QT.01 - THE EFFECTS OF CAMPTOTHECIN IN *TRYPANOSOMA CRUZI* PROLIFERATION AND ULTRASTRUCTURE SUGGEST ITS PARTICIPATION IN DNA REPAIR

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Trypanosoma cruzi contains an unique mitochondrion with an enlarged portion termed kinetoplast where is harbour the kDNA. The nucleus presents compartments, as the nucleolus, and a condensed chromatin associated with the nuclear envelope. The topological state of the DNA is modulated by topoisomerases, which act by reverting supercoilings of the double stranded DNA during replication, transcription and repair, thus representing an important target in chemotherapy. According to this, camptothecin, a topoisomerase type I inhibitor, has been widley used in cancer research, once it is effective against cell proliferation by DNA damage that leads to DNA repair or apoptosis. Our previous works showed that camptothecin strongly inhibits the proliferation of *T. cruzi* epimastigote and causes a remarkable unpacking of nuclear chromatin. In this study, we compared the proliferation between wild-type cells and protozoa over-expressing the *TcRad51* gene, that is involved in DNA repair, after removal of the drug from the culture medium. Thus, cells were cultivated in medium containing different drug concentrations (1, 5, 10 and 50 µM) and samples were collected after each 24 hours for counting on Neubauer's chamber or for processing to transmission electron microscopy. Our data showed that removal of camptothecin, after treatment with 1µM drug for 24h, restored cell proliferation. With respect to the transfected cells, protozoa over-expressing Rad51 gene presented a higher resistance to camptothecin, since in this case the number of viable protozoa was two fold higher when compared to the wild-type cells. These results suggest that DNA repair mechanisms are activated, with participation of the RAD51 enzyme, after cell treatment with camptothecin. Supported by CNPq.

QT.02 - TETRAZOLIUM SALTS BASED METHODS AS TOOLS FOR QUANTITATIVE EVALUATION OF ANTI-PARASITE CHEMOTHERAPY.

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Genome projects brought much information about metabolic pathways and enzymes that are good targets for neglected diseases chemotherapy, increasing the need for sensitive and reliable high throughput screening of drugs in parasites. The widely used tetrazolium salt MTT, form insoluble formazan crystals that need to be solubilized. In contrast, the second generation, MTS and XTT are reduced by metabolically active organisms, producing hydrosoluble colored formazans. The water soluble tetrazolium methods require an intermediate electron transfer reagent, 5-methyl-phenazinium methyl sulfate (PMS), that is reduced by agents produced by viable cells, NADH and NADPH. The reduced PMS, transfer its electrons to the tetrazolium salts, producing water soluble reduced formazan, proportional to viable cells number. We improved the detection limit by removing growth culture medium, and compared MTT, XTT and MTS tetrazolium assays using a density curve of *T.cruzi* epimastigotes in buffer saline/glucose 10 mM. The MTS/PMS method was faster and displayed the highest sensitivity, detecting 10^5 epimastigotes/ml (n=3). To validate the MTS/PMS method for anti-parasite chemotherapy studies, epimastigotes were treated with oligomycin, followed by evaluation of mitochondrial potential with Rho123 (2µg/ml) by flow cytometry. Oligomycin at 10µg/ml affected 72.7% ± 7 (n=2) epimastigotes population, compatible with the MTS/PMS method that displayed 67% ± 7 reduction of viable cells. The IC_{50} for the drug amiodarone in epimastigotes was 10.5 ± 2 (n=2) corroborated the IC_{50} in the literature, 9 µM. The MTS tetrazolium method was also tested in Giardia lamblia and demonstrated a detection limit of 10^5 trofozoites/ml (n=2). The MTS/PMS tetrazolium method was also used to evaluate the effect of the anti-helminth drug albendazol in *Caenorhabditis elegan*. This is the first report of the MTS/PMS tetrazolium based method for Giardia, helminthes and *T.cruzi*. The MTS/PMS reduction is associated not only with mitochondria function, but also with cytoplasm and with non-mitochondrial membranes.

Financial support: CNPq and FAPERJ.

QT.03 - EXPLORING PLANTS AND FUNGI FOR DRUG DISCOVERY AND DEVELOPMENT AGAINST CHAGAS DISEASE

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One of the major challenges for drug discovery and development from natural products, especially in the third world, is the lack of standardization, reproducibility and traceability of the produced data. The Program of Technological Development for Health (PDTIS) of the Oswaldo Cruz Foundation (FIOCRUZ) has stimulated building and consolidation of Technological Platforms (TP) in order to improve research and development of health products with quality. The Bioprospecting TP (RPTA 10A) has more than 8,000 plant and fungi crude extracts deposited. All collected plants have a voucher deposited at the Herbarium BHCB and all fungi sample were deposited in the culture collection of UFMG. The extracts were concentrated, dissolved in DMSO at 20mg/ml and as a microtiter film at 10ug/well and kept at -20^{0}C. All procedures are guided by the Standard Operating Procedure of RPT10A. With the aim to detect bioactive extracts against *T.cruzi,* the crude extracts at microtiter plates were *in vitro* assayed at the Chagas Disease TP (RPT11F) using *T. cruzi* (Tulahuen strain) expressing the *Escherichia coli* beta-galactosidase gene (Buckner et al. 1996). Briefly, trypomastigotes were left for 2h to infect L929 fibroblasts seeded in tissue culture micro plates. After 48h, the medium was discarded and replaced by fresh medium and test compounds. After 7 days, chlorophenol red beta-D-galactopyranoside was added to the plates, incubated overnight and the absorbance measured at λ_{570} nm. Benznidazole at its IC_{50} (1µg/mL or 3.8µM)) was used as positive control. The results are expressed as percentage of *T.cruzi* growth inhibition. Bioactivity ≥ Benznidazole was observed in 70 extracts (0.8%). The IC_{50} of 20 extracts showed 4 from plant and 2 from fungi with IC_{50} varying from 1.4 to 6.6µg/mL. The remaining 14 extracts were cytotoxic for the L929 fibroblasts.The results found are encouraging to explore plants and fungi as source of anti-*T.cruzi* drugs. Supported by PDTIS/FIOCRUZ, FAPEMIG and CNPq

QT.04 - INCREASE OF REACTIVE OXYGEN SPECIES BY DESFERRIOXAMINE DURING EXPERIMENTAL CHAGAS' DISEASE

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Oxidative stress is common in inflammatory processes associated with many diseases including Chagas' disease. Work undertaken by our group has demonstrated that within the interaction *T. cruzi*/host in a murine model, changes in iron stores may be a factor favorable to the host. The antioxidant activity of DFO (Desferrioxamine) has already been proven, but until now no work is proposed to evaluate the antioxidant capacity of this iron chelator associated with *T. cruzi* infection. Based on this, this study aims to evaluate the oxidative stress together with components of the antioxidant system in animals treated or not with DFO through the measurement of thiobarbituric acid reactive species (TBARS), protein carbonyl (PC), and serum nitric oxide (NO) and antioxidant defenses through the measurement of superoxide dismutase in serum (SOD) and total glutathione (GT) in liver were determined on 0, 7, 14 and 21 days post-infection (dpi). Swiss mice (n=48) infected or not with the Y strain of *T. cruzi* were divided into four groups: (C) control, (I) infected, (DFO) treated with DFO and (IDFO) infected and treated with DFO. Among the five measurements performed treatment with DFO decreased GT and increased SOD activity in IDFO group. We observed an increase in NO production at 21 dpi and PC at 7 dpi in IDFO group. An increase in TBARS levels at 7 and 21 dpi for the animals of I and IDFO group were observed, respectively. Within the parameters used to evaluate the oxidative status, DFO has a capacity to provide protection while increasing the production of ROS, indicating that the mode of action of the drug involves a positive contribution to the host together with an effect that is not beneficial to the parasite. Supported by FAPEMIG (PPM, Redes Toxifar and Bioterismo), CNPq and UFOP.

QT.05 - EVALUATION OF TRYPANOCIDAL AND CITOTOXICITY ACTIVITY OF N-ALKYL STERS OF GALLIC ACID IN *T. CRUZI* AND HEPG2 CELLS

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Gallic acid (3,4,5-trihydroxybenzoic acid) is a plant phenol obtained by the hydrolysis of tannins. Esters of gallic acid have different uses, such as, antioxidant additives in foods and cosmetics, besides having antibacterial activity, as a synergistic action with antibiotics, antifungal and antiviral activities. In this study we evaluated the activity of gallic acid and nonyl gallate against the *Trypanosoma cruzi* parasites, and the cytotoxicity of nonyl gallate in HepG2-hepatoma cell line, which is used as a model for studying the human liver. The nonyl gallate was obtained by structural modifications of gallic acid. The substances were tested for trypanocidal effect on *T. cruzi* (Y strain) epimastigote forms by the MTT technique, and calculating the citotoxicity index (IC_{50}). Because of the higher activity of nonyl gallate (2.0 µM) when compared with benznidazole (33.6 µM), respectively, both molecules were also evaluated in HepG2 cells. The nonyl gallate citotoxicity in HepG2 cells presented a IC_{50} of 57.67 µM, which was much higher when compared with the IC_{50} in *T. cruzi* (safety index, SI = 28.8). In contrast, gallic acid showed no toxic effects in *T. cruzi* ($IC_{50} \geq$ 300 µM), showing that the esterification on this molecule can produce a very potent trypanocidal effect. These results show that the nonyl gallate would be an interesting molecule for further studies *in vivo* to treat Chagas' disease.
Supported by Capes, FUNDUNESP and FAPESP.

QT.06 - *TRYPANOSOMA CRUZI* ABC TRANSPORTER: GENE STRUCTURE AND ROLE IN DRUG RESISTANCE

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Benznidazole (BZ) therapeutic failures are widely documented in Chagas disease and have been associated to variations in the drug susceptibility of *Trypanosoma cruzi* strains. DNA microarrays indicated that one ABC transporter gene (*Tc*ABCG) was overexpressed in BZ-resistant strains, as compared to susceptible strains. This was confirmed by real time RT-PCR. *Tc*ABCG shares high similarity with ABCG genes of *Leishmania* and *T. brucei*. The goal of this study was to further characterize the association of *Tc*ABCG with BZ resistance and analyze the structural characteristics of this gene in *T. cruzi* strains. The effect of known ABC inhibitors on the drug sensitivity was investigated. In four analyzed strains 20 µM and 30 µM verapamil decreased the IC_{50} to BZ by 38% and 50%, respectively. On the other hand, cyclosporin-A and fumitremorgin-C had no effect. *T. cruzi* strains will be transfected with the *Tc*ABCG gene to verify if overexpression of this protein increases BZ resistance. Single Nucleotide Polymorphisms (SNPs) in ABC transporter genes were associated with alterations of gene expression and/or functionality. The *Tc*ABCG gene of six strains was cloned and sequenced (six clones of each strain). Esmo and Non-Esmo haplotypes of CL Brener *Tc*ABCG display 41 nucleotide variations, of which 28 are synonymous. *Tc*ABCG of two DTUII strains (one susceptible and one resistant) shows predominance of the Esmo haplotype and 2 synonymous SNPs. In three DTUI BZ-resistant strains *Tc*ABCG exhibits alternation of Esmo and Non-Esmo variations and a total of 17-20 SNPs. Most of the SNPs are identical in the three strains and occupy the same positions. The structure of *Tc*ABCG gene in one DTUV strain revealed at least three different "allele" types. Type I and Type II display, respectively, the Esmo and Non-Esmo haplotypes, whereas Type III displays Esmo stretches followed by Non-Esmo stretches, suggesting intragenic recombination. Support: FAPESP; CAPES; CNPq.

QT.07 - EVALUATION OF THE THERAUPETIC PERSPECTIVES OF THE *L*-THIAZOLIDINE-4-CARBOXYLIC ACID, A PROLINE ANOLOGUE, ON MICE INFECTION BY *TRYPANOSOMA CRUZI.*

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Trypanosoma cruzi is dependent on proline for a variety of processes such as energy metabolism, host cell invasion, differentiation and resistance to osmotic, metabolic and oxidative stress. *L*-thiazolidine-4-carboxylic acid (T4C), a proline structural analogue that inhibits the proline uptake, diminishes the ability of *T. cruzi* to deal with several of the stresses that the parasite naturally undergoes throughout its life cycle. From these results, we hypothesized that T4C interferes with proline-dependent mechanisms of resistance to stress conditions, reducing the virulence and supporting a role for proline in the *in vivo* infection. Experiments conducted to test this hypothesis showed that the parasitemia peak was reduced in 49% in the infected mice that were treated with a unique dose of T4C (100 mg/Kg). In addition, histological analysis of lung, heart, bladder, skeletal muscle, spleen and intestine revealed that only the latter showed a reduction (90,3 %) of the number of amastigotes nests when the animals were treated with 150 mg/kg. These results were confirmed by quantitative PCR. Besides, the toxicity of T4C was also evaluated. T4C was inoculated i.p. (0, 50 or 200 mg/kg) in a single dose, and the body weight of animals were followed up for 40 days. At day 12 after treatment it was observed that mice treated with 200 mg/kg had their weight reduced in 6.3 % ($p<0.05$), and the survival of this group was diminished in 20% with respect to control. When T4C was administrated daily for 10 days (200 mg/Kg), treated animals showed a progressive weight reduction and only 60% of the animals survived the treatment when compared to the controls. The present results suggest that T4C-treatment contributes to reduce the virulence of *T. cruzi* infection, in accordance to our hypothesis, but it was toxic in doses over 150 mg/kg.

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QT.08 - THE ANTI-*TRYPANOSOMA CRUZI* EFFECT OF DRUGS USED TO TREAT PSYCHIATRIC DISORDERS

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The search for new therapeutic alternatives against *Trypanosoma cruzi*, the etiological agent for Chagas´ disease, is a main issue due to the serious drawbacks of currently used drugs Nifurtymox and Benznidazole (high toxicity, low efficiency and emergence of resistant strains). Several amino acids have several roles in the biology of trypanosomatids, participating in the energetic metabolism, host cell invasion, resistance to different stresses and differentiation. In previous work we demonstrated that *T. cruzi,* metabolizes glutamate and GABA. In this study, we analyzed the effects of interfering with the metabolism of these amino acids by using the GABA analogue 3-(acetylamino)-1-propanesulfinic acid (Acamprosate), a drug currently in use to treat alcohol dependence. The effect of this drug on epimastigotes growth was evaluated by using concentrations of Acamprosate ranging from 0.25 to 150 μM, showing a dose-response effect on epimastigote growth with an IC_{50}= 0.9±0.043 μM. We also evaluated the possible interferences of Acamprosate with the ability of the parasite to resist stressing conditions like high temperature, acidic pH, nutrient starvation and oxidative stress. We observed a significant diminution of parasite survival ($p<0.05$) when treatment was combined with nutrient starvation or oxidative stress. Finally, the trypanocid effect of this drug on infected mammalian cells (CHO-K_1) was evaluated. It was observed that Acamprosate diminished 65 % the trypomastigote bursting when cells were treated with non-toxic concentrations of this drug (between 0.25 and 150 μM). All these data suggest that Acamprosate may act in the GABA-glutamate metabolism and interferes with mechanisms of resistance to stress that *T. cruzi* naturally faces along its life cycle. Finally, Acamprosate could be an interesting therapeutic drug against *T. cruzi* infection by itself or if combined with others.

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QT.09 - EVALUATION OF EFFECTS OF AN ANTIPSYCHOTIC DRUG (LEVOMEPROMAZINE) IN *TRYPANOSOMA CRUZI* FORMS

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Chagas' disease, caused by the protozoan *Trypanosoma cruzi*, is a relevant parasitic disease in the Americas. The current chemotherapy relies on Nifurtimox and Benznidazole, which present serious drawbacks (high toxicity, low efficiency and emergence of resistant strains). Trypanothione reductase (TR), an enzyme involved in the resistance to oxidative stress, constitutes an attractive target for chemotherapeutic research in relation to Chagas' and other trypanosomatid-caused diseases since this enzyme is present in the parasite but not in mammalian hosts. In this work, we investigate the effect of phenotiazine (levomepromazine), a drug currently in use to treat schizophrenia, which showed a potent inhibitor activity for (TR). Levomepromazine interfered on epimastigotes growth. Growth curves were performed using drug concentrations ranging from 75 to 1000 μM, showing a dose-response effect on epimastigote growth with an IC_{50}= 405±2.43 μM. We also evaluated the possible interferences of levomepromazine with the ability of the parasite to resist stressing conditions like high temperature, acidic pH, nutrient starvation and oxidative stress. We observed a significant diminution of parasite survival ($p<0.05$) when treatment was combined with nutrient starvation or oxidative stress. Also we evaluate the trypanocid effect of this drug on infected mammalian cells (CHO-K_1). It was observed that levomepromazine diminished 75% the trypomastigote bursting when cells were treated with non-toxic concentrations of this drug (between 75 and 1.000 μM). All these data suggest that levomepromazine may inhibit the TR function, interfering with essential biological functions of this enzyme, such as mechanisms involved in the resistance to stress that *T. cruzi* naturally faces along its life cycle. The fact that levomepromazine selectively inhibit the parasite infection *in vitro*, with little compromise of the viability of host cells is promising to propose the use of this drug (already applied to the treatment of a psychiatric disorder), to treat Chagas´ disease.
Supported by CNPq and FAPESP.

