our model is concerned on the essential physics of the problem and connected with experimental observables for a target protein. The essential ingredients used are: hydrophobic effect and stereo-chemical potential. We employed the Monte Carlo method for simulation of model. Since the founding of the Biological Physics Area within the Pharmaceutical Sciences Graduation Program from the Faculty of Pharmaceutical Sciences of Ribeirão Preto, the *in silico* study of protein folding is being developed as one of the group's priorities.

Keywords: Computational simulation; Molecular dynamics; Monte Carlo Method.

FQ 03 - AGGREGATION STATE OF CHITIN AND CHITOSAN NANOPARTICLES AT DIFFERENT DEGREES OF ACETYLATION

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Chitin, a (1 \rightarrow 4)-linked N-acetyl- β -D-glucosamine is widely distributed in nature as the main component in the exoskeleton of crustaceans, insects and fungi. Chitosan, its main derivative, is generally produced by alkaline deacetylation of chitin. These biopolymers have been widely developed for use as antimicrobials, biomedical materials, cosmetics, agricultural materials and principally as decontaminant of residual waters containing pesticides and heavy metals. These various applications require information about the degree of acetylation (DA) and distribution of acetyl groups along the biopolymer chain. The possible effect of the DA on chitosan solubility has been discussed previously, although still a lot of contradiction in the literature. Despite the experimental efforts during the last decade, the behavior of chitosan at molecular level remains elusive due to the intrinsic nature of these polymers. In order to overcome this limitation, a variety of molecular simulation techniques have been employed to evaluate the influence of the number and the distribution of the acetyl groups on the aggregation state of chitin and chitosan nanoparticles in aqueous environment. In this study, a set of explicit-solvent 20-ns molecular dynamics simulations of chitosan nanoparticles were performed at different degrees of acetylation (0%, 40%, 60% and 100%) at neutral pH. Two setups were considered for the systems with 40% and 60% of acetylation, random and block where acetyl groups were evenly distributed or aggregated in a confined region, respectively. The starting filaments in all nanoparticles were modeled as hydrated crystals with filaments in a 2-fold helix conformation. Analyses of these simulations reveal that the presence of acetyl groups in chitin and chitosan filaments contribute to the intra-chain hydrogen bonds stabilization between adjacent sugars monomers, decreasing the solubility of these biopolymers in water and stabilizing a crystal like configuration. The nanoparticles up to 40% of acetylation are moderately soluble in neutral pH, showing more stable aggregates when acetyl groups are in block distribution. This happens due to the increase of inter-chain hydrogen and hydrophobic interaction between chitosan fibers. Chitosans with 60% or more of acetyl groups are insoluble and present similar degrees of swelling, in agreement with experimental observations. The spatial distribution of the N-acetyl and the hydroxylmethyl seem to be responsible to trap water molecules in a well-defined orientation resulting in a peculiar microsolvation environment around the polysaccharides filaments. The simulations also suggest that neither the inter-chain hydrogen bonds nor the hydrophobic interactions, created by the acetyl groups, were responsible for the chitosan aggregation. On the other hand, the solubility of chitin and chitosan are strongly affected by the solvent orientation around the polysaccharide filament and this probably is the main cause of aggregation of these biopolymers.

Keywords: Chitin; Chitosan; Degree of acetylation; Solubility; Molecular dynamics.

Financial support: CAPES; CNPq; DOE Office of Advanced Scientific Computing Research through the project "Data intensive Computing for Complex Biological Systems".

FQ 04 - ASSOCIATION OF CHLOROQUINE INTO MIXED MICELLES SDS/TRITON X-100: EFFECT OF SUPERFICIAL CHARGE OF THE MICELLE

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Introduction: Most drugs used in the malaria treatment, such as chloroquine (CQ), cause phototoxic side effects in skin and eyes (regions rich in melanin), that is attributed to the complex formed by chloroquine-melanin. Chloroquine is also used in the treatment of lupus erythematosus, rheumatoid arthritis and Aids. However, not much is known about the mechanisms responsible for photosensitivity caused by this drug. CQ is a prototropic drug that, depending on pH, can exist in different forms: neutral, monoprotinated or diprotinated. The mixed micelles of SDS and Triton-X-100 will be used to mimetic the biological membrane, which presents a surface density of charge that changes with the location in the body. **Objective:** The goal of this work is to contribute to the elucidation of the antimalarial action mechanisms in biological systems. **Materials and Methods:** The critical micellar concentrations (CMC) of mixed micelles formed by SDS (anionic surfactant) and Triton-X-100 (non-ionic surfactant) (α_{SDS} =0; 0.2; 0.4; 0.5; 0.6; 0.8 and 1.0 - final concentration 50 mM) were determined, using pyrene as fluorescent probe, in order to know the concentration which is formed the micellar aggregate in each pH. The pKa, of CQ, in mixed micelles were obtained by simulation of the curves of absorbance versus pH. The binding constants (Kb) of CQ in mixed micelles were obtained through the simulation of the curves of fluorescence versus concentration of surfactants in the mixture. The wavelengths for excitation and emission were equal to 342 and 390 nm, respectively, and slits of 5nm. All the experiments were performed in water and borate/citrate/phosphate buffer, 50 mM,

in pH 5, 7.5, 9 and 13. The β -parameter for mixed micelles SDS/Triton-X-100 was determined by Rubingh's Method (Rubingh, D.N. Solution Chemistry of Surfactants; Mittal, K.L., Ed. Plenum Press: New York, 3, 337, 1997). **Results and Discussion:** CMC values decreases with the molar fraction of Triton-X-100 for all micellar compositions, independent of the pH. The negative value for the β parameter (-3.05) showed the existence of synergism between the surfactants used. The pK_a1 of the CQ, in homogeneous medium, was 8.55, while in micelles formed by SDS or Triton X-100, the pK_a1 were 9.61 and 7.95, respectively. This behavior indicates the superficial charge of micelle can select the different forms of the CQ. For mixed micelles, it was observed the pK_a1 decreases slowly with molar fraction of SDS. The association of CQ with mixed micelles decreases with the molar fraction of Triton-X-100 reducing the binding constant, K_b . **Conclusion:** The results obtained for pK_a1 and K_b suggest the electrostatic effect is the most important force, responsible for modulation of the prototropic forms of CQ and their association with micelles.

Keywords: Malaria; Fluorescence; Micelles; Chloroquine.

FQ 05 - CRYSTAL STRUCTURE OF RECOMBINANT PORCINE S100A12 AT 1.38Å RESOLUTION

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Porcine S100A12 (also known as calgranulin C) is a member of the S100 proteins, family of small acidic calcium-binding proteins characterized by the presence of two EF-hand motifs. These proteins are involved in many cellular events such as the regulation of protein phosphorylation, enzymatic activity, protein-protein interaction, Ca^{2+} homeostasis, inflammatory processes and intermediate filament polymerization. Here, we report the crystal structure of porcine S100A12 solved at 1.38Å resolution. The structure has been solved by single anomalous scattering (SAD) techniques using the iodine anomalous signal. For our crystallographic studies, S100A12 has been crystallized using vapour-diffusion techniques in sitting drops at 295 K using ammonium sulfate as precipitant. The growth of prismatic crystals was visible after one day with crystal size reaching up to 0.3 x 0.5 x 0.5 mm. Iodide derivative crystals were obtained by using rapid cryo-soaking techniques. Diffraction data were collected from frozen crystals (at 100 K), using a wavelength of 1.30Å for native crystals and 1.54Å for iodide derivatives crystals. X-ray diffraction data were collected at the protein crystallography beam line WO1B-MX2 of the Brazilian Synchrotron Light Laboratory, LNLS, Campinas, Brazil, using a MarMosaic 225 detector. Data processing and scaling were performed using the programs MOSFLM and SCALA from the CCP4 package. Collaborative Computational Project 4, 1994 Collaborative Computational Project, Number 4, 1994. The CCP4 suite: programs for protein crystallography. Acta. Cryst. D50, 760-763. CAGC native crystals diffract to 1.38Å resolution and belong to the triclinic space group P3121, with unit-cell parameters $a=b=51.70$ and $c=65.93$ Å. Data from iodide derivative crystals were collected up to 1.7Å resolution. Derivative crystals belong to the same space group as native crystals with similar unit cell parameters. We used the AutoSol tool from the PHENIX package to setting up inputs, analyzing and scaling the data, finding heavy-atom (anomalously-scattering atom) sites, scoring of heavy-atom solutions, phasing, density modification, and preliminary model-building and refinement. The final structure refinement was performed using the program REFMAC (CCP4), with the native diffraction data set at 1.38Å resolution. The final model presented an R factor and R free of 17.5% and 19.7%, respectively, with good overall stereochemistry. The structure reveals important differences in conformation and secondary structure compared to the human protein which will be used to correlate the difference in function and specificity observed for this class of proteins in different species.

Keywords: Porcine S100A12; Crystal structure; SAD technique.

Financial support: FAPESP.

FQ 06 - ELETRODE MATERIALS CHEMICALLY MODIFIED: BIOLOGICAL SYSTEMS APPLICATIONS

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Introduction: The immobilization of biological macromolecules (e.g., proteins, enzymes, antibodies, receptor, peptides, DNA, etc.) on a transducer surface in a controlled manner that fully preserves their biological activity is one of the major challenges in making a functioning biosensor. Coupled to this area, the study of the electrochemical behavior of redox enzymes has received a great deal of attention, driven in many cases by the desire to construct practical, self-contained enzyme electrodes for biosensor applications. Closely related to such studies has been the use of various types of electroactive polymers employed in conjunction with redox enzymes for immobilization of the enzyme itself and for the acceleration of electron-transfer kinetics. In this way, modification of carbon surfaces is an important objective in electrochemistry and material science. More generally, active attention is currently paid to covalently modified electrodes for catalytic or analytical purposes and also in view of biotechnological applications. Covalent bonding (chemisorption) of the modifier to the carbon surface can be initiated via the direct electrochemical oxidation of amines, electrochemical reduction of aryl diazonium salts, or by gentle thermolysis or photolysis of the aryl diazonium salts. Alternatively, covalent bond formation can be activated by chemical means for example a Friedel-Crafts acylation on graphite and carbon nanotube recently studied in our laboratory. Another platform that we are exploring, is the electropolymerization of of N-(3-pyrrol-1-yl-propyl)-4,4'-bipyridinium (PPB). The interaction between the PPB layer and maltose binding protein fusion is through a nonquaternized pyridine nitrogen (in the PPB film). **Objective:** The aim of our laboratory is to obtain novel electrode materials to analyze and/or study molecules of interest in pharmaceutical sciences, food, agriculture and biological systems as well. **Materials and Methods:** All electrochemical experiments were carried out with a BAS CV-27 potentiostat. Data were recorded on a SoltecXY recorder. A three electrode conventional electrochemical cells were employed. A glassy carbon disk (area) 0.003 cm² or a gold disk (area) 0.006 cm² electrode was used as the working electrode. Prior to use, the electrode was polished with 1 μ m diamond paste (Buehler) and rinsed thoroughly with water and acetone. A sodium chloride saturated Ag/AgCl and a coiled Pt wire were used as reference and counter electrodes, respectively. The electrochemical behavior of Nimesulide (Nimes) and reduced nimes (NimesH) were carried out in PB pH 7 / 0.1 mol L⁻¹. **Results and Discussion:** Electropolymerized films of PPB on glassy carbon electrodes retain their redox activity in aqueous solutions. Further modification with MBP-NR (nitroreductase) gave rise to electrodes that exhibited very high electroactivity for the reduction of trinitrotoluene TNT and dinitrotoluene DNT. However, PPB electrodes modified with wild type NR did not exhibit such activity. **Conclusion:** Results from biosensor research suggest that the presence of specific interactions between the PPB layer and MBP fusions might represent a general way to immobilize enzymes onto surfaces with a high retention of activity. Electrochemical behavior of nimesulide in presence of NADH suggests that the oxidation reaction of NADH is made by nitrogroup in nimes. Graphite and carbon nanotube functionalized have applications in electroanalysis that carbon paste electrode has not.

Keywords: Chemically modified electrodes; Biosensor; Redox drugs; Bioelectrochemistry.

FQ 07 - HETEROLOGOUS EXPRESSION AND KINETIC ANALYSIS OF RECOMBINANT FUMARATE HYDRATASE FROM *Leishmania major*

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Leishmania major Friedlin (LmjF) is a protozoan parasite responsible for *Leishmaniasis* that currently threatens 350 million men, women and children in 88 countries spread around the world and whose genomic sequence has been recently elucidated. In the present work, we have cloned,

overexpressed, purified and kinetically analyzed the product of the gene from LmjF chromosome 24: LmjF24.O320, which encodes a protein with putative fumarate hydratase activity (*LmFH-1*). Fumarate hydratase, also called fumarase, catalyses the stereospecific reversible hydration of fumarate to malate. Recent studies in trypanosomatids, utilizing *Trypanosoma brucei* as model, suggest that fumarases are essential for survival of these parasites. *LmFH-1* has been cloned in pET28a vector using *L. major* genomic DNA as a template. The predicted enzyme from *L. major* was overexpressed in *Escherichia coli* strain BL21(DE3) as a histidine-tag fusion protein. Cells were grown in Luria-Bertani (LB) medium supplemented with kanamycin (30 µg/ml) and protein expression was induced by IPTG (0,25mM). *LmFH-1* was purified to homogeneity by affinity chromatography in Ni-NTA (QIAGEN) column using a step gradient of imidazole. The final product was homogeneous in SDS-PAGE gel electrophoresis. Preliminary kinetic studies are performed by monitoring the product formation through spectrophotometric analysis at 250 nm. Our studies have demonstrated that *LmFH-1* is readily oxidized by oxygen leading to the loss of protein activity. For this reason, all steps, from purification to enzymatic assays, have to be performed in anaerobic conditions. Kinetic experiments are now in progress in order to determine the catalytic constants.

Keywords: Fumarate hydratase; *Leishmania major*; Kinetic.

Financial support: FAPESP.

FQ 08 - INTERACTION OF CEL9A WITH CELLULOSE FIBERS BY COMPUTER SIMULATIONS

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Biofuels are sought as an alternative to reduce the world's dependence on non-renewable resources (petroleum, natural gas, coal). The most common fuel nowadays is ethanol, currently extracted from the corn grain (starch) and sugar cane (sucrose). The breakdown to sugars from cellulosic biomass constitutes a logical and environmental sustainable option of energy source. Plant cell-wall degrading enzymes, cellulases, are produced by a variety of fungi and bacteria. However, the use of cellulases to convert cellulosic biomass into liquid fuel involves the cost-effective production of efficient enzymes. Glycoside hydrolases process polysaccharides relatively inefficiently. The rate-limiting step in hydrolysis is not the catalytic cleavage, but the disruption of a single chain from its native matrix, which is often inaccessible to the active site of the appropriate enzymes. For this reason, enzymes in aqueous solutions have difficulty to act on the insoluble, highly ordered cellulose matrix. The interaction of the cellulases with the cellulose matrix is however mediated by a single scaffoldin-borne cellulose-binding module (CBM), overcoming the problem of cellulose binding. In fact, an enhancement in the catalytic activity for several cellulases has been observed upon attachment of CBMs. It has also been reported that the deletion of the CBM from the family IIIc rendered the enzyme almost completely inactive. Therefore, the understanding of the molecular level interactions between protein and substrate is essential for the design of enzymes with high catalytic efficiency. In order to characterize such interactions, molecular docking and molecular dynamics simulations were used to investigate the binding of a dextran chain in a crystal-like conformation to the carbohydrate-binding module (CBM) of Cel9A from *Thermobifida fusca*. This bacterium synthesizes a total of six cellulases: three endocellulases, two exocellulases and Cel9A, which acts as endo- and exocellulase. Among those, Cel9A is responsible for a higher soluble oligosaccharide output from insoluble cellulose and was the first enzyme identified to comprise both domains, catalytic and CBM. The dual characteristic of this enzyme makes it an ideal model since it allows for the simultaneous probing of carbohydrate recognition/attachment and catalytic activity. The simulations show that the fiber binds to the CBM in a single and well-defined configuration *in-line* with the catalytic cleft, supporting the hypothesis that this CBM plays a role in the catalysis by feeding the catalytic domain with a polysaccharide chain. The results also expand the previously known list of residues involved in the binding. The polysaccharide-protein attachment is shown to be mediated by five amine/amide-containing residues. E478 and E559 residues were found not to interact directly with the sugar chain; instead they seem to be responsible to stabilize the binding motif via hydrogen bonds.

Keywords: Cellulase, Cel9A; CBM-cellulose interaction; Molecular docking; Molecular dynamics.

FQ 09 - INVESTIGATION OF FLUOR TIN OXIDE (FTO) AS EGFET FOR PH SENSOR

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Many chemical and biological processes depend on pH value, what makes it one of the commonest laboratory measurements. A recent development in pH measurement was the introduction of ion-sensitive field effect transistor (ISFET) technology as an alternative to the glass electrode. Recently, the extended gate field effect transistor (EGFET) was introduced as an alternative for the fabrication of ISFET. The EGFET is a device composed of a conventional ion-sensitive electrode and a MOSFET device, which can be applied to the measurement of ion content in a solution. Therefore, the EGFET is fabricated connecting the sensitive membrane to a commercial MOSFET. This structure has a lot of advantages when compared to ISFET because the sensitive membrane can be optimized without the fabrication of the transistor. In this work, we investigated the commercial tin oxide doped with fluor (FTO) as sensitive membrane to EGFET. The device is separated in two parts. The sensitive part is made of a commercial FTO film, with surface area of 1 cm², deposited on a 1mm-thick glass. The system is completed with a commercial CD4007UB MOSFET. The commercial FTO shows a low resistivity and a crystalline phase, both determined using conductivity set-up and by X-ray diffraction experiments, respectively. So far, it has been known that an amorphous structure is desirable to obtain high sensitivity. Despite of the a crystalline phase, we have fabricated the FTO as EGFET for pH sensor and carried out experiments in order to obtain the response of the device inserted into solutions with pH values from 2 up to 12. In this range, we have quantified a sensitivity of 50 mV/pH, which may have large potential applications as pH and biosensors. In addition, both the film and the structure of the sensor are cheaper and easier than common techniques.

Keywords: pH value; Fluor tin oxide; Extended gate field effect transistor.

Financial support: FAPESP.

FQ 10 - STUDIES OF THE INCUBATION TIMES OF PRIONIC DISEASES BY DYNAMICAL MONTE CARLO METHOD

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Introduction: Prions are the infectious agents responsible for a group of fatal neurodegenerative disorders. An pathogenic isoform of the prion protein (PrP^{Sc}) generated by a posttranslational process involving the conversion of alpha-helices into beta-sheets of the normal cellular prion protein (PrP^C) is believed to be the main component of this infectious agents. This conversion of a normal PrP^C into an abnormal isoform PrP^{Sc} kinetically behaves as an autocatalytic process. To better understand this kind of abnormal protein propagation, many analytical models has been proposed. Thus, we studied, using the Monte Carlo method, the distribution of the incubation periods in some of these neurodegenerative disorders such as bovine spongiform encephalopathy well-known as mad-cow disease (BSE), Variant Creutzfeldt-Jakob disease (vCJD) and murine scrapie, an experimental murine prionic disease. The probabilities of the incubation time distribution of these diseases were considered lognormal. **Objectives:** The aim of this study was to investigate some aspects of the toxicity and replication of the prionic diseases by comparing the results of computational simulations with the

incubation time periods of BSE, vCJD and murine scrapie, previously established. **Methods:** Computational simulations using a Dynamical Monte Carlo method (DMC) and the diffusion-limited aggregation model (DLA) were worked out. At first, we evaluate the Eigen model through computational simulations using the DMC. Following the results, we studied the toxicity of the prionic diseases using the DMC and the DLA model, considering that PrP^C converting in PrP^{Sc} just when there is contact (autocatalysis) and free PrP^{Sc} are allowed to diffuse randomly to their nearest neighbor sites in a square lattice, while isolated PrP^{Sc} or aggregate of PrP^{Sc} are fixed. **Results:** The more important parameter in the equation of the prionic kinetic is the Michaelis-Menten constant or the autocatalytic term. We get the fitting in different profiles of the distribution of the incubation periods (lognormal to BSE and vCJD and lognormal with a second peak to murine scrapie). **Conclusion:** The autocatalysis is an essential mechanism to the prionic kinetic and the spontaneous conversion of PrP^C in PrP^{Sc} can be neglected. Based on the DLA model, to BSE and vCJD (diseases with natural occurrence) we can conclude that and the toxicity in these diseases are caused by formation of amyloid plaques. To murine scrapie, we find the toxicity is caused by formation of amyloid plaques in addition to depletion of PrP^C. Changing the initial and terminal simulation parameters we fitting all diseases studied, in spite of the model to be very simple. Moreover, the lognormality is a result of the diffusive process. The concentrations of PrP^C must be low, less than 1% and the number of PrP^{Sc} must be less than 10 to lognormality take place.

Keywords: Prions; Transmissible Spongiform Encephalopathies (TSEs); Autocatalytic process; Monte Carlo; Diffusion-Limited Aggregation model.

Financial support: FAPESP.

NU 01 - ANTIBACTERIAL ACTIVITY OF SPRAY-DRIED ALECRIM PIMENTA ESSENTIAL OIL AND EXTRACT AGAINST *Listeria monocytogenes*

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Listeria monocytogenes is a foodborne pathogen that causes listeriosis, an infection that targets mainly immunocompromised individuals and pregnant women. Novel methods to inhibit *L. monocytogenes* in foods are of great interest, since this bacterium is ubiquitous and may persist in food processing environments for years. Essential oils of plants and their derivatives have wide application as flavouring ingredients in a variety of foods and beverages, and are also used for the control of pathogens carried by food, thereby improving safety of foods. In this work, spray-dried preparations of essential oils (FO1 and FO2) and hydroalcoholic extract (FE1 and FE2) from the plant *Lippia sidoides*, popularly called alecrim pimenta, were tested at different concentrations against *L. monocytogenes* ATCC 19115. The spray-dried preparations of essential oil and extract were introduced in Brain Heart Infusion broth (BHI) at concentrations of 3, 12.5, 6.25, 12.5, 25, 50 and 100 mg/ml to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) by standard macrodilution technique. For the analysis of spray-dried preparations of essential oil control tubes were prepared containing: 1) BHI broth, Tween 80 0.5% and *L. monocytogenes*, 2) BHI broth, erythromycin 5000 mg/ml and *L. monocytogenes*. For the analysis of spray-dried preparations of extract were prepared control tubes including: 1) BHI broth, dimethyl sulfoxide 2% (DMSO) and *L. monocytogenes*, 2) BHI broth, erythromycin 5000 mg/ml and *L. monocytogenes*. The tests and controls were done in triplicate and incubated to 37°C for 24 hours. The results showed a MIC of spray-dried preparations of FO1, FO2, FE1 and FE2 against *L. monocytogenes* of 12.5 mg/ml. The MBC of spray-dried preparations of FO1 and FO2 of 25mg/ml, FE1 and FE2 of 12.5 mg/ml. The results showed that different formulations of spray-dried (FO1 and FO2; FE1 and FE2) had antilisterial activity similar, and that the value of MBC differed between essential oil and extract. This study indicates the potential of the spray-dried preparations of essential oil and extract of *L. sidoides* to be used as a barrier to listerial growth.

Keywords: Lippia sidoides; Listeria monocytogene, Spray dried.

Financial support: FAPESP.

NU 02 - CISPLATIN-INDUCED GENOTOXICITY AND ITS MODULATION BY PASSION FRUIT PULP IN SHR RATS

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Several components of the diet are able to inhibit carcinogenic processes and are important factors in the cancer prevention, due antigenotoxic effects. Evidences in literature have showed promissory action of bioactive compounds from leaves of passion fruit (*Passiflora edulis* Sims) that acts as antioxidants, immunomodulators and anticarcinogenics. The antioxidative capacity of passion fruit pulp is not well characterized in the literature. This study has aimed to investigate the protective effects of passion fruit pulp against the *in vivo* chromosomal damage induced by the antineoplastic drug cisplatin (cDDP), a known genotoxic agent, in spontaneously hypertensive rats (SHR). The *in vivo* bone marrow micronucleus in polychromatic erythrocytes (MNPE) test was performed to assess chromosomal damage. Fifty male SHR rats were divided into eight groups included the negative control; a positive control (cDDP 0.5 mg/kg b.w, intraperitoneally); three groups treated with three different doses of passion fruit pulp (orally) for four days and three groups associated the fruit pulp for four days prior the administration of cDDP. The results indicated no increase in the mean number MNPE, in the three doses tested (pulp I, II and III), compared with the negative control (p>0.05). These results indicate that the fruit pulp had no clastogenic and/or aneugenic effect when administrated orally in SHR rats. These results were accompanied by a slight decline in the polychromatic erythrocytes-normochromatic erythrocytes (PCE/NCE) ratio. The data showed that fruit pulp itself was not genotoxic in the rat micronucleus assay. These data show that the fruit pulp in the three tested doses did not affect the cellular proliferation of the red cells and therefore were not cytotoxic in the tested conditions. In addition, treatment with combinations of fruit pulp and cDDP resulted in lower MNPEs frequencies than those observed for animals treated with cDDP alone. In the animals treated with cDDP the number of MNPEs was significantly increased compared to negative controls (p<0.05). The protection against cDDP-induced genotoxicity by passion fruit pulp may be due to inhibition of free radicals and increased antioxidant status. These promising data obtained *in vivo* can contribute to the inclusion of this fruit in future trials in studies of chemopreventive agents in humans.

Keywords: Passion fruit pulp; Cisplatin; Micronucleus.

Financial support: FAPESP; CNPq.

NU 03 - CLIMATERIC PHYSICALLY ACTIVE WOMEN INGESTING THEIR ROUTINE DIET OXIDIZE MORE CARBOHYDRATES THAN LIPIDS

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Introduction: The Brazilian female population who enter the climacteric is increasing, justified by the increase in women's life expectation, and it is known that the proportion of obese is also increasing in Brazil, called nutritional transition. The largest proportion of obese may be explained in part by a possible drop in the substrates energy oxidation rate in this population. However, this possible drop is questionable by some studies, and in particular, there is a lack of knowledge in overweight climacteric women. Thus, the goal of this work was to study the influence of a Brazilian routine diet

in the substrates energy oxidation in climacteric women. **Methods:** Regularly attended patients were recruited from the Climacteric Clinic of Clinical Hospital of Ribeirão Preto (HCFMRP/USP). The patients were aged between 39 to 65 years, excluding those suffering from diabetes mellitus, hypertension, use of hormone replacement therapy, hypo-or hyperthyroidism. Anthropometric measurements, food frequency and physical activity questionnaires and indirect calorimetry were made. **Results:** According to the food questionnaire, the predominant diet was characterized by a mixed diet with lipids above the recommended levels. The carbohydrate oxidation rate after a 12 hours fasting was higher than the lipids (0.11 ± 0.067 and 0.070 ± 0.0176 grams per minute, respectively, $p=0.014$). The carbohydrate oxidation rate per kilogram of body weight showed significant positive correlation with the resting energy expenditure ($p=0.001$), carbohydrate intake in grams ($p=0.008$), and negative with the lipid oxidation ($p=0.001$). The fat oxidation rate per kilogram of body weight showed significant negative correlation with body mass index ($p=0.001$), waist circumference ($p=0.005$) and the total daily caloric intake ($p=0.03$). **Conclusion:** A high carbohydrate intake and oxidation rate can contribute to weight gain in climacteric, as the reduced lipid oxidation rate as found in the present study.

Keywords: Climacteric; Substrate oxidation; Indirect calorimetry.

Financial support: FAPESP.

