

**QT001 - MEMANTINE, AN ANTAGONIST OF NMDA GLUTAMATE RECEPTOR, AFFECTS CELL PROLIFERATION, DIFFERENTIATION, INTRACELLULAR CYCLE AND CAUSES AN APOPTOSIS-LIKE DEATH IN *TRYPANOSOMA CRUZI*.**

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Chagas disease, caused by the protozoan parasite *Trypanosoma cruzi*, affects approximately 10 million people in endemic areas of Mexico, Central and South America. The currently available chemotherapy is limited to two compounds: Nifurtimox and Benznidazole. Both drugs reduce the symptoms of the disease and the mortality of infected people when used in the acute phase, but its efficacy in the chronic phase (phase in which the majority of cases are diagnosed) is still controversial. Moreover, these drugs have several side effects. *T. cruzi* is able to utilize carbohydrate and amino acids as carbon and energy source. Our group has studied the involvement of glutamate in parasite resistance to thermal, oxidative and metabolic stress. The aim of this study was to evaluate the interference of Memantine (MM), an antagonist of the glutamate receptor in the NCS in mammals, in the life cycle of *T. cruzi*. MM had a trypanocidal effect, inhibiting the proliferation of epimastigotes (IC<sub>50</sub> 172,6 µM). Besides, it interfered with metacyclogenesis (reduced around 30%), and affected the energy metabolism of the parasite decreasing the levels of ATP. Also, MM triggered mechanisms that led to an apoptosis-like cell death of epimastigotes: exposure of extracellular phosphatidylserine, increase of the production of reactive oxygen species, decrease of ATP levels, increase of intracellular Ca<sup>2+</sup> and morphological changes. Moreover, MM interfered with the intracellular cycle of the parasite infection, specifically with the amastigote stage (IC<sub>50</sub> 31µM). Interestingly, the stages of the parasite life cycle that require more energy (epimastigote and amastigote) are more affected, as well as the processes of differentiation and cell invasion. In spite of the fact that the obtained IC<sub>50</sub> for MM is higher than that described for Benznidazole, MM presented interesting characteristics to be used as leader compound for the design of drugs with optimized trypanocidal activity. **Supported by:**FAPESP, CNPq and USP

**QT002 - METALOCOMPLEXE FEHP(SO<sub>4</sub>) REDUCES THE DEVELOPMENT OF *TOXOPLASMA GONDII* IN LLC-MK2 CELL LINE**

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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. 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, drugs known as metalocomplexes have shown several interesting biological activities. A new l-oxo di-iron complex is active against DNA of target cell, mimicking a nuclease and metallohydrolase enzymes. These new metalocomplexes also display antibacterial activity against *Staphylococcus aureus*. The metalocomplex tested in this work came from the reaction of FeSO<sub>4</sub>·7H<sub>2</sub>O with the ligand HPCINOL (1-(bis-pyridin-2-ylmethyl-amino)-3-chloropropan-2-ol) resulting in the following complex [Fe(HPCINOL)(SO<sub>4</sub>)<sub>2</sub>·l-oxo<sub>6</sub>H<sub>2</sub>O]·1. Here, we report the outcomes of the cytotoxicity test with this metalocomplex during the interaction of *T. gondii* tachyzoites with LLC-MK2 cells. The drug did not arrest host cell growth, but was able to decrease the infection index of *T. gondii* with the IC<sub>50</sub> in the micromolar range by 48 hours of infection. Transmission electron microscopy showed damage to the endoplasmic reticulum and mitochondrion of the parasite and the appearance of inclusions at the cytoplasm similar to amylopectin granules, typically found in bradyzoites. The presence of cysts of bradyzoites was confirmed after 6 days of treatment by scanning electron microscopy and staining with *Dolichos biflorus* lectin, specific to bradyzoites cyst wall. We are investigating whether these effects involve the generation of oxidative stress caused by the treatment with the metalocomplex. These results suggest that FeHP(SO<sub>4</sub>) is a compound that induces encystment and kills intracellular tachyzoites of *T. gondii*. **Supported by:**CAPES, CNPq, Faperj

**QT003 - MELATONIN, THE HORMONE OF DARKNESS, MODULATES *LEISHMANIA (L.) AMAZONENSIS* INFECTION**

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In addition to the classical circadian role, melatonin has other biological functions and is produced by other tissues besides pineal gland. Among these is the anti-inflammatory role, acting directly as an antioxidant, or modulating the expression of genes involved in the mounting of an inflammatory response. Recent studies demonstrated that during *E. coli* or zymosan stimulus, the pineal gland stops melatonin synthesis, allowing neutrophils and leukocytes transmigration this way favoring inflammatory response. Besides, the activated immune competent cells produce melatonin at high concentrations, also contributing for inflammation resolution. This shuttle of melatonin synthesis site was named Immune-Pineal Axis (IPA). Here, we evaluated melatonin role and IPA in the context of *L. amazonensis* infection. Syrian hamsters infected with  $10^5$  *L. amazonensis* in the footpad revealed that infection progression depends on the daytime of inoculation. The lesion observed 8 days after parasite inoculation at nighttime was 1/3 of the lesion produced by inoculation at daytime. At 15<sup>th</sup> and 22<sup>th</sup> day this difference diminished to approximately 2/3. Notably, hamsters treatment with luzindole, an antagonist of melatonin receptors, reversed the attenuation of *Leishmania* infection progress after parasite inoculation at nighttime, indicating the existence of a mechanism mediated by melatonin through its receptors. Pre-treatment of BALB/c peritoneal macrophages in culture with melatonin (3 nM, 1 hour) significantly reduced the number of infected macrophages and the amount of amastigotes per infected macrophage, corroborating the melatonin role on *Leishmania* infection. We also verified that *Leishmania* does not affect melatonin production by pineal gland, in contrast to what was shown during a resolvable bacterial infection. Altogether, we showed that the IPA is another host mechanism subverted by *Leishmania* improving its survival and melatonin is able to attenuate *Leishmania* infectivity. **Supported by:**Fapesp, CNPq

**QT004 - LEISHMANICIDAL ACTIVITY OF HYDROXYETHYLPIPERAZINES AND HYDROXYETHYLAMINES USED AS PRECURSORS IN SYNTHESIS OF HIV PROTEASE INHIBITORS**

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In spite of the advances in last decade, control of leishmaniasis remains in the order of day and the search for new treatment alternatives is an actual priority. Although pentavalent antimonials have saved thousands of lives of *Leishmania*-infected patients in the last 70 years, they were developed in an empirical basis and their toxicological profiles are unacceptable nowadays. In this treatment, poor therapeutic responses and adverse effects are common. Several studies have recently demonstrated the leishmanicidal activity of HIV protease inhibitors. This study aims to evaluate the leishmanicidal activity of news hydroxyethylpiperazines and hydroxyethylamines, used as precursors in the synthesis of HIV protease inhibitors. *L. amazonensis* promastigotes were cultured in the presence of the compounds, in concentrations up to 200  $\mu$ M for 72 hours and quantified colorimetrically by MTT assay. Best compounds showed an IC<sub>50</sub> of approximately 4  $\mu$ g/mL. The activity of the compounds was also evaluated in intracellular amastigotes. The infectivity index was determined by light microscopy. Treated macrophages showed a significant reduction in the levels of infection when compared to the control group. Best compounds showed an IC<sub>50</sub> of approximately 1  $\mu$ g/mL. To evaluate the toxicity, murine macrophages were incubated with the compounds for 72 hours. The effect on the viability of the macrophages was quantified by MTT and the LD<sub>50</sub> values were higher than 15  $\mu$ g/mL, indicating a selectivity index more than 10-fold. Our results indicate that these precursors of the synthesis of HIV protease inhibitors are promising prototypes for the treatment of leishmaniasis. Furthermore, leishmaniasis is considered an opportunistic disease in patients infected with HIV-1. In this context, although we have only demonstrated in this work the antileishmanial activity, these compounds may add new insights in the study of the effect of HIV protease inhibitors in coinfecting patients. **Supported by:**CNPQ/PAPES, CAPES

**QT005 - ANTILEISHMANIAL EFFECTS OF 2'-OH FLAVANONE AGAINST POTASSIUM ANTIMONY TATRATE - RESISTANT AND SENSITIVE PROMASTIGOTES AND AMASTIGOTES FORMS OF L. AMAZONENSIS**

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2'-OH flavanone (2'-OH) belongs to a class of flavonoides, the flavanones, which are known for their antitumor and anti-inflammatory properties. In this study, we evaluated the effect of 2'-OH against *L. amazonensis* proliferation in potassium antimony tartrate-sensitive promastigotes, potassium antimony tartrate-resistant promastigote and intracellular amastigotes. Promastigotes were treated with different concentrations of 2'-OH (3-96µM) for 24h, demonstrating an inhibition in a dose-dependent manner, with an IC<sub>50</sub> of 20.96 µM. To investigate mitochondrial damage, promastigotes were treated with 2'-OH (96µM) for 24h, incubated with JC-1 and we observed a marked decrease in mitochondrial membrane potential. It is known that mitochondrial damage is able to increase reactive oxygen species (ROS). ROS levels increased 1.76 compared to the control when treated with 96µM. Pre-incubation of *L. amazonensis* promastigotes with catalase and catalase polyethylene glycol not protect the cells from inhibition promoted by 2'-OH. Furthermore, *L. amazonensis*-infected macrophages were incubated with different concentrations of 2'-OH for 72 h demonstrating a dose dependent-manner decrease on infection index with an IC<sub>50</sub> of 2.30 µM. The toxicity for macrophages was also tested. The 2'-OH concentration used was non cytotoxic, presenting a selectivity index of 75.39. *L. amazonensis* potassium antimony tartrate-resistant cells were tested with concentrations of 2'-OH (12-96µM) showing an IC<sub>50</sub> of 24.84µM, close to the IC<sub>50</sub> of *L. amazonensis* potassium antimony tartrate-sensitive cells. These results suggest that 2'-OH is effective against both *L. amazonensis* forms, without being toxic for macrophages. 2'-OH causes a mitochondrial dysfunction that lead to an increase of ROS levels. Besides, 2'-OH was able to inhibit potassium antimony tartrate-resistant parasites, leading us to point out the 2'-OH- flavanone as a possible candidate for the chemotherapy of leishmaniasis. **Supported by:**FAPERJ;CNPQ/PAPES;IOC/FIOCRUZ

**QT006 - ACTIVITY OF A SESQUITERPENE LACTONE IN NANOCAPSULES FOR TREATMENT OF THE CHRONIC CHAGAS DISEASE**

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Chagas disease is a neglected zoonosis endemic in Latin America. The only drug currently available in Brazil is Benznidazole (BZ) which causes severe side effects and presents low therapeutic efficacy especially in the chronic phase of the infection. LIC is a sesquiterpene lactone isolated from *Lychnophora trichocarpa* which presents *in vitro* and *in vivo* tripanocidal effect (acute phase). This substance is cytotoxic and highly lipophilic demanding a pharmaceutical formulation to carry it. Nanocapsules (NC) allow controlled release of substances, selectivity for the target tissue and reduction of toxicity. This work evaluated the therapeutic efficacy of LIC in NC formulations for treatment of the chronic experimental Chagas disease in murine model. The NC formulations containing LIC (LIC-NC) were produced and physico-chemically characterized. Swiss female mice were i.p. infected with 500 blood trypomastigotes of the Y strain of *Trypanosoma cruzi*. Mice were treated p.o. and IV for 20 consecutive days with LIC-NC-conventional (oral dose 5.0mg/kg/day, IV dose 2.0mg/kg/day), LIC-NC-stealthy (oral 5.0mg/kg/day; IV 2.0mg/kg/day) and BZ (oral 100mg/kg/day; IV 50mg/kg/day). Untreated infected mice were the control. Treatment was started 90 days post-infection. Both NC formulations showed physico-chemical characteristics suitable for IV and p.o. administrations. After treatment, mice were evaluated to assess parasitological cure. Treatment efficacy was verified by hemoculture, PCR and ELISA. Infected mice treated with LIC-NC-conventional showed 33% of parasitological cure IV and 30% p.o. Infected mice treated with LIC-NC-stealthy showed 50% of parasitological cure p.o. and 55% IV. Infected mice treated with BZ were not cured. Data demonstrate the effect of LIC-NC *in vivo* on the chronic Chagas disease and the high efficiency of NC formulations. The work demonstrates that LIC is a potent substance for treatment of Chagas disease and improved therapeutic regimes must be explored. **Supported by:**FAPEMIG