QT.10 - 243 2 BIOTHERAPIC 17DH OF *TRYPANOSOMA CRUZI*: EFFECT *VERSUS* AGE

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Trypanosoma cruzi infection in mice is well known, making this is a good model for understanding the effect of ultra-diluted medicines. The objective of this work was to evaluate the effect of biotherapic-17DH-*T. cruzi* at different ages of mice infected with the parasite. In a double-blind, controlled, randomized by draw assay, 109 Swiss male mice, 4 or 8 weeks old were divided into groups: control treated with water-alcohol solution-7% (CI-4=34 animals/CI-8=21 animals) and treated with biotherapic-17dH (BIOT-4=33 animals or BIOT-8=21 animals). It were used 1400 trypomastigotes intraperitoneally, *T. cruzi Y*-strain. The biotherapic-17dH was prepared by adding 0.9mL of blood with *T. cruzi* (10^7 trypomastigotes/mL) to 9.1mL of distilled water in laminar flow. The following dilutions were made in water-alcohol solution-7%. Microbiological control and biological risk *in vivo* were performed. Treatment: 0.2mL/20 consecutive days/oral route. Parasitological parameters were compared using the program Statistica-7.0. Work approved by the Ethics Committee for Animal Experimentation/UEM. Parasitemia (0.5718) and mortality (0.9136) did not differ between treated and control group in 4 weeks animals. For animals of 8 weeks, the treated group had a higher parasite peak ($p=0.0424$) and total parasitemia ($p<0.005$) than control. The mortality started later in BIOT-8, but was not significantly different from CI-8 ($p=0.8815$). Considering the parasitemia of the 8th day of infection, animals in both group CI-8 and BIOT-8, could be classified as: high, medium and low sensitivity to infection. The difference between BIOT-8 and CI-8 has been identified in animals classified as high and especially medium sensitivity to infection ($p=0.001$ and $p=0.041$). Mortality for each mentioned extract followed the same pattern observed for all BIOT-8 group. Ultra diluted drug has different effect on 4 or 8 weeks old mice and on the group of animals with 8 weeks there is individuality of response to ultra diluted medication.
Supported by PROAP CAPES

QT.11 - Synergistic effect of Semicarbazones/Thiosemicarbazones and Benznidazole on *Trypanosoma cruzi*

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A specific treatment, with more efficiency and less toxicity for neglected diseases, as Chagas' disease and Leishmaniasis, is the main objective of several studies in this field. It has been demonstrated a synergistic effect when reference drugs and new compounds have been associated and used against those pathologies. In a previous study we evaluated the activity of three thiosemicarbzazones (HTIO, 2MEOTIO, VATIO) and two semicarbazones (OVASEMI, 2MEOSEMI) against *Trypanosoma cruzi* (Y strain) and *Leishmania amazonensis* (LTBOO16 strain) with promising results. Based in those data, in the present work we assayed the activity of those compounds associated to Benznidazole, in several concentrations (from respectives LDs50/24 hours to decreasing dilutions), against *Trypanosoma cruzi* trypromastigote bloodstream forms using a viability cellular assay (MTT) and it was observed a significant **increase in** the activities in 24 hours of incubation. Among those thiosemicarbazones and semicarbazones plus Benznidazole, HTIO, 2MEOTIO and VATIO **showed a LDs50** significantly lower, while for 2MEOSEMI no difference was observed as compared to semicarbazones alone, which is interesting considering that the last one was the most active when assayed alone against *T. cruzi*. Concerning semicarbazone, OVASEMI presented only slight diminishing in the LDs50. Further experiments are been realized with a larger concentration range for the reference drug (Benznidazole) and the drugs test.
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QT.12 - TRYPANOCIDAL ACTIVITY AND SELECTIVITY OF NEW FUNCTIONALISED CYNAMIL-*N*-ACYLIDRAZONES

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Chagas disease, caused by *Trypanosoma cruzi*, is a widely distributed debilitating human illness, representing an important health problem. The available chemotherapy for this disease is unsatisfactory; therefore there is an intense effort to find new drugs (Soeiro et al., 2009). Natural products are a rich potential source of drugs since they contain a countless quantity of molecules with a great variety of structures and pharmacological activities (Hoet et al., 2004). Polyphenolics have been known to exert diverse biological effects and those containing a cinamoyl moiety, like caffeic acid, were described as potential antiparasitic agents (Hamilton et al., 2005). In this context, new α,β-unsaturated N-acylhydrazones were designed as trypanocidal candidates by the molecular hybridization of caffeic acid with an acylhydrazone derivative, a potent cysteinyl protease inhibitor (Rodrigues et al., 2002) in order to enhance the trypanocidal activity by the incorporation of the acylhydrazone subunit. Here we investigated the *in vitro* effect of α,β-unsaturated N-acylhydrazones on bloodstream trypomastigotes of *T. cruzi* and the possible toxic effects of the compounds on mammalian host cell (peritoneal macrophages). Six of them showed activity (IC_{50}/1d) at concentrations below 180 µM, being the most active **HD24**, with IC_{50} = 59.3 µM. Our results demonstrate the promising activity on the derivatives against *T. cruzi*, especially **HD24**, justifying further others assays to elucidate the mechanism of action of these compounds and the *in vivo* activity. Supported by FAPERJ, CAPES and CNPq

QT.13 - ALTERATION OF *Trypanosoma cruzi* CELL MEMBRANE INTEGRITY INDUCED BY ELATOL

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Chagas disease, caused by the protozoan *Trypanosoma cruzi*, represent an important public health problem. The available drug for treatment of this infection is ineffective during the chronic phase of disease, causing serious side effects. In this context, research groups are focusing their studies on biological effects of compounds extracted from shellfish, fish and plants to find new medicines. Recently we described the antiproliferative effect of elatol, a secondary metabolite extracted from red macroalgae *Laurencia dendroidea*, present in Brazilian coast in trypomastigote forms of *T. cruzi*. Thus, the goal of this study was to evaluate the alterations caused by elatol on cell membrane plasma integrity and mitochondrion function of trypomastigote forms of *T. cruzi* in a way to try to elucidate possible mechanism of elatol action. For this, trypomastigotes were treated with elatol at concentrations 0.25, 0.5, 1.0 and 2.0 µg/mL and evaluated by flow cytometry using rhodamine 123 and propidium iodide (PI). It was observed a positive staining for propidium iodide, indicating a possible alteration of cell membrane integrity and no depolarization of mitochondrial membrane. These results confirm the ultrastructural alterations caused by elatol in trypomastigote forms of *T. cruzi* described in a previous study made by our group. Therefore, it is possible to suppose that the tripanocidal action of elatol maybe involve its effect on the plasma membrane of the parasite leading cell death.
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QT.14 - MONITORING OF CURE BY PCR FOR MICE INFECTED WITH *TRYPANOSOMA CRUZI* AND TREATED WITH DIFFERENT BENZNIDAZOLE SCHEDULES

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The polymerase chain reaction (PCR) was used to monitor cure of mice infected with *Trypanosoma cruzi* and submitted to treatment with benznidazole (BZ). For this, 21 mice, male, 28 days were intraperitoneally inoculated with $1x10^4$ blood trypomastigotes (BT)/animal of *T. cruzi* Y strain (DTU TcII). Ten were orally treated with BZ (Rochagan, Roche ®) 100mg/kg/day and 11 constituted the not treated control group (NT). Of the ten treated animals, five received the drug for 20 consecutive days (TBZ-20) and five for 60 days (TBZ-60). From the 4th day of infection (di), parasitemia and cumulative mortality were recorded. In assessing the cure, flesh blood examination (FBE), blood culture (BC) and PCR 30 days after treatment were used. Further blood samples for PCR were collected at different times post-treatment: two and 15 days (TBZ-20 group), and six months (TBZ-60). The mortality rate was 0% for the treated groups and 100% (33 di) for the NT group. Cure rates were 40% (2 / 5) and 100% (5 / 5), respectively for TBZ-20 and TBZ-60 groups. TBZ-20 group was 100% agreement between the results of FBE, BC, and PCR performed with two, 15 and 30 days post-treatment. TBZ-60 group was also 100% agreement between the FBE, BC and PCR performed six months after treatment. However, in all animals TBZ-60, *T. cruzi* DNA was detected in samples collected 30 days after treatment. These results were confirmed with further DNA extractions and repetition of reactions. TBZ-20 animals showed cure rate similar to that of literature and animals TBZ-60 an index higher, which reclassify the Y strain as sensitive to BZ. Explanations for the unusual PCR results (positive 30 days and negative 6 months post-treatment) for the TBZ-60 group are searched while performing serology. Supported by CNPq

QT.15 - BIOLOGICAL EFFECTS OF A CYSTEIN-RICH SECRETORY PROTEIN ISOLATED FROM THE *CROTALUS VIRIDIS VIRIDIS* SNAKE VENOM ON *TRYPANOSOMA CRUZI* TRYPOMASTIGOTES.

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Snake venoms have been used as pharmacological tools for drug development. Treatment of Chagas' disease, caused by *Trypanosoma cruzi*, is based on drugs that exhibit toxic effects and limited efficacy. Therefore the search for new drugs is a lining research to be exploited. This work shows the purification of a novel protein belonging to cysteine-rich secretory protein (CRiSP) family, isolated from *Crotalus viridis viridis* (Cvv) snake venom, and its effects over *T. cruzi* and murine muscles. The crude venom extract was loaded onto to a reverse phase analytical (C8) column using a high performance liquid chromatographer. A linear gradient of water/acetonitrile with 0.1% trifluoroacetic acid was used. The peak contained the isolated protein (confirmed by SDS-PAGE and mass spectrometry) was collected, lyophilized, ressuspended in distilled water and its protein content measured. The isolated protein exhibited a molecular mass of 24,893.64 Da and the MS/MS-derived sequences are nearly identical to the protein Catrin and related snake venoms CRiSPs. Trypomastigotes obtained from LLC-MK_2 cells cultures were incubated with 0.6 to 4.8 μg/ ml of the protein, and the effect on the cells lysis was evaluated by counting with a Neubauer chamber. The treatment caused a significant reduction (36% to 88%) in the parasites living after 24h, with a LD_{50} of 0.93 μg/ ml. The mainly morphological alterations observed by transmission electron microscopy were at the plasma membrane, with blebs exhibiting different shapes and sizes, and in lysosomes related organelles, which were often enlarged. No myotoxicity was observed in isolated murine gastrocnemius muscles treated with 0.93 μg/ ml, where only basal creatine kinase release was detected. This work presents, for the first time, the purification of a protein from Cvv venom belonging to the CRiSP family with trypanocidal and no myotoxical effects, and which could be a promissory compound to Chagas' disease treatment.
Suportted by CAPES, CNPq and FAPERJ.

QT.16 - ANTITRYPANOSOMAL ACTIVITY OF DIFFERENT SYNTHETIC COMPOUNDS AGAINST EPIMASTIGOTE FORMS OF *Trypanosoma cruzi*

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Chagas disease is present in almost all Latin American territory and represents a serious health problem, by reaching millions of people and lack of effective and appropriate treatment. Benznidazole, the only available drug for the treatment of this infection is not effective in chronic phase, in addition to having high toxicity. The search for new drugs that are able to cure or even prevent the disease progress is necessary and a priority. The purpose of this study was to report the antitrypanosomal activity from synthetic compounds, being 8 nitroketene *N,S*-arylaminoacetals and 8 2,3-disubstituted-quinoxaline derivatives. In 24-well plates, $1x10^6$ parasites were inoculated in LIT medium supplemented with 10% of fetal bovine serum in different concentrations of compounds. After 96 h of incubation at 28°C the parasites were counted and the growth inhibition determined. The results showed that 13 of the 16 substances tested showed activity against epimastigotes at concentrations below 50 μg/mL, and the most effectives were 6-methoxy-3-(methylsulfonyl)-2-phenylquinoxaline, *N*-[1-(methylthio)-2-nitroethenyl]-benzenamine and 4-fluoro-*N*-[1-(methylthio)-2-nitroethenyl]-benzenamine with IC50 of 0,34, 2,40 and 4,50 μg/mL, respectively. However, *in vitro* and *in vivo* studies are necessary to elucidate the mechanism of action of these compounds.
Supported by CAPES, CNPq, FINEP, PRONEX/Fundação Araucária, FAPESP.

QT.17 - DEPOLARIZATION OF MITOCHONDRIAL MEMBRANE INDUCED BY EUPOMATENOID-5 ON TRYPOMASTIGOTE FORMS OF *Trypanosoma cruzi*

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Chagas disease, an infection caused by the protozoan *Trypanosoma cruzi,* is present in almost all Latin American territories. Since it has been discovered, the pharmacological treatment for this infection is unsatisfactory due to limited efficacy and toxic side effects. Therefore, the study for new pharmacological agents is a priority. Eupomatenoid-5 isolated from leaves of *Piper regnellii* var. pallescens has already been described as a tripanocidal agent, making this compound a new alternative for Chagas disease treatment. Thus, the goal of this study was to investigate a possible mechanism of action of eupomatenoid-5 through the study of membrane plasma integrity and mitochondrion function of trypomastigote forms of *T. cruzi*. Previously, it was found that the EC_{50} of eupomatenoid-5 on trypomastigote forms was 100 µg/mL. Then, the trypomastigotes were pre-treated with eupomatenoid-5 at concentrations of 50 and 100 µg/mL for 2 hours and analyzed by flow cytometry using rhodamine 123 and propidium iodide (PI). The results showed depolarization of mitochondrial membrane in all concentrations tested. However, it had no effect on staining for propidium iodide in the tested concentrations, indicating that the cell membrane integrity was not changed. On this basis, our results show that the trypanocidal action of eupomatenoid-5 may be associated with mitochondrial dysfunction which could lead cell death. Further studies to elucidate better the mechanism of action of eupomatenoid-5 on *T. cruzi* death are been performed.
Supported by CAPES, CNPq, FINEP, PRONEX/Fundação Araucária.

QT.18 - DESFERRIOXAMINE, AN IRON CHELATOR, DECREASES MORTALITY AND PARASITEMIA IN *Trypanosoma cruzi* INFECTED MICE THROUGH DIRECT ACTION ON PARASITE

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Although DFA is known to reduce the intensity of *T. cruzi* mice infection, the mechanism underlying this effect is still unclear and may involve host and parasite factors. To investigate the impact of DFA on mice disease outcome, on *T. cruzi* biology and on host biomarkers, disease and parasitological studies were performed. The effect of DFA in disease outcome was verified by parasitemia and mortality studies as well as host iron metabolism, blood cells and lymphocyte subsets analysis. To evaluate the activity of DFA directly on parasites we tested culture growth inhibition and performed mobility, membrane integrity and apoptosis assays. DFA treated animals presented lower cumulative mortality rate in long term infection and lower parasitaemia in both short and long term infection. No effect was observed in iron metabolism markers, erythrogram, leukogram, lymphocyte subsets, except for an increase in lymphocyte counts at $7^{\underline{th}}$ d.p.i. DFA inhibited amastigotes and trypomastigotes growth in fibroblast culture, decreased parasite mobility, induced minor parasite apoptosis but did not change viability measured by trypan blue staining. Beneficial DFA effects on mice *T. cruzi* infection may be due to trypanostatic effect, independently of interference on host iron metabolism and with minor effects on lymphocyte subpopulation counts.
Supported by CAPES, FAPEMIG, UFOP, UFMG and CPqRR.

QT.19 - INDUCTION OF RESISTANCE TO THIOSSEMICARBAZONE AND BENZNIDAZOLE IN *Trypanosoma cruzi* AND ITS ASSOCIATION WITH P-GLYCOPROTEIN

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Chagas disease, caused by the protozoan *Trypanosoma cruzi*, represents an important health problem in Latin America. Specific treatment for this pathology is up to now not efficient and present high toxicity. Furthermore, studies have shown that resistance to benznidazole (Bz) for a variety of *T. cruzi* strains, results in failure of patients treatment. One of the mechanisms related to drug resistance in different pathogenic protozoa is the transport of drugs across the membrane by ATP-binding cassette (ABC) transporters. Recently, a member of the ABC superfamily, P-glycoprotein (Pgp), have been described in *T. cruzi* although its function has not been characterized. In the present study, the effects of (2-methoxy-styryl)-thiosemicarbazone (2-MEOTIO), a synthetic compound presenting activity on trypomastigote and amastigote forms of *T. cruzi* and Bz (reference drug) were investigated on *T. cruzi* epimastigotes (Y strain). The IC_{50} values observed for 2 MEOTIO and Bz were 230 ± 20,2 µM and 182,1 ± 10,7 µM, respectively. Both drugs were then used to induce resistance in *T. cruzi* epimastigotes. After at least 5 passages under drug pressure, It was obtained resistant parasites as demonstrated by the increase of the ICs_{50} for 2 MEOTIO (401,8 ± 33,7 µg/mL) and Bz (474,1 ± 15,7 µM). In order to verify the influence of P-gp, in the mechanism of drug resistance in *T. cruzi* it was analyzed the efflux of Rhodamine 123 (Rho-123) by resistant and wild-type epimastigotes. Parasites were incubated with Rho-123 (a fluorescent probe) in the presence or absence of verapamil (Pgp inhibitor) and the Rho-123 fluorescence was analyzed on a FAC-Scan flow cytometer. It was observed a significant time-dependent reduction of Rho-123 fluorescence in resistant parasites in comparison with wild-type. The results suggest participation of Pgp in *T. cruzi* resistance induced by benznidazole and (2-methoxy-estiryl)-thiosemicarbazone.
Supported by FAPERJ, CAPES and CNPq.

QT.20 - EFFECT OF EFAVIRENZ AND NEVIRAPINE ON THE PROLIFERATION OF *TRYPANOSOMA CRUZI* EPIMASTIGOTES

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Chagas' disease is a zoonosis caused by the haematic protozoan *Trypanosoma cruzi*. The therapeutic possibilities rely on two drugs, nifurtimox and benznidazole whose efficiency is highest during the acute phase of the disease. In addition, these drugs are highly toxic, with systemic side effects on patients. From the above, it is clearly necessary to validate new drugs that allow more effective treatment. The aim of this work was to evaluate the activity of two antiretroviral drugs, efavirenz and nevirapine, on *T. cruzi* growth. These drugs belong to the first generation of NNRTIs (non-nucleoside reverse transcriptase inhibitors). Used in combination with other antiretroviral drugs, these compounds have become a cornerstone for the treatment of HIV-1 infection. Efavirenz and nevirapine presented IC_{50} values of 45 ± 0.98 µM and 75 ± 1.65 µM for the Hep3B cell line, respectively. For the HeLa cells the values were 39 ± 1.05 µM and 67 ± 0.95 µM, respectively. The effect on *T. cruzi* proliferation was evaluated in the epimastigote form, cultured in media LIT. The efavirenz and nevirapine concentrations are ranged between 1 and 100 µM using rotenone (60 µM) and antymicin (0.5 µM) mixture as positive controls. The data were obtained daily, monitoring the absorbance change to 620 nm in 96-well-plates during 7 days. The dose-response on growth showed for efavirenz and nevirapine, IC_{50} values of 54.13 ± 0.75 µM and 62.86 ± 0.07 µM respectively. When comparing the cell growth in control and treated parasites, statistical differences were found ($p<0.01$). Our *in vitro* results showed that efavirenz and nevirapine affects the cell growth of epimastigotes from *T. cruzi.* Further experiments will be done to confirm its therapeutic potential. Possible synergism between the two drugs and their potential effect on different stages of the life cycle of *T.cruzi* will also be analyzed. Supported by FAPESP, CNPq and USP.

QT.21 - VITAMIN C EFFECTS IN MICE EXPERIMENTALLY INFECTED WITH *Trypanosoma cruzi* QM2 STRAIN

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Studies on new perspectives for the human treatment of Chagas' disease, concluded that the combined administration of 500mg/day of vitamin C and vitamin E 800UI/dia was able to halt the progression of oxidative stress caused by the disease. However, several studies describe the use of ascorbic acid by *T. cruzi* to protect itself from free radicals produced by immune cells of host. Thus, 60 mice were infected by *T. cruzi* QM2 strain and divided into six groups: G1, G1', G2, G2', G3 and G3', and G1, G2 and G3 for the acute phase and G1', G2 'and G3' to the chronic stage. G1 and G1' received 8.6 x 10^{-4} mg vitamin C, G2 and G2', 7.14 x 10^{-3} mg of vitamin C, and G3 and G3' were the placebo groups. The study of the acute phase showed statistically significant differences between G1 and the other groups at various count days of the parasitemia evolution, and until the 11^{th} day multiplying parasite was slower in G1, but at 22^{th} day it has parasitemia greater than G2 and G3, and from 36^{th} day stabilizes its parasitemia at higher levels, however there was no significant difference in histopathological analysis. In the chronic phase was not significant difference in histopathological analysis between the groups. So, it was found that even with a significant difference in parasitemia during the acute phase, the administration of two different vitamin C doses was not able to protect mice and likely contain the oxidative stress caused by free radicals formed by metabolism of oxygen and nitrogen, establishing the characteristic lesions of Chagas' disease.
Supported by Fapesp.

QT.22 - THE INVOLVMENT OF RESERVOSOMES, GOLGI AND AUTOPHAGY IN TRIAZOLIC NAPHTHOQUINONE MECHANISM OF ACTION IN *TRYPANOSOMA CRUZI* EPIMASTIGOTES

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The current chemotherapy for Chagas´ disease caused by the pathogenic protozoan *Trypanosoma cruzi* is based on two nitroderivatives, but its variable efficacy and serious side effects require the development of alternative drugs for the treatment of this disease. In this framework, among several naturally occurring quinones, emerge the naphthoquinones with a broad distribution in the plant kingdom and involved in oxidative processes. In the last decade, our group has been synthesized and screened on bloodstream trypomastigotes, 80 derivatives of quinones, being a novel triazolic naphthoquinone (**TN**) derived from nor-lapachol, the most active compound. Here, we evaluate the ultrastructural effect of this compound in epimastigote forms of *T. cruzi*. Scanning electron microscopy showed important morphological alterations in **TN**-treated epimastigotes (3-9 µM) such as retraction of the posterior region of the body and the presence of multiple flagella. Transmission electron microscopy pointed to a remarkable disorganization in reservosomes morphology, a severe Golgi disruption as well as blebs in the flagellar membrane and the formation of concentric membranar structures in the cytosol in treated parasites. 9 µM **TN** also triggered an extensive autophagic process, being endoplasmic reticulum profiles observed surrounding cytosolic portions and organelles such as reservosomes. Our scanning electron microscopy data showing parasites treated with 3-9 µM with multiple flagella, together with flow cytometry experiments indicated the partial blockage of cytokinesis, leading to the cell cycle arrestment. Our ultrastructural data together with the cell cycle analysis strongly suggests that **TN** blocks cytokinesis and impairs the parasite proliferation. Further electron microscopy assays must be performed to evaluate the targets of this compound in bloodstream trypomastigotes, a clinical relevant form of *T. cruzi*.
Supported by CNPq, FAPERJ and IOC/FIOCRUZ.

