TB020 - EICOSANOID BIOSYNTHETIC PATHWAY IN PATIENTS WITH TEGUMENTARY LEISHMANIASIS

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Eicosanoids display an important role during Leishmania infection. Indeed, prostaglandin E2 (PGE2) has been shown to benefit parasite survival whereas Leukotriene B4 (LTB4) enhances intracellular parasite killing by host cells. However, whether this dichotomy is relevant in the context of Localized Cutaneous (LCL) and Mucosal Leishmaniasis (MCL) remains unknown. Here, we assess circulating levels as well as in situ RNA expression of mediators from the eicosanoid pathway in patients with LCL patients or MCL from an endemic area in Brazil. Plasma levels of LTB4 were higher in MCL patients than in those with LCL (median: 353pg/ml, IQR: 246-541 vs. 212pg/ml, 127-289; P=0,01). In converse, MCL individuals exhibited lower levels of PGE2 than LCL patients (median: 185pg/ml, IQR: 104-261 vs. 959pg/ml, 770-1160; P=0,001). LTB4/PGE2 ratio was 5-fold higher in MCL samples, suggesting that MCL is prone to skew the eicosanoid balance towards the leukotriene pro-inflammatory activity. Importantly, assessment of selected genes from the eicosanoid pathway by nanostring analyses from lesion biopsies from MCL and LCL patients underlined that expression of Cyclooxygenase 1, Prostaglandin E2 Synthase, prostaglandin E receptor 3 (PTGER3) and 5-Lipoxygenase was dramatically increased in LCL lesions compared with MCL. These findings uncover a previously unappreciated biosignature that can distinguish LCL from MCL patients based on the differential expression of biomarkers from eicosanoid pathways. Furthermore, these distinct expression profiles have potential implications on the understanding to the pathogenesis of the diverse clinical forms of tegumentary leishmaniasis investigated here, which can lead to development of new therapeutic strategies. **Supported by:**FAPESB, CNPq, FIOCRUZ **Keywords:**Mucosal leishmaniasis ; leukotriene; prostaglandin

TB021 - ACTION OF AQUEOUS EXTRACT FROM ROOTS OF PHYSALIS ANGULATA ON PROTOZOAN LEISHMANIA (LEISHMANIA) INFANTUM CHAGASI

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Leishmania (Leishmania) infantum chagasi is the protozoan that causes visceral leishmaniasis (VL) in Brazil. The disease affects viscera such as, liver, spleen, intestines and bone marrow. It is considered the clinical forms most severe among of leishmaniasis, lead to death, especially children and the elderly. The pentavalent antimony are first-line treatment, however, are toxic and invasive. In that way, search for new medicaments with a natural origin and a high efficacy against the parasite without toxicity and side effects are needed. In this context the Physalis angulata plant, which is widely used in folk medicine for its diuretic, antiinflammatory and analgesic effects, was recently shown its leishmanicidal activity against Leishmania (Leishmania) amazonensis. Thus, this study aim to evaluate the effects of aqueous extract from roots of Physalis angulata (AEPa) on Leishmania (Leishmania) infantum chagasi. The promastigotes were treated with AEPa by 24-96 hours at concentrations of 50, 100 and 200 µg/mL. The growth curve was performed to evaluate whether the extract had the capacity to reduce the number of promastigotes in vitro. Morphological analysis of the treated cells with AEPa were determined by light microscopy (LM) and transmission electron microscopy (TEM). AEPa promoted a reduction of 69% (50 µg/mL), 76 % (100 µg/mL) and 90% (200 µg/mL) on promastigotes (IC50 = 64.97 µg/mL) when compared to the control. In addition, morphological analysis by LM and TEM showed that AEPa caused morphological changes in promastigote forms. AEPa (100 µg/mL) induced apoptosis death in promastigotes forms of L (L.) chagasi and this effect is mediated by increased of reactive oxygen species (ROS). Thereby, this study revealed that AEPa is able to induce morphological changes and death by apoptosis in promastigotes forms of L. (L.) chagasi. **Supported by:**This research was supported by CNPq (Brazil), CAPES (Brazil), INBEB (Brazil), FAPERJ and FAPESPA.
Keywords: Physalis angulata; antileishmanial properties; natural products