NU 04 - PROMOTION OF LIVER STEATOSIS IN RATS BY HIGH-FAT AND/OR HIGH PROTEIN DIETS

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Introduction: The hepatic steatosis is a macrovesicular fat accumulation, that represents a liver alteration. Studies with normal rates are showing they become obese and develop hepatic steatosis when eat high fat diets *ad libitum*. When changed, the diet composition could lead to hepatic steatosis and compromise the liver, so it's very important to find out the diets action. **Objective:** We analyzed the effects of different kinds of diets in promotion of liver steatosis in rats and the possible adverse effects in the lipid peroxidation and antioxidants levels, in correlation with the grade of steatosis. **Methods:** We used 30 male *Wistar* rats, divided in 3 groups: Control Diet AIN-93 (CD); High Fat Diet (HFD) with 50% of saturated fat and High Fat and Protein Diet (HFPD) with 50% of saturated fat and 40% of protein. In the beginning the animals had a diet adaptation period, then, each group received the diets and water *ad libitum* during 28 days. The animals were killed after the 28 days of diet, we collected the liver and blood to biochemical analysis: vitamin E, GSH, TBARS and Glucose. The grade of hepatic steatosis was assayed by histological analyses and by total liver fat. **Results:** Concerning the average intake of the diet at the first and the last weeks, there were no statistical differences between the three groups ($p>0.05$). The rats livers average weight was significantly different among the three groups ($p<0.05$). The glycemic levels of the group HFPD were significant higher than the others ($p<0.05$). The average liver fat levels of the groups HFPD and HFD were significant higher comparing with the control group, and, the average value of HFPD group was significantly higher than the HFD group, too. The total hepatic MDA average value of the HFPD group was significantly higher. However, the free hepatic MDA average value and the vitamin E average value of the control group were significantly higher than the HFD group, which were significantly higher than the HFPD group. The glutathione average value of the control group was significantly higher than the HFD group. The histologic analysis of the liver show absence of hepatic steatosis in control group, mild steatosis for the HFD group and for the HFPD group the level of steatosis vary between mild, moderate and severe. **Conclusion:** We showed through this study that high fat diet can increase hepatic steatosis, while the other diet, high fat and protein is worse than high fat diet only, due to higher frequency of hepatic steatosis cases found.

Keywords: Steatosis; Liver; Rats High-fat; High-protein; Lipid peroxidation.

NU 05 - THE IMPORTANCE OF MICROSCOPY IN THE FOOD QUALITY CONTROL PERFORMED AT THE ADOLFO LUTZ INSTITUTE

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The Adolfo Lutz Institute (ALI), the Public Health Laboratory of the State of São Paulo, Brazil, is subordinated to the State Health Secretariat through the Coordination of Disease Control. Its section of Food Microscopy at the Bromatology and Chemistry Sector is involved in food hygienic-sanitary control aiming to determine contamination levels of foreign materials and impurities, whether prejudicial or not to the population at large. It also establishes the food identity and quality by characterizing authentic histological (composition) or foreign (fraud) elements. This study aimed to evaluate through microscopy standards the sanitary profile of food analyzed at the Regional Laboratories of Ribeirão Preto, Santo André and Campinas of ALI in the State of São Paulo in the period of January, 2006 to December, 2007. The 504 different food samples evaluated, originating from control or orientation analysis, were collected by the Municipal Sanitary Vigilance or requested to the Adolfo Lutz Institute by private parties. Microscopic analysis were performed according to the procedures described in the publication of the Association of Official Analytical Chemists (AOAC, 2000) for the investigation of foreign materials (dirt) and the food composition or fraud verified by identification of histological elements (Rodrigues et al., 1999). In all cases samples were evaluated according to current legislation. Among the food samples examined, 37% showed discordant microscopic analysis, 3.2% having misidentified histological elements due to fraud. Coffee samples (7.7%) had impurities like seed hulls and plant twigs and the other 24% rejected showed other foreign materials, macroscopic and/or microscopic. Of the total specimens included in the study, 1.6% contained rodent fur and droppings, cockroach droppings, flies and metal particles considered prejudicial to human health and were, thus, in disaccord to the ANVISA/MS Resolution RDC nº 175/2003. Considering SVS/MS Regulation nº 326/1997, 22.4% of samples contained impurities indicating lack or inadequate maintenance of Good Manufacturing Practices. By verifying how elements, like insect fragments, hair strands, amorphous material, filamentous and yeast-like fungus, larvae, and insect webs, ended up mixed in food could be a good indicator of the sanitary conditions in food production and handling. Although these impurities may not be considered potentially prejudicial to the consumer, the data obtained through microscopical analysis furnishes subsidies to the analysis of critical points in the productive food chain, indicates where are implanted and maintained Good Manufacturing Practices as well as allows more effective control by the Sanitary Vigilance bodies, this being an important tool in the offering of safe food to the consumer. Last but not least it guarantees health promotion and protection for the whole population.

Keywords: Microscopic analysis; Food; Foreign materials; Legislation; Public health.

PN 01 - ALTERATIONS IN THE RENAL PARAMETERS CAUSED BY CHRONIC INGESTION OF TEA LEAVES OF THE YACÓN (*Smallanthus sonchifolius* - ASTERACEAE)

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Smallanthus sonchifolius (Poepp. & Endl.) H. Robinson (Asteraceae) is a perennial herb popularly known as yacón. It is originary of the Andes, where its tuberous roots are used as food and its leaves are used in the folk medicine for the treatment of diabetes. The hypoglycemic activity of the aqueous extract of the leaves of this plant has been demonstrated in a recent study. Phytochemical investigations of the foliar extracts have shown the

presence of phenolic compounds, sesquiterpene lactones, kaurane-type diterpenes and flavonoids. There is a great amount of sites in the Internet recommending the use of yacón for the treatment of diabetes. Its utilization as food or in the traditional medicine is growing in some countries, including Brazil. However, studies on the medicinal efficacy of specimens cultivated in Brazil are scarce, while studies which prove the lack of toxicity both in prolonged and in large amount use of the tea of the leaves are do not exist. Thus, the aim the present work was to evaluate if the tea of the yacón leaves present toxicity in chronic oral ingestions. Plants were collected in São Paulo, Brazil. Tea infusion was prepared by pouring 1000 ml of boiling water onto each 20 g of dried yacón leaves. The extraction continued by 20 min while cooling. Similar water extracts are the common form of administration used in popular medicine. Male and female *Wistar* rats were divided into groups of six animals (three of each sex). The tea infusion was orally administered using a gastric tube (gavage) at doses of 10, 50 and 100 mg/Kg of rat per day. Controls received water. Body weights and food intake were measure weekly. After 90 days, the animals were anesthetized and blood was collected for hematological and biochemical analyzes. The data was expressed as mean±S.E.M and analyzed by one-way ANOVA following Tukey's test. The experimental protocol was approved by commission of ethic in animal experimentation of the University of de São Paulo State (Protocol number 07.1.636.53.5). Body weights and hematological parameters have not change after chronic ingestion of the infusion. The biochemical analyzes demonstrated that serum levels of hepatic parameters were normal, while triglycerides, creatinine and glucose were increased in relation to control group. These results pointed to renal abnormalities, since studies about chronic renal disease demonstrated that human patients in this clinical stage present these abnormalities. However, only after histopatological studies will be possible to confirm the renal injury. In case of confirmation of the renal injury, the results may indicate that the anti-diabetic effect of tea of yacón is transitional, and that chronic ingestions could worsen the state of diabetic patients who use the plant.

Keywords: *Smallanthus sonchifolius*; diabetes; renal abnormalities.

Financial support: CAPES; FAPESP.

PN 02 - AN INTEGRATED PROCESS TO EXPLORE THE BIOTECHNOLOGICAL POTENTIAL OF *F. oxysporum* 152B

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The classical biotechnology offers the opportunity to produce fine chemicals and natural products with wide application in different industries. It was recently demonstrated that the fungal strain *F. oxysporum* 152b produces an extracellular alkaline lipase with an activity of ~10 U after 72 h of cultivation. Meanwhile, a parallel study has indicated that this strain is also able to biotransform *R*-limonene to produce the flavor compound *R*- α -terpineol, that reached, in optimal conditions, ~2.4 g.L⁻¹ after 72 h. This work studies the unification of these processes and describes the co-production of alkaline lipase and *R*- α -terpineol by *F. oxysporum* 152b. The lipase production was carried out in 250 mL-conical flasks with 50mL of synthetic medium (w/v) (1.5 % peptone, 0.5 % yeast extract, 0.3 %K₂HPO₄, 0.04 % MgSO₄.7H₂O, 1 % olive oil, pH 6), incubated in shaker at 30°C/160 rpm for 72 h. In the sequence, the biomass was separated by filtration, resuspended in water and incubated at 30°C/200 rpm with 0.5 % (v/v) of *R*-limonene for the biotransformation trials. The lipase activity was determined in the filtrate, based on the degradation of p-nitrophenyl-laurate. The results have confirmed that the biomass resultant from the lipase production has presented a *R*-limonene-biotransformation activity and that *R*- α -terpineol was produced in a concentration equivalent to the traditional process. The lipase activity (14 U) in this study was similar to that obtained in the former work. Therefore, it was confirmed that an integrated process for the production of an alkaline lipase and *R*- α -terpineol is feasible for the strain *F. oxysporum* 152b.

Keywords: alkaline lipase; biotransformation; *R*-limonene; *R*- α -terpineol.

Financial support: FAPESP; CNPq; CAPES.

PN 03 - ANALYSIS OF ACETILCOLINESTERASE INHIBITING SUBSTANCES IN PLANT *Eclipta alba*

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Eclipta alba (L) popularly known as grass button, is a cosmopolita plant, with antirust, antihepatotoxic, antiinflammatory action. Studies take in them to observe its action against degenerative illnesses for increase of the enzyme acetilcolinesterase (AChE). This plays an important role in the cholinergic mechanism, catalyzes hydrolysis of asetic acid the ACh substratum in and hil. It is found in sinapses of the central nervous system (SNC), parassimpático nervous system (SNP) and junction to neuromuscular. The increase of the AChE cause degenerative, dysfunction of the neurotransmitters in the brain contributing for the loss of cells in pró-brain basal, that al is followed by the loss of the neurotransmitter of acetilcolina. Studies show that the inhibition of the enzyme (AChE), increases the concentration of the acetilcolina in sinapse, with this diminishes or delays the symptoms associates to the illness of Alzheimer. Objective of our study was to prove action of *Eclipta alba* as inhibiting of AChE. The methodology of Ellman was used to measure activity of AChE e the used solutions had been DTNB, ACTI and AChE enzyme. Through a fraction of aerial parts of *Eclipta alba* (phase Acetate) was made a Chromatography in Column Clássica (CCC) using sephadex LH 20, after congregated the samples, got 4 fractions that had been applied the plate of thin-layer chromatography (TLC) and eluted in 20ml Chloroform/ Methanol in the ratio of (9: 1), after eluted the plates of CCDC, one was disclosed with sulphuric Vanilina for comparison and to another one with solution DTNB + ACTI (1: 1) and left to act during 3 minutes. After drying the AChE enzyme was sprayed in the TLC acting during 10 minutes. A white halo in fraction 3 was observed, thus being able to prove that *Eclipta alba* (phase Acetate) presents substantiates inhibitors of Acetilcolinesterase. These inhibitors are useful for the treatment of degenerative diseases and can be used as molecules and so can leading future drugs.

Keywords: *Eclipta alba*; acetilcolinesterase.

PN 04 - BIOTRANSFORMATION OF SOY (*Glycine max*) BY FERMENTATION PROCESS AND BY ADDITION OF β -GLYCOSIDASE

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Introduction: There is a growing scientific interest about the soy chemical compounds characters and the relation between their consume and benefices effects to human health. Around the interest substances, the isoflavones are specially abundant in this vegetable and characterize the soy like a functional food. Around the isoflavones, the glycosylated form (most common in soy) like genistin and daidzin show smaller biological activity then theirs aglycones forms (genistein and daidzein). Studies indicate that the soy fermented products contain bigger amounts of aglycones isoflavanoids than theirs glycosides. In these products, the isoflavanoids can be hydrolyzed by the β -glycosidase enzyme activity during the microorganism fermentation. This enzyme hydrolyzes the glycosides phenolics compounds improving the free polyphenols concentration and the biological activity. **Objective:** To Characterize the soy biotransformation by fermentation process with *Aspergillus awamori* semisolid culture and compare with the soy biotransformation by enzymatic process using a rich β -glycosidase aqueous extract. Material and methods: Soy biotransformation by semisolid fermentation with 10⁷ spores of *Aspergillus awamori* and by the addition of enzymatic aqueous extract from *Aspergillus awamori* culture. Determination of antioxidant activity by the DPPH* method and quantification of free polyphenols, by Folin-Ciocalteu method, determination of genistein and daidzein content by HPLC. **Results:** The soy biotransformation by fermentation improve gradually and significantly, by time incubation fuctionthe antioxidant activity, free polyphenols and

aglycones isoflavonoids (genistein and daidzein) amount. Was gotten an improve more than 3 times the antioxidant activity and the total polyphenols amount after 96 hours of incubation and an improve of 2 and 3 times the daidzein and genistein content, respectively. The soy enzymatic biotransformation wasn't able to promote a proportional improve in free polyphenols and antioxidant activity compared with the biotransformation by fermentation, however, was noted a more significant improve of aglycones isoflavonoids. **Conclusion:** The results demonstrate that the two alternatives for soy biotransformation are efficient to improve the aglycones isoflavonoids amounts, but they distinguish one from the other about the rise of the antioxidant activity and free polyphenols.

Keywords: *Glycine max*; *Aspergillus awamori*; biotransformation; isoflavonoid; antioxidant.

PN 05 - CHARACTERIZATION OF THE EXTRACELLULAR COMPOUNDS RELEASED FROM *Rubus fruticosus* CELLS DURING A HYPERSENSITIVE RESPONSE

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The plant-pathogen interactions trigger a series of signals that are not yet completely understood. One of the mechanisms is the hypersensitive response (HR), which is characterized by cell death in the infection site in order to prevent pathogen proliferation. Our previous studies with different elicitors demonstrated the correlation between the formation of reactive oxygen species (ROS) and cell wall degradation. Here, we characterized the extracellular components released and the modifications of the monosaccharide composition in cell wall during a hypersensitive response in *Rubus fruticosus* (blackberry-black). Cells were elicited with 1 $\mu\text{mol/L}$ salicylic acid (SA), methyl jasmonate (MeJA) or acid polysaccharide (rhamnoglucuronogalactan, F-I). After 1 h, they were decanted with Tris-HCl buffer. The supernatant was withdrawn (fraction E) for the quantification of phenolic compounds and total protein, as well as β -D-galactosidase and β -D-glucosidase enzymatic activities. They were subsequently disrupted releasing the intracellular components (fraction I) and the cell wall fraction was hydrolysed, reduced and acetylated; a liquid-gasose chromatography analysis was carried out on an OV225 column. The main constituents of neutral sugars in the cell wall of *R. fruticosus* were glucose (55-61%), arabinose (22-29%) and mannose (13.8-15%). Minor constituents were fucose (0.65-1.2%), galactose (0.5-0.8%), xylose (0.5-0.8%) and rhamnose (~0.5%). AS decreased the rhamnose and fucose concentrations; F-I both decreased the percentage of mannose and glucose and increased rhamnose and fucose. MeJA, in turn, increased the percentage of rhamnose, xylose and galactose. The time-course curves for β -D-galactosidase and β -D-glucosidase activations in fraction E were most effective for MeJA (300 and 230%), while F-I and AS inhibited β -D-galactosidase. The total protein increased in the presence of F-I and MeJA. F-I and AS increased extracellular phenolic compounds, although they decreased them in the fraction I. MeJA was unable to change the synthesis of either intracellular or extracellular phenolic compounds. The data suggest that F-I and AS modulate the defense responses of plants through a via different that of MeJA.

Keywords: *Rubus fruticosus*; β -D-galactosidase; β -D-glucosidase; hypersensitive response.

Financial support: CNPq.

PN 06 - ESTABLISHMENT OF CELL SUSPENSION CULTURE OF *Bauhinia forficata* LINK FOR PHENOLIC COMPOUNDS AND PROTEIN BIOACTIVE PRODUCTION

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The medicinal plants have been used as a major source of prevention and treatment of diseases for the poor population in the developing countries. The bioactive compounds have been found in leaves of *Bauhinia forficata* for diabetes. The aim for this work was to establish a protocol to obtain the *B. forficata* cell culture and to produce the phenolic compounds and proteins with hipoglycaemic activities. Maximum response to callogenesis induction (77%) was developed using leaves as explants on MS medium, supplemented with 1 mg/L 2,4-dichlorophenoxyacetic acid (2,4-D) and 1 mg/L Kinetin plus sucrose at 3% (w/v). The cell suspension culture was established by transferring an inoculum of fresh callus (2g) to 40 mL liquid MS medium. The effect of methyl jasmonate (MeJA) and salicylic acid (AS), at 1 $\mu\text{mol/L}$, in the cell culture was evaluated by monitoring the proteins and phenolic compounds, to colorimetric methods, in the extra and the intra cellular medium at 0, 3, 6, 9 and 12 days. In the extracellular fractions, the best time of protein and phenolic compounds induction, with MeJA and AS, was at 6 and 9 days, the values were increased to 105 and 106.5%, respectively. In the intracellular medium, MeJA demonstrated the highest effect for phenolic induction 132.6%, at 6 days. Additionally, the protein profile, obtained from intracellular medium of suspension cell culture and the leaves of *B. forficata*, was analysed by polyacrylamide gel electrophoresis (SDS-PAGE) of MW markers from 2.5 to 45 KDa. The results demonstrated one band to 6 KDa, for which the literature suggests a hipoglycaemic activity. In conclusion, the conditions utilized were efficient for obtaining cell suspension culture of *B. forficata*. Moreover, MeJA and AS can be used as elicitors of phenolic compounds and proteins in this cellular system.

Keywords: *Bauhinia forficata*; cell culture; hipoglycaemic compounds.

Financial support: CAPES and CNPq.

PN 07 - EVALUATION *in vitro* OF BASAL AND METABOLISM-MEDIATED CYTOTOXICITY OF SOLAMARGINE AND SOLASODINE

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Member of the *Solanaceae* family of plants synthesize secondary metabolites, including glycoalkaloids, both during growth and during post harvest marketing, presumably to protect themselves against phytopathogens. Solamargine is a glycoalkaloid that is found in eggplants (*Solanum melongena*) and at least other 100 *Solanum* species. Studies attempted to define the absorption, toxicity, and metabolism of glycoalkaloids indicates that biotransformation of the parent glycoalkaloids to the aglycon and several unknown metabolites takes place both in the digestive tract and in other organs. Solamargine and the aglycon solasodine were considered *in vitro* for its basal cytotoxicity and metabolism-mediated cytotoxicity with the incorporation of an S9 fraction using a microculture neutral red assay. The exposure of McCoy cells (CRL 1696) to these alkaloids demonstrated the role of sugar moiety in the cytotoxicity, with IC50 of 17,2 $\mu\text{g/mL}$ and 235 $\mu\text{g/mL}$ for solamargine and solasodine, respectively. In presence of S9 (hepatic microsomal fraction and cofactors), the cytotoxic midpoint for solamargine did not differ from that attained without metabolizing system, indicating that the S9 mixture failed to metabolically activate this compound. Unlike solamargine, the presence of S9 in the incubation mixture increased the cytotoxicity of solasodine. The aglycone is more hydrophobic (less water-soluble and more fat-soluble) than the glycoalkaloid that has both hydrophobic and hydrophilic character. These differences explain the basal cytotoxicity observed for both alkaloids.

Keywords: Solamargine; solasodine; cytotoxicity; S9 metabolic activation system.

PN 08 - EVALUATION OF THE ANTIULCEROGENIC POTENTIAL OF THE AQUEOUS PORTION OF *Byrsonima intermedia* A. JUSS

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Introduction and Objectives: The specie *B. intermedia* (Malpighiaceae), known popularly as murici-small, is a bush of the Cerrado considered medicinal for property antiulcer and astringent in the diarrheas and dysenteries. This work seeks to evaluate the potential antiulcerogenic of the aqueous portion (AcoAq) obtained starting from the leaves of *B. intermedia* in different experimental models *in vivo* and the possible mechanisms of gastroprotector action of this portion. **Materials and Methods:** Male rats (*Wistar*) were used with corporal weight of 150-200g (n=7-8). The gastroprotector effect was evaluated in groups of animals submitted to the previous treatments with: AcoAq (50, 100 or 200 mg/kg, p.o.), Salina 0,9% (it controls negative) and Carbenoxolona (100 mg/kg) as positive control. The gastric lesions were induced by absolute ethanol (1 mL/animal). The action mechanisms of gastroprotector of AcoAq (100 mg/kg) were evaluated as: the participation of the compounds sulfhydryl in the gastroprotection (NEM-blocking of the formation of the compounds sulfhydryl - 10 mg/kg), involvement of the nitric oxide (L-Name - inhibitor of the nitric oxide sintase enzyme - 70mg/kg) and quantification of the total glutathione in the gastric mucous membrane (nmol/g). One hour after at oral administration (absolute ethanol), all the animals were died and the stomachs examined for counting of the index of lesion ulcerative (ILU) or for the quantification of the glutathione levels that were expressed in the form of standard mean±error and submitted to the analysis of variance (ANOVA). **Results:** AcoAq presented effective gastroprotection in the doses of 100 and 200 mg/kg (41,0 ± 9,6 and 38,8 ± 5,9, respectively) reducing from a significant way to the lesions ulcerative when compared to the animals treated with the vehicle Salina (147,0 ± 5,7). The effect gastroprotectorive of AcoAq was completely reverted by the previous administration of the inhibitor of the synthesis of nitric oxide (99,86±10,5) in comparison with the animals that received only AcoAq (24,28±4,3). The gastroprotective action of AcoAq (39,7±8,5) was also reverted by the previous administration of the blocking of formation of the compounds sulfhydryl (166,2±24,7) indicating therefore that ACoAq exert influence on gastroprotection for the invigoration of the compounds sulfhydryl as well as for nitric oxide activation in the gastric mucous membrane. The portion is also responsible for stimulate the expression of the glutathione antioxidant levels in the gastric mucous membrane of animals pré-treaties with AcoAq (559,7±64,3) when compared to the negative control (371,4±27,4) indicating therefore the action multifactorial of AcoAq. **Conclusion:** The aqueous portion of *B. intermedia* presented potent gastroprotective effect, corroborating with popular indication. The gastroprotective action feels for the activation of the compounds sulfhydryl, nitric oxide and glutathione that exert influence the protection together of the barrier mucous front to severe harmful agents.

Keywords: antiulcer; *Byrsonima intermedia*; ethnopharmacology.

Financial support: BIOTA/FAPESP.

PN 09 - EVALUATION OF THE EFFECT OF NEEM (*Azadirachta indica*) SEEDS EXTRACT IN CELL CULTURE OF *Rubus fruticosus*

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A wide variety of properties has been attributed to the neem (*Azadirachta indica*) extracts such as antimicrobial, anti-inflammatory and insecticide. But, there are only few studies regarding the changes in plants by using neem extracts. The plants protect themselves against pathogen attack. One of these reactions is the hypersensitive response (RH), which is characterized by formation of reactive oxygen species (ROS) and cell walls reinforcement. The present study evaluated the neem seed extract actions on the hypersensitive response in *Rubus fruticosus* cell culture. The powdered seeds were submitted to two consecutive extractions with ethanol:water (1:1, v/v) at room temperature for 10 minutes, yielding E1 and E2 fractions. The solvent was evaporated and the aqueous extracts were concentrated and lyophilized, resulting in two samples, LE1 and LE2. The latter was used for analyses by high performance liquid chromatograph (HPLC) in C-18 column (4.6 x 250 mm), with acetonitrile-water (4:6 v/v) as mobile phase, flow rate 1 mL/min, monitored at 214 nm. The principal compound of this fraction is azadirachtin, 1.1 µg/mL. This is the main secondary metabolite in the neem seeds. The *Rubus fruticosus* cells (1.8g) were incubated in sodium citrate buffer containing LE2, up to 10 mg/mL, 1h, at room temperature. After this period of time, the phenolic compounds and reducing sugar were determined in the extracellular and intracellular medium by colorimetric methods. Also, the effect of this fraction on the production of ROS, in intact cell of *R. fruticosus*, was analyzed using 2,7-dichloro-fluorescein diacetate probe (LEE et al., 1999; MURATA et al., 2001). The treatments with LE2 fraction increase phenolic compounds and decrease ROS, in the intracellular medium, while increase its concentration. Conversely, phenolic compounds and reducing sugars decrease, in the extracellular medium, in these conditions. The data showed the protector effect of LE2 at the concentrations used here, in *R. fruticosus* cell culture. Therefore, these results suggest that the neem extract contributes to the ability to self-defense in the plant, in parallel with its repellent activity, on the insect.

Keywords: Neem; *Azadirachta indica*; *Rubus fruticosus*; Elicitors; Hypersensitive response.

Financial support: CAPES.

PN 10 - EVALUATION OF THE PERIPHERAL ANALGESIC ACTIVITY OF THE CRUDE ETHANOL EXTRACT OF *Protium spruceanum* (BENTH.) ENGLER

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The family Burseraceae is composed of 16 varieties and more than 800 species distributed over tropical and subtropical areas of the planet. The genus *Protium* is the principal genera of the family Burseraceae and one of the most commons in Latin America. It includes approximately 135 species bringing together from herbal to arboreal species. Most of the species of *Protium* are found in the amazon region and have, as exudates of the leaves and stem, a resin or aromatic gum used in the incense production, known as "breus". In folk medicine it's used as analgesic, expectorant, healing agent and anti-inflammatory. Some studies confirmed, in part, some pharmacologic activities described in folk therapy, like ant itching, gastro protective, acaricidal activity and, principally, anti-inflammatory and analgesic. In the present study, it was evaluated the peripheral analgesic activity of the brutish aqueous and crude hidroalcoolic extract (EEB) of *Protium spruceanum* (Benth.) Engler, by the abdominal writhes test in mice, according to the protocol of Collier, et al., modified. This experiment is based in the counting of the number of abdominal writhes induced by acetic acid (0,8%, 0,1 mL/10g), in determined interval of time. The plant material was collected in the local authority of Lavras - MG, Brasil, and identified by investigators of Universidade Federal de Lavras. The EEB was obtained by exhaustive maceration/percolation in ethanol 92,8%GL, and the solvent was removed in a rotary evaporator under reduced pressure at temperature of 45°C, subsequently this EEB was resuspended in aqueous solution with Tween 80 for animals' administration. For the realization of the writhes test, were utilized *Wister* mice, with approximately weight of 30g (±5g), supplied for the Biotério of UFOP. The mice were maintained in boxes with food and water *ad libitum*. And the doses of 200, 300 and 400 mg/kg were administered into the peritoneal cavity 30 minutes before the injection of the nociceptive agent. To the negative control group was administered the vehicle solution in what EEB was solubilized. After the administration, by intraperitoneal injection, of 200, 300 and 400 mg/kg doses, it was possible to observe an inhibition percentage of the

writhes in the order of 18.09%, 17.29% and 64.10%, respectively, what indicates significantly analgesic activity. To the positive control group was administered a solution of dipirone 200mg/kg (75.18% of inhibition). Analyzing these results it's possible to suggest a confirmation of the analgesic activity to the EBB of this specie's leaves. The dose of 400mg/kg presented a better result, however, it is necessary subsequent studies and experiments to complement this information and find an active fraction or isolate a responsible substance for this activity.

Keywords: *Protium spruceanum*; Burseraceae; peripheral analgesic activity.