QT.23 - INHIBITING EFFECTS OF GALLIC ACID AND ESTERS DERIVATIVES ON *Trypanosoma cruzi* TRYPANOTHIONE REDUCTASE

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Trypanothione reductase (TR) is a key enzyme in the trypanothione-based redox metabolism of pathogenic trypanosomes. Due its absence in mammals, were glutathione reductase (GR) takes place, TR represents a potential drug target. Since gallate esters were reported to be active against *T. cruzi*, the aim of this work was to assess their potential as inhibitors of *T. cruzi* TR (*Tc*TR). For that, *Tc*TR was cloned and expressed in *E. coli* and the purified active enzyme was evaluated against gallic acid (GA) and its esters as well as against a commercially available *S. cerevisae* GR. The *Tc*TR ORF was PCR amplified from *T. cruzi* Y strain genomic DNA, cloned into pET14b and expressed in *E. coli* BL21(DE3) by IPTG (1mM) induction at 37°C for 4 hours. After purification of the soluble recombinant protein by affinity columns (Ni-NTA) under non-denaturing conditions, *Tc*TR inhibition assays were performed using different concentrations of gallates, 40mM Hepes (pH 7.5), 1mM EDTA, 150µM NADPH, 1µM trypanothione, 25µM of DTNB and 230ng of purified *Tc*TR. *Tc*TR and GR activities were assessed spectrophotometrically at 412nm and 340nm, respectively, using clomipramine (12.5-100µM) and carmustine (10µM) as inhibition controls. Among six compounds, only GA and decyl gallate were able to inhibit *Tc*TR activity, showing IC_{50} values of 45.6 and 78.9µM, respectively. This GA-induced inhibition was higher than clomipramine control (IC_{50} of 63.6µM), showing a selective *Tc*TR inhibition. Along with an efficient heterologous expression of *Tc*TR, the results reveal that GA is a prominent prototype for the development of new molecules with selective trypanocidal activity. Supported by CNPq, FINEP and UFSC.

QT.24 - EFFECT OF *APIS MELLIFERA* VENOM ON *TRYPANOSOMA CRUZI* INTRACELLULAR CYCLE

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Chagas' disease chemotherapy is based on drugs that exhibit toxic effects and limited efficacy such as Benznidazole. Therefore, new chemotherapeutic agents from natural sources are a lining research to be exploited. Honeybee (*Apis mellifera*) venom consists of many biologically active compounds and has been reported to exhibit anticancer effects. In the present study we analyzed the effect of *A. mellifera* venom on *T. cruzi* intracellular cycle. Briefly, LLC-MK_2 cells were incubated in the presence of 0.1, 0.5, 1 e 2 µg/ ml of *A. mellifera* venom for 5 days. Coverslips were collected daily, fixed and stained with Giemsa to analyze the venom effects on host cells. None of these dose was toxic to them. To analyse the effect on the parasite intracellular cycle, host cells were infected with tissue culture trypomastigotes at a 10:1 parasites: host cell ratio in the absence of the venom. After 24 h interaction, cells were washed and different venom concentrations (0,025- 0,4 µg/ ml) in RPMI medium were added to distinct wells, and then incubated for 24 to 96 h at 37°C. The percentage and number of infected cells, and the number of amastigotes per 100 cells were daily evaluated. The venom's presence during the intracellular development of *T. cruzi* caused a significant reduction in the percent (32 to 76 %) and number (17.5 to 56%) of infected cells, after 24h, reaching 100% and 69% after 96h of incubation with 0.4 µg/ ml, respectively. The parasites number per 100 cells was 59 to 87.9% after 24 h, reaching 68 to 95.1% after 96h. Our data demonstrate that *A. mellifera* venom gains access to the host cells cytoplasm where it was effective against the intracellular forms of *T. cruzi*. Further studies are underway to investigate the venom targets in *T. cruzi* amastigotes forms.
Supported by CNPq, CAPES and FAPERJ.

QT.25 - ULTRASTRUCTURAL STUDY OF *Trypanosoma cruzi* TREATED WITH POSACONAZOLE PLUS AMIODARONE

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Chagas disease, caused by the protozoan *Trypanosoma cruzi,* represents an important public health problem, with overall prevalence estimated in 10-16 millions of cases. There is no effective treatment for the prevalent chronic form of Chagas' disease. Posaconazole, an ergosterol biosynthesis inhibitor acting at cytochrome P 450-dependent lanosterol C14 demethylase (CYP51), has potent and selective anti-*T. cruzi* activity *in vitro* and *in vivo* and is currently entering clinical trials for the specific treatment of this condition. On the other hand, the antiarrhythmic drug amiodarone has been shown to have also direct activity against *T. cruzi*, disrupting the parasite's Ca^{2+} homeostasis and inhibiting ergosterol biosynthesis at the level of lanosterol synthase (Benaim et al, 2006). Furthermore, it was shown that amiodarone acts synergistically with posaconazole against the clinically relevant form of the parasite, intracellular amastigotes. These results have now been reproduced our laboratory. Epimastigotes and intracellular amastigotes were treated with IC_{50} and IC_{90} of posaconazole and fixed with 2.5% glutaraldehyde. For transmission electron microscopy (TEM), cells were post-fixed in osmium tetroxide, dehydrated in acetone, embedded in Epon and observed by TEM. For field-emission scanning electron microscopy, parasites were dehydrated in ethanol, critical-point dried in CO_2, and observed. Epimastigotes treated with posaconazole and observed by field-emission scanning electron microscopy displayed extensive loss of integrity of the plasma membrane, with blebs formation and parasite body retraction. Observations by TEM showed intense changes in organization of microtubules in the flagellum, alterations in the Golgi complex organization and extensive autophagic vacuolization. In addition, analysis of intracellular amastigotes treated with posaconazole by TEM showed alterations in the Golgi complex organization and plasma membrane shedding. Further microscopic assays are being performed to study alterations in the ultrastructure of *T. cruzi* amastigotes and trypomastigotes treated with amiodarone plus posaconazole, to better understand the synergic effects of these drugs. Supported by CNPq, CAPES and FAPERJ.

QT.26 - ACTIVITY OF AN INHIBITOR OF TRANSFORMING GROWTH FACTOR BETA SIGNALING DURING THE ACUTE PHASE OF *TRYPANOSOMA CRUZI* INFECTION

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Transforming growth factor beta (TGF-β) is a key mediator in the pathogenesis of cardiac remodeling during Chagas` disease. High TGF-β levels are associated with extensive fibrosis in acute and chronically *T. cruzi*-infected humans and mice. We recently reported that a TGF-β type I receptor inhibitor, SB-431542, partially inhibits *T. cruzi* invasion, reduces amastigote numbers per infected cell and inhibits the differentiation into trypomastigotes at the end of the intracellular cycle in cardiomyocytes. Our present aim is to investigate the role of a novel orally active TGF-β type I receptor inhibitor, GW-788388, in attenuating heart damage and cardiac dysfunction, using a *T. cruzi* mouse infection model. In our experimental model of acute infection, male Swiss mice were inoculated with the *T. cruzi* Y strain and evaluated by clinical, parasitological and histopathological investigations up to 30 days post-infection (dpi). One dose of GW-788388 was orally administered at 3 or 13 dpi. Our results show that GW-788388 treatment significantly increased mice survival rates and reduced the number of circulating parasites, as compared to non-treated mice. Moreover, GW-788388 also reduced tissue lesions, showing lower cardiac parasitism and numbers of cardiac inflammatory cells. Interestingly, blocking of TGF-β intracellular signaling at 13 dpi increased the mortality of treated animals, suggesting that TGF-β could have, at this stage of infection, an important regulatory role in the inflammatory process. Thus, we believe that inhibition of the exacerbated biological effects of TGF-β might represent an attractive strategy to combat the severity of this disease. Supported by Fiocruz and CNPq.

QT.27 - EFFICACY OF BENZIDAZOLE TREATMENT IN CHRONIC CANINE CHAGAS DISEASE: HISTOPATHOLOGY AND FUNCTIONAL ANALYSIS

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The analysis of available information reveals that the efficacy of benznidazole treatment in chronic chagasic infection is doubtful. In this study we evaluated the effect of Bz-treatment on the cardiac alterations using dogs infected with Berenice-78 strain as experimental model. The infected animals were divided in two experimental groups: (i) 12 dogs were Bz-treated at 7.0 mg/kg bid (Q12) for 60 days during the chronic phase; (ii) 12 dogs were maintained as non-treated control. Another 8 animals were maintained as non-infected control group. For cardiomegaly and systolic or diastolic function evaluation the animals were examined by echocardiography in the 1st and 12th month post-treatment. The parameters fractional shortening, Left Atrium (LA) volume, Left-ventricle (LV) ejection fraction, diastolic volume and systolic diameter were measured. After this evaluation a half of animals were euthanized in the same period for histopathological analysis of heart tissue. Bz-treatment led to a reduction of around 20% to 36% of inflammatory cells and intra-fascicular collagen deposition when compared to non-treated animals in the first month post-treatment. Additionally, all animals evaluated showed echocardiographic parameters similar to non-infected animals. Differently, 12 months post-treatment the intensity of cardiac lesions were similar to treated and non-treated animals and significantly larger than those detected in non-infected animals. Also, the echocardiographic parameters, related with cardiomegaly (LV and LA volume, LV systolic diameter) and diastolic function (LA volume), were similar among treated and non-treated animals and significantly higher than those observed in non-infected animals. Interestingly, the Bz-treatment was able to prevent alterations related to cardiac functions (LV ejection and shortening fraction), such this parameters were similar to treated and non-infected animals. Taken together, the results indicate that Bz-treatment performance during the chronic phase of the dogs' infection is efficient in preventing cardiac lesions immediately after the treatment and the systolic cardiac function long-time post-treatment. Financial Support: CNPq, FAPEMIG, UFOP.

QT.28 - EVALUATION OF THE BENZNIDAZOLE THERAPY EFFICACY OF BLOCKING HOST CELL APOPTOSIS IN DOGS INFECTED BY *Trypanosoma cruzi*

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The identification of the deleterious effects of apoptotic cells and their effect on *T.cruzi* replication provide a new conceptual framework for the pathogenesis of Chagas disease. However, several questions remain unsolved, including the role of apoptosis in cardiac inflammation and the therapeutic efficacy of blocking host cell apoptosis. In the present study, we investigate the effect of the Benznidazole (Bz) treatment in the occurrence of apoptosis in peripheral blood mononuclear cells (PBMC) and in cardiomyocytes of dogs infected with VL10 strain (resistant to Bz-terapy). Infected animals were divided in two experimental groups: (i) 5 dogs were Bz-treated at 7.0 mg/kg bid (Q12) for 60 days; (ii) 5 dogs were maintained as non-treated control. Another 5 animals were maintained as non-infected control group. The animals were treated during the acute phase, and after treatment the blood was collected for PBMC analysis and the animals were euthanized for heart tissue evaluation. The results showed that infected and Bz-treated animals presented a higher proliferative response (proliferative index:1.96±0.63), similar to infected control (1.95±0.24) and significantly higher to no-infected group (0.95±0.24). Differently, the apoptosis index was truly influenced by Bz treatment, while the apoptotic index among treated animals (1.36±0.45) and non-infected (1.26±0.42) was similar and significantly ($p<0.01$) smaller to the one detected in the PBMC of those infected and non-treated animals (2.63±1.1). Differently, cardiomyocytes apoptosis (analyzed by TUNEL method) was not influenced by Bz-treatment, as the apoptotic index showed similar value among Bz-treated (13.13±0.45) and non-treated (12.17±2.04) infected animals, and significantly higher to non-infected animals (9.42±0.57). Additionally, similar intensity of inflammatory infiltrated and fibrosis area were detected among Bz-treated and non-treated animals. Taken together, these results confirm a causal link between apoptosis and heart damage and suggest that influence of the Bz-therapy in cell apoptosis is related with the cell type evaluated, PBMC or cardiac. Supported by: FAPEMIG and CNPq

QT.29 - EXPERIMENTAL CHEMOTHERAPY WITH COMBINATIONS OF ITRACONAZOLE PLUS BENZNIDAZOLE IN MURINE MODELS OF CHAGAS' DISEASE

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We report the effects of benznidazole (Bz) acting alone or in combination with Itraconazole (Itz) in murine models of Chagas' disease. The following treatment arms were selected: Itz 100, 75 and 50 mpk (mg/kg/day); Bz 100, 75 and 50 mpk; Bz/Itz combination: 50/50; 75/75 and 100/100 mpk of each of the drugs. Female Swiss mice, 18-20g, 10 animals/group, were infected with 5×10^3 bloodstream trypomastigotes of Y strain. Treatment was administered orally for 20 consecutive days, beginning at 4 days after inoculation. Parasitological cure was assessed by parasitemia at and up to 60 days after treatment through optimal microscopy and blood PCR. All Bz-treated animals survived for 60 days after treatment. In contrast, only animals that received 100 mpk of Itz survived during same period, while 50% and 60% of animals treated with 50 and 75 mpk of Itz died up to 50 days post-infection. Although there was significant reduction in the level of parasitemia after treatment, parasitological cure was not achieved in all treatment groups. Parasitological cure was documented in 60% (6 of 10) of mice treated with 100mpk of Bz; whilst there was no cure among animals treated with Bz 50 and 75 mpk or Itz 50, 75 and 100mpk. Bz/Itz combination treatment induced parasitological cure in 80% (2 of 10), 70% (7 of 10) and 20% (2 of 10) of mice that received 100, 75 and 50mpk of Bz/Nfx combination therapy, respectively. These results suggest a beneficial effect of the combination, as parasitological cure was observed in a higher proportion of animals treated with the drugs in association than in animals receiving the same dose of each of the drugs in monotherapy. Supported by DNDi, UBS Optimus Foundation, Fapemig, CNPq and UFOP.

QT.30 - EFFICACY OF BENZNIDAZOL OR NIFURTIMOX AND POSACONAZOLE COMBINATION IN EXPERIMENTAL CHAGAS DISEASE

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As there are limited prospects for the near-term introduction of new compounds for the treatment of Chagas disease, an alternative strategy involves the identification of candidate drugs among those already available on the market that could be used in combination with the aim of increased efficacy, safety and potential shortened duration of treatment regimens. We have investigated the anti-*Trypanosoma cruzi* efficacy of combinations of Posaconazole(Ps) and Benznidazol(Bz) or Nifurtimox(Nfx) in mice infected with the *T. cruzi* Y-strain using a rapid treatment protocol, in which each animal received drug suspension by gavage for 7 days. In the initial phase, the effects of half and one-fourth of the curative dose (CD)of each drug alone on the evolution of the infection in mice were evaluated and compared with animals treated with known CD those for long-term treatment (e.g., 100 mpk of Bz, 50 mpk of Nfx and 20 mpk of Ps). The analysis of parasitemia and mortality rate after treatment showed that all compounds had a dose-dependent trypanocidal effect with significantly lower parasitemia levels and mortality rate with CD of Bz, Nfx and Ps compared with those receiving half and one-fourth of the CD. In a second phase of data analyses, the activities of drugs combinations were compared with results of the CD of each drug alone. The results showed a clear beneficial effect of the Bz/Ps or Nfx/Ps combinations, as animals that received the half and one-fourth doses of these drugs in association presented a significant reduction of mortality and parasitemia levels in comparison to those that received the same dose of each drug alone. These data will be confirmed in experiments with 20-day treatment in acute murine model. Supported by DNDi, UBS Optimus Foundation, Fapemig, CNpq and UFOP.

QT.31 - COMBINED TREATMENT OF HETEROCYCLIC ANALOGUES AND BENZNIDAZOLE UPON *TRYPANOSOMA CRUZI IN VIVO*

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Chagas disease caused by *Trypanosoma cruzi* is an important cause of mortality and morbidity in Latin America but no vaccines or safe chemotherapeutic agents are available. Combined therapy is envisioned as an ideal approach since it may enhance efficacy by acting upon different cellular targets, may reduce toxicity and minimize the risk of drug resistance. Thereafter, we investigated the activity of benznidazole (Bz) in combination with the diamidine prodrug DB289 and in combination with the arylimidamide DB766 upon *T. cruzi* infection *in vivo*. The oral treatment of *T.cruzi*-infected mice with DB289 and Benznidazole (Bz) alone reduced the number of circulating parasites compared with untreated mice by about 70% and 90%, respectively. However, the combination of these two compounds decreased the parasitemia by 99% and protected against animal mortality by 100%, but without providing a parasitological cure. When Bz (p.o) was combined with DB766 (via ip route), at least a 99.5% decrease in parasitemia levels was observed. DB766+Bz also provided 100% protection against mice mortality while Bz alone provided about 87% protection. This combined therapy also reduced the tissular lesions induced by *T. cruzi* infection: Bz alone reduced GPT and CK plasma levels by about 12% and 78% compared to untreated mice group, the combination of Bz with DB766 resulted in a reduction of GPT and CK plasma levels of 56% and 91%. Cure assessment through hemocultive and PCR approaches showed that Bz did not provide a parasitological cure, however, DB766 alone or associated with Bz cured ≥13% of surviving animals. Our data support additional studies with other diamidines and arylimidamides alone or in combination with other drugs with the goal of identification of new candidate therapies for the treatment of Chagas disease.
Supported by: FAPERJ-APQ1 and Pensa Rio,CNPq, PAPES V/FIOCRUZ and The Consortium for Parasitic Drug Development (CPDD).

QT.32 - BIOLOGICAL EFFICACY OF NEW ARYLIMIDAMIDES UPON *Trypanosoma cruzi IN VITRO*

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Chagas disease (CD) is a tropical neglected illness affecting mostly the poorest people living in endemic areas of many Latin American countries and also occurring in Europe and North America, due to immigration. Current treatment is based on benznidazole (BZ) or nifurtimox that are indicated for the therapy of all acute cases and early chronic patients, during infection or reagudization processes after transplantation and immune suppression cases as well as for cases caused by laboratory accidents. The aim of this work is to investigate the in vitrotrypanocidal effects of novel arylimidamides (AIAs - DB667, DB709, DB745B, DB749 and DB946) against *T. cruzi*. All the AIAs tested exhibited significant trypanocidal effects, giving dose and time-dependent activity against bloodstream trypomastigotes and intracellular amastigotes (Y strain), and exhibiting IC_{50} values ranging from 15 nM up to 2.48 µM. DB745 also exerted striking effects upon different parasite stocks, including those naturally resistant to benznidazole such as YuYu and Colombiana strains, displaying higher efficacy than the reference drugs (BZ and gentian violet). Our data clearly demonstrates the trypanocidal effect of the novel AIAs, showing that this class of compounds exhibits the potential to provide new leads compounds for CD therapy.
Supported by: FAPERJ, CNPq , CPDD and PAPES/FIOCRUZ.

QT.33 - INHIBITORY ACTIVITY AGAINST *Trypanosoma* AND *Leishmania*: A KNOWN DRUG REVISITED.