TB022 - LQB-166 HAS ACTIVITY AGAINST *LEISHMANIA BRAZILIENSIS IN VITRO* AND SHOWS THERAPEUTIC EFFECTS ON INFECTED BALB/C MICE

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Naphthoquinones are bioactive molecules that can interfere with several cellular processes and have described anti-tumor and anti-protozoa activity. The aim of this study was to evaluate the antiparasitic effect of the lapachol analogue LQB-166 against L. braziliensis, modulation of the host cell, toxicity and therapeutic effects. Promastigotes of L. braziliensis were treated with LQB-166 (0-800µM) for 48h and counted under a microscope. Monolayers of peritoneal macrophages from SW mice were infected with L. braziliensis promastigotes (5:1), incubated with RPMI medium for 24h and treated with LQB-166 (0-800µM) for 48h. The macrophages were stained and the percentage of infected macrophages and intracellular amastigotes counted under a microscope. In the supernatant cytokine production was measured by CBA kit. The toxicity was evaluated on non-infected macrophages by MTT assay. For the in vivo experiments, BALB/c mice were infected with 106 promastigotes of L. braziliensis in the footpad and treated with LQB-166 by intralesional route 1 week after the infection for 2 weeks (200µM/3x/week). The parasite load was estimated by limiting dilution of the footpad. The naphthoquinone showed no toxicity on macrophages (LC50=3200 µM). IC50 on promastigotes forms was estimated in 34µM. On intracellular amastigotes forms IC50 was estimated in 193µM. The selectivity index was determinated in 16. LQB-166 at 200 and 400µM was capable of increase the pro-inflammatory (IL-6, TNF, MCP-1) and anti-inflammatory (IL-10) cytokine production by macrophages. The in vivo treatment with LQB-166 was capable of significantly control the lesion progress and decreases the parasite load on the footpad. These data indicate that LQB-166 is not toxic, inhibit intracellular amastigotes of L. braziliensis and is active in vivo on BALB/c mice infected. We are currently investigating the action of LQB-166 in vivo using hamsters as experimental model for L. braziliensis. **Supported by:**CAPES
Keywords: Naphthoquinone; leishmania braziliensis; balb/c

TB023 - 5-HYDROXY-2-HYDROXYMETHYL-GAMMA-PYRONE INDUCES DEATH OF LEISHMANIA (LEISHMANIA) AMAZONENSIS VIA THE GENERATION OF REACTIVE OXYGEN SPECIES

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The leishmaniasis are infectious diseases caused by kinetoplastid protozoa. The drugs used for the treatment of leishmaniasis as the antimony, pentamidine and amphotericin present severe limitations due to the emergence of resistant strains and various side effects to patients. The 5-hydroxy-2-hydroxymethyl-gamma-pyrone (HMP) is a secondary metabolite synthesized by some species of fungi from the genera Aspergillus, Penicillium and Acetobacter, recently was demonstrated its ability to activate macrophages and antileishmanial activity. However, the effect of HMP in human macrophages and during infection with Leishmania (Leishmania) amazonensis is unknown. Thus, this study aim to evaluate the biological action of HMP on human macrophages and during interaction with L. (L.) amazonensis in vitro. Human monocytes were obtained from peripheral blood, isolating the cells was performed by density gradient Histopaque®1077, maintained in culture for 6 days to occur differentiation into macrophages and treated for 24 hours with 50 µg/mL HMP. Analysis by light microscopy of macrophages showed a significant increase in the cytoplasmic area, increase in cytoplasmic projections and vacuoles. HMP promoted activation of human macrophages through of the increased production of reactive oxygen species (ROS), but not nitric oxide. Regarding the action of HMP on the protozoan Leishmania, it was observed that the HMP showed antiproliferative activity against intracellular forms of L. (L.) amazonensis. After 24 hours of treatment with HMP was observed a reduction of 79.80% of the L. (L.) amazonensis. HMP treatment induced an increase of ROS production in infected macrophages. No cytotoxic effect was observed in human macrophages treated with HMP. These results demonstrate that HMP seems to have immunomodulatory effect by promoting the activate macrophages by enhancing ROS production, besides presenting leishmanicidal effect on amastigote forms of L. (L.) amazonensis. **Supported by:**CNPq (Brazil), CAPES (Brazil), INBEB (Brazil), FAPERJ and FAPESPA.
Keywords: Macrophages; hmp; leishmanicidal action