PN 11 - *in vitro* ASSAYS FOR ANTILEISHMANIAL ACTIVITY OF DIFFERENT *Artemisia annua* L. EXTRACTS

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Almost 350 million people live in areas of active transmission of *Leishmania* occurring two million of new cases per year, among which are reported 1.5 million of the cutaneous form of the disease. Cutaneous leishmaniasis can be spontaneously cured after few months or can evolve to diffuse cutaneous or mucocutaneous relapsing cutaneous forms according to the agent. *L. amazonensis* is one of the main agents of the diffuse cutaneous leishmaniasis, which is usually unresponsive to all usual treatments. Therefore, new drugs are urgent needed. In last decades, a great number of crude drugs and natural compounds showed to be a promising source of antiprotozoal metabolites. The tea of *Artemisia annua* L. has been used for centuries, in China, to treat malaria. The bioactive compound artemisinin has been deeply studied. In our investigation, two traditional preparations, from this species, such as infusion and ethanol extract, were tested *in vitro* against *L. amazonensis* for evaluation of the potentialities of *A. annua* as an antileishmanial agent. The inhibitory concentrations of 50% of the parasites (IC₅₀) were determined as well as the cytotoxic concentrations of 50% on human epithelial cells HEP-2 (CC₅₀), for further Selective Index calculation. Flowering tops (full flowered) of the *Silves-1 A. annua* hybrid were collected in CPQBA-UNICAMP, SP. Infusion from dried and powdered parts was lyophilized. The ethanol extract (EE) was prepared by percolation. Fractionation of the EE was performed with n-hexane, chloroform, ethyl acetate and methanol. The promastigotes of *L. amazonensis* (MHOM/BR/73/M2269) were cultured at 25 °C, in M199, incubated 48h, with MTT testing for viability. In the preliminary screening, extracts were tested at 1,600 µg/mL, and fractions, at 300 µg/mL per well (96 microplate). For IC₅₀, fractions and artemisinin were assayed, respectively, at 3-300 and 30-120 µg/mL. Assays on HEP-2 (ATCC CCL 23) were performed with 30-3,000 µg fraction/mL. Results indicated that the infusion was inactive, while ethanol extract killed 80% of the parasites. Among the most effective EE fractions, the ethyl acetate was more active (IC₅₀: 8.9 µg/mL) than artemisinin (IC₅₀: 13.8 µg/mL), and the n-hexane fraction was less active (IC₅₀: 19.2 µg/mL). All the fractions showed low cytotoxicity (CC₅₀ > 1,100 µg/mL) [artemisinin CC₅₀ > 100 µg/mL]. In conclusion, although artemisinin was active against promastigotes, the activity of EE fractions should not only be due to this metabolite, still its content was around 1.2 % (w/w). Furthermore, the low toxicity presented by the active fractions is a good sign of a promising continuation in search for the compounds responsible for the antileishmanial activity.

Keywords: *Artemisia annua*; artemisinin; *Leishmania amazonensis*; ethanol extract; infuse.

Financial support: CNPq; CAPES/PROAP; Dr. Pedro Mellilo de Magalhães (CPQBA-UNICAMP) (for providing the studied specimen).

PN 12 - ISOQUINOLINE ALKALOIDS BIOGUIDED FRACTIONATION FROM TWO *Annona* SPECIES (ANNONACEAE) FOR *in vitro* ANTIPROTOZOAL ACTIVITY

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Neglected tropical diseases still remain a severe Public health problem that affects about one billion people, killing or disabling almost one sixth of the world's population. Limited access to drugs, resistance and low safety of the existing drugs are the major limitations for the treatment and control of leishmaniasis and Chagas disease. Our previous works signalized the promising antiprotozoal potential of the total alkaloid fraction from two Annonaceae species: *Annona coriacea* Mart and *A. squamosa* L. In search for new antiprotozoal compounds, the aim of this research was to identify bioactive fractions and compounds from these species. Leaves of *A. coriacea* (AC) and *A. squamosa* (AS) were collected at Águas de Santa Bárbara in the Ecological Reserve of the Instituto Florestal de São Paulo (W: 49°14' 40"7", S: 22°46' 9"15") and in a cultivated field at Sabino (W: 21° 33' 75"2", S: 49° 34' 96"3"), respectively, at 345 and 480 Km from São Paulo, in the São Paulo State. Voucher specimens were deposited in the Herbarium of Instituto de Biociências, Universidade de São Paulo. Dried and powdered leaves (1000 g) were exhaustively extracted, with ethanol (conc.) by cold percolation and the solvent was evaporated under reduced pressure. The total alkaloid (TA) fractions were obtained from the EtOH residues (250g AC, 96g AS), following the usual acid-base partition methods. TA (7g AC, 1,8g AC) were fractionated by column chromatography on silica gel 60 with a CH₂Cl₂/MeOH gradient. Fractions were tested for the *in vitro* activity on promastigote forms of *Leishmania* (L.) chagasi (M6445 strain) at 200µg/mL, according to the technique described by Tempone et al (*Phytomedicine* 12, 382, 2005). Chromatographic analysis of the fractions was done and successive fractionation steps are in progress, similarly bio-monitored. Assays indicated that six among 15 and eight of the 14 fractions, respectively, from AC an AS, ranged from 2 to 50% MeOH, are highly active, causing 100% deaths at the tested concentration. Results are in agreement with the TA antiprotozoal activity found on previous screening. Furthermore it was seen that the high level of activity remained with fractionation, stimulating isolation of the bioactive components with further tests on the parasite amastigotes and also for trypanocidal activity.

Keywords: isoquinoline alkaloids; bioguided fractionation; *Annona coriacea* Mart.; *Annona squamosa* L.; *Leishmania* (L.) chagasi.

Financial support: FAPESP; CNPq.

PN 13 - MODULATORY ACTIVITY OF FLAVONOIDS IN THE HUMAN NEUTROPHIL OXIDATIVE METABOLISM AND PHAGOCYTOSIS STIMULATED VIA FC-GAMMA AND COMPLEMENT RECEPTORS

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Introduction: Tissue damage found in some inflammatory and autoimmune diseases involves excessive neutrophil ROS production, triggered by immune complexes (ICs) via Fc-gamma (Fc_γR) and complement receptors (CR). Natural products are being investigated in the treatment of diseases caused by over-ROS. The flavonoids is the most important group from the phenolics and diverse among the natural products. So, modulation of both the effector potential of these receptors and ROS generation are important to the maintenance of body homeostasis. **Objectives:** Evaluation of the modulatory effect of the flavonoids quercetin, galangin, myricetin and kaempferol in the oxidative metabolism and phagocytosis the human neutrophil stimulated via Fc_γR and CR classes of membrane receptors. Methods and **Results:** Human neutrophils were isolated by the gelatin method. The tested flavonoids inhibited the neutrophil ROS production, measured by the luminol- and lucigenin-enhanced chemiluminescence assays, in a concentration-dependent manner. Such negative modulatory effect was not dependent on the class of membrane receptor stimulated but it was dependent on their chemical structure and the inhibitory potency of the compounds decreased in the following order: galangin > kaempferol > quercetin-myricetin. At concentrations near the IC₅₀, the flavonoids did not interfere with the neutrophils phagocytosis of IgG-ovalbumin ICs (evaluated by transmission electron microscopy). **Conclusion:** The flavonoids tested

herein had a selective and structure dependent negative modulatory effect in the ROS generation process, without impairing the phagocytic processes. These results provide a possible application of flavonoids studied as a drug of natural origin in diseases that focuses on the neutrophils with, for example, rheumatoid arthritis, targeting the selection of new therapeutic targets for a more appropriate treatment of these diseases.

Keywords: Neutrophil; Phagocytosis; Immunoconplexes; Flavonoids; ROS.

Financial support: FAPESP.

PN 14 - PREPARATION AND CHARACTERIZATION OF LIPOSOMES CONTAINING FLAVONOIDS - EFFECT OF LIPID COMPOSITION ON THE ENTRAPPMENT EFFICIENCY

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Flavonoids are a group of polyphenols found in fruits, vegetables, seeds, flowers and red wine. A variety of pharmacological properties have been attributed to these phenolic compounds, such as anti-inflammatory, antiviral, antitumor which are often associated with their oxidative, ROS scavenging and enzyme inhibitory actions. However, because of their low solubility in water, administration of flavonoids through the systemic route is unpractical and therefore, development of an appropriate flavonoid-carrier is of great importance in the clinic. Due to specific structural properties, liposomes have been known to have appreciable potential as drug carrier for a variety of compounds, incorporating hydrophilic and hydrophobic drugs into aqueous phase and phospholipid layer, respectively, offering a powerful tool to improve therapeutics effects and to reduce toxicity. For these reasons, the aim of the present study is (1) prepare large unilamellar liposomes (LUVs) containing flavonoids; (2) evaluate the effect of lipid composition (content and type of cholesterol) on the entrapment efficiency of compounds and (3) to characterize the vesicles based on size and zeta potential measurements. The ethanol injection method was used for preparing the LUVs containing or not flavonoids (quercetin, myricetin, kaempferol and galangin). Vesicles are prepared using different lipid composition, including hydrogenated soybean phosphatidylcholine and cholesterol (SPC/chol) or SPC and cholesteryl ethyl ether (SPC/ chol-OET) system. The untrapped flavonoid was separated from LUVs by ultracentrifugation or dialysis. The flavonoid incorporation in LUVs was determined by UV-vis spectrophotometry at specific λ_{nm} for each compound, using a calibration curve. The physicochemical characterization of vesicles was performed by laser diffraction using the Zeta Sizer Nano-ZS equipment (Malvern Instruments). The best performance concerning flavonoid incorporation was achieved with the SPC/ chol-OET system, considering the encapsulation efficiencies up to 70-90% for all compounds while for SPC/ chol, the entrapment was about 35 and 71% for myricetin and galangin, respectively, showing a relation between structure of flavonoid and its incorporation in LUVs. The content of chol or chol-OET also affected the incorporation of flavonoid, which is higher for galangin and kaempferol when low proportions of cholesterol were used. All LUVs obtained had a mean vesicle diameter varied from 150 to 250nm. Considering our results, flavonoid-containing LUVs can be easily prepared by ethanol injection method, which is simple, of low cost and produce vesicles with high incorporation efficiency of all compounds tested. Moreover, the lipid composition can affect the flavonoid incorporation by vesicles, an important point to be considered on selecting a suitable preparation in further investigations.

Keywords: Liposome; Flavonoid; Preparation; Characterization.

Financial support: FAPESP.

PN 15 - RAPID DIFFERENTIATION OF THE SPECIES OF "GUACO" - *Mikania laevigata* AND *Mikania glomerata*, BY GC-MS

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Mikania glomerata Sprengel and *Mikania laevigata* Schultz Bip. (Asteraceae, Eupatorieae, Mikaniinae) popularly known as "guaco", they have been a lot studied due to the medicinal activities of the same ones. They are used for the treatment of the fever, rheumatism, flu and respiratory tract diseases. The monographs of both species are in Brazilian Pharmacopoeia. *M. laevigata* a lot of times it is confused with *M. glomerata*, and it has been sold as the last. This occurs mainly of the south of Brazil, where this vegetable has its largest dispersion area. The chemical evaluations of the leaves rinse extracts of *M. glomerata* and *M. laevigata* were realized. The dried and undamaged leaves were extracted by 30 seconds with dichloromethane. Aliquots these extracts were filtered and concentrated, for later analysis by GC-MS. This method is advantageous due to the small time spent in the process of extraction, and also due to obtain extracts with little complex mixtures. The qualitative analysis of those extracts allowed to identify as majority substances, coumarin and lupeol, in *M. laevigata*, and lupeol and a substance no identified in *M. glomerata*. The standardization of the analytical method in GC-MS allowed the differentiation of the two species. Samples of leaves of "guaco" of several areas of Brazil were also analyzed, and their identities were proposed.

Keywords: guaco; *Mikania glomerata*; *Mikania laevigata*.

PN 16 - THE CHEMICAL PROFILES AND CYTOTOXIC ACTIVITIES OF ENDOPHYTIC FUNGI ARE CHANGED BY MIXED CULTURES

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Introduction: Microorganisms interact with other in the natural environment and these interactions can be the driving force for the production of secondary metabolites. Endophytic fungi live inside the plant tissues in symbiotic relationship with their plants host. However, little is known about the relationship among the endophytic microorganisms. So, we hypothesized that culturing endophytic fungi together might be seen as an effective way to discover new natural products. The endophytic strains *Papulospora immersa*, *Alternaria tenuissima*, *Nigrospora sphaerica* and *Phoma betae* produced cytotoxic activities in single cultures and were selected to grow in binary cultures. **Objectives:** The aim of this work was to verify the influence of mixed cultures on both chemical profiles and cytotoxic activities produced by four endophytic fungi strains. **Materials and Methods:** The four strains were cultured alone and in dual mixtures in a two-step process. In the first step fungi were cultured individually for 5 days in a rich medium (yeast extract, malt extract, dextrose, triptone). In the second step, the mycelia obtained from each fungus were inoculated in dual combinations in PDB medium and allowed to grow for 14 days. Metabolite extraction has been made with HP-20 resin. The extracts from dual and single cultures were chemically analyzed by TLC and HPLC, and tested against the cancer cells lines SF295 (brain), HCT-8 (colon) and MDAMB-453 (breast) at 100 $\mu\text{g}/\text{mL}$. **Results:** The HPLC analyses showed significant differences in the profiles of *A. tenuissima* and *N. sphaerica* when they were cultured in single and mixed cultures. One compound was produced only in the mixed culture between of *A. tenuissima* and *N. sphaerica*. Interestingly, this fungal combination also showed higher cytotoxic activity against MDAMB-453 cells than individual cultures. In addition, the production of the cytotoxic diterpene aphidicolin by *N. sphaerica* has not been changed either by *P. immersa* or by *P. betae*. However, aphidicolin production was decreased when *N. sphaerica* was cultured together with *A. tenuissima*. Finally, *P. betae* appears to decrease cytotoxic activity produced by *A. tenuissima* against SF295 and HCT-8, since the mixed culture extract showed lower cytotoxicity compared to the single *A. tenuissima* culture. **Conclusions:** our preliminary results have shown that the mixed cultures approach led to both different chemical diversity and cytotoxic activities from those obtained in single cultures. This approach might represent a promising strategy to discover new bioactive natural products.

Keywords: Endophytic fungi; mixed cultures; cytotoxicity.

Financial supports: FAPESP.

PN 17 - THE PROTECTIVE ROLE OF *Lychnophora mart* (BRAZILIAN ARNICA) IN 1,2-DIMETHYLHYDRAZINE INDUCED EXPERIMENTAL COLON CARCINOGENESIS

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Introduction: Aberrant crypt foci (ACF) and colon rectal mucosal epithelial cell proliferation (PCNA) have been shown to be increased in patients with colon cancer and have been largely used for early detection of factors that influence colorectal carcinogenesis in rats. It is known that the colonic carcinogenesis is associated to an increased COX-2 expression, which leads to the excessive production of prostaglandin E2 (PGE2) that in turn forms a stimulatory loop with many biologic functions, including that of increasing cell proliferation. **Objective:** As vast amount of the native Brazilian plant species have not yet been chemically or biologically evaluated. The present study was aimed in collaborating with the search for medical properties of the native Brazilian flora, and designed specifically to evaluate *Lychnophora ericoides* (LE) for its potential inhibitory properties against the formation in the colon of putative preneoplastic lesions. **Materials and Methods:** Fifty male *Wistar* rats were randomly divided into five groups. The groups G1 to G4 were given four injections of the carcinogen 1,2-dimethylhydrazine (DMH). The group G2 received *Lychnophora ericoides* (LE) extracts for 6 weeks. The groups G3 and G4 received LE for 4 weeks and two weeks respectively, at the post initiation and initiation phases of colonic carcinogenesis. The group G5 was the control. **Results:** Forty-two days after the first injections of DMH for the neoplastic induction, we observed a statistically significant decrease in number of aberrant crypt foci (ACF), COX-2 expression and an attenuation of the increase in cell proliferation (PCNA) induced by DMH in all the LE-treated groups. **Conclusion:** Thus, we concluded that *Lychnophora ericoides* extracts were effective against the development of cancer. These data suggest that LE has a protective influence on the process of colon carcinogenesis, suppressing both the initiation and the promotion of colonic carcinogenesis.

Keywords: Aberrant crypt foci; cell proliferation; COX-2; 1,2-dimethylhydrazine; *Lychnophora ericoides*.

PN 18 - TYROSOL QUANTIFICATION IN *Glomerella cingulata* EXTRACTS AFTER DIFFERENT CULTURE CONDITIONS

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Introduction: Tyrosol has been identified as an auto regulatory molecule that presents important implications on the dynamics of growth and morphogenesis in *Candida albicans*. Interestingly, we have isolated tyrosol from several bioactive endophytic fungi cultures. So, we hypothesized it might have some quorum-sensing role in those microorganisms. **Objective:** To verify the applicability of a validated HPLC method quantification of tyrosol in liquid Czapek medium. **Materials and Methods:** Analytical HPLC analyses were carried out using gradient mobile phase, starting with acetonitrile:water (1:9) and increasing up to acetonitrile (100%) in 30 minutes, 1 mL min⁻¹ flow rate, in a ZORBAX® ODS column (4.6 x 250 mm, 5µm), 25° C. The extraction of the Czapek medium after *G. cingulata* cultivation was efficiently carried out with EtOAc pH 7.0 at 0, 2, 4, 6, 8, 10, 15 and 20 days of incubation using four different types of culture conditions. **Results:** Correlation between tyrosol production in µg/mL with different culture conditions was satisfactorily obtained. Cultivation at pH 5.0, 30 °C, 120 rpm for 6 days was shown to be the most promising culture condition for tyrosol production. **Conclusion:** Tyrosol quantitative method validation previously established showed to be applicable for the analysis of tyrosol in liquid Czapek medium.

Keywords: Tyrosol; *Glomerella cingulata*; endophytic fungi; HPLC.

Financial support: FAPESP.

PN 19 - EVALUATION OF THE ANTI-ALLERGIC ACTIVITY OF FLAVONOIDS FROM *Bidens sulphurea* USING RBL-2H3 CELLS BASED BIOSENSOR

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Introduction: Mast cells participate in many biological processes responsible by allergic diseases such as asthma and rhinitis. The RBL-2H3 cells line has been used as a model to study the regulated secretion in mast cells. These cells, stimulated by an antigen, release chemical mediators of allergic response as b-hexosaminidase enzyme (b-hex) which is used as a biological signal in this cell-based biosensor model. The biological assay to quantify b-hex can be used to monitor the potentiality of organic molecules, as flavonoids, to inhibit degranulation and allergic processes. **Objective:** The goal of this work is to study the antiallergic activity of flavonoids isolated from *Bidens sulphurea* (Asteraceae), a plant very common in Brazilian territory. **Methodology:** Cells were sensitized overnight with anti-DNP-IgE in a 96 well plate. In the next day, they were washed using Tyrode's buffer and stimulated by the specific antigen, DNP-BSA, in the presence of flavonoids. The b-hex were quantified using a fluorometric substrate, named methylumbelliferone, measured in a fluorescence microplate reader, at wavelengths equal to 360 and 450 nm for excitation and emission, respectively. **Results and Discussion:** Quercetin (IC₅₀=4.6µM) has shown potent inhibitory activity. However, when glycosides are substituted on C-3 position of the flavonoid, it can be observed a reduced activity as found to 3-O-b-D-galactopyranosylquercetin (IC₅₀=8.6µM), 3-O-b-D-glycopyranosylquercetin (IC₅₀=7.5µM), 3-O-(6"-trans-cafeoil)-b-D-galactopyranosylquercetin (IC₅₀=10.4µM) and 3-O-b-D-xylopyranosylquercetin (IC₅₀=23µM). Comparison of the last one and structures containing -CH₂OR group on 5' position of the glycosides revealed that the activities can be improved, but it is still smaller than quercetin. The same behavior was observed for luteolin (IC₅₀=12.5µM) when compared to 6-C-α-D-glycopyranosylluteolin (IC₅₀=22.3µM - 46% inhibition) and 8-C-α-D-glycopyranosylluteolin (IC₅₀=22.3µM - 40% inhibition). Besides, it was also observed, for glycosilated quercetin, the stereochemistry of hydroxyl group on C-4 position of the glycoside doesn't cause a significant change on antiallergic activity of the flavonoid. **Conclusion:** Flavonoids isolated from *Bidens sulphurea* can inhibit b-hexosaminidase release and allergic response.

Keywords: β-hexosaminidase; Flavonoids; Mast cells; Biosensor; *Bidens sulphurea*.

QM 01 - SOLUTION-PHASE SYNTHESIS AND CYTOTOXIC ACTIVITY OF PROLINE-CONTAINING DIKETOPIPERAZINE

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Diketopiperazines (DKPs) are peculiar heterocyclic systems presenting spatially defined amino acid side chains around a proteolysis-resistant peptide-mimicking scaffold, thus constituting a rich source of new biologically active compounds. Despite many naturally occurring analogs, additional chemical variety can be assessed by combinatorial chemistry, through a number of conventional procedures that lead to intramolecular cyclization of selected dipeptides. Proline-containing amide bonds adopt a cis conformation, which favours head-to-tail ring closure, contributing for the study of DKP formation reactions. In addition, proline amino acid is extensively present in DKPs isolated from natural products which show a wide spectrum of biological properties. The aim of this work was to synthesize cyclo(L-Pro-L-Ser) from conveniently protected amino acid building blocks by solution-phase protocols and to evaluate the cytotoxic activity over tumoral cell lines. The synthesis of the DKP of interest involved the amino deprotection of Nfmoc-L-

Ser benzyl ester with 20% piperidine in DMF, followed by coupling reactions of the resultant L-Ser-OBn (94%) with the amino acid FMoc-L-Pro in the presence of the coupling reagents PyBOP, HOBT and the base DIEA, dissolved in acetonitrile, being obtained the dipeptide FMoc-L-Pro-L-Ser-OBn in 80% yield. In the final step, the dipeptide was cyclized in the presence of 20% piperidine in DMF, given that after the well known FMoc cleavage, the dipeptide presumably reacts intramolecularly by nucleophilic attack of its free amino group towards its terminal carbonyl with displacement of OBn. Stirring the reaction mixture at room temperature for 15 h furnished cyclo(L-Pro-L-Ser) in 68% yield, whilst the cyclization reaction carried out in open vessels under microwave conditions in a microwave oven (200 W) at 163°C led to the total consumption of the starting material after 4 min and to the generation of the correspondent DKP as the major product in high and satisfactory yield of 82%. Structures were assigned by ¹H and ¹³C NMR and confirmed by HRMS. Cyclo(L-Pro-L-Ser) was subjected to *in vitro* cytotoxicity assays against the following human tumoral cell lines: MDA-MB435 (breast), SF-295 (glioblastoma) and HCT-8 (colon), showing respectively 15,2 ± 11,3%; 27,8 ± 4,2% and 62,8 ± 1,3% cell growth inhibition at 5µg/mL, as judged by the classical MTT method. In conclusion, compared to the conventional procedure, microwave-assisted synthesis provided cyclo(L-Pro-L-Ser) in higher yields and in shorter reaction times, although both methods have successfully furnished the desired product. The proline-containing DKP showed low cytotoxicity for human breast cancer and glioblastoma, but a high level of cell growth inhibition was observed for HCT-8 human colon tumoral cells.

Keywords: diketopiperazine; proline; intramolecular cyclization; microwave-assisted chemistry; cytotoxicity.

Financial support: Laboratório de Oncologia Experimental research group from Universidade Federal do Ceará and FAPESP.

QM 02 - SYNTHESIS AND ANTI-*Mycobacterium Tuberculosis* ACTIVITY OF RUTHENIUM COMPLEXES

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Introduction: WHO estimates that 5% of the 9 million new cases of tuberculosis notified each year are resistant to the standard treatment: a 6-month course of the 'first-line drugs' isoniazid and rifampicin. Eastern Europe and parts of China were hard struck by such multi-drug resistant tuberculosis (MDR-TB). Baku, Azerbaijan, held the record with a hair-raising 22.3% of new tuberculosis cases being MDR-TB (WHO, 2008). **Objective:** Screening the Ruthenium complexes to discover new drugs that can be used to treat disease caused by *M. Tuberculosis* (MDR-TB). **Material and Methods:** According to the methodology of Collins & Franzblau (Antimicrob. Agents Chemother. 41, 1004-1009, 1997), the anti-*M. Tuberculosis* activity of the tested compound and standard drug isoniazid were determined in triplicate by using of the Microplate Alamar Blue Assay (MABA). Complexes cis-[RuCl₂(P-P)]₂, P-P = dpmp or dppe, and [Ru(pic)₂(PPh₃)₂] were synthesized following the literature procedures (Bautista et al 1991). Characterization data for these complexes were in agreement with the previously published data. **dpmp:** Yield: 91 mg (78 %). IR: 1654 (ν_{as} C-O); 1341 (ν_s C-O). UV-Vis (CH₂Cl₂): λ_{max} - nm (ε): 250 sh (47000), 337 (5600). Calc. for C₅₈H₄₈P₅NO₂F₆Ru: C = 59.16; H = 4.25; N = 1.23. Found: C = 58.78; H = 4.15; N = 1.00 %. ³¹P{¹H} NMR: δ (ppm) P_A = -15.5; P_B = 1.8; P_C = -22.0 and P_D = -7.7. E_{1/2} (Ru^{II}/Ru^{III}): 1.49 V. dppe: Yield: 96 mg (80 %). IR: 1666 (ν_{as} C-O); 1337 (ν_s C-O) UV-Vis (CH₂Cl₂): λ_{max} - nm (ε): 262 sh (30500), 318 (3300). Calc. for C₅₈H₅₂P₅NO₂F₆Ru: C = 59.80; H = 4.50; N = 1.20. Found: C = 59.60; H = 4.90; N = 1.13 %. ³¹P{¹H} NMR: δ (ppm) P_A = 58.1; P_B = 52.1; P_C = 46.3 and P_D = 46.3. E_{1/2} (Ru^{II}/Ru^{III}): 1.51 V. **Results:** The complexes presented MIC values, 0,78 µg/mL for the dpmp; 0,26 µg/mL for the dppe and >50 µg/mL for the [Ru(pic)₂(PPh₃)₂]. **Conclusion:** Some the complexes presented MIC values better than those observed for some commonly used to treat disease caused by *M. tuberculosis*.

Keywords: Antimycobacterial agents; Mycobacterium tuberculosis; MABA; Ruthenium (II) complexes.

Financial support: CNPq, CAPES, FINEP, PRONEX and FAPESP.

QM 03 - SYNTHESIS OF NEW ACETYLCHOLINESTERASE INHIBITORS DESIGNED BY MOLECULAR HYBRIDIZATION

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Introduction: Alzheimer disease (AD) is a severe and incurable neuropathology characterized for progressive disturb in memory and other cognitive functions, affecting social and occupational behavior. Hitting 25 millions of people in the world, AD is considered the main cause of degenerative demency in individuals over 60 years old. With life expectancy increase of worldwide population, illnesses associate to longevity such as AD and Parkinson, perform a big challenge where the development of more efficient and safe therapeutic action becomes the main target for the scientific staff. Based on evidences that AD occurs with decrease of central cholinergic function, effective new drugs have been planned as acetylcholinesterase inhibitors, which led us to design new carbamate-acylhydrazone derivatives with innovator molecular framework, drawn by molecular hybridization between donepezil and rivastigmine, two commercial drugs for AD treatment. **Aim:** Synthesis and pharmacological evaluation of a new series of hybrid compounds, designed to act as symbiotic compounds, showing both anti-inflammatory and acetylcholinesterase inhibitors. **Methods and results:** A convergent approach was proposed to the synthesis of the target compounds, using 3-hydroxybenzoic acid (1) and 4-piperidine methanol (2) as starting materials. In the first stage of the synthetic sequence, carboxylic acid 1 was converted into the correspondent methyl ester (3), by reaction with MeOH/H₂SO₄ in 95% yield. The ester 3 was then reacted with hydrazine hydrate to furnish the key-intermediate 3-hydroxy-benzohydrazide (4). In a second synthetic sequence, 4-piperidine methanol (2) was used as starting material to the preparation of the key N-benzylpiperidine-4-carbaldehyde (5) by reductive amination of 2 with benzaldehyde, followed by oxidation with PCC/DMSO, in 45% yield, after two steps. The convergent synthetic sequence was completed by the condensation of aldehyde 5 and hydrazide 4, to furnish N'-(1-benzylpiperidin-4-yn) methylene-3-hydroxybenzohydrazide (6). In a final step, compound 6 could be converted in the target carbamate-hybrid compounds by reaction with various substituted isocyanides. **Conclusion:** The synthetic route planned to the preparation of the target compounds was optimized and led to the advanced key-intermediate 6 by a convergent manner, in good global yield. At the moment, we are concluding the synthesis of the desired carbamate series to evaluate the anti-inflammatory and anti-cholinesterase profile, and possible pharmacophoric contributions of the carbamoyl moiety in the acetylcholinesterase inhibition.