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Neglected diseases are in its majority tropical infectious pathologies transmitted by insect vectors or by contaminated water and soil. The World Health Organization lists a total of fourteen main tropical neglected diseases among which are leishmaniose, sleeping sickness, malaria, Chagas disease, schistosomiasis and dengue. Regardless of all the knowledge already reached about those infectious diseases, they still present high worldwide morbidity and mortality rates. Moreover, the investment in drug research and development to its treatment is stills low, when compared to other diseases. The development of novel drugs for the Kinetoplastida parasites (*Leishmania, Trypanosoma cruzi* or *Trypanosoma brucei*) is the main focus of several research efforts. We have tested the use of a known human rheumatoid arthritis drug that is believed to interact selectively with selenoproteins. We have shown that this proteins are vital components to organisms belonging to Kinetoplastida order. *In vitro* experiments with this drug have shown a LD_{50} of 75µM and 2.88µM for the trypomastigote and epimastigote forms of *T. cruzi* respectivelly. Moreover, this drug is effective against *Leishmania* and *T. brucei* cells. The *in vivo* experiments, following the World Health Organization protocols, are been conducted to verify the efficacy of this compoind as a treatment for Chagas disease as a less toxic alternative to the current treatment Benznidazol.
Supported by grants from CNPq and FAPESP.

QT.34 - THE NEOLIGNAN GRANDISIN PRESENTS ACTIVITY AGAINST AMASTIGOTES OF *Leishmania chagasi*

Caldas, J.P.A.[1], Amorim, F.M.[1], Néris, P.L.N.[1], Penha, A.R.S.[1], Barbosa-Filho, J.M.[2], Oliveira, M.R[1*].

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Leishmaniasis treatment relies mainly on pentavalent antimonials, amphotericin B and pentamidine, expensive and highly toxic treatments that require prolonged parenteral administration. Thus, there is an urgent need for developing new drugs to replace those in current use. The plants are valuable sources of new medicinal agents. Lignans are an important group of plant metabolites with several biological activities documented. The objectives of this study were to evaluate the cytotoxicity of the neolignan grandisin on murine macrophages (MØs), and its antiamastigote activity against *Leishmania* (*L.*) *chagasi*. MØs were incubated in the presence or absence of grandisin at 37ºC in a 5% CO_2 atmosphere for 24h, and their viability was assessed by MTT reduction and trypan blue exclusion assay. MØs were infected with stationary-phase *L. chagasi* and incubated as aforementioned with different concentrations of grandisin (200 and 400 µg/mL) for 24, 48 and 72h. The cells were dyed, and the amastigotes were counted under light microscopy. Cell supernatants were analyzed for nitric oxide (NO) and tumour necrosis factor (TNF)-α production. Cytotoxicity of grandisin (concentrations up to 800µg/mL) was not detected by MTT reduction assay. Cell viability measured by trypan blue exclusion assay resulted in a CC_{50} of 729.79µg/mL. The treatment of infected MØs with grandisin at 400µg/mL, for 24h, significantly reduced the percentage of infected cells (29.87%). At 48h, infection was reduced by 21.19% (200µg/mL) and 33.67% (400µg/mL). After treatment for 72h, a further reduction of infection was observed at 200 (45.65%) and 400µg/mL (55.65%). The antiamastigote activity of grandisin correlated with NO production at 24 (at 400µg/mL), 48 and 72h (at 200 and 400µg/mL). No correlation was found between NO and TNF-α production. These data demonstrate grandisin presents antiamastigote activity against *L. chagasi*, little cytotoxicity for MØs, and is able to modulate MØs infection by inducing NO production.
Supported by CAPES.

QT.35 - LEISHMANICIDAL ACTIVITY OF *Momordica charantia* (CUCURBITACEAE)

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In spite of the advances in last decade, such as miltefosine registry and the development of new formulations of amphotericin B, control of leishmaniasis remains in the order of day and the search for new treatment alternatives is an actual priority. Thus, the study of large chemical potential of Brazilian biodiversity can be an interesting approach to the development of new treatment alternatives. This study aimed to evaluate the leishmanicidal activity of *Momordica charantia* (Cucurbitaceae), originally known for its use in cooking and medicine. Specimens of *M. charantia* were collected, air parts were dried, macerated and subjected to extraction with ethanol by dynamic maceration. *Leishmania amazonensis* promastigotes were cultured in the presence of several concentrations of crude extracts and their subsequent partitions up to 100 µg/mL for 72 hours and quantified colorimetrically by MTT assay. Under these conditions, the ethanolic partition was the most active, with IC_{50} of 4.5 µg/mL. This sample was sequentially fractionated and the partition in ethyl acetate (MSØAc) was significantly more active, with IC_{50} of 6.12 µg/mL. The fractionation of this partition led to a fraction rich in triterpenes (F7), with IC_{50} of 2.1 µg/mL. Again, this partition was fractionated and the activity was concentrated in three fractions, with IC_{50} of 1.50 µg/mL, 1.08 µg/mL and 1.16 µg/mL. Five pure substances were obtained from these fractions, all with IC_{50} around 5.0 µg/mL. The activity of MSØAc and F7 fractions was evaluated in intracellular amastigotes. The infectivity index was determined by optical microscopy. F7 and MSØAc showed a significant antiamastigote activity, with IC_{50} of 1.8 µg/mL and 2.3 µg/mL, respectively. The structural identification of the isolated active compounds may lead to a new antileishmanial prototype. Supported by Papes/CNPq.

QT.36 - EVALUATION OF A COMBINED MEGLUMINE ANTIMONIATE AND TAMOXIFEN THERAPEUTIC SCHEME FOR LEISHMANIASIS

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Tamoxifen is an antiestrogen used for the treatment of breast cancer. Recently, our group has demonstrated that this drug is effective against *Leishmania in vitro*, reduces the parasite burden in *L. amazonensis* and *L. braziliensis*-infected BALB/c mice and in hamsters infected with *L. chagasi*. The aim of this study is to assess the effect of the association of tamoxifen and meglumine antimoniate (Glucantime®), a drug regularly used in the clinical practice as the first choice treatment for cutaneous leishmaniasis. Our assays were performed after infecting the basis of the tail of BALB/c female mice with 1 million *L. amazonensis* stationary-phase parasites. Fifty days post-inoculation, animals were distributed in different groups (n=6-7/group) for the following i.p. treatment protocol for 15 days: 1) saline solution; 2) 20 mg/kg/d tamoxifen; 3) 10 mg/kg/d Glucantime; 4) 20 mg/kg/d Glucantime; 5) 10 mg/kg/d Glucantime plus 20 mg/kg/d tamoxifen and 6) 20 mg/kg/d Glucantime plus 20 mg/kg/d tamoxifen. The ulcerated area and lesion size were measured weekly up to 65 days after the interruption of the treatment. Two weeks after the end of treatment, the response in groups receiving the association of tamoxifen and Glucantime was comparable to groups receiving Glucantime alone. The pattern of response was maintained up to 9 weeks after the end of treatment and was also observed when the ulcer size was evaluated. Experiments testing this association in different dose schemes are being carried out to confirm these initial observations, which indicate that the association of Glucantime and tamoxifen in vivo does not show antagonic or synergic properties. On the other hand these drugs may show an additive effect in the treatment of cutaneous leishmaniasis in this experimental model. Other studies are also being conducted to establish the effectiveness of Amphotericin B-tamoxifen association in *L. amazonensis*-infected mice.
Support: FAPESP, CNPq.

QT.37 - EVALUATION OF MECHANISM OF ACTION, IN VITRO AND IN VIVO ACTIVITIES OF LQB118, A NEW ANTILEISHMANIAL PROTOTYPE

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Leishmaniasis is one of the most neglected diseases, affecting the poorest segments of populations, resulting in substantial morbidity and mortality in 2 million people worldwide. Nevertheless, there is scant investment for development of new effective and safe drugs. In previous studies, we reported that the naphthopterocarpanquinone LQB118 is a potent *in vitro* antileishmanial agent. Here, we investigated the mechanisms involved in cell death of *L. amazonensis* promastigotes treated with LQB118 and the ability of this compound in controlling lesions in murine model. Morphological alterations induced by LQB118 in promastigotes were evaluated by TEM and SEM. To evaluate the biochemical pathways involved in parasite death, loss of mitochondrial membrane potential (rhodamine, JC1 and mitocapture assays), ROS production (H_2DCFDA), lipid peroxidation (TBARS), phosphatidylserine exposure (annexin V) and DNA fragmentation (TUNNEL) were analyzed. The concentration of LQB118 used for in vitro assays ranged between 1.25 and 10.0 µM. In vivo studies were performed in *L. amazonensis* infected mice (license LW07/2010). Our SEM and TEM results showed that LQB118 induced drastic morphological changes, as mitochondrial damage (increase of electron-density, swelling and cristae disorganization), nuclear changes (chromatin condensation and disorganization of the nuclear envelope) and rarefaction of the cytoplasm. Incubation of promastigotes with LQB118 induced dose-dependent generation of reactive oxygen species, lipid peroxidation and loss of mitochondrial membrane potential, which were accompanied by phosphatidylserine exposition and DNA fragmentation. LQB118 was effective in controlling lesions in leishmaniasis murine model by all tested routes of administration (subcutaneous, intraperitoneal and oral), with activity similar to the reference drugs Pentostam and Glucantime. In the present study, we demonstrated that LQB118 exerts its leishmanicidal effect by interfering in mitochondrial activity and triggering several events which suggest programmed cell death apoptosis-like. Altogether, our results indicate that LQB118 is a promising prototype for the treatment of leishmaniasis. Supported by FAPERJ, CAPES, PAPES/CNPq.

QT.38 - CYTOTOXICITY AND ANTILEISHMANIAL ACTIVITY OF THE NEOLIGNANS BURCHELLIN AND GRANDISIN

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Leishmaniasis chemotherapy, for over 60 years, relies on pentavalent antimonials, amphotericin B and pentamidine, which are toxic and prone to drug resistance. New drugs for the treatment of leishmaniasis are necessary, and the plant kingdom is a valuable source of potential medicinal agents, as the lignans. The objectives of this study were to evaluate the antipromastigote activity of the neolignans burchellin and grandisin against *L. chagasi*, to evaluate farnesyl pyrophosphate synthase of *L. chagasi* (*Lc*FPPS) as a possible molecular target of the neolignans and to investigate their cytotoxicity on MDCK cells. Logarithmic phase *L. chagasi* promastigotes were incubated in Schneider medium ($1x10^6$ cells/mL) with burchellin and grandisin (0-100µg/mL) for 72h at 25ºC. The cultures were diluted and quantified in a neubauer chamber under light microscopy. The crystallized structure of *Lc*FPPS is unavailable, so homology modeling using PDB 1YHL of *Trypanosoma cruzi* FPPS was carried out to obtain a model for molecular docking calculations, which was done using the CCDC Gold Suite v.4.0 with the ChemScore function. MDCK cells cultured in RPMI medium were seeded into 96-well plates ($1x10^5$ cells/mL), treated with the neolignans (0-400µg/mL) and submitted to lactate dehydrogenase (LDH) assay. Burchellin and grandisin inhibited *L. chagasi* growth with an IC_{50} of 16.54 and 7.17µg/mL, respectively. In molecular docking calculations, burchellin scored 21.45, by establishing 1 hydrogen bond and 3 coordinations with the active site of *Lc*FPPS, and grandisin scored 6.76 by establishing 4 hydrogen bonds with it, suggesting this enzyme as a possible molecular target of burchellin. No increment of LDH activity was detected on MDCK cells, implying neither burchellin nor grandisin exerts citotoxicity by altering cell membrane integrity in the concentrations tested (400µg/mL). The data demonstrate burchellin and grandisin present a specific cytotoxicity for *L. chagasi*, with *Lc*FPPS as a possible molecular target of burchellin. SUPPORTED by CAPES.

QT.39 - CHITOSAN COMPLEXES PRESENT ANTILEISHMANIAL ACTIVITY IN EXPERIMENTAL MODELS *IN VITRO* AND *IN VIVO*

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The drugs commonly used for the treatment of leishmaniasis are administered via parenteral route and present high toxicity for humans. Taking into account that the search for more effective treatments for leishmaniasis is necessary, the objectives of this study were to evaluate the antileishmanial activity of chitosan complexes in experimental models *in vivo* and *in vitro*, as well as to evaluate the cytotoxicity of the complexes on mammalian cells. Logarithmic-phase *Leishmania (L.*) *chagasi* promastigotes were incubated in Schneider medium ($1x10^6$ cells/mL) with chitosan for 72h at 25ºC. The cultures were diluted and quantified in a neubauer chamber under light microscopy. Murine macrophages (MØs) were infected with stationary-phase *L. chagasi* and incubated with chitosan at 37ºC in a 5% CO_2 atmosphere for 72h. The cells were dyed, and the amastigotes were counted under light microscopy. MØs and Madin-Darbin Canine Kidney (MDCK) cells cultured in RPMI medium were seeded into 96-well plates ($1x10^5$ cells/mL), treated with chitosan and submitted to lactate dehydrogenase (LDH) assay. The left hind footpad of Swiss mice was subcutaneously infected with stationary-phase *L. amazonensis* ($1x10^7$ cells/mL). The mice were orally treated with 50mg/kg of chitosan for 8 weeks, after which lesion size and parasite load was analyzed. Chitosan presented antipromastigote activity against *L. chagasi*, with an IC_{50} of 88.7µg/mL, and at 50µg/mL reduced the percentage of *L. chagasi*-infected MØs (30%). Additionally, this carbohydrate did not demonstrate cytotoxicity on MØs and MDCK cells at concentrations up to 400µg/mL for 24h. In Swiss mice infected on the footpad with *L. amazonensis*, treatment with 50µg/kg of chitosan reduced lesion size and parasite load in both the footpad and the popliteal lymph nodes. It can be concluded that chitosan complexes present a potentiality in the therapeutic of leishmaniasis, characterized by antileishmanial activity *in vitro* and *in vivo*.
Supported by CAPES.

QT.40 - RESVERATROL SYNERGIZES WITH AMPHOTERICIN B TO INHIBIT *Leishmania amazonensis*

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Leishmaniasis is a disfiguring and potentially fatal disease caused by parasites of the genus *Leishmania*, which affects around 350 million people worldwide. Leishmaniasis treatment relies mainly on antimonials and amphotericin B that present high toxicity, elevated cost and parasite resistance. Recently, miltefosine was approved for visceral leishmaniasis treatment, but teratogenicy limits its use. All these facts stimulate the search for new anti-leishmanial agents, and natural products constitute an important source of such compounds. Besides, combination therapy for leishmaniasis treatment has been advocated as a way to reduce treatment duration and cost, and limit the emergence of drug resistance. The aim of this study is to validate the anti-leishmanial activity of the natural product resveratrol, alone, or in combination with amphotericin B. Resveratrol, a polyphenolic compound present in wine and grapes has biological activities, such as anti-inflammatory, anticancer and antioxidant. Our previous results have shown that resveratrol presented an anti-*L.amazonensis* activity with an IC_{50} of 27µM for promastigotes and of 42µM for amastigotes. Morphological analysis by optical microscopy demonstrated that resveratrol treated promastigotes (100µM for 48h) presented an irregular number of nucleus and flagella. Corroborating these finds, alteration in the cell cycle was also observed in these parasites analyzed by flow cytometry after propidium iodide stained. Here, by isobolografic analysis, we describe that the combination of resveratrol (R) with amphotericin B (A), showed a synergistic effect for promastigotes (13R + 0.01µM A) as well as for amastigotes 13R + 0.0002µM A) of *L.amazonensis*. Treatment of peritoneal murine macrophages with 600µM of resveratrol was not toxic (75% of viable cells, detected by the XTT assay). Our results confirmed the anti-*Leishmania amazonensis* effect of resveratrol and add its synergic association with amphotericin B, pointing them as possible substances for further studies of drugs combination therapy *in vivo*.
Supported by FAPERJ, CNPq.

QT.41 - EVALUATION OF THE LEISHMANICIDAL POTENTIAL OF QUINOLINE DERIVATIVES ON *LEISHMANIA MAJOR*

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Leishmaniasis is one of the most important neglected tropical diseases caused by parasites of the genus *Leishmania*. The current chemotherapy for leishmaniasis is limited, because the drugs are toxic and most to be used parenterally for prolonged period. Several drugs based on the quinoline structure have improved the therapy of protozoa diseases. In order to find new drugs against leishmaniasis, we evaluate the *in vitro* antileishmanial activity of the quinoline derivatives. The compounds Methyl 3β-(*N*-[(7-chloroquinolin-4-yl)amino]propylamynomethyl)-1*H*-1,2,3-triazol-1-yl)]7α-12α-dihydroxy-5β-cholane-24-oate **(1)**, Methyl 3β-(*N*-[(7-chloroquinolin-4-yl)amino]buthylaminomethyl)-1*H*-1,2,3-triazol-1-yl)]7α-12α-diidroxi-5β-cholane-24-oate **(2),** 6-chloro-*N*-(4-(di(prop-2-ynyl)amino)butyl)naphtalen-1-amine **(3)**, Platinum(II) Complex from *N*-(2-(di(prop-2-ynyl)amino)ethyl)-7-chloroquinolin-4-amine **(4)** and 6-chloro-*N*-(2-(prop-2-ynylamino)ethyl)naphtalen-1-amine **(5)** were tested against promastigote and amastigote forms of *L major.* The viability of promastigote forms and mammalian cells were determined by tetrazolium-dye (MTT) colorimetric method. The results in promastigotes were expressed as the concentration inhibiting parasite growth by 50 percent (IC_{50}) after 72 h of incubation period. For anti-amastigote activity, peritoneal macrophages were infected with promastigotes of *L.major* and treated with the compounds for 72 h. The survival index of amastigote proliferation was obtained multiplying the percentage of infected macrophages by the mean number of amastigote forms per infected cell. All compounds showed activity against promastigote forms of *L. major* (IC_{50} of 32.10 µM, 25.60 µM, 1.80 µM, 19.70 µM and 20.60 µM for **1**, **2**, **3**, **4** and **5** compounds, respectively). Only compounds **3** and **4** showed a significant activity against intracellular amastigotes, with an inhibition of survival index of 78% and 95% at 100 µM respectively, compared to control. The compounds were not toxic to macrophages at the highest inhibitor concentration tested. The present results stimulate further investigations of this class of compounds for the rational design of new chemotherapy agents for leishmaniasis.
Supported by UFJF, CNPq and FAPEMIG.

QT.42 - EFFECTS OF METHANOLIC EXTRACTS ON SEVERAL *LEISHMANIA* SPECIES

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Leishmaniasis represents an important public health problem with significant rates of morbidity and mortality. The chemotherapeutic agents used for its treatment exhibit high toxicity and drug resistance are frequently encountered. Plants extracts or plant-derived compounds represent an important alternative source of new antileishmanial agents. Therefore, was analyzed the activity of methanolic extracts against *L. amazonensis*, *L. chagasi* and *L. major*, as well as cytotoxic effects on mammalian cells. Methanolic extracts were obtained from of *Achillea millefolium*, *Casearia cf. silvestris*, *Piptocarpha cf. macropoda*, *Casearia sylvestris*, *Vernonanthura divaricata* and *Samanea tubulosa.* Anti-promastigote assay and cytotoxicity test were checked using the tetrazolium-dye (MTT) colorimetric method. The results of anti-promastigote activity are expressed as the concentration inhibiting parasite growth by 50 percent (IC_{50}) after 72h of incubation period. Among the extracts analyzed, *C. cf. silvestris* was active against *L. amazonensis*, *L. major* and *L. chagasi*, displaying IC_{50} values of 3.3 µg/mL, 6.5 µg/mL and 6.5 µg/mL, respectively. *P. cf. macropoda* displayed activity against *L. amazonensis* and *L. major* (IC_{50} of 6,9 and 7,0 µg/mL, respectively). *C. sylvestris*, *V. divaricata*, *A. millefolium* and *S. tubulosa* showed activity against only one *Leishmania* species (*L. amazonensis* with IC_{50} of 38.1 µg/mL; *L. amazonensis* with IC_{50} of 46.2, *L. major* with IC_{50} of 29.6 µg/mL and *L. chagasi* with IC_{50} of 12.4 µg/mL, respectively). *A. millefolium*, *S. tubulosa*, *C. sylvestris* and *V. divaricata* were not toxic to macrophages at the highest inhibitor concentration tested (111 µg/mL). *C. cf. silvestris* and *P. cf. macropoda* showed moderate cytotoxicity against mammalian cells, indicating poor selectivity. These results encourag us to continue the experiments in order to isolate and identify the bioactive compounds.
Supported by UFJF, CNPq and FAPEMIG.

