QT001 - **IN VIVO SUSCEPTIBILITY TO BENZNIDAZOLE OF TRYPANOSOMA CRUZI ISOLATES FROM THE STATES OF AMAZONAS AND PARANÁ, BRAZIL**

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Chagas disease is an emerging anthropozoonosis in the Amazon and still do not know the response to chemotherapy of mice experimentally infected with *Trypanosoma cruzi* from this region. Due to its great biological and genetic diversity, drug susceptibility of *T. cruzi* may vary according to the parasite genotype circulating in a given geographic area. Our goal was to determine the in vivo susceptibility to benznidazole (BZ) of *T. cruzi* isolates from the states of Amazonas (AM) and Paraná (PR). Were studied 16 isolates: 12 obtained from four different regions of the AM, eight from acute phase-AP patients, three from triatomine *Rhodnius robustus*, and one from reservoir *Didelphis marsupialis*; four isolates were obtained from chronic phase-CP patients living in PR, three autochthonous and one allochthonous from Minas Gerais (MG). For each isolate, 20 Swiss mice were inoculated via IP with 10,000 blood trypomastigotes per animal. Ten were treated from 5 days after inoculation by the oral route with BZ (Lafepe) 100mg/kg/day for 20 consecutive days. The remaining 10 animals comprised the untreated control group. For monitoring the cure were performed fresh blood examination, haemoculture, and PCR. Six isolates from AP patients originating from Santa Isabel do Rio Negro (SIRN)-AM (2), from triatomines of Apuí-AM (1) and Coari-AM (1), and from CP patients of Virgem da Lapa-MG (1) and Maringá-PR (1), were considered susceptible to BZ (67 – 100% of cure); six isolates from AP patients from Coari (2) and SIRN (2), CP patients from Maringá (1), and opossum of Manaus (1), showed intermediate sensibility (43 – 50% of cure); and four isolates from AP patient of Coari (2), triatomine from Apuí (1), and CP patient from Maringá (1), were resistant to BZ (27 – 30% of cure). The in vivo susceptibility to BZ ranged both for isolates from the same location and the same host, and the phenotype of drug resistance was detected even in natural populations of parasite that circulates in the Amazon.
Supported by:CNPq and Fundação Araucária

QT002 - **OVEREXPRESSION OF THE ABC TRANSPORTER GENE (TCABCG) PROMOTES AN INCREASED RESISTANCE TO BENZNIDAZOLE IN TRYPANOSOMA CRUZI**

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Numerous clinical studies have shown that the efficacy Benznidazole (BZ) and Nifurtimox (NF) in Chagas disease treatment varies according to the geographical area, probably due to differences in drug susceptibility among T. cruzi strains. Several ATP Binding Cassette (ABC) transporters play an important role in drug resistance, mediating the transport of drugs away from their targets inside the cell. Previous data of our group indicate that one ABC transporter gene (TcABCG) is overexpressed in BZ-resistant strains, as compared to susceptible strains. The goal of this study was to further verify the association of TcABCG with BZ resistance. For this purpose, the genes of CL Brener (BZ-sensitive) and VL10 (BZ-resistant) strains were cloned in the pROCKNeo vector, which allows the integration of the target gene in the T. cruzi beta-tubulin locus by homologous recombination. After electroporation in CL Brener epimastigotes, the transfectants were selected in culture media containing 200 µg/mL G418. Drug selection for 8 weeks resulted in no parasites being detected in mock-transfected cultures. 22% and 48% increase of the IC50 to BZ was observed, respectively, for pROCKNeo.CL and pROCKNeo.VL transfectants. Interestingly, the transfectants also showed 35% (pROCKNeo.CL) and 85% (pROCKNeo.VL) increased resistance to NF. In the transfected cultures the relative abundance of TcABCG transcripts was determined by real time RT-PCR. A 3.7-fold increase of the transcript level was observed only in the parasites transfected with the CL Brener ABC gene. To verify the integration of the recombinant vectors in CL Brener genome, Southern blots were hybridized with 32P-labeled probes for TcABCG and neomycin resistance genes. Data suggest that pROCKNeo.CL was integrated in the tubulin cluster, whereas pROCKNeo.VL was integrated in the TcABCG locus, replacing CL Brener TcABCG gene. Studies are in progress to confirm this hypothesis.
Supported by:FAPESP; CAPES; CNPq.

QT003 - **INH1 INDUCES AUTOPHAGIC CELL DEATH OF LEISHMANIA AMAZONENSIS IN INFECTED MACROPHAGES**

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Leishmaniasis is a neglected endemic disease with a broad spectrum of clinical manifestations. Pentavalent antimonials have been the treatment of choice for the past 70 years and, due to the appearance of resistant cases, the efficacy of these drugs has come under scrutiny. The present study aimed to investigate the leishmanicidal effect of INH1 (isoniazid) in vitro. This drug is currently being tested in clinical trials for the treatment of other pathologies, but its effect on Leishmania infection has never been evaluated. This study represents the first attempt to assess the effectiveness of INH1 using in an in vitro model of macrophage-Leishmania interaction. In order to evaluate the effect of INH1 on the course of infection, infected macrophages were treated with INH1 and the percentage of infection was determined by light microscopic observations. Our results show that treatment with INH1 resulted in a reduction of up to 98% of the number of macrophages infected with L.amazonensis in a dose- (25-500 nM) and time-dependent manner. Intracellular parasite death occurs independently of the production of NO and superoxide, and becomes irreversible at 4h following INH1 treatment. Then, pro-inflammatory cytokine production was determined using a Cytometric Bead Array. Interestingly, treatment with INH1 also provokes a reduction in pro-inflammatory cytokine production, including TNF-α, IL-12 and MCP-1. Electron microscopy analysis of intracellular parasites in INH1-treated macrophages revealed the presence of many autophagic vacuoles and myelin figures, indicating that parasite cell death is dependent on autophagy. No nuclear or mitochondrial alterations were observed, which implies that apoptosis is not involved in intracellular parasite death. Our findings suggest that INH1 may lead to the development of a new generation of anti-leishmanials, since this drug has high potency under low dosage and reduces both pro-inflammatory response and oxidative molecule production.
Supported by:FIOCRUZ/ CNPq - 306672/2008-1

QT004 - **EVALUATION OF MECHANISM OF ACTION, IN VITRO AND IN VIVO ACTIVITIES OF PMIC4, A NEW ANTILEISHMANIAL HYDROXYETHYLPIPERAZINE**

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In spite of the advances in last decade, such as miltefosine approval and the development of new formulations of amphotericin B, control of leishmaniasis remains in the order of day. Several studies have recently demonstrated the leishmanicidal activity of HIV protease inhibitors. This study aims to evaluate the in vitro and in vivo leishmanicidal activity of a new hydroxyethylpiperazine (PMIC4) used as a precursor in the synthesis of HIV protease inhibitors, and to investigate its mechanism of action. L. amazonensis promastigotes were cultured in the presence of PMIC4 for 72 hours and quantified by MTT assay. PMIC4 showed an IC50 of approximately 23 µM. The activity of PMIC4 was also evaluated in intracellular amastigotes. PMIC4 showed a significant antiamastigote activity, with IC50 lesser than 5 µM. Assays were performed to detect if PMIC4 is able to inhibit the activity of aspartic proteases in promastigotes of L. amazonensis. Different concentrations of PMIC4 were not able to inhibit this enzymatic activity. To evaluate if PMIC4 disturbs the composition of the parasite membrane, L. amazonensis promastigotes were treated or not with PMIC4 and their neutral lipids were extracted and analyzed by TLC. The densitometric analysis revealed that the bands related to the major Leishmania sterols (ergosterol isomers) became less intense proportionally to elevation of PMIC4 concentration, with simultaneous enhancement of intensity of unidentified bands, suggesting precursors accumulation. In vivo studies were performed in L. amazonensis infected BALB/c mice (license LW07/2010). PMIC4 was effective in controlling lesions by subcutaneous and oral routes. Altogether, our results suggest that the orally active PMIC4 is a promising prototype for the treatment of leishmaniasis and that it exerts its selective leishmanicidal effect by changing the composition of the parasite membrane sterols, which are essential for the survival of Leishmania.
Supported by:FAPERJ, IOC/FIOCRUZ, PDTIS, CNPq e PAPES

QT005 - **NANOPARTICLES INCREASE THE TOPICAL ANTI-LEISHMANIAL ACTIVITY AND INNATE MECHANISMS INDUCTION OF CHALCONE CH8**

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With the purpose to develop a topical therapy against cutaneous leishmaniasis, we encapsulated a chalcone (CH8) with anti-leishmanial activity previously described by us, in phosphatidilcoline conventional lipossomes (CH8-LC) and peguilated lipossomes (CH8-LP), aiming to improve its cutaneous penetration and eficcacy. *In vitro*, BALB/c infected macrophages with *L. amazonensis* were incubated during 48 h at 37^{oC}/4% CO2 with the drugs mentioned above. Such macrophages had their parasite load decreased by 49% and 36% comparing to the untreated control, when treated with free CH8 (15 µM) and CH8-LC (15 µM of free CH8) respectively. No effect was observed in CH8-LP, LC and LP without CH8. To investigate the role of CH8 free and CH8-LC in innate mechanism we quantified NO by Griess reagent, ROS by H_2DCFDA dye and intracellular pH by Lysotracker Red dye. We detected that both free CH8 and CH8-LC were capable to increase ROS and NO release (5x and 2x; 6x and 5x respectively). But when analyzing the effect of these drugs on intracellular acidification, we verified that free CH8 increased the intracellular pH by 1,3x, and the CH8-LC decreased by 1,5x. This more acidic intracellular environment caused by CH8-LC may contribute to a better lysosomal enzymes action and parasite control. *In vivo*, BALB/c mice were infected with 2x10^6 *L. amazonensis* GFP-promastigotes in the ears. After 12 days of infection, mice were treated by free CH8 and CH8-LC, with a total of 8 intralesional doses, 2x/week (3,3 µg/dose), or daily topical treatment for 30 days (6,6 µg/dose). Similarly to the *in vitro* results, intralesional and topical CH8-LC treatment controlled the parasite load similar to intralesional free CH8. Together these results show that conventional lipossome raises the efficacy of free CH8 through a non-invasive topical administration, and prompt leishmanicidal mechanisms of macrophages (ROS, NO and pH), contributing to the decrease of parasite burden during the leishmania infection.

Supported by:CNPq, CAPES

QT006 - **SYNERGISTIC ACTIVITY OF CARBOLINE N-BUTYL-1-(4- DIMETHYLAMINO) PHENYL-1,2,3,4–TETRAHYDRO-β-CARBOLINE-3-CARBOXAMIDE) ASSOCIATED WITH REFFERENCE DRUGS OVER TRYPANOSOMA CRUZI**

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Chagas' disease, is caused by Trypanosoma cruzi. The available drug is benznidazole (BZ), partially effective and in current therapeutic concentration this drug is toxic and must be taken for long periods. Therefore new drugs must be found. Carboline compound showed previously a good in vitro trypanocidal effect. In the present study was evaluated the effect of a carboline C4 associated with other drugs at low concentrations BZ, ketoconazole, amphotericin B over epimastigote and trypomastigote forms. Epimastigote forms from a logarithmic phase culture were suspended to 106 cell/mL in a 24 well plate. Each well was filled with a 1 mL suspension containing the parasite, LIT medium, 10% fetal calf serum (FCS), over trypomastigote was used DMEM, 10% FCS and 107 parasites/mL. In both cases the compounds were tested alone or in combination so that serial dilutions of the experimental drugs were performed and each concentration and combination was tested in triplicate. The checkerboard technique was used to analyze the results, and also was calculated fractional inhibitory concentration (FIC) for each drug and then calculated the fractional inhibitory concentration index (FICI) for the drugs combination, where the sum of these fractions is 1, is addictive; when the sum is <1, is synergistic; when the sum is >1, is antagonistic . The results were plotted on an isobologram. The C4-BZ association showed a synergistic effect against the epimastigote and trypomastigote forms with a FICI: 0.47 and 0.71. The C4-amphotericin B combination also showed a synergistic effect with FICI: 0.72 and 0.77. However the combination of C4 with ketoconazole resulted in antagonism with FICI: 2.05 and 1.53 respectively. The results suggest that C4 has a capacity to interact with other drugs and this interaction increased its activity, indicating that these drugs could be used in lower doses, reducing the possible side effects of its use. These results support news studies in vitro and in vivo.

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QT007 - **ACTIVITY OF 3-ARILIDENEINDOLIN-2-ONAS (TFMDI), A SIRTUINS INHIBITOR AGAINST TRYPANOSOMA CRUZI**

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Trypanosoma cruzi is the etiological agent of Chagas disease. At present, treatment of Chagas disease involves the use of compounds such as nifurtimox and benznidazole which are poorly tolerated and unsatisfactory because of the frequent toxic side effects. Therefore, it is important to find new active compounds as well potential parasite targets. The sirtuins are histone deacetylase enzymes present in prokaryote and eukaryote cells, which are associated with stress resistance, longevity, genomic stability and energy metabolism. In this study we evaluated the effect of 3-arilideneindolin-2-onas (TFMDI), a sirtuins inhibitor, against T. cruzi. This compound inhibited proliferation of epimastigote and amastigote forms, showing IC50 of 7 µM and 1.1 µM, respectively. Against trypomastigote form, the compound exhibited a LD50 of 1.1 µM. TFMDI demonstrated low potential toxicity to peritoneal macrophages, with CC50 of 90 µM, being more selective for amastigote (around 81 times) than macrophages. This compound induced alterations of kinetoplast size in treated epimastigote forms as observed by light microscopy. Moreover, it inhibited parasite cell division, especially during cytokinesis. These alterations also were confirmed by scanning electron microscopy. Thin sections of treated epimastigotes observed by transmission electron microscopy (TEM) revealed a loss of chromatin condensation, presence of several electron-lucent and autophagic vacuoles, Golgi apparatus and kDNA disorganization. The same kDNA alterations were observed in treated trypomastigote. Furthermore, loss of cytoplasm organelles, presence of swelled mitochondrion and myelin-like figures also were visualized. Taken together, our observations show that TFMDI is a potent inhibitor of T. cruzi proliferation (in epimastigote and amastigote forms) interfering mainly on cellular cycle, and display lytic activity against trypomastigote forms.
Supported by:CNPq, CAPES, FINEP and FAPERJ.

QT008 - **IN VIVO IMAGING MODELS OF AFRICAN TRYPANOSOMIASIS TO SUPPORT DRUG DISCOVERY PROGRAMS**

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Human African Trypanosomiasis (HAT) or sleeping sickness remains highly prevalent in sub-saharan Africa. Chemotherapy of HAT is associated with toxicity, complex dosing regimens with subsequent compliance issues, and emerging drug resistance. Clearly, new treatments are urgently needed. Current models for assessing drugs against the second stage disease, when the CNS is invaded, are cumbersome and time consuming. This project aims to 1) develop improved approaches for drug screening by the use of fast and robust *in vivo* imaging systems and 2) enhance our understanding of how and when trypanosomes cross the blood-brain barrier to become established in the brain. We have generated strains of *Trypanosoma brucei* expressing bioluminescent proteins for *in vivo* imaging of whole mice using a sensitive camera (IVIS). This approach allows the longitudinal monitoring of parasite distribution and burden in infected mice through the full course of infection. Fluorescently-labelled *T. brucei* were imaged through the thinned skull in vivo with two-photon microscopy to localise trypanosomes in the brain in high resolution and investigate their interaction with cells of the BBB. Magnetic resonance imaging was used to assess how *T. brucei* lines affect blood-brain barrier integrity over time. Using these reporter *T. brucei* lines and the various imaging modalities we monitored disease progression *in vivo* in an acute stage 427 model and a second stage GVR35 murine model. Greater sensitivity of detection was achieved using our *in vivo* imaging approach compared with standard haemocytometer techniques and the localisation of trypanosomes in various organs including the brain was detected. Two-photon microscopy revealed the concentration of *T. brucei* GVR35 in the subarachnoid space. Using these imaging techniques, we have demonstrated effects of known trypanocidal drugs on regional parasitaemia, demonstrating the value of this approach for future drug discovery.

QT009 - **INSIGHTS INTO THE MECHANISM OF ACTION OF CAMPTOTHECIN, A TOPOISOMERASE I INHIBITOR, IN TRYPANOSOMA CRUZI**

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Trypanosoma cruzi, the aetiological agent of Chaga's disease, presents a single nucleus and an unique mitochondrion with an enlarged portion named kinetoplast, that contains the kDNA. The topological state of DNA is modulated by topoisomerases, that revert supercoilings during replication, transcription, recombination and repair, thereby representing an interesting target in chemotherapeutic studies. Topoisomerase I inhibitors have been widely tested in tumor cells, but few studies show its effects on trypanosomatids, specially on *Trypanosoma cruzi*. Thus, in this work we evaluated the effects of different topo I inhibitors, camptothecin and its two derivatives, topotecan and irinotecan, on proliferation and ultrastructure of *T. cruzi* epimastigotes. For this purpose, cells were cultivated in culture medium containing different drug concentrations and samples were collected after each 24 hours (until 96 hours of cultivation) for counting on Neubauer's chamber or for processing to transmission electron microscopy. Cell proliferation was highly inhibited after treatment with camptothecin, which presented a low IC50 value, whereas topotecan and irinotecan did not cause significant growth impairment. Transmission electron microscopy analysis revealed that camptothecin promoted nuclear ultrastructural alterations, as a remarkable unpacking of the perinuclear chromatin. Based on these data, our next step is to investigate the effects of this compound in the production of reactive oxygen species and on the cell cycle arrest. Taken together, our data emphasize the essential role of topoisomerase I on cell proliferation and nuclear ultrastructural organization and reinforce the idea that topoisomerases constitute a promising target for antitrypanosomal chemotherapy.
Supported by CNPq and FAPERJ.