Keywords: Acetylcholinesterase Inhibitors; Alzheimer Disease; Medicinal Chemistry; Molecular Hybridization.

TF 01 - A HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC ASSAY FOR CIPROFLOXACIN HYDROCHLORIDE OPHTHALMIC SOLUTION

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Introduction: Ciprofloxacin is a fluoroquinolone antibiotic with a broad spectrum of activity used in the prophylaxis and treatment of a wide range of bacterial infection. Ciprofloxacin is the most widely prescribed fluoroquinolones in the world, followed by ofloxacin. The objective of the current study

was to development and subsequent validate of a simple, sensitive and precise reversed-phase HPLC method for the determination of ciprofloxacin hydrochloride in ophthalmic solution form. **Materials and Methods:** Ciprofloxacin hydrochloride reference substance (assigned purity 100%) was supplied by EMS Sigma Pharma group, Brazil. Ciprofloxacin hydrochloride ophthalmic solutions were obtained commercially. The ophthalmic solutions were claimed to contain 3.5 mg mL⁻¹ of drug and boric acid, sodium citrate, disodium edetate (EDTA), benzalconium chloride and water as excipients. The mobile phase consisted of acetic acid 2.5%, methanol and acetonitrile (70:15:15, v/v/v). High purity water Milli-Q was used as solvent. The liquid chromatographic system, used in the present study, consisted of a Waters chromatograph equipped with Waters 1525 pump binary grade, Rheodyne Breeze 7725i injection valve with a 20 µL loop and UV-Vis Waters 2487 detector. The separation was performed at temperature of 21 ± 2°C, on a reversed phase Symmetry Waters C₁₈ column (250 mm x 4.6 mm i.d., 5 µm particle size). UV detection of the analyte was carried out at 275 nm. The flow rate of 1.5 mL min⁻¹ was maintained. **Results and Discussion:** Linear calibration plots for the ciprofloxacin hydrochloride assay method were obtained over the calibration range of 1.0-6.0 µg mL⁻¹. The correlation coefficient obtained was 0.9994 (n = 6), with slope and intercept were 119062 and 38613, respectively. The results exhibited an excellent correlation existed between the peak area and concentration of the analyte. The precision and robustness were assessed as repeatability, intermediate precision and varying the conditions room temperature and analyst. The RSD% values of the measurements was 3.39 and 3.77% for precision and intermediate precision, respectively, and 2.02% for robustness, confirming good precision and robustness of the proposed method. The accuracy of the method was established by recovery experiments. This study was employed by addition of known amounts of ciprofloxacin hydrochloride to the samples. The recovery experiments for ciprofloxacin hydrochloride show medium recovery of 100.11%. Specificity was described by ability of the method to measure the ciprofloxacin hydrochloride response in the presence of its excipients in the formulations. **Conclusion:** Analytical method for ciprofloxacin hydrochloride in ophthalmic solution using high-performance liquid chromatography with ultraviolet detection was developed and validated. The method is highly sensitive, simple, precise, accurate and selective in pharmaceutical preparations. The results indicated that the proposed method may be recommended for determining ciprofloxacin hydrochloride in ophthalmic solutions.

Keywords: Fluoroquinolone; HPLC; ophthalmic solution; ciprofloxacin.

Financial support: CAPES; CNPq; FAPESP.

TF 02 - ALECRIM-DO-CAMPO MICROEMULSION MOUTHRINSE: DEVELOPMENT, CHARACTERIZATION AND *in vitro* EVALUATION UPON VIRULENCE FACTORS OF *S. mutans* ASSOCIATED WITH DENTAL CARIES

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Introduction: The use of antimicrobial mouthrinses to help the plaques supragingival control and gingivitis has been shown to significantly contribute to patients' daily oral hygiene. Formulations based on microemulsions have several interesting characteristics such as the ability to protect labile drugs, their release control, and to increase their solubility and bioavailability. It has been demonstrated that extracts from *Baccharis dracunculifolia* (Bd), a Brazilian shrub species, popularly known as "alecrim-do-campo", show anticariogenic properties, which suggests its appliance into oral care products. However, Bd extracts are poorly water soluble. **Objective:** In this work, we have developed a microemulsion in which Bd extract may be incorporated to produce a clear solution in an alcohol-free mouthrinse. **Materials and Methods:** Four Bd extract - containing microemulsion systems (1) ethil oleate/Labrasol®/Plurol® (LP), (2) isopropyl miristate/PEG 40/Sorbytol (PSM), (3) ethil oleate/PEG 40/Sorbytol (PSO) and (4) ethil oleate/*Baccharis dracunculifolia* essential oil/PEG 40/Sorbytol/Glycerol (PSG), were prepared in aqueous mixtures at room temperature, using the conventional titration technique. The pseudo-ternary phase diagrams were prepared at room temperature. The systems were characterized by optical microscopy, stability, rheology, pH, Light Scattering and electrical conductivity. The inhibitory effect on bacterial acid production was evaluated through the potentiometric measurement of pH from bacterial suspensions treated with serial concentrations of Bd mouthrinse (0.016 a 0.32 µg/mL). For the antibacterial activity assay, the inhibitory effect on bacterial growth and the minimum inhibitory concentrations (MIC) were determined. **Results:** Two regions were identified in the first diagram (bifasic system and microemulsion) while three regions were identified on the others (bifasic system, emulsion and microemulsion). All systems displayed an oil-in-water microemulsion region. The system chosen for the mouthrinse presented viscosity (12.40 cP), droplet size (19.53nm), conductivity (145.70 µS/cm), final pH (5.89), and relative density (1.0425g/mL). The inhibitory effect values obtained for the bacterial acid production were 12.56 - 53.38%. The IC₅₀ value was 0.25mg/mL. The MIC value was 560µg/mL. **Conclusion:** The Results indicate that these systems could be an interesting Bd vehicle for a mouthrinse and suggest that the final rising formulation has antimicrobial properties superior to the rough hydroetanolic extract, with low cytotoxic effect, turning it credible to clinical evaluation.

Keywords: mouthrinse; microemulsion; *Baccharis dracunculifolia*; *S. mutans*; carie.

Financial support: CNPq.

TF 03 - CHARACTERIZATION OF GRANULES CONTAINING SOLID DISPERSIONS OF CURCUMIN/LACTOSE /GELUCIRE® 50/13 OBTAINED BY HOT MELT GRANULATION

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Introduction: A large number of drugs present low solubility in water, thus their application in therapy is restricted due to low bioavailability. Hence, several researches have been developed aiming to overcome this problem. Solid dispersions have shown to be an interesting method for enhancing the solubility of poorly soluble drugs. The greatest challenge in applying this approach is to select the polymeric system, which must present low melting point and high compatibility with the drug and which also allows the processing at low temperatures. Polymeric excipients such as long chain PEG, PVP and Gelucire® have proved to be suitable in the production of these formulations. The preparation of solid dispersions by hot melt coating technique is an alternative to improve the solubility of many drugs and yield solid dispersions with reproducible physico-chemical properties. **Objective:** The aim of this work was to evaluate the pharmacotechnical properties of granules composed of spray-dried lactose and ternary dispersions containing curcumin. **Materials and Methods:** The study followed a 2² full factorial design in order to evaluate the influence of the ratio of Curcumin:Gelucire® 50/13 (mixture A), and the proportion of Lactose:mixture A on the characteristics of the dried powder, such as angle of repose, Hausner Factor, Carr Index, particle size distribution and granulation yield. **Results and Discussion:** Granules repose angles were always below 35°, indicating good flow. The compressibility parameters varied in an acceptable range, the Hausner Factor was below 1.25 and Carr Index was below 12%. The yield during the Fluidized Bed Hot Melt Granulation process varied from 57 to 65%. Particle size distributions were used to predict d50, which varied from 590 to 910 µm for the conditions studied. The results of the analysis of variance (ANOVA) showed that the drug/carrier and lactose content in the granules did not affect significantly their densities, flowabilities and compressibilities. This result is interesting since it indicates that curcumin/carrier content in granules can be increased without affecting their pharmacotechnical properties. Furthermore, all solid dispersions showed adequate flow and compressibility properties, which are important to predict the behavior of the granules during die filling and how well it will be compressed, and the formulation stability.

Keywords: Hot Melt; flowability; compressibility; solid dispersion; curcumin.

Financial support: FAPESP.

TF 04 - CHARACTERIZATION OF SOLID DISPERSION PIROXICAM WITH POLYETHYLENE GLYCOL

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Introduction: One of the most effective methods to improve the solubility of poorly water-soluble drugs is their incorporation into solid dispersions. Piroxicam, PX, is a non-steroidal anti-inflammatory drug and it is a poorly water-soluble drug, classified as class 2 in the Biopharmaceutical Classification System. PX association with hydrophilic materials to improve solubility has been extensively studied and a recent alternative is the hot melt technique, where drug is melted together with soluble carriers. **Objective:** The aim of this work was to evaluate solid dispersions and physical mixtures of PX and polyethylene glycol 4000 (PEG 4000) or polyethylene glycol 6000 (PEG 6000), and to evaluate the physical and chemical interactions and drug polymorphism. Materials and Methodology: Solid dispersion (SD) and physical mixtures (PM) containing piroxicam and PEG 4000 or 6000 were prepared at the ratios of 1:1, 1:4 and 4:1. SD were prepared by the melting method. A calorimeter DSC 50 Shimadzu was used to obtain thermograms at the temperature range of 0-300°C, at a heating rate of 10°C/min and under nitrogen atmosphere with flow rate of 50ml/min. The infrared spectra were obtained in a FT IR Nicolet Protege 460 the samples were prepared in KBr discs. X-ray diffraction studies were taken in X-ray diffractometer D5005 Siemens, between 2 to 50°. **Results:** Infrared spectroscopy and X-ray diffraction analysis did not indicate interactions among PX and PEGs 4000 and 6000 in SD and PM, neither an evidence of drug polymorphism. The peaks size changed proportionally to the contents of drug and carrier in the solid dispersion, e.g., when drug ratio was higher (4:1), the curves showed a shape similar to the profile of pure drug. DSC thermograms showed a possible interaction among PX and PEGs 4000 and 6000 at the 1:1 ratio in SD and PM, because changes in melting points and fusion enthalpy occurred when compared to values for drug and carriers alone. **Conclusion:** The carriers PEGs 4000 and 6000 are useful to prepare solid dispersions containing PX, as demonstrated by the results in the analysis of the DSC, infrared spectroscopy and X-ray diffraction. No interactions occurred, neither an evidence of drug polymorphism. The possible interaction showed in the DSC 1:1 ratio was not confirmed by other analysis.

Keywords: piroxicam; solid dispersion; DSC; infrared spectroscopy; X-ray diffraction.

Financial support: CNPq; FAPESP.

TF 05 - COMPARATIVE STUDY BETWEEN ACID-BASE TITRATION AND FLAME PHOTOMETRY FOR DETERMINATION OF LI₂CO₃ WITH AS RAW MATERIAL AND PRODUCT DONE

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The lithium carbonate (Li₂CO₃) is often referred to as drug "antimaniac" but in many parts of the world is regarded as a drug "stabilizing of the metal condition" because of its action in the primary prevention of the mood swings in patients with bipolar affective disorder (manic-depressive). Commercially can be found with the name of CARBOLITUM® (300 mg) or CARBOLITUM CR® (450 mg), the latter being prolonged action. For quality control of Li₂CO₃ as raw material, as well as finished product, the United States Pharmacopeia (USP) recommended the use of acid-base titration. However, this procedure spends large amounts of reagents and is more subject to error. In this sense, it is necessary to seek simple techniques that allow smaller generation of waste and decrease the amount of material and spent determinations that are as effective as the methodologies that already exist. Under these conditions, we propose to evaluate the use of a flame photometry as alternative technique for determining the lithium carbonate in raw material, as well as finished product. For comparison performed all analyses (n = 4) using the official method recommended (USP) and then comparing the results obtained with the method proposed. For this, use the Student's t test. For a significance level of 5% and 3 degrees of freedom, the critical value (tabled) from t is 2.35. Obtained a value of t_{calculated} equal to 2.08 for raw material. For the finished product this value was 0.95. Thus, in both situations have to t_{calculated} < t_{tabled}, so that the results using both methods are statistically equal. The main advantages, the photometry of flame generates little amount of waste, is faster and simpler than the method recommended. So it appears that it is feasible the use of a flame photometry for determination of Li₂CO₃ in both types of samples.

Keywords: lithium carbonate; flame photometry.

TF 06 - DEVELOPMENT AND EVALUATION OF ZINC PHTHALOCYANINE LOADED PCL NANOPARTICLES FOR PHOTODYNAMIC THERAPY USE

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Nanocarriers as polymeric nanoparticles (Nps) have been used to overcome drugs insolubility problems and improve bioavailability and target. Zinc phthalocyanine (ZnPc) is a second generation of photosensitizer used in cancer photodynamic therapy. However, it is hydrophobic and insoluble in acceptable physiological solvents. The objective of this work is the development and characterization of poly-ε-caprolactone (PCL) nanoparticles to load ZnPc for photodynamic therapy use. Nps were prepared using nanoprecipitation method. Nanoprecipitation involves the dispersion of the polymer and the photosensitizer in acetone, which is added into an aqueous phase containing a surfactant under moderate stirring. The acetone was removed by evaporation under reduced pressure and the suspension of Nps was centrifugated and freeze-dried. Nps were prepared with different amounts of polymer, surfactant, organic solvent and aqueous phase to modify the particle size. Size distribution and morphology were analyzed by laser light scattering and transmission electron microscopy (TEM). Encapsulation efficiency was determined by a fluorescence emission method. The fluorescence emission intensity was correlated with the ZnPc concentration in acetone (µg/ml) to obtain the standard curve. The standard solutions were excited at 602 nm and the fluorescence emission spectra recorded between 630 to 730 nm using a spectrofluorimeter. ZnPc standard solution and ZnPc loaded Nps were also analyzed for fluorescence emission for spectroscopic characterization. The smallest nanoparticles were obtained from two samples prepared in triplicate containing 50 mg (A) or 100 mg PCL (B), 25 ml acetone, 0.3 mg ZnPc and 50 ml aqueous phase (1% surfactant). The Nps have a mean diameter of 259.1 ± 2.1 nm (A) and 341.2 ± 4.5 nm (B), a narrow size distribution with a polydispersive index of 0.11 ± 0.03 (A) and 0.11 ± 0.02 (B), smooth surface and spherical shape. The process yield was 33.5 ± 1.8% (A) and 62.3 ± 4.5% (B). The intensity of fluorescence emission of ZnPc correlated linearly with the concentration over the 0.02 - 0.1 µg/ml ZnPc range. The precision of the standard curve was below 5%. The correlation coefficient was 0.9999, with excellent linearity. The encapsulation efficiency was 45 ± 3.7% (A) and 57 ± 1.9% (B). ZnPc loaded Nps maintains its spectroscopic behavior after encapsulation. Nanoprecipitation is an adequate method to encapsulate ZnPc in PCL particles with nanometric size and satisfactory encapsulation efficiency.

Keywords: nanoparticle; zinc phthalocyanine; PCL; photodynamic therapy; cancer.

TF 07 - DEVELOPMENT AND VALIDATION OF A METHOD FOR DETERMINATION OF DICLOFENAC SODIUM IN PLASMA BY HPLC

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Diclofenac sodium is a non-steroidal anti-inflammatory drug (NSAID) with pronounced analgesic and antipyretic properties. It is widely used in the long-term treatment such as rheumatoid arthritis, and osteoarthritis. Nevertheless, it produces a relatively high incidence of gastrointestinal side effects such as irritation, ulceration and eventually perforation in the gastric wall. Several analytical methods have been described for the quantification of diclofenac sodium in plasma based on different extraction procedure. In this study, the development and validation of a high-performance liquid chromatography (HPLC) method for determination of diclofenac sodium concentration in plasma for pharmacokinetic studies is described. The procedure used for sample preparation was based on the liquid-liquid extraction by using a mixture of hexane:diethyl ether (1:1, v/v) as extracting solvent and the analysis of diclofenac sodium was carried out on a C-18 analytical column (125 mm \times 4.8mm i.d., 5 μ m particle size, LinchroCART®) using a mobile phase consisting of acetic acid (pH 2.5, 0.7 mol/l):acetonitrile (1:1, v/v), at a flow-rate of 1 ml/min. The method showed a recovery of 100.3% and the coefficients of variation in the precision and accuracy studies were below 12%. The proposed method presented quantitation limit of the 0.1 μ g/ml and was linear up to a concentration of the 60 μ g/ml and it is enough for application to pharmacokinetic studies.

Keywords: Diclofenac sodium; HPLC; Validation.

TF 08 - EFFECT OF CATIONIC POLIMER FORMULATION ON THE IONTOPHORETIC DELIVERY OF DOXORUBICIN

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Introduction: Doxorubicin (DOX) and its bioactive derivatives are among the most widely used anticancer drugs in chemotherapy treatment. Due to the various side effects, topical chemotherapy could be an interesting alternative to treat skin cancer with reduced toxicity. Nevertheless, the superficial lipophilic layer of the skin, the stratum corneum, constitutes a major barrier to the cutaneous delivery of hydrophilic and charged molecules such as DOX. The application of iontophoresis, to drive molecules into the skin, can improve DOX skin penetration. **Objectives:** The aim of this work was to investigate DOX percutaneous absorption and retention in the skin with the application of iontophoresis. The convective flow contribution to the overall electrotransport of DOX was also elucidated for non-ionic gel of hydroxyethylcellulose (HEC) and cationic gel of chitosan. **Materials and Methods:** DOX 0.5% was dispersed in water, HEC and Chitosan gel. All formulations contained 119 mM of NaCl and pH 5.5. Experiments were performed *in vitro* using vertical, flow-through diffusion cells, dermatomed porcine skin and Ag/AgCl electrodes. DOX transport from anode compartment was followed over a period of 6h at a constant current of 0.5mA/cm². In addition, so that electroosmotic and electrorepulsive contributions of DOX delivery could be distinguished, the DOX formulations also contained the electroosmotic marker acetaminophen, at the same molar concentration as DOX. **Results:** It was observed that iontophoresis of DOX increased significantly the skin permeation and skin retention of the drug. The electroosmotic flow was dramatically reduced when DOX was added to the non-ionic gel indicating that the drug interacts with negative charges of the skin. Interestingly, electroosmosis was also significantly reduced when the iontophoresis of the chitosan gel, in the absence of DOX, was performed. Consequently, electroosmotic marker transport from this gel almost disappeared when the positively charged drug was added to the cationic gel. Chitosan seems to interact with negative charges of the skin, hence, reducing electroosmotic flow but also releasing DOX from interactions with these sites and thus improving its diffusion to deeper skin layers. **Conclusions:** Iontophoresis increased significantly not only the permeation, but also the skin retention of DOX. Despite the fact that iontophoresis of chitosan gels decreased significantly the electroosmotic flow, it improved DOX diffusion through deeper layers of the skin, probably by competing with the drug for stratum corneum negative charge sites.

Keywords: Doxorubicin; Iontophoresis; electroosmotic flow.

TF 09 - EFFICACY OF DERMOCOSMETIC FORMULATIONS CONTAINING DIMETHYLAMINOETHANOL (DMAE) AND ACETYL HEXAPEPTIDE-3

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Amongst the many active substances launched in the cosmetic market to treat and prevent skin ageing, dimetilaminoetanol (DMAE) and acetyl hexapeptide-3 should be emphasized, since these substances have been widely used in cosmetic formulations for that purpose. However, there are few studies in scientific literature that have conclusive data about the effectiveness and mechanism of action of these substances in the skin ageing. So, the aim of the present study was the clinical efficacy evaluation of dermocosmetic formulations containing DMAE or acetyl hexapeptide-3 by using Skin Biophysical Techniques. For this purpose, four formulations containing batyl alcohol and lecithin or C12-20 acid PEG-8 ester stabilized by different polymers (hydroxyethylcellulose or acrylate copolymer) were prepared. They were supplemented or not (vehicle) with 10% of a solution containing 0.05% acetyl hexapeptide-3 or 3% DMAE acetamidobenzoate and submitted to stability tests and sensorial analysis. For clinical efficacy studies, formulations were applied to the volar forearm and face of 40 female volunteers. Skin conditions in terms of skin moisture and skin mechanical properties (skin visco-elasticity and anisotropy) were evaluated by using Skin Biophysical Techniques (Corneometer® CM 825, Cutometer® SEM 575 and Reviscometer® RV600, respectively) before and after a 14 and 28-day period of daily application. In the stability tests and sensorial analysis, it could be observed that the formulation containing C12-20 acid PEG-8 ester and acrylate copolymer was the most stable and showed the best sensorial attributes, and consequently was chosen as the vehicle for efficacy studies. The skin moisture evaluation showed that all formulations, containing or not the active substances under study provoked an enhancement in face skin water content of the stratum corneum. On the other hand, the formulations containing acetyl hexapeptide-3 altered skin anisotropy because they reduced the coefficient of variation (CV) of multidirectional RRTM (resonance running time measurements taken in 4 different directions 0°, 45°, 90° and 135°), which is a parameter inversely proportional to the speed of propagation of the emitted ultrasound wave at the skin surface and can suggest an increase of the skin firmness. The formulations under study did not provoke any significant alteration in skin visco-elasticity. Under the present experimental conditions it was possible to conclude that acetyl hexapeptide-3 can be used in cosmetic formulations in order to enhance anti-ageing effects of cosmetic formulations, since this substance reduced CV values of multidirectional RRTM, improving skin anisotropy.

Keywords: dimetilaminoetanol; acetyl hexapeptide-3; skin; Skin Biophysical Techniques.

TF 10- EVALUATION OF DELIVERY SYSTEMS: CYCLODEXTRINS AND LIPOSOMES IN ANTISOLAR PREPARATIONS

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The harmful effects of sunlight ultraviolet radiation, have led to the widespread use of topical sunscreens. However, recent *in vitro* and animal studies have reported estrogen-like activity for some sunscreens, showing systemic absorption for these molecules. Thus, to reduce the toxic effects of sunscreens, it has been used the inclusion of sunscreen in different release systems like *cyclodextrins* (CD) and *liposomes* (Lipo). The main purpose of this work consists of including the sunscreen agent *octylmethoxycinnamate* (OMC) in release systems CD and Lipo; the development of *Structure XL* @gel cream formulations containing the release systems; and the assessment of the effectiveness of four formulations developed (formulation with 8% w/w of free OMC, formulation with β -CD/OMC complex, formulation with Lipo/OMC complex and formulation with 50% of β -CD/OMC + 50% of Lipo/OMC). The method used to include OMC in the β -cyclodextrin cavity was the Kneading method. The sunscreen liposomal preparation was obtained by swelling the dry phospholipids/OMC film. *In vitro* SPF was measured using the methodology of Mansour (1986). Where, absorbance values of each preparation were determined from 290 to 320 nm, using a Shimadzu UV-1601 UV-visible spectrophotometer. *In vivo* SPF and water resistance were carried out using the methodology of COLIPA (2006). The test products were applied in the amount of 2 mg/cm² in the back of the volunteer's. UV Simulator Model 601 was used as the source of radiation. Each sample was tested in 10 human volunteers (age 18-42, gender: female) of skin phototypes I, II and III. The water resistance has been determined by evaluating the subject after two 20-min immersions with moderate activity in water. *In vitro* SPF values: formulation with free OMC = 14.65 \pm 0.09, with β -CD/OMC complex = 14.80 \pm 0.510, with Lipo/OMC complex = 15.05 \pm 0.53 and with both complexes = 14.67 \pm 0.26. There is no significant statistical difference between *in vitro* SPF values for all formulations developed (P = 0,600). *In vivo* SPF values: formulation with free OMC = 8.4 \pm 1.7, with β -CD/OMC complex = 8.5 \pm 1.5, with Lipo/OMC complex = 11.0 \pm 1.3 and with both complexes = 11.6 \pm 1.6. After immersions *in vivo* SPF values: formulation with free OMC = 7.3 \pm 1.6, with β -CD/OMC complex = 6.5 \pm 0.9, with Lipo/OMC complex = 10.3 \pm 2.2 and with both complexes = 9.5 \pm 1.4. There is no significant statistic difference among the formulation with both complexes (β -CD/OMC + Lipo/OMC) and with Lipo/OMC complex and between formulation with free OMC and with β -CD/OMC complex (P>0.05). The formulation containing the complex Lipo/OMC had higher SPF *in vivo* and resistance to water, due to the ability of liposome to interact and penetrate in the stratum corneum.

Keywords: octylmethoxycinnamate; *cyclodextrins*; liposomes; SPF; water resistance.

Financial support: Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES); Farmácia Universitária/UFRJ; LabCQ/UFRJ.

TF 11 - EVALUATION OF LIQUID CRYSTAL FORMULATION FOR PHOTODYNAMIC THERAPY: STABILITY AND TESTS

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Photodynamic Therapy (PDT) is a medical treatment which employs the combination of light and a drug (photosensitizer) to bring about a cytotoxic or modifying effect to cancerous or otherwise unwanted tissue. It is a great promise thanks to its dual-selective mode of action. Firstly, a photosensitizer of negligible dark toxicity is introduced into the body and accumulates preferentially in rapidly dividing cells. When the drug reaches an appropriate ratio of accumulation in diseased *versus* healthy tissue, a carefully regulated light dose is shone onto the diseased tissue. Light activates the drug and elicits the toxic action. The amount of light needs to be large enough to cause the desired response in the tissue, but small enough to spare the surrounding (and incidentally illuminated) healthy tissue from extensive damage. Shortly after treatment, the damaged cells become necrotic, or suitably modified. The aim of this work was the pharmaceutical development of liquid crystal formulations containing monoolein, oleic acid and water, seeking the increase of the cutaneous penetration of the chlorin in the skin, for skin cancer PDT. Studies of formulation for application in PDT with desirable characteristics of permeation and retention on skin were carried out through ternary diagrams monoolein/oleic acid/water, on different temperatures and times. One formulation was then chosen and turbidimetry of the sample was monitored through 72 hours. Permeation and retention *in vitro* tests were also conducted for the formulation associated with a chlorin, using fluorescence intensity to quantify the chlorin. It was observed through ternary diagrams that most of the formulations tested showed up to be stable over time tracked (2 weeks). Was then chosen a formulation of hexagonal phase, which also showed good stability through turbidimetry during monitored time (72 hours). Permeation and retention *in vitro* tests showed that the drug remained trapped in the stratum corneum (SC) and [epidermis + dermis] and was not detected in the permeation solution. The formulation tested presented good characteristics for application on photodynamic therapy, using the chlorin as photosensitizer.

Keywords: Photodynamic Therapy; liquid crystal; ternary diagram and photosensitizer.