TB024 - SILVER NITRATE MODULATES ANTIMONY SUSCEPTIBILITY THROUGH AQUAGLICEROPORIN-1 IN *LEISHMANIA*

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Antimony (Sb) resistance in leishmaniasis chemotherapy has become one of the major control disease challenges in this spreading worldwide public health problem. Since the plasma membrane pore forming protein aquaglyceroporin 1 (AQP1) is the major route of trivalent antimony (SbIII) uptake in Leishmania, functional studies are relevant to characterize drug transport pathway in the parasite. Indeed, this will support the rational drug development approaches urgent needed to overcome resistance. In this regard, the role of silver nitrate SbIII susceptibility using a competition assay approach was evaluated. In order to investigate AQP1 involvement in this process, overexpressing-AQP1 *L. guyanensis* and *L. braziliensis* mutants were obtained. The level of AQP1 was 3 to 4-fold higher in all transfected clones analysed than in the non-transfected cells as assessed by western blotting. Functional analysis showed that *L. braziliensis* and *L. guyanensis* clones overexpressing AQP-1 were about 2-fold more susceptible to SbIII than their non-transfected parental lines. Competition experiment using silver nitrate prior to SbIII exposure with their respective EC50 increased parasite growth especially in AQP1-overexpressing *Leishmania* (*Viannia*) parasites. This is the first report showing an evidence of drug susceptibility modulation by silver nitrate and SbIII combination mediated by AQP1. In addition, our functional analysis revealed that AQP-1 is involved in the antimony-resistance phenotype of *L. braziliensis* and *L. guyanensis*. **Supported by:**FAPEMIG, CNPq, CAPES, PROEP/CNPq/FIOCRUZ and UNICEF/UNDP/World Bank/WHO.
Keywords: Leishmania spp; antimony-resistance; aquaglyceroporin 1

TB025 - *ANNONA ACUTIFLORA*: A SOURCE FOR OBTAINING ANTI-LEISHMANIA SUBSTANCES.

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Leishmaniasis is a neglected disease caused by parasites of the Leishmania genus. The drugs used for its treatment are pentavalent antimonials, amphotericin B and miltefosine. For all of them high toxicity, elevated cost and parasite resistance have been reported. Natural products constitute an important source for obtaining substances with leishmanicidal potential. Here, bio-guided assay were performed to identify compounds with potential leishmanicidal activity in stalk extracts from *Annona acutiflora*, of the *Annonaceae* family. We evaluated the leishmanicidal potential of hexane (HEX), dichloromethane (DCl), butanolic (BT) and acetate (AC) fractions against promastigotes of *Leishmania amazonensis*. Our results indicate HEX and DCl fractions as the most active with IC_{50s} of 8.1 and 11.8 µg/mL, respectively. Evaluating the toxicity for macrophages by XTT method, we found that BUT and AC fractions were not toxic to the host cells, and HEX and DCl fractions presented CC_{50s} 22 and 23 µg/mL, respectively. Next, the anti-amastigote activity was tested and IC_{50s} of 3.3, 4.6, 67.4 and 22.0 µg/mL, were obtained for HEX, DCl, BT and AC fractions respectively, after 24h treatment. The selectivity index (CC_{50} for peritoneal macrophages / IC_{50} for intracellular amastigotes), obtained for the fractions HEX, DCl, BT and AC were 6.7, 5.0, >2.9 and >9.1, respectively. HEX and DCl fractions alter the cell cycle, increasing 11.3 and 6.0 times the G0 phase of promastigotes. To evaluate the HEX and DCl fractions capacity to modulate the microbicidal mechanism of macrophages, nitric oxide (NO) production was tested. Our data shows that at the IC_{50s} of the fractions decreased NO production in infected macrophages stimulated with IFN-γ and LPS. Our data support the use of HEX, DCl fractions of the *Annona acutiflora* as a source of new anti-leishmanial compounds.
Supported by:CAPES, FAPERJ e CNPq
Keywords: Leishmania amazonensis; annona acutiflora; natural products

