Quimioterapia- Chemotherapy

QT01 - Characterization of genes encoding enzymes involved in antioxidant defense in *Trypanosoma cruzi* populations susceptible and resistant to benznidazole

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Antioxidant defense in trypanosomatids have been indicated as a potential target for chemotherapy. This system is based on low molecular weight thiol trypanothione, which maintain the reduced intracellular environment by the action of trypanothione reductase (TR). The pathways that metabolize the hydrogen peroxide to water molecules surrounding the cytosolic (cTXNPx) and mitochondrial (mTXNPx) tryparedoxin peroxidase enzymes and ascorbate peroxidase (APX). In the present work, the genes encoding cTXNPx, mTXNPx and APX antioxidant enzymes were characterized in 10 benznidazole-resistant and susceptible *Trypanosoma cruzi* strains.
T. cruzi populations susceptible and resistant to benznidazole (BZ). Northern blot and real-time RT-PCR analyses revealed that the levels of TcTXNPc and TcTXNPm mRNA were two-fold higher in the *T. cruzi* population with *in vitro*-induced resistance to BZ (17LER) than its drug-susceptible counterpart (17WTS). The levels of mRNA for both genes were similar among the other *T. cruzi* samples studied. No amplification of these genes was observed in the parasite genome. In silico analyses indicated that cTcTXNPx and mTcTXNPx genes present eight and two copies, respectively, dispersed in the parasite genome. Western blot analysis using anti-cTcTXNPx and anti-mTcTXNPx polyclonal antibodies showed that the expression levels of these native proteins were similar for all samples, except the 17LER, which displayed two-fold greater expression. In addition, the oxidized mTcTXNPx protein (50 kDa) demonstrated 5.5-fold greater expression in the 17LER population than 17WTS. Phylogenetic analysis of cTcTXNPx and mTcTXNPx proteins from *T. cruzi* and other trypanosomatids showed that they are closely related to their homolog in *T. brucei*. The *T. cruzi* APX enzyme is considered a good target for drug because it is absent in mammalian hosts. The mRNA level and copy number of the TcAPX gene showed no significant difference between *T. cruzi* populations susceptible and resistant to BZ. The TcAPX gene presents two copies dispersed in the parasite genome and it is located in one chromosomal band in all *T. cruzi* strains analyzed. TcAPX protein showed greater similarity to APX from *Leishmania* than plants. The expression level of TcAPX protein was two-fold higher in the *in vivo*-selected resistant BZR *T. cruzi* population than its drug-susceptible counterpart BZS. Based on our results, we suggest that the *T. cruzi* population with *in vitro* resistance to BZ exhibits an increase in TcTXNPx protein levels together with other enzymes associated with peroxide metabolism, such as the previously described TcFeSOD (Nogueira et al., 2006), protecting these resistant parasites against oxidative stress.
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QT02 - The trypanocidal activity of α- and β-lapachone derivatives

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The available chemotherapy for Chagas disease, caused by *Trypanosoma cruzi,* is unsatisfactory, therefore there is an intense effort to find new drugs for treatment of this disease. In our laboratory we analyzed the effect on bloodstream trypomastigotes of 19 new naphthoquinones, aiming to establish a correlation between the chemical structure and the trypanocidal activity. The derivatives were prepared via methylene and aryl *o*-quinone methides (*o*-QMs) generated *in situ* by Knoevenagel condensation of 2-hydroxy-1,4-naphthoquinone (lawsone) and formaldehyde or arylaldehydes followed by hetero Diels-Alder reaction with substituted styrenes. Quinones have been extensively studied for their variety of biological effects, including antitumoral, and their biological profile is based on their *ortho* or *para*-quinonoid moiety that generally accepts one and/or two electrons (redox cycling) to form the corresponding radical anion or dianion species *in situ*. The parasites were treated for 24h in DMES medium containing 5% blood at 4°C. The compounds presented a broad spectrum of activity, presenting five derivatives IC_{50}/24 h in the range of 22 to 63 μM, while the parent compound β-lapachone, the corresponding value was 48.4 ± 6.6 μM. The most active compounds will be further investigated in relation to their mode of action and *in vivo* effect.
Supported by CNPq, Faperj and Fiocruz

QT03 - The phospholipid biosynthesis pathway: A hope to Chagas disease chemotherapy

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Chagas disease, caused by *Trypanosoma cruzi,* is a public health problem that affects 12 million people in Latin American, and has at least 60 million people living at risk of contamination. The current treatment has demonstrated unsatisfactory results and ineffectiveness to acute stage and prevalent chronic stage, besides side effects. Lysophospholipid analogues (LPAs) were designed as potential antimetabolites of phospholipid metabolism, and were shown to display activity against tumor cells and pathogenic trypanosomatids. The phosphocholines miltefosine, ilmofosine and edelfosine, have been found to be active against *T.cruzi in vitro*, inducing plasma membrane alterations in epimastigote and trypomastigote forms. Our group has been testing new phospholipid analogues (TC19, TC70, TC104, TC95 and TCAN26) to evaluate their effects against *T.cruzi* infections aiming to a better treatment for Chagas disease. Our previous results, in epimastigote forms, showed IC_{50} values of 100nM in TCAN26 and TC70, 5 times lower than IC_{50} obtained with other phospholipid compounds tested, being able to inhibit epimastigote proliferation. Induction of drastic structural alterations such as shortening of the flagellum after treatment with TCAN26 and TC70 for at least 24 hours were observed by scanning electron microscopy. Recently, fluorescence microscopy assays, using anti-tubuline antibodies and DAPI, confirmed these alterations and also showed that some kinetoplasts were rounded, as seen in trypomastigote forms. When trypomastigote were treated with the effective analogues (cited above) at 50nM, 100nM, 1,0μM and 3,0μM for 24 hours, a decrease in trypomastigote number (about 70% of reduction) was observed. Transmission electron microscopy analysis demonstrated that 100nM TCAN26 is able to induce kinetoplast disturbance and mitochondrial swelling, besides membrane blebs and autophagic structures. In the intracellular replicative form, amastigote, our preliminary results showed a parasite's multiplication reduction in infected peritoneal macrophages, even after 24 hours of treatment with TC70 and TCAN26.
Supported by: CAPES, FAPERJ and CNPq

QT04 - HISTOPATHOLOGICAL EVALUATION OF HEART LESIONS DURING CHRONIC PHASE OF *TRYPANOSOMA CRUZI* INFECTION IN DOGS AFTER ACUTE ETIOLOGICAL TREATMENT WITH BENZNIDAZOL

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The efficacy of the etiological treatment of chagasic patients with drugs including Nifurtimox and Benznidazol in parasitological cure or prevention of the development of chronic Chagas disease is very controversial. The aim of this study is to evaluate the benefit of the etiological treatment administered during acute phase in the prevention of severe heart lesions with the evolution for chronic disease. For this, 14 dogs were infected with Colombian or Y strains, resistant and partially resistant to Benznidazol respectively. The treatment schedule was 7 mg/ Benznidazol/Kg administered in two doses daily for 45 days, 12–22 days after *T. cruzi* detection. Hemoculture, PCR and serological tests were performed and demonstrated that benznidazole treatment induced 100% of the cure in animals infected with Y strain and 0 % in those infected with Colombian strain. Untreated and treated animals were euthanasied 180 days after treatment and heart fragments were collected for morphometric analysis in Hematoxylin-Eosin, Masson Trichromic and anti-*T. cruzi* immunohistochemistry preparations. No tissue parasites were observed, independent of the treatment. Morphometric evaluation of inflammatory infiltrate in untreated animals infected with Y or Colombian strains and Colombian treated animals showed a similar number of cells. However, in treated animals infected with Y strain, a significant reduction of inflammatory cells was observed. On the other hand, the fibrosis was higher in treated not cured Colombian group when compared to untreated group. These data showed that the effectiveness of Benznidazol therapy in preventing the cardiac lesions may be related with the *T. cruzi* population considered.
Supported by CNPq and FAPEMIG.

QT05 - Antiprotozoal activity of lignans isolated from *Phyllanthus amarus*

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The Kinetoplastida order includes the genus *Trypanosoma* and *Leishmania*, both digenean parasites that alternate between two different hosts: a mammal and a vector insect. These parasites are the causative agents of Chagas' disease and Leishmaniasis, respectively. The currently drugs available for treatment of these infections are unsatisfactory due to limited efficacy and toxic side effects, making the search for more specific pharmacological agents a priority. Genus *Phyllanthus* has been investigated to determine the constituents with pharmacological activities that frequently are attributed to lignans, glycosides, flavonoids, alkaloids, ellagitannins and phenylpropanoids. *Phyllanthus amarus Schumach & Thonn* (Break-stone) is commonly used as hepatoprotective and to treat diabetes and hypertension. Pharmacological evaluation showed that this plant possesses anti-inflammatory, antimicrobial, antimutagenic, anticarcinogenic, and antiviral activity. Three Lignans (phyllantin, hypophyllantin and nirantin) obtained from hexanic extract of *P. amarus* were screened for their antiprotozoal activities against *L. amazonensis* and *T. cruzi*. The cytotoxic activity against mammalian cells lines was evaluated by sulphorodamine B technique. Proliferative forms of *L. amazonensis* and *T. cruzi*, as well as trypomastigotes of *T. cruzi* were treated with several concentrations of the lignans and the parasite growth was determined by counting. The lignan nirantin was more active than phyllantin and hypophyllantin with antiproliferative effects for promastigote (IC_{50} 8.5 µg/mL) and axenic amastigote (2.7 µg/mL) of *L. amazonensis* and epimastigote (9.0 µg/mL) of *T. cruzi*. All lignans were inactive against trypomastigote forms. The toxicity for cells and the activity against the parasites were compared by using the selectivity index (SI) ratio (CC_{50} for cells/IC_{50} for parasite). Nirantin showed to be more toxic to parasites than to mammalian cells with SI at 46.7 for *L. amazonensis*, while for *T. cruzi* the SI was 27.2. The nirantin showed good activity against the parasites and represents an exciting advance in the search for new antiprotozoal agents.

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QT06 - MIGRATION OF *ACANTHAMOEBA* INTO AN ORGANOTYPIC SKIN MODEL AND TREATMENT WITH MILTEFOSINE

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Acanthamoeba spp. are the causative agents of *Acanthamoeba* keratitis (AK) on one hand and of often fatal opportunistic infections such as skin lesions, pneumonitis and granulomatous amoebic encephalitis on the other hand. Treatment is problematic, particularly in case of the opportunistic *Acanthamoeba* infections where there is currently no drug of choice. Miltefosine is known to be highly effective against *Acanthamoeba* and other amphizoic amoebae *in vitro* and due to the lack of an established medication miltefosine has recently been applied for topical treatment of *Acanthamoeba* skin lesions leading to rapid and complete healing of the lesions.

In the current study an organotypic skin model was adapted for investigating the migration of *Acanthamoeba* into the skin and for evaluating the suitability of miltefosine for the topical treatment of *Acanthamoeba* infections. Moreover, the susceptibility of other important opportunistic pathogens to miltefosine and synergistic and adverse effects of combinations of miltefosine with other anti-*Acanthamoeba* substances were revealed. Acanthamoebae were shown to penetrate the skin within 48 h, while treatment with miltefosine prevented this penetration. Moreover, it was shown that miltefosine in dilution can be stored over a period of 6 months at 4°C without any loss of activity. The toxicity of anti-*Acanthamoeba* effective concentrations of miltefosine lies considerably below those of substances used for AK treatment.

Miltefosine has been successfully used for the oral and topical treatment of leishmaniosis and may also be a promising new candidate for the topical treatment of *Acanthamoeba* infections.

QT07 - EFFECTS OF TOMATIDINE ON THE ENERGY METABOLISM AND ULTRASTRUCTURE OF *Leishmania amazonensis*

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Some plants produce substances for their own defense against pathogens and predators. In *Lycopersicon* species, such as tomato *L. esculentum*>, the main antimicrobial compound is the steroidal glycoalkaloid α-tomatine. The loss of saccharide side chain of tomatine produces the aglycone tomatidine. The leishmaniasis are clinically different infectious diseases caused by flagellated protozoan of the genus *Leishmania*, which have a cosmopolitan distribution and afflict millions of people worldwide. In the present study we shown that tomatidine inhibits the growth and promotes alterations in the ultrastructure of *Leishmania amazonensis*>. Through transmission electron microscopy was shown that cells treated with tomatidine presented remarkable lesions, such as alterations in the mitochondrial structure and vacuolization. LDL endocytosis was significantly increased in cells treated with tomatidine. It was also observed that cells treated with tomatidine presented loss of membrane potential and depletion of ATP levels. In previous studies we found that tomatidine had similar effects on growth and ultrastructure on *Phytomonas serpens*, a tomato parasite, with a concomitant inhibition of the ergosterol biosynthesis. These results indicate that tomatidine seems to have similar mechanism of action on the different trypanosomatids and should be considered as new promissory drugs against parasite diseases.
Key words: *Leishmania*, tomatidine, sterol biosynthesis inhibitors.

QT08 - LEISHMANICIDAL ACTIVITY OF COPAIFERA SPP. ESSENTIAL OILS

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Current treatment for leishmaniasis include toxic, expensive, side-effecting drugs as pentavalent antimonial, amphotericin B and pentamidine, Searching for new agents to fight against this neglected disease is imperative, and natural products may represent a source to find new drugs with this purpose. In this study, the in vitro leishmanicidal activity of four commercial copaíba oils (Copaifera spp; C1 to C4) was evaluated. The content of sesquiterpenes and diterpenes as well as the individual constituents were chemically quantified by GC-FID, using caryophyllene or copalic acid as the external standard. The oils were assayed against Leishmania amazonensis on promastigotes and amastigotes, as well as host cell citotoxicity and nitric oxide production by the macrophages. Promastigotes in logarithmic growth phase (106 parasites) were cultured in the presence or absent of 50 µg/ml of each sample and the activity was evaluated by cell counting at different time points. The leishmanicidal activity was also assayed in murine macrophage infected with L. amazonensis (10 parasites/cell) and treated with different concentrations of the oils during 24 h. The host cell citotoxicity was assayed by XTT method and nitrite oxide production in the supernatants of macrophages was measured by the Greiss reaction. C2 and C3 showed the higher activity against promastigotes while C1 and C4 were more active against amastigotes (IC50 6.6 and 3.8 µg/ml, respectively for C-1 and C-4). None of them was toxic for the host cells at the concentrations used. Therapeutic indices calculated were C1 12 and C4 24,5. These preliminary results suggest that the death of the parasites was not caused by the increasing production of nitrite oxide by the macrophages. The efficacy against the parasites was correlated with the chemical composition of the copaiba samples.