Financial support: CNPq; FAPESP.

TF 12 - *in vivo* PHOTOCHEMOPROTECTIVE EFFECT OF QUERCETIN INCORPORATED IN W/O MICROEMULSION BY INHIBITION OF METALLOPROTEINASES ACTIVITY INDUCED BY UV IRRADIATION

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Introduction: It has been demonstrated that ultraviolet exposure leads to sustained elevation of matrix metalloproteinases (MMPs), a family of proteolytic enzymes which contribute to skin photoaging and in skin carcinoma to the spreading of metastatic cells by specifically degrading skin collagen and elastin. Molecular mechanisms underlying the induction of MMPs after exposure to UV irradiation have been proposed. It has been suggested that UV irradiation activates growth factor receptors, which induce the activation of protein kinase cascades, like the MAPK cascade. The activation would be followed by an increase in the expression of the c-Jun and c-Fos and formation the AP-1 complex, a MMPs transcription regulator. Topical treatment with antioxidants may interrupt the activation of MAP kinase pathways, and thus inhibit UV-induced MMP expression in human skin. **Objectives:** Therefore, firstly the present work investigated the *in vivo* effectiveness of w/o microemulsion containing quercetin (ME+Q), a well-known antioxidant, in inhibiting the proteinase secretion/activity increase induced by UVB irradiation in hairless mice skin. Then, finally the quercetin photoprotective mechanism of action against UV induction of MMPs was evaluated. **Materials and Methods:** To evaluate the *in vivo* topical effectiveness, ME+Q was applied on the dorsal skin of hairless mice 1 h before, immediately before and after exposure to UVB irradiation (2.87 J/cm²). Then its effectiveness was evaluated by the measurement of its capability to decrease the cutaneous proteinase secretion/activity induced by UVB irradiation using SDS-PAGE zymography. The investigation of quercetin photoprotective mechanism of action against the secretion/activity of UV-induced MMPs was done using primary human keratinocytes as an *in vitro* model and by evaluation of quercetin effect against UV-induced (100 mJ/cm²) activation of MAP kinases (ERK, JNK, and p38), using Western blot analysis, and against UV (50 mJ/cm²) induction of c-Fos, c-Jun, MMPs-1, 3 and 9 mRNA levels, using real-time RT-PCR analysis. **Results:** SDS-PAGE zymography detected inhibition of proteinase secretion/activity increase induced by UVB irradiation when ME+Q was applied. Furthermore, supporting these results, the control dosage of total proteins in the skin detected no significant statistical difference among the samples. On the other hand, the pretreatment of primary human keratinocytes with methanolic solution of quercetin (20 μ g/mL) had no blocking effect on UV activation of all three MAP kinases evaluated and

consequently c-Jun and c-Fos mRNA upregulation was not prevented. Furthermore, this flavonoid did not inhibit the increase in mRNA levels of MMPs-1, 3 and 9 induced by UV irradiation. **Conclusion:** Based on the current observations, it seems that *in vivo* photoprotective effects of quercetin incorporated in w/o ME are mediated mainly by inhibition of MMPs activity once no effect of this flavonoid was observed against the UV-induced AP-1 activation and consequently increase in MMPs expression.

Keywords: Quercetin; W/O microemulsion; UV irradiation; Metalloproteinases.

Financial support: CAPES; FAPESP; NIH.

TF 13 - INFLUENCE OF EMULSIFICATION TEMPERATURE IN THE FORMATION OF NANO-EMULSIONS CONTAINING ANDIROBA SEED OIL

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Purpose: To determine the influence of emulsification temperature in the formation of nano-emulsions by low energy method in the system containing Andiroba seed oil (*Carapa guianensis*), PEG-40 castor oil and sorbitan monooleate. **Methods:** Emulsions were yielded by low energy emulsification method. Blends of surfactants PEG-40 castor oil 3.85% (w/w) and sorbitan monooleate 1.50% (w/w) were dissolved in the oil phase (andiroba seed oil 15% w/w). Oil and aqueous phases were separately heated to the temperatures: 25, 35, 45, 55, 65, 75, and 85°C. Water phase (80.0% w/w) was slowly added into oil phase. The mixer speed was set at 600 rpm by 20 minutes. After 24 hours emulsions were submitted to macro and microscopic analyses and droplet size were measured by photon correlation spectroscopy using Coulter Delsa 440 SX. Those analyses were repeated after 15 days. **Results:** After 24 hours the emulsions prepared at 25°C showed creaming and light phase separation, and mean droplet size of 1800±155 nm. Emulsions prepared at 35 and 45°C showed milky aspect, multiple globules, mean droplet size of 918±84 nm and 1990±255 nm respectively, while those prepared at 55 to 75°C, presented macroscopic characteristics of nano-emulsions such as bluish reflection and low viscosity. In microscopic analyze these emulsions showed small and uniform droplets with mean around of 264±32, 262±29, and 272±30 nm respectively. Emulsion prepared at 85°C presented the same characteristics, but demonstrated instability (flocculation) in microscopic analyses, the mean droplet size was 237±45 nm. After 15 days at room temperature (25°C) there was a not significant change in the droplet sizes, but macroscopically it was observed creaming in emulsions prepared at 35 and 45°C, and phase separation in that prepared at 25°C. **Conclusions:** Due to obtained results in can be concluded that temperature of emulsification plays an important role in the shape and size of droplets, considering that there is an optimum temperature to the formation of nano-emulsions in the studied system.

Keywords: nano-emulsion; andiroba seed oil; *Carapa guianensis*; low energy method.

TF 14 - LEUKOTRIENE B₄-LOADED MICROSPHERES: A NEW THERAPEUTIC STRATEGY TO MODULATE CELL ACTIVATION

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Introduction: Leukotriene B₄ (LTB₄) is a potent inflammatory mediator that also stimulates the immune response. In addition, it promotes polymorphonuclear leukocyte phagocytosis, chemotaxis, chemokinesis and modulates cytokines/chemokines release. Regarding chemical instability of the leukotriene molecule, in the present study we assessed the immunomodulatory activities conferred by LTB₄ released from biodegradable poly-lactic coglycolic acid (PLGA) microspheres (MS). A previous oil-in-water emulsion solvent extraction-evaporation method was chosen to prepare LTB₄-loaded MS. **Objective:** To assess the activity of microencapsulated LTB₄ during *in vitro* and *in vivo* assays. **Materials and Methods:** Previously characterized LTB₄-MS were employed to assess the inflammatory effects of microencapsulated LTB₄ within the mice cremasteric microcirculation by the use of intravital microscopy (IM). To extend these events to human, human umbilical vein and artery endothelial cells (HUV_{EC}s and HUA_{EC}s, respectively) were stimulated with LTB₄-MS or LTB₄ in solution. Nitrites and MCP-1 production were measured by Griess reaction and ELISA, respectively. Peroxisome proliferator-activated receptor- α (PPAR- α) expression was detected by Western Blot. **Results:** IM within the mice cremasteric microcirculation showed that after 4 h intrasacrotal injection of 0.1 ml of LTB₄-MS, significant increases in leukocyte rolling flux (72.0 ± 5.2 vs. 46.0 ± 1.7 cells/min), adhesion (4.0 ± 1.0 vs. 1.2 ± 0.5 cells per 100 mm vessel) and emigration (7.3 ± 0.6 vs. 1.3 ± 1.0 cells per field) followed by significant decreases in the leukocyte rolling velocity (14.1 ± 0.9 vs. 25.9 ± 3.3 mm/s) were detected vs. values obtained in the control group (saline). HUV_{EC}s stimulation with LTB₄-MS for 4h provoked a significant increase in nitrites and MCP-1 levels when compared with those obtained with medium or unloaded MS. Similar results were obtained after 4 and 24h HUA_{EC}s stimulation. LTB₄-MS also activated PPAR- α receptor in a different manner when compared to unloaded MS or LTB₄ in solution, especially when a specific antagonist of the membrane receptor was used. **Conclusion:** LTB₄-loaded MS preserve the biological activity of the encapsulated mediator indicating their use as a new strategy to modulate cell activation, especially in the innate immune response.

Keywords: Leukotriene B₄; Biodegradable microspheres; Inflammatory mediators; Cell activation.

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TF 15 - MAGNETIC TABLET DISINTEGRATION: COMPARISON OF BIOSUSCEPTOMETRY, WATER UPTAKE AND DISINTEGRATION FORCE

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Introduction: Despite increasing interest on modified release systems, conventional tablets are still the most popular solid dosage forms due to ease of manufacture, convenience of dosing and stability. Disintegration of compressed tablets is an important parameter and is influenced by the properties of the excipients, such as particle size distributions and compression force. In experimental determination of tablet disintegration an official *in vitro* apparatus is used, however it does not describe satisfactorily the disintegration properties. Alternate Current Biosusceptometry (ACB) has become an alternative method for pharmaceutical research and has demonstrated the capability to be used as a tool in quality control for pharmaceutical products. **Objectives:** The aim of this study was to quantify the tablet disintegration process by Biosusceptometry AC associated with an apparatus to measure water uptake (WU) and disintegration force (DF). **Materials and Methods:** ACB bases its functioning on induction coils for recording the magnetic flux variation obtained from the response of a magnetic material when an alternating magnetic field is applied; the data is processed to generate images which are segmented to quantify the disintegration. The apparatus for WU and DF measurements consists in a precision balance coupled to a force transducer which registers the amount of water that leaves a recipient placed on the balance and the DF produced due the water penetration. Tablets (0.5g ferrite and 0.375g excipients) were produced by direct compression (10, 20, 30, 40 and 50 kN) on a single punch tablet machine. To

investigate the relationship among the data, the graphic profiles from ACB images, DF and WU were analyzed using a modified Weibull distribution to evaluate the curve shape (?) and the time needed to reach 63.2% of the maximal value ($t_{63.2\%}$). **Results and Discussion:** Compression force plays an important role for tablet manufacturing process and it is a well-known parameter that influences the disintegration time. The preliminary results showed that the disintegration time is highly influenced by the compression force, which results on a time delay on the evaluated parameters as the compression force arises. **Conclusion:** All the data presented a high level of correlation among the three techniques, i.e., the evaluation of the disintegration process using ACB has an advantage of being radiation-free and low cost. Besides, this preliminary study demonstrated that ACB has enough sensibility to perform quality control of solid dosage forms. This methodology might be able to assess *in vivo* studies for pharmaceutical parameters.

Keywords: AC Biosusceptometry; compression force; tablet disintegration.

Financial support: CAPES; FAPESP; Blanver Farmoquímica Ltda.

TF 16 - METHOD VALIDATION FOR THE DETERMINATION OF RACTOPAMINE CHLORIDE IN RAW MATERIAL BY HPLC/UV

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The quantity of drugs used in animal production has grown exponentially, mainly due to new forms of intensive livestock. In special, the use of β -adrenergic agonists as growth promoters have grown considerably in recent years. Among the β -adrenergic agonists, ractopamine (RAC) is the most used as a nutrient repartitioning agent by diverting nutrients from fat deposition in animals to the production of muscle tissues. It promotes reduction of fat, increased muscle mass, and improved feed utilization efficiency in swine, cattle, and turkeys. The goal of this research was to optimize and validate a method for determination of the RAC chloride in raw material by HPLC/UV for using in quality control in veterinary industries. The best optimized HPLC conditions obtained were: Gemini C18 column Phenomenex® (250 mm x 4.6 mm I.D., 5.0 mm particle size) at room temperature under isocratic conditions, acetonitrile/sodium acetate buffer (25:75, v/v) as mobile phase at a flow rate of 1.0 mL min⁻¹, UV detector at 275nm. Before starting the study for method validation, it is necessary to realize System Suitability through a series of tests to ensure that equipment used is able to generate results of precision and accuracy acceptable. The method shows theoretical plates (M) > 6290, retention time (T_r = 5.2 min.) with CV = 0.03%, area with CV = 0.18% and tailing factor (T_f) 10% < 1.16. After verifying that the system was in compliance, the method validation was performed. Some validations parameters obtained were: linearity 160-240 $\mu\text{g mL}^{-1}$ ($r \geq 0.99$), limit of detection of 2.2 $\mu\text{g mL}^{-1}$, limit of quantification of 7.5 $\mu\text{g mL}^{-1}$, coefficients of variation and relative errors in precision and accuracy studies (within-day and between-day) were below 2%. In addition, the stability study showed that the sample were stable (CV < 0.2%), the robustness (flow rate, column and temperature) presented CVs < 2.0%. A simple and rapid high-performance liquid chromatography method using UV detection was developed and validated for the determination of RAC in raw material.

Keywords: ractopamine chloride; HPLC.

Financial support: Ouro Fino Saúde Animal; FAPESP; CNPq and CAPES.

TF 17 - NITRIC OXIDE RELEASE BY VISIBLE LIGHT IRRADIATION OF AN AQUEOUS SOLUTION OF A NITROSYL RUTHENIUM COMPLEX IN THE PRESENCE OF QUANTUM DOTS

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Introduction: Nitric oxide (NO) is a radical molecule which presents fundamental roles in biochemical processes, including cardiovascular control, neuronal signaling and as an agent for defense mechanisms against microorganisms and tumors. Therefore efforts have been employed in the development of metallic complexes, such as $[\text{Ru}(\text{terpy})(\text{Bdq})\text{NO}](\text{PF}_6)_3$, which are able to release NO after light irradiation. Quantum Dots (QDs) have recently garnered the spotlight as imaging agents and diagnostics. But, knowing that QDs are nanocrystals which present energy-transfer properties, papers have reported their potential therapeutic effect mainly as cytotoxic agents mediated by UV irradiation. Therefore, as energy donors, QDs could even improve the nitrosyl ruthenium NO release after light stimuli. **Objectives:** The objective of this work was to assess the release of NO by visible light irradiation of an $[\text{Ru}(\text{terpy})(\text{Bdq})\text{NO}](\text{PF}_6)_3$ aqueous solution in the presence of QDs. **Materials and Methods:** 20 mL of $[\text{Ru}(\text{terpy})(\text{Bdq})\text{NO}](\text{PF}_6)_3$ aqueous solutions at 40 $\mu\text{g/mL}$ containing or not QD (CdS-cadmium sulfide) at the same concentration were irradiated in a glass flask using mercury lamp for 180 minutes. NO release was assessed by a NO sensor electrode (Nometer) immersed in the solutions. In addition, samples were withdrawn and submitted to HPLC analysis in order to determine $[\text{Ru}(\text{terpy})(\text{Bdq})\text{NO}](\text{PF}_6)_3$ content. **Results:** It was observed that the light irradiation did lead to NO release from the Nitrosyl Ruthenium complex alone or associated with QDs during the assessment by Nometer. In the HPLC analysis, in both experiments it was observed a gradual decrease in the $[\text{Ru}(\text{terpy})(\text{Bdq})\text{NO}](\text{PF}_6)_3$ content, inferring the NO release, since the NO release from this ruthenium complex leads to the formation of another complex. In the absence of QDs, it was observed that after 60 and 180 min of light irradiation, the Nitrosyl Ruthenium complex released around 6,413 mmols and 43,857 mmol of NO, respectively. In contrast, when QDs were present, the Nitrosyl Ruthenium complex had already released around 28,217 mmol already in the first 60 min of irradiation and 53,332 mmol of NO after the 180 min. Therefore, the QDs presence, induced a burst of NO release from the Nitrosyl Ruthenium complex of around 5-fold after 60 min of light irradiation. However, after 180 min the increase in NO release was around 1.5-fold. **Conclusion:** The presence of QDs in an aqueous solution of $[\text{Ru}(\text{terpy})(\text{Bdq})\text{NO}](\text{PF}_6)_3$ did improved the NO release after light irradiation, inferring that this system may be suitably employed in pharmacological therapies, such as in the Photodynamic Therapy (PDT).

Keywords: Nitric Oxide; Nitrosyl Ruthenium complex; Quantum Dots.

Financial support: CNPq

TF 18 - OBTENTION OF PROPOLIS EXTRACT AND *in vitro* EVALUATION OF ITS ANTIOXIDANT EFFECT IN ASSOCIATION OR NOT WITH VITAMIN E ACETATE

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The skin, after intense exposure to the sun, shows immediate signs, as well as chronic and permanent damage, from aging to the incident of cancer. To protect the skin of early aging there are several classes of active substances and products to be used during or after exposure, as the sunscreens, and antioxidant substances. Propolis is a resin obtained mainly from the bee *Apis mellifera*, and its chemical composition varies due to biodiversity, where more than 300 constituents have been identified, such as phenols and flavonoids, which have antioxidant potential, among others, and that make the propolis an important object of study for many different pharmaceutical and cosmetic applications, including antiaging purpose. A substance proposed for association with propolis is the vitamin E acetate, once it is known for its antioxidant potential against the free radicals. The

objectives of the present study were to obtain the extract of propolis and to evaluate the antioxidant effect of propolis in association or not with the vitamin E acetate. Green propolis obtained from the south of Minas Gerais (Brazil) was macerated in absolute ethanol. The extract obtained was hydrolysed and analysed by thin-layer chromatography (mobile phase: methanol: distilled water: formic acid (40:57:3); spray solution: ethanolic solution of 5% aluminum chloride and 5% ferric chloride; pattern: quercetin). The antioxidant capacity was evaluated by the method of ferric thiocyanate of four different solutions in ethanol: butylhydroxytoluene 0.01%; extract of propolis 5%; vitamin E acetate 2%; mixture of propolis extract 5% and vitamin E acetate 2%. The presence of quercetin in the propolis extract was confirmed. All samples showed antioxidant potential. The propolis extract provided an inhibition higher when compared with the control, the vitamin E acetate higher and the mixture of propolis extract with vitamin E acetate provided an inhibition of oxidation higher compared with the control, in other words, both propolis and acetate tocoferila have antioxidant effect and these substances in association were more effective. Under the present experimental conditions, we observed that the obtained extract showed quercetin in its composition. Both propolis extract and vitamin E acetate presented antioxidant effect, and the association had the best antioxidant potential. Thus, this result can be regarded as very favourable in terms of prevention of premature aging of the skin when these compounds are added in a topical formulation, because it indicates the possibility of protecting the skin from the reactive oxygen species and other factors, such as environmental pollution.

Keywords: propolis; vitamin E acetate; antioxidant.

TF 19 - OPPORTUNITY RECOGNITION IN THE VETERINARY PHARMACEUTICAL INDUSTRY

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Brazil is one of the five largest veterinarian markets throughout the world and a major exporter of beef and chicken. The country concentrates the second largest population of pets in the world. It is increasingly expanding the market for animal health because the growing population of pets as "family members", health and careful monitoring particularly for vaccination and reducing the use of antibiotics to promote growth. So a growing demand for veterinary pharmaceuticals has driven the development of new products and technologies in the industry, making the ability to innovate a fundamental requirement for the competitive edge of enterprises. The need for introduction of new drugs and / or drugs to a multitude of diseases / emerging zoonoses, control of pests and diseases as cancer, mainly in the pet segment, coupled with the need for innovation to support businesses in the market highlight the importance of identifying opportunities. As a result, the goal of work is to identify the factors that influence the link technology-market in the veterinary pharmaceutical industry, identifying the market conditions governing the effect of these factors and analysing the external environment of the industry. Data collection through Research Desk, i.e., bibliographic searches in magazines, publications and technical expertise, dissertations and theses, information from national government, laws, unions, professional associations, etc. Also theoretical concepts were explored in academic books and scientific papers that formed the theoretical basis for the grounds and construction of the project, and for setting goals. **Results:** The trends in technological innovations in products and processes are similar to those of human therapeutic as verified by newer drugs launched as: the Reconcile (floxetine hydrochloride) launched by Eli Lilly for dogs suffering from separation anxiety, and Sientrol (dirlotapide), of Pfizer Inc, to reduce canine obesity. The regulatory framework in the country, set up by the Ministry of Agriculture and Livestock (MAPA), is in the process of improvement verified by the constant meetings between representatives of the National Association of Pharmaceutical Laboratories (ALANAC) and MAPA. These changes in legislation may be sources of opportunities. Also there was a trend in the use of phytomedicines in the treatment of cattle in the management of the so-called organic beef. The objective was achieved with the development of a range of pharmaceutical and veterinary source identification of opportunities for new products and business. The main sources are: converging technologies, changes in the industry; customers; demographic changes; technological change; legislative and regulatory environment; competitors; economic environment; patents, scientific articles; fairs, exhibitions and conferences.

Keywords: Opportunities recognition; veterinary pharmaceutical industry; product innovation; entrepreneurship.

Financial support: CNPq.

TF 20 - PHARMACEUTICAL TECHNOLOGY IN THE *Schistosoma*

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Introduction: The number of carriers of *Schistosoma mansoni* infection in Brazil was estimated based on the results of parasitological examinations of feces carried out by the FNS (National Health Foundation) in 2006, as well as population data from 18 states collected by the IBGE - Brazilian Institute of Geography and Statistics. This information allowed the number of carriers of schistosomiasis mansoni to be estimated at 7.1 million in 2006 and 6.3 million in 2007. These figures may not reflect the true situation since the population sample used was not originally selected for this purpose. The absence of precise data indicates the need for an adequate national survey of the prevalence of schistosomiasis, which continues to be an important endemic parasitic disease. Praziquantel (class II drug) has been shown to be highly effective against all known species of *Schistosoma* infecting humans. The pharmaceutical technology, e.g. formulation of solid dispersions is a promising approach to enhance the dissolution rate and solubility of drugs belonging to class II of the Biopharmaceutical Classification System. Often the amorphous state of the drug is preferred in solid dispersions, since it shows improved solubility and dissolution rate in comparison to the crystalline material. A relatively recent approach for obtaining pharmaceutical materials in pure physical forms is represented by technologies based on supercritical fluids (SCF). By proper adjustment of the operating conditions as well as physical and chemical parameters (pressure, temperature, drug concentration, flow and nature of supercritical fluid and organic solvent) materials of a desired crystal form can be generated. Advantages for the use of SCF technology are its high versatility, the flexibility in offering alternative processing approaches, the possibility to avoid or minimize the use of organic solvents, and the ability to reach peculiar processing conditions (i.e., changes in pressure and in the rate of solvent evaporation) which would otherwise be difficult to obtain with traditional processes. Furthermore, the original concept of SCF as a "green" alternative has become very important as the regulatory requirements for the use and residual contents of volatile organic compounds in the drug product become more and more restrictive. The aim of the present study was, therefore, to increase the solubility of solid dispersions of praziquantel in pharmaceutical carriers using SCF pharmaceutical technology. **Materials and Methods:** To evaluate the SCF pharmaceutical technology were carried solid dispersion using SCF pharmaceutical technology of the drug and pharmaceutical carriers. **Results:** The solid dispersions of praziquantel in pharmaceutical carriers using SCF pharmaceutical technology were useful for the solubility of the drug. **Conclusion:** The SCF pharmaceutical technology solid dispersion employing supercritical fluid is important for increase the solubility of insoluble drugs.

Keywords: Pharmaceutical Technology; *Schistosoma*.

TF 21 - POTENCIAL OF POLYAMIDOAMINE DENDRIMER AS A DRUG CARRIER FOR PROTOPORPHYRIN IX: SOLUBILITY STUDIES

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Introduction: Polyamidoamine dendrimers (PAMAM) are hyperbranched, ordered, monodispersed polymers. Above the fourth generation, dendrimers adopt a globular structure with large hollow cavities inside. These molecular boxes may be utilized as drug delivery vehicles because of their ability to form

complexes via molecular encapsulation, covalent and non-covalent interactions. PAMAM dendrimer have high surface charge density that make possible that each macromolecule attach numerous drug molecule. Furthermore, their hydrophobic interior can bind hydrophobic drugs, increasing in this way their water solubility. Protoporphyrin IX (PpIX) is an efficient photosensitizer for Photodynamic Therapy (PDT). However, it is highly hydrophobic and forms aggregates in water environment. Therefore, PpIX has a limited skin penetration for the treatment of topical skin tumours by PDT. The present study evaluates the efficacy of dendrimer PAMAM G4.5 to form complexes with PpIX and increase its solubility for further skin penetration studies. **Materials and Methods:** Solubility studies were carried out using the Higuchi rotating bottle method. Solubility of PpIX in the presence of 0,2% of PAMAM G4.5 was first studied in function of time and pH (7 and 4). Then the solubility profile of PpIX as function of PAMAM G4.5 concentration was obtained adding an excess of PpIX to HEPES buffer solutions at pH 7 in vials containing increasing amounts of the dendrimer. The vials were rotated at 37° C for 4 days. After equilibration, the samples were filtered and analyzed by HPLC. **Results:** Solubility studies of PpIX in the presence of PAMAM G 4.5 0,2% in function of time showed that the complex reaction attain its equilibrium in 4 days. After this time, PAMAM G4.5 increased around 32 times the aqueous solubility of PpIX at pH 7. At this pH, PAMAM G4.5 has its carboxylic groups at the surface negatively ionized but the tertiary amines inside its cavity are positively charged. Therefore, its probably that the negative PpIX interact electrostatically with the positive groups inside the dendrimer. At pH 4 a slight, but not statistically significant, increase in the PpIX solubility was observed. This may occurs because the internal and central amine groups are protonated, favouring electrostatic interactions between PpIX and the amine groups. The solubility profile of PpIX as a function of increasing concentration of the G-4.5 PAMAM dendrimer in aqueous vehicle at pH 7.0. The phase-solubility profile showed a trend similar to the relatively infrequent Higuchi's AN-type curve (negative deviation from the linearity). The solubility of PpIX increased linearly at lower concentrations (up to 0.05% w/v) and above this concentration the increase was no longer linear, presumably due to self-association of dendrimers at higher concentrations. **Conclusion:** In conclusion, PAMAM G4.5 increased significantly the solubility of the high lipophilic PpIX in buffer solution at pH 7 and 4.

Keywords: Protoporphyrin IX; PAMAM; polyamidoamine dendrimer; solubility studies.

Financial support: FAPESP.

TF 22 - PREFORMULATION STUDIES FOR TOPICAL ADMINISTRATION OF A NITROSYL RUTHENIUM COMPLEX BY IONTOPHORESIS

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Introduction: The use of compounds capable of releasing NO, as the nitrosyl ruthenium complex $[Ru(terpy)(Bdq)NO](PF_6)_3$ (Bdq = 1,2 benzoquinonedimine; terpy = terpyridine), has recently become a very active area of research. This ruthenium complex is able to release NO under light or electrochemical stimulation, and its topical application is an alternative for skin cancer treatment. However, the physical chemical characteristics of this compound, as high molecular weight (947g.mol⁻¹) and positive charge, limits its skin passive diffusion, but the iontophoresis technique may improve its skin penetration. Therefore, the purpose of this work was to verify the drug stability in the presence of a weak electrical current as used in iontophoresis experiments and to evaluate NO delivery from a solution (pH 7.4) and from a hydrophilic gel containing this complex. **Materials and Methods:** The stability of $[Ru(terpy)(Bdq)NO](PF_6)_3$ at 200µg.mL⁻¹ in aqueous solution at different pHs (7.4, 5.0 and 4.0) was evaluated in the presence of an electrical current of 0.4 mA and Ag/AgCl electrodes. The drug was put in contact with the positive electrode and its remaining concentration was analyzed after 6 h of the current passage. NO release from nitrosyl ruthenium complex formulations (solution and hydrophilic gel at 40µg.mL⁻¹) under laser irradiation at 355nm and at 532nm was also evaluated by UV-Vis spectrum variation analysis and by an amperometric technique that detects directly the NO released. **Results:** Stability studies showed that $[Ru(terpy)(Bdq)NO](PF_6)_3$ solutions at pH 7.4 are electrically stable for at least 6h. Nevertheless, a decrease of ~10% in the drug remained concentration was observed in pH 4 and in pH 5 solutions. The UV-vis absorption spectral change after different doses of irradiation of the complex solution with a laser at 355nm or 532nm showed a decrease on the shoulder at 360nm and an increase in 510nm peak with the improvement of dose irradiation. This peak is concerning to the aqua complex $[Ru(H_2O)(bdq)(terpy)]$ formed after NO release. Therefore, NO release from the complex with light irradiation was demonstrated. Its also shows that NO is released easier (with low irradiation doses) when the complex solution is irradiated at 355 nm. *In situ* NO formation after nitrosyl ruthenium complex formulations irradiation is showed by the NO sensor. The signal recorded by the NO sensor rose quickly when photolysis was initiated, then decreased when the light was turned off. **Conclusion:** The NO is released from the complex after light irradiation at 355nm and at 532nm. Moreover the $[Ru(terpy)(Bdq)NO](PF_6)_3$ is stable in the presence of a weak electrical current when its dispersed in a pH 7.4 formulation suggesting the possibility to delivery this drug topically using iontophoresis.