TB026 - BIOPHYSICAL CHARACTERIZATION OF THE 1-DELTA-PYRROLINE-5-CARBOXYLATE DEHYDROGENASE FROM *TRYPANOSOMA CRUZI*

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Chagas' Disease afflicts the population present in countries of emergent and poor economy of America and belongs to the same group as Sleeping Sickness and the leishmanias, classified by the World Health Organization as neglected tropical diseases. Chagas' disease is caused by the flagellated parasite *Trypanosoma cruzi* that has a complex life cycle, going from an invertebrate host to a vertebrate one. In order to survive and proliferate in these host changes, *T.cruzi* must adapt itself to osmotic and oxidative stress, changes in the environment composition and changes in energy sources. To this adaptation, the amino acid L-proline has a prominent role playing important and essential participation that affect the protozoan life cycle, such as the mitochondrial metabolism, the host-cell invasion and metacyclogenesis. *T.cruzi* 1-Delta-Pyrroline-5-Carboxylate Dehydrogenase (TcP5CDH) is involved in the catabolism of proline holding a major role in the conversion of Pyrroline-5-Carboxylate to L-gluatamate and, thus, shows itself as a possible molecular target for new drug development. This work aims at the molecular characterization of TcP5CDH by biophysical techniques such as Dynamic Light Scattering, Circular Dichroism and X-ray Crystallography. A homologous modeling was performed using Modeller 9.14 with PDB proteins from *Homo sapiens*, *Mus musculus*, *Saccharomyces cerevisiae* and *Bacillus tuberculosis*; structures with sequence identity >40% and coverage >90% were chosen. The recombinant TcP5CDH cloned in pET28a vector was obtained from *E.coli* CodonPlus strain and purified by affinity chromatography and size exclusion chromatography (16/60 Sephacryl S-500) to remove any remaining contaminants. DLS was performed with 0.50, 0.75, 1.5 and 3.0 mg/ml of TcP5CDH and revealed an apparent hydrodynamic radius compatible with a trimeric organization. At 3.0 mg/ml a tetramer/hexamer is observed with a hydrodynamic radius estimated as approximately between 120 and 180 nm respectively. **Supported by:**CAPES
Keywords: Trypanosoma cruzi; 1-delta-pyrroline-5-carboxylate dehydrogenase; structural biology

TB027 - INVESTIGATION OF MILTEFOSINE EFFICACY IN A MOUSE MODEL OF INFECTION WITH LUCIFERASE-EXPRESSING *LEISHMANIA BRAZILIENSIS*

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Leishmania braziliensis is the main species responsible for cutaneous and mucocutaneous leishmaniasis in Brazil. Miltefosine is the only oral drug available for leishmaniasis treatment and although not yet approved in Brazil, it is widely used in India against visceral leishmaniasis. Recent clinical trials in cutaneous leishmaniasis patients in Brazil have shown higher efficacy for miltefosine than for meglumine antimoniate, the main drug used in Brazil. To investigate miltefosine's activity against this species further, we developed a BALB/c experimental model employing a *L. braziliensis* line expressing luciferase. The transgenic line expressing luciferase was generated through homologous recombination into the ribosomal DNA locus of the parasite and was used to evaluate the progression and parasite burden of the disease. When infected with this transgenic line, BALB/c mice presented persistent lesion with no cure after 4 months of infection. Miltefosine treatment was administered at 5 or 15 mg/kg/day for 15 consecutive days. No bioluminescence was detected in treated groups at the end of the treatment, suggesting that animals were completely cured. However, 3 months after the end of treatment, relapses were observed in animals treated with 5 mg/kg/day miltefosine. These results indicated that parasites persist in vivo after treatment and suggest that relapses in patients treated with miltefosine may occur. **Supported by:**FAPESP, CNPq and CAPES
Keywords: Miltefosine ; cutaneous leishmaniasis ; experimental model