QT010 - **A NOVEL TRIAZOLIC NAPHTHOFURANQUINONE INDUCES AUTOPHAGY OF RESERVOSOMES AND IMPAIRMENT OF MITOSIS IN TRYPANOSOMA CRUZI.**

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Chagas' disease, caused by the protozoan *Trypanosoma cruzi*, represents a serious health problem in Latin America, and the available chemotherapy based on two nitroderivatives is not satisfactory. In folk medicine, natural products including naphthoquinones have been employed for the treatment of different parasitic diseases. In the pursuit of alternative drugs for Chagas' disease, in the present work we investigated the mechanism of action of the triazolic naphthoquinone TN, the most active against *T. cruzi* trypomastigotes among a series of naphthofuranquinoidal compounds. TN was active against bloodstream trypomastigote, epimastigote and intracellular amastigote forms of the parasite, resulting in a time- and dose-dependent inhibitory effect. The treatment with TN led to important morphological alterations in epimastigotes such as the appearance of well-developed endoplasmic reticulum profiles surrounding specially reservosomes, an indicative of autophagy, as well as an intense reservosome disruption, blebbing of the flagellar membrane, Golgi cisternae disorganization, the presence of cytosolic concentric membranar structures and abnormal multiflagellar parasites. Interestingly, no ultrastructural damage was detected in the mitochondrion of TN-treated epimastigotes. Flow cytometry analysis demonstrated that TN induced a reduction in the percentage of duplicated DNA parasites impairing mitosis in an early point. A discrete increase in ROS production and the maintenance of mitochondrial membrane potential observed in treated parasites by the same technique, together with no morphological damage in this organelle strongly suggests the TN mechanism of action in a mitochondrion-independent pathway.
Supported by:FAPERJ, CNPq, FIOCRUZ.

QT011 - **THE EFFECT OF MK-801 ON THE PROLIFERATION OF TRYPANOSSOMA CRUZI EPIMASTIGOTES**

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Chagas' disease is caused by the flagellate protozoan Trypanosoma cruzi. Despite being discovered over a century, the available therapies are not efficient. Only two drugs are currently in use: nifurtimox and benznidazole. Both are more effective when used in the acute phase, being their effectiveness in the chronic phase controversial. Besides, the treatment with these drugs presents several side effects and requires long treatment times. T. cruzi is able to use carbohydrates and amino acids as carbon and energy sources. Epimastigotes derive their energy preferably from L-proline, L-aspartic and L-glutamic oxidation. The uptake of glutamate was characterized by our group, and in this process, a relevant GABA transport and metabolism was also identified. The aim of this study was to evaluate the effect of MK-801 (an antagonist of N-methyl-D-aspartate (NMDA), a glutamate receptor in glutamatergic synapses) on the proliferation of T. cruzi epimastigotes. The cells were grown in LIT medium at pH 7.5 at 28 ° C, in 96-well plates, treated or not (negative control) with different concentrations of MK-801 (0.1 - 1 mM). Antimycin (0.5 mM) and Rotenone (60µM) were used as positive control for growth inhibition. Cell growth was determined by following-up for 8 days the variation of cultures absorbencies at 620 nm. The absorbencies read at the 5th day of growth as a function of MK-801 concentration, were used to construct dose-response curves and calculate the IC50 value (0.3 ± 0.02 mM). The results show that in vitro MK-801 affects the growth of epimastigotes of T. cruzi indicating that its target, probably a glutamate receptor, can be relevant for therapy. The interference of drugs in different stages of T. cruzi, as also a possible synergism with stressful situations (pH, temperature, oxidative and metabolic) will be analyzed.
Supported by:FAPESP, CNPq and USP

QT012 - **APIS MELLIFERA BEE VENOM GENERATES DIFFERENT DEATH PHENOTYPES ON TREATED TRYPANOSOMA CRUZI EPIMASTIGOTES AND TRYPOMASTIGOTES.**

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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 enzymes, peptides and biogenic amines and has been reported to exhibit remarkable anticancer effects, often presenting an apoptosis-like death phenotype. Our previous results exhibited that *A. mellifera* venom can affect growth and ultrastructure of all *Trypanosoma cruzi* morphological forms, without host cells damage. Investigating the treated epimastigotes and trypomastigotes ultrastructure led us to believe the occurrence of different programmed cell death pathways. To investigate this hypothesis, we treated epimastigotes and trypomastigotes forms with their respective IC_{50} (0.67 µg/ml) and LD_{50} (0.1 µg/ml) for 24 h, and labeled with monodansyl cadaverine (MDC), an autophagy biochemical marker, and with TUNEL reaction, a specific in situ marker of DNA fragments of apoptotic cells. Our results confirmed our suspicion by the strong MDC labeling only in treated epimastigotes forms, besides the frequent presence of structures suggestive of autophagosomes (observed by transmission electron microscopy). On the other hand, an increased TUNEL staining was only detected in treated trypomastigotes, plus the abnormal nuclear chromatin condensation and kDNA disorganization. These data confirmed the high activity of the bee venom over *T. cruzi* parasites, which displayed different death phenotypes. Our findings confirmed the great potential of natural products such as *A. mellifera* venom as a source to screen e develop new drugs for the treatment of neglected diseases, such as Chagas' disease.
Supported by:CNPq, CAPES and FAPERJ.

QT013 - **DERIVATIVES OF NAPHTHOQUINONES: TRYPANOCIDAL ACTIVITY, MECHANISM OF ACTION AND SELECTIVITY**

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Since its discovery by Carlos Chagas more than a hundred years ago Chagas disease still represents a serious challenge to public health, including its peculiar epidemiology, characterised by a variety of risk factors, and the lack of effective chemotherapeutic schemes. The available chemotherapy for this disease is unsatisfactory, therefore there is an intense effort to find new drugs for treatment of this disease, justifying our work to evaluate the activity of naphthoquinones on *Trypanosoma cruzi*, the etiological agent of Chagas disease. Naphthoquinones are present in several families of higher plants, being considered privileged structures in medicinal chemistry. They are involved in a variety of biological activities, including trypanocidal activity, being able to generate ROS by a redox cycling. In this context, 16 derivatives of naphthoquinones were assayed on trypomastigotes of parasite and due to higher and excellent activity of 4 of them, they were selected to testing on the proliferation of epimastigotes and intracellular amastigotes. Some derivatives inhibited cardiac muscular cells infection by *T. cruzi* and reduced the proliferation of intracellular parasites in both cardiac cells and macrophages. The naphthoquinones were more active on bloodstream trypomastigotes and epimastigotes than on intracellular forms. Our results demonstrate the promising activity of some naphthoquinones on *T. cruzi.* At the moment we are investigating their mechanism of action, through transmission electron microscopy and evaluating ROS generation and mitochondrion membrane potential by flux cytometry. In the future we intend to assay *in vivo* and perform *in vitro* studies with other napthoquinones to correlate the activity with the chemical structure, aimed at designing more effective and selective compounds on the parasite and potential candidates for new drugs for Chagas disease.
Supported by:Capes, Faperj, CNPq e IOC

QT014 - **COMPARISON OF THE LEISHMANICIDAL EFFECT OF A PALLADACYCLE COMPLEX ON BALB/C AND C57BL/6 MICE INFECTED WITH LEISHMANIA (LEISHMANIA) AMAZONENSIS.**

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Previous results from our laboratory demonstrated the leishmanicidal activity of some palladacycle compounds and among them Pd(N,N-dimethyl-1-phenethylamine-1,2-ethanebis(diphenylphosphine) (DPPE 1.2) showed an effective action against L. (L.) amazonensis infection in vitro. In vivo treatment of L. (L.) amazonensis-infected BALB/c mice with DPPE 1.2 reduced 99% of parasite burden compared to non-treated mice. T CD4+ and T CD8+ lymphocytes were analysed by FACS in popliteal lymph nodes of infected mice and a significant increase of both T lymphocyte populations was observed in animals treated with DPPE 1.2 compared to non-treated controls. These findings indicated that the leishmanicidal activity of this palladacycle complex could be involved with activation of cellular immune responses. However, despite of the significant reduction of parasite burden in animals treated with DPPE 1.2, a high number of amastigotes was still observed in animal lesions. Considering that the BALB/c strain represents an experimental model of extreme susceptibility to L. (L.) amazonensis, the leishmanicidal activity of DPPE 1.2 was also tested in C57BL/6 mice. This strain shows a different pattern of disease when infected with L. (L.) amazonensis, displaying a lower parasite load and lesion chronicity. Comparison of the leishmanicidal effect of DPPE 1.2 in BALB/c and C57BL/6 mice infected with L. (L.) amazonensis as well as the expression of CD4+ and CD8+ lymphocytes in these two strains is currently in progress.
Supported by:CNPq AND FAPESP

QT015 - **EVALUATION OF MELITTIN ACTIVITY OVER TRYPANOSOMA CRUZI EPIMASTIGOTES AND TRYPOMASTIGOTES FORMS.**

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The *Apis mellifera* venom contains several components with a wide variety of pharmaceutical properties, such as melittin, which comprises 40-50% of the venom's dry weight. Previous studies demonstrated its leishmanicidal, anti-microbial and anti-tumor activities. Chagas' disease, caused by *Trypanosoma cruzi*, is estimated to affect 16-18 million people in Central and South America, and the patients' treatment is based on drugs such as Benznidazole, which exhibits toxic effects and limited efficacy. Therefore, new chemotherapeutic agents from natural sources, is a lining research to be exploited. This study displays the melittin activity against *T. cruzi* (CL Brener clone) epimastigotes and trypomastigotes, and over the host cells. Four-day-old culture epimastigotes were cultivated for 4 days in LIT medium containing 1.34, 2.68 and 5.36 µg/ml of melittin. Tissue culture trypomastigotes were incubated in RPMI medium containing 0.1, 0.2 and 0.4 µg/ml melittin for 1 day. Effect of this compound on epimastigotes growth and trypomastigotes lysis was evaluated by counting with a Neubauer chamber. The treatment resulted in a reduction of parasites number, in a doses-dependent manner. The IC_{50} for epimastigotes inhibition growth is 2.44 ± 0.39 µg/ml and LD_{50} for trypomastigotes lysis is 0.14 ± 0.05 µg/ml. To test the melittin citotoxicity to the host cells, peritoneal macrophages were treated or not with 1 and 5 µg/ ml for 48h and examined by the MTS assay. The formazan precipitate did not occurred in the cells treated with 5 µg/ ml, with a significant absorbance reduction compared to untreated cells. To analyze the melittin's effect on the cell morphology, treated parasites are under processing for transmission electron microscopy. Our data demonstrate that melittin was effective against the epimastigote and trypomastigote forms of *T. cruzi* at concentrations non-toxic to host cells. Further studies are underway to investigate the possible intracellular targets of the peptide.

Supported by:CNPq, CAPES, FAPERJ e Pronex

QT016 - **THE EFFICACY OF ARYLIMIDAMIDES AGAINST TRYPANOSOMA CRUZI IN VITRO**

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American trypanosomiasis, which was discovered by the Brazilian Carlos Chagas in 1909, affects people predominantly from poor rural areas of America Latina. Antitrypanosomal therapy is effective in the acute and early chronic phases of the disease and the cure rates vary according to the geographical area, patient age and dose prescribed. Aromatic diamidines and analogs (such as AIAs) were suggested to interfere with DNA-targeted-enzymes and/or through the direct inhibition of transcription, triggering parasite death. The present study showed the efficacy *in vitro* of seven novel AIAS (DB1850, DB1852, DB1853, DB1862, DB1867, DB1868, and DB1890) upon the relevant forms of Chagas disease, bloodstream trypomastigotes and intracellular amastigotes *in vitro*. Our data corroborated previous studies related to the outstanding effect of AIAs such as DB745 and DB766 against *T.cruzi* with superior activity when compared to Bz, representing then potential candidates for blood prophylaxis since many of these amidines, especially DB1853 (IC_{50}=0.14µM) presently studied, maintained a high efficacy at 4ºC in mice blood. The excellent antitrypanosomal activity against *T. cruzi* stimulate further studies in in vivo models and justify the screening of new analogs aiming to help establish a useful alternative therapy for Chagas disease.

Supported by: FAPERJ, CNPq, DECIT/SCTIE/MS, MCT by CNPq, and PAPES/FIOCRUZ.

QT017 - **THE LEISHMANICIDAL EFFECT OF THE ORGANOTELLURANE RF07 ON LEISHMANIA (LEISHMANIA) CHAGASI INFECTION.**

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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. The leishmanicidal effect of the organotellurane RT01 was demonstrated against promastigote and amastigote forms of L. (L.) amazonensis (Lima et al., 2009, Korean J. Parasitol. 47:213-218). Previous results from our laboratory showed that the organotellurane RF07 at 0.75 µM inhibited 76% of L. (L.) chagasi growth in infected macrophages whereas did not show macrophage toxicity. The possible inhibitory action of RF07 on L. (L.) chagasi cysteine proteinases was also analysed by spectrofluorimetric assays and results indicated that RF07 inhibited the cathepsin L of L. (L.) chagasi. These results encouraged us to test the effect of RF07 on L. (L.) chagasi infection in vivo. Golden hamsters were infected by intraperitoneal route with L. (L.) chagasi amastigotes and 1 month post-infection 65 µg/day of RF07, 2.4 mg/day of glucantime or sterile PBS were administered by the intraperitoneal route for 15 days. Animals were sacrificed 15 days after treatment. The parasite burden was evaluated by amastigote counts in imprints of spleen stained with Giemsa and by the limiting dilution method. Results showed that the treatment with 1 mg of RF07 inhibited 99% of parasite load in hamsters infected with L. (L.) chagasi, while an inhibition of 75% was observed with 36 mg of glucantime. Data from the literature showed a protective effect of a tellurium compound against a parasitic infection in BALB/c mice concurrently to the activation of cellular immune responses (Rosenblatt-Bin et al., 1998, Cel. Immunol, 184:12-25). In order to estimate the possible cellular immune activation induced by the treatment with RF07, the expression of IFN-γ and IL-10 by real time PCR in L. (L.) chagasi-infected hamsters at the end of treatment is currently evaluated.

Supported by:CNPq e FAPESP

QT018 - **STUDIES ON THE IN VITRO EFFECTS OF ERGOSTEROL BIOSYNTHESIS INHIBITORS ON LEISHMANIA AMAZONENSIS**

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Leishmaniasis is a parasitosis caused by organisms of the Leishmania genus, which is associated with significant rates of morbidity and mortality throughout the world. Pentavalent antimonials remain the mainstay of chemotherapy but in several regions miltefosine, amphotericin B and pentamidine are also used. However, these new treatments are still not fully satisfactory and thus there is a need for safer and more efficacious anti-Leishmania agents. Trypanosomatids have an essential requirement for ergosterol and other 24-alky sterols, which are not present in their mammalian hosts. We have previously demonstrated that amiodarone, an antiarrhythmic drug, is active against both proliferative stages of Leishmania amazonensis and also interferes with ergosterol biosynthesis, (Macedo-Silva, S. T, et al.; Molecular Biology International, 2011). Thus, we decided to investigate the effects of posaconazole, another ergosterol biosynthesis inhibitor, alone and in combination with amiodarone on the proliferation of this parasite in vitro. Posaconazole induced growth inhibitory effects similar to those of amiodarone on Leishmania amazonensis promastigotes and intracellular amastigotes, with IC50 values of 3.18 µM and 2.32 µM, respectively. Promastigotes incubated in the presence of posaconazole displayed an intense alteration in cell morphology. Transmission electron microscopy demonstrated the presence of several ultrastructural alterations, the mitochondrion being the organelle most affected by the treatment, exhibiting intense swelling, loss of the matrix content and the presence of internal membrane profiles. The presence of autophagic structures was also observed. Further studies are in progress to evaluate the potential synergic effects of combinations of amiodarone and posaconazole against intracellular amastigotes. In summary, this study could open the possibility of a novel combination therapy for the treatment of Leishmaniasis.

Supported by:CNPq, CAPES, and FAPERJ.

QT019 - **LEISHMANICIDAL EFFECT OF ATOMARIC ACID FROM THE BROWN ALGAE STYPOPODIUM ZONALE AND ITS SYNTHETIC ESTER DERIVATIVE**

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Leishmaniasis is a neglected disease caused by Leishmania affecting 12 millions of people worldwide. The available treatment is costly, present side effects and induces resistance. Currently WHO and DNDi are stimulating studies to find new compounds for chemotherapy development against leishmaniasis. Here we evaluated the anti-*Leishmania amazonensis* effect of atomaric acid from the brown algae Stypopodium zonale and its synthetic derivate, the atomaric acid ester (AAE). Evaluating the anti-promastigotes activity we demonstrated that treatment with atomaric acid and AAE at 50µM were able to decrease in 96h the growth of parasite in 100 and 86%, respectively. To verify the leishmanicidal effect of these compounds in intracellular amastigote forms we treated infected macrophages with the compounds and measured parasite survival. Our results showed that both atomaric acid and AAE have a dose-dependent effect with IC50 90µM for atomaric acid and 64µM for AAE. In order to test the safety of these compounds for host cells, their viability was assessed using XTT and Trypan blue exclusion assays. Both methods showed that cells remained viable after treatment with the same concentrations that induced a strong leshmanicidal effect. Since nitric oxide (NO) production is an effective mechanism against the parasite, we tested if the compounds were able to modulate NO activity in macrophages. The level of NO was not changed when macrophages stimulated or not with IFN-GAMMA were treated with atomaric acid. On the other hand a significant increase in NO was observed after AAE treatment in non-activated macrophages. Interestingly, this effect was not seen in IFN-GAMMA activated-macrophages. This study adds seaweeds as a source of new compounds for new drug discovery against leishmaniasis.
Supported by:Supported by: CAPES, FAPERJ and CNPq

QT020 - **RESISTANCE TO MILTEFOSINE AND TREATMENT FAILURE IN LEISHMANIA CHAGASI INFECTION**

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Oral miltefosine has recently been licensed for used in India for the treatment of visceral leishmaniasis (VL). This drug is an alkylphosphocholine, initially developed as an anticancer agent that also shows activity against *Leishmania*. During a clinical trial to evaluate the efficacy of miltefosine in the treatment of Brazilian VL patients we detected around 50% of relapse. In order to evaluate whether this treatment failure was due the resistance of *Leishmania* strains, we studied the sensitivity of strains isolated from VL patients treated with miltefosine presenting different outcomes. The patients were treated with 2.5 mg/Kg/day of miltefosine during 28 or 42 days and the response to treatment was evaluated by repeating the bone marrow aspiration in the end of treatment. All patients were followed up to six months and they were considered cured if there were no signs and symptoms of relapse. In this study, we evaluated 27 strains which were typing as *L. chagasi* using PCR-RFLP. Bone marrow aspirates obtained at diagnosis, after the treatment and at relapse were used as the source of material for primary culture of promastigotes. Promastigotes within eight passages from isolation were used to infect mouse peritoneal macrophage, and cultivated in medium containing different concentrations of miltefosine. For each test we calculated a 50% effective concentration (EC_{50}). All strains obtained from responsive patients showed a mean EC_{50} of 5 υM and were considered susceptible to miltefosine, whereas most of the isolates (77%) from non-responsive patients showed EC_{50} >15 υM, which correspond to the highest drug concentration tested, and were considered drug resistant. We also evaluated strains isolated at time of failure of ten unresponsive patients. Seven strains were resistant to miltefosine before treatment and maintained the phenotype of resistance after the treatment, suggesting primary resistance. On the other hand, three strains which were susceptible before treatment became resistant after treatment, suggesting secondary resistance. Our results demonstrated that the treatment failure of VL caused by *L.chagasi*, using miltefosine, can be due to the resistance of parasite to the drug.
Supported by:FAPES (Grant 36316326/2007), CAPES e Zentaris.