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QT09 - HYDROXIMETHYLNITROFURAZONE: *IN VIVO* EVALUATION OF ITS TRYPANOCIDAL AND LEISHMANOCIDAL ACTIVITY.

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Chagas disease is treated with Benznidazol (BZL) or Nifurtimox, both toxic and ineffective in patients during the chronic phase. Similarly, meglumine antimoniate (MA) which cures American Tegumentary Leishmaniasis (ATL) presents adverse effects and numerous cases of resistance. In completely randomized murine models of Chagas disease and ATL we evaluated the new compound hydroximethylnitrofurazone (NFOH) as an alternative to current treatment to these maladies.
For Chagas disease, 48 Swiss female mice were infected intraperitoneally with 1000 trypomastigote forms/mouse. From day 5 post-infection, they received 60 oral doses of 60 mg/Kg/day BZL, 150 mg/Kg/day NF (parental compound), or 150 mg/Kg/day NFOH, in 5% NaCl - 9 % Tween 80 suspension (control group). At 30 and 240 days post-infection conventional PCR and ELISA were performed. Circulating parasites were detected in the control group, causing 66% mortality, compared to 0% NFOH and 10% BZL. PCR and IgG titres were negative at 240 days post-treatment in treated mice; the control group remained positive. While NF was lethal at the administered dose, NFOH caused lower mortality than BZL being also effective in controlling the disease.
For ATL model, 35 Swiss male mice were subcutaneously infected in the right footpad with 10000 *Leishmania (L) amazonensis* promastigote forms/mouse. At 5 days post-infection, they received 30 doses of 300 mg/Kg/day, 150 mg/Kg/day NFOH, or 200 mg/Kg/day MA in 5% NaCl - 9 % Tween 80 (control group). Granulome size was measured from days 49 to 126 post-infection. Lesion development was inhibited between days 49 - 69 (NFOH, 150 mg/Kg/day), and 49 – 63 (MA). At later dates, lesions were similar to those of the untreated mice. 300 mg/Kg/day NFOH was similar to the control group.
Although NFOH showed promising results as an anti-chagasic, its effect as a leishmanocidal drug is not so clear.
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QT10 - BIOLOGICAL, ULTRASTRUCTURAL EFFECT AND SUBCELLULAR LOCALIZATION OF AROMATIC DIAMIDINES IN *Trypanosoma cruzi*

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Chagas disease, caused by the protozoan *Trypanosoma cruzi*, is a public health problem in endemic areas of Latin American. Currently, no vaccines or safe chemotherapeutic agents are available which justifies the search for more efficient and less toxic drugs. Pentamidine and related dications are DNA minor groove-binders which exhibit broad spectrum antiprotozoal activity. Based on these observations, our aim was to evaluate the *in vitro* efficacy of six dicationic compounds - DB1645, DB1582, DB1651, DB1646, DB1670 and DB1627 - against relevant forms of *T.cruzi*: bloodstream trypomastigotes (BT) and intracellular parasites. Intracellular localization and cellular targets of these compounds in treated parasites were also analyzed by fluorescence and transmission electron microscopy (TEM). DB1645, DB1582 and DB1651 were the most active against BT showing IC_{50} values ranging between 0.15 to 6.9 µM. All compounds displayed low toxicity towards mammalian cells and DB1645, DB1582 and DB1651 were also the most effective against intracellular parasites, with IC_{50} values ranging between 7.3 to 13.3 µM. All compounds localized in parasite nuclei and KDNA (with greater intensity in the later structure). Interestingly, two of them, DB1582 and DB1651 also concentrated in punctated non-DNA-containing cytoplasmic organelles possibly acidocalcisomes. TEM revealed striking alterations in mitochondria and kinetoplast of treated parasites, besides important disorganization of microtubules, with the occurrence of multiple axoneme structures. Our data provide further information regarding the activity of this class of compounds upon *T. cruzi* which should aid future design and synthesis of more potent agents that could be used for Chagas disease therapy. Supported by: FIOCRUZ, CNPq, FAPERJ, DECIT-SCTIE-MS and MCT by CNPq, PAPES V-FIOCRUZ. Funding to DWB by the Bill and Melinda Gates Foundation is gratefully acknowledged.

QT11 - 1,4-Benzoxazine analogues arrest *Toxoplasma gondii* proliferation *in vitro*

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T. gondii is the aetiological agent of Toxoplasmosis. Although being a widespread disease, there are few treatments available and, the first choice regimen, sulfadiazine plus pyrimethamine, is usually associated to many side effects. Thus, the study of new drugs for toxoplasmosis is quite important. In the present work, the antiproliferative effect of 5,7,8-trimethyl-1,4-benzoxazine compounds has been evaluated on *T. gondii* infected LLC-MK_2 cells *in vitro*. An initial test with 7 analogues possessing different substituents on the 5,7,8-trimethyl-1,4-benzoxazine scaffold (no substitution; C2; C3; C4; C6; C2 and C6) was performed. With exception of the non-substituted 5,7,8-trimethyl-1,4-benzoxazine, all the other compounds demonstrated excellent activity against *T. gondii*, with IC_{50} values at micromolar range. The analogue with substitution at C2 and C6 was the most active one, presenting the lowest IC_{50}: 3.1μM and 0.8nM after 24 and 48h, respectively. The ultrastructural analysis by transmission electron microscopy of infected cells, treated with the C2 and C6 disubstituted 5,7,8-trimethyl-1,4-benzoxazine compound, demonstrated several cellular damages, with the mitochondrion and the apicoplast being the principal organelles affected. Currently, studies involving a set of 15 new C2 and C6 disubstituted 5,7,8-trimethyl-1,4-benzoxazine derivatives are underway.
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QT12 - INITIAL STUDIES OF AMIODARONE AND POSACONAZOL ON *Leishmania amazonensis*

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Leishmaniasis is a parasitosis caused by organisms of the *Leishmania* genus which are associated with significant rates of morbidity and mortality throughout the world. The mainstay of chemotherapy employed is based on pentavalent antimonials as first line of compounds, and in special cases, on miltefosine, amphotericin B and pentamidine. However, they are very unsatisfactory and there is an urgent need for safer and more efficacious anti-*Leishmania* agents. Posaconazole and Amiodarone are two novel compounds with potent effects in *Trypanosoma cruzi,* interfering directly with the ergosterol metabolism. Amiodarone also acts in the Ca^{2+} homeostasis leading to several effects on the mitochondrion physiology. We report here preliminary results of Posaconazole and Amiodarone on *Leishmania amazonensis*. The IC_{50} values found for promastigote forms were about 100 nM and 5 μM to Posaconazole and Amiodarone, respectively. The IC_{50} values found for amastigote forms were around 1 μM to both compounds. Citotoxicity assays revealed that the maximum concentration for the macrophages were about 15μM and 50μM to amiodarone and posaconazol, respectively, indicating that amiodarone is less selective than posaconazole. Cells incubated in the presence of both compounds displayed an intense alteration in the morphology of the promastigotes which showed a rounded shape and became swollen. Transmission electron microscopy demonstrated the presence of several alterations. The mitochondrion was the main organelle affected with the treatment, presenting an intense swelling, loss of the matrix content and alterations in its membranes. In addition, we also observed the presence of several large vacuoles containing part of the cytoplasm and membrane profiles resembling to autophagic structures. Taken together, these results indicate that Amiodarone and Posaconazole are promising compounds against *Leishmania*-parasites. Further studies are in progress to evaluate the synergetic effects of these drugs against intracellular amastigotes trying to decrease the concentrations of the treatments.
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QT13 - EVALUATING OF ANTILEISHMANIAL POTENCIAL OF A LOW MOLECULAR WEIGHT BASIC MYOTOXIN AGAINST *L. (L.) AMAZONENSIS*

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Introduction – Crotamine is a small and strongly basic peptide capable of cause spastic paralysis in hind limbs of mice and rats probably by acting on sodium channels of skeletal muscles. Antileishmanial activity of crotamine has been previously described, however its mechanism of action against parasites has not been well understood. **Objective** – The aim of this work was evaluate the mechanism of antileishmanial activity of Crotamine. **Methods** – Crotamine was isolated from *Crotalus durissus cumanesis* venom through a molecular exclusion HPLC column. This peptide was submitted to the treatment of (a) concentrated acetic acid and (b) incubation with DTT followed by 4-vynilpyridine aiming tertiary structure changes. Conformational alterations were confirmed by spectrophotometry. Native and treated samples were evaluated over promastigotes forms of *L. (L.) amazonensis* in a range from 6.2 to 100.0 µg/mL then its survival index was estimated by Neubauer chamber counting after 24 h. **Results** – Spectrophotometry profile of treated and no treated proteins revealed alterations in their tertiary structure. Antileishmanial activity consequently changed from a dose-dependent manner (IC50 = 50.0 µg/mL with native crotamine) to a no dose-dependent manner with treated peptides: Crot + vynilpyr (IC5O = 25.0 µg/mL) and Crot + AcAcid (IC50 not determinate). **Conclusion** – The results together with literature data suggest that crotamine antileishmanial activity remains on its N-terminal region. This aminoacid sequence shares its cationic charges properties with C-terminal region of PLA_2 myotoxins which possess antitumor, microbicidal and leishmanicidal activities. This work corroborates to show that crotamine could be a promising candidate for leishmaniasis chemotherapy.
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QT14 - POTENTIAL *IN VITRO* ANTILEISHMANIAL ACTIVITY OF PLANTS EXTRACTS

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Infections caused by protozoa of the genus *Leishmania* are a major worldwide health problem, with high endemicity in developing countries. Despite a lot of studies to find new antileishmanial drugs, first line chemotherapy is based on pentavalent antimonials which are toxic and prone to drug resistance. Phytotherapy has received considerable attention in the search for alternatives to chemotherapy in parasitic diseases control, such as leishmaniasis. In order to find new drugs against leishmaniasis, we analyzed the anti-*Leishmania* activity and cytotoxic effects on mammalian cells of methanolic extracts. Methanolic extracts were obtained from *Leonorus sibiricus, Cymbopogon citrates* (leaves)*, Trembleya parviflora, Plectranthus neochilus* (leaves) and *Tropaeolum majus* (leaves and inflorescence). We analyzed the cytotoxicity on mammalian cells and activity against promastigote forms of *L. amazonensis, L. chagasi, L. braziliensis and L. major.* The antileishmanial activity and cytotoxicity were determined using the tetrazoliumdye (MTT) colorimetric method. The results of anti-promastigote activity are expressed as the concentration inhibiting parasite growth by 50 percent (IC_{50}) after three days of incubation period. Among the extracts analyzed, *L. sibiricus* were active for promastigotes of *L.amazonensis*, *L.major* and *L.braziliensis* with IC_{50} of 53.54 µg/ml, 56.30 µg/ml and 32.10 µg/ml, respectively. *C. citrates* showed an activity against *L.amazonensis* and *L.chagasi* promastigote forms with IC_{50} of 28.17 µg/ml and 12.5 µg/ml, respectively. *T. parviflora* displayed an activity against *L.amazonensis, L.braziliensis* and *L.chagasi* with IC_{50} of 20.87 µg/ml, 24.53 µg/ml and 41.48 µg/ml, respectively. *P. neochilus*, *T. majus* did not show activity against the promastigote forms of the species tested. *T. parviflora* extracts showed moderate cytotoxicity on mammalian cells while the others were not toxic. The results show a good *in vitro* activity of some methanolic extracts and reinforce the need to investigate their activity against intracellular amastigotes.
Supported by UFJF, CNPq and FAPEMIG.

QT15 - Synergistic effect of metronidazole and pyrantel pamoate on *Giardia lamblia*

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Giardia lamblia is a pathogenic protozoan, and is considered the earliest branching eukaryote, presenting as the main characteristic, the capacity of trophozoite forms to adhere in host intestinal epithelium, infecting different mammals including dogs, cats and humans. The current treatment of *G. lamblia* infection is based on metronidazole (Mz), a drug that acts as an alternative electron acceptor, and its reduction in the anaerobic metabolism, promoting DNA impairment. In veterinary treatment, one of the best options is pyrantel pamoate (Pm), whose mode of action has not been elucidated yet. Extensive efforts are being directed to the development of different strategies for *Giardia* treatment, avoiding side effects to the host tissues and organs. The increasing resistance of the parasite to the clinical compounds, together with the recognizable efficiency of combined treatments on other intestinal parasites, support the employment of a combined treatment for protozoa diseases as an interesting alternative. In this framework, we have evaluated the synergistic effects of Mz and Pm combination on trophozoites and on its adherence to intestinal epithelial cells *in vitro*. The treatment with Mz or Pm was effective on trophozoites *in vitro*, with IC_{50}/ 24h values of 5.3 ± 0.9 µM and 13.8 ± 1.4 µM, respectively. As a preliminary analysis to evaluate the synergistic effect of Mz and Pm, three different combinations were employed. The observed Fractional Inhibitory Concentration (FIC) values were under 0.5 µM in all conditions, thus corresponding to a synergistic activity. The combinations of 5.3 µM Mz + 0.4 µM Pm and 13.8 µM Pm + 0.1 µM Mz also induced a remarkable reduction in adhesion percentage, with an observed inhibition in the range of 85-90% and 52-59%, respectively. The low cytotoxicity of the single compounds, similar to their combinations, associated to the strong synergistic effect of Mz and Pm, encourage us to further investigate their combined effect in *in vivo* models.
Supported by CNPq, FAPERJ and IOC/Fiocruz.