TB028 - EASY, CHEAP AND ACCESSIBLE IMAGE-BASED WAYS TO COUNT INTRACELLULAR LEISHMANIA AMASTIGOTES FOR DRUG SCREENING PURPOSES

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Many drug screening techniques used for discovery of new antileishmania compounds are performed on promastigotes and axenic amastigotes, which are not the appropriate models due to biochemical differences when compared to the intracellular forms. Fluorescence- or reporter gene-based assays are also available, however they are expensive, require specific equipment and training, and usually can't assess the distribution of amastigotes per macrophages. The gold standard technique is still the microscopic manual counting method, which is vastly used even though is laborious and dependent on experience. Open source softwares like INsPECT and the ImageJ plugin ITCN are used to automated cell counting, but they only work properly on fluorescence images. Here, we propose alternative ways using ImageJ for cell counting. The method is based on Giemsa-stained infected cell images using ImageJ's cell counter plugin. Each cell type is marked up and the count is displayed separately. Alternatively, 8-bit images had the background removed and the threshold adjusted for automated counting of single color. The count was performed considering pixel size (30-120 or 300-infinity) and circularity (0-1.0). A workflow was written on javascript and resulted on 163:29 counts of parasites:host cell against 161:32 obtained from manual counting. However, this automated counting is limited due to background noise and contrast of each image. Overall this method is accessible, only requiring an optical microscope and a simple camera, it is more reliable, it doesn't require previous experience. Using this approach we calculate the EC50 of amphotericin b (0.1 µM) and miltefosine (1.5 µM), compatible with reporter gene-based approaches. Moreover, image counting is less harmful ergonomically, less wearing and the data can be stored for further recount. Thus, the manual ImageJ counting is the best alternative to the microscopic determination of intracellular parasites. **Supported by:**CNPq, FAPEMIG, CAPES
Keywords: Leishmania; drug screening; cell counting

TB029 - LEISHMANICIDAL POTENTIAL OF PROPOLIS EXTRACT FOR THE TREATMENT OF CUTANEOUS LEISHAMANIASIS

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Cutaneous Leishmaniasis is important to public health problem due to the increasing number of cases. To date, treatment is still based on antimonials, that pose important toxic side effects. Propolis is a natural compound widely used in popular medicine, displaying activity against different infectious agents. The aim of this study was to test the in vitro leishmanicidal effect of different propolis extracts against Leishmania braziliensis promastigotes and against intracellular amastigotes. First, stationary-phase L. braziliensis promastigotes were incubated with the aqueous, alcoholic or glycolic propolis extracts (10, 50 and 100µg/ml) for 120h. Our data showed that all extracts exhibited a dose-response effect on the viability of L. braziliensis promastigotes. Moreover, all extracts were able to induce morphological changes in parasites, as determined by SEM and TEM microscopy after 96 hours of incubation with 50µg/ml of extracts. However, only the glycolic extract induced cellular alterations indicative of death of L. braziliensis promastigotes. In assays using macrophages, cell viability was assessed by Trypan blue staining and by MTT of cells following incubation with extracts (10 and 100µg/ml) for 24h. None of the extracts induced cytotoxic effects on murine cells. For the evaluation of leishmanicidal effect against amastigotes, murine macrophages were infected with L. braziliensis and treated with the extracts (10 and 100µg/ml) for 24h. Antileishmanial activity was highest at 100µg/ml, as measured by promastigote viability. The results show the effectiveness of propolis extracts against L. braziliensis in vitro, and, in particular of the glycolic extract. This result coupled with low cell toxicity, makes the glycolic extract of propolis a strong candidate for the development of additive treatment for cutaneous leishmaniasis caused by L. braziliensis. **Supported by:** FAPESB; CPqGM – FIOCRUZ-BA; APIS FLORA INDL. COML. LTDA.
Keywords: Propolis; leishmaniasis; macrophage

TB030 - DIARYLUREAS AFFECTS REPLICATION AND MORPHOLOGY OF *TRYPANOSOMA CRUZI* AND *T. BRUCEI* VIA EIF2α PHOSPHORYLATION.