**QT007 - SCREENING OF DRUGS AGAINST *TRYPANOSOMA CRUZI* USING DRUG REPOSITIONING APPROACH**

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Tropical neglected diseases caused by protozoan parasites as Chagas disease, cause high morbidity and / or mortality in developing countries. Affecting large population marginal to the global economic process it is not seen as a potential market. Thus, the therapy of Chagas disease remains very limited with only two toxic drugs as benznidazole and nifurtimox. Considering the need for the discovery of new therapies, the pharmacological screening of approved drugs in clinical use for other purposes, known as drug repositioning, represents a promising approach for the search of new drugs. The objective of this study was to evaluate the antitrypanosomal potential of fifteen drugs belonging to different pharmacological classes, as antihistaminic, antiinflammatory, antiprotozoal, calcium channel blockers, among others. We also evaluated the cytotoxicity against mammalian cells (NCTC clone 929). The viability of cells was determined by Alamar Blue® at 570 nm. Among the tested drugs, the nitro-derivative nitazoxanide and the antidepressant cloxazolam showed activity against trypomastigotes of *T. cruzi*, with IC<sub>50</sub> values of 15.16 µM and 81.63 µM, respectively. Both drugs showed trypanocidal effect killing 100% of parasites at the highest tested concentration of 200 µM. Nitazoxanide and cloxazolam showed toxicity to NCTC cells, showing IC<sub>50</sub> values of 68.14 µM and 270.50 µM, with selectivity indexes of 4.49 and 3.31, respectively. The study of approved drugs in clinical use is a cost-effective strategy for neglected parasitic diseases, and may contribute to the selection of new therapeutics for Chagas disease. **Supported by:**CAPES/CNPq

**QT008 - THE LONG ROAD TOWARDS FINDING BETTER CHEMOTHERAPIES FOR CHAGAS DISEASE IS BEING PAVED**

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New therapeutics are critically needed for the treatment of Chagas' disease due to limitations of the available treatments by significant toxicities, limited efficacy, and resistance. The Center for Discovery and Innovation in Parasitic Diseases (CDIPD) and the Small Molecules Discovery Center (SMDC) at the University of California San Francisco (UCSF) developed a High-Content High-Throughput Screening (HC-HTS) assay targeting the causative agent of Chagas' disease, *Trypanosoma cruzi*. The assay format allows screening against *T. cruzi* intracellular amastigotes, the form of the parasite that produces the human disease. Several strategic partnerships have been established with renowned institutes, including the Genomic Institute of the Novartis Research Foundation (GNF), the BROAD Institute and Scripps Research Institute, to discover and develop new chemotherapies for Chagas Disease. The approach involves iterative strategy based on the newly developed methodologies for screening for anti-*T. cruzi* compounds combining medicinal chemistry, compound efficacy testing *in vitro* and *in vivo*, *in vitro* ADMET (adsorption, distribution, metabolism, excretion, toxicity) testing and *in vivo* PK (pharmacokinetics) evaluation to develop new pre-clinical candidates for treatment of Chagas disease. For the moment, we have screened a few hundred thousand compounds, with more than two thousand confirmed hits identified. After clustering the hits based on chemical structure similarities, priority scaffolds have been identified and are being carried out in a hit-to-lead chemistry phase. Snapshot pharmacokinetics have been made on more advanced hits, and *in vivo* efficacy has been observed in mouse models. The ultimate goal of this project is to select chemotype series that possess lead-like properties for a downstream lead optimization program through an iterative medicinal chemistry approach, selecting compounds for Pre-Clinical Candidate Characterization by 2015. **Supported by:**Gates Foundation

QT009 - **REVEALING THE CAMPTOTHECIN MECHANISM OF ACTION IN THE  
TRYPANOSOMA CRUZI EPIMASTIGOTE FORM**  
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Chagas' disease, one of the most neglected tropical diseases in the world, affects approximately 8 million people in Latin America. This illness is caused by *Trypanosoma cruzi*, a parasite that has a single nucleus and a unique mitochondrion with an enlarged portion (named kinetoplast) that harbors the mitochondrial DNA (kDNA). The DNA topology is modulated by topoisomerases that act during replication, transcription and repair reverting positive and negative supercoilings of the double-strand. Trypanosomatids topoisomerases are distinct from human enzymes, what encourages their use as targets in chemotherapeutic studies. In the present work, we evaluated the effects of camptothecin, a topoisomerase I inhibitor, considering *T. cruzi* epimastigote proliferation, ultrastructure, cell cycle, DNA lesions, mitochondrial activity and apoptosis. Our data showed that camptothecin caused a strong proliferation inhibition and only the lowest drug concentration used (1µM) led to a reversible effect on cell growth, ultrastructure and phosphatidylserine exposure. At ultrastructural level, treated parasites presented unpacking of the nuclear heterochromatin and the swelling of the mitochondrion. Camptothecin also promoted cell cycle arrest at G2/M and caused nuclear DNA lesions. On the other hand, some protozoa entered in early apoptosis, but they do not progress to late apoptosis, which may indicate that the parasites stay alive in a "senescence-like" state. We also observed that treated cells presented higher levels of reactive oxygen species and loss of mitochondrial membrane potential, what may be associated with apoptosis. Thus, this work indicates that the camptothecin mechanism of action comprises linked events that block the cell cycle, affect DNA organization and mitochondrial activity, which may result in apoptosis. **Supported by:**CNPq e FAPERJ

QT010 - **IN VITRO LEISHMANICIDE EFFECT OF 18-ETHOXYCORONARIDINE ON  
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. Leishmaniasis treatment relies mainly on antimonials and amphotericin B that present high toxicity, elevated cost and is not effective. Natural products constitute an important source of useful compounds for the treatment and control of different infectious diseases. In previous studies the anti-leishmanial effect of coronaridine, a natural occurring indole alkaloid, and of 18-methoxycoronaridine, a synthetic coronaridine derivative were demonstrated (Delorenzi et al 2001; 2002). In this study we demonstrated the leishmanicidal effect of 18-ethoxycoronaridine (18-EC), also a synthetic coronaridine derivative. Our results have shown that 18-EC presented an anti-*L.amazonensis* activity with an IC<sub>50</sub> of 20.5µg/mL for promastigotes and of 6.7µg/mL for amastigotes. The cell cycle of promastigotes was changed after treatment with 20.5µg/mL of 18-EC, where we observed an increase of the G0 and decrease of the G1 and G2 phases. Our data demonstrated low cytotoxicity for macrophages treated with 18-EC at concentrations until 100µg/mL. To evaluate if the leishmanicidal activity of 18-EC was due to increase of Nitric Oxide (NO) production, an important mediator of parasite death in phagosomes, we verify the level of NO by the Griess method. Our data shows that 18-EC at 6.7µg/mL do not modulate NO production in macrophages, stimulated or not with IFN-γ. In conclusion, our results demonstrate the safety for macrophages and the anti-*L. amazonensis* activity of 18-EC, indicating this compound as a promising candidate for future studies regarding treatment of Leishmaniasis. **Supported by:**CAPES, FAPERJ, CNPq, MACKPESQUISA; HEBRON FARMACÉUTICA

**QT011 - TRICLOSAN AFFECTS *TOXOPLASMA GONDII* DIVISION AND APICOPLAST MORPHOLOGY**

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*Toxoplasma gondii* is a world spread parasite, which infects about one third of human population. Although the infection is usually asymptomatic in the majority individuals, *T. gondii* is one of the most important pathogens affecting immune-compromised patients and congenitally infected newborns. The treatment of toxoplasmosis is restricted to few options of treatment, which are often associated to side effects. Thus, the identification and characterization of new pathways are extremely important for developing new treatments for toxoplasmosis. The apicoplast is a plastid-like organelle, which performs several important metabolic functions. The pathways harbored by apicoplast are potential drug target as they are prokaryote in origin and often have significant differences of the corresponding mammalian pathways. One of these pathways is the fatty acid synthesis (FAS) which in prokaryotes consists in a system of independent enzymes (type II) and is different from the multifunctional polypeptide found in animals (type I). The triclosan is well-known inhibitor of enoyl-ACP reductase enzyme of FAS type II (FAS II). Although triclosan activity against *T. gondii* has been widely studied, the characterization of the alterations at cell and ultrastructural levels have not been studied yet. In this work, we evaluated the effect of triclosan in *T. gondii* utilizing transmission electron microscopy (TEM) and fluorescence microscopy. First, monolayers of LLC-MK<sub>2</sub> cells infected with tachyzoites of RH strain of *T. gondii* were treated with different concentrations of triclosan and the IC<sub>50</sub> obtained were 0.83µg/mL (2.87µM) and 0.26µg/mL (0.9µM) after 24 and 48 hours of treatment, respectively. The ultrastructural effects evaluated by TEM of parasites treated with 1µg/mL for 24 and 48h showed that triclosan arrested cell division, leading to the formation of gathered daughter cells or rounded cells presenting diverse profiles of inner membrane complex (IMC) and the apicoplasts in such cells had also, altered morphology. Observations of treated parasites by fluorescence microscopy utilizing antibodies for the IMC (anti-IMC1 antibody) and apicoplast (anti-Cpn60 antibody) confirmed cell division arrestment and showed that treatment with triclosan causes enlargement of the apicoplast. **Supported by:**CNPq, FAPERJ

**QT012 - SYNTHETIC PROTOTYPES PYRAZOLYL BENZENESULFONAMIDE DERIVATIVES WITH POTENTIAL ANTILEISHMANIAL ACTIVITY**

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Leishmaniasis is a disease of public health significance and worldwide distribution. This study evaluated the antileishmanial activity of ten newly synthesized molecules, pyrazolyl benzenesulfonamide derivatives, against extracellular forms of *Leishmania amazonensis*. We also evaluated possible cytotoxic effects of these compounds on experimental murine peritoneal macrophages. The effective concentration of each compound producing 50% of maximum possible response (EC<sub>50</sub>) and cytotoxicity concentration to 50% of cell (CC<sub>50</sub>) were determined in 24 hours. Synthetic compounds D1 to D10 showed values of EC<sub>50</sub>/24h between 5.3 µg/mL and 146.0 µg/mL. Additionally, CC<sub>50</sub>/24h values ranged from 15.6 µg/mL to 261.7 µg/mL. The selectivity index (SI) of the synthetic compounds was determined corresponding to values between 0.21 and 9.25. Compared to the values of the reference drug Pentamidine (EC<sub>50</sub> = 7.6 µg/mL, CC<sub>50</sub> = 19.5 µg/mL and SI = 2.56), it was selected the synthetic compound D6 (EC<sub>50</sub> = 28.3 µg/mL, CC<sub>50</sub> = 261.7 µg/mL and SI = 9.25) to therapeutic activity evaluation in experimental murine model. BALB/c mice were infected with promastigotes of *Leishmania amazonensis* in stationary phase. Two weeks later, infected mice were orally treated with compound D6 (5 and 20 mg/kg/day) for 28 days. Control group received Ketoconazole (50 mg/kg/day). Weekly monitoring of body mass was performed. Draining popliteal lymph nodes were collected at the end of the treatment to evaluate the parasite load. The animals treated with pyrazolyl benzenesulfonamide derivative D6 have not altered body mass and not shows obvious toxicity. Furthermore, reduction of parasitic load in draining popliteal lymph node indicates potential therapeutic of pyrazolyl benzenesulfonamide derivative D6. **Supported by:**PROPPI/UFF

**QT013 - POTENTIAL ANTILEISHMANIAL ACTIVITY OF EXTRACTS AND FRACTIONS OF ALGAE OF THE GENUS *PLOCAMIMUM***

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Leishmaniasis are diseases of worldwide distribution, caused by obligate intracellular protozoa of the genus *Leishmania*. It is considered a reemerging disease and neglected by the pharmaceutical industry. The current chemotherapy used in leishmaniasis treatment is pentavalent antimonials. However, they present several side effects, they are expensive, besides increasing parasites resistance cases and needs patient hospitalization. Therefore, there is a great effort in the search for new drugs. The objective of this study was to evaluate the potential in vitro antileishmanial activity and cytotoxicity of two extracts and two purified fractions of algae of the genus *Plocamium* on *Leishmania* and peritoneal macrophages. For in vitro test was used promastigotes of *L. amazonensis* at a concentration of  $1 \times 10^7$  parasites/mL. The extracts were tested in 96-well plates over a wide range of concentration. For in vitro cytotoxicity test was used peritoneal macrophages from BALB/c mice. The cells were cultured in complete DMEM at 37°C with 5% CO<sub>2</sub>, with extracts and reference drug, pentamidine. After 24 hours of incubation in both tests, cell viability was determined using the technique of MTT using the ELISA reader, filter 545 nm. Thereafter, the EC<sup>50</sup>/24h or CC<sup>50</sup>/24h values were determined by linear regression analysis. The experiments were performed three times in triplicate, and the data were statistically analyzed using Student's t-test and ANOVA, using as parameter of significance  $p < 0.05$  (InStat Graph Pad). Crude extracts of the two available values EC<sup>50</sup>/24h were as follows: the eluate with dichloromethane=14.75 µg/mL, whereas the eluate with hydroalcoholic=160 µg/mL. The two fractions available EC<sup>50</sup>/24h values were as follows: Fraction A= 27.6 µg/mL and Fraction B=154.3 µg/mL. The cytotoxicity test was performed with the best antileishmanial activity extract and presented results greater than the reference drug. Other tests will be performed to improve results. **Supported by:**FAPERJ, PROPPI/UFF