Keywords: Nitric Oxide; Nitrosyl Ruthenium Complex; NO delivery.

Financial support: FAPESP.

TF 23 - PREPARATION AND CHARACTERIZATION OF SOLID DISPERSIONS OF PRAZIQUANTEL BY HOT MELT

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Introduction: Praziquantel (PZQ) is the first choice in the treatment of schistosomiasis, a serious health problem in Brazil and other developing countries. However, due to its very low water solubility, high doses are required for a proper therapeutical result. PZQ is classified in Class II in the Biopharmaceutics Classification Systems; which means that PZQ also has high permeability, and its dissolution is the absorption rate-limiting factor. Solid dispersions have shown to be a simple method to improve drug solubility, and are usually prepared by solvent evaporation, spray drying or hot melt techniques. **Objective:** The aim of this work was to evaluate solid dispersions of PZQ prepared by the hot melt method using polyethylene glycol (PEG) 4000 and 6000 as hydrophilic carriers for enhancing the solubility and dissolution rates of PZQ. **Materials and Methods:** Physical mixtures, PM, and solid dispersions, SD, were prepared at the ratios of 2:1, 1:1, 1:2, 1:5 and 1:10 (w/w) of PZQ in PEG 4000 and PEG 6000. The solubility of PZQ PM and SD were determined in water under stirring at 37 °C during 24 hours. The PM and SD at the ratios 2:1 e 1:10 were characterized by Fourier transform infrared spectroscopy (IR-FT), differential scanning calorimetry (DSC) and x-ray powder diffraction (XRPD). **Results:** The main result of solubility assay showed that PEG 6000 SD at the ratio 1:10 provided an increase of 100% in PZQ solubility compared to pure drug. The IR-FT results showed that the PZQ and PEG functional groups were maintained, however, significant interactions were evidenced between the drug and the carrier. Also, the DSC confirmed that there is interaction between PZQ and PEG. And the XRPD indicated some changes in crystallinity of both PZQ and PEG in the SD and MP, leading to amorphous state. **Conclusion:** In conclusion, hot melt methods for production of PZQ solid dispersions are an interesting alternative as solvent free, fast and low cost process to increase the solubility of this drug.

Keywords: praziquantel, solid dispersion, peg, hot melt, schistosomiasis

Financial support: FAPESP; CNPq.

TF 24 - SENSORIAL AND STABILITY OF COSMETIC FORMULATIONS CONTAINING POLYSACCHARIDES OF PLANT OR BIOTECHNOLOGICAL ORIGIN

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A new tendency in cosmetic formulations is the use of biotechnological raw materials like polysaccharides, and among them the ones produced by *Klebsiella pneumoniae* as well as their derivatives can be pointed out due to their proposed effects on cell renew, skin moisture and micro-relief. In addition, *Myrtus communis* leaves are rich in flavonoids, and its hydrolyzed extract contains different polysaccharides, which can also act in skin barrier function. On the other hand, in the development of cosmetic products, rheological studies have been largely used due to the possibility to obtain a correct profile of physical stability and to predict an instability process of cosmetic formulations under "accelerated" conditions. The objective of this study was to evaluate the physical stability and to perform sensorial analysis of cosmetic formulations containing polysaccharides produced by *Klebsiella pneumoniae* and/or polysaccharides from *Myrtus communis* hydrolyzed extract. The following formulations were developed: F1: hydroxyethylcellulose (HEC), methylphenyl polysiloxane (MPPS), batyl alcohol and lecithin; F2: HEC, MPPS and behenyl alcohol, sodium stearyl lactylate; F3: HEC, MPPS and C12-20 acid PEG-8 ester; F4: HEC and MPPS; F5: acrylate polymer and MPPS. These formulations were supplemented or not (vehicle) with 10% of polysaccharides produced by *Klebsiella pneumoniae* and/or 3% of polysaccharides from *Myrtus communis* hydrolyzed extract. These formulations were submitted to preliminary stability studies (centrifugation, pH and visual analysis) when stored at room temperature and at 37 and 45°C for a week. In the study of the physical stability by the determination of rheological behavior, the formulations were stored at room temperature, 37 and 45°C, for 28 days. Viscosity, consistency and flow index and thixotropy of the formulations were determined using a Brookfield cone and plate rheometer. The 2 most stable formulations were applied on the volunteers forearm skin, who answered a questionnaire about their perception concerning the cosmetic properties, as well sense of touch, spreadability, skin feeling just after the application (sensorial analysis). Formulations F1, F4 and F5 were considered more stable in preliminary stability tests and were submitted to the determination of rheological behavior. F4 presented more pronounced alterations in all analyzed rheological parameters than F1 and F5, thus, formulations F1 and F5 were considered more stable and were selected for the subjective analysis. F5 presented the higher degree of acceptance, showing the best sensorial. In addition, the presence of polysaccharides produced by *Klebsiella pneumoniae* in both formulations under study reduced their degree of acceptance. In the experimental conditions of this study, it can be concluded that F1 (containing HEC, MPPS, batyl alcohol and lecithin) and F5 (containing acrylate polymer and MPPS) were considered stable, however F5 presented the higher degree of acceptance in subjective analysis, showing the best sensorial properties.

Keywords: Cosmetics; Sensorial analysis; Rheology; *Myrtus communis*; *Klebsiella pneumoniae*.

Financial support: CNPq; FAPESP.

TF 25 - SENSORIAL AND STABILITY OF COSMETIC FORMULATIONS CONTAINING UV FILTER

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Rheological behavior has a fundamental importance in the formulation of sunscreens, because the formation of an evenly distributed film is critically influenced by the flowing properties and in the performance of the formulations, as well, since sunscreen products need to be applied as a thicker film, which must stay on the skin surface. On the other hand, sensorial properties of cosmetic products are key characteristics and many of these properties are often related with rheological properties. Thus, the aim of this work is to evaluate the physical stability (rheology) and to perform sensorial analysis of different sunscreen formulations. Four formulations containing two different polymers and three different types of self-emulsifying bases were developed: Acrylates/C10-30 alkyl acrylate crosspolymer and Cetearyl alcohol ceteth-10 phosphate dicetyl phosphate (F1), HEC and Batyl Alcohol, Stearic Acid, Lecithin (F2), HEC and Behenyl Alcohol, Polyglyceryl-10 Pentastearate, Sodium Stearyl Lactylate (F3) and HEC with Cetearyl alcohol ceteth-10 phosphate dicetyl phosphate (F4). These formulations were supplemented with the following UV filters: octocrylene, benzophenone-3, octyl methoxycinnamate and submitted to preliminary stability studies (centrifugation, pH and visual analysis) when stored at room temperature and at 37°C and 45°C for a week. In the study of the physical stability by the determination of rheological behavior, the formulations were stored at room temperature, 37°C and 45°C, for 28 days. Viscosity, consistency, flow index and thixotropy of the formulations were determined using a Brookfield rheometer. These 4 formulations were applied on the volunteers forearm skin, who answered a questionnaire about their perception concerning the cosmetic properties, as well sense of touch, spreadability, skin feeling just after the application, skin appearance of the formulations (sensorial analysis) and purchase intention. All formulations under study were considered stable in preliminary stability tests and were submitted to the determination of rheological behavior. All formulations under study had pseudoplastic behavior, with a flow index below 1 and lower thixotropy values, which are important characteristics for sunscreen products. Formulation F3 presented more pronounced alterations in all analyzed rheological parameters and showed a pronounced reduction of viscosity after 7 days stored at 45°C. Formulations F1 and F3 presented the higher degree of acceptance, showing the best sensorial attributes, when compared to F2 and F4. In the experimental conditions of this study, it can be concluded that F1 and F3 presented the higher degree of acceptance in subjective analysis, showing the best sensorial properties, however F1 were considered more stable in rheological analysis.

Keywords: Physical stability; sunscreens; cosmetics.

TF 26 - VALIDATION OF ALTERNATIVE APPARATUS FOR *in vitro* DISSOLUTION TEST OF SEMISOLIDS DOSAGE FORMS

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Two factors are accepted by FDA to compare dissolution profiles of solid oral forms (F1: differences $f1 < 15$ and F2: similarities $f2 > 50$) because it has the advantage of characterizing with more precision, the dissolution than those tests based on just one point according to U.S. department of health and human services F.D.A CDER Guidance for industry dissolution testing of immediate release solid dosage forms 1997 and V. Shah et al. 1998. The goal of this work is validation of alternative apparatus for *in vitro* dissolution test using as reference, Vanke!® enhancer cell meets SUPAC SS Guidelines (patent 5.408.865). Hence, semisolid forms containing 1% of caffeine were prepared in order to study the profiles of the drug from the topical dosage forms. The caffeine release studies (n= 3) were done by using a SR8 Dissolution Test System - Hanson Research, 500 ml of medium with buffer saline pH 7.4 at 37°C with rotation speed at 50 rpm and synthetic membrane for both apparatus. The alternative apparatus was a PVC tube with 4cm² surface area used together with apparatus 1 (basket shaft) without basket, and apparatus reference was apparatus 2 (paddle) over vanke! enhancer cell. After finishing the assay, the percentage of the caffeine released across synthetic membrane was calculated following the equations proposed by MOORE & FLANNER and the difference (f1) and similarity (F2) value factors obtained were 2.67 and 86.6, respectively. Those results indicate that there is no different significant relevance between the profiles of caffeine release through membrane by different apparatus, suggesting that both apparatus can be equivalents to be used *in vitro* release studies of semisolids dosage forms.

Keywords: dissolution; apparatus; semisolid dosage forms; caffeine.

TF 27 - DEVELOPMENT AND VALIDATION OF METHODOLOGY FOR QUANTITATIVE ANALYSIS OF ENOXAPARIN SODIUM

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Heparin is the most used anticoagulant worldwide for the treatment of deep vein thrombosis (DVT) and its major complications such as pulmonary embolism and chronic venous insufficiency. Enoxaparin sodium is a fractionated heparin (FH) obtained from unfractionated heparin (UH) (extracted from porcine intestinal mucosa) through benzylation followed by alkaline depolymerization. Its molecular weight is more homogeneous, ranging from 2,000 to 8,000 Da (average 4,500). Its anticoagulant effect is more predictable than UH with less risk of bleeding. Its therapy does not require constant laboratory monitoring for most patients. There is a great shortage of studies published about methodology of quantitative analysis of both intact FH and UF by HPLC. The analysis is complicated because it is a macromolecule sulphated, acidic, negatively charged and it has variable molecular weight. In this work, a method for the analysis of enoxaparin sodium based on turbidimetry was developed and validated. The methodology consists of a reaction of sulphated groups existing in the enoxaparin molecule with the quaternary ammonium groups of the cetylpyridinium chloride after heating at 37°C for one hour, resulting in an insoluble precipitate in water and turbidity of the reaction environment. This turbidity is directly proportional to concentration of enoxaparin present in the sample. The samples were analyzed using a spectrophotometer in several wavelengths to achieve greater sensitivity and range of application. The method was linear in the range 30.0 to 300.0 µg mL⁻¹, 10.0 to 100.0 µg mL⁻¹ and 5.0 to 75.0 µg mL⁻¹ with wavelengths 500, 340 and 290 nm, respectively. The linear correlation coefficient was higher than 0.99. The values found showed maximum deviation of 12% on the nominal value and precision intra and inter-assays showed coefficient of variation below 3% and 5% respectively. The proposed method was validated for linearity, specificity, precision, accuracy and robustness and it was shown to be useful for the analysis of quantitative enoxaparin sodium for the wavelengths studied.

Keywords: Enoxaparin sodium; quantitative analysis; turbidimetry.

Financial support: CAPES; FAPESP.

TF 28 - DEVELOPMENT AND VALIDATION OF A METHOD TO ANALYSE THE PRODRUG HYDROXYMETHYLNITROFURAZONE (NFOH) IN HUMAN PLASMA BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC-LLE)

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Chagas disease is still a serious Public Health problem and considered extremely neglected because it afflicts exclusively populations in developing countries. Benznidazol (Rochagan[®], Roche) is the only drug available in the Brazilian market. Among the potentially anti-Chagas compounds under study, the prodrug hydroxymethylnitrofurazone (NFOH) has shown lower toxicity and higher tripanomicide activity than nitrofurazone (NF, active compound) in all stages of development of the parasite. In 2006, preclinical toxicity studies showed that NFOH presented an LD50 higher than 2000 mg in rats. This value is considered safe by the World Health Organization (WHO) and promising for continued testing. In this context, the purpose of this work was to investigate the *in vitro* stability of NFOH. To this end, an analytical methodology, not yet described in literature, was developed and validated for the determination of NF and NFOH in human plasma by LLE-HPLC-UV. NF and NFOH were extracted from samples of human plasma at pH 11 with ethyl acetate. The organic layer was filtered with anhydrous sodium sulfate and dried under nitrogen. The extracts were re-dissolved in sodium carbonate : acetonitrile (79:21) and analysed by HPLC, using: a C18 (250 mm x 4.6 mm I.D., 5µm) column, sodium acetate/acetic acid buffer at pH 4.6: acetonitrile (79:21) as mobile phase and detection at 365 nm. The response of the LLE-HPLC-UV method for NF and NFOH was linear over a dynamic range from 0.20-10.0 µg/mL, with correlation coefficients of NF: 0.9978, NFOH: 0.9942. The accuracy and precision of the extraction procedure was evaluated by means of the recovery of five replicates (inter-day) and ten replicates (intra-day) of fortified plasma samples. Mean recoveries for NF ranged from 86 to 98% on the same day and 84 to 98% intra days and coefficients of variation of $\leq 3.0\%$ on the same day and $\leq 4.0\%$ inter- days. For NFOH, the mean recoveries were 88 to 96% on the same day and 90 to 96% intra - days with coefficients of variation being $\leq 11.0\%$ on the same day and $\leq 13.0\%$ between days. The quantification limits of the method were: 0.19 and 0.12 µg/mL for NF and NFOH, respectively. The stability assays in frozen plasma indicated that NF remains stable for up to 22 days (limit of the test) and NFOH begins to convert on the second day. These results indicate that plasma samples with NFOH should be prepared on the day of the experiment. The efficiency of the methodology was demonstrated by the adequate validation of the parameters obtained for the quantification of NF and the prodrug NFOH, thus permitting its application in pharmacokinetic studies.

Keywords: *Trypanosoma cruzi*, hydroxymethylnitrofurazone, analytical methodology, human plasma.

Financial support: CAPES.

TF 29 - METHOD VALIDATION FOR THE ANALYSIS OF NITRENDIPINE ENANTIOMERS IN STANDARD SOLUTION

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Nitrendipine is a potent vasodilator with antihypertensive activity whose effects are related to calcium channel inhibiting properties. This drug has one asymmetric center and is administered as a racemate (equimolar enantiomers mixture). Considering the interests in studying the pharmacologic activity of chiral drugs because of differences in pharmacokinetics and pharmacodynamics properties observed between enantiomers, a method was developed and validated for the analysis of nitrendipine enantiomers by HPLC. The resolution of the nitrendipine enantiomers was achieved with a Chiral AGP column (150 x 4 mm I.D., 5 µm particle size), and the mobile phase used was sodium phosphate buffer:isopropanol:acetonitrile (85:7:8, v/v/v), at a flow rate of 0.65 mL/min and detection wavelength of 227 nm. Some validation parameters were studied. Linearity was assessed with concentration of both enantiomers in the range of 2-100 µg/mL (correlation coefficient of 0.993 and 0.9909 for each enantiomer). The precision and accuracy of the method were tested in within-day and between-day studies. The within-day precision and accuracy of the assay were determined by analysis of standard solution (n=3) at concentrations of 10.0, 40.0 and 100.0 µg/mL of each enantiomer. For the evaluation of between-day precision and accuracy the same concentrations were analysed, in triplicate, on five consecutive days. The coefficients of variation for precision studies, as well as the accuracy values, were below 10% at the three concentration levels tested. The quantification limit was 2 mg/mL for both nitrendipine enantiomers.

Keywords: Nitrendipine; validation; enantiomers; HPLC; chiral.

TX 01 - ANTITUMORAL, ANTIPARASITE AND BACTERICIDAL EFFECTS OF AN L-AMINOACID OXIDASE FROM *Bothrops atrox* SNAKE VENOM

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Introduction: BatroxLAO is an L-amino acid oxidase enzyme isolated from *Bothrops atrox* snake venom, which presents multifunctional activity. It is an acid glycoprotein (approximate *M_r* and *pI* of 67,000 and 4.4, respectively) and displays high specificity toward hydrofobic amino acids. LAOs from snake venom catalyze oxidative deamination of L-amino acids producing the corresponding α -keto acids, hydrogen peroxide and ammonia, which are responsible for their toxic effects. These enzymes present antiprotozoal activities in *Trypanosoma cruzi* and in different species of *Leishmania* (*Leishmania amazonensis*, *Leishmania braziliensis*, *Leishmania donovani* and *Leishmania major*). *Leishmania* causes a spectrum of diseases ranging from self-healing ulcers to disseminated and often fatal infections, depending on the species involved and host's immune response. These enzymes also display bactericidal activity against both Gram-positive and Gram-negative bacteria. LAOs from snake venom can induce apoptosis in different cell lines. **Methods and Results:** The cell viability of *Trypanosoma cruzi* and *Leishmania* sp. was investigated after treatment with BatroxLAO performed by MTT assay. The addition of BatroxLAO directly to *T. cruzi* as well as to promastigotes of different *Leishmania* species resulted in a dose-dependent parasite death. This effect was almost completely abolished by the addition of catalase, suggesting that the release of H₂O₂ is directly involved with the parasiticidal effect of the enzyme. *Leishmania* species were more sensitive to the action of this LAO than *T. cruzi*. BatroxLAO displays bactericidal activity against *E. coli* and *S. aureus*. The minimum inhibitory concentration (MIC) was visually determined through the macrodilution method using 4x10⁵ CFU (colony-forming units)/mL incubated with 24 and 48 μ g of purified enzyme for 30 and 60 min. This protein also promotes apoptosis in HL-60 (human promyelocytic leukemia), B16F10 (murine melanoma) and PC12 (murine adrenal gland pheochromocytoma) cells. This effect was determined by flow cytometry assay. When LAO was incubated with catalase on cells, the effect of BatroxLAO was not observed. BatroxLAO presents approximately 20% of cell death by apoptosis toward PBML (peripheral blood mononuclear cells). These observations suggest that cancer cell lines respond differentially to BatroxLAO exposure when compared with their normal counterparts. The apoptotic effects were inhibited by catalase, suggesting that they are mediated by H₂O₂ production. **Conclusion:** One of the priorities in tropical medicine research has been the development of efficient drugs for treatment. The understanding of LAO mode of action on parasites may trigger the design of new drugs or therapeutical approaches. Additional protocols need to be developed to study the mechanisms of BatroxLAO action and its interaction with cancer cells. BatroxLAO is an interesting enzyme that provides a better understanding of the ophidian envenomation mechanism, and has a biotechnological potential as a model for therapeutic agents.

Keywords: L-amino acid oxidase; snake venom; antiparasite; bactericidal; apoptosis.

Financial support: FAPESP.

TX 02 - BIOCHEMICAL AND PHARMACOLOGICAL CHARACTERIZATION OF EXTRACTS FROM SEA ANEMONES

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Introduction and Objective: Sea anemones are sessile coelenterates that protect themselves against predators by releasing different types of polypeptide toxins. So far, only a few studies analyzed the composition of extracts from these marine animals. Therefore, this work had as objective the biochemical and pharmacological characterization of the extracts from sea anemones. **Materials and Methods:** The protein profile of sea anemone extracts was evaluated by SDS-PAGE. The PLA₂ activity was analyzed using indirect hemolytic assay on agar gel containing egg yolk as phospholipid substrate supplemented with red blood cells. The hemolytic activity was evaluated using erythrocyte suspensions from human blood. The edema inducing activity was evaluated by injection of marine anemone extracts in paw of mice and measured with a low-pressure pachymeter. The myotoxic assay was carried out with intramuscular injection of different doses of marine anemone extracts in mice, collecting blood from their tail to determine the CK levels. Hemorrhagic activity was quantitatively estimated by intradermic (i.d.) injection of different doses of the extracts in mice. After 2h, the animals were euthanized and had their skins removed for the measurement of the hemorrhagic halos. The caseinolytic activity was tested incubating anemone extracts for 30min in solution of 0.1 M Tris-HCl pH 9.0 containing 1% casein, followed by addition of TCA for the precipitation of intact proteins and analysis in spectrophotometer (280nm). **Results and Conclusion:** The electrophoretic profile of several extracts showed significant differences in protein composition, but all showed a high concentration of low molecular weight proteins, possibly phospholipases A₂ and peptides, mainly because all the extracts showed to be active in lysing phospholipid substrates in *in vitro* test. The anemone extracts demonstrated similar PLA₂ activity, but the extracts of *S. helianthus*, *P. homomalla* and *B. annulata* showed to be 10% more active. Regarding edema, only the extracts of *P. homomalla* and *S. helianthus* were able to induce this effect. The proteolytic activity on casein was higher for the extracts of *B. granulifera* and *B. annulata*. The direct hemolytic activity was dose-dependently induced by all the tested extracts and hemorrhage was only induced by extracts of *B. granulifera*, *M. alicornis* and *P. homomalla*. The extracts of *C. gigantea* and *S. helianthus* showed pronounced myotoxicity, while *B. annulata* and *B. granulifera* showed no myotoxic effect. The study of animal venoms is very important for the discovery of novel molecules that can be used, in the future, in medical clinic.

Keywords: Toxins; sea anemones extracts; biochemical characterization.

Financial support: FAPESP; CNPq; FCFRP-USP.

TX 03 - BIOCHEMICAL CHARACTERIZATION OF A PHOSPHOLIPASE A2 INHIBITOR FROM *Bothrops jararacussu* SNAKE PLASMA

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Introduction and Objective: The objective of this work was to isolate and characterize a myotoxin inhibitory protein of the α -type that neutralizes the enzymatic, toxic and pharmacological activities of phospholipase A2 snake venoms. **Materials and Methods:** The myotoxin inhibitor, denominated α BjussuMIP, was isolated from plasma of the *Bothrops jararacussu* by affinity chromatography using the myotoxin Lys49-BthTX-I connected in Sepharose 4B CNBr-activated. Toxins and different proportions of α BjussuMIP were previously incubated to analyze its inhibitory effect. Phospholipase activity was tested by indirect hemolytic method. Edema and myotoxicity were evaluated by subplantar and intramuscular injections, respectively, in Swiss mice. The inhibitory activity on the effects of toxins was also analyzed with supplementation of antiserum with α BjussuMIP. **Results and Conclusions:** α BjussuMIP showed to be an acid glycoprotein with a *M_r* of 24.500 for the monomer and 120.000 for the oligomer, determined by SDS-PAGE and gel filtration, respectively. The α -type inhibitor did not lose its ability to neutralize the enzymatic activity of the PLA₂s after incubation at different temperatures and pH values. The analysis of the circular dichroism spectra indicated that no significant alterations in the secondary structure of α BjussuMIP complexed with

the BthTX-I occurred. α BjussuMIP presented inhibitory properties against basic snake venom PLA₂s. The myotoxic effect induced by Lys49 and Asp49 myotoxins was inhibited by α BjussuMIP in 70% and the edema induced by Lys49-PLA₂ was also inhibited. Additionally, α BjussuMIP was able to supplement the antivenom in *in vivo* tests. Therefore, this α -type inhibitor of PLA₂s showed to be effective against toxic and pharmacological effects induced by snake venom PLA₂s, suggesting that these PLLs can be useful in the treatment of snake envenomations or human illnesses in which these enzymes are involved.

Keywords: *Bothrops jararacussu*; inhibition; phospholipase A₂; α BjussuMIP; snake plasma.

Financial support: FAPESP; CNPq; FCFRP-USP.

TX 04 - BMOOTX-I, A NEW ACIDIC MYOTOXIC AND ANTI-PLATELET PLA₂ ISOLATED FROM *Bothrops moojeni* SNAKE VENOM

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Introduction and Objective: Phospholipase A₂ (PLA₂, EC 3.1.1.4), a major component of snake venoms, specifically catalyzes the hydrolysis of fatty acid ester bonds at position 2 of 1,2-diacyl-sn-3-phosphoglycerides in the presence of calcium. This study reports the purification and biochemical characterization of BmooTX-I, a new myotoxic acidic phospholipase A₂ from *Bothrops moojeni* snake venom. **Materials and Methods:** The purification of this enzyme was carried out through three chromatographic steps (ion-exchange on DEAE-Sephadex, molecular exclusion on Sephadex G-75 and hydrophobic interaction on Phenyl-Sepharose). The degree of purity was determined in the presence and absence of β -mercaptoethanol by Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) stained with coomassie blue and isoelectric focusing assayed with pH gradient. PLA₂ activity was determined by indirect hemolytic activity assayed in agarose gel, using egg yolk emulsion as substrate supplemented with human erythrocytes and the myotoxic activity was assayed using the CK-UV kinetic kit. Platelet aggregation was triggered with collagen or ADP after pre-incubation of platelets with isolated BmooTX-I for 2 minutes by an aggregometer. **Results and Conclusions:** BmooTX-I was found to be a single-chain protein of 15,000 Da and pI 4.2. It displayed a high phospholipasic activity and platelet aggregation inhibition induced by collagen or ADP. Myotoxicity was also induced by BmooTX-I. Acidic myotoxic PLA₂s from *Bothrops* snake venoms haven't been much explored and the knowledge of its structural and functional features will be able to contribute for a better understanding of their action mechanism regarding enzymatic, pharmacological and toxic activities.

Keywords: acidic phospholipase A₂; *Bothrops moojeni*; myotoxin; platelet aggregation inhibitor.

Financial support: FAPESP; CNPq.

TX 05 - *Bufo paracnemis*- ISOLATED ALKALOID INDUCES SWELLING IN RAT BRAIN MITOCHONDRIA

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Neurodegenerative diseases like Parkinson's or Alzheimer's diseases are disorders with no known cure. Recent genetic advances suggest that mitochondrial dysfunction may be an early event involved in such disorders. Mitochondrial dysfunction and associated bioenergetics failure can lead to abnormal cellular ion homeostasis, as a result of which cells undergo swelling and cellular disruption, eventually leading to cell death. Drugs that target the mitochondria may therefore represent the best hope. The venoms/toxins of different animals have been shown to possess neuroprotective effects. Amphibian skin secretions have provided a wide range of biologically active compounds, such as biogenic amines, steroids, alkaloids, peptides and proteins. In order to investigate the action of partially purified *Bufo paracnemis* toxin (Bp1) on mitochondrial permeability, we isolated brain mitochondria from *Wistar* rats (n=3, 200g). For this purpose, it was used a procedure that combine differential centrifugation and discontinuous Percoll density gradient centrifugation. Changes in mitochondrial volume were measured by absorbance at 540 nm. Our results indicate that low concentrations (0,005 μ g/ml-5 μ g/ml) of Bp1 induce, *in vitro*, swelling and alteration of the membrane potential in rat brain mitochondria.