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Some disubstituted urea compounds from the class of N,N′-diarylureas activate the mammalian heme-regulated inhibitor kinase, inhibiting translation initiation due to the phosphorylation of the eukaryotic translation initiation factor (eIF2). We have previously found that three derivatives, BTdCPU, I-m6, I-17, affected *Trypanosoma cruzi* multiplication. By using epimastigotes, we obtained an IC50 of 3 µM for I-17, 5 µM for BTdCPU and 10 µM for I-m6. We then asked whether these compounds could act on eIF2 phosphorylation by using parasites over-expressing the wild-type version, or versions containing each one or both of the two possible phosphorylation sites of the alpha subunit (eIF2α) replaced by alanines. We observed that the *T. cruzi* expressing the mutated versions including the double mutant eiF2 were more resistant to I-17, exhibiting no morphological changes in the presence of the drug, as compared to parasites overexpressing the wild type eIF2. In addition, I-17 disturbed the polysome profile. In addition, the I-17 compound at 2 µM inhibited the proliferation of intracellular amastigotes of *T. cruzi* and bloodstream trypomastigotes of *Trypanosoma brucei*, while mammalian cells toxicity was only observed at concentrations above 10 µM. As in the case of *T. cruzi*, *T. brucei* bloodstream trypomastigotes with one the phosphorylatable threonine of eIF2α replaced by alanine were also more resistant to I-17. We concluded that some N,N'-diarylureas could be used to inhibit replication of *T. cruzi* via eIF2 phosphorylation. The target eIF2α kinase and whether these compounds affect other parasite enzymes remain to be elucidated. **Supported by:**FAPESP and FAPERJ
Keywords: Eif2α kinases; drug treatment

TB031 - A POLYPROTEINS-BASED VACCINE DERIVED FROM *LEISHMANIA INFANTUM* EMPLOYED TO PROTECT AGAINST VISCERAL LEISHMANIASIS

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Visceral leishmaniasis (VL) is a major health problem in the world. Life-long immunity to disease in recovered patients has motivated the development of studies for vaccination against disease. Aiming to select new candidate antigens to compose an effective vaccine against VL, in the present study, a multiproteic vaccine composed by three *Leishmania* sp. proteins, LiHyp1, LiHyp6, and IgE-dependent histamine-releasing factor (HRF) proteins; expressed in the amastigote or promastigote stages of *Leishmania infantum* was evaluated in BALB/c mice against the challenge infection with this parasite species. The LiHyp1 (LinJ.35.1290), LiHyp6 (LinJ.36.0580), and HRF (LinJ.24.1560) DNA coding sequences were cloned, and the recombinant proteins were purified and used in the experiments. The immunogenicity and protective efficacy of the multiproteic vaccine (composed by three recombinant proteins) combined with saponin, was evaluated in BALB/c mice, which were lately infected with *L. infantum*. The vaccinated mice with the multiproteic vaccine plus saponin presented a high and specific production of IFN-gamma, IL-12, and GM-CSF after in vitro stimulation with the proteins, which was maintained after infection. These animals presented significant reductions in the parasite burden in the liver, spleen, bone marrow, and draining lymph nodes; when compared to the control groups (saline and saponin). Protection was associated with an IL-12-dependent production of IFN-gamma, produced both by CD4+ and CD8+ T cells, and by high levels of nitric oxide. Moreover, a decrease in the IL-4 and IL-10 levels was also observed. The multiproteic vaccine plus saponin, as compared to the individual proteins plus adjuvant, induced a better protection against infection. The study showed new antigens that could be combined in a multiproteic vaccine and used to protect against VL. **Supported by:**FAPEMIG,CNPq, INCT NanoBiofar, PRPq/UFMG
Keywords: Multiprotein-based vaccine; hypothetical proteins; visceral leishmaniasis