**QT014 - ACTION OF THE PTEROCARPANOQUINONA LQB-118 ON HAMSTERS AND HUMAN MACROPHAGES INFECTED WITH *LEISHMANIA BRAZILIENSIS*.**

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We showed previously that LQB-118 has therapeutic effect on hamsters *L.braziliensis* infected by oral and intralesional. The aim of this study was to investigate the action of LQB-118 on amastigotes of *L.braziliensis* using hamster and human macrophages and evaluate a possible modulation of macrophages. Peritoneal macrophages were infected hamsters or not and incubated with LQB-118 at 5-20µM for 24 and 48h. Human PBMCs were isolated from healthy volunteers using Ficoll-Hypaque and after 72h of culture were infected and treated with LQB-118 at 5-40µM for 48h. The monolayers were stained and amastigotes were counted to determining the index of infection. The survival of amastigotes was evaluated based on its ability to differentiate into promastigotes after removal of LQB-118 and reincubation to Schneider plus 20% fetal bovine serum at 28°C for 5-7 days. To detect DNA fragmentation *in situ* monolayer of macrophages infected hamster treated and TUNEL kit was labeled and visualized in fluorescence microscopy. In the hamster macrophages LQB-118 showed dose-dependent inhibition in the index of infection of 37,67 and 93% at concentrations of 5,10 and 20µM, respectively. Pretreatment of macrophages hamsters 24 hours before infection was even more effectively reducing the index of infection at 62,89 and 95% at the same concentrations. We found that only the amastigotes were stained with TUNEL Kit being the core of macrophages labeled with DAPI, showing the viability of the host cell. In human macrophages observed a reduction of 26,45,59 and 73% infection index at concentrations of 5,10,20 and 40µM, respectively, with no apparent toxicity to the host cell. These results indicate that LQB-118 has antiparasitic action and induces DNA fragmentation in intracellular amastigote *L.braziliensis*, without toxicity to the host cell. We are currently evaluating the expression and cytokine production by macrophages treated with hamster and human LQB-118. **Supported by:**CNPq e FAPERJ

**QT015 - ANTI-LEISHMANIA ACTIVITY OF LQB-182, A NOVEL SYNTHETIC PTEROCARPANOQUINONE DERIVATED FROM LQB-118**

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LQB-182 is a novel pterocarpanoquinone derived from synthetic pterocarpanoquinone LQB-118. The precursor molecule presents antitumor and antiparasitic activity against *Leishmania amazonensis* and *L. braziliensis* *in vitro* and *in vivo*. The aim of this study was to evaluate the activity of LQB-182 against *L. braziliensis* and compare with LQB-118. LQBs were tested on promastigotes and intracellular amastigotes forms. Promastigotes forms were treated with molecules (20µM/72h) and the number of parasites was counted daily under a microscope. In order to verify if the inhibitory effect on the cell growth was reversible, after 72h of treatment the cells were washed and reincubated with culture medium (72h). The anti-amastigote activity was evaluated by treating monolayers of peritoneal macrophages of SW mouse infected with *L. braziliensis* with pterocarpanoquinones (3-40µM/72h). After this monolayers were stained and the number of amastigotes counted under microscope. To investigate the viability of the parasites after the treatment, we evaluated the capacity of the remaining amastigotes to differentiate into promastigotes forms after the substitution for Schneider's medium and reincubation (48h/28°C). When molecules were removed, the inhibitory effect was not reverted and the promastigotes forms previously treated with LQBs-118 and 182 at 20µM. had their growth inhibited in 83% and 80% respectively. The IC50 values for LQB-118/LQB-182 were respectively 9,93µM/16,7µM. The percentage of viable promastigotes differentiated was under 2% (p<0,001). These data indicate that LQB-182 has activity against *L. braziliensis*, however not more than its precursor molecule, chemically related, presenting only a change in the position of the furan ring. The molecules showed no toxicity to the host cells until 160µM. We are currently investigating the capacity of LQB-182 modulate the production of cytokines, nitric oxide and reactive oxygen species by macrophages **Supported by:**FAPERJ

**QT016 - ANTIPARASITIC ACTIVITY OF LAPACHOL ANALOGUES AGAINST *LEISHMANIA BRAZILIENSIS***

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Naphthoquinones are bioactive molecules that can interfere with several cellular processes and have described anti-tumor and anti-protozoa activity. The aim of this study was to evaluate the direct and indirect antiparasitic effect of the naphthoquinone lapachol and its synthetic analogues (LQB 180, LQB 181, and LQB 209) on intracellular amastigotes of *L. braziliensis*, modulation of the host cell and toxicity. Monolayers of peritoneal macrophages from normal swiss mice were infected with *L. braziliensis* promastigotes (5:1) and incubated with naphthoquinones for 72h at 37°C/5%CO<sup>2</sup>. The macrophages were stained and the percentage of infected macrophages and intracellular amastigotes counted under a microscope. Nitric oxide (NO) production was measured (Griess method) in the supernatant. Naphthoquinones toxicity was evaluated on non-infected macrophages by MTT assay. The naphthoquinones showed no toxicity on macrophages at the concentration tested (200 µM). On intracellular amastigotes forms all naphthoquinones tested decreased significantly (p <0.05) the infection index. Lapachol presented an IC50 of 73µM and the IC50 of LQBs 180, 181 and 209 was estimated in 63µM, 116µM and 66µM respectively. None of the molecules increased NO production by infected macrophages. These data indicate that lapachol and its analogues inhibit intracellular amastigotes of *L. braziliensis*, and the addition of two methoxy groups in LQB 180 improved the anti-amastigote activity comparing to lapachol. We are currently investigating the modulation of macrophages by naphthoquinones. **Supported by:**CAPES

**QT017 - IN VITRO LEISHMANICIDAL AND APOPTOTIC EFFECTS OF *LIPPIA GRACILIS* ESSENTIAL OIL AND CARVACROL ON *LEISHMANIA CHAGASI* PROMASTIGOTES**

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The current drugs used to treat leishmaniasis are not so much suitable due to resistance reported, high toxicity and side effects. So, new therapeutic strategies are urgently required. Plant essential oils are emerging as alternative sources for chemotherapeutic compounds, including leishmanicidal ones. In this study, leishmanicidal and apoptotic effects of *Lippia gracilis* essential oil (LGEO) and carvacrol were investigated. Promastigotes of *Leishmania chagasi* were incubated in presence of increasing concentrations of essential oil and carvacrol for 24 h. Cell density was determined by counting in a Neubauer chamber. The IC<sub>50</sub> was determined by regression analysis. Evaluation of alterations on promastigotes membrane permeability was based on propidium iodide (PI) labeling and promastigote apoptosis assessment was carried out using annexin V/PI labeling. All these analyses were performed with an Attune Cytometer. The incubation of promastigote of *L. chagasi* with both LGEO and carvacrol, its major compound, efficiently inhibited the parasite growth. The IC<sub>50</sub> were 85.2 and 86.9 µg/mL for LGEO and carvacrol respectively. Analyses of promastigotes morphology by flow cytometry showed that LGEO and carvacrol induce a reduction on promastigotes size. A gradual increase in PI-positive cells was observed, indicating changes in membrane permeability as result of LGEO and carvacrol treatments. In addition, the percentage of LGEO-treated promastigotes positive only for annexin V gradually decreased with the increase of oil concentration. However, the number of cells that were both annexin V- and PI-positive increased, indicating a late-apoptotic effect. On the other hand, the cell dead caused by carvacrol shows a necrotic pattern and degradation of promastigotes treated with 60 µg/mL of carvacrol was evidenced. Thus, we can conclude that the leishmanicidal effect of *L. gracilis* essential oil is mediated through apoptosis while dead induced by carvacrol has necrotic features. **Supported by:**CAPES/CNPq/FAPITEC-SE

**QT018 - QUERCETIN CAUSES DEATH BY REACTIVE OXYGEN SPECIES IN INTRACELLULAR AMASTIGOTE FORM OF *LEISHMANIA AMAZONENSIS***

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Leishmaniasis, a parasitic disease caused by protozoa of the genus *Leishmania* and presents extensive mortality and morbidity. Quercetin is the most common flavone in the human diet. This compound has a wide range of reported biological effects, including antioxidant, antimicrobial and antiprotozoal activities. The present study reports the mechanism of the antileishmanial activity of quercetin against the intracellular amastigote form of *L. amazonensis*. Macrophage murine were infected with promastigote forms of *L. amazonensis* and after 72 hours, the *L. amazonensis*-infected macrophages were then incubated in the absence or in the presence of quercetin (3, 6 and 12µM) for 72h. Treatment with quercetin reduced the infection index in *L. amazonensis*-infected macrophages in a dose-dependent manner (p<0.05), with an IC<sub>50</sub> value of 3.4µM, reaching at 74.8% in the highest concentration (12µM). The concentrations of quercetin employed in this assay had no cytotoxic effects on the macrophage (IC<sub>50</sub> = 80.2µM) presenting a selectivity index of the 16.8. After 72h, quercetin increased ROS generation in *L. amazonensis*-infected macrophage in a dose-dependent-manner but did not increase ROS in uninfected macrophages, suggesting that the increase in ROS could be specific to cells infected with intracellular amastigotes. The level ROS was 1.5-fold higher in 12µM quercetin-treated *L. amazonensis*-infected macrophages than in non-treated *L. amazonensis*-infected macrophages. Additionally, a linear correlation (R<sup>2</sup>=0.96) between the percent inhibition of the infection index and ROS production upon treatment with quercetin was observed. The present results demonstrate a specific antileishmanial activity of quercetin against intracellular amastigotes of *L. amazonensis* by ROS production. Taken together, these results suggest that ROS production plays a role in the mechanism of action of quercetin in the

control of intracellular amastigotes of *L. amazonensis*. **Supported by:**FAPERJ; CNPq/PAPES; IOC/FIOCRUZ

**QT019 - QUERCETIN CAN INDUCE REACTIVE OXYGEN SPECIES PRODUCTION IN HAMSTERS PERITONEAL MACROPHAGES INFECTED WITH *LEISHMANIA BRAZILIENSIS* AND IS EFFECTIVE AGAINST *LEISHMANIA MAJOR***

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The flavonoid quercetin has a wide range of biological effects, including antioxidant, anti-inflammatory and antimicrobial activities. Quercetin can act as pro-oxidant generating ROS in cells. Previous studies demonstrated the effect of quercetin *in vitro* and *in vivo* in *Leishmania amazonensis*. We had already demonstrated that quercetin inhibited the growth of intracellular amastigotes of *Leishmania braziliensis* and its therapeutic activity *in vivo* using golden hamster as infection model. The aim of this work is to investigate ROS production by quercetin in *Leishmania braziliensis* infected macrophages and its effect in *Leishmania major*. ROS production was measured by H<sub>2</sub>DCFDA. The anti-amastigote activity of *L. major* was performed using BALB/c infected peritoneal macrophages. ROS production was increased when hamsters macrophages were treated with 100 and 50 µg/ml in a dose- and time dependent manner, without endangering the host cell viability. Pre-treated macrophages before infection was increased when compared to controls, while macrophages treated before and after infection showed a decrease of ROS production (p < 0,05) that can be associated to the inhibition of *L. braziliensis* intracellular amastigotes, that is more effective when macrophages were treated 24h before infection inhibiting 50 and 60% at 100 and 50 µg/ml, respectively. Quercetin inhibited the proliferation of *L. major* intracellular amastigotes in 79% 53% and 44% at 100, 50 and 25 µg/ml, respectively, after 48h of treatment. When macrophages were treated 24h before infection, quercetin inhibited 82%, 75% and 25% at 100, 50 and 25 µg/ml. Taken together, our results suggest that quercetin can induce the production of ROS in hamsters peritoneal macrophages infected with *L. braziliensis*, as part of its mechanism of action and is effective against *L. major* amastigotes that belongs to another subgenus of *Leishmania*, indicating quercetin as possible candidate for the chemotherapy of Leishmaniasis. **Supported by:**CAPES AND FAPERJ

**QT020 - ACTIVITY OF EXTRACTS OF *PSIDIUM GUAJAVA*, *SOLANUM LYCOPERSICUM*, *COLACASIA ESCULENTA*, *KALANCHOE PINNATA* AND ITS CLONES *IN VITRO* AGAINST *LEISHMANIA BRAZILIENSIS***