QT.43 - STUDY OF THE POTENTIAL RESISTANCE OF *Leishmania amazonensis* TO A THIOSEMICARBAZONE DERIVATIVE.

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The chemotherapy of leishmaniasis is based until now in drugs which are not totally efficient and present severe side effects and in some case are able to induce resistance to treatment. This resistance could be related to the volume of the drug (dose and frequency and the time of administration, among other factors. The mechanism of resistance have been associated to the increase expression of a transmembrane protein (Pgp), that act as a efflux pump for a wide spectrum of drugs and depends on energy (from ATP) and must be phosphorylated to be active. As part of our research program on chemotherapy against diseases caused by trypanosomatids we have been studied several thiosemicarbazones and semicarbazones derivatives, which have a medical interest because of their capacity of inhibit the growth of several pathogens. Studies concerning its biological activity show that these compounds are active against trypanosomatids, such as *T. cruzi, T. brucei* and *Leishmania sp.*. In the present work, it was used a thisemicarbazone [(3-methoxy-4-hydroxy-stiryl)-thisemicarbazone], that showed to be very active against *Leishmania amazonensis* promastigotes and Pentamidine as a reference drug. Parasites were grown in Schneider's medium, pH7.2, temperature of 26^0C and resistance was induced in the presence of the compound for several passages in culture. During this process, it was evaluated the potential acquired resistance by new screening in each passage (new LD_{50}), besides the assay of infectivity of the parasites through complement lyses test (to detected metacyclic forms) and *in vitro* infection. The results showed that a significant increase in the LD_{50} was observed at passage number 10 and the parasites were able to maintain its infectivity, even after several passages in culture.
Supported by: CNPq/FAPERJ/CAPES/FIOCRUZ-PDTIS/FIOCRUZ-IOC

QT.44 - INHIBITION OF *Leishmania (Leishmania) amazonensis* ARGINASE BY CONSTITUENTS OF PLANT *Cecropia pachystachya*

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Arginase is a manganese metalloenzyme that catalyzes the hydrolysis of L-arginine to L-ornithine and urea. In *Leishmania*, the arginase is responsible for the production of ornithine, a precursor of polyamines required for the proliferation of the parasite. The plant *Cecropia pachystachya* is used in folk medicine to treat asthma and hypertension. The extracts of *C. pachystachya* can inhibit rat liver arginase and recombinant arginase from *L. (L.) amazonensis*. Previous characterization of the aqueous extract of leaves of *C. pachystachya* showed the presence of chlorogenic acid, (+)-catechin, (-)-epicatechin, isoquercitrin and isovitexin. These five molecules were tested against recombinant arginase from *Leishmania*. The inhibition assays were performed in 50 mM of substrate L-arginine pH 9.6 containing 20 µM of either inhibitor. Between the five inhibitors studied molecules, the highest percentage of inhibition was observed for chlorogenic acid and (-)-epicatechin, which showed 67% and 66% inhibition, respectively. The simulation with the program Dock 6.0 was performed for these five molecules and showed an interaction with the active site of arginase of *Leishmania* that differs from that obtained for the interaction of rat liver arginase. Based on our results, these molecules could be used as prototypes for the molecular development of a new drug against leishmaniasis.
Supported by FAPESP and CNPq.

QT.45 - ANTILEISHMANIAL ACTIVITY OF THIOPURINE DERIVATIVES CONTAINING TRIAZOLE AND STEROID

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The term leishmaniasis refers to a wide variety of clinical syndromes caused by the *Leishmania* species. The primary chemotherapy of leishmaniasis has been based on the use of pentavalent antimonial drugs. Other medications, such as pentamidine, amphotericin B and paromycin are used as secondary options in resistant cases, despite their high toxicity to the host. However, these drugs are not orally active, requiring long-term parenteral administration and displaying serious side effects. Protozoan parasites are unable to synthesize purines *de novo* and this fact represents potential alternatives for drug design in the treatment of parasitic disease. We reported herein the *in vitro* antileishmanial activity and cytotoxicity on mammalian cells of 6-thiopurine derivatives containing 1,2,3-triazole and steroid substituents. All the compounds were assayed against promastigote forms of *L. amazonensis*, *L. major* and *L. braziliensis*. Antileishmanial activity and cytotoxicity on macrophages were determined using the tetrazolium-dye (MTT) colorimetric method. The results in promastigotes were expressed as the concentrations inhibiting parasite growth by 50 percent (IC_{50}) after a three days' incubation period. Among the five compounds tested only one, the 6-thiopurine/bile acid conjugates (6-(3'-colic esther)thiopurine), showed activity against promastigotes of *Leishmania* species. Interestingly, despite the biochemical differences existing between the parasite species, this compound showed activity against the three species of *Leishmania* tested (IC_{50} of 22.8, 13.9 and 17.3 µM for *L. amazonensis, L. braziliensis* and *L. major*, respectively). None of compounds showed cytotoxicity against mammalian cells. These results confirm the antileishmanial activity of thiopurine derivatives and lead to new perspectives about the chemotherapy of this disease.

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QT.46 - EVALUATION OF THE ANTILEISHMANIAL ACTIVITY OF LIPOPHILIC AROMATIC AMINOALCOHOLS

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Leishmania and others protozoa belonging to the trypanosomatid family have distinct polyamine metabolisms compared to mammalian cells, opening the possibility of identifying new targets for antileishmanial drug development. Our purpose is to explore the leishmanicidal effect of thirteen aminoalcohols prepared by the reaction of aromatic halides and aromatic glycidyl ethers with aminoalcohols, aliphatic amides and aliphatic amines. These type of compounds, bearing a covalent bonded aliphatic chain attached to an aminoalcohol fragment, could interact with membrane lipids and be transported into the cytoplasm where they can possibly interfere with the lipid or polyamine transport or metabolism of the parasite. All compounds were assayed against promastigotes of *L. amazonensis, L. major* and *L. chagasi.* The viability of the promastigote forms was determined by the tetrazolium-dye (MTT) colorimetric method. The results are expressed as the concentration inhibiting the parasite growth by 50% (IC_{50}) after a 3-day incubation period with the compounds tested. Among the compounds assayed, five compounds displayed a good activity against *L. amazonensis*, seven compounds were active against *L. major,* and only two were active against *L. chagasi.* The results point to the importance of lipophilicity for antileishmanial activity: the two most active compounds were *N*-decyl aminoalcohol and N-dodecanoyl ethylenediamine (IC_{50} of 0.7 µM and 5.2 µM for *L. major*, respectively). None of the less lipophilic compounds was active. These results confirm the antileishmanial activity of these lipophilic aromatic aminoalcohols and further studies will be done in amastigote forms model.

Supported by FAPEMIG, CAPES, UFJF and CNPq.

QT.47 - IMMUNOCHEMOTHERAPY IN BALB / C MICE INFECTED WITH *Leishmania (L.) amazonensis*

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The aim of the present study was to evaluate the efficacy of the treatment of BALB/c mice infected with *L. (L.) amazonensis* with a palladacycle complex, [Pd(N,N-dimethyl-1-phenethylamine-1,2-ethanebis(diphenylphosphine), DPPE 1.2, associated to the immunization with a recombinant cysteine proteinase from *Leishmania (Leishmania) chagasi*, rLdccys1. Fifteen days after infection with *L. (L.) amazonensis*, BALB/c mice received three doses of 50 µg rLdccys1 plus *Propionibacterium acnes* as adjuvant by subcutaneous route with a 7 days interval. Concomitantly, the animals received 120 µg DPPE 1.2 for 30 days. During the treatment the animal infection was evaluated by measuring the diameter of foot lesions and 10 days after end of the treatment the animals were sacrificed and the parasite burden was also evaluated by the limiting dilution method. A reduction of 99.6%, 99.1% and 97.6% was observed in animals treated with *P. acnes* + DPPE1.2, *P. acnes* + rLdccys1 + DPPE1.2 and DPPE 1.2 alone, respectively, compared to controls that received PBS. T $CD4^+$ and T $CD8^+$ lymphocytes also were analysed in popliteal lymph nodes by FACS during and in the end of the immunochemotherapy. After the second immunization there was a significant increase of both T cell populations in animals that received either DPPE 1.2, DPPE 1.2 plus *P. acnes* or DPPE 1.2 plus *P. acnes* +rLdccys1, indicating that the leishmanicidal activity of this palladacycle complex could be involved with activation of cellular immune responses. The effect of DPPE 1.2 on the activity of *L. (L.) amazonensis* cysteine proteinase was also studied and results demonstrated that the drug inhibited 75% of the cathepsin B activity of *L. (L.) amazonensis* amastigotes. Taken together, these results opened perspectives to evaluate the role of cellular immunity activation and inhibition of *Leishmania* cysteine proteinase activity in the leishmanicidal effect of DPPE 1.2.
Supported by FAPESP and CNPq.

QT.48 - EVALUATION OF THE LEISHMANICIAL ACTIVITY OF A HYPERVALENT ORGANOTELLURIUM COMPOUND AGAINST *Leishmania (Leishmania) chagasi*

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Organotellurium compounds display several biological activities, such as antioxidant properties, antihelmintic and antibacterial activity. More recently, organotelluranes have been studied as irreversible cysteine proteinase inhibitors. Previous experiments performed in our laboratory showed the *in vitro* leishmanicidal effect of the organotellurane RF07 on *L. (L.) chagasi*, the etiological agent of American visceral leishmaniasis, encouraging us to test the *in vivo* leishmanicidal activity of RF07. Treatment of Golden hamsters infected with *L. (L.) chagasi* with RF07 at 300 µM resulted in a decrease of 75% of the spleen parasite burden. The possible inhibition of RF07 on the activity of *L. (L.) chagasi* cysteine proteinase was also analysed by a spectrofluorometry assay performed in the absence and presence of DTT, an agent that mimics the reducing environment found within the macrophage parasitophorous vacuoles. In the absence of DTT, RF07 showed a significant inhibition of proteolytic activity on substrates used for detection of all cathepsin-like cysteine proteinases (L, B, K, V and S), as well as for those specific for cathepsin B and cathepsins K, V and S. On the other hand, in the presence of DTT, RF07 significantly reduced the hydrolysis of the substrate used by all cathepsin-like cysteine proteinases, whereas it did not inhibit the enzyme activity on substrates specific for cathepsins B, K, V and S. These results indicated that RF07 inhibited the cathepsin L of *L. (L.) chagasi*. There is a body of evidence that shows *Leishmania* cathepsins B and L as virulence factors implicated in the amastigote survival within the parasitophorous vacuoles of the vertebrate hosts. Thus, our data showed the efficacy of RF07 for killing *L. (L.) chagasi* and suggest that its leishmanicidal mechanism could be through the inhibition of cathepsin L of *L. (L.) chagasi* amastigotes, opening perspectives to explore this hyphotesis in our model. Supported by FAPESP and CNPq.

QT.49 - HIGH TARGETING OF ANTIMONY TO THE LIVER OF DOGS WITH VISCERAL LEISHMANIASIS FROM MEDIUM SIZED (400 NM) LIPOSOMES

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In the 1970s, a major advance occurred when it was found that liposome-encapsulated antimonial drugs were hundreds of times more effective than non-encapsulated drug for the treatment of visceral leishmaniasis (VL). Recent observation that reduction of liposome diameter from 1200 nm to 400 nm improved the targeting of antimony (Sb) to bone marrow of infected dogs suggested the small sized liposomes are more effective in promoting parasitic reduction. In this work, we investigated the influence of reduction in mean vesicle diameter from 400 to 200 nm on the pharmacokinetics of liposome-encapsulated Sb in dogs with VL. Two liposome formulations differing in their mean size were prepared. Formulation 1 (LP1) was obtained by the dehydration-rehydration, in the presence of cryoprotectant sucrose (diameter 410±75 nm, encapsulation 40±4%). Formulation 2 (LP2) was obtained by further extrusion of the liposomes through 200 nm-pore membrane (diameter 175±25 nm, encapsulation 34±3%). The formulations were applied as intravenous bolus injection at 4.2 mg Sb/Kg body weight (LP1) or 6.5 mg Sb/kg (LP2), plasma pharmacokinetics were evaluated and Sb were determined in liver, spleen and bone marrow after 24 h. LP1 exhibited a significantly shorter plasma half-life of Sb than LP2 (27±8 h vs. 127±24 h). Surprisingly, even though a higher dose of Sb (LP2), a lower level of Sb was found in the liver, and similar levels were found in the bone marrow and spleen. Our data suggests that saturation of the mononuclear phagocyte system (MPS) took place with LP2, because of higher lipid dose and total vesicle surface area, resulting in a reduced liver capture efficiency and slower plasma elimination of Sb. In conclusion, our data indicates that medium sized liposomes (400 nm) are more effective in the targeting of Sb to the infected sites of dogs with VL than small sized liposomes (200 nm).
Supported by CNPq and Fapemig.

QT.50 - DETERMINATION OF *IN VITRO* ANTILEISHMANIAL ACTIVITY OF COPAIBA OIL FROM *Copaifera lucens*

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Leishmaniasis is a group of infectious diseases caused by different protozoa species of the genus *Leishmania*. Generally, the chosen drugs for these diseases are the pentavalent antimoninals, but this drug has high toxicity. Due to this context there is a great need to search new drugs for leishmaniasis treatment. This present study had the purpose to evaluate *in vitro* copaiba oil obtained from *Copaifera lucens* against *Leishmania amazonensis*. Materials and methods: It was evaluated the antiproliferative activity of copaiba oil against promastigotes and axenic amastigotes. The cytotoxic effect of copaiba oil against macrophages J774G8 cells was determined using the colorimetric sulforhodamine-B method. Results: Copaiba oil had significant activity both on promatigote and axenic amastigote forms with IC_{50} values of 22.0 µg/mL and 4.0 µg/mL respectively. The cytotoxicity assay showed that copaiba oil obtained from *C. lucens* has low toxicity against macrophages J774G8 cells with CC_{50} values of 40.0 µg/mL. Conclusion: The effect of copaiba oil obtained from *C. lucens* showed significant activity against *L. amazonensis* parasite.
Suported by: CNPq, FINEP, CAPES, and PRONEX/Fundação Araucária

QT.51 - 5-HYDROXY-2-HYDROXYMETHYL-γ-PYRONE (HMP), OBTAINED FROM *ASPERGILLUS* FUNGI HAS ANTILEISHMANIAL ACTIVITY *IN VIVO*

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Parasites of genus *Leishmania* are transmitted by the sandflies and infect cells of the mononuclear phagocyte lineage of their vertebrate hosts. The chemotherapy is one of the most effective treatments for this disease. Although a number of antileishmanial drugs are available, these drugs are in general toxic, expensive and require long-term treatment. New drugs isolated from plants and microorganisms have shown leishmanicidal action. The 5-hydroxy-2-hydroxymethyl-γ-pyrone (HMP), produced by some species of *Aspergillus* fungi, has bacteriostatic activity and it effectively inhibits the formation of L-DOPA (3,4-dihydroxy-L-phenylalanine) from tyrosine in the process of melanin biosynthesis. Previous studies of our group showed that HMP was straightly involved with antileishmanial activity *in vitro* and could be useful as selective source for the new antileishmanial agent. However, *in vivo* antileishmanial activity of HMP and its effects are unknown. HMP ointment treatment was initiated after 5 weeks of infection. Control and vehicle groups were also done. Tissue samples were collected and analyzed for histopathological, collagen stain and transmission electron microscopy (MET) techniques. Topical treatment with HMP-ointment decreased the parasite burden observed at histopathological and MET analysis when compared with control group. Healing process observed, suppressing ulcer dissemination. In addition, many collagen fibers were disposal in infection site of HMP-treated animals and absence or few cellular infiltrated were observed. These results demonstrated that HMP effectively inhibits the growth of parasites in lesion sites and does not have cytotoxic effects on the host cells *in vitro*. Thus, HMP may have a great potential as antileishmanial agent. Supported by CAPES, CNPq/UFPa, CNPq/MCT/CT-INFRA/CT-PETRO (Processo nº 620179/2008), MCT/CNPq/FNDCT/CAPES/FAPERJ.

QT.52 - DEVELOPING NEW TREATMENTS FOR LEISHMANIASIS

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Leishmaniasis is a worldwide problem affecting 12 million people, with 2 million new cases being reported each year, of which 1.5 million cases are of the cutaneous form of the disease. Drugs used currently for leishmaniasis treatment are administered parenterally, are associated with various side effects and do not result in sterile cure. More importantly, some strains of the parasite have started to develop resistance against treatment. Anti-cancer agents are emerging as an alternative treatment against parasites. Our lab previously tested several anticancer compounds against *Leishmania* and observed that one such drug, tamoxifen, when administered by the intraperitoneal route, exhibits antileishmanial activity *in vivo*. Our project is focused on developing and testing topical tamoxifen formulations to be used as adjuvants or sole agents in the treatment of cutaneous leishmaniasis. The infection of BALB/c mice with *L. amazonensis* at the basis of the tail was chosen as the experimental model. Topical administration of tamoxifen as an ethanolic solution for 2 weeks, starting 30 days after infection resulted in decrease of lesion size. Nanoemulsions (NE) containing tamoxifen were developed and characterized for size and entrapment efficiency (EE). High EE (101 ± 0,4 %) and low particle size (191,5 ± 2 nm) were obtained using a hot homogenization method. Sepigel was used to gel the tamoxifen-loaded NE to facilitate the topical application. The activity of these nanoemulsions, containing various concentrations of tamoxifen, is being evaluated in comparison with the ethanolic formulation. Based on the previously observed drug's activity against the parasite and on reports of tamoxifen as a modulator of wound healing, we expect these topical formulations to contribute to the resolution of leishmaniasis ulcers. Funding: PNPD/CAPES 02847/09-4; FAPESP; CNPq.

QT.53 - ANTILEISHMANIAL ACTIVITY OF IMIDAZOLIDINE DERIVATES ON PROMASTIGOTE E AMASTIGOTE FORMS OF *LEISHMANIA* SPECIES

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Leishmaniasis is caused by parasites of the genus *Leishmania*, which causes illness ranging from skin lesions to systemic infections. Treatment relies on pentavalent antimonials, which have very toxic effects. So, the need to new efficient and safe drugs is urgent. Imidazolidines have shown biological properties, including leishmanicidal activity. In this work, some imidazolidine derivatives were assayed against promastigote and amastigote forms of *Leishmania*. Promastigote forms *of L. amazonensis* and *L. major* were used and the parasite viability was checked by MTT method. The results in the promastigotes were expressed in concentrations inhibiting parasite growth by 50 percent (IC_{50}) after three days incubation. For anti-amastigote activity, peritoneal macrophages infected with promastigotes of *L. amazonensis* were used. The antiparasitic effect of the compounds was evaluated by counting the intracellular amastigotes after 72 hours of treatment. The compounds showed a strong activity against *Leishmania* without cytotoxicity for macrophages. Among the compounds assayed, the ethylenediamine derivative 1,2-Bis(p-methoxybenzyl)ethylenodiamine (**4**), and the compounds 1,3-Bis(p-methoxybenzyl)imidazolidines (**5**), 2-(phenyl)-1,3-Bis(p-methoxybenzyl)imidazolidines (**6**), 2-(4'-metoxiphenyl)-1,3-Bis(p-methoxybenzyl)imidazolidines (**7**) and 2-(2'-hydroxyphenyl)-1,3-Bis(p-methoxybenzyl)imidazolidines (**11**) showed activity against *L. amazonensis* and *L. major* promastigotes (IC_{50} values of 1.86 µg / mL and 1.77 µg / mL for the compound **4**, 4.66 µg / mL and 2.42 µg/mL for the compound **5**, 13.57 µg/mL and 4.05 µg/mL for the compound **6**, 9.04 µg/mL and 2.97 µg/mL for the compound **7,** and 12.47 µg/mL and 6.73 µg/mL for the compound **11**, respectively). The compounds **4** and **5** showed the best activity on intracellular amastigotes of *L. amazonensis*, with an IC_{50} value of 2.0 µg/mL and 9.4 µg/mL, respectively. The leishmanicidal activity can be related with inhibition of polyamine synthesis and cellular penetration across biological membrane. These results suggest that these compounds have promising antileishmanial potential and may contribute to the development of new leishmanicidal agents. Supported by FAPEMIG, UFJF and CNPq.