Keywords: Neurodegenerative diseases; mitochondria; neurotoxin; bioenergetic; swelling.

Financial support: FAPESP.

TX 06 - CARVEDILOL EXHIBITS PROTETOR EFFECT AGAINST RENAL MITOCHONDRIAL INJURY CAUSED BY CISPLATIN IN RATS

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Introduction and Objectives: Cisplatin is one of the most widely used anticancer agents; however, it has many adverse reactions, mainly nephrotoxicity. Mitochondria are the main targets of the platinum compounds; therefore studies on antioxidant agents that reduce these adverse effects are necessary. Carvedilol (CV) is a drug widely used in the clinical practice because of its anti-hypertensive properties, but it also has antioxidant activity. There are reports of its protective action against the heart damage induced by doxorubicin. In the present study we will evaluate the protective effect of carvedilol against the renal mitochondrial damage induced by cisplatin. **Materials and Methods:** Male rats (200 to 250g) were divided into 4 groups of animals (n = 6). Group 1 (Control) received injections of saline and DMSO, group 2 (CV + CISP) was treated with carvedilol (1 mg / kg, ip) immediately before the injection of cisplatin (10 mg / kg, ip) followed by two daily injections of carvedilol (1 mg / kg, ip); group 3 (CV) was treated with carvedilol (1mg/kg ip) for 3 days, group 4 (CISP) received only one injection of cisplatin (10 mg / kg, ip). After 72 hours the animals were sacrificed. The mitochondrial fraction was obtained through differential centrifugation. The mitochondrial function was evaluated by oxygen consumption, formation of the mitochondrial membrane potential and calcium uptake. **Results:** The ratio of respiratory control (RCR) in the CV + CISP group was 4.3 \pm 0.51, which was significantly different from the CISP group (3.0 \pm 0.15), but similar to the control group (4.4 \pm 0.8). Results of both tests were significantly different from those of control groups (data shown in representative traces). Renal function was assessed by plasma urea and creatinine levels. Our results showed reduced levels of creatinine and urea in the CV + CISP group (0.33 \pm 0.11 mg / dL and 38.8 \pm 6.1 mg / d, respectively) as compared to the CISP group (3.0 \pm 0.2 mg / dL and 291.6 \pm 10.2 mg / d, respectively), but similar to control (0.47 \pm 0.1 mg / dL and 57.8 \pm 1.9 mg / dL, respectively). The results of the CV group were similar to control. All results were statistically evaluated by t non-parametric Student t test, with level of significance p < 0.05. **Conclusion:** Based on the partial results presented, it was concluded that the treatment with carvedilol was able to protect against the renal mitochondria dysfunction of rats treated with cisplatin, therefore, reducing the kidney damage, as showed by the significantly decreased levels of plasma urea and creatinine.

Keywords: mitochondria; cisplatin; carvedilol; nephrotoxicity.

Financial support: FAPESP.

TX 07 - COMPARATIVE FUNCTIONAL AND STRUCTURAL CHARACTERIZATION OF THREE L-AMINO ACID OXIDASES FROM *Bothrops* SNAKE VENOMS

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Introduction and **Objectives:** L-Amino acid oxidases (LAAO, EC 1.4.3.2) are flavoenzymes that catalyze the stereospecific oxidative deamination of L-amino acids to the corresponding α -ketoacid with the production of hydrogen peroxide and ammonia. The present work describes a comparative analysis of three LAAOs isolated from the venoms of different *Bothrops* snakes: BjarLAAO-I from *B. jararaca*, BmoolLAAO-I from *B. moojeni* and BpirLAAO-I from *B. pirajai*. **Materials and Methods:** BjarLAAO-I and BpirLAAO-I were isolated by three chromatographic steps (Sephadex G-75, Benzamidine Sepharose and Phenyl Sepharose), while BmoolLAAO-I was isolated by only two steps (CM-Sepharose and Phenyl-Sepharose). The purity level of the enzymes was then verified in reverse phase HPLC and SDS-PAGE in both denaturing and non-denaturing conditions. Additionally, deglycosylation and activity of the LAAOs were monitored after treatment with PNGase F. The isoelectric focusing and Edman degradation methods were carried out to determine the pl and N-terminal amino acid sequences, respectively. The stability of the enzymes at different temperatures and pH values was analyzed using L-Leu as substrate. Bactericidal activity on *E. coli* and *S. aureus*, antiparasite effects on *Trypanosoma cruzi* and *Leishmania* species and cytotoxic effects on tumor (JURKAT and SK-BR-3) and normal cell lines (macrophages) were also evaluated. **Results:** The three LAAOs are dimeric, acid glycoproteins and were isolated with high purity levels. BjarLAAO-I showed pl 5.7, Mr of 55.000 for the monomer and 110.000 for the dimer, while BmoolLAAO-I and BpirLAAO-I showed similar pl values (4.7 and 4.9) and Mr of approximately 65.000 for the monomers and 130.000 for the dimers. These enzymes showed to be more stable at pH values of 7-8 and temperatures of 4-37°C, and after N-deglycosylation, differences in their Mr, but not in their enzymatic activities, were observed. Also, a high N-terminal sequence homology was observed among them. The LAAOs were able to promote dose-dependent cytotoxicity on the analyzed bacteria, parasites and tumor cell lines, and all these effects showed to be partially related to the production of hydrogen peroxide, as they were diminished in the presence of catalase. **Conclusions:** Snake venom LAAOs are interesting multifunctional enzymes, not only for a better understanding of the ophidian envenomation mechanism, but also as models for potentially novel therapeutic agents.

Keywords: L-amino acid oxidases; *Bothrops* snake venoms; bactericide; antiparasite; antitumor.

Financial support: CAPES; FAPESP; CNPq.

TX 08 - DETERMINATION OF LEAD CONTENTS IN MEDICINAL PLANTS BY USING A PRE-CONCENTRATION FLOW INJECTION THERMOSPRAY FLAME FURNACE ATOMIC ABSORPTION SPECTROMETRY SYSTEM

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Lead contents in soil are critically influenced by anthropogenic activities and also by the transportation of this metal by the air. Both dry and moist depositions can be considered important vials of contamination. Despite the fact lead is naturally found in plants as a result of capitation and incorporation processes, it is very difficult to establish its original concentration levels. This is a consequence of the generalized contamination in the different terrestrial biomes throughout the centuries by lead. Chemical products and fertilizers used in agriculture are among the main sources of lead contamination. Regardless its concentration, this metal is seriously dangerous since lead presents cumulative effect in the living organisms. Atomic absorption spectrometry is traditionally used for metal analysis and it is a technique characterized by speediness, operational simplicity, low level of interferences and low cost. However, its sensitivity is many times limited due to the low nebulization efficiency considering only 5 to 10 % of the produced aerosol reaches the flame. The goal of this work is to establish lead contents in medicinal plants largely consumed by the population by using a pre-concentration flow injection thermospray flame furnace atomic absorption spectrometry system (FI-TS-FFAAS). The proposed system allowed overcoming technique low sensitivity by increasing nebulization efficiency since the whole solution sample was introduced into the flame and also by increasing residence time in the flame. To optimize the system, the eluent flow rate, distance between pre-concentration column and ceramic capillary from the thermospray device and signal integration time were evaluated. FI-TS-FFAAS system allowed achieving an increase of 200 fold in terms of sensitivity when compared to the conventional FAAS system (flame atomic absorption spectrometry). The obtained results also demonstrated the system presents good stability and robustness. Good analytical features such as r.s.d. $\leq 4,95\%$ and analytical throughput of 48 h⁻¹ were attained. Finally it should be emphasized the analyte could be determined at ng mL⁻¹ level, such achievement could not be obtained in the conventional FAAS system.

Keywords: lead; medicinal plants; pre-concentration; FI-TS-FFAAS.

TX 09 - EVALUATION OF THE MUTAGENIC AND ANTIMUTAGENIC POTENTIAL OF AÇAÍ (*Euterpe oleracea* MART) PULP ON *Swiss albinus* MICE RETICULOCYTES BY THE MICRONUCLEUS TEST

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Açaí (*Euterpe oleracea* Mart.) is a native fruit from Amazonian region widely consumed by the local population in the form of juice, candy, ice cream or in nature. It is a fruit with great energy values, rich in fat, vitamin E, iron and fiber. It has an elevated antioxidant capacity due to its high content of anthocyanins and tocopherols, with protective effect against the development of coronary disease and suggests protection against the development of cancer. The aim of this study is to evaluate the mutagenic and antimutagenic potential of açaí pulp by the micronucleus test in peripheral blood cells from *Swiss albinus* mice, a toxicogenetic test widely recognized by regulatory agencies. The animals were divided into eight treatment groups, being a negative control, three different concentrations of the pulp by gavage (3.33; 10.00 and 16.67 mg/kg body weight), a positive control (doxorubicin 90 mg/kg body weight, intraperitoneally) and 3 groups receive the açaí pulp by gavage (3.33; 10.00 and 16.67 mg/kg body weight) and doxorubicin (90 mg/kg body weight, intraperitoneally). We collected the blood from caudal vein, 24 hours after the treatment, and the animals were submitted to euthanasia. Micronuclei were scored on Feulgen/fast-green stained. The frequency of micronucleus was verified in 2,000 reticulocytes per animal. The results were submitted to One-way analysis of variance test (ANOVA) and the Tukey-Kramer multiple comparison test. The results showed that açaí pulp itself was not mutagenic. In animals treated with açaí pulp and doxorubicin, the number of micronuclei was significantly decreased compared to animals receiving doxorubicin alone. In conclusion, the açaí pulp demonstrated a protective effect against the chromosomal damages caused by doxorubicin at all concentrations tested in these conditions. The antimutagenic potential must be better investigated by others assays and using others conditions and we suggest the identification of anthocyanins compounds and other polyphenols in açaí pulp.

Keywords: Açaí; *Euterpe oleracea* Mart; Micronucleus test.

Financial support: FAPESP; CNPq; Ricaeli Industria e Comércio de Polpas Ltda.

TX 10 - EVALUATION OF THE USE OF HUMAN HAIR FOR BIOMONITORING THE DEFICIENCY OF ESSENTIAL AND EXPOSURE TO TOXIC ELEMENTS

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Introduction: Monitoring the nutritional status of essential elements and assessing exposure of individuals to toxic elements is of great importance for human health. Thus, the appropriate selection and measurement of biomarkers of internal dose is of critical importance. Due to their many advantages, hair samples have been widely used to assess human exposure to different contaminants. However, the validity of this biomarker in evaluating the level of trace elements in the human body is debatable. The aim of this paper was to evaluate the use of hair as a biomarker of Sr, Zn and Cu deficiency and/or Pb exposure. Thus, the relationship between the level of these elements in hair with their levels in whole blood or plasma was obtained in an adult Brazilian population. **Materials and Methods:** Hair, blood and plasma were collected from 280 adult volunteers (São Paulo, Minas Gerais and Pará) for metal determination. 4-mL blood sample from each participant. Blood samples were collected in trace-metal-free evacuated tubes. On the same day as blood collection, hair samples were taken from the occipital area of the head, close to the scalp. The lock of hair was stapled at the base and stored in labeled Ziploc bags. Hair samples were cut into 1 cm lengths and washed before analysis. The plasma fraction was then pipetted into an eppendorf tube (2 mL volume) previously cleaned in a 100 clean room and was immediately frozen at -20°C before analysis. Trace elements levels were determined by Inductively Coupled Plasma Mass Spectrometry. **Results:** Manganese, copper, lead and strontium levels in blood varied from 5.1 to 14.7, from 494.8 to 2383.8, from 5.9 to 330.1 and from 11.6 to 87.3 $\mu\text{g/L}$, respectively. Corresponding levels in hair varied from 0.05 to 6.71, from 0.02 to 37.59, from 0.02 to 30.63 and from 0.9 to 12.6 $\mu\text{g/g}$. Trace element levels in plasma varied from 0.07 to 8.62, from 118.2 to 1577.7 and from 2.31 to 34.2 $\mu\text{g/L}$ for Mn, Cu and Sr, respectively. There was a weak correlation ($r=0.22$, $p<0.001$) between lead levels in hair and blood. Moreover, copper and strontium levels in blood correlate with those levels in plasma ($r=0.64$, $p<0.001$ for Cu) and ($r=0.22$, $p<0.05$ for Sr). However, for Cu, Mn and Sr there was no correlation between levels in hair and blood. **Conclusion:** Our findings suggest that while the idea of measuring trace elements in hair is attractive, hair is not an appropriate biomarker for evaluating Cu, Mn and Sr deficiency or Pb exposure.

Keywords: Hair; metals; Biomarkers; Toxic; Exposure.

Financial support: FAPESP.

TX 11 - EVALUATION OF THE PRESENCE OF BARIUM, LEAD AND CHROMIUM IN THE GROUNDWATER COLLECTED IN THE AQUIFER BAURU

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Introduction: The occurrence of metals in groundwater is a great concern all over the world because of the diversity of toxic effects and wide distribution in the environment. Between these compounds, we can cite barium, lead and chromium that can induce diverse effects such neurotoxicity and carcinogenicity. The Bauru aquifer has great importance for economic aspects for the State of São Paulo is given because of its long occupation, which is approximately 42% of the area of the state (western region). Its average thickness is about 75 meters, which reaches its maximum thickness of 300 meters on the plateau at Marília city. It has been considered that the aquifer Bauru has high vulnerability. Therefore the contamination may occur due to the presence of these elements in the soil related to a variety of sources. **Objective:** So, the objectives of this project were: the validation of the method of atomic spectrometry by inductively coupled plasma ICP-OES; and the evaluation of presence of these compounds in samples of groundwater collected in aquifer Bauru. **Materials and Methods:** The methods of analyses were based on 21^a Edition of Standard Methods for the Examination of Water and Wastewater APHA AWWA WEF 13120 Metals by plasma emission spectroscopy. The readings were made in following a wavelength: Ba (455,40nm), Cr (267,72nm) and Lead (220,35nm). **Result:** We collected samples in 56 sites located in 55 municipalities in the western region of the state of São Paulo. Almost all the samples analyzed were in accordance with the Brazilian law (Portaria 518-MS) in relation to the metals. Only 10 sites showed values above to the established in the legislation: one for Barium and nine for Chromium. The threshold Values allowed for these metals are respectively, of 0.7mgBa.L⁻¹ and 0.05 gCr.L⁻¹. **Partial Conclusion:** The technique employed is valid for the monitoring of groundwater in relation to the presence of barium, chromium and lead, allowing analysis of trends of contamination. Apparently the lead is not a relevant contaminant in the aquifer Bauru. The presence of barium in Bauru aquifer should be evaluated in a more careful, and examine whether there is trend of increasing concentration. Concentrations of chromium detected are relevant in the aquifer Bauru and their presence should be monitored and an investigation about the sources of this contamination should be carried out. Water that contains chromium should not be use for human consumption, according to Ordinance 518, without appropriate treatment for removal of that metal.

Keywords: aquifer Bauru; barium; chromium; lead.

TX 12 - GENOTOXIC AND ANTIGENOTOXIC EFFECT OF CURCUMIN AGAINST CISPLATIN TOXICITY IN PC12 CELLS EVALUATED ON COMET ASSAY PARAMETERS

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Cisplatin is an antineoplastic drug used on the treatment of many cancer types. However, it is intrinsic the development of various side effects, including neurotoxicity, resulting from its toxicity to healthy cells. Numerous efforts are being made in attempt to prevent or even control the toxic effects induced by cisplatin with special attention being given to the supplementation during chemotherapy treatment with antioxidant compounds. The curcumin is a polyphenolic compound with a broad spectrum of biological activities and neuroprotective properties, demonstrated in several models, strongly related to its antioxidant potential. The multiple mechanisms of action of the phenolic compounds and their neuroprotective properties, credited to its reactive oxygen species inhibition, indicate a potential inhibition of neurotoxicity that develops during the chemotherapy treatment with cisplatin, given its well known ability to generate reactive oxygen species. Since the generation of reactive oxygen species intracellular can result in DNA damage by direct action of reactive species or indirect, through degradation products of the lipid peroxidation, our studies evaluated the potential protection of curcumin against the genotoxicity induced by cisplatin in PC12 cell cultures, analyzing DNA single strand breaks, double strand breaks, and alkali-labile sites by the comet test. Before the antigenotoxic evaluation of the curcumin, a cytotoxic and genotoxic test were conducted where it was observed that when in high concentrations (above 16 $\mu\text{g/ml}$) curcumin is cytotoxic to the PC12 cells. The protective effect of curcumin was evaluated at the pre-treatment of PC12 cell cultures with non-toxic levels (1.0, 2.5 and 5.0 $\mu\text{g/ml}$). Although there was not obtained statistical differences in the results, the data indicates a protective effect of the curcumin against the damage caused to the DNA of cells PC12 by cisplatin. The positive results obtained in this study combined with the pre-existing data about the protective effects of the curcumin encourage some more new research on possible benefits of using curcumin in combination to chemotherapy.

Keywords: curcumin; cisplatin; genotoxicity; antigenotoxicity.

Financial support: CAPES; CNPq.

TX 13 - HISTOLOGY OF THE REPRODUCTIVE SYSTEM OF *Brontocoris tabidus* (HETEROPTERA: PENTATOMIDAE) AFTER EXPOSITION TO THE DELTAMETHRIN

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Introduction: Predators as *Brontocoris tabidus* (Signoret, 1852) (Heteroptera: Pentatomidae) are important natural enemies of eucalyptus pests and they present better performance with prey and this plant. Pest Management Programs (IPM) reduces the impact of chemical insecticides on natural enemies through using less aggressive ones. This work evaluated the impact of three deltamethrin concentrations on the reproductive system of males and females of *B. tabidus*. **Materials and Methods:** This work was developed at the Laboratory of Biological Control of Insects of the Institute of Applied Biotechnology to Agriculture (BIOAGRO) of the Federal University of Viçosa (UFV) in Viçosa, Minas Gerais State, Brazil. Egg masses of *B. tabidus* were obtained from the laboratory (26±2°C, 60±10% RH, 12h photophase). Second instar nymphs of this predator were put on eucalyptus plants and they returned to the laboratory in the thirty instar to be submitted to treatments. The experiment was developed in an entirely, casualized, design with three deltamethrin dilutions in distilled water as the treatments (T1= 0.0165%, T2= 0.033 and T3= 0.066%) with three replications. The control (T4) had, just, distilled water. Eucalyptus leaves impregnated with this insecticide and one *Tenebrio molitor* (Coleoptera: Tenebrionidae) pupae were individualized, during 24 hours, in Petri dishes identified and lined, with ten nymphs of *B. tabidus* each one, which survival was obtained every 12 hours, during 48 hours, and joined, per treatment, in organza bags identified and fixed on eucalyptus plant until the emergence of its adults when their reproductive system were extracted for histological studies on the impact of this insecticide. **Results:** The tunica propria of the follicles were thicker at the center than in the extremities with decreasing values from T4<T1<T2<T3. The cysts, germarium and maturation areas, besides the follicle were less organized, atypical, round and non differentiated espermatogonias, without scourge, varied sizes, retarded development and maturation area were larger in T1 than in the T4. The females had immature ovaries and cysts. The tunica propria in the T2 had homogeneous thickness but it increased in the germarium and in the tunica externa that presented a vast net of associated windpipes. Germarium and tropharium had less dense areas in the treatments with deltamethrin and many intercellular spaces. The gonad in the T2 were similar to the control. **Discussion:** The deltamethrin affected the testicles and ovaries of *B. tabidus*, what could reduce its reproduction and use in the biological control. Males were more affected, probably because they have developed gonads, different stages of cellular maturation and matured spermatozoids, besides resistance levels to this insecticide. The tunica propria was most affected with varied thicknesses and discontinuity. Histology in the T2 was similar to the control, indicating that this insecticide can be applied in this concentration with this predator.

Keywords: Impact; Insecticide; Histology; Heteroptera; Reproduction.

Financial support: National Counsel of Technological and Scientific Development (CNPq); Coordination for Improvement of Higher-Level Manpower (CAPES); and Foundation for the Support of Research in the State of Minas Gerais (FAPEMIG).

TX 14 - INHIBITORY EFFECTS OF A NOVEL SYNTHETIC QUINOLINONE AGAINST SNAKE VENOMS AND ISOLATED TOXINS

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Introduction and Objective: Snake venoms are complex mixtures of proteins, which exert a wide variety of pharmacological effects. Important classes of natural compounds like the quinolinones, found in medicinal plants, are widely used as antibacterial and antimycobacterial drugs to treat various infectious diseases and possesses low toxicity. In this study, we describe the inhibitory potential of a novel synthetic quinolinone, 2-hydroxymethyl-6-methoxy-1,4-dihydro-4-quinolinone (C2), against snake venoms and isolated toxins. C2 was synthesized from commercially available anilines. **Materials and Methods:** **Hemorrhagic activity** - Male Swiss mice (18-22g) were injected i.d. in the back with inhibitor/venom or metalloprotease (w/w). Mice were killed 3h after injection, and the diameter of the skin hemorrhage zone was measured. **Proteolytic activity on casein** - Snake venom or isolated metalloprotease with the inhibitor were incubated with casein for 30 min at 37°C. The reaction was stopped with trichloroacetic acid and then centrifuged (2,000xg) for 5 min at 25°C. Proteolytic activity was estimated spectrophotometrically at 280 nm. **Coagulant activity** - Plasma (0,2mL) was incubated for 30 min at 37°C with venom/inhibitor, and the clotting times determined. **Fibrinogenolytic activity** - Evaluated through polyacrylamide gel electrophoresis using bovine fibrinogen (80µg) preincubated with different doses of inhibitor and venom/toxins. **Myotoxic activity** - Male Swiss mice (18-22g) were injected i.m. in the right gastrocnemius with preincubated inhibitor and venom/toxins. Blood was collected 3h after the injections. Plasma creatine kinase activity was determined using the Bioclin Kit (Bioclin, Brazil). **Edema-inducing activity** - Edema was induced by subplantar injection of venom or toxins incubated with inhibitor at different ratios (w/w) in the right paw of male Swiss mice (18-22g). The progression of edema was evaluated 30 and 60 min after injection with a low pressure pachymeter. **PLA₂ activity** - Determined by indirect hemolytic activity assayed in agar gel, using egg yolk emulsion as substrate supplemented with human erythrocytes. C2 was tested after incubation with venoms or toxins at different ratios (w/w). All control groups received PBS, DMSO, venom/toxins or inhibitor alone. **Results and Conclusion:** C2 was able to inhibit the hemorrhage induced by different bothropic venoms and P-III metalloprotease. The fibrinogenolytic activity induced by *B. jararacussu* venom and two isolated proteases (BjussuSP-I and BjussuMP-II) and the caseinolytic activity of class P-I and III metalloproteases (neuwiedase and BjussuMP-II) isolated from *Bothrops neuwiedi* and *B. jararacussu* venoms, respectively, were also inhibited by C2. Clotting activity induced by isolated thrombin-like enzymes from *B. jararacussu* and *Crotalus d. terrificus* venoms (BjussuSP-I and Giroxin) were totally inhibited after incubation at different ratios. Myotoxic, edema and phospholipasic activities were also partially inhibited. Thus, further studies with C2 could lead to its use as complement in the treatment of snakebite envenomations.

Keywords: 4-quinolinone; anti-snake venom; proteases; myotoxins; antiserum.

Financial support: FAPESP; CNPq.

TX 15 - ISOLATION AND PARTIAL CHARACTERIZATION OF A NEW SCORPION TOXIN FROM *Tityus serrulatus* VENOM

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Tityus serrulatus scorpion venom (Tsv) is a complex mixture composed of a wide array of substances. It is a rich source of small neurotoxic proteins, interacting specifically with various ionic channels in excitable membranes. The symptomatology of *Tityus serrulatus* envenomation is the result of primary effects of neurotoxins on ion channels, causing a massive release of neurotransmitters (catecholamines, acetylcholine and others), and leading to the stimulation of the autonomic nervous system. Many Tsv constituent toxins have been described, however, the venom displays in addition, several compounds which were not isolated or studied so far, what makes it a yet poorly explored source of several compounds showing important pharmacological actions. The aim of the present study was the isolation and partial characterization of a new toxin of the fraction XII-A from Tsv. The venom was extracted and fractionated by ion exchange chromatography on a column of CM-cellulose-52, which was equilibrated and eluted with ammonium bicarbonate buffer (pH 7.8), furnishing 13 main fractions. Fraction XII-A was submitted to a reverse-phase (C18) high performance liquid

chromatography (RP-HPLC) and the isolated protein, named XII-A 2, is a new toxin. Polyacrylamide gel electrophoresis (PAGE) showed that XII-A 2 is a basic protein and confirmed its purity. The N-terminal amino acid sequence was obtained by automated Edman degradation in a protein sequencer. Partial N-terminal sequence [15 amino acid residues] showed that XII-A 2 is different from the previously deposited sequences in NCBI, but it is homologous to TsKappa (85% of identity among the residues 5 to 11), a very high potent ligand for small-conductance potassium channels. The chromatographic profile in RP-HPLC, a single band by PAGE and the N-terminal sequence of XII-A 2 confirmed its high purity level. In conclusion, XII-A 2 is a new basic scorpion toxin, probably selective for K⁺ channels. Scorpion toxins selective for K⁺ channels represent excellent tools for investigating the physiological contribution of ion channels to cell and organ behavior and for probing and correlating ion channel structure and function.

Keywords: scorpion venom; *Tityus serrulatus*; neurotoxins; ion channels; protein structure.

Financial support: FAPESP; CNPq.

TX 16 - LIPID PEROXIDATION AND DNA DAMAGE IN RATS EXPOSED TO METHYLMERCURY AND ITS ASSOCIATION WITH SELENIUM

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Methylmercury (MeHg) is the most malignant form of the Hg, and the exposure to this element may cause damage to nervous, immune, kidney and cardiovascular system, besides induces genetic damage. Oxidative stress has been proposed as the main mechanism to explain the toxicity of MeHg. Selenium (Se), an essential trace metal, participates as an antagonist under the toxic effects of many heavy metals, including Hg, and has antioxidants properties. Thus, the purpose of this study was to evaluate the effects of MeHg on lipid peroxidation (MDA evaluation) and DNA damage, in rats chronically exposed to low doses of this metal. Moreover it was observed the effects of the co-administration of Se. Animals were divided in groups as following: (I) control; (II) MeHg (100 µg/day); (III) MeHg (100 µg/day) + Se (2 mg/L) and (IV) Se (2 mg/L), with 8 rats/group, treated during 100 days. MDA levels were determined by High Performance Liquid Chromatography technique and DNA lesion by Comet Assay. Mean MDA levels in groups I, II, III and IV were, respectively: 22.8 ± 2.0; 26.6 ± 4.1; 22.6 ± 5.6 and 17.8 ± 2.9 µM. DNA damage was represented by a mean score, from 0 to 300; the results to the groups I, II, III and IV were, respectively: 7.4 ± 3.1; 126.4 ± 13.1; 86.7 ± 13.1 and 9.7 ± 2.9. In relation to the lipid peroxidation bioindicator, MDA, there was not a significative difference between control and MeHg treated group, neither between control and MeHg + Se treated group; however a significantly difference was found in Se treated group when compared with all others groups (p<0.05), showing that Se could indirectly minimized the lipid oxidation. The results of Comet assay presented a significantly difference in DNA migration in MeHg group when compared with the control group (p<0.0001). Besides, the association of Se with MeHg has also promoted a DNA injury when compared with control group; however this lesion was approximately 30% lower when compared with MeHg treated group, evidencing a significative protective effect of Se on DNA damage (p<0.001). No difference was found between control group and Se treated group. Thus, these findings can provide the oxidative and genotoxic properties of low doses of MeHg, although we did not found significative difference in MDA levels. It could occur by an adaptive mechanism of the organism, since it was a chronic treatment. On the other hand, DNA damage was well-defined and the Se co-association exposure reduces the DNA injury.