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The aim of this study was to evaluate *in vitro* the anti-Leishmanial potential of plants extracts against *Leishmania braziliensis* (Lb). We evaluated aqueous extracts of *Kalanchoe pinnata* (Kp) grown in the habitat (Kp-habitat), under blue light (Kp-blue), white light (Kp-white) or ultraviolet light (Kp-UV), *Psidium guajava* (Pg), *Solanum lycopersicum* (Sl) and *Colacasia esculenta* (Ce). Promastigotes forms were cultured with 0-500 µg/ml of extracts for 96h/28°C and parasites were counted daily using a Neubauer chamber. To perform the tests with amastigotes, monolayers of mice peritoneal macrophages were infected with promastigotes of Lb and incubated with 0-500 µg/ml of extract for 48 and 96h/37°C/5%CO<sub>2</sub>. Nitric oxide was measured by Griess reagent on supernatants. Kp extracts has little effect on promastigotes growth, inhibiting 27,4% (Kp-blue), 17,35% (Kp-habitat) and 1,66% (Kp-white) at 500 µg/ml. Sl inhibited 59% at 125 µg/ml and Ce inhibited 61% at 250 µg/ml. About amastigotes there was no improvement in the effect varying growing conditions of Kp, but the effect was time-dependent. The treatment with 500 µg/ml Kp-habitat showed a reduction in infection index by 24% and 50% at 48h and 96h, respectively. Pg extract (500 µg/ml) inhibited 46% (p < 0,05); Sl extract (125 µg/ml) inhibited 61% (p < 0,05); Ce extract (125 µg/ml) inhibited 43% of infection index. None of the extracts tested was able to stimulate nitric oxide production by macrophages. In macrophages pretreated for 24h before infection, Kp-habitat extract was able to inhibit 54, % (p < 0,05) of infection index, suggesting effect on macrophage independent of nitric oxide production. Only Ce extract (500 µg/ml) and Sl extract (500, 250 and 125 µg/ml) decrease

macrophage viability. Although further studies are required, Kp and Pg extracts showed promising anti amastigote activity on Lb. The production of cytokines by macrophages and also the death of the parasite through apoptosis is being investigated. **Supported by:**CNPq/FAPERJ

**QT021 - SUBACUTE TOXICITY AND EFFICACY OF LQB-118 ON EXPERIMENTAL VISCERAL LEISHMANIASIS.**

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Previously, we demonstrated that the pterocarpanquinone LQB-118 was effective in experimental cutaneous leishmaniasis via oral delivery, and that the mechanism of action involves induction of oxidative stress with characteristic events of cell death via apoptosis in *Leishmania amazonensis*. In continuing this study, we evaluated the effect of LQB-118 on experimental visceral leishmaniasis, the subacute toxicity and the early events involved in triggering death in *Leishmania*. Treatment of *L. infantum*-infected mice showed a dose dependent reduction of parasite load in the liver (ED<sub>90</sub>=3,4mg/kg/dia) and spleen (ED<sub>90</sub>=5,7mg/kg/dia). In the subacute toxicity evaluation (10 and 50 mg/kg) did not induce variation in weight gain and in biochemical parameters of liver and kidney functions. Unexpectedly, a slight change in leukocytes count was observed, suggesting that additional studies are needed. To assess the early effects of LQB-118, promastigotes were incubated with LQB-118 and antioxidants. Cell viability and mitochondrial membrane potential were not reversed, although the ROS production was, suggesting that ROS production is not the major cause of cell death. Interestingly, when promastigotes or intracellular amastigotes of *L. amazonensis* are incubated with LQB-118, an intense mitochondrial activity is produced in the early hours, evidenced by the strong reduction of Alamar Blue. Inhibitors of complex I, II or III failed to reverse this effect in promastigotes, only the inhibitor of complex IV was able to reduce this phenomenon. Concomitantly to the higher Alamar Blue reduction, we also observed an increase in intracellular ATP; however, after 48 h the mitochondrial activity fails, decreasing the intracellular ATP. In conclusion, our findings indicate that LQB-118 acts on different clinical forms of leishmaniasis, with no signs of toxicity at therapeutic dose, through the induction of a selective disability on the mitochondrial activity of the parasite. **Supported by:**FAPERJ, PAPES IV and CAPES

**QT022 - LEISHMANICIDAL ACTIVITY OF NEW THIOSEMICARBAZONES**

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Leishmaniasis is caused by protozoa of the genus *Leishmania* and constitutes a serious problem of public health. The drugs used in the clinic are toxic and often ineffective. Thus, a rational search for new therapeutic alternatives is necessary. Thiosemicarbazones are classes of compounds with broad pharmacological profile, presenting antiinflammatory, antitumor, antiviral, antibacterial and antimalarial activity. In this study, we investigated the anti-*Leishmania amazonensis* (MHOM/BR/77/LTB0016) activity of ten thiosemicarbazones derivatives. The treatment of the parasites with the thiosemicarbazones resulted in the dose-dependent growth inhibition of the promastigote forms. Among all derivatives, the compounds TSC TT and TSC I were the most active with IC<sub>50</sub> 16.4 and 27.7µM after 72 hours of incubation. These compounds showed high inhibitory activity against intracellular amastigotes, with IC<sub>50</sub> of 17 and 22µM respectively and presented low cytotoxicity on mammalian cells. Based on *in vitro* activity exhibited by these compounds, we considered these molecules to be promising novel prototypes for drug development against leishmaniasis. Thiosemicarbazones are known inhibitors of cruzipain, the major cysteine protease in *T. cruzi*, which has proven to be essential for infection, replication and metabolism of the parasite. Furthermore, this class of substances can also inhibit cathepsin L in trypanosomatids. However, the mechanism of action of TSC TT and TSC-I remains to be evaluated.

**QT023 - MODULATION OF ROS PRODUCTION AND INCREASED MICROBICIDAL ACTIVITY IN MACROPHAGES TREATED WITH GLUCANTIME**

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The pentavalent antimonial drugs are generally used as first choice for the treatment of leishmaniasis, however its mechanism is not fully understood. Presents toxic action only against intracellular amastigotes because the antimony must be reduced to the trivalent form by the host cell and inhibits the glycolytic pathway, fatty acid and trypanothione reductase of parasite. Recently was described that Glucantime enhances phagocytosis and TNF- $\alpha$  production by monocytes in human cutaneous leishmaniasis. The aim of this study is to evaluate a possible modulation of macrophages. Monolayers of peritoneal macrophages from Balb /c mice were treated for 24 hours with Glucantime (0.1, 1 and 10 mg / ml) before infection with *Leishmania braziliensis*. After 48 hours cells were stained and infection index was estimated by counting under the microscope. The production of nitric oxide (NO) was measured in supernatants by Griess method and the production of reactive oxygen species (ROS) by macrophages was measured fluorimetrically using H2DCFDA dye. Alternatively, to ensure that Glucantime was fully eliminated by macrophages after treatment, cells were washed and re-incubated for 24 hours with only culture medium and subsequently infected. The pre-treatment of macrophages with Glucantime was able to reduce the infection index in 48,72 and 85% ( $p < 0,01, p < 0.001$  and  $p < 0.001$ ) at 0.1, 1 and 10 mg/ml, respectively. When the macrophages were re-incubated with culture medium before infection the reduction of the infection index was 11%, 67% and 95% (ns,  $p < 0.001$ ,  $p < 0.001$ ). We did not observe an increase in NO production by macrophages. However macrophages pre-treated for 24h with Glucantime at 1 and 10mg/ml were able to enhance the production of ROS ( $p < 0.05$ ). These results indicate that Glucantime can be modulating the microbicidal ability of macrophages. The profile of cytokines produced by macrophages is being investigated. **Supported by:**FAPERJ

**QT024 - BIOLOGICAL EVALUATION OF THE POTENTIAL EFFECTS OF ALKYLATING AGENT 3-BROMOPYRUVATE DURING TOXOPLASMA GONDII-HOST CELL INTERACTION**

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*Toxoplasma gondii* is the causative agent of toxoplasmosis and infects a wide range of hosts, including man. In humans, the disease typically presents asymptotically, but can cause damage in immunocompromised individuals. The most commonly used therapy, and likely more effective is the synergistic combination of pyrimethamine and sulfadiazine. However, this therapy is commonly associated with several limitations. Thus, the study of new compounds that act on the parasite becomes necessary, associated with the study of the cell biology of the parasite. In this work, the action of the alkylating agent 3-BromoPyruvate (3-BrPA) was tested in epithelial cell lines LLC-MK2 infected with tachyzoite forms of *T. gondii* RH strain. One of the aspects analyzed was the effect of the compound on the proliferation of the parasite. Cultures infected at a ratio 3:1 parasite: cell were treated for 24 h with 3-BrPA (5 and 10  $\mu$ M). The results showed that the proliferation of *T. gondii* was inhibited by about 58% at 5  $\mu$ M of 3-BrPA. Using concentration of 10  $\mu$ M of 3-BrPA in 5:1 ratio parasite-host cell, followed by treatment for periods of 24 and 48 h showed a reduction in the number of parasites of 55% and 61%, respectively. Interactions after 6 days of treatment with 10  $\mu$ M of 3-BrPA were observed by scanning electron microscopy and the images revealed changes in the organization and the number of parasites into parasitophorous vacuoles in several cells, compared to the control which showed typical structures in rosettes due to the multiplication of tachyzoites. The analysis of these cultures processed for cytochemical showed changes on the parasitophorous vacuole membrane, which began to express Nacetylglactosamine residues, as revealed by incubation of the cells with DBA lectin, indicator of cyst wall formation. Thus, we suggest that

the 3-BrPA compound may be useful to study the *T. gondii* interconversion and its action mechanisms that determine the induction of cystogenesis. **Supported by:** Faperj, CAPES, CNPq

**QT025 - TREATMENT OF TACHYZOITES OF *TOXOPLASMA GONDII* STRAIN ME49 USING PTEROCARPANQUINONE LQB118 AND METALOCOMPLEX FEHP(SO<sub>4</sub>)**

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*Toxoplasma gondii* is the causative agent of toxoplasmosis, the most common parasitic infections to humans and other warm-blooded animals, that affects one-third of the world population in its chronic form. *T. gondii* are classically classified in three different genotypes, designated type I, II and III, correlating with virulence and epidemiological occurrence. The actual treatment against toxoplasmosis is based on the combination of drugs pyrimethamine and sulfadiazine. These therapies are not able to eliminate the parasite from the infected host and are associated with considerable toxicity and side effect. Therefore, drugs with less toxicity to the host, higher efficacy against tachyzoites and is able to cause bradyzoites conversion are urgently necessary. Pterocarpanquinones resulting from molecular hybridization between pterocarpan and lapachol have several biological activities such as antitumoral, antiviral and are able to control *Leishmania amazonensis* growth. Our group has investigated the anti-*Toxoplasma* effect of these compounds that are able to induce the conversion of tachyzoites of *T. gondii* (RH strain, type I) to bradizoytes. In addition previous studies with new drugs known as metalocomplexes have shown several interesting biological activities. In this work, we used these compounds already active against tachyzoites of *T. gondii* (RH strain), the LQB 118 pterocarpanquinone and the metalocomplex FeHP(SO<sub>4</sub>) for treatment of tachyzoites of the ME49 strain (type II), order to verify the effect of compounds on cistogenics strains, approaching a model most common infection of this parasite, the chronic infection. Our results shown that both compounds are able to control the growth of ME49 tachizoytes in low concentrations, in the micromolar range. Further experiments are necessary to better characterize the action of these compounds in *T. gondii* of the ME-49 strain. **Supported by:** CAPES, CNPq, Faperj

**QT026 - *PHYSALIS ANGULATA*, AN INDUCER OF THE PRODUCTION OF REACTIVE OXYGEN SPECIES ON *LEISHMANIA (L.) AMAZONENSIS* PROMASTIGOTES AND ITS HOST CELL**

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Leishmaniasis are infectious diseases caused by protozoa of the genus *Leishmania* and transmitted by phlebotomine sandflies. This protozoa is unicellular, showing only one mitochondrion, and its mitochondrial DNA is located in a region called kinetoplast. The most effective treatment for leishmaniasis is the chemotherapy and besides the high cost, these drugs are toxic and require a long period of treatment. Currently, some herbal products are considered an important alternative source of a new leishmanicidal agent, which includes the plant *Physalis angulata*, a plant widely used in popular medicine and has been shown by our group potent leishmanicidal action on amastigotes and promastigotes of *Leishmania amazonensis*. In the present study we observed an increased production of reactive oxygen species (ROS) in promastigotes of *Leishmania (L.) amazonensis* treated with 100 µg/ml of the root extract from *Physalis angulata* (REPa) in 48 (47,67%) and 72-hour (81,42%) period compared to controls grown for 48 (26,01%) and 72 (26,93%) hours. REPa promoted the activation of macrophages through the production of ROS in cells infected with *L. amazonensis* and treated with the concentration of 100 µg/mL (75%). No cytotoxic effect was observed in host cell treated with REPa when compared to the untreated control. Thereby, this study revealed that root extract from *Physalis angulata* is able to activate macrophages through the production of ROS and has antileishmanial properties. **Supported by:** CAPES, CNPq/UFPA, CNPq/MCT/CT-INFRA/CT-PETRO, Instituto Nacional de Biologia Estrutural e Bioimagem