QT16 - TOPOISOMERASE INHIBITORS AND DNA BINDING DRUGS AFFECT CELL PROLIFERATION AND ULTRASTRUCTURE OF *TRYPANOSOMA CRUZI* EPIMASTIGOTE FORMS

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Protozoa of Trypanosomatidae family present a unique mitochondrion which contains an enlarged region, the kinetoplast, composed of catenated DNA (kDNA). DNA topoisomerases catalyze changes in the topology of DNA during replication, transcription and repair, thus representing an important target in chemotherapy. In this work, we analyzed the effects of topoisomerases I and II inhibitors and DNA binding drugs in the proliferation and ultrastructure of *Trypanosoma cruzi* epimastigote forms. For this purpose, cells were cultivated in medium containing different drug concentrations: 1, 5, 10 and 50uM to Camptothecin; 1, 5 and 10uM to Rebeccamycin; 50, 100, 200 and 300uM to Merbarone; 5, 10, 20 and 50uM to Mitoxantrone; 2, 10, 20 and 50uM to Berenil and 5, 10, 20, 40 and 100uM to Distamycin. Samples were collected after each 24 hours for counting on Neubauer's chamber or for processing to transmission electron microscopy until 72 hours of cultivation. Our results showed that Camptothecin caused higher growth inhibition, when compared to Rebeccamycin, which is also an eukaryote topoisomerase I inhibitor. Electron microscopy analysis showed that Camptothecin treated cells presented the typical kDNA arrangement, however the nuclear condensed chromatin disappears after drug treatment. Merbarone and Mitoxantrone, which are eukaryote topoisomerase II inhibitors, did not affect nuclear or kinetoplast ultrastructure, nevertheless Mitoxantrone promoted the appearance of lipids inclusions and loss of content in the trypanosomatid reservosomes. Protozoa treated with Berenil and Distamycin, which are DNA binding drugs, showed reduced cell proliferation, but only Berenil treated cells presented mitochondrial swelling and changes on kDNA compactation. Our results showed that Camptothecin was the most effective drug against *T. cruzi* proliferation, presenting an IC_{50} of 2,08µM. Taken together, our data reinforce the idea that topoisomerases constitute a promising target for antitrypanosomal chemotherapy.
Supported by Capes and Faperj.

QT17 - EFFECTS OF AMINOQUINOLINE DERIVATIVES ON *PLASMODIUM BERGHEI*

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Introduction: The high incidence of malaria and the drug-resistant strains of the parasite have turned this disease into a problem of major health importance. One of the aims to control it is the research for new antimalarial agents, such as quinoline derivates. They compose a big group of antimalarial agents, besides being already largely used, and they inhibit the formation of β-haematin (malaria pigment), which is lethal to the parasite. **Material and methods:** In order to assess antimalarial activity, six aminoquinoline derived drugs were tested *in vivo* using the 4-day suppressive test at 10mg/Kg each. The compounds were commonly named: RAF19MP [7-chloro-4-(6-mercaptopurine)-quinoline] ***(1)***, ART34 [4-amino-7-chloro-*N*-(2-(prop-2-inilamino)etil) quinoline] ***(2)***; ART28 [4-amino-7-chloro-*N*-(2-(prop-2-inilamino) propil) quinoline] ***(3)***; ART33 [4-amino-7-chloro-*N*-(2-(prop-2-inilamino) butil) quinoline] ***(4)***; ART31 [cis-dichloro(*N*-(7-cloroquinolin-4-il)-propanodiamine)platinum(II)] ***(5)*** and ART32 [4-amino-7-chloro-*N*-(2-(prop-2-inilamino) hexil) quinoline] ***(6)***. **Results:** On day 5th of the trial, the inhibition of parasite multiplication (ipm) was 0% for all compounds, except for ***2*** and ***5***, which were 4% and 12%, respectively. On day 7th, the ipm was: 41.2, 17.6, 50.0, 58.8, 61.8 and 47.1 % for the compounds ***1, 2, 3, 4, 5*** and ***6***, respectively. On day 9th, ipm was: 46.0, 63.5, 61.9, 58.7, 69.8 and 73.0 % for the compounds ***1, 2, 3, 4, 5*** and ***6***, respectively. The control treated group (chloroquine 200mg/kg) presented an ipm of 100% on 5th, 7th and 9th days. Moreover, the parasitaemias of most groups started lower than the untreated group and the surviving times of ***2***, ***5*** and ***6*** were longer than the untreated group. **Conclusion:** The aminoquinoline derived compounds, specially ***5***, may be objects of further researches for new antimalarial agents.
Supported by UFJF (BIC), Fapemig, CNPq.

QT18 - EFFECTS OF AMINOQUINOLINES AND *N*-ALKYL DIAMINE COMPOUNDS ON *PLASMODIUM BERGHEI*

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Introduction: Malaria is a global health problem, especially due to the increasing resistance of parasites strains. Chemotherapy has been the mainstay of malaria control strategies and the current ones rely on combination of drugs, what demands urgent search for new antimalarial agents. Two broad groups of agents that deserve pronounced attention are quinoline and amine derivates, since both act in crucial points: the first ones bind the haemoglobin, hampering the parasite survival, and the second ones interfere with essential cellular processes, such as growth, differentiation and macromolecular biosynthesis. **Material and methods:** In order to evaluate antimalarial activity, three compounds were obtained by means of organic synthesis and tested *in vivo* in a murine model using the 4-day suppressive test at 10mg/Kg each. They were two quinoline derivatives: N-(3-(9H-purin-6-ylthio) propyl)-7-chloroquinolin-4-amine ***(1)*** and (R)-4-((3S,5S,7R,10S,12S,13R,17R)-3-(4-((7-chloroquinolin-4-ylamina)methyl)-1H-1,2,3-triazol-1-yl)-hexadecahydro-7,12-dihydroxy-10,13 dymethyl-1H-cyclopenta[a]phenanthren-17-yl)pentanoic acid ***(2)*** and a N-alkyl diamine (N-dodecyl-ethylenodiamine) ***(3)***. **Results:** The results are expressed as the inhibition of parasite multiplication (ipm) percentage for the compounds previously code named as ***(1)***, ***(2)*** and ***(3)***. On day 5 of the trial, it was 24.4% for ***(1)***, 0% for ***(2)*** and 46.3% for ***(3)***. On day 7: 21.4% for ***(1)***, 31% for ***(2)*** and 83.3% for ***(3)***. On day 9: 26.8% for ***(1)***, 0% for ***(2)*** and 70.7% for ***(3)***. The control treated group (chloroquine 200mg/kg) presented an ipm of 100% on 5th, 7th and 9th days. The survival time of the group treated with ***(3)*** was longer than the untreated group. **Conclusion:** Both groups of compounds compose potential sources of antimalarials, concerning their points of action. Thus, these – specially ***(3)*** – may be objects of further research for new antimalarial agents, as we have looked forward to achieving.
Supported by UFJF (BIC), FAPEMIG and CNPq.

QT19 - Production, characterization and *in vivo* anti-*Trypanosoma cruzi* activity of Cubebin Poly (d,l-lactic-co-glycolic acid) microparticles

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Trypanosoma cruzi, etiologic agent of Chagas' disease, affects approximately 18 million inhabitants on the American continent and up to days alternative trypanocidal drugs with no side effects are necessary. In this study, PLGA microparticles was prepared and characterized in order to sustain Cubebin release for treatment of Chagas disease. A high performance liquid chromatography (HPLC) method to determination of Cubebin in pharmaceutical formulations was developed and validated. The trypanocidal effect of microparticles contain Cubebin was evaluated *in vivo*. Microparticles were prepared by emulsion/evaporation and showed a mean diameter of 3,798μm ± 1,945μm, with smooth surface and spherical shape. The encapsulation efficiency was 81% and the results of the validation demonstrated that the developed method was useful to quantify Cubebin on PLGA microparticles, showing to be accurate, reproducible, with coefficient of variation and exactness less than 3% and a linear response (r = 0.9998) over the concentration ranging between 1 – 20 μg/mL. The developed system maintained drug release with Higuchi kinetics. The preparation method showed to be suitable, since the morphological characteristics, efficiency yield and *in vitro* released, were satisfactory. *In vivo* assays showed significant reduction of parasitaemia after administration of PLGA Cubebin microparticles in mice infected. Thus, the developed microparticles seem to be a promising system for sustained release of Cubebin.
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QT20 - Anti-Trichomonas activity of Methyl Jasmonate

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Trichomonas vaginalis, an extracellular aerotolerant protozoan, is the cause of trichomoniasis, the most prevalent non-viral sexually transmitted infection in the world. Infection by *T. vaginalis* is associated with serious adverse health consequences to women that include infertility and predisposition to cervical neoplasia. Trichomoniasis among men can cause urethritis and, more recently, *T. vaginalis* was found to be related to prostate cancer. Metronidazole has been the drug of choice for *T. vaginalis* infection. However, there has been an increase in the recognition of metronidazole-resistant trichomoniasis. Jasmonates are a group of small lipids produced in plants and function as stress hormones. Recently, researchers found that jasmonates are directly cytotoxic to several types of cancer cells. Previous studies showed the effects of jasmonates on parasites such as *Plasmodium falciparum* and *Schistosoma mansoni.* Jasmonates has direct mitochodriotoxic effects, suggesting that mitochondria are the target organelles of methyl jasmonates. The present work aimed to examine whether jasmonates are able to damage cells lacking mitochondria, e.g., the unicellular parasite *Trichomonas vaginalis.* When *T. vaginalis* were exposed to 3 μM and 6 μM of MJ, a significative growth arrest was seen, mainly at 6μM of the drug. Parasite samples from the experiments were processed to scanning electron microscopy, and treated cells showed a reduced size when compared to control cells. Several cells were also seen internalizing their flagella, differently from the control cells, that showed all flagella externalized and a pear shape. Transmission electron microscopy showed that MJ induced morphological changes including autophagic structures, altered hydrogenosomes and an undefined and extracted cytoplasm, suggesting a drug-induced necrotic process. In conclusion, this study demonstrates that MJ is a promising candidate for the treatment of trichomoniasis. Supported by AUSU, CAPES, CNPq, FAPERJ AND PRONEX.

QT21 - ARRESTED GROWTH OF *TRYPANOSOMA CRUZI* BY THE CALPAIN INHIBITOR MDL28170 AND DETECTION OF CALPAIN HOMOLOGUES IN EPIMASTIGOTE FORMS

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In this paper, we aimed to explore the effects of the calpain inhibitor III (MDL28170) and to detect calpain-like molecules (CALPs) in epimastigote forms of *Trypanosoma cruzi* isolate Dm28c. MDL28170 at 70 μM promoted a powerful reduction in the growth rate after 48 h. The IC50 value was calculated to be 31.7 μM. This inhibitor promoted an increase in the cellular volume, but not cell lysis, resulting in a trypanostatic effect. *T. cruzi* CALPs presented a strong cross-reactivity with anti-*Drosophila melanogaster* calpain and anti-cytoskeleton-associated protein from *Trypanosoma brucei* antibodies, and labelling was found mainly intracellularly. Furthermore, an 80 kDa reactive protein was detected by Western blotting assays. No significant cross-reactivity was found with anti-human brain calpain antibody. The expression of CALPs was decreased in cells kept for long periods in axenic cultures in comparison to a strain recently isolated from mice, as well as in MDL28170-treated cells, the latter being paralleled by an increased expression of cruzipain. Different levels of CALPs expression were also detected in distinct phylogenetic lineages, like Y strain (lineage TCI), Dm28c (TCII) and INPA6147 strain (Z3 zymodeme). These results may contribute for the investigation of the functions of CALPs in trypanosomatids.
Supported by: MCT/CNPq, CEPG/UFRJ, FAPERJ, FIOCRUZ.

QT22 - MILTEFOSINE INDUCES PROGRAMMED CELL DEATH IN *LEISHMANIA AMAZONENSIS* PROMASTIGOTES

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Leishmaniasis remains a major health problem of the tropical and subtropical world. The pathology is manifested in cutaneous, mucocutaneous or visceral forms. Dramatic increases in the rates of infection and drug resistance and the non availability of safe vaccines have highlighted the need for identification of novel anti-leishmanial agents and their modes of action. Miltefosine has proved to be a potent oral treatment for human visceral leishmaniasis, as observed in *Leishmania donovani.* In the present study, we have demonstrated that in promastigotes of *Leishmania amazonensis,* the etiological agent of cutaneous leishmaniasis, miltefosine exerts its leishmanicidal effect by triggering a programmed cell death. At the concentration of 30 μM, this compound promoted the loss of plasma membrane integrity as detected by binding of annexin V and propidium iodide, the arrest of cell-cycle at the sub G_0/G_1 phase, the fragmentation of genomic DNA into oligonucleosomal fragments and the DNA nicking shown by deoxynucleotidyltransferase-mediated dUTP end labeling (TUNEL). These data suggest that miltefosine was able to induce programmed cell death in *Leishmania amazonensis* promastigotes. The identification of the death-signaling pathways activated in miltefosine-treated parasites appears to be essential for a better understanding of the molecular mechanisms of action in these parasites.
Supported by: CNPq, FAPERJ, FIOCRUZ and CEPG/UFRJ.