QT021 - **EVALUATION OF THE LEISHMANIAL ACTIVITY OF RESVERATROL AND ITS SYNERGIC ASSOCIATION WITH AMPHOTERICIN B AGAINST LEISHMANIA AMAZONENSIS**

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Leishmaniasis a disease that affects 12 million people worldwide is caused by protozoa of the genus Leishmania. The discovery of new drugs for leishmaniasis treatment is a pressing concern for global health programs. Previous results of our group described the anti-Leishmania effect of Resveratrol and its synergic association with Amphotericin B. Here we focused on investigating the mechanism of action of Resveratrol alone or in association with Amphotericin B. Damage to parasite mitochondria was assessed by the XTT text and mitochondrial membrane potential detection kit (JC-1). A decrease of 37 and 22% in the dehydrogenases activity and 83 and 70% in the mitochondrial potential was revealed, respectively, after promastigote treatment with Resveratrol or with its synergic association with Amphotericin B. Assessing macrophage nitric oxide (NO) production we observed that Resveratrol, Amphotericin B and their synergic association did not induce NO. Actually, Resveratrol reduced around 8 and 44 times and Resveratrol-Amphotericin B association reduced 44 and 5 times the NO production on IFN-γ-activated macrophages and IFN-γ-activated infected-macrophages, respectively. To rule out a scavenger effect as the responsible for the NO decrease, a cell-free system using SNAP as a NO donor in the presence or not of Resveratrol, Amphotericin B and their synergic association was used. Our results demonstrate that these drugs were not able to scavenger NO. Testing the arginase activity of macrophages, we showed that Resveratrol inhibited this enzyme. Interestingly, Resveratrol and Resveratrol-Amphotericin B association induce macrophage production TGF-β and decrease the production of TNF-α. Our results suggest that Resveratrol and its synergic association with Amphotericin B are efficient against *L. amazonensis*, and provide new perspectives for further studies of drugs combination therapy *in vivo*.
Supported by:FAPERJ, CNPq, CAPES

QT022 - **A NEW PTEROCARPANQUINONE THAT AFFECTS TOXOPLASMA GONDII DURING HOST CELL INTERACTION**

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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 an intracellular activity. The most common 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, such as anticancer activity, functioning as agents capable of acting on groups of DNA, preventing the duplication of cancer cells. These derivatives also display antiparasitic activity against *Plasmodium falciparum* and *Leishmania amazonesis*. The derivative we tested in this work resulted from molecular hybridization between pterocarpans and the naphtoquinone with anti-tumoral and anti-parasitic activities lapachol. Here, we report the outcomes of the cytotoxicity test with this derivative during the interaction of *T. gondii* with LLC-MK_2 cells. The compound was able to inhibit intracellular parasite proliferation with an IC_{50} of 2.5 µM. Scanning and Transmission Electron Microscopy analysis showed that the concentrations that damaged the parasite did not affect the host cells. Parasite alterations included the damage of membranes. The derivative was also capable of decreasing the infection index of T. gondii's in with LLC-MK_2. The protozoa that survived tended to encyst. These results suggest that pterocarpanquinones are compounds potentially important for the killing and encystment of *T. gondii.*
Supported by:CNPq, Pronex, Faperj

QT023 - **TRYPANOCIDAL ACTIVITY OF GUAIANOLIDE ISOLATED FROM TANACETUM PARTHENIUM (L.) SCHULTZ-BIP (ASTERACEAE) AND ITS SYNERGISTIC EFFECT WITH BENZONIDAZOLE**

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Chagas` disease is an endemic infection, caused by the protozoan *Trypanosoma cruzi*, which affects 15 million people in Central and South America. It's treatment is today still a challenge, since the only available drug, benznidazole (BZ) posses limited effectiveness and severe side effects. In this context the naturals products could be a source of new drugs with high activity and low toxicity. Chemical and biological studies conducted with *Tanacetum parthenium* (herbaceous plant) showed excellent antiparasitic activity, which is assigned the sesquiterpenes lactones present. This study investigated in vitro trypanocidal activity and the potential toxic effects of guaianolide isolated from *T. parthenium*, as well as possible synergism of this compound with BZ in *T. cruzi*. Trypanocidal activity was assayed on epimastigote cultivated in LIT medium and trypomastigote obtained on tissue-culture in DMEM. The $IC_{50/96h}$ (50% inhibition concentration) and the $EC_{50/24h}$ (50% lyses concentration) was determined by direct microscopic counting of parasites. Guaianolide was also tested for its synergistic effect with BZ against epimastigote form. The cytotoxic activity against $LLCMK_2$ mammalian cells was evaluated by sulphorodamine B technique and then was calculated $CC_{50/72h}$. Guaianolide showed be more toxic to epimastigote and trypomastigote form (IC_{50} 16.8 µM and EC_{50} 5.7 µM) than to $LLCMK_2$ cells (CC_{50} 93.5 µM) with selectivity index of 5.6 and 16.4, respectively. The treatment with guaianolide and BZ, reducing the IC_{50} values and the Fractional Inhibitory Concentration Index (FICI) was 0.63, thus corresponding to a synergistic activity. The high activity displayed by guaianolide, together to the strong synergistic effect with BZ, motivate us to further investigate their combined effect against the blood forms of *T. cruzi*.

Supported by:CAPES, CNPq, FINEP, PRONEX/Fundação Araucária

QT024 - **THE ORAL DIURETIC FUROSEMIDE CAN POTENTIATE THE PARENTERAL EFFICACY OF PENTOSTAM® AGAINST CUTANEOUS LEISHMANIAIS**

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Intramuscularly administered pentavalent antimonials such as Glucantime® and Pentostam® are the first line therapy against cutaneous leishmaniasis despite their toxicity and drug-resistance potential. In previous studies, we showed that specific inhibition of Na^+-ATPase of *L. amazonensis* promastigotes by furosemide, a marketed oral diuretic drug, 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: 1) the furosemide activity in vivo by two routes (intraperitoneal and oral); 2) the synergy with Pentostam®; and 3) efficacy comparing different sites of infection. For that, BALB/c mice were infected in the ear and in the footpad with *L. amazonensis* promastigotes. The treatment was initiated 7 days later. In intraperitoneal treatment, the mice received 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 doses. The oral regime was similar to intraperitoneal, except that it lasted until the end of experiment (day 86). The lesion sizes were measured throughout the infection with a dial calliper. On day 86 of infection, the animals were sacrificed and the parasite load was quantified by Limiting Dilution Assay. The results showed that furosemide was also effective in vivo against *L. amazonensis*; both by the i.p. and the oral routes, but oral efficacy was more pronounced irrespective of the site of infection. The footpad lesions regressed faster than the ear infections, probably due to the lesser vascularization of the later. Combination therapy of oral furosemide and i.p. Pentostam® potentiated the Pentostam® efficacy. Together these results showed that the clinically approved furosemide is potentially effective in the treatment of cutaneous leishmaniasis by oral route.

Supported by:CNPq

QT025 - **PROTEOMIC ANALYSIS OF MILTEFOSINE-RESISTANT LEISHMANIA CHAGASI REVEALS THE POSSIBLE INVOLVEMENT OF PEROXIREDOXIN AND CALPAIN-LIKE PROTEIN**

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Visceral leishmaniasis is a systemic disease that is fatal if untreated and is caused by the *Leishmania donovani* complex, which include the *Leishmania* (*L*.) *chagasi*. Visceral leishmaniasis treatment relies on a few chemotherapeutic drugs including Sb(V), amphotericin B and miltefosine. Miltefosine was recently approved as the first oral drug active against visceral leishmaniasis in India. Miltefosine resistance mechanisms are being elucidated in laboratory *Leishmania* spp. isolates but are less clear in clinical isolates. In this study, we used comparative two-dimensional gel electrophoresis and mass spectrometry methodologies to highlight and identify proteins that are differentially expressed between miltefosine-sensitive (S: S1 and S2) and –resistant (R: R1 and R2) *L.* (*L*.) *chagasi* isolates from kala-azar patients included in clinical trial in Brazil, in order to assess the effectiveness of this drug. We describe here a high-resolution proteome for *L.* (*L*.) *chagasi* promastigotes comprising an average of 459 spots, which corresponds to 5,7% of gene products predicted for *Leishmania* spp. Following comparison of the whole proteome profiles between sensitive and resistant *L.* (*L*.) *chagasi* clinical field strains, 80 differentially expressed spots were detected. Eighteen spots were found to be specific of a sensitive group and only one of a resistant group, while 48 spots changed in intensity between these groups. The others spots were present in a unique isolates (7 in S1, 3 in S2 and 2 in R1) or in three isolates simultaneously (1 in S1, S2 e R1). MALDI/TOF-TOF mass spectrometry allowed the identification of 49 spots (61,3%) corresponding to 32 distinct protein and 7 hypothetical proteins. Among the proteins identified, the comparative proteomics screen highlighted two proteins, peroxidoxin (overexpressed in R group) and calpain-like cisteína peptidase (exclusively detected in S), differentially expressed, suggesting that programmed cell death is reduced in resistant parasite. These data suggest that these proteins may be related to resistance phenotype to miltefosine.Supported by:CNPq/CAPES

QT026 - **EFFECT OF HUMULUS LUPULUS AND ITS DERIVATIVE XANTOHUMOL, IN PROMASTIGOTES AND AMASTIGOTES FORMS OF LEISHMANIA**

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Leishmania are protozoan parasites that are responsible for substantial public health problems, especially in tropical and subtropical regions. Visceral leishmaniasis (VL) is a vector-borne disease caused by obligate intra-macrophage protozoan parasites such as *Leishmania donovani*. VL is a systemic disease that is fatal in the absence of treatment. It is estimated that 88 countries are leishmaniasis - endemic. These diseases affect around 12 million people worldwide. Current treatment is based on chemotherapy, which relies on a handful of drugs with serious limitations such as high cost and toxicity, difficult route of administration and lack of efficacy in endemic areas. In this study, we described the antileishmanial activity of an aqueous extract of the female inflorescences hop plant (*Humulus lupulus L.*) and xanthohumol, a *H. lupulus*, on promastigote and intracellular (amastigotes) forms of two Leishmania species. Our results showed that the aqueous extract of *H. lupulus* killed 60% of *L. amazonensis* and 70% of *L. braziliensis* promastigote forms. The extract was able to reduce the endocytic index (EI) in human THP1 lineage cells infected with *L. amazonensis* (99.3 %) and *L.braziliensis* (84 %). The xanthohumol was able to reduce the endocytic index on *L. amazonensis* (96.5 %) and *braziliensis* (74 %) amastigotes. Assessment of cytokine production in supernatants of THP-1 macrophage culture treated with aqueous extract of *H. lupulus* by ELISA showed an increase of TNF-α production. these results suggest that *H. Lupulus* and xanthohumol could be a potential candidate as antileismanial drug.
Supported by:FAP/FIOCRUZ

QT027 - **ACTIVITY OF SYNTHETIC AMIDINE COMPOUNDS AGAINST TRYPANOSOMA CRUZI IN VITRO**

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Chagas disease (CD) is an important neglected disease that was discovered by Carlos Chagas in 1909 and affects mainly the rural very poor population in endemic areas of Latin America. More than one hundred years after its discovery, CD still poses as a serious public health problem exhibiting lack of therapeutic options, as presently the treatment is mostly based in benznidazole (BZ) and nifurtimox two drugs that display limited efficacy, especially during the chronic phase and considerable side effects. On this basis, our group has evaluated some classes of synthetic heterocyclic compounds, especially aromatic diamidines and their analogs, such as arylimidamides. Presently, the biological activity of dicationic (DB1979, DB1989 and DB1995) and monocationic (DB1996, DB1997, DB1980, DB2001, DB2002, DB2003, DB2004, DB2006 and DB2007) arylimidamides were investigated against bloodstream trypomastigotes (BT) and amastigotes forms of *T. cruzi*. The assays were performed at different temperatures (4C and 37C) and in presence or not of whole blood, also the aiming to evaluate the potential applicability in blood bank prophylaxis. In this later protocol condition (4C with 96% mice blood), all compounds revealed a decrease in their trypanocidal effect. However, their efficacy upon BT and amastigotes showed that dicationic compounds are more effective than the monocationic ones. After that, the cytotoxic potential of each compound was investigated upon uninfected cardiomyocytes. All compounds did not induce considerable loss of cellular viability. Some arylimidamides revealed superior efficacy when compared with aromatic diamidines, being especially more effective than the reference drug (BZ), representing a great promise for future studies, aiming new treatment options.
Supported by: FAPERJ, CNPq, DECIT/SCTIE/MS, MCT by CNPq, and PAPES/FIOCRUZ.

QT028 - **LEISHMANICIDAL ACTIVITY OF PYRAZOL BENZENESULFONAMIDE DERIVATIVES**

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The current drugs used for treatment of leishmaniasis have toxicity, side effects and determine parasite resistance. Leishmaniasis is a disease neglected by the pharmaceutical industry and the search for new therapeutic agents becomes urgent. The biological effects of chemical groups known as Pyrazol Benzenesulfonamide are antiviral, antibacterial, antitumoral and antiinflammatory. The ideal anti-Leishmania drug would be one that eliminated the parasite and, at the same time, modulated the host immune response. In our studies were investigated the in vitro effects of five Pyrazol Benzenesulfonamide derivatives against Leishmania. These compounds were synthesized and characterized by chemical elemental analyses. They have been screened for antileishmanial activities in order to evaluate the possibility of the derivatives to be used as potential chemotherapeutic agents. Infective promastigotes of Leishmania amazonensis (LTB0016) were incubated with and without the test compound in culture medium. Pentamidine was used as the reference drug. After incubation for 24 hours, the biologic activity of Pyrazol Benzenesulfonamide derivatives was assessed by counting the parasites under an optical microscopy. The results in promastigotes were expressed as the effective concentration (EC50) determining cell lyses by 50 percent after a 24 hours incubation period. Of the five compounds, one compound (D5) showed significant leishmanicidal activity with EC50 (2,3 µg/mL) value better than the reference drug (EC50=10µg/mL). The other drugs follows EC50 values D1 (65 µg/mL), D2 (125 µg/mL), D3 (33 µg/mL) e D4 (30 µg/mL). As perspective the toxic effects of compounds on peritoneal macrophages of mice will be evaluated. In additional, these compounds will be analysed on other Leishmania species to confirm their potential as new antileishmanial drugs.
Supported by:proppi/UFF e FAPERJ

QT029 - **TRYPANOCIDAL EFFECT OF HUMULUS LUPULUS AND ITS DERIVATIVE XANTHOHUMOL, IN CHAGAS DISEASE: ACTIVITY ON TRYPANOSOMA CRUZI Y AND COLOMBIAN STRAINS**

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Chagas disease is an endemic disease caused by *Trypanosoma cruzi* that affects about 18-20 million people in Central and South America with more than 100 million at risk of infection. The development of new drugs against Chagas disease is a priority since the currently available medicines have toxic effects, partial efficacy and are targeted just against the acute phase of disease. In this study, we described the trypanocidal effects of an aqueous extract of the female inflorescences hop plant (*Humulus lupulus L.*) and xanthohumol, a derivative of *H. lupulus*, on extracellular (trypomastigotes) and intracellular (amastigotes) infective forms of two *T. cruzi* strains. Our results showed that aqueous extract of *H. lupulus* was lethal on extracelular trypomastigotes forms of both *T. cruzi Y* and *Colombian* strains. Interestingly, *H. lupulus* and xanthohumol was also able to reduce the endocytic index (EI) in human macrophage and THP1 lineage cells infected with *T. cruzi Y* (95 %) and *Colombian* (90 %) strains amastigote forms. Similar effects were observed on T. cruzi amastigote infected Vero cells. The ELISA analysis of supernatant cytokines of macrophages treated with aqueous extract of H. lupulus showed an increase of TNF-α production.. These results suggest that H. Lupulus could be a potential candidate for Chagas disease chemotherapy and xantohumol could be used as an imunoteherapeutic drug as it presents trypanocidal activity on T. cruzi infective forms.
Supported by:FAP/FIOCRUZ

QT030 - **ANTITRYPANOSOMAL ACTIVITY OF BENZALDEHYDE-THIOSEMICARBAZONES DERIVED FROM LIMONENE**

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The Chagas' disease caused by the parasite Trypanosoma cruzi, it's considered an important public health problem in Latin America where millions of people are infected. The treatment is limited to nifurtimox and benznidazole. However, these drugs cause many side effects and are ineffective in chronic phase. So it becomes necessary to intensify studies in search of new drugs that show safe and effective in the treatment of American trypanosomiasis. The aim of this study was to evaluate the trypanocidal activity of benzaldehyde-thiosemicarbazones derived from S-(-)-limonene and R-(+)-limonene. The antiproliferative assay was held in 24-well plates, 1x106 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 in Neubauer chamber and the growth inhibition was determined. Analysis of cytotoxicity was determined by MTT assay in 96-wells plates containing monolayers of LLCMK2 cells were incubated for 72 h at 37 ºC and 5% of CO2 with different concentrations of the compounds. The results showed that 11 of the 13 compounds tested showed activity against epimastigotes at concentrations below 10 µg/mL, and the most effectives were TR02 and TS1 with IC50 of 1.98 and 2.54 µg/mL, respectively. The TS1 compound showed to be less cytotoxic with CC50 of 625 µg/mL and with an index of selectivity of 246. However additional studies on other forms of the parasite as well as other in vitro studies are needed.
Supported by:CNPq, FINEP, CAPES and Fundação Araucária.