**QT027 - CHEMICAL ENGINEERING DERIVED MOLECULE HAS ANTILEISHMANIAL ACTIVITY.**

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American Tegumentary Leishmaniasis (ATL) is a parasitic disease, widely spread in most countries of Latin America, and caused by different species of the genus *Leishmania*. This protozoan is an obligate intracellular parasite that developed mechanisms to subvert the microbicidal activity of macrophages, such as inhibition of superoxide and nitric oxide (NO) production. The chemotherapy is one of the most effective treatments for this disease. Although a number of antileishmanial drugs are available, these drugs are in general toxic, expensive and require long-term treatment. The development of new drugs from computational chemistry area has been an alternative to obtain drugs against molecules targets in parasites. Thus, we consider analyze the activity of LaSOM 158, a heterocyclic molecule similar to benzopyrene which is derived from computational chemistry, against promastigotes of *Leishmania amazonensis* and the host cell. Antiproliferative activity and a dose-dependent inhibition of promastigote growth (87.5% and 100%) was observed when parasites were treated with 100 and 200 µg/ mL of LaSOM 158, respectively. The colorimetric assay (MTT), that measures cytotoxic metabolic viability, showed that this compound presented no cytotoxic effects against macrophages. In addition, morphometric analysis demonstrated that macrophages after 24h of treatment presented an increasing cellular area when treated with 100µg/mL of LaSOM 158, which is characteristic of cellular activation, an important parameter during parasite-host cell interaction. These results demonstrated that LaSOM 158 effectively inhibits the growth of parasites and does not have cytotoxic effects on the host cells. Thus, this compound may hold great potential as an antileishmanial agent. **Supported by:** CAPES, UFPA, CNPq, INBEB

**QT028 - ANTILEISHMANIAL ACTIVITIES OF FLAVONE APIGENIN**

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Leishmaniasis has been reported in 98 countries and affects more than 12 million people worldwide. The development of new, safer, cheaper and orally available drug treatments for leishmaniasis is urgently needed. Apigenin, a naturally occurring plant flavone, is recognized as a bioactive flavonoid. In this present study, we report *in vitro* and *in vivo* effects of apigenin against *Leishmania (Leishmania) amazonensis*. Apigenin inhibited promastigote viability in a dose-dependent manner with 24 h of treatment reaching 74% of inhibition at the concentration of 96µM (IC<sub>50</sub> = 23.68µM). Apigenin induced cell cycle arrest of *L. amazonensis* promastigotes in a dose-dependent manner. A sub-G0/G1 cell cycle phenotype was observed in 33%-63% of the promastigotes incubated with 12-96µM of apigenin. Evaluation of proliferating cells using CFSE staining showed inhibition of cell division of treated promastigotes. Apigenin also inhibited infection index in a dose-dependent manner with 72 h of treatment reaching 71% of inhibition at the concentration of 12µM (IC<sub>50</sub> = 4.33µM) and this concentration had no cytotoxic effects on macrophages. For *in vivo* studies, apigenin was administered orally (1 and 2 mg/Kg/day, every day) to *L. amazonensis*-infected BALB/c mice lasting 38 days. Apigenin reduced lesion size in a dose-dependent manner (P<0.001) and parasite burden (P=0,019) in these mice, without altering serological markers of toxicity (alanine aminotransferase, aspartate aminotransferase and creatinine). Apigenin (2mg/Kg/day) was more effective than meglumine antimoniate in preventing lesion development (ED<sub>50</sub> = 1,84 mg/Kg/day e ED<sub>90</sub> = 3,18 mg/Kg/day). Taken together, our results demonstrate *in vitro* and *in vivo* antileishmanial activity of apigenin on *L. amazonensis*. These characteristics encouraging and suggests the apigenin as a possible prototype for the clinical treatment of cutaneous leishmaniasis. **Supported by:** CAPES, FAPERJ, CNPq and IOC/FIOCRUZ

QT029 - **EVALUATION OF THE EFFECT OF MEMANTINE IN EXPERIMENTAL INFECTION BY *TRYPANOSOMA CRUZI* (IN VIVO AND IN VITRO).**

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The search for new therapeutic drugs is of extreme importance for the specific treatment of Chagas disease. The chemotherapy currently available is unsatisfactory, mainly regarding the effectiveness of treatment in the chronic phase of the disease, as well as in terms of adverse side effects. Therefore, our group is exploring a “piggy-back” strategy. According to this, our laboratory demonstrated that memantine, a drug currently used to treat neurodegenerative diseases, presents a tripanocidal activity against *Trypanosoma cruzi*, the aetiological agent of Chagas disease. Based on this information, in the present work, we evaluated the effect of memantine in the experimental *T. cruzi* infection. BALB/c mice were infected with  $1 \times 10^3$  trypomastigote of Y strain and subjected to treatment with memantine (10mg/kg/day). Treated mice, showed a reduction of the parasitemia of 42.6% ( $p < 0.05$ ) in 8th day after infection (dpi) when compared to the control. At 15th dpi, the tissues were collected for analysis of tissue parasite load by qPCR. This trial showed a reduction in parasite load in some tissues. Since the strain used in our study is reticulotropic (invading preferentially macrophages) we evaluated the effect of this drug on the gene expression of iNOS and IL-10 in adherent peritoneal cells. Our data show that adherent peritoneal cells when stimulated with lipopolysaccharide for 18h, showed a significant reduction the gene expression of iNOS (antagonistic effect) and a significant increase in gene expression of IL-10 (additive effect). Therefore, we suggest that memantine has (besides its trypanocidal activity) an immunomodulatory effect. More experiments are being performed for consolidate the effect of memantine on immune system of mammalian host. **Supported by:** CAPES and FAPESP, CNPq and INBEQMEDI

QT030 - **THE PROLINEMIA, A VIRULENCE FACTOR IN *TRYPANOSOMA CRUZI* INFECTION, LEADS TO AN ANTI-INFLAMMATORY RESPONSE IN PERITONEAL CELLS**

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*Trypanosoma cruzi* is dependent on proline for a variety of processes such as energy metabolism, host-cell invasion, differentiation and resistance to osmotic, metabolic and oxidative stress. Recently we showed *in vitro*, that a proline analogue interferes with its uptake. Moreover, this compound reduces the resistance to stress conditions in *T. cruzi* and decreases the burst of trypomastigotes from host-cells. Here we evaluated the effect of the proline-supplementation on the *ex vivo* infection and two immune-related genes expression of peritoneal cells. The treatment decreased the infection rate and caused a cell volume reduction. The same event was observed in non-infected cells, which could be indicative of apoptosis. PCRq analysis showed, in LPS-stimulated and non-infected cells, a decrease of the inducible nitric oxide synthase (iNOS) gene expression in the presence of proline, while IL-10 gene expression appears to increase. These results suggest that the hyperprolinemia leads to an anti-inflammatory response in peritoneal cells however suppress the amastigotes replication and in turn, masking a possible immunosuppressive effect. **Supported by:** FAPESP, CNPq, INBEQMEDI

QT031 - **IN VITRO ACTIVITY OF PTEROCARPANQUINONE LQB118 ON *TRYPANOSOMA CRUZI***

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Chagas disease is a neglected disease which treatment is restrict and unsatisfactory. Both Nifurtimox and Benznidazole act on trypomastigote and very little on intracellular amastigote. Thus, the development of new drugs is required, mainly to act on amastigote forms. The synthetic pterocarpquinone LQB118 presents antitumoral and antileishmanial activity. The aim of this study was to evaluate in vitro the anti-parasitic effect of LQB118 and derivatives on *Trypanosoma cruzi*. For the evaluation of anti-parasitic effect on intracellular amastigotes, peritoneal macrophages from SW mice were infected with metacyclic trypomastigotes at a ratio of 5:1 and after were treated with LQB118 and their derivatives at 20 µM for 72h/37°C/5%CO<sub>2</sub>. For the evaluation on metacyclic trypomastigotes, parasites were incubated with 20µM of pterocarpquinones for 48h/28°C and after viability was evaluated by motility. Pterocarpquinones also was incubated with epimastigotes for 4 days at 20µM. Macrophages also were pretreated with LQB118 before infection. Controls was done with parasites or parasitized macrophages in culture medium with or without DMSO. The results show that only LQB118 was active inhibiting the infection index into 90% at 20µM (IC<sub>50</sub> 4,2µM). On trypomastigotes, IC<sub>50</sub> was estimated at 38µM. On epimastigotes, LQB118 inhibited 96% the growth of parasites at 20µM, with morphological changes such as rounding of the cell body and loss of flagellum. Citotoxicity of LQB118 to macrophages was evaluated by MTT method (LC<sub>50</sub> 40µM). Peritoneal macrophages pretreated with LQB118 for 24 hours were able to reduce the number of amastigotes after 72 hours of culture in absence of the molecule. Analysis of TUNEL labeling showed that the treatment with LQB118 induced selectively fragmentation of amastigotes's DNA. The results show that LQB118 acts especially on intracellular amastigote. Studies in vivo are necessary to evaluate if LQB118 could be used on treatment of chronic phase of infection. **Supported by:** CNPQ and FAPERJ

QT032 - **INHIBITION OF TRYPANOSOMES OLIGOPEPTIDASE B PROTEASE BY A NOVEL CLASS OF SYNTHETIC NITROALKENES COMPOUNDS**

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Nitroalkenes compounds promote enzymatic inactivation by forming a covalent bond between an electron acceptor, mainly thiol groups of cysteine of enzymes and the ligand. As trypanosome oligopeptidases B possess important roles in both pathogenesis of sleep sickness and Chagas' disease and it is known that, this enzyme possess several cysteine residues exposed in the solvent with possible allosteric roles, we prepared a set of 12 nitroalkene synthetic derivatives to be used as possible inhibitors. The IC<sub>50</sub> values for inactivation were obtained for *Trypanosoma cruzi* and *Trypanosoma brucei* oligopeptidases B and for cruzain using a fluorescent substrate. The inactivation velocities were determined by an irreversible kinetic model, which allows us to obtain all the kinetic parameters in the slow inhibition steps. We found that the nitroalkenes inactivates both oligopeptidases B showing predominantly a two steps reaction behavior with very low *Ki* values (11 to 670 nM) and very high *k4/Ki* value (3.1x10<sup>6</sup> M<sup>-1</sup>.s<sup>-1</sup>). Thus, these inhibitions act in concentration and time-dependent. The differences are related to the size of the inhibitor combined with their different radical groups. A mass spectrometry and sequencing of the inhibited oligopeptidase B are in progress to identify the compounds binding site. The smaller size with a more precise fitting would appear to allow a better interaction with the thiol groups exposed to the solvent. These results indicate that allosteric inhibition of exposed free cysteine is a feasible approach to design potential nitroalkenes hits for drug development for trypanosomiasis. **Supported by:** Capes

**QT033 - EFFECT OF CRUDE EXTRACT OF THE MARINE SPONGE *AMPHIMEDONVIRIDIS* AGAINST *TRYPANOSOMACRUZI* IN VITRO AND IN VIVO**

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*Trypanosomacruzi* is the etiological agent of Chagas disease, an important neglected illness affecting about 8 million people in endemic areas of Latin America. The chemotherapy for this pathology is unsatisfactory, mainly due to its poor efficacy, especially during the later chronic phase and the considerable well-known side effects. Therefore, the need for further research aimed at seeking new natural and synthetic drugs against *Trypanosomacruzi*. In this sense, there are already several reports in the literature pointing marine sponges as a major source of natural products, own or derived from microorganisms them associated, which have importance ecological and biomedical. Thus, the present study investigated the trypanocidal activity of crude extracts from the marine sponge *Amphimedonviridis* against *T. cruzi* in vitro and in experimental infection model of *Swiss Webster* mouse. In studies of the direct action of the compound against the parasite, bloodstream trypomastigotes forms of *T. cruzi* were treated with increasing concentrations of the extract (from 100 to 0.78 µg/mL) during 24 hours of incubation at 37 ° C. The results demonstrated a dose-dependent activity against the parasite, displaying IC<sub>50</sub> value of 7.8 µg / mL, dose capable of killing 50% of the parasites. Given the potential effect of this natural product, we follow the studies evaluating the activity of the compound on the curve of parasitaemia and mortality of animals infected with 10<sup>4</sup> parasites of the Y strain, treated for five consecutive days with the extract at a concentration of 100mg/kg and followed until 20 days post infection. The preliminary results showed no differences in the peak of parasitaemia and in the percentage mortality of the animals treated compared to untreated animals. New treatment schemes and toxicity assay are being conducted to confirm the efficiency of this natural product on the in vivo infection, whose in vitro trypanocidal activity has been promising.