QT23 - EFFECT OF NEW DERIVATIVE AMINOQUINOLINE ON SEVERAL *LEISHMANIA* SPECIES

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Introduction and Objectives: The leishmaniases are neglected diseases that affect approximately 12-million people in the world and are present in developing countries. The drug of choice for the treatment of leishmaniasis is pentavalent antimony, which is expensive, present a high toxicity and requires prolonged administration. Amioquinolines have been shown as promising candidates for the development of future antiprotozoals drugs. Thus, we have tested new aminoquinoline derivatives against New and Old *Leishmania* species. **Material and Methods:** The compounds 4-amino-7-chloro-*N*-(2-(prop-2-inylamino)ethyl) quinolin **(1)**, 4-amino-7-chloro-*N*-(2-(prop-2-inylamino)propyl) quinolin **(2)**, 4-amino-7-chloro-*N*-(2-(prop-2-inylamino)buthyl) quinolin **(3)** and 4-amino-7-chloro-*N*-(2-(prop-2-inylamino)hexyl) quinolin **(4)** were assayed against *L. amazonensis*, *L. braziliensis*, *L. chagasi* and *L. major* promastigote forms. The viability of promastigotes was checked using the tetrazolium-dye (MTT) colorimetric method. The results in promastigotes were expressed as the concentrations inhibiting parasite growth by 50 percent (IC50) after three days incubation period. **Results and Conclusions:** The drugs showed activity for *L. major* (IC50 values of 20.57 µM, 44.98 µM, 80.00 µM and 25.14 µM for **(1), (2)**, **(3) and (4),** respectively), *L.chagasi* (IC50 values of 18.20 µM, 21.96 µM, 9.43 µM for **(1), (3) and (4),** respectively) and *L. braziliensis* (IC50 values of 35.88 µM, 16.71 µM for **(1) and (4),** respectively). None of compounds showed antileishmanial activity for promastigote forms of *L. amazonensis*. The **(1)** and **(4)** compounds with 2 and 6 methylene between diamines groups, respectively, showed the best antileishmanial activity. These results show a good in vitro activity of quinoline derivatives and reinforce the need to investigate their activity against intracellular amastigotes.
Supported by FAPEMIG, CNPq and UFJF.

QT24 - Ether phospholipid-dinitroaniline hibrids as new prototypes against *Leishmania amazonensis*

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Leishmaniasis is one of the most important neglected tropical diseases caused by parasites of the *Leishmania* genus. In Brazil, *Leishmania amazonensis* is responsible for cutaneous and diffuse cutaneous leishmaniasis. The current chemotherapy for the treatment of leishmaniasis is based on Pentavalent Antimonials, Amphotericin B and Pentamidine. In countries like India and Germany, hexadecylphosphocholine, Miltefosine, is the first choice to treat visceral leishmaniasis. However, current drugs are very unsatisfactory and there is an urgent need for safer and more efficacious anti-*Leishmania* agents. Oryzalin is a dinitroaniline herbicide with a known activity against protozoan parasites, but with high IC_{50} and cytotoxicity effects for the host cells. In this work, we study novel compounds that combine the parmacophores of oryzalin and miltefosine which may interact with different molecular targets in *Leishmania amazonensis*. The hybrid compounds TC 95, TC 106 and oryzalin were tested on promastigote forms of *Leishmania amazonensis* presenting IC_{50} values around 2 µM, 25 µM and 20 µM, respectively. Intracellular amastigote forms were more sensitive presenting lower IC_{50} to TC95 and TC106, which were also less toxic for the host cell than miltefosine and oryzalin. After treatment, promastigotes displayed profound alteration in the shape appearing rounded and swollen as visualized by scanning electron microscopy, which also indicated alterations in the flagellar membranes. In addition, transmission electron microscopy revealed that the mitochondrion is the main organelle affected, presenting an intense swelling with loss of the matrix content and disorganization of the mitochondrial membranes. Sometimes, lysis of the mitochondrion was also observed. In addition, alterations in the flagellar membrane, Golgi complex, increase in the number of lipid bodies and appearance of structures typically found in autophagic processes were observed. Taken together, these results indicate that these hybrid molecules are promising compounds against *Leishmania sp*. Supported by FAPERJ, CNPq and CAPES.

QT25 - Experimental chemotherapy for Chagas' disease targeting the protease cruzain

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Chagas' disease, caused by *Trypanosoma cruzi*, remains the leading cause of cardiopathy in Latin America with about 10-12 million people infected. Classic clinical manifestations derive from infection of muscle cells leading to progressive cardiomyopathy, while some patients develop megasyndromes. A very aggressive clinical course including fulminant meningoencephalitis has been reported for chagasic patients in the background of immunodeficiency. This includes either patients with HIV co-infection or receiving immunosuppressive therapy. Two drugs are approved for chemotherapy of Chagas' disease, nifurtimox and benznidazole. Both have significant limitations due to common and serious side effects as well as limited availability.

The major parasitic cysteine protease cruzain is a validated target of effective chemotherapy. As gene deletion of cruzain appears to be lethal, a *T. cruzi* clone expressing less than 1% of wildtype cruzain activity was studied. Cruzain-deficient parasites have a significantly prolonged growth rate and are 40 fold less infectious than parental wild type *T. cruzi.* Most significantly, cruzain-deficient *T. cruzi* cannot establish infection in immune competent mice. Our results show that cruzain proteolytically degrades the host cell nuclear factor NF-□B preventing cell activation (our unpublished data).

A variety of cysteine protease inhibitors with different chemical structures cure *T. cruzi* infection of mammalian cells *in vitro* and in animal models of infection. The dipeptide inhibitor *N*-methyl-Pip-F-homoF-vinyl sulfonyl phenyl (*a.k.a.* K11777, K777) is currently in pre-IND discussion with the USFDA. Treatment with K11777 rescued experimental mice from both acute lethal and chronic *T. cruzi* infection, and was effective even in the background of immunodeficiency. K11777 was effective against different *T. cruzi* isolates as well as against Nifurtimox and/or Benznidazole resistant parasites (our unpublished data). An additive effect with Nifurtimox was observed. Extensive pharmacokinetic and toxicology studies carried out by SRI International under NIH sponsorship estimated the maximum tolerated dose at >150 mg/kg weight. K11777 is now a drug candidate for chemotherapy of Chagas' disease.

QT26 - Development of a Image-based High Throughput Screening assay to identify small molecule targeting the intracellular amastigote stage of Trypanosoma cruzi

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We have develop, optimize and validate an Image-Based High Throughput Drug Screening (HTS) to screen small molecule against the intracellular (amastigote) stages of the parasite *T. cruzi*, the etiological agent of Chagas' disease. T. cruzi-infected BESM cells, grown in cell culture 96-well assay plates, were then incubated with test compounds for 72 hours. The cultures were fixed with paraformaldehyde and stained with nuclear dye DAPI. Assay plates were automatically imaged using the GE's IN Cell Analyzer 1000 with image analysis based on size difference between the DAPI-stained host cell nuclei and parasite kDNA. A reduction in the ratio of T. cruzi / host cell provides a quantitative measure of parasite growth inhibition, while a decrease in the host nuclei count indicates toxicity of the test compound. The HTS assay allows the evaluation of effective compounds with both laboratory and field isolates reflecting the parasite genetic diversity. Different host cells including macrophages and cardiomyocytes can also be used to better model human infection. This assay was tested by screening a library of FDA-approved drugs to identify clinically applicable trypanocidal compounds and was able to identify several compounds within the list that have been previously reported as trypanocidal. The automated fluorescence microscope based HTS assay provides a powerful tool to screen libraries of small molecules, analyze the effect of compounds at the level of single cells and determine cytotoxicity levels for parasites and host cells and allow the rapid selection of physiologically relevant hits.

QT27 - EVALUATION OF BLOOD DISTRIBUTION OF MEGLUMINE ANTIMONIATE-CONTAINING LIPOSOME WITH PHOSPHATIDYLSERINE IN MICE

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Visceral Leishmaniasis is a severe disease, caused by intramacrophage protozoa parasite *Leishmania* sp., and is fatal if left untreated. *Leishmania* resides mainly in the liver and the spleen and multiplies. Pentavalent antimonials, though toxic and their mechanism of action being unclear, remain the first-line drugs for leishmaniasis. Effective therapy of leishmaniasis could be achieved by delivering antileishmanial drugs to these sites of infection. Liposomes associated formulations of antileishmanial agents provide more effective therapies with considerably reduction of toxicity and adverse side effects. Moreover, they have some attractive biological properties, especially to be fast eliminated from the blood and captured by the cells of reticulo-endothelial system. The aim of the present study was to develop meglumine antimoniate-containing liposome with phosphatidylserine to achieve targeted delivery to the *Leishmania* site infection and compare its pharmacokinetic with free meglumine antimoniate in healthy mice. Meglumine antimoniate was neutron irradiated (IMA) inside the IEA-R1 nuclear reactor to produce antimony radiotracers, ^{122}Sb and ^{124}Sb. Liposome formulation was prepared from phosphatidylserine, cholesterol and phosphatidylcholine in the molar ratio 1:4:5. IMA was encapsulated in freeze-dried empty liposome (FDEL-IMA). Healthy mice received a single intraperitoneal dose of both drugs. At different times after injection blood samples were collected and activity measured in a NaI (Tl) scintillation counter. Analysis of the curves of the concentrations in blood after administration of FDEL-IMA and IMA showed that both formulations produced similar pharmacokinetic curves with two compartments, a distribution in the central compartment and other associated to drug equilibrium and excretion. Blood antimony concentration showed to be higher in mice that received IMA, but this concentration was reduced faster than when FDEL-IMA was administrated. The development of liposome formulations should be a new alternative for the chemotherapy of leishmaniasis as they are used to deliver pharmaceuticals into cells or even inside individual cellular compartments. This research was supported by Brazilian Agency CNPq/MCT.

QT28 - 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(N,N-dimethyl-1-phenethylamine-1,2-ethanebis(diphenylphosphine), DPPE 1.2. The compound was first tested on the growth of *Leishmania (Leishmania) amazonensis* promastigotes in axenic medium. These experiments showed that three days of incubation with 156 nM DPPE 1.2 completely blocked growth of *L. (L.) amazonensis* promastigotes. The effect of this compound was then tested on *L. (L.) amazonensis* amastigotes by the treatment of infected mouse peritoneal macrophages. DPPE 1.2 was added at 100 nM to 2,500 nM 24 h after macrophage infection by *L. (L.) amazonensis* amastigotes and the cultures were examined after 72 h. A significant, dose-dependent decrease in infection index was observed with DPPE 1.2, with inhibition of 95% for 1,000 nM and at this concentration DPPE 1.2 was not toxic to macrophage cultures as determined by the MTT assay. Several concentrations of DPPE 1.2 were also tested *in vivo* in *L. (L.) amazonensis*-infected BALB/c mice. Glucantime was also used for *in vivo* experiments as a positive control. The animal infection was evaluated by measuring the foot lesion diameter and by determination of parasite burden by the limiting dilution method. A reduction of 97% in *L. (L.) amazonensis* infection was observed when infected mice were treated for 1 month with 120 μg of DPPE 1.2 by subcutaneous administration in foot lesions, whereas a reduction of 99.7% was obtained with 24 mg of glucantime. Hepatic and renal functions were normal in mice treated with DPPE 1.2. Although DPPE 1.2 showed leishmanicidal effect on *L. (L.) amazonensis*-infected BALB/c similar to that exhibited by glucantime, it is important to emphasize that DPPE 1.2 was used in a significant lower concentration (200X). These results point to the potential use of this palladacycle complex as a leishmanicidal drug.
Supported by CNPq and FAPESP.

QT29 - EFFECTS OF INDOLQUINOLINE DERIVATIVES ON SEVERAL LEISHMANIA SPECIES

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Introduction and objectives: Leishmaniasis is caused by species of the genus *Leishmania* and is one of the major global public-health that affects more than 12 million people worldwide with an increasing number of cases each year, especially in developing countries. The treatment for Leishmaniasis in Brazil has been done based on pentavalent antimonials, which have prolonged therapy and caused many toxic effects. Thus, there is an urgent need for effective drugs to replace or supplement those in current use. In rational trials, we analyzed the antileishmanial activity of indolquinoline derivatives including *N-(2-(1H-indol-3-yl)ethyl)-7-chloroquinolin-4-amine* (**1**), *Methyl 2-(7-chloroquinolin-4-ylamino)-3-(1H-indol-3-yl)propanoate* (**2**) and *2-(7-chloroquinolin-4-ylamino)-3-(1H-indol-3-yl)propanoic acid* (**3**). **Materials and methods:** All the compounds were assayed against *L. amazonensis*, *L. braziliensis*, *L. chagasi* and *L. major* promastigote forms and were tested for cytotoxic effects on mammalian cells. Each concentration was screened in duplicate and it was performed in flat-bottomed 96-well plastic tissue-culture plates. The viability of promastigotes and mammalian cells was checked using the tetrazolium-dye (MTT) colorimetric method. The results in promastigotes were expressed as the concentrations inhibiting parasite growth by 50 percent (IC_{50}) after three days incubation period. **Results and conclusions:** Among the drugs assayed, only the compound **3** showed activity against the four species tested and the IC_{50} values were: 30.40 µM, 14.64 µM, 30.96 µM, and 22.17 µM to *L. amazonensis*, *L. braziliensis*, *L. chagasi* and *L. major*, respectively. Probably the acidic group in this molecule favors the antileishmanial activity. Unfortunately, this compound showed high toxicity against mammalian cells, indicating poor selectivity. We are currently synthesizing new indolquinoline analogs which will hopefully show better *in vitro* and *in vivo* antileishmanial activities. Supported by FAPEMIG, CNPq and UFJF.

QT30 - Evaluation of the leishmanicial activity of a hypervalent organotellurium compound against *Leishmania (Leishmania) chagasi*

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Organotellurium compounds display several biological activities, the most described of them are their antioxidant properties. Among the several known classes of tellurium compounds, the hypervalent derivatives (organotelluranes), exhibit antihelmintic and antibacterial activity. More recently, organotelluranes have been studied as irreversible cysteine protease inhibitors. Prior to the present study, the leishmanicidal effect of the organotellurane RT01 was demonstrated against promastigote and amastigote forms of *L. (L.) amazonensis*. These results led us to study the leishmanicidal activity of several organotelluranes on *L. (L.) chagasi*, the etiological agent of American visceral leishmaniasis. Preliminary results showed that one of the organotelluranes tested, RF07, displayed leishmanicidal activity when tested on *L. (L.) chagasi*-infected macrophages. A range of concentration from 0.5 to 2 μM was then tested on infected macrophages and the cultures were examined after 72 h. A significant, dose-dependent decrease in infection index was observed with RF07 treatment, with inhibition of 85% for 0.75 μM and at this concentration RF07 was not toxic to macrophage cultures, as checked by the MTT assay. The kinetics of drug action was determined after incubation of *L. (L.) chagasi*-infected macrophages with 0.75 μM RF07 at different times resulting in inhibition of 97% after 7 days. These results led us to test the leishmanicidal effect of RF07 *in vivo* and experiments of treatment of *L. (L.) chagasi*-infected hamsters with RF07 are currently in progress.
Supported by CNPq and FAPESP.