Keywords: methylmercury; selenium; oxidative stress; DNA damage.

Financial support: São Paulo State Foundation for Scientific Research (FAPESP, Brazil) and the Brazilian National Council for Scientific and Technologic Development (CNPq).

TX 17 - MANGANESE SPECIATION AS DETERMINANT OF ITS GEOCHEMICAL BEHAVIOUR AND TOXICITY: ALTO DO PARANAPANEMA BASIN AS A CASE STUDY

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Manganese (Mn) is an essential nutrient, but previous reports indicate that this metal is neurotoxic for humans and toxic to aquatic organisms. Several hypotheses have been offered to explain such activity, however no precise toxicological mechanisms have been established. Therefore, the effects of metal speciation on the mechanisms of toxicity are relevant in the case of Mn. During the last decade, levels of Mn in the Alto do Paranapanema (ALPA) basin (Sao Paulo State, Brazil) have been higher than that allowed by Brazilian environmental regulations. For that reason, the present work aimed to study Mn speciation as determinant of both its geochemical behaviour and toxicity. Field experiments conducted at ALPA between August 2006 and April 2007 suggest that most Mn present is of natural origin; however, it poses a moderate to high environmental risk. Sediment samples contained Mn in five fractions: exchangeable Mn > iron and manganese hydr(oxides) > Mn bound to carbonates > Mn bound to silicates > Mn bound to organic matter. In the water column, particulate Mn (oxides, hydroxides and/or adsorbed on clays) was the main physical form found, followed by labile organic and inorganic species (eg, H₂O; OH⁻; Cl; SO₄²⁻; citrate; pyrophosphate) and non-labile species. In addition, moderated and high relationship between Mn species of the sediments and the waters suggests the release to the water column of Mn from the sediment and/or particulate by different mechanisms. These data can provide a basis for the management of Mn at ALPA. At the same time, they allowed us to suggest a set of species [MnCl₂, MnCit, [MnCit]⁻, [MnPy]²⁻] to be assayed on primary cell cultures of neurons. It is shown that Mn species at concentrations above essentiality are neurotoxic, as a consequence of imbalances in cell energy metabolism and/or induction of oxidative stress. Both phenomena can be partially controlled with ascorbic acid and D,L-lactate. Additionally, these results imply that the oxidation state of manganese plays an important role in mediating its cytotoxicity.

Keywords: Manganese; Geochemical; Speciation; Neurotoxicity; Neuroprotection.

Financial support: FIS P1061212 (Ministry of Health, Spain); USP and FAPESP-Brazil (06/00001-3); and CNPq - Brazil (201362/2007-4).

TX 18 - MITRAGYNINE ANALYSIS IN RAT PLASMA BY LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY

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Mitragynine is an indole alkaloid extracted from *Mitragyna speciosa* Korth (Rubiaceae) (known as Kratom in Thailand) which presents analgesic and antitussive properties comparable to codeine. Unlike codeine, mitragynine did not produce emesis or dyspnea, was not blocked by nalorphine, and had much less respiratory depression. Interestingly, it could suppress the opioid withdrawal syndrome. *Mitragyna speciosa* Korth, has been wide available as an economical alternative to other opioid-replacement medications which can be obtained without a prescription. In this way, studies are required to understand the efficacy and adverse effects related to this emerging psychoactive supplement and also to investigate the potential use of

mitragynine to treat opioid addiction or as an analgesic. A selective and highly sensitive high-performance liquid chromatography-tandem mass spectrometry (LC-MS-MS) was developed and validated to quantify mitragynine in rat plasma. Plasma samples were extracted with hexane-isoamyl alcohol (99:1 v/v) and the recoveries were about 95% and 90% for mitragynine and amitriptyline (internal standard), respectively. Mitragynine and the internal standard were resolved on a Lichrospher® RP-18 SelectB column using 20mmolL⁻¹ ammonium acetate-acetonitrile-formic acid (70:30:0.5 v/v/v) at a flow rate of 1.2mL/min as mobile phase. Multi reaction monitoring detection was performed by electrospray ionization in positive ion mode. Protonated ions [M+H]⁺ and their respective ion products were monitored at the following transitions: 398>174 for mitragynine and 278.4>90.8 for internal standard. Method validation showed relative standard deviation (precision) and relative errors (accuracy) lower than 15%. The quantification limit was 0.2ngmL⁻¹ and the linear range was 0.2-1000ngmL⁻¹. The method was successfully applied to pharmacokinetic studies of mitragynine after oral administration to rats.

Keywords: mitragynine; *Mitragyna speciosa*; rats; pharmacokinetics; LC-MS/MS.

TX 19 - MUTAGENIC EFFECT EVALUATION OF SNAKE VENOM PROTEINS ON HUMAN LYMPHOCYTES

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Introduction and Objective: Snake venom proteins show high potential as tools for study of diseases as Cancer, Alzheimer, Parkinson and blood clotting disturbances and represent models for the elaboration of new medicines (Captopril and Ancrod, anti-hypertensive and fibrinolytic agents, respectively). These toxins can induce effects involved in several cell processes. The micronucleus assay has been used as a marker for mutagenic potential evaluation of chemical and natural agents. The objective of this work was to evaluate the frequency of micronuclei in binuclear lymphocytes after treatment for 24h with different isolated snake venom toxins. **Materials and Methods:** Human peripheral blood was collected from healthy subjects (18-35 years) and cultivated in RPMI medium with 20% fetal bovine serum and 2% phytohemagglutinin A at 37°C in stove with 5% CO₂. After 24h, the treatment was carried out with different toxins in 3 pre-determined non-cytotoxic concentrations. Cytochalasin B (6 µg/ml) was added 44 h after the beginning of the cultivation. After 72 h of culture, the cells were washed under appropriate conditions for the isolation of lymphocytes, the slides were prepared and stained (5% Giemsa), being analyzed by light microscope counting 1000 binuclear cells/individual/treatment. **Results and Conclusions:** Isolated PLA₂s from *Crotalus durissus terrificus*, *Bothrops jararacussu* and *Apis mellifera* venoms (CB PLA₂s, BthTX-I, BthTX-II and PLA₂ of *Apis mellifera*) were assayed at 5, 15 and 30 µg/mL. Increase in the number of micronuclei in 1000 binuclear cells was detected only with BthTX-II, with 6 cells presenting micronuclei. The culture treated with cisplatin, an antitumoral drug used as positive control, presented 13 binuclear lymphocytes with micronuclei in 1000. Although these proteins belong to the class of venom PLA₂s and present structural similarities, their toxic and pharmacological activities are different. As a conclusion, only BthTX-II from *B. jararacussu* is able to induce mutagenicity in the tested concentrations, and may present distinct mechanisms of action when compared to the other tested toxins. Thus, BthTX-II may be used as a promising tool for further studies in the genetic toxicology area.

Keywords: Toxins; snake venoms; micronucleus assay; mutagenicity.

Financial support: FAPESP; CNPq; FCFRP-USP.

TX 20 - NANDROLONE DECANOATE EFFECTS ON AGGRESSION OF THE RATS NICOTINE EXPOSED

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Anabolic-androgenic steroids are abuse drugs commonly used by athletes and young population with the goal of increased performance, appearance, or muscle mass, and their use have been reported to cause aggressive tendencies in both laboratory-based animal models and in human clinical situations. In worldwide is estimated that 1.2 billion people currently smoke tobacco, and nicotine is the main addictive component of tobacco that motivates its continued use in spite of its harmful characteristics and anxiety effects provoked by use cessation. Both substances are consumed mainly for man in seemed aged group. This project, using a resident-intruder paradigm model examined the concomitant effects of nicotine (NIC) and a typical anabolic-androgenic steroid, nandrolone decanoate (NAN) on the rat aggression. *Wistar* male rats, 60 days old received one of the following treatments: canole oil (control), nandrolone decanoate (5mg/kg, im., two times week), nicotine (2mg/kg, sc., five times week) and nandrolone + nicotine. After 24 hours the last nicotine administration, aggressive behavior was evaluated using the resident-intruder paradigm, measuring latency of the first bite (T1B), frequency of attacks (TNA) and duration of time spent in attacks (TTA). Both, animals treated with NIC, NAN and NIC + NAN showed reduction in T1B compared with the control group. The TNA was not altered significantly however was observed an increased TTA in animals treated with NIC, NAN and NIC + NAN. The findings together suggest that both animals treated with NIC or NAN had aggressive behavior enhanced. The anabolic-androgenic hormone effect can be linked to a increase in aggressive behavior in the NAN animals. As animals were available during nicotine withdrawal by a 24 hs period, anxiety and irritability originated could have contributed to increase aggressive behavior. The group exposed to nicotine plus nandrolone co-administration had no significant difference when compared to NIC or NAN. The results would be suggestive that the abnormal aggression it may be by different ways to nicotine and nandrolone decanoate.

Keywords: aggression; nandrolone decanoate; nicotine; rats; resident-intruder paradigm.

TX 21 - ORGANOPHOSPHORUS-INDUCED DELAYED NEUROPATHY: A STUDY WITH TOCP IN HENS

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Organophosphorus-induced delayed neuropathy (OPIDN) has been known since 1930s when tuberculosis patients were treated with phosphoreosate. OPIDN is caused by single doses of certain esters organophosphorus compound including phosphates, phosphonates and phosphoramidates. It is characterized by degeneration of long and large diameter axons in central and peripheral nervous system that develops 8-14 days after exposure. Neuropathy target esterase (NTE), a neural protein with an esteratic activity, is believed to be the molecular target for OPIDN. The animal model for neuropathy studies is hen due to its sensitivity and the development of clinical signs similar to those seen in man. Tri-ortho-cresyl phosphate (TOCP) is an organophosphorus ester capable of producing OPIDN in hens without to produce severe acute effects. It's the prototype of studying this kind of neurodegeneration. Some diseases states, caused by chemicals agents, are thought to occur through a similar sequence of events, it is often referred to as *Wallerian-like* axonal degeneration. Digestion of the axon appears to be an all-or-none event effected through endogenous proteases that appear to be activated through increased levels of intracellular free calcium. The aim of this study was to investigate the mechanism of toxic action of

organophosphorus esters compounds after a single dose of 500 mg/kg of TOCP administrated orally by gavage. To reach this aim we used the following strategy: monitoring the levels of calcium in plasma and sciatic nerve of hens at six, 12, 24, 48 hours and eight, 14 and 28 days after TOCP; monitoring the activity of lymphocytes NTE at six, 12, 24, 48 hours and eight, 14, 28 days after TOCP. Chickens were purchased from the poultry farming cooperative of Guataparã. Twenty four isabrown leghorn chickens were divided into six groups with four hens in each group, weighed from 1.4 to 2.3 kg, were bred for eggs and were more than 18 months old. They were acclimated at least one week prior to the start of the experiment (24 ± 2 °C, light and dark cycles, food and water *ad libitum*). The toxicant was administered orally by gavage after fasting for 10 - 12 h. To evaluate enzymatic activity in the lymphocytes it has been necessary 10 milliliters of blood and to evaluate the levels plasmatic and sciatic nerve of calcium both were necessary 40 μ l of plasma or a mix of nervous tissue in saline how to describe on liquiform labtest kit. After twenty four hours TOCP inhibited 85 % lymphocytes NTE activity and has caused severe ataxia in isabrown chickens. After 12 hours TOCP, has occurred a decrease in the plasmatic calcium. These results have shown that certain organophosphorus esters produce OPIDN after single doses and have caused imbalance in calcium homeostasis. The authors are deeply grateful to cnpq and unesp.

Keywords: OPIDN; NTE; TOCP; calcium.

TX 22 - PHENOBARBITAL AND MITOCHONDRIAL TOXICITY: EFFECTS ON MITOCHONDRIA ISOLATED FROM RAT LIVER

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Classical antiepileptic drugs have been extensively used for the treatment of epilepsy, one of the most frequent disorders of the central nervous system which affects nearly 1.5% of the world population. Idiosyncratic hepatotoxicity is a well-known complication associated with anticonvulsants treatment. It may occur as an isolated event or as a part of the spectrum of a multisystem disorder. Therefore, one possible mechanism for the idiosyncratic reaction is the accumulation of toxic arene oxide metabolites due to a defect in the epoxide hydrolase-mediated detoxification. They can also directly affect cell biochemistry, causing cell death. Little is known about the cause of delayed, non-immune forms of idiosyncratic hepatotoxicity. The direct toxicity of reactive metabolites may involve mitochondrial dysfunction, one of the most important mechanisms of drug-induced liver injury. Mitochondria are the central point where the different signals responsible for initiating hepatocyte cell death converge, irrespective of whether the cells ultimately die by apoptosis, necrosis or autophagy. Therefore, we investigated, *in vitro*, the cytotoxic mechanism of phenobarbital, unaltered and bioactivated, in the hepatic mitochondrial function. The murine hepatic microsomal system was used to produce the anticonvulsant metabolites. The bioactivated drug affected mitochondrial function causing decrease in state 3 respiration, started at 200 μ M and the IC_{50} was of approximately 750 μ M, phenobarbital-bioactivated also presented an uncoupling effect characterized by increased oxygen consumption during state 4 respiration. Such effect was observed from 50 μ M ($p < 0.01$) and reached the maximum value at 500 μ M, RCR, markedly decreased from 50 μ M. at 100 μ M, was 95% lower as compared to control; ATP synthesis, was able to decrease IC_{50} for this effect was 750 μ M and membrane potential, increase in state 4 respiration as well as impairment of Ca²⁺ uptake /release and inhibition of calcium-induced swelling. As an unaltered drug, was able to affect mitochondrial respiration (except state 4 respiration) ATP synthesis and membrane potential, the bioactivated drug assayed in the same concentration caused a more potent effect, leading to mitochondrial inability to phosphorylate and to resume to the pre-ADP potential; however, Ca²⁺ uptake/release as well as swelling induction were not affected. Our results strongly suggest that mitochondrial toxicity is one of the mechanisms involved in idiosyncratic hepatic damage associated with phenobarbital. In fact, hepatic mitochondrial toxicity induced by phenobarbital has not been previously demonstrated. The knowledge of potential hepatotoxicity as well as the delineation of possible mechanisms involved can provide important tools for the diagnosis, treatment and prevention of idiosyncratic hepatic damage, a real challenge in optimizing AAED therapy.

Keywords: Mitochondria; Aromatic antiepileptic drugs; Hepatotoxicity; Phenobarbital; Arene oxides.

TX 23 - RELATION BETWEEN STEREOCHEMICAL OF AMINO ACID RESIDUES IN PRIMARY AND SECONDARY STRUCTURES OF TOXINS AND NON-TOXINS PROTEINS

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Secondary structures are very common in proteins and consist of local inter-residues interactions. The most common secondary structures are helices and b-sheets (formed by strands). Here we are analysed the composition stereochemical of the a.a. (by a steric pattern simplified (Small/Big) and pattern simplified to polarity (Hydrophobic/Polar)) to helices and strands with the composition stereochemical of the a.a. at the primary structure of two groups of proteins both comprises 35 amino acid residues (a.a.), the toxins and the non-toxins. The first group (with eight toxins) is much structurally different of second (with thirty-one proteins) due to its high compactation, many disulfide bridges and strands. Toxins have been studying mainly owing to they have become a serious problem of Public Health in many countries besides by its potential to development of new medicines. Due to major number initially are analyzed the non-toxins being possible observed that helices utilize very well (of linear form) both steric and hydrophobic ingredients, on the other hand strands utilize well only its steric component. The linear behavior show that as more a.a., for example Big, are disposable by primary structure of the protein, as a.a. of same type (Big) are used by secondary structure. Despite structurally different the toxins show like behavior of non-toxins. These results show that the protein use very efficiently the composition stereochemical of its a.a. but differently at helices and strands. It is possible still propose a simple physicochemical theory to secondary structures, same with its structural complexity, as a.a. sequence different, assorted composition of secondary structure elements and varied functions.

Keywords: toxins; secondary structures; steric constraints; hydrophobicity.

TX 24 - RENAL GENOTOXICITY AND METHYLATION PATTERN OF TP53 GENE IN RATS EXPOSED TO METHIONINE-RICH DIET

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Introduction: To understand the interaction between nutrient and genome, it is necessary to consider epigenetics mechanisms which are responsible for regulating genes expression and they do not involve changes in DNA sequence. Methionine (Met) is a diet donor of methyl groups. Excessive intake of Met could result in changes on metabolism and methylation reactions. DNA methylation occurs specifically in the C5 position of

cytosines lying 5' to guanosine in so-called CpG dinucleotides. Global hipomethylation and gene-specific hipermethylation are involved in genomic instability and could result in genotoxicity. **Objective:** This study intends to evaluate if Met on diet result on changes in DNA methylation and genotoxicity in rat kidney. **Materials and Methods:** *Wistar* rats (n = 20) received commercial diet (Control) or commercial diet plus Met 2% (Met) during six weeks. Kidney was excised and used to determine genotoxicity by comet assay. DNA methylation pattern of the promoter region of *TP53* gene was assessed by COBRA method. This method is based on differential conversion of cytosine to uracil in single-stranded DNA by treatment with sodium bisulphite, leaving 5-methyl cytosine unmodified. After PCR amplification, promoter region of *TP53* fragment was digested with restriction enzymes: *HpyCH4* IV was used to determine methylation pattern and *Tas I* was used to validate sodium bisulphite conversion. **Results:** *TP53* gene was hipomethylated in Control group, and diet Met exposure did not change renal methylation pattern. Met treatment decreased DNA damage on kidney. It was observed reduction of 34.2%, 55.4% and 48.73% on %DNA in tail, tail Moment and Olive Moment respectively when compared with Control group (p<0.05). So, Met reduced genomic instability. **Conclusions:** Our results showed that diet with Met does not induce genomic instability and it does not alter *TP53* methylation pattern in rat kidney.

Keywords: DNA Methylation; Methionine; TP53; Comet Assay; Rat.

Financial support: CNPq and FAPESP

TX 25 - SIMULATION OF THE *in vivo* OXIDATION OF AZO DYE SUDAN III USING A ESPECTROELETROCHEMICAL METHOD

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Introduction: Azo dyes (N=N) represent the largest group of synthesized organic compounds. Sudan dyes are azo-dyes that are widely used in industrial applications, research and in the past these compounds were used as a food colorant in several countries but it has been recommended as unsafe, because it causes tumors in the liver or urinary bladder in rats, mice, and rabbits, and is considered a possible carcinogen and mutagen for humans. Sudan III is a suspected human carcinogen and have been classified as group 3 by International Agency of Research on Cancer (IARC) and are thus banned from use in food in the EU. Despite its danger, there are no studies in the literature relating Sudan III carcinogenicity or mutagenicity of the dye itself or its metabolites. Azo colorants are biologically active through their metabolites. Cytochrome P450 (CYP) play a critical role in the metabolism of numerous carcinogens, the activity of this system can occasionally result in their activation into a reactive species generating DNA adducts, which is believed to be one of the primary steps in the initiation of chemical carcinogenesis. CYPs were assumed to play a role in the oxidative metabolism of the Sudan I, but no information were found about the role of CYP isoforms for Sudan III. **Objective:** Investigate the importance of the oxidation reactions for the mutagenicity of the Sudan III metabolites using spectroelectrochemical method and metabolic activation (S9) in the same conditions applied for Ames test. **Materials and Methods:** In this work, a spectroelectrochemical method was developed *in vitro* to simulate the cleavage of the azo bond (N=N) by oxidation reactions. The chemical splitting of the dye was performed by oxidation using chemical electrodes in solution and with S9. The equipment was capable of donating electrons from the solution, causing oxidation reactions on the dye. The mutagenicity of the Sudan III will be evaluated with the Salmonella/microsome using the strains TA98 and TA100, with and without S9. For the Sudan III metabolites, the test will be performed just in the absence of S9. **Results:** The experiment involving the electrochemical oxidation of the Sudan III dye showed that the dye was oxidized at 5,5X10⁻⁵ mol L⁻¹ at oxidative potential + 1,5 V for 40 minutes, DMSO was used as solvents. The oxidation was characterized by the reduction of colour intense and by the modification of the spectrophotometric profile. The Ames test is still being performed. **Conclusion:** During the electrochemical oxidation of Sudan III a radical was probably formed, assuming that the mutagenicity may vary when compared to the original dye. On the obtained solutions, the colour became lighter as the dye was oxidized, this may indicate that the bond (N=N) was cleaved, and possibly aromatic amines were formed.

Keywords: Sudan III; mutagenicity; electrochemical oxidation; CYP-450.

TX 26 - THE IMPORTANCE OF THE OXIDATION IN THE BIOTRANSFORMATION OF AZO DYES DISPERSE RED 1, DISPERSE RED 13 AND DISPERSE ORANGE 1

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Introduction: Azo dyes are aromatic compounds with one or more -N=N groups, represent the largest class of dyes applied in the textile, food and cosmetic industry. The release of these compounds into the environment is undesirable, not only because of their color but also because many azo dyes and their breakdown products are toxic and/or mutagenic to life. Mammalian enzymes in the liver and other organs can catalyze the reaction of azo bond and the nitroreduction of the nitro group; however, it has been shown that the intestinal microbial azoreductase and nitroreductase play a more important role in this type of metabolism. In both cases, if N-hydroxylamines are formed they are capable of causing DNA damage. The dyes can be oxidized also to N-hydroxyderivatives by P450 enzymes. The N-hydroxy radicals can be further acetylated by enzymes such as the O-acetyltransferase generating electrophilic nitrenium ions capable of reacting with DNA to form adducts. However, little is known about the direct oxidation of the azo dyes by the CYP isoforms. The azo dyes Disperse Red 1, Disperse Red 13 and Disperse Orange tested positive for the Micronucleus Test (with HepG2 and lymphocytes) and Salmonella mutagenicity assay. However, for the Ames test in the presence of S9, a reduction in the mutagenic activity of the dyes was observed. **Objectives:** To elucidate oxidation mechanism by S9 of the dyes Disperse Red 1, Disperse Red 13 and Disperse Orange 1. **Materials and Methods:** The oxidation by S9 of Disperse Red 1, Disperse Orange 1 and Disperse Red 13 dyes in solution was monitored by measurement of the absorbance changes at specified time intervals using a Hewlett Packard 8453 diode array spectrophotometer operated in the 200-1200 nm wavelengths, after the electrochemical oxidation and S9 reaction. The Salmonella/microsome assay with the strain TA98 and YG1041 with and without S9 is in progress for the evaluation of the mutagenic activity of oxidation products. **Results:** The obtained solutions incubated with S9 showed that the colour of the dyes became lighter as the dye was oxidized by S9, also it may indicate that the bond (N=N) was cleaved, because of the reduction on the spectral absorbance. **Partial Conclusions:** The chemical studies showed that the azo bond was cleaved after oxidation by S9.

Keywords: Disperse Red 1; Disperse Red 13; Disperse Orange 1; oxidation; Ames test.

TX 27 - TRACE ELEMENTS DETERMINATION IN BLOOD BY DYNAMIC REACTION CELL INDUCTIVELY COUPLED PLASMA MASS SPECTROMETRY (DRC-ICP-MS)

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Introduction: The increased interest in the role of trace elements content in biological fluids has influenced the research of analytical techniques particularly suitable to this purpose. The low levels of many of such elements in the human fluids and the complexity of the matrix created physical and

spectral (polyatomic) interferences. The Dynamic Reaction Cell™ (DRC) technology applied to the inductively coupled plasma mass spectrometry (ICP-MS) proposes another opportunity for the resolution of the interferences without loss of sensitivity. A fast biomonitoring method for the determination of Cr, V, Tl, Pb, Cu, Al, Zn and Co in whole blood by using a direct injection of the diluted samples in DRC-ICP-MS is presented. **Objective:** Provide a fast, sensitive and reliable method for the quantification of trace elements in blood by using a DRC-ICP-MS. **Materials and Methods:** Before analysis by matrix-matched calibration, samples were diluted 1:50 v/v in a solution containing HNO₃ 0.5% and Triton X-100® 0.01%. **Results:** Due to spectroscopic interferences on the determination of ⁵²Cr and ⁵¹V, the use of DRC-ICP-MS was fundamental. The reaction cell conditions (RPq and gas reaction flow), using ammonia (NH_{3(g)}) as reaction gas, were evaluated for the elimination of ArC⁺ and ClOH⁺ interferences on chromium at mass 52 and OCl⁺ on vanadium at mass 51. Rhodium, indium and yttrium were evaluated as possible internal standards. Data validation are provided based on the analysis of blood samples from the trace elements intercomparison program operated by the *Institut National de Sante' Publique du Quebec*, Canada (QMEQAS07B06). Results showed that the use of NH_{3(g)} eliminated the interferences and provided lower limits of detection. **Conclusion:** Need the use of DRC for the direct determination of chromium and vanadium in blood. For Tl, Pb, Cu, Co, Al and Zn determination was necessary the ICP-MS mode only.

Keywords: DRC-ICP-MS; interferences; blood samples; trace elements; rapid determination.

Financial support: FAPESP.

TX 28 - ZINC PHTHALOCYANINE TETRASULFONATED AND PHOTODYNAMIC THERAPY: EFFECTS ON MITOCHONDRIA AND SYNAPTOSOMES ISOLATED FROM RAT'S BRAIN CORTEX

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In this study was investigated the phototoxic effect of Zinc Phthalocyanine Tetrasulfonated (ZnPcS4) in isolated brain cortex mitochondrial and synaptosomes from male *Wistar* rats. ZnPcS4 has been used in treatment of tumors of brain in photodynamic therapy. In the dark study (with the dye and without irradiation), the ZnPcS4 did not change mitochondrial parameters evaluated (state 3 and 4, of respiratory control ratio (RCR) and mitochondrial membrane potential) at concentrations until 10 µmoles/L. At 15 and 25 µmoles/L, it was observed that the dye showed a dose dependent: inhibitory effect on state 3 of respiration and RCR. The state 4 and mitochondrial membrane potential were not altered for the dye in this concentrations. When the mitochondria was incorporated with 5 µmoles/L of ZnPcS4 and irradiated in the 600 and 1800mJ of potency it was observed and inhibitory effect on state 3 of respiration, reaching the maximum effect with 1800mJ of potency. On 200mJ irradiation was unable to promote any effect. The similar conduct was observed when was analyzed the state 4 of respiration. The mitochondrial irradiation in the presence of dye on the potency 600 and 1800mJ, provoked significant increase in the oxygen consumption by mitochondria, this effect can be considered like one uncoupled (oxygen consumption enlarging without ADP). It was observed with appreciation on the mitochondrial RCR, leading to values very close to 1, is important to say that in this conditions the mitochondrial are uncoupled. Similar to respiratory parameters, the membrane potential was affected since to irradiation of 600mJ, that was enough to inhibit formation of potential. One of the possibilities for occurring the membrane potential collapsed, is that this fact occurs by the mitochondrial membrane potential (MPT). It is can be inhibit by cyclosporin A a immunomodulator. Our results demonstrated that the cyclosporin A showed be effective to inhibit the mitochondrial membrane potential collapsed demonstrating that on our experimental conditions, the effects phototoxic over membrane potential can be mediated, at least specifically, by the MPT induction. Concluding, our resulted demonstrated that in the brain cortex mitochondrial and synaptosomes isolated are intensively affected when was submitted by the ZnPcS4 photodynamic effect *in vitro*, with inhibition of the energetic metabolism.

Keywords: Photodynamic therapy; mitochondria; synaptosome; Mitochondrial Membrane Potential.

Financial support: FAPESP.