**QT034 - EVALUATION OF TRYPANOCIDAL ACTIVITY OF NATURAL PRODUCTS FROM THE BARK OF *CROTON CAJUCARA* BENTH ON *TRYPANOSOMA CRUZI***

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The use of plants for medicinal purposes - to treat, cure and prevent diseases - is one of the oldest forms of medical practice. The WHO (2008) reaffirmed that 80% of the population of developing countries depend on medicinal plants to access basic health care. *Croton cajucara* Benth, an Amazonian species, stands out for its potential medicinal value. Its bark is widely used in folk medicine for several disorders. The increase of the parasite resistance to current chemotherapies stresses the importance of new active plant components as novel antiparasitic agents in the search for lower toxicity and greater selectivity. The aim of this study was to evaluate the effects of special metabolites and extract fractions obtained from the bark of *C. cajucara* against *Trypanosoma cruzi*.

The crude hydroalcoholic extract of *C. cajucara* bark provided a fraction rich in fixed oil (CC-EHA), F1-7 (sesquiterpenes and CTN), F25-27 (sesquiterpenes and diterpenes) and F28 (mixture of diterpenes). The terpenes (DCTN, CTN and AAA) were isolated and characterized. The evaluation of the antiparasitic activity against *T. cruzi* trypomastigotes was done in LIT medium supplemented with 10% fetal bovine serum. Cultures in 96-well microplates were incubated at 26°C for 24, 48 and 72h, in the presence or absence of fractions and terpenes dissolved in DMSO. Live parasites were counted in a Neubauer chamber. Assays were performed in triplicate in 3 independent experiments; benznidazole was used as control.

The percentage of live parasites indicated DCTN and CTN as the most active at the concentration of 9.38mg/ml, showing only 1.96 and 0% of live trypomastigotes in 72h culture, respectively. Fractions also showed important results: F28 was the most active since at a concentration of 3.125mg/ml, it led 98% of the parasites to death in 72h culture. The results proved that diterpenes and fixed oil fractions obtained from the bark of *C. cajucara* are a new alternative for Chagas disease chemotherapy. **Supported by:** CAPES CNPQ FAPERJ PPGCTIA

**QT035 - *IN VITRO* INVESTIGATION OF THE EFFICACY OF NOVEL DIAMIDINES AGAINST *TRYPANOSOMA CRUZI***

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Chagas' disease (CD), caused by the intracellular protozoan parasite *Trypanosoma cruzi*, is a neglected tropical disease, representing a serious health problem in Latin America. The available chemotherapy based on two nitroderivatives (benznidazol (Bz) and nifurtimox (Nf)) is not satisfactory, especially during the later chronic phase. Aromatic diamidines are DNA minor-groove binders exhibiting considerable activity against several pathogens. As a part of a search for new therapeutic opportunities to treat CD, pre-clinical studies were performed to characterize the antichagasic activity of ten novel amidine compounds (DB2238, DB2239, DB2240, DB2242, DB2243, DB2246, DB2247, DB2248, DB2249 and DB2250), as well as their cytotoxic effect upon uninfected cardiac cells. Our findings demonstrated that up to 32  $\mu$ M, all of the compounds studied did not cause significant loss of cardiac cell viability as evaluated by Alamar blue assays. Seven compounds (DB2239, DB2240, DB2246, DB2247, DB2248, DB2248, DB2249 AND DB2250) gave activity equal or greater than Bz (  $IC_{50}$  = 2 to 8 mM) against bloodstream trypomastigotes (Y strain) after 24h exposure. However, when these compounds were screened against intracellular forms (Tulahuen strain transfected with B-galactosidase gene), none were more effective than the reference drug (Bz). Further in vitro and in vivo studies with new analogs are needed to identify new anti-*T.cruzi* candidates to establish a useful alternative therapy for Chagas disease. **Supported by:**Fiocruz, Fundação Carlos Chagas Filho de Amparo a Pesquisa do Estado do Rio de Janeiro, Conselho Nac

**QT036 - REPOSITIONING HISTAMINE H1-RECEPTOR-ANTAGONISTS: AN *IN VITRO* STUDY AGAINST *LEISHMANIA (L.) INFANTUM***

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In the search for novel drug candidates against neglected parasitic diseases as leishmaniasis, the drug repurposing approach continue to be an excellent opportunity to reduce time and costs of research. Leishmaniasis is included among the most important tropical diseases and represent one of the major causes of mortality and morbidity of parasitic diseases in developing countries. In this work, by using the drug repurposing approach, we studied the antileishmanial effect of histamine H1-receptor-antagonist drugs in clinical use for psychosis (quetiapine) and as anti-histaminics (loratadine, hydroxyzine and ketotifen). By using the fluorimetric assay rezasurin and MTT, the H1 antagonists showed  $IC_{50}$  values in a range concentration of 13 to 84  $\mu$ M against promastigotes of *Leishmania infantum*. No activity against intracellular amastigotes was verified. Cytotoxicity studies using the mammalian cells NCTC showed moderate toxicity, with  $IC_{50}$  values ranging from 69 to 229  $\mu$ M. The investigation of the lethal mechanisms of H1 antagonists on Leishmania involves: i) inhibition of the MTT reduction caused by the action the H1 antagonists, suggesting a leishmanicidal effect on promastigotes; ii) plasma membrane permeation induced by loratadine and to a lesser extent hydroxyzine, resulting in a rapid influx of the vital dye SYTOX Green; ii) depolarization of the mitochondrial membrane potential induced by loratadine and to a lesser extent, hydroxyzine and quetiapine, revealed by the fluorescent probe Myotracker Red; increased generation of reactive oxygen species (ROS) induced by hydroxyzine, resulting in the oxidation of the fluorescent probe H<sub>2</sub>DCF-DA. The studied histamine H1-receptor antagonists induce different lethal effects in Leishmania and might have the mitochondria as potential target. Future studies could use these drugs for the discovery of new prototypes for leishmaniasis. **Supported by:** FAPESP and CNPq.

**QT037 - INHIBITION OF *LEISHMANIA (LEISHMANIA) AMAZONENSIS* AND RAT ARGINASES BY GREEN TEA EGCG, (+)-CATECHIN AND (-)-EPICATECHIN: A COMPARATIVE STRUCTURAL ANALYSIS OF ENZYME-INHIBITOR INTERACTIONS.**

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Polyamine (PA) have a central role in proliferation, differentiation, and antioxidant mechanisms in *Leishmania*. Antioxidant mechanisms in trypanosomatids use the PA spermidine to synthesize trypanothione. Trypanothione protects the parasite from oxidative stress by promoting the removal of reactive nitrogen species, reactive oxygen species and other reactive species produced by the host's defense system. Arginase is the first enzyme of the PA pathway and was considered a target to control *Leishmania* infection. The roles of arginases in infection were studied in mutants containing a knockout of ARG-L gene. Food polyphenols show bioactivities that contribute to human health. Balanced food intake enriched with polyphenols from vegetables, green tea, wine and fruits can prevent cardiovascular diseases. In addition to the known antioxidant activity attributed to green tea (-)-epigallocatechin-3-gallate (EGCG), this compound paradoxically contributes to lethal mitochondrial damage in *L. (L.) amazonensis*. EGCG is also active against *Leishmania (Leishmania) donovani*, *Trypanosoma brucei rhodesiense* and *Trypanosoma cruzi*. However, the molecular target has not been defined. In this study, EGCG, (+)-catechin and (-)-epicatechin were tested against recombinant arginase from *Leishmania amazonensis* (ARG-L) and rat liver arginase (ARG-1). The compounds inhibit ARG-L and ARG-1 but are more active against the parasite enzyme. Enzyme kinetics reveal that EGCG is a noncompetitive inhibitor of the ARG-L while (+)-catechin and (-)-epicatechin are competitive inhibitors. The most potent arginase inhibitor is (+)-catechin (IC<sub>50</sub>=0.8 μM) followed by (-)-epicatechin (IC<sub>50</sub>=1.8 μM), gallic acid (IC<sub>50</sub>=2.2 μM) and EGCG (IC<sub>50</sub>=3.8 μM). Docking analyses showed different modes of interaction of the compounds with the active sites of ARG-L and ARG-1. Due to the low IC<sub>50</sub> values obtained for ARG-L, flavanols can be used as a supplement for leishmaniasis treatment. **Supported by:**FAPESP

**QT038 - DOCKING ANALYSIS AND INTERACTION KINETICS OF TRIAZOLOPYRIMIDINE DERIVATIVES INHIBITING ARGINASE FROM *LEISHMANIA (LEISHMANIA) AMAZONENSIS***

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Arginase is a glycosomal enzyme in *Leishmania* that is involved in polyamine and trypanothione biosynthesis. The central role of arginase in *L. (L.) amazonensis* was demonstrated by the generation of two mutants: one with an arginase lacking the glycosomal addressing signal and one in which the arginase coding gene was knocked out. Both of these mutants exhibited decreased infectivity. Thus, arginase seems to be a potential drug target for *Leishmania* treatment. In an attempt to search for arginase inhibitors, twenty-nine derivatives of the [1,2,4]triazolo[1,5-a]pyrimidine system were tested against *L. (L.) amazonensis* arginase *in vitro*. The [1,2,4]triazolo[1,5-a]pyrimidine scaffold containing R<sub>1</sub> = CF<sub>3</sub> exhibited greater activity against the arginase rather than when the substituent R<sub>1</sub> = CH<sub>3</sub> in the 2-position. The novel compound 2-(5-methyl-2-(trifluoromethyl)-[1,2,4]triazolo[1,5-a]pyrimidin-7-yl)hydrazinecarbothioamide (30) was the most potent, inhibiting arginase by a non-competitive mechanism, with the K<sub>i</sub> and IC<sub>50</sub> values for arginase inhibition estimated to be 24 ± 1 μM and 15.9 ± 0.6 μM, respectively. Docking analysis showed that the hydrazinecarbothioamide substituent in compound 30 forms a hydrogen bond with Asp141, which is involved in manganese cofactor coordination with the active site of the arginase enzyme, and with Asn143, His154 and Asp194 in the active site. These results can guide the development of new drugs against leishmaniasis based on [1,2,4]triazolo[1,5-a]pyrimidine derivatives targeting the arginase enzyme. **Supported by:**FAPESP - FAPERJ

**QT039 - ACTIVITY OF A PALLADACYCLE COMPLEX AGAINST *LEISHMANIA*  
(*LEISHMANIA*) *AMAZONENSIS***

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The present study evaluated the leishmanicidal activity of one palladacycle complex, [Pd<sub>2</sub>(S(-)C<sub>2</sub>,N-DMPA)<sub>2</sub>(m-DPPE)]Cl<sub>2</sub>, DPPE 1.1. The compound was first tested on the growth of *L. (L.) amazonensis* promastigotes in axenic medium and as a control drug parasites were grown in the presence of amphotericin B. After 96 h of incubation the IC<sub>50</sub> values for DPPE 1.1 and amphotericin B were 2.72 nM and 15.06 nM, respectively. The effect of this compound was also tested on *L. (L.) amazonensis* amastigotes by the treatment of infected mouse bone marrow macrophages and Glucantime was used as a control drug. DPPE 1.1 was added at 125 nM to 1,000 nM 24 h after macrophage infection by *L. (L.) amazonensis* amastigotes and the cultures were examined after 5 days. A significant, dose-dependent decrease in infection index was observed with DPPE 1.1, with inhibition of 78%, 73%, and 95%, respectively, 3, 5, and 7 days after treatment. The IC<sub>50</sub> of DPPE 1.1 and Glucantime (expressed as µg/ml of pentavalent antimony [Sb<sup>V</sup>]) on *L. (L.) amazonensis* amastigotes was 93,01 nM (0.090 µg/ml) and 178.5 µg/ml, respectively. The macrophage cytotoxicity of DPPE 1.1 on bone marrow macrophages was evaluated by the MTT assay and a CC<sub>50</sub> of 603 nM was determined, resulting in the selectivity index of 6.48. The stability at -20°C of DPPE 1.1 prepared in 100% of DMSO was also demonstrated. Studies on leishmanicidal effect of DPPE 1.1 *in vivo* are currently in progress in BALB/c and C57BL/6 mice infected with *L. (L.) amazonensis*. **Supported by:**FAPESP