QT031 - **FEHP(SO4) INDUCE NECROSIS IN TOXOPLASMA GONDII DURING HOST CELL INTERACTION**

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Toxoplasma gondii, the agent of Toxoplasmosis, is an obligate intracellular protozoan able to infect a wide range of vertebrate cells. Therefore, drugs to control this parasite must have an intracellular activity. In previous studies, a new l-oxo di-iron complex showed biological activities against DNA mimicking a nuclease and metallohydrolase. These new metallo- complexes also displayed antibacterial activity against *Staphylococcus aureus*. The metallo complex tested in this work comes from the reaction of FeSO4 _ 7H2O with the ligand HPClNOL (1-(bis-pyridin-2-ylmethyl-amino)-3-chloropropan-2-ol resulting in a complex [Fe(HPClNOL)(SO4)]2-l-oxo _ 6H2O 1, in which the iron ions are coordinated by two pyridines, one amine and one alcohol group from the ligand HPClNOL, an oxo bridge and monodentate sulfate ion (Parrilha et al., 2008). Here, we report the outcomes of the cytotoxicity test with this drug during the interaction of *T. gondii* with LLC-MK2 cells. The compound was able to inhibit intracellular parasite proliferation with an IC50 of 2.5 µM. Moreover, treatment was able to induce tachyzoites conversion into bradyzoites. These results indicate that this compound stress the parasite inducing stage conversion.
Supported by:FAPERJ/CNPQ

QT032 - **ACTIVITY OF ESSENTIAL OILS ON TRYPANOSOMA CRUZI EPIMASTIGOTES**

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Treatment for Chagas' disease, caused by *Trypanosoma cruzi,* is based on benznidazole and nifurtimox, drugs with low efficacy that may cause a number of side effects leading to discontinuation of treatment. One alternative to combat *T. cruzi* is the search for natural compounds such as plant essential oils (EO) that have activity against this parasite. To evaluate the activity of different EOs, *T. cruzi* epimastigotes, strain Dm28c, were grown in 96-well plates containing LIT medium and different concentrations of essential oils. Parasites viability was evaluated after incubation for 24h at 28°C by the MTT colorimetric method. The spectrophotometry readings were used to calculate the IC_{50} (concentration that inhibits culture growth by 50%). In an initial screening, better results were obtained with EOs of *Cinnamomum verum* (cinnamon) IC_{50} = 31.38 µg/ml; *Myrocarpus frondosus (*cabreúva) IC_{50} = 58.29 µg/ml; *Eugenia uniflora* (Surinam cherry) IC_{50} = 72.42 µg/ml; *Citrus limon* (lemon) IC_{50} = 93.94 µg/ml; *Pinus* sp. (Pine) IC_{50} = 114.43 µg/ml and *Melissa officinalis* (citronela) IC_{50} = 128.31 µg/ml. Preliminary results on the cytotoxicity of these OEs against Vero cells grown in 96-well plates containing DMEM + 2.5% FBS for 24 hours in a humidified atmosphere at 37°C and 5% CO2, were also obtained by the MTT colorimetric method, suggesting that the EOs of cinnamon, lemon and cabreúva are cytotoxic at concentrations above 100 µg/ml. On the other hand, the CC_{50} (cytotoxic concentration that inhibits culture growth by 50%) for the Surinam cherry EO was 343.89 µg/ml (selectivity index SI = 4.75). Further experiments will be conducted to confirm the results regarding the OEs cytotoxicity, allowing the choice of OE with best SI. Furthermore, experiments will be conducted to evaluate the effect of EOs on intracellular amastigotes and trypomastigotes of *T. cruzi* in vitro.
Supported by:CNPq, Fiocruz

QT033 - **PHOTOANTIMICROBIAL EFFECT OF A SILICON PHTHALOCYANINE AGAINST LEISHMANIA MAJOR PROMASTIGOTES**

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Photodynamic therapy (PDT) and photodynamic antimicrobial chemotherapy (PACT) has potential application in various areas of medicine, such as therapies against cancer, skin diseases, and antimicrobial activity. The effects of PACT for treatment of cutaneous leishmaniasis have been investigated and phthalocyanines have been tested and identified as promising photosensitizers for CL therapy. To evaluate the phototoxic effect of Phtalocyaminato[bis(dimethylaminoethanoxy)] silicon (NzPc) on *Leishmania major*, promastigotes (MRHO/SU/1959/P) were cultured in M199 medium with 10% FBS, 2,5 µg/mL of hemin, 1% de penicilin/streptomicin, and Hepes 0.1M at 26 ^{o}C. Promastigotes in stationary phase (1x106 parasites/well) were treated with NzPc at 0.5 or 1.0 µM for one hour. They were irradiated with GaAlAs Laser diode semiconductor (λ 659 nm, 40mW - KONDOTECH®) at 5 or 10 J/cm^2. Controls groups included: untreated promastigotes, promastigotes incubated with photosensitizer without irradiation, and parasites only irradiated. Cell viability was determined by the trypan blue dye exclusion method. Parasites survival after treatment was monitored by the growth curve. The results were analyzed using ANOVA (BioStat 5.0) and were considered significant if $p < 0.05$. Our results showed a significant reduction of viable parasites ($p<0.01$) when submitted to PACT. The mortality was 56% for promastigotes treated with 1.0 µM and 5 J/cm^2 and 85,7 % for parasites submitted to PACT with 0.5 µM and 10 J/cm^2. The most effective treatment (98.3% of deaths) was observed for PACT with 1.0 µM e 10 J/cm^2. The viability of promsatigotes was not reduced by Laser in the energy densities tested and photosensitizer alone.Our results suggested that the NzPC has potential for use as a treatments against cutaneous leismaniasis. Experiments with amastigotes and *in vivo* models should be conducted to characterize the mechanisms and the therapeutic potential of this photosensitizer.
Supported by:Fundação Vale Paraibana de Ensino-FVE

QT034 - **ANTILEISHMANIAL ACTIVITY OF THE ISOPRENOIDS FARNESOL AND GERANYLGERANIOL**

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Leishmaniasis is a worldwide parasitic disease caused by protozoa of genus Leishmania, endemic mostly in developing countries, where causes severe social-economical losses. As the current treatment induces several side effects and drug resistance is frequently encountered, it is necessary to develop new antileishmanial agents. Farnesol (F-OH) and geranylgeraniol (GG-OH) are the two major isoprenoids intermediates in the mevalonate pathway. Interestingly, in a specific concentration range, they inhibit the proliferation and induce death in mammalian cancer cells. Furthermore, F-OH and GG-OH present fungicidal and trypanocidal activities, respectively. In the present work, our purpose is to evaluate the effect of F-OH and GG-OH against promastigotes and amastigotes of Leishmania amazonensis. Promastigotes were incubated with the compounds in Schneider's medium at 26ºC, using pentamidine as reference drug. The parasite viability was evaluated after 72h using the tetrazolium-dye (MTT) colorimetric method. For intracellular amastigote assay, L. amazonensis infected murine macrophages were treated with the compounds. After 72 h at 37^{o}C, the cells were stained and counted by light microscopy. Farnesol (IC_{50} = 71,6µM) and GG-OH (IC_{50} = 29,4µM) presented activity against promastigote forms. Interestingly, F-OH was more potent against intracellular amastigotes (IC_{50} = 59,6µM), while GG-OH presented IC_{50} values higher than 200 µM. Taken together, these results showed that F-OH inhibits significantly the proliferation of intracellular amastigotes at concentration which do not affect the mammaliam host cell and provided new perspectives on drug development against Leishmaniasis.
Supported by:INSTITUTO OSWALDO CRUZ

QT035 - **PYRIMETHAMINE-LODED LIPID CORE NANOCAPSULES TO IMPROVE THE DRUG DELIVERY FOR THE TREATMENT OF TOXOPLASMOSIS**

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Toxoplasma gondii is a world spread protozoan and an important pathogen associated with many medical areas, such as: pediatrician, ophthalmology and cases of immunocompromised patients with AIDS. The cerebral toxoplasmosis is the most important and common clinical presentation on immunocompromised patients and one of the main cause of morbidity and mortality on those patients. The first choice treatment of toxoplasmosis consists in an association of pyrimethamine (PYR) and sulfadiazine. However many patients do not tolerate the sulfa-based treatment. A second option is the association of PYR with other drugs, such as clindamycin or azithromycin. The administration of PYR is frequently associated to many side effects that can compromise the continuation of treatment. The development of innovative formulations for the treatment of the disease using commercial drugs is very promising and a fast manner for the discovery of new or better treatments. Herein we propose an innovative technology based on the nanoencapsulation of PYR aiming an improvement on drug delivery and dose reduction for the treatment of toxoplamosis. Lipid-core nanocapsule constituted of a dispersion of lipids as core, made of capric/caprilic triglyceride and sorbitan monostearate, surrounded by a biodegradable polymeric wall of poli(ε-caprolactone) was the chosen system. The PYR-loded lipid core nanocapsules present a mean size of 210 nm and drug concentration of 0.5 mg/mL. The homogeneity of the PYR nanocapsules was characterized using scanning electron microscopy and the atomic force microscopy. The cytotoxicity of encapsulated PYR was evaluated by the treatment of LLC-MK2 lineage and mouse peritoneal macrophage with different concentrations (0.1 – 5 µg/ml). These experiments were performed concomitantly with the incubation of equal concentrations of non-encapsulated drug and demonstrated that cells had similar tolerance for PYR encapsulated and non-encapsulated. The i.p. treatment of Swiss mice acutely infected with RH and EGS strain with different doses (5.0 – 10mg/kg/day) of encapsulated PYR resulted in significant increasing of the survival rate when compared to the animals that were just treated with the same doses of PYR non-encapsulated. Thus, the encapsulation increased PYR efficacy being an alternative for the reduction of the PYR dose, which could also reduce the side effects associated to the treatment.
Supported by:CNPq, CAPES and FAPERJ

QT036 - **LEISHMANICIDAL ACTIVITY OF SORANJIDIOL, AN ANTRAQUINONE ISOLATED FROM HETEROPHYLLAEA PUSTULATA**

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Leishmaniasis is a disease caused by a protozoan Leishmania sp. According to WHO, about 1.5 million new cases of cutaneous leishmaniasis occur each year worldwide. The treatment is based on antimonials compounds and amphotericin B, the last is used on resistant parasite strain; however, these drugs have high toxicity and are not fully effective. Many studies using natural products have been done to find out new chemotherapeutic agents against Leishmania sp. Anthraquinones (AQs) with photosensitizing and antimicrobial properties have been isolated from a phototoxic plant, Heterophyllaea pustulata Hook f. (Rubiaceae); one of the predominant metabolites was purified and identified as soranjidiol. In this study, we investigated the leishmanicidal effect of soranjidiol in vitro. We analysed the cytotoxicity of this compound against peritoneal macrophages of BALB/c mice by means the XTT assay and trypan blue exclusion test, which showed that soranjidiol did not affect cell viability until 100µM and the cell membrane integrity. In addition, macrophages phagocytic capacity was also not altered in comparison with untreated macrophages. However, soranjidiol showed toxic effects against amastigotes inside infected-macrophages. Our results showed an amastigotes survival inhibition of 59.4% at 10µM and 63.2% of 100µM of soranjidiol inside infected-macrophages. In order to investigate one possible mechanism of action, we evaluated if soranjidiol was able to modulate NO production in LPS/IFN-g activated-macrophages. Our results showed that soranjidiol at 10µM inhibited NO production in 17.4% and 63.85% at 50µM compared to untreated activated macrophages. Our results provide new perspectives for novel compounds for leishmaniasis treatment.
Supported by:CNPq, FAPERJ

QT037 - **TOPICAL ADMINISTRATION OF TAMOXIFEN REDUCES LESIONS AND ULCERATION IN A CUTANEOUS LEISHMANIASES MODEL**

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The number of patients with cutaneous leishmaniasis increases steadily with over a million new cases reported each year. Current treatments are costly and have a number of secondary effects. Furthermore, the appearance of resistant strains stresses the importance of discovering new treatments against this disease. Our lab has determined that the anticancer drug tamoxifen exhibits antileishmanial activity in vitro as well as in vivo. Until now, the in vivo data has been gathered from infected rodents that received systemic treatment by intraperitonial injection. These animals exhibit a significant improvement in terms of clinical symptoms as well as parasite burden. This project aims at investigating whether tamoxifen is effective in the treatment of experimental leishmaniasis if applied topically at the site of the lesion and whether topical treatment can be used in combination with drugs currently used to treat leishmaniasis. BALB/c mice infected with *L. amazonensis* were treated with 2 topical formulations: an ethanolic solution containing 1% tamoxifen or nanoemulsions composed of triglycerides containing various concentrations of tamoxifen. Both formulations effectively reduced the progress of lesions and the development of ulcers. These results suggest that tamoxifen administered topically effectively reduces the clinical symptoms of the disease. We are currently testing the possibility of using these topical formulations in association with meglumine antimoniate (Glucantime®), the current treatment of choice. Infected mice treated with Glucantime® by the intraperitoneal route in combination with topically applied tamoxifen (1% in ethanol), exhibited a slightly better response to treatment than those animals treated solely with Glucantime® or with the ethanolic formulation of tamoxifen. These preliminary tests show that while the drugs do not have an additive effect, they also do not interfere with one another and thus can be used concurrently.
Supported by:PNPD/CAPES;FAPESP;CNPq

QT038 - **EVALUATION OF ACTIVITY AGAINST PHYTOMONAS SERPENS EXTRACTED FRACTIONS OF PIPER CRASSINERVIUM, 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, infecting restricted sites such as fruit and laticifers. The aim of this study was to evaluate the antiparasitic activity of five fractions of P. crassinervium against Phytomonas serpens. The fractions were obtained by macerating of dried leaves of Piper crassinervium and was evaluated the activity of these fractions against promastigotas forms of P. serpens using microdilution plate. The protozoa were treated with fractions and the parasite growth in Warren medium supplemented with 10% fetal calf serum, incubated at 28°C/48h and determinated by counting. 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 (IC50). The results demonstrated that the dichloromethane, ethyl acetate and methanol fractions of P. crassinervium have strong effect over the proliferation of P. serpens, with IC50 of <10 μg/mL, <10 μg/mL and 13 μg/mL, respectively. The Hexane and Hexane-Dichloromethane fractions showed low antiproliferative activity against the protozoan with IC50 of 84 μg/mL and 47 μg/mL, respectively. The results demonstrate substantial selectivity of Piper crassinervium analyzed against P. serpens. However future studies should be continued investigation with scanning electron microscopy and cytotoxicity .
Supported by:CNPq, Fundação Araucária.

QT039 - **IMMUNOMODULATORY ACTIVITY OF PTEROCARPANOQUINONE LQB118 ON MACROPHAGE INFECTED WITH LEISHMANIA BRAZILIENSIS**

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Infection of macrophages with intracellular parasites such as Leishmania induces a negative modulation of the production of proinflammatory cytokines and nitric oxide. We showed previously that the pterocarpanoquinona LQB118 has antiparasitic direct effect on promastigotes and intracellular amastigotes of Leishmania braziliensis by apoptosis. LQB118 treatment of hamsters infected with L. braziliensis controlled the lesions and increased intradermal response to antigens of the parasite. The aim of this study was to evaluate the immunomodulating effects of LQB118 on murine macrophages. Monolayers of peritoneal murine macrophages infected with L. braziliensis (at a ratio of 5 parasites/macrophage) for 4h at 37°C/5%CO2 were incubated with LQB118 (0-20µM) for 24-48h /37°C/5%CO2. After staining with Giemsa, the number of intracellular amastigotes was counted under a microscope. Production of cytokines and nitric oxide were performed on culture supernatants after 24 or 48h. Cytokynes production was determined by ELISA and nitric oxide by Griess method (determination of nitrite). We observed that uninfected macrophages treated with 5, 10 and 20 µM LQB118 for 24 hours did not change in the production of IL-10 and IL-12. However the production of TNF-α and NO were increased by two times compared to control. In infected macrophages, the treatment with LQB118 (5, 10 and 20µM) for 24h was able to decrease the production of IL-10 ($p < 0.01$) and increase IL-12 ($p < 0.01$) and nitric oxide ($p < 0.01$) in a dose-dependent manner. The production of IL-12 was increased by ten times in relation to control at 20µM of LQB118. The treatment of macrophages infected for 48h did not alter the production of TNF-α, IL-10 and nitric oxide compared to control. However there were dose-dependent increase in IL-12 production ($p < 0.01$). The inhibitory action of the growth of intracellular amastigotes was 80%, 60% and 10% at 20, 10 and 5µM of LQB118, respectively. These data indicate that LQB118 is able to modulate the macrophage to a proinflammatory state that may contribute to the observed antileishmanial effect in vitro and in vivo. We are currently evaluating the immunomodulating action of LQB118 on murine lymphocytes. Supported by Faperj.
Supported by:Faperj

QT040 - **ANTIPROMASTIGOTE EFFECT OF CONYZA BONARIENSIS ESSENTIAL OIL ON LEISHMANIA AMAZONENSIS AND LEISHMANIA CHAGASI**

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Leishmaniasis is a worldwide health problem affecting more than 12 million people in 88 countries caused by the protozoan parasites of the genus Leishmania. The disease is spread through sandflies of the genus Phlebotomus in the Old World and Lutzomyia in the New World. The species of Leishmania that we focused on for this study was L. amazonensis and L. chagasi which are the pathogens involved in cutaneous and diffuse cutaneous (L. amazonensis) and visceral (L. chagasi) Leishmaniasis in the New World. Conyza bonariensis is a worldwide distributed plant from the family Asteraceae (Compositae), present in rural areas in Brazil. The C. bonariensis essential oil (CBO) is also being investigated by our group for anti-fungal activity. The GC analysis revealed the presence of an acetylene derived molecule (called LME), as a major compound. The aim of this work is to determine the antipromastigote effect of CBO against L. amazonensis and L. chagasi, comparing their susceptibility. The cells (5.106 promastigotes/mL) were cultured in 199+ 10% FCS (Cultilab, Brasil) and incubated with the drug at a concentration of 100 µg/mL in test tubes and left to culture over a period of 96 hrs, counting the viable cells every 24 hrs in hemocytometer. Our results suggest that CBO had a strong antipromastigote effect for both species due to the rapid inhibition of growth and no further growth after 24 hours for both species, inhibiting more than 84% and 90% for L. amazonensis and L. chagasi respectively. No damage was observed for host cell macrophages as measured by their spreading and adherence to glass surface.
Supported by:MACKPESQUISA; HEBRON FARMACÊUTICA

QT041 - **ANTILEISHMANIAL ACTIVITY OF SYNTHETIC COMPOUNDS AGAINST LEISHMANIA AMAZONENSIS**

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Leishmaniasis, caused by different parasites of genus Leishmania sp., represents an important public health problem, affecting more than 12 million people. Pentavalent antimonials are still the first-chose drugs for the treatment of leishmaniasis. However, these substances cause serious toxic effects leading to patients discontinuing treatment and the emergence of drug-resistant strains. In this work, we investigated the antileishmanial activity of eight nitrokene N,S-arylaminoacetals and eight 2,3-disubstituted-quinoxaline derivatives against promastigote forms of Leishmania amazonensis. In vitro biological activity was evaluated on 24-well plates when 1x106 promastigotes were treated with several concentrations of the compounds for 72 h. The antiproliferative effect was determined by direct counting of the cells in a Neubauer chamber. Fourteen compounds tested showed antileishmanial activity with IC50 (50% inhibition concentration) between 1.0 and 43.0 µM. The most effective compounds were 6-methoxy-3-(methylsulfonyl)-2-phenylquinoxaline (SULA), 2-(4-methylphenyl)-3-phenyl-quinoxaline (12B) and 2-(4-methoxyphenyl)-3-phenyl-quinoxaline (12D) with IC50 of 1.2, 8.9, and 12.8 µM, respectively. The less effective compounds were 3-phenyl-7-methoxi-2-methylsulphanylquinoxaline (4A) and 3-phenyl-2-methylsulphanylquinoxali ne (4B) with IC50 of 110.0, and > 396.3 µM. Our studies revealed that some of these compounds have a chemical structure with important antileishmanial activity .