QT31 - EVALUATION OF THE LEISHMANICIDAL ACTIVITY OF LIPOSOMES CONTAINING A SUBSTANCE ISOLATED FROM *Combretum leprosum* FRUITS AGAINST *Leishmania amazonensis*

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Cutaneous leishmaniasis is an important dermatological infection, not only dut to its high prevalence, but mainly because of the therapeutical difficulties, and the deformities and sequels that the disease can cause. Although the effectiveness of antimonial drugs, cases of failure and toxicity in leishmaniasis treatment are frequent. Studies on new antileishmanial compounds and other forms of application, such as liposome carrier systems, may contribute to increase the efficacy of the treatment. Previous results from our laboratory have shown that CLEFT, a substance isolated from the fruit of *Combretum leprosum*, has a potent in vitro activity against *L. amazonensis*. The objective of this study was to evaluate the in vitro and in vivo antileishmanial activity of CLEFT-encapsulated liposomes. Murine peritoneal macrophages have been infected in vitro with *L. amazonensis* promastigotes, and the infected cultures were treated with liposomal CLEFT. The drug in the liposomal formulation showed a high antileishmanial activity in vitro, reducing the amount of intracellular parasites by approximately 33%, 50% and 56% after 24h, 48h and 72h, respectively. The number of parasites in macrophages treated with pentamidine decreased around 47% after 72h, but this drug has revealed to be highly toxic to cells in culture. For the in vivo assay, BALB/c mice were inoculated in the right hind footpad with 10^6 L. amazonensis promastigotes. Six weeks post-infection, mice were treated with CLEFT-encapsulated liposome intraperitoneally for 7 days. The progression of the injury was monitored daily per 8 weeks. BALB/c mice treated with pentamidine decreased the lesion size in 20%, but animals treated with liposomal CLEFT have shown a lesion size decrease of about 50% when compared to untreated mice. These results demonstrate that the CLEFT is effective against *L. amazonensis* and may represent an alternative for the development of new antileishmanial drugs, thus contributing to the advancement of new treatments for this disease.
Supported by CNPq.

QT32 - EVALUATION OF LEISHMANICIDAL ACTIVITY OF A TRITERPENE FROM *Combretum leprosum* ASSOCIATED WITH GLUCANTIME IN VITRO

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Leishmaniasis is an illness caused by several species of the Leishmania parasite. *Leishmania amazonensis* causes cutaneous injuries of difficult healing that can evolve for cutaneous diffuse leishmaniasis. The chemotherapy is the only form of control of leishmaniasis and there is still no commercial vaccine available for this disease. The natural products of the Brazilian flora compose a valuable tool in the research of antileishmanicidal agents. The chromatographic analysis of the ethanolic extract of *C. leprosum* fruits led to isolation of a triterpene that presented a high leishmanicidal activity. To evaluate the possibility of a combined use of triterpene and Glucantime, murine peritoneal macrophages were infected with *L. amazonensis* promastigotes and treated with those drugs. The infected cells had been dealt with 5µg/mL of triterpene and 300µg/mL of glucantime and monitored for 24, 48, 72 and 96h. After these periods, the rate of infection was scored. After 24h, it was observed no significant differences among the tested drugs. However, at 48h, the phagocytic index presented a reduction of 50%, in the triterpene-treated cultures and in the cultures treated with triterpene combined with Glucantime. After 72h, this reduction was of 85%, and after 96h of 90%. Glucantime alone did not present difference in relation to the control. The used concentrations of drugs not present cytotoxicity on host cells. These results show that the triterpene from *C. leprosum* is capable to inhibit the intracellular growth of *L. amazonensis* in vitro and that its association with glucantime did not modify its action, demonstrating that triterpene can be a powerful tool for development of new drugs for leishmaniasis.
Supported by CNPq

QT33 - *TRYPANOSOMA CRUZI* ABC-TRANSPORTER GENE AND SUSCEPTIBILITY TO BENZNIDAZOLE

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Benznidazole (BZ) is one of the two drugs used to treat Chagas disease. Nevertheless, therapeutic failures of BZ were reported, which were mostly attributed to different susceptibilities of *T. cruzi* strains to this drug. A few genes were described to be involved in BZ resistance induced *in vitro*, but not in the natural resistance phenotype. The differential gene expression between naturally susceptible and resistant *T. cruzi* strains was assessed by DNA microarray hybridization with slides covering ~12,000 CL Brener ORFs (PFGRC). Among the probes up-regulated in the resistant strain one ABC-transporter gene was detected, which showed high similarity with homologous ABC-transporters (sub-family G) of *Leishmania* species and *T. brucei*. The goal of this study was to obtain further evidence that the *Tc*ABCG-transporter is related to BZ resistance in *T. cruzi*. An *in vitro* test was standardized to quantify BZ susceptibility in epimastigotes of ~20 parasite isolates. The IC50 varied from 7.6±3.6 to 55.2±3.5 µM BZ. Real time RT-PCR assays indicated that the transcript abundance of the *Tc*ABCG-transporter was, on average, three-fold higher in seven naturally resistant strains (24<IC50<55 µM BZ) as compared to five sensitive strains (IC50 <13 µM BZ). We investigated whether known ABC-transporter inhibitors altered BZ susceptibility. The ABCB/PGP/MDR1 inhibitor verapamil showed IC50 of 56.1±2.3 µM for epimastigote forms. Addition of 30 µM verapamil decreased BZ IC50 by 51% (CL Brener and VL10 strains) and 77% (007 human strain). The ABCC/MRP1 inhibitor cyclosporin A showed IC50 of 22.0±5.6 µM. Addition of 5 and 10 µM cyclosporin A did not modify BZ IC50s in the three above-mentioned strains. The effect of fumitremorgin C, a specific inhibitor of ABCG/MXR/BCRP, will be assayed. In the future, *T. cruzi* strains will be transfected with the *Tc*ABCG gene to verify if over-expression of this protein increases BZ resistance. Support: FAPESP; MCT/CNPq.

QT34 - 4-AMINO-7-CHLORO-*N*-(PROP-2-INYL)QUINOLINE AS A PROMISING LEISHMANICIDAL AGENT: ACTIVITY AGAINST PROMASTIGOTES AND AMASTIGOTES OF DIFFERENT *LEISHMANIA* SPECIES.

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Introduction: The drugs currently in use for the treatment of leishmaniasis display high toxicities. Several researches have been carried out to develop new protocols and chemotherapies for leishmaniasis treatment. Indolquinoline compounds have shown great promise for development of future antiprotozoals drugs. **Objectives:** The aim of this work was evaluate the in vitro antileishmanial activity of the 4-amino-7-cloro-*N*-(prop-2-inil)quinoline against promastigotes and intracellular amastigotes of *Leishmania*. **Methods:** The compound 4-Amino-7-chloro-*N*-(prop-2-inyl)quinoline was assayed against *L. amazonensis*, *L. braziliensis*, *L. chagasi* and *L. major* promastigote forms by MTT colorimetric method. For anti-amastigote activity peritoneal macrophages infected with promastigotes of *L. amazonensis* were used. The antiparasitic effect of the substance was evaluated by counting the intracellular amastigotes after 24, 48 and 72 hours of treatment. **Results:** The compound showed activity against all *Leishmania* species promastigotes (IC_{50} values = 5.27 µM, 5.13 µM, 23.00 µM and 26.75 µM for *L. amazonensis*, *L. braziliensis*, *L. chagasi* and *L. major*, respectively). Furthermore, the indolquinoline derivative inhibited amastigotes of *L. amazonensis* in a time- and dose-dependent manner, reaching 60% of amastigote-forms death in 72 hours at 25 µM. The compound was not toxic to macrophages up to the highest inhibitor concentration tested (114 ug/ml). **Conclusion:** These results suggest that this compound has promising antileishmanial potential and may contribute to the development of new leishmanicidal agents. Supported by FAPEMIG, UFJF and CNPq.

QT35 - EVALUATION OF LEISHMANICIDAL ACTIVITY OF DICHLOROMETHANE AND METHANOL EXTRACTS OF *ROLLINIA MUCOSA* IN VITRO

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Leishmaniasis is one of the diseases of protozoal origin that are widespread in tropical and subtropical regions of the world where they represent an important health problem. The chemotherapeutic agents used for the treatment of leishmaniasis, besides being expensive, show serious side effects such as cardiotoxicity, renal toxicity, teratogenicity, causing resistance to the parasite. In the fight against leishmaniasis, plant extracts or plant-derived compounds represent an important alternative source of new antileishmanial agents. *Rollinia mucosa* (Annonaceae) is a tropical tree that produces large edibles fruits and several medicinally active metabolites. Chemical and pharmacological studies of this species have reported the presence of alkaloids, acetogenins and lignans, with antiprotozoal, antimicrobial and antifungal activity. In the search of new leishmanicidal agents, it was evaluated the effect of dichloromethane and methanol extracts of leaves of *Rollinia mucosa* in cultures of promastigotes of *Leishmania amazonensis in vitro*. Promastigotes of *Leishmania amazonensis* had been cultivated in presence or absence of 100; 50; 25; 12 and 6,0 µg/mL of extracts, for five days at 23°C. The pentamidine was used as negative control. Methanol and dichloromethane extracts inhibited in about 100% the growth of promastigotes in the concentrations of 100 and 50 µg/ml. The dichloromethane extract in the concentrations of 25 and 6 µg/ml, presented inhibition of 47% and 24% respectively. The methanol extract, in the concentration of 25 µg/ml, had an inhibition of only 23%. The other used concentrations, presented no effect on the promastigotes growth. The cytotoxicity of extracts was evaluated in cultures of mice peritoneal macrophages and it did not present toxic effect on these cells. Results showed that both extracts of *Rollonia mucosa* inhibit the proliferation of promastigotes forms of *Leishmania amazonensis in vitro* suggesting the use of this species in the elaboration of a potential leishmanicidal drug.
Supported by FAPEAM, CNPq.

QT36 - EFFECT OF *APIS MELLIFERA* VENOM ON *TRYPANOSOMA CRUZI* EPIMASTIGOTES AND TRYPOMASTIGOTES

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Chagas' disease chemotherapy is based on drugs that exhibit toxic effects and limited efficacy such as Benznidazole and Nifurtimox. Therefore, new chemotherapeutic agents from natural sources are a lining research to be exploited. Honeybee (*Apis mellfera*) venom consists of many biologically active enzymes, peptides and biogenic amines and has been reported to exhibit anticancer effects. The present study shows that the *A. mellifera* venom can affect growth and ultrastructure of *T. cruzi* epimastigotes and trypomastigotes. Four-day-old culture epimastigotes (Y strain and CL-Brener clone) were cultivated for 4 days in LIT medium containing 0.2 to 200 µg/mL of the venom. Trypomastigotes (CL-Brener clone) were obtained from infected LLC-MK_2 cells cultures and then incubated in RPMI medium at 37°C, containing the same concentrations for 1 day. Effect of this compound on epimastigotes growth and trypomastigotes lysis was evaluated by counting with a Neubauer chamber. To analyze the effect on the cells morphology, treated trypomastigotes were processed for transmission electron microscopy (TEM). The ED_{50} for epimastigotes inhibition growth was 0.85 µg/mL and 0.67µg/mL for Y strain and CLB clone respectively, and for trypomastigote lysis was 0.1µg/mL after 1 day. Loss of cell viability was confirmed by detection of morphological alterations, like swelling of plasma membrane, changes on cell shape and loss of cytoplasm content. Treated epimastigotes are under TEM processing. Our data demonstrate that *A. mellifera* venom was effective against the epimastigote and trypomastigote forms of *T. cruzi*. Further studies are underway to investigate the possible intracellular targets of the drug.
Supported by CNPq, CAPES and FAPERJ.

QT37 - EFFECT OF *CROTALUS VIRIDIS VIRIDIS* VENOM PARTIAL PURIFIED FRACTIONS ON *TRYPANOSOMA CRUZI* TRYPOMASTIGOTES.

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Snake venoms are complex mixtures of proteins, nucleotides and inorganic ions and their toxins have been used as pharmacological tools for drug development. Chagas' disease, caused by *Trypanosoma cruzi*, affects 16-18 million people in Central and South America and the treatment is based on two drugs that exhibit toxic effects and limited efficacy. Therefore the search for effective compounds for disease chemotherapy is a lining research to be exploited. Our previous works showed that *Crotalus viridis viridis* crude venom is effective against *T. cruzi*, and it is capable to access the host cells cytoplasm at concentrations that only kills the intracellular parasite form. The aim of this study was initiate the investigation of the activity of *C. v. viridis* venom partial purified fractions in order to start the screening of the bioactive molecules responsible for the protozoan killing. The crude extract venom was loaded onto to a reverse phase analytical (C8) column using a high performance liquid chromatographer (RP- HPLC). A linear gradient of water/acetonitrile with 0.1% trifluoroacetic acid was used. The obtained peaks were pooled into 3 fractions according to the chromatogram profile. They were lyophilized, ressuspended in distillate water and their protein content measured by Bradford method. Trypomastigotes obtained from LLC-MK_2 cells cultures were incubated in RPMI medium containing 0.625 to 10 µg/mL of each fraction, and the effect on the cells lysis was evaluated by counting with a Neubauer chamber. The fraction 1 did not present any effect; fractions 2 and 3 caused a significant reduction (95% to 100%) in parasites number after 24h of incubation. Further studies are underway to isolate and characterize each compound presented in these two active fractions, and verify the mode of action against the protozoan by electron microscopy analysis.
Suportted by CAPES, CNPq and FAPERJ.