QT.54 - IN VITRO ACTIVITY OF AQUEOUS EXTRACT OBTAINED FROM ROOTS OF *PHYSALIS ANGULATA* ON *LEISHMANIA (L.) AMAZONENSIS* PROMASTIGOTES AND HOST CELL

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The leishmaniasis is an infectious disease caused by various species of the protozoan parasites in the genus *Leishmania*. The drugs of choice for the treatment of this disease are the pentavalent antimonials, which show high toxicity. Natural products from plants represent an important source of new antileishmanial compounds. Thus, we consider interesting to analyze an aqueous extract from roots of *Physalis angulata,* an annual herb widely used in popular medicine, against promastigotes of *L. amazonensis*. In the present study we showed that extract inhibited 83.5 % and 100% the promastigotes growth in the concentrations of 50 and 100 µg/ mL, respectively. In addition, ultrastructural analysis showed significant morphological changes in promastigotes. On treated promastigotes with 50 µg/mL of the extract were observed some vacuoles in flagellar pocket membrane and alterations in flagellar membrane. Treated promastigotes in the concentration of 100 µg/ mL showed morphological alterations such as myelin-like figures into the flagellar pocket, duplication of kinetoplast DNA, some vesicles inside the flagellar pocket and alterations on shape and swelling of kinetoplast. The tetrazolium-dye (MTT) colorimetric method and Mitochondrial Membrane Potential Detection Kit (JC-1) showed that this compound presented no cytotoxic effects against mammalian cells. These results demonstrated that aqueous extract of *Physalis angulata* effectively inhibits the growth of parasites and does not have cytotoxic effects on the host cells. Thus, this study revealed that extract from *Physalis angulata* has antileishmanial properties. Supported by PIBIC/CNPq/UFPA; MCT/CNPq/ICT (Grant number 16/2008).

QT.55 - LEISHMANICIDAL ACTIVITY OF MARINE ALKALOID ANALOGUES

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Leishmaniasis is a zoonotic disease that is considered endemic in 88 tropical countries, which is caused by protozoa of the genus *Leishmania*. The World Health Organization estimates that approximately 12 million people are infected and the annual incidence of the disease is approximately 1.5 to 2 million. Currently the treatment of this disease is based on chemotherapy with antimonial derivatives, but these compounds show severe toxicity to patients and require intravenous administration, and even the appearance of cases of drug resistance. Thus, the necessity of discovering new compounds that present high leishmanicidal activity and low toxicity is clear. Results describing the anti-parasitic potential of compounds derived from marine natural products, led us to examine the leishmanicial activity and cytotoxicity on mammalian cells of some synthetic marine alkaloid 3-alkylpyridinium analogues. All the compounds were assayed against promastigote forms of *L. amazonensis* and *L. braziliensis*. Antileishmanial activity and cytotoxicity on macrophages were determined using the tetrazoliumdye (MTT) colorimetric method. The results in promastigotes were expressed as the concentrations inhibiting parasite growth by 50 percent (IC_{50}) after a three days' incubation period. In general, the alkaloid 3-alkylpyridinium analogues tested were more active against promastigotes of *L. amazonensis.* Among ten compounds tested, four compounds, 3-(3-(9-azidononacyloxy)propyl)pyridine (**6**) and its corresponding *N*-benzyl salt (**7**), *N*-benzyl salt of methyl *N*-[1,1-dimethylethoxy)carbonyl]-*N*-[9-[3-(3-pyridinyl)propoxy]nonyl]-alaninate (**8**) and the 3-pyridinepropanol Zincke´s salt (**9**), showed significant activity against promastigotes of this *Leishmania* species (IC_{50} of 23.92 µM, 2.88 µM, 1.09 µM and 14.95 µM, respectively). The compounds showed moderate toxicity in mammalian cells. These results confirm the antileishmanial activity of these synthetic marine alkaloid analogues and further studies will be done in amastigote forms model. Supported by FAPEMIG, CNPq and UFJF.

QT.56 - USE OF PLGA MICROPARTICLES FOR SINGLE-DOSE TREATMENT OF CUTANEOUS LEISHMANIASIS

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The conventional treatment of cutaneous leishmaniasis is based on multiple parenteral injections with antimonial or anfotericin B drugs. In this study, we propose to develop a localized single-dose treatment for this disease using poly-(lactide-co-glycolide) PLGA microparticles (mps) loaded with anfotericin B. 50:50 PLGA mps were prepared with 10 % (w/w) anfotericin B by the multiple emulsion method, followed by solvent evaporation, aqueous washing and dried. Morphological and physical parameters were recorded. Mice were infected in the ear with fluorescent *Leishmania amazonensis* promastigotes, and on day 26 they received a subcutaneous injection with 50 ug of anfotericin B: i) in the free form; ii) in PLGA mps; iii) in liposomal formulation (Ambisome®). Controls received empty mps or 10 ul of saline alone. The lesion sizes and parasite loads were measured with a dial calliper and fluorimetry, respectively. Toxicological parameters (AST, ALT and creatinin) were measured in the serum using commercial kits.The drug encapsulation ratio was 89.7% as measured by HPLC. Drug loading did not affect the zeta potential (~ -11 mV) and the size (~ 5.5 µm) of the microparticles. In vivo, despite the transient effect of Ambisome®, only the PLGA formulation controlled the lesion growth throughout infection. Free drug or empty microparticles were not effective. On day 70 of infection, the parasite loads were significantly smaller in the animals treated with drug-loaded PLGA mps but not with free drug. No changes in AST, ALT and creatinin were observed at the completion of the experiment.Loading of anfotericin B in PLGA microparticles may promote a sustained drug release in the lesion site leading to a durable and safe therapeutic effect. These findings support this new approach for single-dose localized treatment of cutaneous leishmaniasis.

QT.57 - LEISHMANICIDAL ACTIVITY OF ESSENTIAL OILS FROM *Myrcia splendens* AND *Protium hebetatum*

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Leishmaniasis is a widespread tropical disease, with high prevalence around the world. According to WHO, about 4000 new cases are reported every year. The current treatments rely mainly on antimonials and amphotericin B that are unsatisfactory due to their toxic side effects, high costs, and increasing problems with drug resistance. Different approaches have been used to identify novel chemotherapics against Leishmania sp. parasites, and one strategy has been the analysis of naturally occurring plant-derived compounds. In this study we analyzed the effects of two essential oils, extracted from *Myrcia splendens*, identified on the experiments as oil 6 (O6) and *Protium hebetatum* identified as oil 8 (O8) on *Leishmania amazonensis* in vitro. Antileishmanial activity of O6 and O8 were evaluated in vitro on promastigotes culture as well as on amastigotes-infected macrophages. Besides that, we analysed citotoxicity of them for peritoneal macrophages's in vitro through XTT assay. Our results showed that such as O6 as O8 inhibited promastigotes growth in vitro in a dose-dependent manner. It was observed around 31,18%; 42,83 and 50% of promastigotes growth inhibition for 0,1µg/mL, 1µg/ml and 10µg/mL of O6, respectively and 27,25%; 47,67% and 57,44% for 0,1µg/mL, 1µg/ml and 10µg/mL of O8. Moreover, both essential oils (O6 and O8) were able to reduce amastigotes survival inside macrophages in a dose-dependent manner. At 10µg/mL O6 inhibited 42% of amastigotes survival, while O8 10µg/mL inhibited around 52%. At 10µg/mL, neither O6 nor O8 were cytotoxic to peritoneal macrophages as evaluated by the XTT test, after 24 hour of treatment. Furhther experiments will be performed in order to identify possible mechanisms of action of them. Anyway, our results were correlated with the essential oils chemical compositions and point both essential oils as efficient compounds against *L. amazonensis* and provide new perspectives for novel compounds for leishmaniasis treatment.
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QT.58 - ANTILEISHMANIAL ACTIVITY OF THIOSEMICARBAZONE DERIVATIVE FROM LIMONENE COMPLEXED WITH COPPER

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Leishmaniasis is a neglected tropical parasitic disease resulting from infection of macrophages by intracellular parasites of genus *Leishmania sp*., which represents an important public health problem. The treatment for leishmaniasis depends on a limited number of drugs, and the basic treatment consists on the administration of the pentavalent antimonials derivatives. However, the use of this drug has been showed serious toxic effects. This way is very important to the development of efficient and safe new drugs for leishmaniasis treatment. Here we investigated the antileishmanial activity of the compound [Bis [N- 4 - [*R*-1-metil-4-(1-metiletenil)-cicloexeno]-*o*-clorobenzaldeidotiossemicarbazonato)] derivative from limonene complexed with copper, denominated as TSZ against the protozoan *Leishmania amazonensis*. Effects of TSZ on parasites were evaluated on axenic and intracellular amastigote forms, and its cytotoxicity to $J774_{G8}$ murine macrophages. We also used scanning electron microscopy (SEM) to evaluate the effect of TSZ on the morphology of promastigotes, and flow cytometry using Rhodamine 123 as a fluorescent marker to evaluate mitochondrial membrane potential. TSZ showed activity against *L. amazonensis*, with IC_{50} values of 7.25 ± 2.22 µg/ml and 8.15 ± 0.21 µg/ml for axenic and intracellular amastigote forms, respectively. TSZ showed cytotoxicity against machophages $J774_{G8}$ at CC_{50} of 17.6 ± 4.21 µg/ml. The cytotoxicity of the compound to $J774_{G8}$ macrophages and its activity against the protozoa were compared using the selectivity index (SI) ratio (CC_{50} for $J774_{G8}$ macrophages/IC_{50} for protozoa). When observed by SEM, TSZ caused alterations dose-depedent in the shape and size of the parasites, included cellular disintegration. By flow cytometry was observed that Rhodamine 123 showed decreased fluorescence in parasites treated with TSZ, indicating a decreased in the mitochondrial membrane potential. These results could be followed up by *in vivo* testing. Acknowledgements: This study was supported by grants from CNPq, FINEP, CAPES and Fundação Araucária.

QT.59 - STUDIES OF NOVEL 1,2,3 TRYAZOLE DERIVATIVES AGAINST *LEISHMANIA AMAZONENSIS*

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Leishmaniasis refers to various clinical syndromes caused by intracellular parasites from the genus Leishmania. This disease is an increasing public health problem in Brazil. Most of the antileishmanial drugs currently in use for treatment from the long time established antimonials to the recently introduced miltefosine have side effects or parasite resistance.The need of development of new drugs is necessary. The *in vitro* antileishmanial activity of a series the benzyl piridyl, furanyl ou tiofenyl-1-(phenylamine)-5-methyl-1*H*-1, 2,3-tryazol-4-carbohydrazide sustituted are evaluated against *Leishmania amazonensis* ((MHOM/BR/77LTB 0016 strain) promastigotes and intracellular amastigotes.forms. Promastigotes were evaluated by. counting the parasites in Neubauer´s chamber. Intracellular amastigotes activities were measured by microscopical counting of percentage of amastigote/macrophage.Most of nitro furanyl tryazole derivative exhibited good activity (IC_{50}=0.2-2.µM) against the promastigotes forms.In addition, the results showed that these furanyl tryazole derivatives were less active for amastigote (IC_{50}=15- 360µM) than for promastigotes. The references antileishmanial agents pentamidine showed active for both amastigotes and promastigotes forms (IC_{50}<2.0 µM). It can be suggested that others factors would be associated of this specific 1,2,3 tryazole structure. Further experiments are being carried out in order to define a mechanism of action besides a chemical structure and biological activity.
Supported by CNPq/PDTIS/PAPES/IOC-FIOCRUZ./ UFF

QT.60 - ANTILEISHMANIAL ACTIVITY OF AERIAL PARTS FROM *Porophyllum ruderale* (Jacq.) *Cass.*

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Leishmaniasis is a group of parasitic diseases caused by different species of *Leishmania* which affects around 2 million people *per annum*. The available therapy still causes serious side effects. On northwest of State of Paraná, Brazil, water and alcohol-water extracts of *Porophyllum ruderale* (Jacq.) *Cass.* have been used popularly as treatment over lesions caused by *Leishmania* sp.. This study includes the extraction process, bioassay-guided fractionation by liquid chromatography method and the *in vitro* antileishmanial activity. The dichloromethane extract, fractions and subfractions of aerial parts from *P. ruderale* (Jacq.) *Cass.* were evaluated against promastigotes of *Leishmania amazonensis* and cytotoxicity against murine macrophages J774G8. Antileishmanial assay were performed in 24-well microplates and IC_{50} (50% growth inhibitory activity) values was determined by direct count in a Neubauer chamber. The viability of the macrophages was determined by MTT method. Cytotoxic concentration of 50% viable cells (CC_{50}) was calculated by linear regression analysis. Dichloromethane extract, fraction 6 and subfractions 6.16 and 6.16.12 were the most actives against promastigotes of *L. amazonensis* with IC_{50} values of 57±13.11, 19,50±6,36, 13.5±0.71 and 3.05±0.64 µg/mL, respectively. Cytotoxic concentrations (CC_{50}) for dichloromethane extract, fraction 6 and subfractions 6.16 and 6.16.12 were, respectively, 215±21.21, 39.5±7.78, 70±7.07 and 38±4.24 µg/mL. These results suggest that *P. ruderale* (Jacq.) *Cass.* has interesting antileishmanial activity and low cytotoxic effect against murine macrophages J774G8. Studies have been done to understand the mechanism of action of this plant and extracts.
Supported by UNIOESTE, CNPq, CAPES, FINEP and PRONEX/Fundação Araucária

QT.61 - EFFICACY OF CHALCONE-CONTAINING ELASTIC LIPOSOMES FOR TOPICAL TREATMENT OF CUTANEOUS LEISHMANIASIS

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Previously, we reported that the introduction of a NO_2 group into the molecule of a natural chalcone (CH8) improved its efficacy when tested subcutaneously in *Leishmania amazonensis*-infected BALB/c mice, and rendered the drug more effective than Pentostam. Aiming at developing a CH8 formulation for topical application, the CH8 chalcone was encapsulated in conventional liposomes (CL) and in polyoxyethylene glycol (PEG)-functionalized liposomes (PL) for greater cutaneous permeation. BALB/c mice were infected in the ear with promastigotes of GFP-transfected *Leishmania amazonensis*. After 12 days, they were topically treated twice a day, during 30 days with the following formulations of CH8: a) CL, 6.6 μg CH8/ dose; b) PL 6.6 μg CH8/ dose; or lanete cream, 200μg CH8/dose. Controls received empty liposomes or lanete cream. Alternatively, intralesional treatments were carried out twice a week for the same period of time with CH8 in liposomal formulations or in PBS at 3.3 μg CH8/dose. Measurement of lesion size growth showed that on day 42 post infection, encapsulation in CL rendered the topically applied CH8 more effective than when presented in lanete cream in a dose 30-fold higher, comparable to intralesional CH8. The parasite burden in animals given topical CH8 in CL was 90% smaller than in untreated controls. Interestingly, all treatments with CH8 in CL were more effective than CH8 in PL. Permeation through polycarbonate membranes demonstrated the superior elasticity of the CH8 in CL in relation to PL, and this was confirmed in isolated pig skin by tape-stripping and Franz cell HPLC techniques. These results show that intrinsic physical-chemical properties of the antileishmanial CH8 confer enhanced elasticity and skin permeability to CL, simplifying the liposomal preparation process, and increasing topical drug efficacy against cutaneous leishmaniasis. Supported by FAPERJ and CNPQ.

QT.62 - EFFICACY OF MILTEFOSINE IN THE TREATMENT OF MURINE MODEL OF CUTANEOUS LEISHMANIASIS BY *Leishmania amazonensis*

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Leishmaniasis is one of the most important neglected tropical disease caused by parasites of the *Leishmania* genus. In Brazil, *Leishmania amazonensis* is responsible for cutaneous and diffuse cutaneous leishmaniasis. The current chemotherapy for the treatment of leishmaniasis is based on Pentavalent Antimonials, Amphotericin B and Pentamidine. Recently, Miltefosine has been implemented as the first oral treatment for visceral leishmaniasis in India. However, when Miltefosine was tested in some species of the New World, the results were very unsatisfactory. Then, we decided to study the efficacy of Miltefosine in the treatment of BALB/c murine models infected with *Leishmania amazonensis*. BALB/c mice were inoculated with infective promastigotes of *L. amazonensis* through subcutaneous injections at the base of the tail. After the development of lesions, mice were divided into ten groups: control and vehicle groups, Miltefosine-treated groups (2,5; 5; 10; 20; 30; 40; 50 mg/kg/day), and Glucantime® group (50 mg/kg/day). Miltefosine was administrated by oral gavage, while Glucantime by intraperitoneal route, both treatments for 21 days. The efficacy of Miltefosine was evaluated by measuring the size of the lesions, and the presence of parasite in lesions stained with Giemsa. The result obtained suggested a dose-dependent response, where the size of the lesions decreases significantly with the increase of the Miltefosine doses. In a few days after the last doses, it was observed a gradual increase in the lesions size in mice treated with 5 and 10 mg/kg/day, while in the other groups, only one mouse developed a new lesion after three months of the end of treatment. Evaluation of the lesions stained with Giemsa confirmed a significant reduction of the parasite burden in the Miltefosine-treated groups. Thus, this study shows that miltefosine is effective against experimental cutaneous leishmaniasis caused by *L. amazonensis* in mice and suggests that further studies should be carried out in patients. Supported by CNPq, FAPERJ, and CAPES.

QT.63 - EVALUATION OF LEISHMANICIDAL ACTIVITY OF *PENICILLIUM WAKSMANII* PRODUCTS

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Leishmaniasis is a neglected disease prioritized by WHO Program of Tropical Diseases (TDR). In the face of this public health problem, treatment and control alternatives are necessary, with development of more efficient and less toxic new drugs. In this study, we demonstrated the leishmanicidal activity of products from the *Penicillium waksmanii* fungus on *Leishmania amazonensis* promastigotes. Tests were performed incubating *L. amazonensis* promastigotes for 72 hours in the presence of 0-100 µg/mL of extracts of several polarities from culture supernatants of *Penicillium waksmanii*. The activity was colorimetrically evaluated by MTT (Thiazolyl Blue Tetrazolium Bromide) assay. ICs50 were calculated through logarithmic regression analysis. We observed that some extracts from supernatants of *Penicillium waksmanii* presented interesting antipromastigote activity, with emphasis on the extracts PW2 and PW7, both with IC50 around 25 µg/mL. These extracts will be purified and further analyzed on intracellular amastigotes to confirm their potential as new antileishmanial drugs. Supported by PAPES/PIBIC/CNPq.

QT.64 - COMBINED FUROSEMIDE AND PENTOSTAM THERAPY AGAINST CUTANEOUS LEISHMANIASIS

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Intramuscularly administered pentavalent antimonials such as Glucantime® and Pentostam® are the first line therapy against cutaneous leishmaniasisdespite their toxicity and drug-resistance potential. In previous studies, we showed that specific inhibition of Na+-ATPase of *L. amazonensis* promastigotes by furosemide led to parasite killing (De Almeida-Amaral, E.E *et al*., 2008). After determining that the antileishmanial activity of furosemide is extended to the intracellular parasite forms, in this work we proposed to investigate whether furosemide is active in vivo in infected mice, and whether the drug acts synergistically with Pentostam aiming at reducing the antimonial toxic dose. BALB/c mice were infected in the ear with *L. amazonensis* promastigotes-GFP. After 7 days of infection, the animals were treated intraperitoneally with a total of 14 daily doses of 50 mg/kg of furosemide, 20 mg/Kg of Pentostam®, or a combination of furosemide plus Pentostam, in the same dose. Controls received PBS. The lesion sizes were measured throughout the infection with a dial calliper. In the end of the experiment, the animals were parasites were quantified both by fluorimetryand by Limiting Dilution Assay. The results indicated that furosemide is effective in vivo against *L. amazonensis* and that the combination therapy with Pentostam® further decreased lesion growth. Supported by CNPq.