**QT040 - LEISHMANICIDAL EFFECT OF SYNTHETIC TRANS-RESVERATROL ANALOGS**

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Leishmaniasis is a public health problem in at least 98 countries and its therapy is based on pentavalent antimonials, pentamidine, amphotericin B and miltefosine. However, these drugs have limitations, such as toxicity, difficult administration and high cost, which may limit their use. All this, associated to the few advances made in relation to the development of new drugs and therapeutic approaches for this disease, stimulates search of new alternatives for the treatment of leishmaniasis. Natural products are potential source of novel active molecules that may provide structural template for drug discovery. Stilbene-based compounds show antitumor, antioxidant, antihistaminic, anti-inflammatory and antimicrobial activities. The aim this study was to evaluate the effect of Pterostilbene (PT), Piceatannol (PI), Polydatin (PO) and Oxyresveratrol (OX) against *Leishmania amazonensis*. In this study we first evaluated the cytotoxicity of trans-resveratrol analogs for host cells by the XTT method. Our results demonstrated low cytotoxicity of PT, PI, PO and OX for murine macrophages. However, our results show that PT, PI, PO and OX presented an anti-*L. amazonensis* activity with an IC<sub>50</sub> of 18µM, 65µM, 95µM and 65µM for promastigotes, respectively, while for intracellular amastigotes the IC<sub>50</sub> were 33.2µM, 45µM, 29µM and 30.5µM, respectively. Only PI was able to alters the cell cycle of the parasite, increasing 5 times the G0 phase and decreasing 1.7 times the G2 phase, besides change the mitochondrial membrane potential (ΔΨ<sub>m</sub>) of the parasite, and also increase the number of annexin V positive promastigotes, which suggests death by apoptosis. In summary, our results show the anti-*Leishmania amazonensis in vitro* activity of trans-resveratrol analogs, and also demonstrated PI capacity to induce apoptosis in the promastigotes, which suggest these compounds as promising candidates for future studies regarding treatment of leishmaniasis. **Supported by:**CAPES, FAPERJ e CNPq

QT041 - **TRYPANOSOMA CRUZI MITOCHONDRIAL SWELLING AND MEMBRANE POTENTIAL COLLAPSE AS PRIMARY EVIDENCE OF THE MODE OF ACTION OF NAPHTHOQUINONE ANALOGUES**

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Naphthoquinones are privileged structures in medicinal chemistry due to the biological effects associated with the induction of oxidative stress. The present study evaluated the activities of sixteen naphthoquinone derivatives on *Trypanosoma cruzi*. Four compounds, the naphthoquinone (NQ1) and three juglone derivatives (NQ8, NQ9 and NQ12), were the most active. These compounds were also tested on epimastigotes and intracellular amastigotes. Bloodstream trypomastigotes were more susceptible to NQ8, whereas epimastigotes were more susceptible to NQ1. Ultrastructurally, NQs induced alterations in the mitochondrion, the development of autophagic features and flagellar blebbing. NQ8 caused a remarkable reduction in TMRE fluorescence and totally disrupted the mitochondrial membrane potential (DYm) of about 20% at its IC<sub>50</sub> value. This naphthoquinone led to an increase in the percentage of DHE+ parasites, which is indicative of ROS production and confirms the effect on DYm. We hypothesized that the strong redox effect of NQ8 was due to the presence of the acetyl group in the chemical structure that facilitates this quinone reduction, however the existence of more than one mechanism of action and the suppression of ROS generation by the detoxification system of the parasite could not be discarded. **Supported by:**CNPq, FAPERJ and FIOCRUZ

QT042 - **AZOLES DERIVATIVES WITH POTENTIAL ANTILEISHMANIAL ACTIVITY IN BALB/C MICE**

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Leishmaniasis is an endemic disease that represents suffering for people in developing countries. There are current problems such as toxicity and resistance of parasites to drugs. The search for new treatment is urgent. Mouse is a model of the study to immune response and treatment of leishmaniasis. Some strains of mice have increased resistance or susceptibility to leishmaniasis. The objective of this work was to study the potential therapeutic action of a synthetic derivative Imidazole with confirmed *in vitro* activity against promastigotes forms of *Leishmania*. Susceptible BALB/c mice were infected with 10<sup>6</sup> promastigotes forms of *L.amazonensis*. Infected animals were orally treated with DMSO (control group), reference drug Ketoconazole (50mg/Kg), and different doses of Imidazole 45mg/Kg, 30mg/kg and 15mg/kg. Cutaneous lesion was measured weekly and the analysis of parasite load was performed by limiting dilution of the homogenized popliteal lymph node at end of treatment. Infected treated mice with doses of 15 and 45mg/Kg demonstrated significant reduction in the size of the cutaneous lesions (P = 0.0113) compared to the control group or infected treated with ketoconazole. Mice treated at a dose of 30mg/kg have not controlled the evolution of cutaneous lesion but showed a lower parasite load (p <0.0021). Preliminary histopathology demonstrated an intense inflammatory infiltrate consisting of macrophages, lymphocytes, neutrophils and eosinophils in all groups, but with a lower incidence in the infected treated groups at the dose of 15 and 45 mg/kg. Those two groups had lower macroscopic and microscopic alteration in the epidermis and dermis. However, infected treated group at a dose of 30mg/kg demonstrated inflammatory cells at cutaneous lesion with lower intensity of vacuolated macrophages with amastigotes forms. It is necessary efforts to continue this study with new approaches to better understand immune response to a new potential drug to leishmaniasis. **Supported by:**PROPPI/UFF

QT043 - ***IN SILICO* EVALUATIONS AND ANTI-PROMASTIGOTE ACTIVITY OF THREE INDOLE ALKALOIDS FROM *TABERNAEMONTANA* GENUS AGAINST *LEISHMANIA AMAZONENSIS***

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Leishmaniasis still a worldwide health problem affecting more than 12 million people around the world. Although the wide distribution in more than 88 countries, few advances are observed in Leishmaniasis treatment. The first-line choice still the Antimonials. Miltefosine, and new Anphotericin B formulations, were the two major advances in Leishmaniasis treatment. Alkaloids are among the most active natural molecules from many diseases including Leishmaniasis. Computational software analysis, called *in silico* evaluations help to predict if a molecule behaves as drug, analyzing determining its druglikeness and drug-score. The aim of this study is for the first time evaluate the anti-promastigote activity of three indole alkaloids isolated from species of *Tabernaemontana* genus, Ibogamine (IBOG), Tabersonine (TABS) and 12-Methoxy-Nb-Methylvoachalotine (MVOA) against *Leishmania amazonensis* and also make *in silico* analysis comparing the druglikeness and drug-score of them. These last evaluations showed that all compounds attend Lipinski "Rule of 5" (LIPINSKI, 2001). The druglikeness for TABS, IBOG and MVOA were 2.68, 1.44, 0.45, respectively. This result suggests the alkalonine ring confers better properties to be a drug in TABS, although the isoquinuclidinic ring in IBOG also confers the same. The drugs showed a relevant inhibition in promastigote growth of 97.5, 96 and 67.4% when treated with 50mg/mL of IBOG, TABS and MVOA, respectively, after 72 hr of treatment. The IC<sub>50</sub> (Inhibitory Concentration of 50%) for promastigotes were of 15.2, 29,9 and 44.8µg/mL for IBOG, TABS and MVOA suggesting the isoquinuclidinic ring of IBOG is most important for leishmanicidal effect, which corroborates the previous observation with other Iboga-type alkaloids Coronaridine and 18-MC. In conclusion, our results stimulate the better characterization the leishmanicide effect of these compounds using amastigote forms and other *Leishmania* species, besides the investigation of their toxicity. **Supported by:** MackPesquisa, CNPq, FAPERJ, Hebron Farmacêutica

QT044 - **EFFECT OF 2",3"-DIHYDROOCHNAFLAVONE BIFLAVONOID ISOLATED FROM *LUXEMBURGIA NOBILIS* ON *TRYPANOSOMA CRUZI* Y STRAIN.**

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*Trypanosoma cruzi* is the causative agent of Chagas disease which affects 18 million people in Latin America. However, drugs that effectively destroy the parasites without causing side effects to patients are still not available. Natural products have been considered potential alternative chemotherapy agents. 2",3"-dihydroochnaflavone is a biflavonoid isolated from *Luxemburgia nobilis* (Ochnaceae), a shrub that is distributed throughout southeastern Brazil. The antitumor activity of this compound was previously attributed to Ochnaceae family members by Daniel *et al.* (2007), thus in this work we investigated the potential use of this biflavonoid as a chemotherapeutic agent against Chagas disease. Our results showed that this compound greatly affects *T. cruzi* Y strain epimastigotes proliferation after treatment using different concentrations 15, 30, 45 and 60 µg/ mL for four days. This effect was not dose dependent and resulted in a IC<sub>50</sub> of 4.8µg/ mL after 96h of treatment. Parasites treated with 15 µg/ mL of biflavonoid were able to reestablish growth after drug removal and addition of fresh medium, indicating a reversible effect of this compound, which was not observed with higher drug concentrations. Additionally, we demonstrated that the different tested doses of 2",3"-dihydroochnaflavone were not toxic to macrophages or lymphocytes of mice, since at least 80% of viability was maintained after cell treatment as revealed by Trypan Blue assays. Preliminary ultrastructural analyses by transmission electron microscopy indicated that treatment with 5 and 15 µg/ mL of the drug promoted mitochondrial swelling on *T. cruzi* epimastigotes. Our next step is to verify the biflavonoid effect in the intracellular amastigote form and if the drug affects trypomastigotes release from infected cells. **Supported by:** CNPq & FAPERJ

**QT045 - POTENTIAL ANTILEISHMANIAL ACTIVITY OF NATURAL PRODUCTS**  
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Leishmaniasis are infectious diseases of public health importance classified according to their clinical manifestations: cutaneous and visceral leishmaniasis. Despite their high incidence leishmaniasis are considered by the World Health Organization as neglected diseases by pharmaceutical industries. The treatment is principally based on pentavalent antimonials, amphotericin and pentamidine. However, treatment is not ideal due to side effects, the high cost of production and increasing number of cases of resistance to the parasite. So, it is necessary to search for new drugs. Brazil has a great biodiversity of species of plants and their potential medicinal may be explored. Extracts derived from Brazilian plants offer new possibilities to obtain new active compounds against *Leishmania* with perspective to development new drugs. Studies prove the highlight of *Clusia Fluminensis* in Brazilian folk medicine. The main phenolic compound present in the genus *Clusia* is a bi-flavonoid that have antioxidant, antifungal, anti-allergic and other biological activities. The objective of this study is to evaluate the potential leishmanicidal activity of extracts of *Clusia fluminensis* on promastigotes forms of *Leishmania amazonensis*. Material and Methods: Cell viability was measured by MTT colorimetric assay and counting in Neubauer chamber by optical microscopy. Then, 10<sup>7</sup> promastigotes forms of *L. amazonensis* were tested in a screening of crude extracts of *C. fluminensis* to obtain EC<sub>50</sub>/24h. Preliminary results showed that three crude extracts of *C. fluminensis* had promising leishmanicidal activity and all these extracts were eluted in hexane solvent, with respective values of EC<sub>50</sub>/24h followed 3, 4 and 24 µg/mL. Conclusion: These preliminary assessments are promising and needs to be explored with prospect of developing new drugs against leishmaniasis. **Supported by:**CNPQ,FAPERJ,PROPPI/UFF

**QT046 - IN VIVO TRYPANOCIDAL ACTIVITY IN A MURINE MODEL OF ACUTE CHAGAS DISEASE BY A DITHIOCARBAZATE COMPOUND**  
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Introduction: American Trypanosomiasis or Chagas disease is endemic in Latin America. According to the World Health Organization (WHO), 12 million people are infected with the *T. cruzi*, resulting in an annual death toll of 50,000. The development of new antichagasic drugs is needed since the only one in current use in most of the countries, benznidazole<sup>®</sup> presents several undesirable side effects and is effective mainly in the acute form of the disease. Here we report that 5-hydroxy-3-methyl-5-phenyl-pyrazoline-1-(S-benzylthiocarbamate) (H<sub>2</sub>bdtc) exhibits high *in vivo* trypanocidal activity when compared to benznidazole<sup>®</sup>. Objective: The present work reports *in vivo* trypanocidal activity in a murine model of acute and chronic Chagas disease. The activity of aqueous and encapsulated H<sub>2</sub>bdtc is described. Method: The mice were infected with Y strain trypomastigote form of *T. cruzi* and distinct groups were treated with either benznidazole<sup>®</sup>, free H<sub>2</sub>bdtc or encapsulated-H<sub>2</sub>bdtc. After treatment, histological analysis, serum activity of Creatine Kinase Isoform MB (CK-MB) and PCR analysis were performed. Results: This work revealed that mice that received H<sub>2</sub>bdtc loaded in SLNs doesn't show cardiac lesion while free H<sub>2</sub>bdtc showed 50 % reduction. We have also found that *in vivo* treatment with encapsulated-H<sub>2</sub>bdtc was significantly more effective in reducing the parasite burden compared with the other groups for the acute phase. Conclusion: We found that killing the parasites is most likely the mechanism by which this compound acts in reducing tissue lesions and enhancing mice survival. **Supported by:**FAPESP and CNPQ