QT38 - Biological activity of *Copaifera* sp. against amastigotes of *Trypanosoma cruzi*

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Fabaceae is the third largest family of angiosperms with about 700 genera, including the *Copaifera* genus, and over 18,000 species. Chagas disease, a vector-transmitted infectious disease, is endemic to Latin America and it is caused by the protozoa parasite *Trypanosoma cruzi*. Since its discovery, a hundred years ago, no efficient drug has been developed and the eradication of the intracellular form of the parasite is still far from being achieved. The present work reports the activity of exsudate oils obtained from 8 species of *Copaifera* (*C. reticulata*, *C. multijuga*, *C. martii*, *C. cearensis*, *C. paupera*, *C. langsdorfii*, *C. officinalis*, *C. lucens)* against amastigotes of *T. cruzi* and their toxicity to human erythrocytes. In 24-well plates, LLCMK_2 cells were plated in round cover slips in a way to form confluent monolayers which were subsequently infected with trypomastigote forms of the parasite for 24 h. After the internalization and differentiation processes, the cell monolayers containing the amastigotes were incubated with 5 and 10 µg/ml of the oils for 96 h. The cells were then fixed with methanol and stained with Giemsa. The index of amastigote proliferation was determinated by counting the number of infected cells and the intracellular parasites. Haemolytic assay was performed incubating 100 µg/ml of the oils together with a 4% suspension of human red blood cells. After 2 h of incubation at 37 °C the absorbance of the supernatant was obtained by spectrophotometric reading at 540 nm. The majority of the oils reduced the amastigote index by more than 70% at 5 µg/ml, compared to control, while at 10 µg/ml both *C. martii* and *C. officinalis* were able to eliminate the intracellular forms. No haemolytic effect was observed. The promissory results show that the copaiba oils have important compounds which may be considered for chemotherapeutic purposes.
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QT39 - SECONDARY METABOLITE OBTAINED FROM *ASPERGILLUS* FUNGI HAS ANTILEISHMANIAL ACTIVITY

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Parasites of the genus *Leishmania* are transmitted by the sandflies and infect cells of the mononuclear phagocyte lineage of their vertebrate hosts. The chemotherapy is one of the most effective treatments for this disease. Although a number of antileishmanial drugs are available, these drugs are in general toxic, expensive and require long-term treatment. New drugs isolated from plants and microorganisms have shown leishmanicidal action. Thus, we consider interesting to analyze a secondary metabolite produced by some species of *Aspergillus*, *Penicillium* and *Acetobacter* fungi against promastigote and amastigote of *L. amazonensis in vitro.* This metabolite has bacteriostatic activity and it effectively inhibits the formation of L-DOPA (3,4-dihydroxy-L-phenylalanine) from tyrosine in the process of melanin biosynthesis. However, antileishmanial activity and effects on host immune cells are not known. Firstly, the secondary metabolite acted decreasing of 62% on the growth of *L. amazonensis* promastigotes at 50µg/mL (IC_{50} 30.6µg/mL). Another aspect observed was the growth inhibition of 79% (IC_{50} 13.3µg/mL) in amastigotes when macrophages were treated and infected *in vitro* at the same concentration. Ultrastructural analysis of infected cells showed that the metabolite induced morphological alterations in the promastigotes, such as some vesicles bodies into the flagellar pocket, an intense intracellular vacuolization and swelling of the mitochondrion. The colorimetric assay (MTT), that measures cytotoxic metabolic viability, showed that this compound presented no cytotoxic effects against mammalian cells. These results demonstrated that secondary metabolite effectively inhibits the growth of parasites and does not have cytotoxic effects on the host cells. Thus, this compound is involved straightly with antileishmanial activity and could be useful as selective source for the new antileishmanial agent.
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QT40 - EFFECTS OF THIOSEMICARBAZONE DERIVATIVE ON *Leishmania amazonensis*

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Infection by protozoan parasites of the *Leishmania genus* represents an important public health problem. The basic treatment consists on the administration of the pentavalent antimonials derivatives. However, the use of these drugs shows serious disadvantages because of their toxic effects, emphasizing the importance of developing new, effective, and safe chemotherapies against leishmaniasis. Thiosemicarbazones and thiosemicarbazides are particularly interesting, as well as their metal complexes, due to their biological properties. Studies have shown that among essential metals, copper complexes have the greatest activity. In the present work, we have investigated the antileishmanial activity of the compound [Bis [N- 4- [*R*-1-metil-4-(1-metiletenil)-cicloexeno]-*o*-clorobenzaldeidotiossemicarbazonato)] derivative from limonene copper complexes, denominated as TSZ against the protozoan *Leishmania amazonensis*. The copper (II) complex was obtained by reacting a thiosemicarbazone methanol solution with $CuCl_2.2H_2O$ in a 1:1 ligand:metal molar ratio. The resulting solids were washed with ethanol, afterwards with chloroform, and then finally dried. To evaluate the effects of this compound in the protozoan growth, we have performed *in vitro* assays with promastigote forms,. The 50% inhibition concentration (IC_{50}) was determined by direct counting of the cells in an Neubauer chamber. Ultrastructural alterations of promastigote forms treated with TSZ were analysed by transmission electron microscopy (TEM). The TSZ showed activity against *L. amazonensis*, with IC_{50} values of 3,5 µM. Analysis of TEM showed the presence of autophagic process to remove damaged organelles, vacuolization and extraction of the cytoplasm, swollen mitochondrial, chromatin disorganization, and formation of exocytic projections in the flagellar pocket. These results show that TSZ may represent a good candidate for the development of a new leishmanial drug.
Acknowledgements: This study was supported by grants from CNPq, FINEP, CAPES and Fundação Araucária

QT41 - ANTILEISHMANIAL ACTIVITY OF ELATOL OBTAINED FROM BRAZILIAN RED SEAWEED *Laurencia obtusa*

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Leishmaniasis is a human disease caused by organisms of the *Leishmania* genus which are associated with significant rates of morbidity and mortality throughout the world. Currently, the drugs used for treatment of leishmaniasis are highly toxic and present limited efficacy. This research aimed to assess the *in vitro* activity of Elatol, isolated from the Brazilian red seaweed *Laurencia obtusa,* against *L. amazonensis.* Elatol is a secondary metabolite used by algae as source of chemical defenses against other organisms. The antiproliferative effect of Elatol was determined by direct counting of the cells in a Neubauer chamber. The survival index was determined, to evaluate the effect of the Elatol on intracellular amastigotes. Morphological and ultrastructural alterations of promastigote forms treated with Elatol were analysed by scanning electron microscopy (SEM) and transmission electron microscopy (TEM). The antiproliferative assays showed the 50% inhibitory concentration (IC_{50}) values for promastigote of 6.0 µg/mL. The internalization index reflected a decrease of 75.0, 65.0, and 57.0% of growth intracellular amastigote when treated with 5.0, 1.0, and 0.5 µg/mL, respectively. Cells treated with Elatol revealed alterations in the shape and size when observed by SEM. These alterations were progressive with increasing drug concentrations, and eventually included the disintegration of the cell of parasites. TEM showed mitochondrial damage, presence of myelin-like figures, cytoplasmatic extraction and formation of exocytic projections in the flagellar pocket. These results suggest that Elatol has promising antileishmanial potential for the leishmaniasis. Moreover, Elatol had significant activity against intracellular amastigotes, replicative forms present in the vertebrate host.

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QT42 - STRUCTURE-ACTIVITY RELATIONSHIP OF 1,3-DISUBSTITUTED-β-CARBOLINES DERIVATIVES AGAINST *Leishmania amazonensis*

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The leishmaniasis is an infectious disease of multifaceted clinical manifestations caused by protozoan parasites of the genus *Leishmania*. Advances have been made in the development of new drugs for the treatment of leishmaniasis. However, the basic treatment for the disease consists in the administration of pentavalent antimonials; which unfortunately show considerable toxicity for the patients. The requirements for a new drug include low toxicity, high efficacy, and an affordable price. The β-carboline compounds showed many biological activities, such as antiproliferative to cancer cells and parasiticidal. In the present search we evaluate the antileishmanial activity of the twenty-four 1,3-disubstituted-β-carbolines on *L. amazonensis* promastigotes. The methyl 1-(substituted phenyl)-β-carboline-3-carboxylates **4a-h** were prepared through a Pictet-Spengler condensation of the L-tryptophan methyl ester **2** with appropriate aromatic aldehydes and subsequent oxidation of methyl tetrahydro-β-carboline-3-carboxylates **3a-h**. Conversion of the derivatives **4a-h** to the corresponding 1-(substituted phenyl)-3-*N*-butylcarboxamide **5a-h** and -3-*N*-pyrrolidilcarboxamide β-carbolines **6a-h** was carried out by reaction with butylamine and pyrrolidine, respectively. Promastigotes were treated with several concentrations of the compounds and the parasite growth was determined by counting. Treatment of promastigote with the compounds **4a-h**, **5a-h**, and **6a-h** resulted in growth inhibition of the parasite. The most effective compounds were **4b,e,g** (containing in position-1 the groups *p*-methoxy phenyl, *m*-methoxy-*p*-hydroxy phenyl and *o*-chlorine phenyl, respectively), **5a,b,g** (containing in position-1 the groups *p*-dimethylamino phenyl, *p*-methoxy phenyl and -*o*-chlorine phenyl, respectively), **6a,c,g** (containing in position-1 the groups *p*-dimethylamino phenyl, phenyl and -*o*-chlorine phenyl, respectively) with IC_{50} (50% inhibition concentration) between 0.2 and 9.0 µM. Our studies revealed the antileishmanial activity of β-carbolines derivates, such as the chemical structure had important biological activity.

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QT43 - Effect of crude extracts obtained from Piperaceae family on promastigote forms of *Leishmania amazonensis*

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Leishmaniasis is a disease caused by a number of species of protozoa of the genus *Leishmania* and it represents an important public health problem in more than 88 countries around the world, where 350 million people live at risk of infection with 2 million new cases annually. *Leishmania amazonensis* is the most important agent of diffuse cutaneous leishmaniasis and is widely found in South America. Natural products from plants offer vast and valuable sources of bioactive compounds and have been traditionally used in folk medicine to treat illnesses from headaches to parasite infections. Piperaceae family is found in tropical countries and has been reported to exhibit antimicrobial, antifungal and antiprotozoal activity. Twenty-five crude extracts obtained from 8 species of Piperaceae were screened for antileishmanial activity against promastigote forms of *L. amazonensis* WHOM/BR/75/JOSEFA strain. The parasites were treated with several concentrations of the crude extracts (1, 10, 50, 100, 500, and 1000 µg/mL) and 50% growth inhibition concentration (IC_{50}) was determined by counting in Neubauer hemocytometer. The species that showed IC_{50} < 30 µg/mL were: chlorophorm extract from leaf (*Piper crassinervium* IC_{50} 13.5 µg/mL), dichloromethanic extract from leaf (*Piper arboreum* IC_{50} 22 µg/mL, *Piper amalago* IC_{50} 29 µg/mL, *Piper dyospirifolium* IC_{50} 29 µg/mL, *Piper aduncum* IC_{50} 18.7 µg/mL, *Piper hispidum* IC_{50} 16.3 µg/mL), dichloromethanic extract from stem, (*Piper aduncum* IC_{50} 6 µg/mL, *Piper xylosteoides* IC_{50} 21 µg/mL), and aqueous extract from stem (*Piper aduncum* IC_{50} 20 µg/mL). The screening of 8 species indicated that 7 showed activity against *L. amazonensis*. The antileishmanial activity presented by the crude extracts obtained from species of Piperaceae deserves further investigations in order to isolate and identify the bioactive compounds.
Acknowledgements: CNPq, Fundação Araucária, FINEP, Pronex, CAPES

QT44 - EVALUATION OF LEISHMANICIDAL ACTIVITY OF A NATURAL PRODUCT ISOLATED FROM *COMBRETUM LEPROSUM* FRUITS *IN VITRO*

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The leishmaniasis is a disease caused by protozoa of the genus Leishmania, transmitted to humans by the bite of female sandflies of Phlebotominae subfamily. This disease affects 12 million people in 88 countries of the world. In Brazil the *Leishmania braziliensis* is the most common ethiological agent of the leishmaniasis. The available antileishmanicidal medicines present problems as toxicities and resistance of the parasites. The currently available treatments include pentamidina and anfotericina B, which cause serious adverse effects. Plants are rich sources of novel compounds that can be used to developed new drugs for almost all diseases. The *Combretum leprosum* is a species of Combretaceae family, known popularly as mofumbo or mufumbo. It is used as healing, antihemorrhagic, anti-inflammatory among others. The present work evaluated leishmanicidal activity of CLEFT-1, a substance isolated from fruits of *Combretum leprosum* and a derivative (acetylated and oxidated) on promastigotes of *Leishmania braziliensis* *L. braziliensis* promastigotes were cultivated in the absence or presence of 10, 5 and 2 µg/mL of CLEFT 01 and its derivative for five days at 23°C. At concentration of 10µg/mL of CLEFT-1 the growth of promastigotes was inhibited in 98%. The concentrations of 5µg/mL and 2µg/mL presented an inhibition of 95% and 78% respectively. The derivative showed a lower activity than CLEFT01, with an inhibitory effect of 77% (10µg/mL), 67% (5µg/mL) and 29% (2µg/mL). The CLEFT 01 and its derivative did not present cytotoxic effect in cultures of murine macrophages. The presented results show that the CLEFT-1 from *C. leprosum* and its derivative has a significant activity against *L. braziliensis* promastigotes, demonstrating the potential of this drug as a leishmanicidal agent.
Supported by CNPq and CAPES

QT45 - EVALUATION OF ACTIVITY OF CRUDE EXTRACTS OF *Piper amalago* AGAINST *Phytomonas serpens*

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Phytomonas are trypanosomatids parasites of many families of plants, including agents of important pathogens of plants with great economic interest. Some species of *Phytomonas* are able to cause lethal diseases, since infection sites restricted products such as fruits and laticifers. Species of the genus *Piper* are best known because of its commercial use and in traditional medicine, because their many biologically active compounds that have antifungal activity, bactericidal, antitumour, and trypanocidal effects, especially *Piper amalago* is used in folk medicine in regions of Mexico such as antimicrobial an antiparasitic . The crude extracts (hydroalcoholic and chloroformic) were obtained by maceration of dried leaves of *P. amalago* and then was evaluated the activity of these extracts against promastigote forms of *Phytomonas serpens* using the microdilution plate. The promastigotes were treated with several concentrations of both extracts (10 to 1000 □g/mL) in Warren medium supplemented with 10% fetal calf serum, incubated at 28 ^{o}C. The growth was determined by counting the parasites with a haemocytometer chamber every 24 h over 7 days and was calculated the 50% inhibitory concentration (IC_{50}). To evaluate the toxicity of the extracts on mammalian cells was used $LLCMK_2$ cells, the citotoxicity assay was performed by sulforhodamine B technique and then was calculated CC_{50} (concentration that lyses 50% of cells). The results demonstrated that the both crude extract of *P. amalago* have strong effect over the proliferation of *P. serpens,* resulting in IC_{50} of 57 µg/mL and 22.5 µg/mL to chloroformic and hydroalcoholic extracts, respectively. The extracts showed a moderated and low toxicity. The chloroformic extract showed a CC_{50} of 199 µg/mL and the hydroalcoholic extract a CC_{50} above 500 µg/mL. These data demonstrate substantial selectivity on the protozoan and stimulate new *in vitro* assays.