De Almeida-Amaral, E.E; Caruso-Neves, C; Pires, V.M.P.; Meyer-Fernandes, J.R. (2008) Leishmania amazonensis: characterization of an ouabain-insensitive Na+-ATPase activity. *Experimental Parasitology,* 118(2):165-171.

QT.65 - EFFECTS OF TOMATIDINE ON THE STEROLS METABOLISM OF *LEISHMANIA AMAZONENSIS* PROMASTIGOTES

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The leishmaniasis are clinically different infectious diseases caused by flagellated protozoan of the genus *Leishmania.* It is known to affect ~12 million people worldwide with approximately 2 million new infections per year. Some plants produce substances for their own defense against pathogens and predators. In *Lycopersicon* species, such as tomato *L. esculentum*, the main antimicrobial compound is the steroidal glycoalkaloid α-tomatine. The loss of saccharide side chain of tomatine produces the aglycone tomatidine. In the present study we shown that tomatidine inhibits the growth and promotes alterations in the ultrastructure of *Leishmania amazonensis*. Through transmission electron microscopy was shown that cells treated with tomatidine presented remarkable lesions, such as alterations in the mitochondrial structure and vacuolization. It was also observed a reduction in oxygen consumption and membrane potential in promastigotes treated. Cells exposed to tomatidine for 48 h presented a complete depletion in the level of endogenous 24-alkylated sterols, such as ergosta 5,7,22-trien-3β-ol (ergosterol) and ergosta 7,22-dien-3 β-ol. However, the treated cells accumulated 24-desalkyl sterols (4-methylcholesta-8,24-dien-3 β-ol and cholesta-8,24-dien-3 β-ol). These results are consistent with an inhibition of 24-sterol methyltranferase (24-SMT), an enzyme that methenylates the steroid at the 24 position during ergosterol and others 24-alkylated sterol biosynthesis. Currently, there is no medication that is both perfectly safe and completely efficacious against leishmaniasis. Tomatidine shows great potential as an antileishmanial agent and should be considered as new promissory drugs against leishmaniasis.
Supported by Capes, CNPq and Faperj.

QT.66 - EFFECT OF *Kalanchoe pinnata* AND ITS FLAVONOID QUERCETIN AGAINST *Leishmania braziliensis in vitro* AND *in vivo*

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Previously, we showed therapeutic effect of the aqueos extract of *Kalanchoe pinnata* and its flanoid quercetin in mice infected with *Leishmania amazonensis*. In the present study, we investigate the effect of *K. pinnata* and quercetin on *L. braziliensis,* the most important dermatropic *Leishmania* species on Brazil. *In vitro* promastigotes *L. brazileinsis* (2,5 x 10^5/ml) were cultivated with 0-100µg/ml of quercetin for 96h/28°C. Parasites were counted daily in a Neubauer chamber. Monolayers of peritoneal murine macrophages infected with *L. brazilieinsis* (at a ratio of 5 parasites/macrophage) for 4h at 37°C/5%CO_2 were incubated with *K. pinnata* aqueous extract or 0-100µg/ml of quercetin for 96h/28°C for 48h/37°C/5%CO_2. Hamster were infected with 10^7 promastigotes of *L. braziliensis* on the footpad. The experimental groups were treated after seven days of infection during eight weeks with 2mg of quercetin or 40mg of aqueous extract of *K. pinnata* by oral route for five time a week. Controls were non treated or treated animals with 8mg of glucantime five time a week by intraperitoneal injections. The lesion size was measures with dial caliper. Delayed-type hypersensitivity (DTH) against total antigen of *L. braziliensis* was evaluated in the ninth week of the infection. *K. pinnata* extract and quercetin not showed effect inhibitory against promastigote form, but inhibited intracelluar amastigote a dose-dependent fashion. *K pinnata* extract and quercetin inhibited 50% and 70% of intracelluar amastigote at 500µg/ml and 100µg/ml, respectively. *In vivo* quercetin was more active in controlling the growth of the lesion than the *K.pinnata* extract. The DTH was increased in all treated animals.These data demonstrate that the extract of *K. pinnata* and quercetin are active against *L. braziliensis* and may be important for antileishmanial drug development. Supported by Faperj.

QT.67 - THE ANTI-LEISHMANIAL PTEROCARPANOQUINONE LQB118 TRIGGERS INDUCTION OF APOPTOSIS IN *Leishmania braziliensis*

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We previouly demonstrated therapeutical effect of LQB118 by intralesional and oral route on hamster infected with *Leishmania braziliensis*. The aim of this study was to evaluate the anti-leishmanial activity in *L. brazileinsis*. Promastigote forms were cultivated with LQB 118 at concentrations of 0-20 µM for 96h/28°C. Parasites were counted daily in a Neubauer chamber. Monolayers of peritoneal murine macrophages infected with *L. brazileinsis* (at a ratio of 5 parasites/macrophage) for 4h at 37°C/5%CO2 were incubated with the LQB118 at 0-20 µM for 48h/37°C/5%CO_2. The supernatants was collected and nitric oxide was determined by Griess reagent. After staining with Giemsa, the number of intracellular amastigotes was counted under a microscope. To assess whether the anti-leishmanial effect was due to apoptosis, promastigotes were treated with LQB118 at 20µM for 24-48h/28°C and then incubated with double staining for annexin V-FITC and propidium iodide. *In situ* detection of DNA fragmentation following treatment of promastigote or intracellular amastigotes with LQB118 for 48h at 20µM was performed using the TUNEL Kit and analysed by fluorescence microscopy. The LQB118 showed a dose-dependent inhibitory effect in both promastigote and amastigote. On promastigote inhibited 100%, 78% and 30%, respectively, at 20, 10 and 5µM. Already on intracellular amastigotes the inhibitory effect was 80%, 60% and 10% at 20, 10 and 5µM, respectively. This effect not was acomppanied by increased of nitric oxide. LQB118 induces phosphatiylserine externalization in *L. braziliensis* promastigotes. The percentage of annexin V-FITC-positive cells increased to 9,06% at 24h and 21,78% at 48h. The negative control (untreated promastigotes) at 24 and 48h was 3.68 and 5.22%, respectively. LQB118 induced increase fluorescence in both promastigote and intracelular amastigote as compared to controls. These results demonstrate that the antileishmanial activity of LQB118 in promastigote and amastigote of *L. braziliensis* was mediated via apoptosis. Supported by FAPERJ.

QT.68 - *TOXOPLASMA GONDII* CELL CYCLE INHIBITION BY A NEW PTEROCARPANQUINONA

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The protozoan parasite *Toxoplasma gondii* is capable of infect almost all warm-blooded and nucleated cells, being responsible for toxoplasmosis disease in humans. In this work, a new pterocarpanquinona, which has recently shown antitumoral activity, is tested in RH strain *T. gondii* crucial cell cycle stages. Invasion assay was performed infecting monolayers of LLC-MK2 cells in the presence of 2,5 or 5µM of the compound. After interaction time, samples were fixed and processed to optical microscopy. The same concentratios were tested during infection development, with samples fixation at 24 and 48 hours post-infection and processed to optical and electron microscopy. On the other hand, to evaluate egress step, calcium ionophore A238187 was used to trigger the evasion of *T. gondii* from host cells, an event of *T. gondii* life cycle that still poorly understood. While invasion and egress were inhibited under the new pterocarpanquinona treatment, by 49 and 33% respectively, no significant changes were observed when the compound was added after interaction, as verified by quantitative and structural assay of *T. gondii* infection development. Supported by CAPES and FAPERJ.

QT.69 - EFFECT OF ITRACONAZOLE AGAINST TISSUE CYSTS OF *TOXOPLASMA GONDII IN VITRO AND IN VIVO*

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Toxoplasma gondii is an important opportunistic pathogen affecting immunocompromised patients with AIDS and other syndromes. The toxoplasmic encephalitis in those patients is responsible for great morbidity and mortality, this clinical presentation of the disease is commonly related to the reactivation of tissue cysts due to a former chronic infection. Although there are very effective drugs against the acute phase of toxoplasmosis, the chronic stage is untreatable and once an individual is infected it remains for the rest of its life. Thus the discovery of potential drugs that affect tissue cysts is very important. Previous studies of our group demonstrated the high susceptibility of *T. gondii* tachyzoites to itraconazole (ITZ) (Martins-Duarte, et. al. 2008). In this work we present data of the effect of ITZ against cell culture infected with encysted bradyzoites analyzed by transmission electron microscopy and the effect of the treatment in chronically infected mice. Monolayers of LLC-MK_2 were infected with low burden of parasites and after 2 weeks of infection were incubated with ITZ 2µM for 48h. Transmission electron microscopy analysis of encysted bradyzoites after treatment with ITZ showed many remarkable effects, as the presence of autophagic bodies observed in many bradyzoites. The presence of lysed parasites was also observed inside cysts after the treatment. Preliminary results have demonstrated that this drug might also be effective against chronic toxoplasmosis in murine models as the treatment with ITZ 10mg/kg reduced the numbers of cysts compared with the control group. Altogether, the results obtained up to now suggest that ITZ may have a direct effect against encysted bradyzoites of *T. gondii* and can be considered a potential drug against chronic phase of toxoplasmosis.
This work was supported by CNPq and FAPERJ.

QT.70 - TREATMENT OF *Toxoplasma gondii* INTERACTIONS WITH LLCMK2 USING A NEW PTEROCARPANQUINONE

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Toxoplasma gondii, the agent of Toxoplasmosis, is an obligate intracellular protozoan able to infect a wide range of vertebrate cells including nonprofessional and professional phagocytes. Therefore, the drugs to control this parasite must have intracellular activity. The most usual therapy for Toxoplasmosis is the combination of sulfadiazine and pyrimethamine, although this treatment is associated with adverse reactions. Because of this, the development of new drugs is necessary. In previous studies, naphthoquinone derivatives showed different biological activities as anticancer, as agents capable of acting in groups of DNA, avoiding that it will double the cancer cells. These derivatives also display antiparasitic activity against *Plasmodium falciparum* and *Leishmania amazonsis*. The derivative that had its activity tested in this work was a synthetic structure analog of naphthoquinone. This work show the outcomes of the citotoxicity test with this derivative during *T. gondii* interaction with nonprofessional phagocytes. For this, LLCMK2 were cultured with RPMi 1640 supplemented 10% Fetal Bovine Serum. Before interactions, cells were cultured in 24 well plates. The interaction was realized in the presence or absence of pterocarpanquinone. The compound was able to inhibit intracellular parasite prolifertation with an IC50 of 2,5 µM. Scanning and Transmission Electron Microscopy analysis showed that the concentrations wich damage the parasite did not afect the host cells. Alterations included damage of parasite membranes. The derivative was also capable of to decrease the infection index during interaction with LLCMK2. These results suggest that naphthoquinones are compounds potentially important for the killing of *Toxoplasma gondii*. Supported by: CNPq, FAPERJ and PRONEX.

QT.71 - EFFECT OF A FUCOSYLATED CHONDROINTIN SULFATE ON CYTHOADHESION OF *Plasmodium falciparum*-INFECTED ERYTROCYTES TO ENDOTHELIAL CELLS

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Severe malaria is characterized by the sequestration of *Plasmodium falciparum*–infected erythrocytes (IEs) in the microvasculature of vital organs. Adhesion of IEs to endothelial cells has a key role in the pathogenesis of life-threatening malaria and could be targeted by anti-adhesion therapy. Therefore, sulfated polysaccharides, such as heparin, chondroitin-4-sulfate (CSA), dextran sulfate, have been tested to prevent severe malaria due to their ability in inhibiting cytoadherence of IEs to host receptors to different extents. However, their uses have been discouraged; since heparin treatment leads to serious side effects, such as intracranial bleedings; and CSA and dextran sulfate, which are extracted from mammals, present a potential risk of carrying harmful contaminants to humans. Indeed, although several compounds have been tested in order to prevent malaria severe forms, none has demonstrated unequivocal evidence in the amelioration of severe malaria outcomes in clinical trials. Here, we showed that fucosylated chondroitin sulfate (FucCS), a highly sulfated polysaccharide isolated from sea cucumber, *Ludwigothurea grisea,* composed of a chondroitin sulfate backbone substituted at the 3-position of the β-D-glucuronic acid residues with 2,4-disulfated α-L-fucopyranosyl branches, is a potent inhibitor of the IEs cytoadhesion to human lung endothelial cells (HLEC) and blocks *P. falciparum* merozoites re-invasion. Inhibition seems to be nonspecific of parasite adhesive phenotype and occurs in a concentration-dependent-manner. Interesting, removal of the sulfated fucose branches on the FucCS practically abolished the inhibitory effect, suggesting a pivotal role played by this compound electrical charge. Furthermore, treatment with FucCS at 1 mg/kg/animal/day showed to improve survival of C57BL/6 mice infected with *Plasmodium berghei* ANKA, an experimental model for cerebral malaria and characterized by a potent inflammatory process. Of note, treated mice did not exhibit visible side effects during therapy. Thus, we propose FucCS as a promising candidate for adjunct therapy to prevent severe malaria outcomes. Supported by FAPESP and Instituto Nacional de Tecnologia em Vacinas (CNPq-INCTV) and CNPq - Doenças Negligenciadas.

QT.72 - ANTIMALARIAL ACTIVITY OF PRIMAQUINE DERIVATIVES AGAINST *PLASMODIUM FALCIPARUM IN VITRO*

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Adequate therapeutic control of human malaria infections with *Plasmodium vivax* includes the use of the anti-relapsing drug primaquine (8-amino-quinoline) together with chloroquine. Primaquine leads to radical cure, causing complete elimination of the exoerythrocytic liver stages, the hypnozoites, responsible for such relapses. Primaquine however is toxic, causing multiple side effects, thus needs to be replaced. In this work, a series of primaquine derivatives thiazolidinones were obtained by chemistry synthesis using the primaquine as precursor. These thiazolidinones were synthesized by one-pot reaction of primaquine, arenealdehydes and mercaptoacetic acid after purification by column chromatography using silica gel and hexane ethyl acetate as solvent. The activity of the compounds was first evaluated against blood forms of *P. falciparum* (cloroquine-resistant clone – W2), maintained in continuous cultures. Two methods were used to evaluate parasite survival: i) incorporation of tritiated hypoxanthine by the live parasites; ii) enzyme-linked immunosorbent assays (ELISA) which provide very sensitive in quantifying the histidine-rich protein 2 (HRP2) produced during parasite development. From the 13 new compounds tested, six were active (IC_{50} <3ug/ml) in both tests; the others were inactive (IC_{50} $\geq$50ug/ml. The cytotoxicity of the active compounds was evaluated against hepatoma cells (HepG2) in cultures, through MTT. All compounds had low toxicity, thus demonstrating high selectivity index (SI=50 to 200), therefore are potential new antimalarials. Primaquine, less active against the blood forms, was more toxic then all derivatives in vitro (SI= <7ug/ml). These compounds are now tested to evaluate whether they will inhibit sporogony of *P. gallinaceum* in *Aedes fluviatilis*. In this model, primaquine inhibits 100% of the sporogony in mosquitos fed in infected chicken treated once with 15mg/ml (Carvalho et al. 1992). Those new antimalarial aiming to replace primaquine, if identified may be useful, especially because these compounds are also active against *P. falciparum*. Supported by CNPq and FAPEMIG.

QT.73 - ANTIMALARIAL ACTIVITY OF BIOPRODUCTS FROM *Aspidosperma* sp PLANTS TESTED IN BLOOD CULTURES OF *Plasmodium falciparum*

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Antimalarials currently used as first-line treatments of human malaria, quinine and artesunate, are originated from medicinal plants, *Cinchona* sp and *Artemisia annua*, respectively. Over 1,000 plant species are used in folk medicine against fever in the world, reinforcing the importance of searching new chemotherapeutic agents from ethnopharmacology. Our malaria group has tested about 60 different species of medicinal plants used in the Amazon and other regions, and described antimalarial activity in 50% of them (Krettli *et al.,*Current Drug Targets*,* 2009*).* Now, we tested crude extracts and fractions of *Aspidosperma* sp plants collected in Alagoas state which was characterized phytochemically by one of us (AEGS). This gender is popularly used in Brazil to treat several diseases including malaria, and it is rich in indole alkaloids. Three species were tested, referred here as APM, APT and APP, to preserve future registration as phytotherapics or patenting. The extracts were obtained from plants stems, leaves, roots, wood barks and tested against blood stages of *Plasmodium falciparum* maintained in continuous cultures, following standard protocols. The parasites growth inhibition rates were evaluated by light microscopy or through incorporation of tritiated hypoxanthine, after 42h of parasite incubation with sample and controls (standard antimalarial). Most crude extracts of *Aspidosperma* sp were active at low dose (IC_{50} ≤ 11μg/ml); APM leaves and APT stem barks were partially active (IC_{50} 22 and 19μg/ml). The APP wood bark extract, the most active (IC_{50} 4μg/ml), was further fractioned in ethyl acetate and aqueous fractions, both being active at low concentrations (IC_{50} 3μg/ml and 7μg/ml). Cytotoxicity tests against HepG2 cells showed that all extracts had low toxicicity, thus high selectivity index (SI = 45 to 294μg/ml). These plants provided potential bioproducts for development of new antimalarials, to be further characterized and tested against *P. berghei* parasites in mice.

Supported by FAPEMIG, CNPQ (Project 575746/2008-4).

QT.74 - ANTIMALARIAL ACTIVITY OF *Kielmeyera variabilis* AGAINST *Plasmodium falciparum* BLOOD PARASITES *IN VITRO*

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About 80% of the populations in developing countries use medicinal plants to treat diseases. The interest in alternative therapies with natural products has been growing. The Brazilian flora has huge pharmacological potential for the discovery of new drugs due to its vast biodiversity. Thus, the screening of compounds obtained from plants through ethnopharmacology is an important strategy aiming new pharmaceutical compounds. *Kielmeyera variabilis* (Clusiaceae) known as “malva-do-campo”, is traditionally used as folk medicine in Brazil to treat several diseases (Alves, TMA. *et al*., 2000, Memorias IOC.). Previous phytochemical studies showed xanthones as the major plant constituent. Such substances are considered as antifungal, antitumoral, antibacterial, tuberculostatic and anti-inflammatory (Pinheiro, L. *et al*., 2003. Memorias IOC.). We now tested 25 plant samples (extracts, fractions, purified substances) from *K. variabilis* against blood forms of *P. falciparum* and used distinct methods for evaluation of antimalarial activity: (a) incorporation of tritiated hypoxanthine, semi-automated; (b) traditional test determining parasitemia through optical microscopy; (c) colorimetric assays with monoclonal antibodies anti-histidine rich protein (HRP-2). Among the extracts of *K. variabilis* tested, ethanolic extract of leaves (EFK) was active (IC_{50} ≤ 10μg/ml); the ethanolic branch extract (EGK) was partially active (IC_{50} ~ 25μg/ml). The active fractions derived from EFK and EGK were EAFK, EHGK and EAGK (IC_{50} 5 to 8μg/ml), whereas other five fractions were inactive (IC_{50} > 25μg/ml). Some subfractions (F1 and L2) and a pure substance (P6→ kielcorin) obtained from the ethyl acetate branch fraction (EAGK) were active, in a preliminary experiment; the subfraction EP5.6 and a pure substance (P3, 5-hydroxy-1,3-dimethoxyxanthone) were partially active. The active fractions will be prepared in larger amounts to be evaluated in mice infected with *P. berghei*. The plant cytotoxicity tests against HepG2 cells in vitro are now undertaken.