**QT047 - LEISHMANICIDAL ACTIVITY OF SEMIPURIFIED FRACTIONS OF *MOMORDICA CHARANTIA* (CUCURBITACEAE)**

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Leishmaniasis is a group of neglected tropical diseases that presents a high morbidity rate, distributed in 98 endemic countries. It is constituted by different clinical forms and the current treatment presents difficulties such as high cost, toxicity and resistance cases. This study aimed to evaluate the leishmanicidal activity of *Momordica charantia* (Cucurbitaceae). Promastigotes of *Leishmania amazonensis* were cultured in the presence of several concentrations of the crude extract and its derived partitions up to 200 µg/mL for 72 hours and quantified colorimetrically by MTT assay. The fractions were tested in promastigotes and the ethyl acetate fraction was significantly the most active. The ethyl acetate partition was fractionated on sephadex and the partition named F7 was significantly more active than the others with IC<sub>50</sub> of 4.5 µg/mL. F7 was fractionated and three pure not yet characterized cucurbitacines were obtained (F1, F3 and F5), with the following IC<sub>50</sub>: 10.88 µg/mL, 11.42 µg/mL and 22.71 µg/mL. To evaluate the activity of the fractions in amastigotes, macrophages were infected with *L. amazonensis* and incubated with several concentrations of the semipurified fractions. The infectivity index was determined by optical microscopy. Macrophages treated with these partitions showed a reduction in the levels of infection when compared to the control group. The most active fraction was the F7, with an IC<sub>50</sub> of 2,1 µg/mL. The cucurbitacines showed the following IC<sub>50</sub>: 3.3 µg/mL, 3.1 µg/mL, 5.9 µg/mL. To evaluate the toxicity, murine macrophages were incubated with the fractions for 72 hours. The effect on the viability of the macrophages was quantified by MTT and the LD<sub>50</sub> values were higher than 30 µg/mL, indicating a selectivity index more than 10-fold. The semipurified fraction F7 was more active than the pure substances, probably due to a synergic effect among its constituents, and it can be a promising phytotherapy treatment for leishmaniasis. **Supported by:**CNPQ/PAPES, FAPERJ

**QT048 - COMPUTATIONAL APPROACHES TO ANALYSIS BIOPHARMACEUTICAL AND TOXICS PROPERTIES OF NEW ANTILEISHMANIAL PYRAZOLYL BENZENESULFONAMIDES DERIVATIVES**

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Leishmaniasis is important disease observed in several geographic regions affecting millions of people. Therapy currently used has low efficacy, high toxicity and cases of resistance of the parasite. The process of discovering and developing a new drug involves an interdisciplinary collaborative research between Computational Biology, Chemistry, Biology, Medicine, among others. Some compounds with sulfonamide or pyrazolyl grouping in its structure have important biological activities such as antibacterial, anti-inflammatory or antileishmanial. The objective of this study was to obtain theoretical predictions about the behavior of 10 new synthetic molecules derived from pyrazolyl benzenesulfonamides with potential antileishmanial activity. Synthetic compounds were analyzed *in silico* through the software Osiris Property Explorer. The software evaluates parameters of toxicity, carcinogenicity, irritability, mutagenicity and reproductive, which are calculated by fragmentation of the molecule and the comparison with a data base. Since good absorption is necessary for oral administration, we analyzed these derivatives according to the rule-of-five developed by Lipinski (2001) and only one of these parameters may be contradicted. None of the 10 compounds analyzed showed theoretical toxic effect, according to the Osiris software. According to Lipinski, three drugs are outside the desired standards for good absorption (D5, D9, and D10), because their high values of ClogP and molecular weight. The cytotoxic tests using murine adherent peritoneal cells pointed out that D5 had worse CC<sub>50</sub> values than pentamidine. *In silico* studies of the parameters of Lipinski's Rule of Five, as well as the drug likeness and drug score indicate that these compounds, especially D6, has potential to be new drug candidates. In studies of antileishmanial activity, some compounds exhibited good results, being more potent than the standard drug pentamidine. **Supported by:**PROGRAD/UFF

**QT049 - SYNTHESIS AND ANTILEISHMANIAL ACTIVITY OF NOVEL TETRAZOLE COMPOUNDS AND THEIR PYRAZOLE-4-CARBONITRILE PRECURSORSSYNTHESIS AND ANTILEISHMANIAL ACTIVITY OF NOVEL TETRAZOLE COMPOUNDS AND THEIR PYRAZOLE-4-CARBONITRILE PRECURSORS**

BECKER, K.M.<sup>1</sup>; FARIA, J.V.<sup>2</sup>; DOS SANTOS, M.S.<sup>3</sup>; BERNARDINO, A.M.R.<sup>2</sup>; LEON, L.L.<sup>1</sup>; RODRIGUES, R.F.<sup>1</sup>; CANTO-CAVALHEIRO, M.M.<sup>1</sup>

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Leishmaniasis is a protozoan disease that affects about 12 million people in the world, particularly in subtropical and tropical regions, causing a serious public health problem. Current chemotherapy of leishmaniasis is unsatisfactory. Efficacious and safe new drugs are needed. In the present work,

a new series of 5-(1-aryl-3-methyl-1H-pyrazol-4-yl)-1H-tetrazole derivatives and precursors 1-aryl-3-methyl-1H-pyrazole-4-carbonitrile were synthesized and evaluated *in vitro* as antileishmanial activities against *Leishmania braziliensis* and *Leishmania amazonensis*. In parallel, the cytotoxicity of these compounds was evaluated on RAW 264.7 cell line. The results showed that among compounds assayed the substituted 3-chlorophenyl ( $IC_{50}/24h = 15 \pm 0.14 \mu M$ ) and 3,4-dichlorophenyl ( $IC_{50}/24h = 26 \pm 0.09 \mu M$ ) were the most potent against *L. braziliensis* promastigotes. The reference drug Pentamidine presented  $IC_{50} = 13 \pm 0.04 \mu M$ . In addition, 3-chlorophenyl and 3,4-dichlorophenyl derivatives were less cytotoxic than Pentamidine. However, the tetrazole derivatives and also pyrazole-4-carbonitriles precursors differs against each of the tested species and were more effective against *L. braziliensis* than on *L. amazonensis*. These results reinforces the 5-[1-(3-chlorophenyl)-3-methyl-1H-pyrazol-4-yl]-1H-tetrazole derivative as potential antileishmanial agent. However, further studies must be performed.

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**QT050 - INHIBITION OF LEISHMANIA AMAZONENSIS ARGINASE BY ISOLATED COMPOUND FROM ETHANOLIC EXTRACT FROM PLANT PLATHYMENIA RETICULATA BTH.**

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The plant *Plathymania reticulata* is used in Brazilian ethnomedicine as antiinflammatory and has leishmanicidal activity against promastigotes from *Leishmania amazonensis*. Arginase is the first enzyme of the polyamine (PA) pathway and was considered a target to control *Leishmania* infection. Enzymes of the PA synthesis pathway are considered to be important targets for drug development against leishmaniasis. PAs have a central role in proliferation, differentiation, and antioxidant mechanisms in *Leishmania*. Antioxidant mechanisms in trypanosomatids use the PA spermidine to synthesize trypanothione. Trypanothione protects the parasite from oxidative stress by promoting the removal of reactive nitrogen species reactive oxygen species and other reactive species produced by the host's defense system. The aim of this study was to evaluate the inhibition of the arginase enzyme by *P. reticulata* ethanolic extract (EE). Recombinant arginase was expressed, purified and was used to inhibition tests. EE was prepared in 50% ethanol/water and was characterized by HPLC using RP-Amide (Ascentis) column with a linear gradient (10-30%) of acetonitrile. Preparative thin layer chromatography (TLC) was used to isolate the major constituent of EE. A sigmoidal model (log  $IC_{50}$ ) was used to determine the  $IC_{50}$ . The isolated compound by TLC was analysed by HPLC and shows a retention time of 27.15 minutes, and a characteristic UV/VIS spectra of polyphenols. The ethanolic extract inhibits 95% of arginase activity at 500  $\mu g/mL$  and the isolated compound showed an  $IC_{50}$  of the  $2.7 \pm 0.4 \mu g/mL$ . In conclusion, *P. reticulata* contains a compound that is a potent arginase inhibitor and can be responsible to leishmanicidal activity of the plant extract. **Supported by:** PIBIC - RUSP

**QT051 - EVALUATION OF THE THERAPEUTICAL EFFICACY OF A SESQUITERPENE LACTONE IN A NANOSTRUCTURED FORMULATION ON EXPERIMENTAL CHAGAS DISEASE**

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TP is a lipophilic substance active in vitro against *T. cruzi* isolated from *Lychnophoras* (Arnica). The use of nanostructured (NS) formulations as a carrier for lipophilic compounds improves its therapeutic efficacy and reduces toxicity. The only drug available to treat Chagas disease is benznidazole (BZ). This work evaluates the activity of TP compared to BZ on the treatment of the acute phase of the infection with *T. cruzi* Colombian strain, resistant to treatment, in murine model. TP was isolated and characterized previously, as well as its NS polymeric formulations, one with conventional polymer (NSTP1), other with stealth polymer (NSTP2). Young female Swiss mice 20g of weigh were intraperitoneally inoculated with 10000 blood trypomastigotes of Colombian strain. The intravenous treatment started in the 7th day of infection for 20 consecutive days. The mice were divided in groups: NSTP1 (2mg/kg/day), NSTP2 (2mg/kg/day), free TP (2mg/kg/day), BZ (50mg/kg/day) and negative controls. Parasitemia was evaluated daily by fresh blood exam for 30 days and the survival was registered. Parasitological (hemoculture and PCR) and serological (ELISA) tests assessed treatment efficacy. Only NSTP2 group presented subpatent parasitemia in all mice (100%) and the other groups showed patent parasitemia. Percentages of 100%, 75% and 62.5% of the animals treated with NSTP2, NSTP1, BZ respectively, survived and were necropsied in the 6<sup>th</sup> month of infection. No survival was observed in animals treated with free. The negative controls survived for 20 days. Parasitological and serological tests showed that only NSTP2 was able to cure 62.5% of the animals, while NSTP1 and BZ did not cure. The polymeric constitution of the formulations improved the pharmacokinetic parameters of TP, maintained its high concentration in plasma and increased its efficacy. This work evidences TP's efficacy in NS formulations for treatment of experimental Chagas disease.

**QT-052 - Antileishmanial activity of lapachol analogues synthesized using Suzuki-Miyaura coupling methodology.**

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Considered by World Health Organization (WHO) a neglected disease, leishmaniasis affects over 12 million people in the world per year. Its treatment receives low investment from the pharmaceutical industries, requires long periods of administration and presents low efficacy, resistance, toxicity and high cost. Quinones represent a very important class of molecules which is directly related to many metabolic processes in the cell and present trypanosomicidal effect. The purpose of this study is to evaluate the activity of 12 derivatives from lapachol in promastigotes and intracellular amastigotes of *L. amazonensis* and their toxicity in murine macrophages. In tests of antileishmanial activity, we observed that lapachol, as described in the literature, showed activity on promastigotes, but the IC<sub>50</sub> on amastigotes was higher than the maximum evaluated concentration. The substitution of the prenyl group for aromatic ring groups originated a series with less toxicity, compared to lapachol, reflected by the increase in the LD<sub>50</sub> value on the host cell. From this series, LQB-180 presented the best selectivity index, becoming the most promising prototype. Addition of the carbamate group potentiated the leishmanicidal activity when compared to lapachol; however, the toxicity on mammalian cell increased proportionally. When both groups, aromatic and carbamate, were added together in the same core, the new series presented similar results. This series had the effect on amastigotes potentiated, compared with other substitutions groups; however, toxicity remained high. Thus, it was shown that the addition of the carbamate group in R<sub>3</sub> increases the leishmanicidal activity, and the prototypes (LQB-180) and (LQB-270) (R<sub>1</sub>=R<sub>2</sub>=OMe) were the most promising. This structure-activity study reveals that the series with addition of a carbamate group in R<sub>3</sub> needs further studies to improve its selectivity. **Supported by: CNPq/PAPES, PIBIC**