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QT46 - ANTITRYPANOSOMAL AND ANTILEISHMANIAL ACTIVITY OF *Bixa orellana*

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Leishmaniases and Chaga's disease are endemic in Latin America and affect million of people. They're caused by the protozoa *Leishmania* and *Trypanosoma cruzi*, respectively, and their treatments are very limited. Treatment of Chaga's disease is based in benznidazole and nifurtimox and leishmaniasis in amphotericin B and pentamidine that present unpleasant side effects. Due to this lack of effectiveness and security of the treatment, new drugs are still urgently needed and many compounds, naturals and synthetics, have been investigated as potential new agents. Previous pharmacological studies have revealed that *Bixa orellana* extracts showed antiprotozoal, antihelmintic, and platelet antiaggregant activity. Its methanolic extract has just demonstrated a good activity against promastigote forms of *L. amazonensis*. In this work, hidroalcoholic fraction (crude extract) and an oily fraction were extracted from *B. orellana* seeds. The fractions were evaluated against promastigote of *L. amazonensis* and epimastigote forms of *T. cruzi*. The protozoa were treated with several concentrations of the compounds and the parasite growth was determinated by counting in Neubauer chamber. The IC_{50} (50% growth inhibition concentration) values for *L. amazonensis* were 20 and 137 µg/mL for the oily and hidroalcoholic fraction, respectively. For *T. cruzi* the IC_{50} values were 23 and 507 µg/mL, respectively. This indicates that the hidroalcoholic fraction was more active to *L. amazonensis* than to *T. cruzi*. Additionaly, the oily fraction was more active than the hidroalcoholic fraction to both protozoa. These results encouraged us to continue the experiments with the oily fraction and the crude extract against *L. amazonensis* and *T. cruzi,* with i*n vitro* and *in vivo* studies.

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

QT47 - *IN VITRO* TRIPANOCIDAL EFFECT OF *EICHORNIA CRASSIPES* ON *TRYPANOSOMA CRUZI* DM28C

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The family *Pontederiaceae* includes popular aquatic plants called Aguapé, being *Eichhornia crassipes* the most popular specie in Brazil. *Eichhornia crassipes* has been predominantly used for phytoremediation against heavy metal contamination, however it also shows antimicrobial activity against bacteria an fungi. We evaluated the effect of the *Eichornia crassipes* ethanolic extract on epimastigotes forms of *Trypanosoma cruzi* (DM28c strain) obtained from axenic cultures. The extract was obtained by maceration of total components from *E. crassipes* followed by extraction with ethanol, at room temperature. The stock solution of *E. crassipes* extract was prepared in 10% DMSO and added asseptically to LIT medium in order to obtain concentrations ranging from 100 to 1000 µg/ml. The growth of epimastigotes forms of *Trypanosoma cruzi* at 28ºC in LIT containing varying concentrations of ethanolic extract from *E. crassipes* was evaluated. The addition of *E. crassipes* ethanolic extract in the culture medium significantly inhibited the growth of epimastigotes forms of *T. cruzi* after 96 hours. The growth inhibition ranged from 48% to 100% in a manner dependent on extract concentration. The final concentration of DMSO never reached 10% when introduced in culture medium and don´t showed inhibition in *T. cruzi* growth in DMSO control. Our future aim is to evaluate fractions obtained from crude extract in order to isolate its most active component.

QT48 - IN VITRO ANALYSES OF THE EFFECT OF AROMATIC DIAMIDINES UPON TRYPANOSOMA CRUZI

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Aromatic diamidines (AD), a class of compounds that includes pentamidine and other related drugs, have been recognized as promising anti-parasitic agents with striking broad-spectrum activity. In the present work we determined: the *in vitro* effect of eleven AD upon the relevant clinical forms of *T. cruzi,* their toxicity to mammalian cells and their sub-cellular localization. Regarding the effect of the AD on bloodstream trypomastigotes, our results showed a dose dependent activity, being the compounds 2, 5, 7 and 9 the most active, with IC_{50} values on the micromolar range (0.7 to 2.7 µM). In the presence of blood, all the AD exhibited a strong reduction of their activity, which can be due to the association with serum components or component instability. Concerning the effect on cardiac cells, only after incubation for 72 h at the concentration of 96 µM, the AD displayed some toxicity, however it did not exceed 34% of viability loss. The treatment of *T. cruzi*-infected cardiomyocytes showed that 10 out the 11 AD displayed similar or higher effect when compared to the direct effect on trypomastigotes. The most active AD were the compounds 2, 5 and 7 leading to total inhibition of parasite bursting at 10.7 µM. Transmission electron microscopy of bloodstream forms treated with compounds 5 and 7 revealed detachment of plasmalemma and nuclear envelope, swelling of mitochondrial cristae besides profound kDNA alterations, leading to its fragmentation. Fluorescence analysis showed that, although all AD localize in the nucleus and preferentially in the kinetoplast, the highest kDNA selectivity was not directly related to the compound efficacy. Our results show the high activity of this group of AD, on both trypomastigotes and intracellular amastigotes forms, with an excellent selectivity index, especially compounds 5 and 7, which merits further *in vivo* evaluation.
Supported by CAPES, CNPq, FIOCRUZ and FAPERJ.

QT49 - HISTOPATHOLOGICAL RESPONSE OF THE EXPERIMENTAL INFECTION BY *TRYPANOSOMA CRUZI* TO THE TREATMENT WITH THE IRON CHELATOR DESFERRIOXAMINE IN ASSOCIATION WITH BENZNIDAZOLE

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The nutritional status of the host can influence host-parasite relationship. Iron ions play an important role in *Trypanosoma cruzi* infection. In this study we evaluated the effects of iron deprivation in association with Benznidazole (Bz) on histopathological response of the infection of mice by Y strain of *T. cruzi.* Male *Swiss* mice were divided into seven experimental groups: (1) not infected and not treated - NINT; (2) infected and not treated - INT; (3) infected and treated with Bz - BZ; (4) infected and treated with Desferrioxamine (DFO) during 21 days DFO (21); (5) infected and treated with Bz and DFO during 21 days DFO+BZ (21); (6) infected and treated with DFO during 35 days DFO (35) and (7) infected and treated with Bz and DFO during 35 days DFO+BZ (35). The animals were sacrificed in the 10^{th} and 16^{th} days after infection (dpi). The average parasitemia value in DFO + BZ (21) was fifty eight times lower than INT group. The cardiac inflammatory process was significantly higher in DFO (35) group when compared with NINT, INT, BZ and DFO (21) groups in the 10^{th} dpi. The cardiac parasitism was significantly higher in INT group in relation to DFO (21) and 35 groups. No significant differences between the groups have been observed in the liver. However the remaining animals of DFO (21) and 35 groups have presented a lower parasitism in comparison to INT group. This study suggests that the prolonged treatment with DFO on *T. cruzi*-infected mice reduces the availability of iron to the infecting parasites, leading to a reduction in parasitemia and mortality. The treatment with Bz eliminated the parasite of the heart, and liver and cardiac inflammatory process was higher in DFO (35). No differences were observed in hepatic inflammatory process in the evaluated groups. Supported by CNPq, FAPEMIG & UFOP.

QT50 - THE SENSITIVITY OF CLINICAL ISOLATES OF *LEISHMANIA CHAGASI* TO MILTEFOSINE

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The current chemotherapy for leishmaniasis is limited, because the drugs are toxic and most have to be used parenterally for prolonged period. Oral miltefosine has recently been licensed for used in India for the treatment of visceral leishmaniasis (VL). This drug is an alkylphosphocholine, initially developed as an anticancer agent that also shows activity against *Leishmania*. During a clinical trial to evaluate the efficacy of miltefosine in the treatment of VL we detected around 50% of relapse. In order to evaluate whether this inefficacy of miltefosine treatment was dependent of resistance by *Leishmania* strains, we studied the sensitivity of strains isolated from patients with VL. All patients had positive parasitological diagnosis by microscopic examination and culture of bone marrow aspiration. The patients were treated with 2,5 mg/Kg/day of miltefosine during 28 days. Response to treatment was evaluated by repeating the bone marrow aspiration at day 28 and the designation of responsive patients were based on absence of signs and symptoms of the disease. All patients were followed up to six months and they were considered cured if there were no signs and symptoms of relapse. In this study, we evaluated 10 isolates which typing as *L. chagasi* using polymerase chain reaction-restriction fragment length polymorphism. Promastigotes within eight passages from isolation were used to infect mouse peritoneal macrophage, and culturing them in medium containing different concentrations of miltefosine. For each test we calculated a 50% effective dose (ED_{50}). All strains obtained from responsive patients showed a mean ED_{50} of 3,6μM, whereas isolates from non-responsive were more heterogeneous with a ED_{50} between 2 μM and >15μM which correspond to the highest tested concentration. These results suggested that the unresponsive can be related to miltefosine-resistant strains. We are currently evaluating the sensitivity of strains obtained after treatment and relapse.
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QT51 - *In vivo* effects of megazol derivatives on *Trypanosoma cruzi*

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Megazol is active against *T. cruzi*, including strains resistant to benzindazole and is a core structure for the design of new drugs for Chagas' disease. We synthesized and assayed the trypanocidal activity of 32 derivatives introducing the arylhydrazone moiety in the heterocyclic framework of megazol. We identified a prototype, Brazilizone A, the 3,4-dihydroxyphenyl derivative (S1), two times more potent than the lead compound, and other seven derivatives S2 to S8 with IC_{50}s/24 h between 11 and 54 µM. These compounds were screened *in vivo*, using a single oral dose of 200 mg/kg at 5 dpi. S1 led to values of body weight, parasitemia and mortality similar to those of the infected control group (GTc). For S2 and S3, it was observed a significant decrease of the parasitemia and mortality. S4 and S5 led to a significant decrease only of the parasitemia peak, while S6 to S8 caused no difference in relation to the GTc. Further experiments were performed only with S1, S2 and S3. Four days after a single dose of 400 mg/kg of each derivative to non infected mice, the levels of urea, GPT and GOT were unaltered in S1-treated mice; for S2 there was an increase of 1.3X in GOT, and for S3 an increase of 2.0X and 1.3X in, GOT and GPT, respectively. The treatment of infected mice with S1 (50 and 100 mg/kg) from 6 to 15 dpi caused no decrease of the parasitemia or mortality, and did not reverse the increase of GPT and GOT caused by the infection. Experiments are underway to investigate the *in vivo* effect of S2 and S3 in the scheme of 10 days-treatment.
Supported by CNPq, Fiocruz and FAPERJ.