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QT.75 - EVALUATION OF NATURAL PRODUCTS USED TO PREVENT MALARIA IN THE AMAZON REGION IN *PLASMODIUM FALCIPARUM* CULTURE

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Over recent years the incidence of malaria has soared to approximately 500 million clinical cases per year. Chloroquine was the first choice antimalarial drug for more than three decades until the emergence of chloroquine resistant *Plasmodium falciparum* strains rendered its application ineffective in many parts of the world. Furthermore, the main drugs developed for malaria and used up to now, quina alkaloids derived drugs and artemisinin were discovered based on traditional use and ethnopharmacological data. In this context, new efforts to search for novel drugs for treating malaria based on popular knowledge are very important. In our studies were evaluated the *in vitro* susceptibility of *P. falciparum* (chloroquine–sensitive and chloroquine-resistant strains) to twenty nine fractions/extracts from species of Euphorbiaceae, Clusiaceae and Rhamnaceae families. The antimalarial activity was assessed by microtiter plate based on SYBR-Green-I assay. The study of cytotoxicity of these fractions/extracts was performed against HEPG2 cell line using the MTT assay. About 30% of the all tested fractions/extracts showed $IC_{50/48h} \leq 2.0\mu g/mL$ with acceptable selectivity index suggesting that they were more specific in their action against the malaria parasite. Based on these results, we can conclude that these extracts/fractions may be interesting as leads for the development of new antimalarials agents.

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QT.76 - INHIBITORS OF *PLASMODIUM FALCIPARUM* LACTATE DEHYDROGENASE AS NEW ANTIMALARIAL DRUG CANDIDATES

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Chloroquine, a drug widely used to treat malaria, is believed to interact with *Plasmodium falciparum* lactate dehydrogenase (*Pf*LDH) – an essential enzyme for parasite survival - as part of the mechanism of drug activity. Results from the literature suggest that the amino acid sequence of *Pf*LDH has residues at the active site that are unique to *Pf*LDH, as compared to the human LDH. Therefore, *Pf*LDH is an attractive target for development of new antimalarials. In our present work the docking energies for inhibition of *Pf*LDH were calculated and compared to drugs described in the literature as useful to treat other protozoan infections (unpublished data). Among 50 drugs studied, six generated stable energy conformations at positions coincident with the binding site of NADH cofactor of *Pf*LDH. Next, we tested whether some of these compounds, i.e. Atorvastatin, Itraconazole and Posaconazole, were able to cause *Pf*LDH inhibition acting as potential antimalarials. *P. falciparum* chloroquine-resistant parasites (W2 clone), maintained in continuous erythrocytes cultures, were incubated with various concentrations of those drugs and controls, following standard protocols previously described, with little modification (Krettli, 2009, Expert Opinion Drug Discovery). The inhibition of parasites growth was evaluated through parasite survival curves and IC_{50} values (50% growth inhibition). The ELISA DELI (*double-site enzyme-linked immunodetection LDH assay*) was used to quantify *Pf*LDH, in parallel with ELISA HRP2 (*histidine rich protein II*) tests. Atorvastatin, Itraconazole and Posaconazole were active (IC_{50}= 5.9μg/ml, 6.7μg/ml and 1.8μg/ml, respectively) in DELI test; and (IC_{50}= 7,7μg/ml, 6.5μg/ml and 3,7μg/ml, respectively) in HRP2 test. Posaconazole was somewhat more active. Itraconazole was also tested in *P. berghei* infected mice (four doses, oral or subcutaneously) being inactive. The reasons for the discrepancy between *in vitro* and *in vivo* tests are being investigated as well as interactions between these drugs and other antimalarials. Supported by CNPq, FAPEMIG and PAPES V-FIOCRUZ.

QT.77 - EVALUATION *IN VIVO* AND *IN VITRO* ANTIMALARIAL ACTIVITY OF CRUDE EXTRACT FROM *Caesalpinia pluviosa* AND ITS DERIVATES

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Malaria is a disease caused by *Plasmodium spp* protozoan that is transmitted by *Anopheles sp*p vector insect. Present in tropical and subtropical areas, this illness is responsible for 1-2 millions deaths and among 300 to 500 million cases reported annually. Quinine and artemisinin are the mainly antimalarials drugs and both are compounds obtained from plants, thus demonstrating the importance of discovering new plant based compounds. However, the increasing resistance of parasites to conventional antimalarial has been observed worldwide. In this sense, *Caesalpinia pluviosa* tree (Sibipiruna) crude extract was purified and separated in seven fractions. All fractions were tested *in vitro* against *P. falciparum*-infected erythrocytes strains, resistant or sensitive to chloroquine, and their toxicity in MCF-7 cells was also determined. The fraction obtained from ethanol 100% solvent exhibited the highest antimalarial activity, and then it was chosen for in vivo tests in *P. chabaudi*-infected mice. When administrated at a concentration of 50 mg/kg/day/animal, this fraction was capable of reducing mice parasitemia up to 91%. Doses superior of 75 mg/kg/day/animal were toxicity to infected animals. Mass spectrometry (ESI-MS/MS) analysis suggested the presence of quercetin on active fraction. Thus, opening perspectives to isolate and/or synthesize this compound and evaluate its specific antimalarial activity.
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QT.78 - EFFECT OF CARBON NANOTUBES, FREE OR ASSOCIATED (FUNCTIONALIZED) TO ANTIMALARIALS, AGAINST *P. falciparum*-INFECTED ERYTHROCYTES

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Globally malaria endangers 2.4 billion people, especially those exposed to infection by *Plasmodium falciparum* and *P. vivax*, which accounted for approximately 100-300 and 70-80 million of annual cases respectively. The strategies being used to combat malaria in the world have not been very effective mainly due to the emergence of parasites strains resistant to conventionally used drugs such as chloroquine. Given the impossibility of malaria control with the current strategies, new formulations capable of fighting parasite infection need to be expanded and their mechanism of action evaluated. In this sense, the use of nanoparticles (NP), structures smaller than 100 nm, are a promising alternative for drug-delivery, in which drug supply is directly delivery to target cells, then reducing dose and side effects. Also, due to its small size NP can overcome anatomical barriers, such as the blood-brain barrier or skin. In fact, carbon nanoparticles or carbon nanotubes (CNT) free or associated with drugs or proteins are able to interact with different molecules or antigens. CNT can, via endocytosis, enter into the cells, therefore modifying development and cell function specifically. Based on these factors, we intend to increase efficiency and reduce the toxicity of conventional antimalarial compounds, such as chloroquine, or experimental, such as violacein, through association (functionalization) to carbon nanotubes (CNT). Preliminary data revealed a synergistic effect of carbon nanotubes functionalized with violacein against *P. falciparum*-infected erythrocytes. The functionalization of carbon nanotubes with violacein increased significantly the effect of this compound. In contrast, when these free structures were added concomitantly and separately the effect does not be observed; thus indicating that functionalization of CNT with violacein generates a third structure with a potent antimalarial activity. Collectively, these data open promising perspectives to use CNT to improve antimalarial activity and, in turn, reducing side effects of experimental and conventional drugs.
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QT.79 - EFFECTS OF AMINOQUINOLINE COMPOUNDS ON *PLASMODIUM BERGHEI*

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Introduction: Malaria consists in one of the world's biggest public health problems nowadays, causing over 500 million clinical cases and 1–3 million deaths per year. Although chemotherapy has been the pillar of malaria control, resistance to both chloroquine and alternative drugs has been reported. Thus, there is urgent need for research and development of new antimalarial agents. Quinoline-containing antimalarials have long been used to combat this disease. Their synthesis is easy, cheap and they compose a very versatile group of compounds considering effectiveness. Their action is based on interfering with the parasite heme detoxification process, what is critical for its survival. **Material and methods:** In order to evaluate antimalarial activity, three aminoquinoline derivates were obtained by means of organic synthesis and tested *in vivo* in a murine model using the 4-day suppressive test at 25mg/Kg each. Giemsa stained blood smears were made on days 5, 7 and 9 after inoculation. The compounds are named as follows: N-(2-(di(prop-2-inyl)amine)ethyl)-7-chloroquinolin-4-amine **(1)**, N-(3-(di(prop-2-inyl)amine)propyl)-7-chloroquinolin-4-amine **(2)**, 7-chloro-N-(4-(di(prop-2-inyl)amine)butyl)quinolin-4-amine **(3)**. **Results**: The results are expressed as the inhibition of parasite multiplication percentage. On day 5, it was 64, 68 and 65% for **(1)**, **(2)** and **(3)**, respectively. On day 7: 52, 59 and 13% for **(1)**, **(2)** and **(3)**, respectively. On day 9: 73, 83 and 60% for **(1)**, **(2)** and **(3)**, respectively. Control treated group (chloroquine) exhibited no parasitemia on those days. **Conclusion**: These compounds represent potential sources for new antimalarial agents and may therefore be objects of further research.
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QT.80 - EVALUATION OF ANTIMALARIAL ACTIVITY OF DERIVATIVES COMPOUNDS OF CHLOROQUINE.

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Since 1960, several studies have shown the high resistance of *Plasmodium falciparum* to chloroquine and other aminoquinolines used as traditional antimalarials, making the search for new drugs urgent. The aim of this study was to evaluate the *in vitro* antimalarial activity of chloroquine derivatives, a drug of low cost, easy synthesis and low toxicity. Seven chloroquine derivatives compounds (DETA and DMA derivatives) were tested against *P. falciparum* blood forms maintained in continuous cultures, following standard protocols. Enzymatic immunoassay with monoclonal anti-HRP2 (histidine rich protein II) which quantifies a histidine and alanine rich protein was used to evaluate parasite growth. The toxicity of the derivatives was evaluated using the MTT colorimetric assay in two cell lines, HepG2 and BGM. All substances tested were very effective, with IC_{50} values (drug concentration that inhibits 50% of parasites growth) varying from 0,125 ug/ml to 1,66 ug/ml, for DETA and DMA derivatives, respectively. They displayed low toxicity thus, especially the DETA derivatives with the highest selective indices (SI= 189 to 7300). The DMA derivates were less active (SI from 11 to 100). Chloroquine tested in parallel displayed SI=555, thus being less active then DETA derivatives. The DETA molecules seem represent promising alternatives for the treatment of chloroquine-resistant malaria but further studies about the efficacy in vivo need to be undertaken before their use against human malaria. Experiments are in progress in mice as well as *in vitro* test using Hypoxantine test.
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QT.81 - EVALUATION OF ANTILEISHMANIAL ACTIVITY OF NIRANTHIN, A COMPOUND ISOLATED FROM *Phyllanthus amarus*

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Infections by protozoan of the genus *Leishmania* are the major world wide health problem, with high endemicity in developing countries. The drugs of choice for the treatment of leishmaniasis are the pentavalent antimonials, which exert renal and cardiac toxicity. Thus, there is a strong need for safer and more effective treatments against leishmaniasis. Genus *Phyllanthus* has been investigated to determine the constituents with pharmacological activities that frequently are attributed to lignans, glycosides, flavonoids, alkaloids, ellagitannins and phenylpropanoids. Pharmacological evaluation showed that this plant possesses anti-inflammatory, antimicrobial, antimutagenic, anticarcinogenic, and antiviral activity. The present study was designated to evaluate the leishmanicidal activity of a lignan (Niranthin) obtained from *Phyllanthus amarus.* Proliferative forms of *Leishmania amazonensis* were treated with several concentrations of the lignans and the parasite growth was determined by counting. The cytotoxic activity macrophage lines was evaluated by sulphorodamine B technique and morphological and ultrastructural alteration was analyzed by electronic microscopy. The niranthin presented a good activity against promastigote, axenic amastigote and intracellular amastigote forms with IC_{50} of 8.5, 2.7 and 8 µg/mL, respectively. The toxicity for cells and the activity against the parasites were compared by using the selectivity index (SI) ratio (CC_{50} for cells/IC_{50} for parasite). Nirantin showed to be more toxic to parasites than to mammalian cells with SI of 46.7 for *L. amazonensi,* moreover niranthin has dose-dependent effect decreasing the infection in macrophages of 81.5% in control for 31.5% in treated cells with 40 µg/mL. In addition, niranthin showed important morphological and ultrastructural alterations, such as swelling of the body, intense exocytic activity in the region of the flagellar pocket, myelin-like figures, and vacuoles in the cytoplasm as compared to control cells. The nirantin showed good activity against the parasite and represents an exciting advance in the search for new antileishmanial agents. **Acknowledgements:** This study was supported through grants from CNPq, FINEP, CAPES, PRONEX/Fundação Araucaria, and FAPESP.

QT.82 - SYNTHESIS AND ANTILEISHMANIAL ACTIVITY OF NOVEL 8-HALOGENS QUINOLINES DERIVATIVES

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Leishmaniasis is a protozoan disease that affects about 12 million people in the world, particularly in subtropical and tropical regions, causing a serious public health problem. Current chemotherapy of leishmaniasis is unsatisfactory. Efficacious and safe new drugs are needed. In the present work, the antileishmanial efficacy of novel 4-(phenylamino)-3-cyano or 3-(dihydro-1H-imidazol) 8-chloroquinolines and 8-fluoroquinolines was determined against *Leishmania amazonensis*. The quinoline rings structure is already established as a template for antiparasitic drugs; this is exemplified by the drug Sitamaquine (8-aminoquinoline derivative), which is currently undergoing clinical trials for its effectiveness in treating visceral leishmaniasis. The quinolines compounds were obtained, in good yields and all the substances were fully characterized by usual methods (IR, ^{1}H, ^{13}C NMR). The antileishmanial efficacy of eight ciano-8-halogens (Cl,F)-quinolines and derivatives was determined *in vitro* against *L. amazonensis* promastigotes. Parasites were cultured with and without the drugs in Schneider´s medium at 25°C, using Pentamidine as the standard drug. After 24 hours incubation, parasite viability was determined using the MTT(tetrazolium blue) assay. The results showed that all quinoline derivatives assayed were very potent in inhbiting promastigotes forms of *L. amazonensis.* This study reinforces that the quinoline ring structure are potential antileishmanial lead compounds for the design and synthesis of similar heterocycle derivatives.
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QT.83 - ANTIMALARIAL TESTS WITH HERBAL MEDICINES COMERCIALLY AVAILABLE SPECIALLY THOSE CONTAINING FLAVONOIDS

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The malaria control with specific drug treatment remains a major strategy to reduce morbidity and mortality caused by the disease. However, it is limited by *Plasmodium falciparum* multi-drug resistant parasites, a barrier to disease control. The discovery and development of new antimalarials, an urgent need at present, is complex and require large investments. We focus our research on the study of marketed herbal drugs, especially those containing flavonoid compounds, since they are related to the antiplasmodial activity of plants like *Bidens pilosa* (Andrade-Neto, Brandão *et al*., 2004). Accuvit, Daflon, Ginkgo, Soyfit and some standard flavonoids commercially available (Hesperidin, Genistein, Quercetin) were tested against blood forms of *P. falciparum* (clone W2, chloroquine-resistant) in parallel with chloroquine, a reference antimalarial. To quantify parasite growth, the enzyme linked immuno sorbet asay (ELISA) was performed using monoclonal antibodies to HRP2 (*histidine rich protein II*). *Pf*HRP2 is present in several cellular compartments and expressed by both knob-positive and knob-negative in infected erythrocytes. Through curves of parasite growth inhibition and IC_{50} values, we observed activity of Accuvit, Soyfit and Quercetin (IC_{50}= 4,9μg/ml, 11,7μg/ml and 17,2μg/ml, respectively). Surprisingly, Gingko, Daflon, Hesperidin and Genistein, referenced flavonoids, were inactive (IC_{50} > 30μg/ml). The activity of Accuvit and Soyfit was also tested in mice infected with *P. berghei,* the results are under analysis. Since Accuvit and Soyfit are active *in vitro* against the human malaria parasite, they might be useful for human malaria treatment in association with other antimalarials. We concluded that the activity of the tested medicines is not related exclusively to flavonoids, thus, possible synergisms between flavonoids and other compounds present in the medicines tested, the case of the multivitamins in Accuvit, need to be clarified. Supported by CNPq, FAPEMIG and PAPES V-FIOCRUZ.

QT.84 - EVALUATION OF ACTIVITY AGAINST *Phytomonas serpens* EXTRACTED CRUDE EXTRACTS OF THREE SPECIES OF *Piper, Piperaceae*

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Protozoa of the genus *Phytomonas* are trypanosomatids parasites of several species of plants known for their important pathogenicity. Studies show that some *Phytomonas* are capable of causing lethal diseases, while others cause less damage to the plant. The aim of this study was to evaluate the antiparasitic activity of crude extract of *Piper aduncum, P. crassinervium* and *P. hispidum* against *Phytomonas serpens*. The extracts (aqueous and chloroformic) were obtained by macerating of dried leaves of three species of *Piper* and then was evaluated the activity of these extracts against promastigotas forms of *P. serpens* using microdilution plate. The promastigotes were treated with different concentrations of extracts (10 to 1000 μg/mL) in Warren medium supplemented with 10% fetal calf serum, incubated at 28°C. The Growth was determined by counting the parasites with a hemocytometer chamber every 24 h for 7 days and was calculated the 50% inhibitory concentration (IC_{50}). To evaluate the toxicity of extracts on mammalian cells was used $LLCMK_2$. Cytotoxicity assay was performed by sulforhodamine B technique and then calculated CC_{50} (concentration that lyses 50% of cells). The results demonstrated that the crude extract of *P. aduncum, P. crassinervium* and *P. hispidum* have strong effect over the proliferation of *P. serpens*, with IC_{50} of 45μg/mL, 16.5 μg/mL and 22 μg/mL to chloroformic extract, respectively. The aqueous extract showed low antiproliferative activity against the protozoan with IC_{50} of 585 μg/mL, 557 μg/mL and 535 μg/mL, respectively. For cytotoxicity the chloroformic extract showed a moderate toxicity with CC_{50} of 39 μg/mL, 210 μg/mL and 87.5 μg/mL, whereas the aqueous extract showed a low toxicity of CC_{50} above 1000 μg/mL for both species of *Piper*. The results demonstrate substantial activity and selectivity of *Piper* species analyzed against the *P. serpens* although future studies should be continued. **Acknowledgements:** This study was supported through grants from CNPq, Fundação Araucária.

QT.85 - BIOGUIDED FRACTIONATION OF TRYPANOCIDAL ASTERACEAE PLANT EXTRACT

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Chagas' disease is endemic in 21 Latin American countries, killing every year, more people in the region than any other parasitic disease, including malaria (DNDi, 2010). The disease manifests in two clinical forms, acute and chronic and for both it is necessary the development of better treatment options. Plant extracts were evaluated in two technological platforms of FIOCRUZ, Bioprospecting (RTP10A) and Chagas Disease (RPT11F), in order to identify new sources of compounds for the treatment of Chagas' disease. The active extract EX6464, from one species of the family Asteraceae, was selected for bioassay-guided fractionation. The ethanol extract of the leaves was obtained by maceration and dried under reduced pressure. An aliquot of 100 µg was fractionated into HPLC using Shim-pack ODS column (4.6 X 250 mm), flow of 1 mL/min, gradient of acetonitrile/water with 0,01% TFA and UV detection at 210 nm. The column effluent was collected in 96-well plate, dried and submitted to the test with the amastigotes of *T. cruzi* (Tulahuen strain expressing beta-galactosidase) in L929 cell line. The infection was made with 10 trypomastigote/cell/2 hours. Two days after the infection, samples were added and incubated for 96 h, when percentage of reduction was calculated. The controls used are DMSO 1% and benzonidazole 1 µg/mL. Activity (85% inhibition) was concentrated in a well that corresponds to the peak with retention time of 2.54 min. In this time of elution water content is high, indicating that polar compounds were responsible for the observed activity. Phytochemical analysis indicates that flavonoids and saponins are present in this fraction. New fractionation in a Sepbox apparatus (multidimensional chromatography) will be performed to obtain the purified active(s) compound(s).
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