Supported by:CNPq, FINEP, CAPES and Fundação Araucária.

QT042 - **IN VITRO EFFECTS OF EPIGALLOCATECHIN-3-GALLATE ON LEISHMANIA AMAZONENSIS**

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Epigallocatechin-3-gallate (EGCG), the most abundant flavonoid in green tea, has been reported to have antiproliferative effects on Trypanosoma cruzi. In the present study, we have evaluated the effect of EGCG on Leishmania amazonensis proliferation and ultrastructure, as well as the activity on intracellular amastigotes development. Promastigote proliferation was verified by direct quantification in a Neubauer chamber. In addition, we examined peritoneal mouse macrophages that were pre-infected with promastigotes for 3 h and were treated daily for 72 h with EGCG. We also evaluated the morphological damage of the treatment of promastigotes with EGCG for 120 h using transmission electron microscopy. Incubation with EGCG significantly inhibited L. amazonensis promastigote proliferation in a time- and dose-dependent manner. Macrophages infected with L. amazonensis and treated with the EGCG presented a significant reduction in the percentage of infected macrophage and the number of intracellular amastigote. Ultrastructural alterations of the mitochondria were observed in promastigote treated with EGCG, being the organelle injury reinforced by the decrease in rhodamine 123 fluorescence. These data demonstrate the effect of EGCG on the promastigote and amastigote forms of L. amazonensis and may suggest mitochondrial collapse as a part of the EGCG mechanism of action.

Supported by:FAPERJ, IOC FIOCRUZ and CNPq

QT043 - **COMPARATIVE ANTIPROMASTIGOTE EFFECTS OF 18-ETHOXYCORONARIDINE AND 19-HYDROXYCORONARIDINE ON LEISHMANIA AMAZONENSIS AND LEISHMANIA CHAGASI IN VITRO**

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Leishmaniasis is a worldwide health problem affecting more than 12 million people in 88 countries caused by the protozoan parasites of the genus Leishmania. The disease is spread through sandflies of the genus Phlebotomus in the Old World and Lutzomyia in the New World. The species of Leishmania that we focused on for this study were L. amazonensis (L. a) and L. chagasi (L. c) which are the pathogens involved in cutaneous and diffuse cutaneous (L. a) and visceral (L. c) Leishmaniasis in the New World. In previous studies the antipromastigote effect of coronaridine (coro), a natural occurring indole alkaloid, and of 18-methoxycoronaridine, a synthetic derived coro molecule were demonstrated (Delorenzi et al 2001; 2002). For this study we demonstrated the antipromastigote effects of 18-ethoxycoronaridine (18 EC), a synthetic coro derivative, and 19-hydroxycoronaridine (19 HC), a natural occurring coro-like compound. The cells (5.106 promastigotes/mL) were incubated with 100 µg/mL of the drugs in test tubes and cultured over a period of 96 hrs, counting the viable cells every 24 hrs in hemocytometer. The growth curve was analyzed for all drugs and both species. Our results suggest that both alkaloids have an antipromastigote effect for L. a due to the rapid inhibition of growth and no further growth after 24 hrs (over 90% and 40% inhibition for 18 EC and 19 HC respectively) and 72 hrs (over 90% and 68% inhibition for 18 EC and 19 HC respectively). This showed that 18 EC had a more pronounced antipromastigote effect than 19 HC for L. a. However, the compounds did not reveal a great effect against L. c; 19 HC and 18 EC caused inhibition for the first 48 hrs (70%, 84% inhibition respectively) but then growth continued as normal. No damage was observed for host cell macrophages as measured by their spreading and adherence to glass surface. The superior activity observed for 18-EC could be due to the presence of the ethoxy group at C-18 position.
Supported by:MACKPESQUISA; HEBRON FARMACÊUTICA; FIPES

QT044 - **EFFECTIVENESS OF THE FLAVONOID QUERCETIN ORALLY IN HAMSTERS INFECTED WITH LEISHMANIA BRAZILIENSIS AND ITS MODE OF ACTION IN VITRO**

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Quercetin is a flavonoid found in plants,which presents various biological effects. In this study, we investigated the effect of quercetin on *Leishmania braziliensis*, the most important dermatropic *Leishmania* species in Brazil. In vitro monolayers of peritoneal murine macrophages were infected with *L. braziliensis* (at a ratio of 5 parasites/macrophage) for 4h at 37°C/5%CO_2 and incubated with 0-100µg/ml of quercetin for 48h/37°C/5%CO2. The supernatants were collected and nitric oxide was determined by Griess reagent. After staining with Giemsa, the number of intracellular amastigotes was counted under a optical microscope. To assess the anti-leishmanial effect due to apoptosis, in situ detection of DNA fragmentation following treatment of intracellular amastigotes with quercetin for 48h at 50 and 100µg was performed using the TUNEL Kit and analysed by fluorescence microscopy. In vivo hamsters were infected with 10^7 promastigotes of *L. braziliensis* on the footpad and were treated after seven days of infection during eight weeks with 2mg of quercetin by oral route (five times a week). Controls were untreated or treated animals with 8mg of glucantime five times a week by intraperitoneal injections. The lesion size was measured with dial caliper. Quercetin inhibited 50% and 70% of intracellular amastigotes at 50 and 100µg/ml, respectively.The production of nitric oxide by macrophages was increased at least two times in comparision to control at 100µg/ml. Quercetin induced DNA fragmentation in intracelular amastigotes when marked with TUNEL suggesting apoptosis. In vivo, quercetin was significantly ($p<0,01$) active in controlling the growth of lesions from the second week of treatment. These results indicate that quercetin has therapeutic effectiveness and may act directly on the parasite and also on the host cells. The production of cytokines by macrophages and lymphocytes is being investigated.
Supported by:FAPERJ

QT045 - **EVALUATION OF AMEBICIDAL ACTIVITY OF THE ESSENTIAL OIL MENTHA CRISPA ON ENTAMOEBA HISTOLYTICA CULTURE**

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Mentha crispa (Lamiaceae) is an aromatic plant popularly known in Brazil as “hortelã da folha miúda”. The essential oil of plants of the genus Mentha has a bactericidal, fungicidal and virucidal activity. Entamoeba histolytica has a worldwide distribution affecting about 500 million people producing extraintestinal and intestinal disorders, representing a risk to health in countries where sanitary barriers are inadequate. Objective: To evaluate the effect of essential oil of M. crispa on cultures of E. histolytica by determining the minimum concentration that inhibits 50% of cultures growth (IC50). Evaluate the toxicological effects of the essential oil in mice. Methods: The M. crispa were collected in the municipality off Itacuruba – PE. The oil was extracted from aerial parts of the plant by hydrodistillation. Two hundred and forty thousand trophozoites of HM1 strain of E. histolytica in logarithimic growth phase were distributed into tubes of 6 mL for association with the essential oil. Five increasing concentrations from 0.015625mg/mL were incubated at 37oC for 48 hours. The tests were performed in triplicate and repeated twice. The inhibition percentage of each association was analyzed by linear regression and the results submitted to analysis of variance. The IC50 and its confidence interval were estimated by inverse regression, considering a confidence level of 95% and 90%. The evaluation of pre-clinical toxicity and determination of the DL50 of the essential oil was performed using Swiss mice by gavage. Results: The essential oil of M. crispa showed amebicidal activity with IC50 of 0.02 mg/mL and DL50 of 0.80 mL/kg. Conclusion: Whereas the results of toxicity showed DL50 greater than IC50, with a good amebicidal activity, the essential oil of Mentha crispa can be exploited in anti-amoeba therapy.
Key words: Entamoeba histolytica; amebicidal effect; Mentha crispa
Supported by:FAPEMIG, CNPq e Hebron

QT046 - **LEISHMANICIDAL EFFECT OF ESSENTIALS OILS FROM LIPPIA SP.**

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Leishmaniasis is a tropical disease caused by *Leishmania spp.* and represents an emergent illness. The drugs currently used are toxic and expensive. The side effects and the appearance of resistant parasites, stimulates the search for new substances with antileishmanial activity, including the prospecting of natural products. In this work, we tested two essentials oils (EOs) extracted from *Lippia sidoides* and *Lippia lycioides* (Verbenaceae). To verify the leishmanicidal effect of these EOs in intracellular amastigotes, infected-macrophages were treated with different concentrations of EOs, named O-10 and O-12. Our results showed that O-10 and O-12 at 50µg/ml inhibited respectively, 84% and 49% the amastigotes survival inside macrophages. The safety of these oils was tested by the XTT assay and macrophage phagocytic capacity. Our results demonstrated by both methodologies that the two oils were not toxic to host cells. Nitric oxide (NO) production is an effective mechanism against the parasite, so we tested if the oils were able to modulate NO production by macrophages. Our results demonstrate that EOs inhibited around 89% the NO production in LPS/activated macrophages in comparison to untreated cells. These results were correlated with the chemical profile of O-10 and O-12. This study adds *Lippia sidoides* and *Lippia lycioides* as a source of new compounds for drug discovery against leishmaniasis.
Supported by:CAPES, FAPERJ, CNPq

QT047 - **LEISHMANICIDAL ACTIVITY OF ESSENTIAL OILS FROM LIPPIA SIDOIDES WITH DISTINCT CHEMICAL COMPOSITION**

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Leishmaniasis is a spectrum of diseases which affect annually about 2 million people worldwide. The current therapy for leishmaniasis is far from satisfactory. All available drugs, including pentavalent antimony, pentamidine and amphotericin B require parenteral administration and are potentially toxic. Moreover, an increase in clinical resistance to these drugs has been reported. In this scenario, plant essential oils used traditionally in folk medicine are emerging as alternatives sources for chemotherapeutic compounds. In this study, in vitro leishmanicidal effects of a thymol- and a carvacrol-rich essential oil from leaves of Lippia sidoides were investigated. The essential oils were extracted by hydrodistillation and their constituents were characterized by gas chromatography coupled to mass spectrometry (GC/MS). The oxygenated monoterpene thymol was the main component of the essential oil from the access LSD-102 (LSEO102) while carvacrol was more abundant in LSD-104 (LSEO104). In order to investigate the leishmanicidal activity, promastigotes of *Leishmania chagasi* were incubated in presence of increasing concentrations of LSEO102 and LSEO104 for 72 hours and cell viability was determined by MTT assay. Both essential oils efficiently inhibited the parasite growth. However LSEO104 presented a higher affectivity, whereas the treatment with this oil resulted in a lower IC50 (54.8µg/mL) compared to the LSEO102 (74.1 µg/mL). Although the relative amount of thymol in LSEO102 and carvacrol in LSEO104 is around 40%, LSEO104 has 6% of thymol in addition to carvacrol content. On the other hand LSEO102 has any carvacrol in its composition. In this way, we can postulate that a synergistic effect of thymol present in the composition of LSEO104 could respond to the better leishmanicidal effect shown by this oil. Nevertheless, further studies are needed to evaluate the individual contribution of each compound in the leishmanicidal properties of *L. sidoides* essential oil.
Supported by:Capes-PROCAD/NF; CNPq

QT048 - **INTRAPERITONEAL EFFICACY OF QUERCETIN OR QUERCETIN PENTAACETATE IN POLIMERIC NANOCAPSULES OF POLI-(E-CAPROLACTONE) ON MURINE CUTANEUOS LEISHMANIASIS**

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The conventional treatment of cutaneous leishmaniasis comprises the use of parenteral antimony for 20-30 days with several side effects. The use of nano and microparticulated systems with antileishmanial drugs can reduce toxicity significantly. We demonstrated the antileishmanial activity of flavonoid quercitrin (MUZIATNO et al, 2006) and its active metabolite quercetin in vivo (MUZITANO et al, 2009). The pharmaceutical development of quercetin is more economically viable. Here, we use a nanoencapsulated formulation of quercetin as an alternative to conventional treatment. A nanoemulsion of lipid nanocapsules of poly (e-caprolactone) (LNC) was prepared with 40 µg/ml of quercetin or quercetin pentaacetate. BALB / c mice (n = 4) were infected with $2x10^6$ promastigotes of *L.amazonensis* in the footpad. The i.p. treatments were from PID 7 until PID 52, on alternated days: a) PBS, b) 500 µg/Kg of quercetin (QE); c) 500 µg/Kg of quercetin pentaacetate (PQE); d) blank lipid nanocapsules (LNC-blank), e) quercetin in lipid nanocapsules (LNC-QE), f) quercetin pentaacetate in lipid nanocapsules (LNC-PQE). The lesion growth was monitored until PID 111. Mice were euthanized and paws were removed for assessment of parasite load by fluorimetry. LNC-blank, LNC-QE or LNC-PQE administration controlled lesion growth during the treatments. After treatments (PID 79), only LNC-PQE controlled the lesion growth (0.397±0.16mm) compared to PBS (0.885±0.28mm). The reduction of lesion was reflected in parasite load (PBS=16210±124UF; LNC-PQE=14040±142UF). Treatments with QE or PQE did not control lesion growth. This sub-optimal dose of free QE or PQE is about 30 times lower than the active dose (15 mg/Kg) and had no effect on cutaneous leishmaniasis. However, the nanoencapsulation of PQE in LNC was effective in controlling the growth of lesion for at least 60 days after treatment. These results indicate that the formulation of PQE in lipid nanocapsules potentiates its antileishmanial effect.
Supported by:CNPq

QT049 - **LEISHMANICIDAL ACTIVITY OF MTB1, MTB2 AND MTB3 IN MURINE MACROPHAGES**

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Leishmaniases are emerging diseases with a broad spectrum of clinical presentations. The recommended course of treatment usually involves pentavalent antimonials, a long-term therapy with the emergence of resistant strains. The search for new anti-leishmanial drugs has certain urgency. The present study aimed to evaluate the efficacy of three new drugs, herein referred to as MTB1, MTB2 and MTB3, to control *L.amazonensis* infection in CBA murine macrophages (MΦ) in vitro. The direct effect of these three compounds on *L.amazonensis* was evaluated in parasite cultures exposed to different concentrations of each drug for 24, 48 and 72h, followed by direct parasite counts. MTB2 (IC50=41.13µM) and MTB3 (IC50=42.45µM) caused a direct inhibitory effect on parasite growth. MTB2 exhibited the best performance, with 50µM inducing a 90% promastigote kill rate after 48h. The effect of each of the three drugs on MΦ viability was assessed using alamarBlue® in cultures incubated with concentrations that ranged from 25-200µM of each of the three drugs for 48h, the IC50 for MTB1 was 88.34µM, for MTB2 was 119.20µM, and for MTB3 was 75.23µM. The effect of MTB2 on parasite infection was evaluated in MΦ infected with *L.amazonensis* and treated with drug concentrations that ranged from 25-100µM for 6, 24 and 48h. The MΦ cultures were then fixed and the percentage of infected cells, and the number of parasites per infected cells were determined by light microscopy. Treatment with 50, 75 and 100µM of MTB2 resulted in a reduction in the number of infected MΦ of 75%, 98% and 99%, respectively. A drastic reduction in parasite load was also observed following MTB2 treatment: 51% at 50µM, 70% at 75µM, and 77% at 100µM. Our findings suggest that further efforts involving these three compounds may lead to the development of a new generation of anti-leishmanial drugs. Proteomic studies will be performed to expose the underlying mechanism involved in MTB2 control of *L.amazonensis* infection.
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QT050 - **GENE AND PROTEIN EXPRESSION ANALYSIS OF THE ABC TRANSPORTER AND ANTIMONY UPTAKE IN NEW WORLD SB-RESISTANT LEISHMANIA SPP.**

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ATP-binding cassette (ABC) transporters are involved in the translocation of a variety of molecules across membranes against concentration gradient. Members of the ABC superfamily has been investigated in *Leishmania* spp. and variations in the levels of protein expression have been suggested as a possible mechanism of drug resistance in this parasite. Here, promastigote forms of susceptible and resistant populations from four different species of *Leishmania*, *L. guyanensis*, *L. amazonensis*, *L. braziliensis* and *L. infantum chagasi* were analyzed for: levels of mRNA ABC gene (*LbrM.23.0230*), P-glycoprotein (PGP) expression levels and determination of intracellular antimony (Sb) level. These populations were selected in vitro to trivalent antimony (SbIII) by step-wise drug pressure and the resistance index varied from 4 to 20-fold higher than of their wild-type counterparts (Liarte & Murta, 2010). Levels of mRNA ABC gene determined by real-time RT-PCR showed an increased expression of 1.8 to 3-fold in all Sb-resistant populations analyzed in comparison with the susceptible ones. In order to investigate whether PGP also was overexpressed in Sb-resistant *Leishmania* populations, we have used a mouse monoclonal antibody (C219-Abcam) directed against the cytoplasmic domain of the mammalian PGP. Western blot analysis showed that C219 recognized a protein of the expected size 170kDa. The intensity of the band from the Sb-resistant populations *L. guyanensis* and *L. amazonensis* was 4.8 and 4.3 higher than of their wild-type counterparts, respectively. The intracellular level of SbIII was quantified by graphite furnace atomic absorption spectrometry. Data showed that levels of Sb in the Sb-resistant populations were 2 to 7-fold lower when compared to its parental sensitive wild-type strains. Further studies will be performed in order to better investigate the role of the ABC transporters in the drug-resistance phenotype in these New World *Leishmania* species.
Supported by:CNPq, FAPEMIG, CPqRR, PDTIS/CPqRR and UNICEF/UNDP/World Bank/WHO/TDR