QT52 - A NEW TOOL FOR HIGH-THROUGHPUT ASSAYS TO SCREEN POTENTIAL DRUGS AGAINST *TRYPANOSOMA CRUZI*

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The two drugs available for the treatment of *T. cruzi* infection, nifurtimox and benznidazole, have several toxic side effects and variable efficacy, contributing to their low rate of use. With scant economic resources available for antiparasitic drug discovery and development, inexpensive, high-throughput assays to screen potential new drugs and existing compound libraries are essential. The objective of this work was to develop and validate an improved method for growth and drug assays in *T. cruzi*. Toward that end, we generated parasite lines expressing the tandem tomato fluorescent protein (tdTomato) by transfection of *T. cruzi* epimastigotes with a pTREX-Neo-tdTomato plasmid. Tomato red parasites were easily observed by flow cytometry or fluorescence microscopy. For drug assays, parasites were plated in 96 well black plates with or without drug and the change in fluorescence intensity as a measure of growth was determined by plate reader each day for 3 days. An IC50 for each compound was calculated. We had similar IC50s for control compound (Benznidazole) as did other previously described methods (e.g. visual counting by hemacytometer and colorimetric assays using AlamarBlue). However the fluorescence-based assay had the added advantages of requiring significantly fewer parasites ($1x10^4$parasites/well vs.10^6/well for AlamarBlue assay), and, because it is not an endpoint assay, parasite growth can be monitored daily, giving more accurate estimation of drug activity. Inter- and intra-assay variation for Benznidazole IC50 was low. Lastly, the method was used to identify new candidate drugs with IC50s equal to or below that of benznidazole. In conclusion, we have developed a consistent and low cost drug testing method for *T. cruzi* does not require any additional indicator reagent, enzymatic process or laborious visual counting. These and similar fluorescent parasite lines should constitute a new tool for faster and high-throughput assays to screen potential new drugs.
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QT53 - ACTIVITY OF β-CARBOLINES DERIVATIVES AGAINST EPIMASTIGOTE FORMS OF *Trypanosoma cruzi*

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Trypanosoma cruzi is the etiological agent of Chagas disease, a debilitating disease that affects millions of people and causes the deaths of 45,000 patients annually. The current treatment for this disease is very limited and available drugs have many side effects. β-carboline compounds already showed biological activities in cancer cells and strong effect parasiticidal. In the search for new trypanocidal agents, we evaluate activity of twenty eight 1,3-disubstituted-β-carbolines on *T. cruzi* epimastigotes. The 1-(substituted phenyl)-β-carboline-3-carboxylic acid (**5a-g**) were prepared through Pictet-Spengler condensation of L-tryptophan methyl ester (**2**) with appropriate aromatic aldehydes, oxidation of methyl tetrahydro-β-carboline-3-carboxylates (**3a-g**) and subsequent hydrolyzation of the compound methyl β-carboline-3-carboxylates (**4a-g**). Conversion of the derivatives **5a-g** to the corresponding 1-(substituted phenyl)-3-*N*-isopropylcarboxamide (**6a-g**), -3-*N*-benzylcarboxamide (**7a-g**) and -3-*N*-cycloexylcarboxamide β-carbolines (**8a-h**) was carried out by reaction of **5a-g** with isopropylamine, benzylamine, and cycloexylamine, respectively. Epimastigotes and $LLCMK_2$ cells were treated with several concentrations of the compounds. The parasite growth was determined by counting and cell viability was evaluated by sulforhodamine B technique. Were calculated the selectivity index (SI), where a value greater than 1.0 considered that the drug is more selective for the parasite. β-Carbolines derivates showed be more toxic to epimastigote than to $LLCMK_2$ cells. The most effective compounds were **6a,d,f,** with SI of 93.4, 94.9, and 122.8, respectively. The SI values for the series **5a-g** (2.5 to 36.2), **7a-g** (1.0 to 5.0), and **8a-h** (2.5 to 70.8) also demonstrated moderate activity against *T. cruzi.* The series of β-carbolines containing *N*-carboxamide group in position-3 showed increased activity when compared to the β-carbolines 3-carboxylic acid derivatives and the series of compounds **6a-g** were the most active of all synthesized compounds.
This study was supported through grants from DECIT/SCTIE/MS and MCT by CNPq, FINEP, and CAPES.

QT54 - CHEMICAL STUDY AND EVALUATION OF LEISHMANICIDAL ACTIVITY FROM THE ETHANOLIC EXTRACT, ELUATE AND ISOLATED SUBSTANCE FROM *Humirianthera ampla* Miers

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Humirianthera ampla Miers. belongs to the Icacinaceae family and , it is known in the folk medicine as surucucaína. Leishmaniasis is a parasitic disease that is found in parts of the tropics, subtropics, and southern Europe. It is caused by infection with *Leishmania* parasites, which are transmitted by the bite of infected sand flies. Chemotherapy of leishmaniasis is quite longstanding, despite the advances in recent years. The drugs used to treat this disease have high toxicity with severe side effects, reasons for looking for new alternatives. This study evaluated various concentrations of ethanolic extract (EE), eluate of acetate ethyl:hexane (EI) and isolated substance (HAP) from the root of the specie *Humirianthera ampla*, against promastigotes forms of *Leishmania braziliensis*. Promastigotes of *Leishmania braziliensis* had been cultivated in absence or presence of 100, 20, 10, 5 and 2.5μg/mL of extracts, eluated and isolated substance for five days at 24°C. The concentration of 100 μg/mL of all fractions of EE, EI and HAP completely inhibited the growth of parasites. The EE in concentration of 5 μg/mL inhibited in 67% the promastigote growth, showing an IC_{50} of 4.2μg/mL. The EI in the concentration of 10μg/mL showed an inhibition of 52% (IC_{50} = 4.44μg/mL). HAP, a substance isolated from *H. ampla*, was identified as a triterpene, with melting point 124-128 °C. It showed an inhibition of promastigote growth about 70%, at concentration of 5μg/mL, with an IC_{50} of 3.8μg/mL. These compounds has no toxic effect in peritoneal macrophages of BALB/c mice at the concentrations of 2.5, 5, 10 e 20μg/mL. In conclusion the ethanolic extract, the eluate and the isolated triterpene inhibited *L. braziliensis* promastigotes growth *in vitro* demonstrating a possible use of compounds from *H. ampla* as potential antileishmanial drug.
Supported by CNPq.

QT55 - *TRYPANOSOMA CRUZI*: INFLUENCE OF IRON DEFICIENCY ASSOCIATED OR NOT WITH BENZNIDAZOLE ON THE SURVIVAL OF INFECTED MICE AND EVALUATION OF BIOLOGICAL AND BIOCHEMICAL PARAMETERS OF ISOLATES PARASITES BY HEMOCULTURE

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Previously it was demonstrated that iron deficiency in mice, followed by infection with *Trypanosoma cruzi*, reduce parasitemia, mortality and injuries resulting from the acute phase. In the present study, the influence of Benznidazole (Bz) therapy in combination with the iron chelator Desferrioxamine (DFO) on the survival of mice inoculated with *T. cruzi* Y strain have been investigated. Swiss male mice (30-days-old; n=120) were distributed in six groups of twenty animals: (1) infected and not treated – INT; (2) infected and treated with Bz – BZ; (3) infected and treated with DFO during 21 days – DFO (21); (4) infected and treated with Bz and DFO during 21 days – DFO+BZ (21); (5) infected and treated with DFO during 35 days – DFO (35) and (6) infected and treated with Bz and DFO during 35 days – DFO+BZ (35). After eighteen months of inoculation it was observed 13.3% of survival (16 mice) distributed as following: 0% for INT, DFO (21), DFO+BZ (21) groups, 20% for BZ and DFO (35) and 40% for DFO+BZ (35) group. The % of cure, determined by ELISA (12,5%) and hemoculture (81.25% negative), was 0%. All positive samples were obtained for DFO+BZ (35) group. Hc positive samples were inoculated in mice for biological and biochemical characterization and kept in exponential growth for isoenzimatic characterization. The GPI profile (glucose-6-phosphate isomerase - E.C. 5.3.1.9, GPI) was similar to the original parental Y strain (T. cruzi II, sublineage 2b). On the other hand, biological parameters were different from the parental strain being observed significant lower parasitemia and mortality. Taken together, these results emphasize that iron alteration can modulated the pathogenicity and virulence of *T. cruzi* in vertebrate host. SUPPORTED BY CNPq, FAPEMIG, UFOP.

QT56 - PHYTOCHEMISTRY STUDY AND EVALUATION OF LEISHMANICIDAL ACTIVITY OF THE ESSENTIAL OIL OF *Copaifera Hayne multijuga in vitro*

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The oil-resin of Copaiba is extracted from trees of the *Copaifera* genus belonging to the *Leguminoseae* family. The volatile oils are produced through distillation by drag of water vapor. Leishmania is a protozoan belonging to the *Trypanosomatidae* family, with two main forms, a flagellated promastigote, found in the gut of the insect vector and a rounded form, called amastigote found in tissues of vertebrate hosts, within the cells. This work aims to evaluate the action of major active components of essential oil of *Copaifera multijuga* against promastigote forms of *Leishmania amazonensis in vitro*. The oil-resin (50 ml) extracted from *Copaifera multijuga* was subjected to a hydrodistillation for 4 hours, using glass Clevenger-like extractants, yielding 10 ml of essential oil (OECM). The OECM major constituents was (*E*)-cariofilene (27,8%), *trans*-cadina-1(6),4-diene (12,1%), δ-cadinane (17,5%), germacrene D (12,3%), epi-zonarene (6,5%), β-sesquiphellandrene (5,9%) e α-humulene (4,0%). To assess the leishmanicidal activity of the Copaiba oil, *L. amazonensis* promastigotes were grown in the presence of 200ug/ml, 150ug/ml, 100ug/ml, 50ug/ml and 25ug/ml of OECM. It was observed that all concentrations were able to inhibit the promastigote growth around 90%. These results indicate that the major constituents of essential oil of *C. multijuga* have leishmanicidal action, inhibiting the proliferation of promastigotes forms of *L. amazonensis in vitro*. Thus, the *C. multijuga* could be used for the development of drugs with leishmanicidal activity.
Supported by CNPq

QT57 - Biological effect and selectivity of dicationic compounds upon *Trypanosoma cruzi in vitro*

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Trypanosoma cruzi is an intracellular obligatory parasite that causes Chagas' disease, an important life-threatening disease affecting millions of individuals in endemic areas of Latin America. However, in the year that we commemorated the centenary of the discovery of Chagas' disease, many challenges are still present, including those related to its chemotherapy. Aromatic dicationic compounds such as pentamidine represent an important class of DNA minor groove-binding ligands that exhibit high antimicrobial activity. This study focused on the *in vitro* activity of ten aromatic dicationic compounds against bloodstream trypomastigotes as well as upon intracellular forms of *T. cruzi*. Our data showed that three compounds, 1MAA119, 25DAP013 and 14SMB013, induced high levels of parasite lysis, showing dose-dependent effects with a low micromolar IC_{50} range. On the other hand, five out of 10 compounds (12SMB032, 10SAB092, 14SMB013, 11SAB081 and 10SAB031) exerted considerable activity against the intracellular forms of the parasite, with low micro and sub-micromolar doses, and also displayed high selective indices (SI values ranging between > 43 and >960). This intriguing activity upon intracellular forms, as compared to bloodstream forms, deserves further analysis, but could represent differences on drug uptake by these different parasite stages and/or different mechanisms of action upon non dividing trypomastigotes and the highly mutiplicative intracellular stages of the parasite. Additional assays to determine the potential toxicity to mammalian cells showed that up to 96 µM, the majority of the dicationic compounds did not induce considerable loss of cellular viability. Fluorescent microscopy analysis demonstrated that although all compounds were localized at a higher extent within the kinetoplast than in the nucleus, no correlation could be found between compound activity and kDNA accumulation. The present results stimulate further investigations of this class of compounds for the rational design of new chemotherapy agents for Chagas' disease.

QT58 - IN VITRO EFFICACY OF MEGLUMINE ANIMONIATE AND AMPHOTERICIN B COMBINATION AGAINST *LEISHMANIA CHAGASI*

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Combination therapy for leishmaniasis could have a role in delaying the development of resistance and shortening the duration of treatment. Here, we report the *in vitro* interactions between meglumine antimoniate (Glucantime) and amphotericin B (AMB) drugs. *In vitro* drug interactions were assessed using a modified fixed-ratio isobologram method. Concentrations of 100 µg/ml for Glucantime and 0.2 µg/ml for AMB were used to prepare fixed-ratio solutions at 5:0, 4:1, 3:2, 2:3, 1:4, and 0:5, which were serially diluted five times in twofold dilutions. Peritoneal macrophages from Balbc mice were plated in culture chamber slides, infected with *L. chagasi* promastigotes at ration of 1:7 and culturing in medium containing different drug concentrations. Drug activity was determined from the percentage of infected cells in drug-treated cultures in relation to nontreated cultures. From the known concentrations of Glucantime and DAMB in the fixed-ratio solutions, EC_{50} and EC_{90} values were calculated by sigmoidal regression analysis. The fractional inhibitory concentrations (FICs) and sum FICs (∑FICs [FIC Glucantime +FIC DAMB]) were calculated as follows: FIC of Glucantime = $EC_{50(90)}$ of Gucantime in combination/$EC_{50(90)}$ of Glucantime alone. The same was applied to DAMB. FICs and ∑FICs were calculated for all fixed-ratio solutions. Mean ∑FICs were used to classify the nature of the interaction. EC_{50s} of Glucantime was 21,5 µg/ml and for DAMB was 0,021 µg/ml. *In vitro* interactions data were analyzed at the EC_{50} and EC_{90} levels. Mean ∑FICs were classified as synergistic with mean ∑FICs of <0.5, as antagonistic with mean ∑FICs of >4, and as additive with mean ∑FICs between >0.5 and <4. The interaction of Glucantime with DAMB was additive with mean ∑FICs of 0,65 to 1.02 and 0,91 to 1,08 at the EC_{50} level and EC_{90} level, respectively. Additional *in vivo* assay will be done to confirm this finding.

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QT59 - PROSPECT OF ANTIMICROBIAL AGENTS PRESENT IN EXTRACTS FROM *RHODNIUS PROLIXUS* EGGS

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The protozoan parasite *Trypanosoma cruzi*, causative agent of Chagas' disease, develop exclusively within the digestive tract of its insect triatomine vectors, never entering into direct contact with hemocytes or hemolymph factors, key elements of insect innate immunity against microbial infections. As a result, *T. cruzi* evolved a mode of transmission that avoid direct contact with lethal components of the vector's immune response and also impair the generation of infected oocytes. Therefore, we hypothesized that oocytes could accumulate sufficient antimicrobial compounds that would not only be toxic to *T. cruzi* but also protect them from other kind of microbial attack, especially after the eggs were deposited in the environment. To test this hypothesis, eggs from *R. prolixus* were grinded in the presence of a protease inhibitor cocktail and the egg yolk extract separated from eggshell and lipid remains by differential centrifugations. The clarified extract was loaded onto C_{18}SepPack and the resin washed with aqueous buffered saline (TMS) followed by stepwise elution with 25%, 50% and 75% aqueous methylformate, 100% methylformate, and hexane. The TMS wash content was precipitated with cold acetone and equivalent amounts of the organic fractions were dried under N_2. After suspension into sterile TMS, the antimicrobial potential of each fraction was evaluated by their anti-proliferative effects on *S. cerevisae* and epimastigote forms of *T.cruzi*. Only the TMS fraction showed a strong dose-dependent activity against yeast and epimastigotes, reaching 88-99% growth inhibition after 48h-72h. Tests using a cell-free system confirmed that the TMS fraction was capable to inhibit the inositol phosphorylceramide (IPC) synthase of both yeast and *T. cruzi*. Because IPC synthase is an essential enzyme for fungal viability and is not present in mammals, the TMS fraction represents an excellent starting point for the prospect of novel antimicrobial agents in extracts from *R. prolixus* eggs.
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