QT051 - **ORAL EFFICACY OF A LIPID NANOFORMULATION OF QUERCETIN IN MURINE CUTANEOUS LEISHMANIASIS**

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The conventional treatment of cutaneous leishmaniasis comprises the use of parenteral antimony for 20-30 days with several side effects. Alternative treatments have been proposed by oral, topical or intralesional routes. The nano and microparticulated systems with antileishmanial drugs reduces toxicity by more acceptable routes to the patient. We demonstrated the antileishmanial activity of flavonoid quercitrin (MUZIATNO et al, 2006) and its active metabolite quercetin in vivo (MUZITANO et al, 2009). The pharmaceutical development of quercetin is more economically viable. Here, we use an oral nanolipid formulation of quercetin as an alternative to conventional treatment. It was prepared a lipid nanoemulsion with quercetin (0.1% w/w). BALB / c mice (n = 4) were infected with $2x10^6$ metacyclic promastigotes of *L.amazonensis* in the footpad. The oral treatments started at PID 7, with 30 daily doses: a) PBS; b) 10 mg/Kg of quercetin (QE); c) blank lipid nanoemulsion (LN); d) quercetin in lipid nanoemulsion (QE-LN); The lesion growth was monitored until PID 101. Mice were euthanized and the paws were removed for assessment of parasite load by limiting dilution. Animals treated with QE or QE-LN controlled lesion growth during the treatment. After treatments, only QE-LN group showed a significant control of lesion growth (0.607±0.204mm), compared to PBS group (2.13±0.153mm). QE did not control the lesion growth after treatment, showing the same growth profile of lesion of PBS group. However, mice treated with QE($1.47x10^{11}$ parasites) and also QE-LN ($1.36x10^8$ parasites) showed a low parasite load compared to PBS ($5.83x10^{14}$ parasites). QE dissolved in PBS was partially effective in cutaneous leishmaniasis, in a dose 1.5 times lower than the effective dose that was used previously by oral route (Muzitano et al 2009). LN improved the effect of QE and seems to be an oral formulation that can be applied by the own patient as an advance in the therapy of cutaneous leishmaniasis.
Supported by:CNPq

QT052 - **LQB-118, A NEW SYNTHETIC NAPHTHOPTEROCARPANQUINONE WITH POTENT ORAL ACTIVITY, INDUCES APOPTOSIS IN LEISHMANIA AMAZONENSIS.**

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Despite the approval of miltefosine and the development of new formulations of conventional drugs had been landmarks in the advancement of chemotherapy of leishmaniasis, little impact has been observed in endemic areas. Aiming at the development of new drugs, the fusion of a synthetic quinone core with a pterocarpan portion yielded a hybrid prototype, LQB-118, which showed activity in vitro in different species of Leishmania. This work intends to demonstrate that LQB-118 is a promising prototype by evaluating its efficacy in an experimental model of leishmaniasis by different routes of administration and by investigating its mechanism of action. The in vivo activity was evaluated in L. amazonensis infected BALB/c mice treated or not with LQB-118 by subcutaneous, intraperitoneal or oral routes. To evaluate the mechanism of action, the following parameters were investigated: mitochondrial activity, production of Reactive Oxygen Species (H2DCFDA, AmplexRed e Mitosox), lipid peroxidation (TBARS), alteration of mitochondrial membrane potential (Rhodamine 123, JC-1, mitocapture) phosphatidylserine exposure (Annexin V) and DNA fragmentation (TUNEL). In vivo administration of LQB-118 by different routes, including oral, resulted in a promising response in controlling the growth of the lesion and parasite load. Furthermore, no signal of resistance or toxicity was observed. Regarding the mechanism of action, LQB-118 induced oxidative stress with increased mitochondrial activity, ROS production, loss of mitochondrial membrane potential and phosphatidylserine exposure in promastigotes of L. amazonensis. We also observed DNA fragmentation both in promastigotes and intracellular amastigotes. Taken together, these results demonstrate that the molecular hybridization of a naphthoquinone core with a pterocarpan moiety yielded an antileishmanial lead compound active by the oral route and suggest that its mechanism of action involves selective apoptosis induction in the parasites.
Supported by:CNPq, CAPES, FAPERJ, PAPES

QT053 - **EFFECT OF ESSENTIAL OIL MENTHA CRISPA ON CULTURES OF TRICHOMONAS VAGINALIS**

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Trichomonas vaginalis, the causative agent of trichomoniasis, an important sexually transmitted infection (STI), predominantly in women where are manifested their morbid effects. It is estimated an annual prevalence of at least 250 million cases in world and the disease can constitute gateway to other STIs including HIV and cervical cancer. Despite the high prevalence, many obscure points still remain, especially with regard to drug resistance in these organisms. Refractory cases to available therapies are frequently reported, signaling toward the development and use of new drugs for the treatment of disease. Medicinal plants occupy a growing part in therapy. Mentha crispa (Lamiaceae) is popularly known in Brazil as "hortelã da folha miúda". In popular medicine, the leaves of plants of this genus has many indications in addition to antiparasitic. Objective: Evaluate trichomonacidal activity of essential oil M. crispa and determine the minimum inhibitory concentration (IC50) of the oil in T. vaginalis cultures. Method: The M. crispa were collected in the city of Itacuruba - PE, a exsiccata was deposited in the Herbarium of Pharmacognosy, of UFPE nº 001507HF HF. The oil was extracted from aerial parts of the plant by hydrodistillation. Sixty thousands trophozoites of JT strain, in logarithimic growth phase were distributed in tubes of 6 mL for association with the essential oil. Five increasing concentration from 0.015625 mg/mL were tested. The cultures were incubated with the drugs at 37 °C for 48 hours. The inhibition percentage of each association was analyzed by linear regression and the results submitted to analysis of variance. The IC50 and its confidence interval were estimated by inverse regression, considering a confidence level of 95% and 90%. Results: The essential oil of M. crispa showed trichomonacidal activity with IC50 of 0.05 mg/mL, ranging from 0.04 to 0.06 with 95% confidence. According to the regression model used was estimated that the drug concentration had significant impact on parasite growth inhibition. Conclusion: This result signalizes the possibility of using the essential oil of Mentha crispa as alternative therapy for trichomoniasis. Supported by:FAPEMIG, CNPq e Hebron

QT054 - **TOPICAL FORMULATIONS OF CHALCONE CH8 FOR CUTANEOUS LEISHMANIASIS TREATMENT**

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Cutaneous leishmaniasis treatment usually involves daily injections for 20-30 days of pentavalent antimonials Pentostam® or Glucantime®, however, high rates of adverse effects and limited efficacy are related. Lipossomal formulations are considered appropriated delivery systems to carry the drug into and across intact skin. Topical treatment is desirable because of its easy application, painless and lack of systemic side-effects. Previous studies revealed that intralesional treatment with chalcone CH8 (8 injections of 6 µg) has the same effect to control the lesion size and parasitic burden than topical application of CH8-loaded on conventional lipossomes (CH8-CvL) at 6.6 µg/dose once a day for 30 days. In this work, new topical formulations containing CH8 were tested against *Leishmania amazonensis*-GFP *in vivo*. We evaluated CH8 in phosphate buffered saline, CH8-CvL in combination or not with collagenase ointment (penetration enhancer), CH8 1% and Paramomicin 15% in lanette cream by topical treatment. At 43 days post-infection, formulations were applied in infected ears once a day for 36 days. Lesion size was measured twice a week and parasitic burden was evaluated in the day 79 post-infection. We observed no effect in CH8 in phosphate buffered saline group, as expected, and a partial reduction of lesion size and parasitic burden by CH8-CvL, CH8 1% and Paramomicin 15% in lanette cream. A strong reduction in lesion size was observed when CH8-CvL was used in association with collagenase ointment. These results indicate the potential use of a new topical formulation of chalcone CH8 in liposome (CH8-CvL) associated to collagenase to treat ulcerated lesions of cutaneous leishmaniasis.

Supported by:CAPES

QT055 - **CHARACTERIZATION OF A GENE ENCODING TRYPAREDOXIN PEROXIDASE (TXP) IN ANTIMONIAL-SUSCEPTIBLE AND -RESISTANT POPULATIONS OF LEISHMANIA BRAZILIENSIS**

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Tryparedoxin peroxidase (TxP) belongs to a widespread family of peroxiredoxins. In parasites, TxP participates of antioxidant defense by metabolizing hydrogen peroxide to water molecules. Data from literature have shown that drug-resistant parasites can increase TxP levels together with others enzymes protecting them against oxidative stress. In this study, we evaluated the levels of transcription of the LbTxP gene and protein expression levels in *L. braziliensis* populations susceptible (LbWTS) and 20-fold more resistant (LbSbR) to potassium antimony tartrate (Sb III). Real-time RT-PCR analyses revealed that the levels of LbTxP mRNA were two-fold higher in the LbSbR drug-resistant population than its drug-susceptible counterpart LbWTS. Protein expression was determined by western blot using polyclonal antibody raised against the TcTxP recombinant protein from *Trypanosoma cruzi*. Alignment analysis between the amino acid sequences this protein from *T. cruzi* showed 84% of identity with sequences from *L. braziliensis*. The antibody anti-TcTxP recognized a 25 kDa peptide in both *Leishmania* populations. The level of expression of this native protein in the LbSbR was 2-fold higher than in the LbWTS population. Studies are being performing to determine whether overexpressing LbTxP in the susceptible population will confer antimony-resistant to these parasites. To overexpress LbTxP, both *L. braziliensis* LbWTS and LbSbR populations were electroporated with pIR1-BSD-LbTxP and plated on semisolid media containing Blasticidin (BSD). Clonal lines were recovered and the presence of the plasmid was confirmed by PCR with primers specific for BSD marker. Western blot analyses showed that the level of expression of the LbTxP protein was 3 to 7-fold higher in the transfected parasites than in the non-transfected parental populations. The susceptibility of these TxP overexpressors parasites to SbIII is being determined. Supported by:CNPq, FAPEMIG, CPqRR, PDTIS/Fiocruz and UNICEF/UNDP/World Bank/WHO/TDR.

QT056 - **CO(II), MN(II) AND CU(II) COMPLEXES OF FLUORQUINOLONES: BIOLOGICAL EVALUATION AGAINST TRYPANOSOMA CRUZI**

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Fluoroquinolones (FQ) are chemotherapic agents with a fluorine atom attached to the central ring system, which represent an important class of synthetic broadspectrum antibiotics. The effect of FQ against protozoan parasites was also reported *in vitro* including *Plasmodium falciparum*, Leishmania spp, and *Trypanosoma cruzi*. This is etiologic agent of the disease Chagas, a disease that leads to morbidity and mortality; that shows no vaccines or safe chemotherapeutic agents are available. In this work, 6 metal complexes of FQ ($[MnCl_2(NOR)(H_2O)_2]$, $[MnCl_2(SPAR)(H_2O)_2]$, $[CoCl_2(NOR)(H_2O)_2]$, $[CoCl_2(SPAR)(H_2O)_2]$, $[CuCl_2(phen)(NOR)]$ and $[CuCl_2(phen)(SPAR)]$) and 2 FQ (sparfloxacin-SPAR and norfloxacin-NOR) were evaluated against both bloodstream trypomastigotes and intracellular forms of *T. cruzi in vitro*. We results showed SPAR and NOR exerted a low trypanocidal effect against bloodstream trypomastigotes exhibiting IC_{50} values for 24h of 114.1±20.4 and 126.8±30.2µM, Mn(II) and Co(II) complexes were not able to improve FQ activity displaying similar or higher IC_{50} values to SPAR and NOR, with values between 114.7 and >200µM (24h). However, when Cu(II)–phen complexes showed a striking increase in efficacy against the parasites, with IC_{50} values of 4.65±0.12 and 4.36±1.44µM for $[CuCl_2(phen)(NOR)]$ and $[CuCl_2(phen)(SPAR)]$, respectively. Importantly, the trypanocidal activity of both Cu(II)–phen complexes was about 2.5-fold higher as compared to benznidazole. Next, we evaluated the activity this compounds against intracellular forms and similarly as noticed with bloodstream trypomastigotes Cu(II)–phen complexes showed the highest activity upon intracellular parasites, showing IC_{50} values of 1.62±0.16 and 2.24±1µM. The potency of metal complexes of FQ justify further trypanocidal screening assays with this compounds in vitro as well as upon experimental models of *T. cruzi* infection. Supported by:FIOCRUZ; FAPERJ (APQ1, Pensa Rio and PPSUS), CNPQ, PDTIS/FIOCRUZ and PAPES V/FIOCRUZ.

QT057 - **LEISHMANICIDAL ACTIVITY OF NEW SYNTHETIC AZOLES**

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Leishmaniasis are caused by protozoan parasites of the genus Leishmania, and their clinical presentation range from skin lesions to visceral impairment, constituting a serious public health problem. The current drugs are toxic and often inefficient, so a rational search for new antileishmanial drugs is necessary. In the present study, the leishmanicidal activity of nine new tetrazoles derivatives (MSN series) was evaluated. The compounds 5-[5-amino-1-(4'-methoxyphenyl)-1H-pyrazole-4-il]-1H-tetrazole (MSN20), 5-[5-amino-1-(3'-bromophenyl)-1H-pyrazole-4-il]-1H-tetrazole (MSN14), 5-[5-amino-1-(2'-chlorophenyl)-1H-pyrazole-4-il]-1H-tetrazole (MSN08), 5-[5-amino-1-(2'-fluorophenyl)-1H-pyrazole-4-il]-1H-tetrazole (MSN09) and 5-[5-amino-1-(4'-fluorophenyl)-1H-pyrazole-4-il]-1H-tetrazole (MSN10) showed activity against Leishmania (L.) amazonensis promastigotes with IC50 of 43, 78, 75, 91, and 102 µM, respectively. Next, the compounds were evaluated on intracellular amastigotes. Murine peritoneal macrophages infected with L. amazonensis were incubated with and without derivatives MSN08, MSN09, MSN10, MSN14 and MSN20 for 72 h. Within this series, derivatives MSN14 and MSN20 showed the highest activity with IC50 of 22 and 25 µM respectively. To evaluate the in vitro cytotoxicity, murine peritoneal macrophages were incubated with and without compounds for 72 hours and cell viability was assessed by MTT. The compounds MSN20 and MSN14 were not toxic for macrophages with LD > 500µM. These results indicate that compounds MSN20 and MSN14 are the most promising of this tetrazole series, with potential to be evaluated in vivo models.
Supported by:CNPQ

QT058 - **IN VITRO ACTIVITY OF NITRONES ON TRYPANOSOMA CRUZI EPIMASTIGOTES**

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Chagas disease has shown an increasing number of cases in the world, even in non endemic countries and the available drugs are not efficient. Thus, there is an enormous necessity for new medical alternatives. Once nitrones had shown a protective role against pathogens, we investigated the effects of six nitrones (LQB121, LQB122, LQB123, LQB131, LQB132 and LQB136) on Trypanosoma cruzi epimastigotes. To evaluate the effect of nitrones upon epimastigote proliferation, cells were maintained in BHI, 10% FCS,and 30µM heme for 7 days at 28ºC. Then, cells were kept for 5 days in the absence (control) or in the presence of 30µM heme (a molecule that improves parasite growth) or increasing concentrations of LQBs. Parasite growth was assessed by cell counting in a Neubauer chamber. Our results showed that the incubation of epimastigotes with LQBs 123, 131 and 132 presented a dose-dependent inhibition of parasite proliferation while the others did not present the same effect. Our group has been showing that reactive oxygen species (ROS) are benefic for epimastigote proliferation then, we evaluated the effect of LQBs on ROS formation. Epimastigotes were loaded with ROS sensitive probes, challenged in the absence or presence of 2mM LQBs, 30µM heme (a ROS inductor) or 1mM urate (an antioxidant control) and ROS production was analyzed by flow cytometry. Interestingly, epimastigotes incubated with LQBs 131 and 136 decreased heme-induced ROS, suggesting an antioxidant role for these molecules, however, LQBs 121, 122, 123 and 132 did not present the same effect. Our results suggest LQBs 123, 131 and 132 as good candidates for new anti-T. cruzi drugs since they presented marked trypanocidal effect upon epimastigotes. Additionally, LQB131 and LQB136 showed antioxidant activity, suggesting that LQB131 can inhibit T. cruzi growth by a mechanism involving the cell redox status. Also, the trypanocidal effect of LQBs upon the clinical relevant forms of T. cruzi remains to be investigated.
Supported by:Supported by CNPq, FAPERJ and PIBIC-CNPq.

QT059 - **SINGLE-DOSE TREATMENT OF CUTANEOUS LEISHMANIASIS USING PLGA MICROPARTICLES LOADED WITH AMPHOTERICIN B OR GLUCANTIME**

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Conventional cutaneous leishmaniasis (CL) treatment is based on multiple parenteral injections with antimonial or amphotericin B drugs, which are highly toxic to the liver, kidneys and heart. In our studies, we developed poly-(lactide-co-glycolide) PLGA microparticles (mps) loaded with amphotericin B (AmB/PLGA) or Glucantime (GLU/PLGA). PLGA (50:50) mps were prepared with 10% (w/w) amphotericin B (AmB) or Glucantime (GLU) by the multiple emulsion method, followed by solvent evaporation, aqueous washing and dried. We observed that in In vitro trial, the mps presented anti-amastigote activity, moderate interference in the nitric oxide production and low cytotoxicity in infected macrophages. In this work, we evaluated the therapeutic activity of mps in murine leishmaniasis model to develop a localized single-dose treatment. Mice were infected in the ear with fluorescent Leishmania amazonensis promastigotes, and on day 25 they received a subcutaneous injection with 50µg of AmB: i) in free form; ii) in PLGA mps; iii) in liposomal formulation (Ambisome®); or 200µg of GLU: iv) in free form; v) in PLGA mps. Controls received empty mps or 10 ul of PBS alone. The lesion sizes were measured by paquimeter and parasite loads by fluorimetry. Toxicological parameters (AST, ALT and creatinin) were measured in serum using commercial kits. We observed that treatment with AmB/PLGA and GLU/PLGA controlled the lesion growth throughout infection, despite the transient effect observed for Ambisome®. Free drug or empty mps were not effective. On day 70 of infection, the parasite load was significantly smaller in mice treated with drug-loaded PLGA mps, however, no effect was observed using free drug. No changes in AST, ALT and creatinin were observed at the end of the experiment. Loading of AmB/PLGA or GLU/PLGA promoted a sustained drug release in lesion site leading to a durable and safe therapeutic effect. These findings support this new approach for single-dose localized treatment of CL.
Supported by:CNPq; Capes; FAPERJ

QT060 - **THERAPEUTIC ACTION OF FUROSEMIDE ON CUTANEOUS LEISHMANIASIS**

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Recently was described that *Leishmania amazonensis* express the enzyme Na^+-ATPase and that its inhibition by furosemide, a drug widely used for treatment of edema and hypertension, induces death of promastigotes. This led us to study the effect of furosemide as an anti-leishmanial drug. *In vitro*, peritoneal macrophages were infected and incubated with furosemide (0; 0,125; 0,25; 1mM). After 48 h/ 37 ºC, the number of amastigotes was valued using optical microscopy. We observed that furosemide reduced the parasite load (IC_{50} 0,06 mM), compared to untreated control, with no cytotoxicity (IC_{50} >1 mM). Then, we analyzed if furosemide induces the cell microbicide mechanisms, analyzing the production of nitric oxide (NO) through Griess reagents, and reactive oxygen species (ROS) using plate fluorimeter and H_2DCFDA dye. Furosemide (158 µM) was capable to induce four times both mechanisms in infected macrophage in comparison to non-treated. These results suggest that furosemide is a potential drug anti-cutaneous leishmaniasis, killing promastigotes and amastigotes without cell toxicity, beyond modulate the innate response on macrophages toward the infection.
Supported by:CNPQ

QT061 - **NEW TRIAZOLES AGAINST LEISHMANIA AMAZONENSIS**

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Leishmaniasis refers to several clinical syndromes caused by intracellular parasites of the genus Leishmania and it is a serious public health problem in Brazil. Most antileishmanial drugs currently used in clinical have side effects and are often inefficient. Thus, a rational search for new drugs is necessary. In this case, the azole antifungal drugs are constantly being studied in these trypanosomatids. The leishmanicidal activity in vitro as well as the mechanism of action of the series benzyl, pyridinyl, thiophenyl or furanyl-1-(phenylamino)-5-methyl-1H-1,2,3-triazole-4-substituted carbohydrazides were assessed in Leishmania amazonensis (MHOM/BR/77 LTB0016 strain) promastigotes and intracellular amastigotes. The promastigotes were assessed by counting the parasites in a Neubauer chamber, and the intracellular amastigotes were quantified by microscopic counting of the percentage of amastigotes/macrophages. Considering that several well known azoles, such as ketoconazole, inhibit ergosterol biosynthesis, we also evaluate if the mechanism of action of these molecules involves disturbance of the parasite membrane composition. L. amazonensis promastigotes were treated or not with the triazolecarbohydrazides and their neutral lipids were extracted and analyzed by TLC. The results showed that the molecule TCH14 presents the better activity, with IC50 of 0,2 µM for promastigotes and 15 µM for intracellular amastigotes. The analysis of the TLC revealed that the ergosterol derivatives were less intense with TCH14 treatment, with simultaneous enhancement of intensity of unidentified bands, suggesting precursor accumulation. Altogether, these results indicate that TCH14 could serve as a new triazole prototype to be evaluated in vivo.

Supported by:CNPQ/PAPES

QT062 - **NEW INSIGHTS ABOUT THE TRYPANOSOMA CRUZI INFECTION IN RHODNIUS PROLIXUS AND MICE BY BIOLUMINESCENT IMAGING.**

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In the search for novel chemotherapy against Chagas disease, selected compounds need to go through trials in animals and vectors. Thus, we genetically modified the Dm28c strain to express firefly luciferase, which allow tracking the progression of infection by detecting the bioluminescence in organs and tissues in real time. Bioluminescence detection method was standardized along the *Trypanosoma cruzi* life cycle, in the vector and mice. Therefore, the firefly luciferase gene was PCR amplified and cloned into the integrative pTREX vector. The pTREX-luc plasmid was mixed with *T.cruzi* epimatigotes, electroporated and tranfectants were selected with G418. Clones of stable transfectants were mixed with luciferin and selected by bioluminescence emission at the SpetramaxM2 plate reader. Thereafter, submitted to *in vitro* metacyclogenesis and used to infect LLCMK2 cells to obtain trypomastigotes expressing luciferase. These trypomastigotes were used to evaluate the progression of infection *in vivo*, in *Rhodnius prolixus* and BalbC mice, by bioimaging using Xenogen IVIS system. Infection kinetics, after luciferin injection in hemolymph or in mice peritoneal cavity, were followed for several days. Fifth instar larvae displayed bioluminescence at the stomach after 1 hour and 48 hours of a bloodmeal containing 2.5 x 10^7 trypomastigotes/ ml. Seven to 16 days post-infection, bioluminescence was seen in small intestine, and 26 days later was concentrated at the rectum, which was corroborated by bioluminescence at the dissected intestines. *T.cruzi* dissemination in mice was observed by bioluminescence emission at the site of inoculation in peritoneal cavity, 10^6 trypomastigotes. After one week, we observed migration to the heart, head and legs. Bioluminescence was more intense in these foci after 15- 20 days, and decline after 24 days, as confirmed by *ex-vivo* evaluation of organs and tissues. This methodology gives new information about the progression and dynamic of infection.

Supported by:FAPERJ, CNPq and CAPES

QT063 - **3-BRPA AFFECT THE MITOCHONDRIAL STRUCTURE OF TOXOPLASMA GONDII DURING INTERACTION WITH LLCMK2**

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Toxoplasma gondii, the agent of toxoplasmosis, is an obligate intracelular protozoan able to infect a wide range of vertebrate cells, including nonprofessional and professional phagocytes. Therefore, drugs to control this parasite must have an intracellular activity. The most common therapy for toxoplasmosis is the combination of sulfadiazine and pyrimethamine, although this treatment is associates with adverse reactions. Therefore, the development of new drugs becomes necessary. This study aims to test the analog of pyruvate (3-BrPA) against the intracellular parasite. 3-BrPA is a drug that inhibit the mithocondrial activity in tumor cells. The functionality of the drug occurs at concentrations nanomolar level. In this study we tested wich had lower concentration of drug activity against *Toxoplasma gondii*. The results showed that the IC50 of this drug is 10µM. The transmission electron microscopy showed mitochondrial alterations with destruction of mitochondrial cristae. Moreover, there is the appearence of granules similar to those of amylopectin granules, suggesting the induction of the conversion of tachyzoites into bradyzoites. These results suggest that the population of the parasite Toxoplasma gondii dies by alteration of mitochondria and that the drug was able to induce the conversion of tachyzoites into bradyzoites.
Supported by:FAPERJ e CNPq

QT064 - **EVALUATION OF THE LEISHMANICIDAL ACTIVITY OF THE LUPANE ISOLATED FROM COMBRETUM LEPROSUM ENCAPSULATED IN LIPOSOME IN VIVO**

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Drug delivery systems are promising pharmaceutical formulations used to improve the therapeutic outcome of drugs. In this study, we developed a liposomal formulation of a lupane [3ß,6ß,16ß-trihydroxylup-20(29)-ene] isolated from fruits of *Combretum leprosum*, whose the pharmacological properties have been shown a promising potential for antinociceptive, anti-inflammatory, antiulcerogenic and anti-leishmanicidal activities. The aim of the present study was to evaluate the efficacy of lupane encapsulated in liposomes in experimental model of cutaneous leishmaniasis caused by *Leishmania amazonensis* in vivo. Liposomes were prepared with DPPC, DPPS and cholesterol at 5:1:4 weight ratio. The lupane (2mg/ml) was added to the lipid mixture, solubilized in chloroform and dried under nitrogen flow. The lipid vesicles were formed homogeneously by the extrusion method. Mice were infected in the right hind footpad with 10^5 stationary growth phase *L. amazonensis* promastigotes. After six weeks, the animals were treated by intraperitoneal injection with lupane encapsulated in liposome for 15 days. The evolution of disease was monitored weekly by measuring footpad thickness with a caliper. The lesion size of mice treated with liposomal lupane showed a reduction of 20% compared with the untreated control group. Three days after the treatment, the peritoneal macrophages of the mice were collected, plated and the production of IL-6, IL-10 and IL-12 was evaluated in supernatants of the cultures after 24h. It was observed a significant reduction in the production of IL-10 by liposomal lupane treated macrophages when compared with untreated control or liposome-treated. And also, there was an increase in the production of IL-6 by liposomal lupane treated macrophages. These results indicate that the liposomal lupane could be involved in the resolution of injuries caused by *L. amazonensis* infection in vivo and we are trying to manipulate liposomal formulation to increase drug effectiveness.
Supported by:CNPq

QT065 - **ASSESSMENT OF LEISHMANICIDAL ACTIVITY IN SYNTHETIC LIPOPHILIC DIAMINES**

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Diamines are amino-hydrocarbons that interfere in polyamines biosynthesis and present several biological effects including anti-parasitic activity. Amine compounds are inhibitors of trypanothione reductase (TR), a key enzyme for the redox balance in trypanosomatids. In this study we evaluate the leishmanicidal activity *in vitro* of 17 synthetic lipophilic diamines. In a preliminary screening for anti-parasitic compounds on promastigotes of *Leishmania braziliensis* (H3 strain), 14 out of 17 compounds tested showed IC50 <20µM and were selected for intracellular activity tests against *Leishmania* amastigotes. The intracellular activity assay was performed in murine bone marrow macrophages. Cells were cultured in 96 well plates and infected with axenic amastigotes (m.o.i. of 10) and incubated at 34°C for 48h in the presence of different concentrations of each compound. Amphotericin B (0.1 µM) and 1% DMSO were used as controls. Leishmanicidal activity was evaluated by determining the percentage of infected cells and the number of intracellular amastigotes in Giemsa stained cell monolayer, experiments were done in triplicates counting 100 cells per assay. Cytotoxicity of the compounds was analyzed by MTT. Recombinant T. cruzi trypanothione reductase (TcTR) inhibition assays were evaluated in 96 well plates and clomipramine was used as standard inhibitor. The IC50 leishmanicidal activity of the compounds varied from 1.68 µM to 23.04 µM and the cytotoxicity varied from 217.6 µM to 312.4 µM. Compounds DP22 and DP22Cl showed the highest selectivity index,131.5 and 141.5, respectively. The compounds DP25Cl and CR40Cl which at (100 µM) inhibited 75% and 44% of the TcTR activity, respectively, showed IC50s of 2.97 µM and 5.59 µM against *Leishmania* parasites and revealed a selective index of 58 and 26.75. These results suggest that lipophilic diamines may be promising molecules for new anti-leishmanial drugs. Supported by:CAPES e CNPq

QT066 - **MORPHOLOGICAL ALTERATIONS OF LEISHMANIA (L.) AMAZONENSIS PROMASTIGOTES TREATED WITH AQUEOUS EXTRACT OF PHYSALIS ANGULATA**

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Leishmaniasis constitutes a complex group of infective parasitic diseases 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. Various plants and plant products have been used as traditional medicine for thousands of years. Physalis angulata is an annual herb distributed in tropical and subtropical regions of the world. Extracts from this plant is widely used in popular medicine as analgesic, antirheumatic, antinociceptive and anti-inflammatory. Thus, we considered interesting to analyze the morphological alterations of promastigote forms of L. amazonensis after treatment with the aqueous extract of Physalis angulata. The extract inhibited 83.5 % and 100% the promastigotes growth in the concentrations of 50 and 100 µg/mL, respectively. We observed by light microscopy that promastigote forms treated with 50 µg/mL of extract showed significant morphological changes characterized by unusual round-shaped, two flagella and reduction of cell body when compared with untreated parasites. In promastigotes treated with 100 µg/mL of the extract were observed a significant reduction in cell number, presence of membranous fragments and reduced cell volume. In addition, ultrastructural observation by scanning electron microscopy confirmed alterations in flagellum and the cell body of the parasite. On promastigotes treated with 50 µg/mL of the extract showed flagellum duplication, multi-septation of the cell body, suggesting interference in the process of cell division. Parasites treated with 100 µg/mL showed cells with rounded appearance, short flagellum and multi-septation the cell body when compared to untreated promastigotes. These results demonstrated that P. angulata aqueous extract promotes morphological parasite alterations. The extract could be useful as alternative source for a new antileishmanial agent. Supported by:CAPES, CNPq/UFPa, CNPq/MCT/CT-INFRA/CT-PETRO (Processo nº 620179/2008), MCT/CNPq/FNDCT/PROCAD-NF CAP

QT067 - **CHARACTERIZATION OF GAMMA-GLUTAMYLCYSTEINE SYNTHETASE AND ORNITHINE DECARBOXYLASE IN FOUR NEW WORLD LEISHMANIA SPECIES SUSCEPTIBLE AND RESISTANT TO ANTIMONY**

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The enzymes gamma-glutamylcysteine synthetase (GCS) and ornithine decarboxylase (ODC) are involved in glutathione and polyamine biosynthesis, the two building blocks of the main cellular thiol trypanothione. In this study, susceptible and resistant populations from four different species of *Leishmania*, *L. guyanensis*, *L. amazonensis*, *L. braziliensis* and *L. infantum chagasi* were analyzed for: levels of mRNA *gsh* and *odc* genes and ODC protein expression levels. These populations were selected “in vitro” to SbIII and the resistance index varied from 4 to 20-fold higher than of their wild-type counterparts (Liarte & Murta, 2010). The levels of *gsh* and *odc* mRNA in these *Leishmania* populations were determined by quantitative real-time PCR. The levels of transcription of the *gsh* gene were 7- and 20-fold higher in the resistant populations *L. guyanensis* and *L. amazonensis* than in theirs susceptible counterparts LgWTS and LaWTS, respectively. No differences in the levels of transcription of that gene were detected between the populations from other species *L. braziliensis* and *L. infantum chagasi*. On the other hand, the levels of transcription of the *odc* gene were 5-fold higher in the Sb-resistant *L. amazonensis* LaSbR population than in the susceptible parental population LaWTS. In the Western blot analysis, anti-LmODC polyclonal antisera from *L. donovani* recognised a 83 kDa protein in all *Leishmania* species analyzed. The level of expression of this polypeptide was approximately 2 and 3-fold higher in the Sb-resistant *L. amazonensis* and *L. guyanensis* populations compared with theirs susceptible counterparts LaWTS and LgWTS, respectively. In contrast, we detected no differences in ODC protein expression levels between other *Leishmania* species investigated. Overexpression of these genes in the susceptible parasites will be performed in order to better investigate the role of the GSH and ODC enzymes in the drug-resistance phenotype in these New World *Leishmania* species. Supported by:CNPq, CAPES, FAPEMIG, PIDTIS/FIOCRUZ, CPqRR and UNICEF/UNDP/World Bank/WHO/TDR

QT068 - **PHARMACOKINETICS OF ANTIMONY IN PEGLATED AND CONVENTIONAL LIPOSOMES: POSSIBILITIES TO IMPROVE THE TREATMENT OF VISCERAL LEISHMANIASIS**

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Much effort has been devoted to the search for treatment of visceral leishmaniasis (VL) in dogs. Reduction of liposome diameter from 1200 nm to 400 nm improved the targeting of antimony (Sb) to bone marrow of infected dogs. Some studies have shown the coupling a peglated phospholipid (PEG-L) to the liposome membrane results in an increase in blood circulation that should be interesting to the treatment of VL. In the present work, we investigated the influence of reduction in mean vesicle diameter from 400 to 200nm and the influence of a PEG-L in the liposome membrane on the pharmacokinetics of liposome-encapsulated Sb in dogs with VL. Four different liposomes were prepared: two conventional liposome (LC) formulations differing in their mean vesicle size and two peglated liposomes (LP) differing in the amount of PEG-L. LC1 was prepared in the presence of sucrose as cryoprotectant (mean vesicle hydrodynamic diameter (R) of 410 nm and drug encapsulation efficiency (EE) of 40%). LC2 was obtained by extrusion of the liposome through 200nm-pore membrane (R=175nm, EE=34%). LP1 were prepared with 3% of PEG-L (R=189nm, EE=26%) and LP2 5% of PEG-L (R=154nm, EE=16%). The formulations were applied as intravenous bolus injection at 4.2mgSb/Kg (LC1), 6.5mgSb/kg (LC2), 5.6mgSb/kg (LP1), 3.7mgSb/kg (LP2), plasma pharmacokinetics were evaluated and Sb levels were determined in the liver and spleen. size reduction and presence of PEG-L increased blood circulation of the vesicles (LC1 24min, LC2 87min, LP1 147min, LC2 189min). Biggest liposomes (LC1) shows a higher accumulation of Sb in the liver, but similar level of Sb was found in spleen. This results may be due saturation of the mononuclear phagocyte system took place when the liposome size is decreased. In conclusion, our data indicates that presence of PEG in liposome membrane increases blood circulation of the vesicles but in the other hand, medium size liposomes are more effective to increase the liver accumulation of Sb.Supported by:CNPq, Fapemig.

QT069 - **CARBOLINE C4 COMPOUND, INDUCES MITOCHONDRIAL MEMBRANE DEPOLARIZATION IN TRYPANOSOMA CRUZI**

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Chagas'disease is an illness without satisfactory treatment until the moment, and there is around 15 million people infected in the Latin America (WHO, 2007). In a previous work carboline compounds displayed a strong activity against all evolutive forms of Trypanosoma cruzi (Valdez et al., 2009). The aim of this work was investigate the possible mechanism of action of carboline C4 compound over epimastigote and trypomastigote forms cultured in vitro, through the study of mitochondrion function and membrane plasma integrity.
The parasites were pre-treated with C4 in the following concentrations; epimastigote (7.0 µg/mL and 30 µg/mL) for 96 h and trypomastigote (45 µg/mL and 90 µg/mL) for 2 h and analyzed by flow cytometry (FACS Calibur®, Becton Dickinson) using rhodamine 123 and propidium iodide (PI). Results: The result obtained in epimastigote and trypomastigote forms stained with rhodamine 123 showed a depolarization of mitochondrial membrane in both concentrations tested, nevertheless over these same parasite forms stained with propidium iodide no alterations were observed, indicating that the cell membrane integrity were not damage.
Taking into account the results obtained, is possible to conclude that the trypanocidal action could be related to mitochondrial dysfunction, leading in consequence of this the cell death. However additional studies to complete elucidate the mechanism of action of carboline C4 over all evolutive forms of T. cruzi are necessary.
Supported by:CNPq, FINEP, Fundação Araucária

QT070 - **IN VITRO ACTIVITY OF EUGENIA CARYOPHYLLATA ESSENTIAL OIL AND ITS MAJOR COMPONENT (EUGENOL) ON LEISHMANIA MAJOR PROMASTIGATES**

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Cutaneous leishmaniasis is caused by different species of *Leishmania*, which occur in 88 countries, it is transmitted to human by Phlebotomine. Although various anti-parasitic drugs are available, the incidence rate has not decreased and Leishmania has been acquiring a high level of resistance against the usually prescribed drugs, making the research of alternative treatment methods necessary. Oil from *Eugenia caryophyllata* have antibacterial, anti-fungal, anti-tumor, analgesic effect and anti-parasitic properties, its major components are eugenol, β-caryophyllene, α-humulene and carvacrol. This investigation has studied the leishmanicidal effect of whole essential oil of *E. caryophyllata* and eugenol on *Leishmania major* promastigotes. The essential oil was extracted from dried bulbs obtained from the local market by hydrodistillation. Promastigotes of *L. major* (strain LV39) were maintained in M199 medium with 4 mg/mL of hemin and 10% FBS plus 1% Penicilin/Streptomicin at 26 °C. Promastigotes of *L. major* were incubated with 10, 20, 40, 80 and 160 µg/mL of essential oil or eugenol. After 72 hours, the promastigotes were washed in M199 and an MTT assay was performed to measure the viability of parasites. The assays were performed in triplicate. The viability assay showed that eugenol have an increasing toxicity effect from 40 to 160 µg/mL, corresponding to 7.9% to 13.6% of inhibition. However the inhibitory effect was not significant when compared to control. The essential oil tested had no significant effect on the viability. Despite these results, essential oil and its major constituents from other plants such as *Piper auritum*, *P. caribaea* and *Chenopodium ambrosioides* were reported as a leishmanicidal, however in a higher concentration than used in this study. Further experiments, with higher concentration of eugenol and essential oil of *E. caryophyllata*, should be done to determine its leishmanicidal properties.
Supported by:Fundação Valeparaibana de Ensino - FVE

QT071 - **EFFECT OF SYNTHETIC HYDROXAMIC ACID DERIVATIVES ON LEISHMANIA SPP.**

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The leishmaniases are parasitic diseases caused by protozoa of the genus Leishmania and can take two distinct forms, a cutaneous that can range from a single lesion to disfiguring lesions and visceral form that affects the liver, spleen and bone marrow compromise their functions.The treatment is based on pentavalent antimonials as first choice and amphotericin B as second choice, with few options for replacement in case of treatment failure, further these drugs are very toxic to host and the parasite resistance emerges as a concern. These are parasites possess mechanisms to invade and remain in the host, among them we can mention the proteolytic enzymes in particular the gp63 a metalloproteinase. The hydroxamic acids have the ability to efficiently form a complex with zinc metalloprotease catalytic site, being used in the design of inhibitors for this enzymatic class. So this work aimed to evaluate “in vitro” the activity of five protease inhibitors derived from hydroxamic acid called by codenames G3C, G3B, G3F G10 and G12 on L. chagasi and L. amazonensis promastigotes and its cytotoxicity to two cell lines. Furthermore, we are evaluating the effect of these drugs in the proteolytic activity of promastigotes grown in the presence of these drugs. The preliminary results show the IC50 values in micrograms per milliliter for L. chagasi ranged from 41.8 to 177 and L. amazonensis between 56.4 to 129.3 and citotoxicity concentration (CC50) values for Raw cells ranged between 36.5 and 75 and L929 cells between 29.1 and 140.2. Then these protease inhibitors may be an option for further study in search of new drugs for the treatment of leishmaniasis.
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QT072 - **THE ANTILEISHMANIAL ACTIVITY OF NEW SEMISYNTHETIC NAPHTHOTHIAZOLS**

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Leishmaniasis is a spectral disease caused by parasites belonging to Leishmania genus, affects millions of people worldwide. Current treatments are unsatisfactory, and in absence of a vaccine, there is an urgent need for effective drugs to replace/supplement those currently in use. The screening for compounds able to inhibit essential biochemical pathways for the parasite survival is a strategy for drug discovery. The enzyme dihydroorotate dehydrogenase (DHOD) catalyzes the oxidation of L-dihydroorotate to orotate, the fourth sequential step in the de novo pyrimidine nucleotide synthesis pathway. Besides, it plays important roles in fumarate metabolism and uridine monophosphate (UMP) biosynthetic pathway. The high druggability index (0.9 in a scale 0 to 1, TDRtargets) and the promising results suggesting DHOD inhibitors as anti-malaric candidates were our motivation to test this enzyme as a potential drug target of new anti-leishmanial drugs. In this scenario, semi-synthetic heterocycles derived from naturally occurring naphthoquinones arises as bioactive compounds potentially useful to inhibit this enzyme. Herein we describe the activity of nine semi-synthetic naphthothiazoles (SSN) derived from 1,4-naphthoquinones against Leishmania and their cytotoxicity against mammalian cells. All synthesized compounds showed a potent activity against the promastigote form of L. braziliensis, L. chagasi, L. major and L. donovani with DL50 ranging from 0.1 to 4.6 µM. For the L. braziliensis amastigotes, all tested compounds reduced by 75% the survival index of parasites in macrophages (6µM). The compounds showed no cytotoxic effects against macrophages when tested in a concentration at least five times the DL50 determined for promastigotes. The results of this study provide new perspectives for development of novel lead compounds with leishmanicidal activity obtained from SSN derived from natural sources of naphthoquinones. The potent and selective activity of SSN against amastigotes in in vitro infection encourages the development of pharmaceutical formulations for further studies in vivo.
Supported by:Fapesp e CNPQ

QT073 - **A CHAGAS' DISEASE TECHNOLOGICAL PLATFORM. IN VITRO AND IN VIVO ANTI- TRYPANOSOMA CRUZI ACTIVITY OF NATURAL PRODUCTS, COMPOUNDS AND DRUGS**

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The two major challenges for drug discovery and development to Chagas's disease are the lack of appropriate *in vitro* and *in vivo* screening protocols and the little interest of the Big Pharmas. The Program of Technological Development for Health (PDTIS) of the Oswaldo Cruz Foundation (FIOCRUZ) has stimulated the building and consolidation of Technological Platforms (TP) in order to improve research and development of health products with quality. Technological Platform (TP) for anti-*T. cruzi* drugs (PlaBio Tc) was created based in the steps, requirements and decision gates for the determination of the efficacy of novel drugs for *T. cruzi* proposed by Romanha *et al.* (2010). In two years of intensive work the PlaBio Tc investigated the trypanocidal activity of the approximately 11,000 samples of natural products, compounds and drugs from nine different providers. In collaboration with the Bioprospecting TP – FIOCRUZ, 10,000 plant and fungi extracts were tested. *In vitro* bioactivity ≥ Benznidazole (BZ) was observed in 20 extracts (0.2 %). Their IC_{50} varied from 0.5 to 15.0 µg/mL. The nine extracts moderately and highly selective (Selectivity Index ≥ 5) were fractionated to identify the active compounds. Furthermore, out of 477 compounds tested, two showed activity similar to BZ and three were 10 times more active, nevertheless cytotoxic. The most active compound was submitted to *in vivo* tests. Unfortunately, the treatment of *T. cruzi* infected mice anticipated death when compared with the experimental groups not treated and treated with BZ, suggesting the necessity of molecular derivatization studies. Altogether the results are encouraging to explore natural products and drugs for treatment of Chagas' disease.

Supported by:PDTIS/FIOCRUZ, FAPEMIG and CNPq

QT074 - **COMPARISON OF DIFFERENT METHODOLOGIES TO EVALUATE DRUG SENSITIVITY IN VITRO IN PLASMODIUM FALCIPARUM BLOOD FORMS**

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Over 60 Brazilian plant species used in traditional medicine to treat fever and/or malaria have been screened at our laboratory against *Plasmodium falciparum* blood cultures, using different tests to evaluate *in vitro* drug sensitivity. We now analyze the levels of activities of crude extracts and fractions from *Aspidosperma* sp (Aristolochiaceae) plants, phytochemicallly characterized, and tested *in vitro* against chloroquine-resistant *P. falciparum* (clone W2). The IC_{50} values (inhibition of 50% parasite growth) were obtained in three different assays: (i) light microscopy; (ii) incorporation of tritiated hypoxanthine by living parasites, and (iii) immunoenzymatic assay anti-HRP2, through dose-response curves. At least two experiments were performed per test, except in the traditional one, performed once or twice. Among 31 compounds tested, ten (32%) were active (IC_{50} <10.0 µg/mL), one (3%) partially active (IC_{50} 10-15.0 µg/mL) and 19 (62%) inactive (IC_{50} >15.0 µg/mL); one (3%) compound showed discrepant IC_{50} values, being either partially active or inactive. We conclude that results were similar in the tests, however, the anti-HRP2 assay data, besides showing reproducible results and less hazardous (compared to the tritiated hypoxanthine assay that involves radioactivity and solid residues to be stored for decades), require less equipment and of lower coast. When compared to traditional microscopy, the anti-HRP2 and the hypoxanthine tests were better, both semi-automated and more precise. In addition, microscopy is more laborious, thus time consuming, requiring individual expertise for parasitaemia determination. Nevertheless, microscopy remains a good choice for anti-*P. falciparum* drug screening if there no other alternative is available.

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QT075 - **EFFECT OF SYNTHETIC QUINONES IN LEISHMANIA CHAGASI AND HERPETOMONAS SAMUELPESSOAI**

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Visceral leishmaniasis is a chronic and neglected disease, caused by protozoa from the genus Leishmania, mainly L. donovani, L. chagasi and L. infantum. This disease affects organs like liver, spleen and bone marrow leading to duty loss. Currently, the available treatments are based on pentavalent antimonials, pentamidine and amphotericin B, as well as miltefosine. Due to the high toxicity of these drugs and the appearance of parasite resistance, treatment failure is often seen, pointing to the need for researches aiming the discovery of new compounds that may be more efficient and less harmful to the host. Herpetomonas are species of monoxenic trypanosomatids frequently used as a model for the study of physiological, biochemical and structural aspects of the Trypanosomatidae family. Despite of its lack of pathogenicity, Herpetomona infection has been suggested to occur as a diffuse cutaneous leishmaniasis in immunocompromised patients. Quinones are oxygenates compounds with a nucleus with two carbonyl interspersed double bonds. More than 1,500 compounds belong to this group and some have demonstrated an effective antimicrobial activity. Therefore, this study aimed to evaluate the effect of two synthetic quinones named ß-norlapachone and OH β-lapachone against L. chagasi and Herpetomonas samuelpessoai. Cytotoxicity assay was performed in mice macrophages and fibroblasts cell lines. For the minimum inhibitory concentration (MIC), promastigotes were incubated in the presence of serial concentrations of the compounds ranging from 0.5 mg/ml to 0.001 mg/ ml. The citotoxicity assay revealed a 50% cytotoxic concentration (CC50) for ß-norlapachone and OH β-lapachone of 5.0 mg / ml and 3.1 mg/ ml for macrophages, respectively; and 4.7 mg / ml and 3.1 mg / ml for fibroblasts, respectively. The MIC, expressed in micrograms per milliliter, for L. chagasi were 26.9 and 55.7; and for H. samuelpessoai 13.5 and 235.7, respectively. Further studies are needed in order to turn the use of these compounds in the treatment of Leishmania infections possible. However the results presented here suggest that quinones may represent an interesting alternative in the development of new therapeutic options for the treatment of leishmaniasis.
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QT076 - **THIAZOLIDINONES, NEW PRIMAQUINE DERIVATIVES WITH LOW TOXICITY, INHIBIT THE SPOROGONY OF MALARIA PARASITE IN MOSQUITOS**

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Primaquine (PQ) is the only drug used in Brazil to prevent P. vivax relapses caused by the hypnozoites. However, PQ is toxic and its metabolites cause severe hemolysis in G6PD deficient patients. In attempts to find PQ substitutes we have used an indirect model to test thiazolidinones (THZ), based on the inhibition of P. gallinaceum (Pg) sporogonic development in A. fluvialitis mosquitoes. Although another test detects directly anti-hypnozoite activity in vitro (Dembele et al, 2011 PlosOne), it requires P. cynomolgi sporozoites, a complex and more expensive simian malaria model, unavailable for us. Eight THZ derived from PQ were tested in mosquitoes fed on chicks with a Pg rising parasitemia (<10%). After the mosquitoes infective blood meal (Time 0h) the blood donor chickens were treated with a single oral dose of the test substance (100mg/Kg), and used again 6h later (time 6h) for mosquito blood feeding. PQ was similarly tested as a positive control. All fed mosquitoes were maintained at 27°C for one week under a sugar diet, then, their midguts were dissected (20/group) and the oocysts counted at the optical microscope. The number of oocysts comparing time 0h with T6, for each compounds showed that THZ N14A and N17B were active inhibiting 90% to 100% sporogony, respectively; P92A and N87B inhibited 75-80% but four other THZ were inactive; PQ completely inhibited sporogony at 15mg/Kg, being the most active drug. But PQ was cytotoxic in vitro against a human hepatoma cell line (HepG2), minimum lethal doses killing 50% of the cells (MLD50) of 116ug/mL whereas N14A and N17B were not toxic (MLD50 >1000ug/mL). The new compounds seem rather promising as a PQ substitute, requiring further assays to explore better their possible anti-hypnozoite activity. In mice infected with P. berghei sporozoites part of these parasites develop in the mouse skin but PQ kills these forms. This protocol should clarify better our data now described, and hopefully be used for THZ studies.
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QT077 - **INHIBITION OF THE HEMOZOIN FORMATION BY CHLOROQUINE-ANALOGUES**

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The high resistance of *Plasmodium falciparum* to most traditional antimalarials, especially to chloroquine and other aminoquinolines, and their worldwide spread, make the search of new drugs urgent. The possible mechanism of action of some new aminoquinoline drugs has been undertaken in the present work. We studied the ability of seven chloroquine-analogues (DMA, DETA) complex or not with metals, to inhibit the hemozoin formation *in vitro*. The inhibition of hemozoin formation *in vitro*, was based in the measurement of free heme after incubation of the compounds with dimeric hematin (synthetic hemozoin). All the compounds inhibited significantly the hemozoin formation *in vitro*, in a dose-response manner. DETA and DMA, alone or complex with metals (Fe and Pt), inhibited the hemozoine formation at lower doses than CQ. Next, we performed docking studies with DETA and DMA, and found that the MolDock Score energies among the chloroquine analogues and the dimeric hematin (synthetic hemozoin) were around –100.00 kcal mol-1. In conclusion, all the aminoquinoline derivatives tested, like chloroquine, are able to interact with dimeric hematin to form a complex, with consequent inhibition of hemozoin formation. All compounds tested are active against *P. falciparum* infected erythrocytes in cultures and P. berghei in mice (Aguiar et al, 2011).These molecules seem promising alternatives for the treatment of human malaria caused by chloroquine-resistant malaria parasites, since they act on a crucial point of the parasite life cycle. Whether they will protect against cerebral malaria and other complications is now being studied in mice infected with severe malaria.

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QT078 - **ANTIMALARIAL ACTIVITY OF POSACONAZOLE, AN INHIBITOR OF PLASMODIUM FALCIPARUM LACTATE DEHYDROGENASE ENZYME, SELECTED BY DOCKING STUDIES**

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The specific drug treatment for malaria remains a major strategy to reduce disease morbidity and mortality. However, human treatment is currently limited by the selection and spread of drug resistant parasites to most available antimalarial drugs, a major obstacle in effective disease control. The process of discovering and developing new drugs is complex and requires great investments, especially in the phase of clinical trials. We have demonstrated the anti-*Plasmodium falciparum* (*Pf*) activity of Posaconazole, a compound used in the prophylaxis and treatment of an extended spectrum of pathogens, commercially available as Noxafil® (Schering-Plough) in U.S. drugstores, as an oral suspension. The compound has been pre-selected for our antimalarial tests by means of molecular modeling computational studies against the parasite enzyme lactate dehydrogenase (*Pf*LDH) (in press, PloS ONE). The *in vitro* incubation of *P. falciparum* culture blood forms with Posaconazole resulted in a significant inhibition of parasite growth, as assessed in immunoenzymatic assay anti-*Pf*LDH (IC_{50} = 7 ug/mL). The drug was also active in *P. berghei* infected mice (71% reduction of parasitemia in relation to non-treated controls). Posaconazole, which represents the latest drug developed among the antifungal triazole derivatives, has good tolerability and, when evaluated in clinical human trials, provided important bioavailability. The drug appears to be safe and well tolerated and should be tested against human malaria in endemic areas of transmission, either alone or in combination with standard antimalarial drugs. Our goal is to reduce drug-resistance and to introduce an effective therapy against human malaria, including *P. vivax* parasites, which seems to become also multidrug-resistant in the recent years, including in Brazil.

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QT079 - **EVALUATION OF KIDNEY AND LIVER CYTOTOXICITY IN DOGS WITH VISCERAL LEISHMANIASIS SUBJECT TO TREATMENT WITH AN ANTI-LEISHMANIASIS EXPERIMENTAL DRUG – ORGANOTELLURIUM COMPOUND (RF07) FROM AN ENDEMIC AREA, TERESINA-PI.**

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In Brazil, Visceral Leishmaniasis (VL) is caused by the protozoan *Leishmania (Leishmania) chagasi*, transmitted by *Lutzomyia longipalpis*. The protozoan has a tropism for the mononuclear phagocytic system and it infects liver, spleen and bone marrow macrophages. The infection affects men and dogs and it is characterized by severe hepatosplenomegaly, weight loss and pancitopenia. The treatment of VL has been prescribed in humans, but it is not effective against canine leishmaniasis. The aims of this study were to analyze the Leishmanicide activity of an experimental organotellurane (RF07) and its kidney and liver cytotoxicity in naturally infected dogs from an endemic area (Teresina-Piauí-Brazil). Sixteen dogs were selected and divided in four groups: Group 1 (N=4) consisted of healthy dogs that received 1 mL/day of intraperitoneal saline for 3 weeks, Group 2 (N=4) was formed by healthy dogs that received 0.5 μg/kg/day intra-peritoneal RF07 for 3 weeks, Group 3 (N=4) consisted of dogs with VL that received 1 mL/day of intraperitoneal saline for 3 weeks and Group 4 (N=4) consisted of dogs with VL that received 0.5μg/kg/day intra-peritoneal RF07 for 3 weeks. After treatment, the animals of Groups 1 and 2 showed no changes in liver and kidney function, which was demonstrated by biochemical parameters (TGO, TGP, alkaline phosphatase, urea and creatinine) and the biopsies of liver and kidney. The animals of Group 3 development symptoms to VL changing the hematological parameters (anemia, leukopenia, thrombocytopenia and decreased hematocrit), a progressive increase of TGO, TGP, alkaline phosphatase, urea and creatinine, and proliferative glomerulonephritis with severe liver fatty metamorphosis. The animals of Group 4 presented diminution for the symptomatology and improvement of renal and liver function, with drastic reduction in the TGO, TGP, alkaline phosphatase and urea. For this last group, there was an increase in the hematologic ranges with an increase of the hematocrit, red blood cells and platelets. Proliferative glomerulonephritis was mild in 3 out of 4 dogs and liver fatty metamorphosis was reduced. The parasitologic exam (myelogram) was negative for one of the four dogs after 3 weeks of treatment. Our findings suggest that the RF07 has the potential for the treatment of canine leishmaniasis.
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