TB032 - MAPPING B-CELL EPITOPES FOR THE TRYPAREDOXIN PEROXIDASE OF LEISHMANIA (VIANNIA) BRAZILIENSIS AND ITS POTENTIAL FOR THE DIAGNOSIS OF TEGUMENTARY LEISHMANIASIS

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The search toward the establishment of novel serological tests for the diagnosis of leishmaniasis and proper differential diagnosis may represent one alternative to the invasive parasitological methods currently used to identify infected individuals. In the present work, we investigated the use of recombinant Tryparedoxin Peroxidase (TP) of Leishmania braziliensis as a potential antigen for the immunodiagnosis of human tegumentary (TL) leishmaniasis. Linear B-cell epitope mapping was performed to identify polymorphic epitopes when comparing orthologous sequences present in Trypanosoma cruzi, the agent for Chagas disease (CD), and the Homo sapiens hosts. TP protein sequence is divergent compared to the T. cruzi and human orthologs. Moreover, was identified one predicted B-cell epitope in TP (score: 1.89) that co-occurs with an intrinsically unstructured region (score: 0.76), which suggests that this protein region has an unfolded structure and is therefore potentially accessible for antibody binding. ELISA demonstrated that TL individuals showed high levels of antibodies against recombinant TP (rTP), allowing identification of infected ones with considerable sensitivity and great ability to discriminate (specificity) between non-infected and CD individuals (100.00% and 100.00%, respectively). Overall, rTP may be a potential antigen for the diagnosis of TL as it has a higher agreement (Kappa index: 1.00, IC95%: 1.00-1.00) with parasitological assays and is better than other reference tests for diagnosing TL in Brazil (accuracy: 100.00%). **Supported by:**CNPq
Keywords: Tegumentary and visceral leishmaniasis; peroxidoxin; b-cell epitope mapping

TB033 - COMPARATIVE EFFICACY BETWEEN AN AMASTIGOTE-SPECIFIC HYPOTHETICAL PROTEIN OF *LEISHMANIA INFANTUM*, AND ITS SYNTHETIC PEPTIDES, AGAINST VISCERAL LEISHMANIASIS

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Visceral leishmaniasis (VL) is a major health problem in the world. The development of new prophylactic measures such as vaccines, it is an attractive alternative to prevent the disease. Aiming to select candidate antigens to compose a vaccine against VL, we have targeted the intracellular amastigote-stage, which is the parasite stage that persists throughout infection with *Leishmania* in the mammal hosts. The present study aims to evaluate a *Leishmania* amastigote-specific hypothetical protein, LiHyV (XP_888524.1), which was identified by a recent immunoproteomic approach performed in *Leishmania infantum*, and two related synthetic peptides (LiPep1 and LiPep2), in an attempt to select a new candidate antigen to compose an effective vaccine against VL. The immunogenicity and protective efficacy of the rLiHyV protein or of synthetic peptide, plus saponin, were evaluated in BALB/c mice before and after the *L. infantum* infection. Spleen cells of mice vaccinated with the rLiHyV protein plus saponin showed a high production of IFN-gamma, IL-12 and GM-CSF, in comparison to the control groups (saline and saponin). The cellular response generated before and after challenge by rLiHyV plus saponin group was typically a Th1 response, with high levels of IFN-gamma, IL-12 and GM-CSF, besides of low levels of IL-4 and IL-10. Animals immunized with rLiHyV plus saponin showed a significant reduction in the number of parasites in the liver, spleen, bone marrow and draining lymph nodes in the infected paws, in comparison to the control groups; however, LiPep1 and LiPep2 plus saponin did not induce protective response against infection. This study showed that an amastigote-specific hypothetical *Leishmania* protein, LiHyV, when combined with a Th1-type adjuvant, could be used as a protective agent to compose a vaccine against VL
Supported by:FAPEMIG, INCT NanoBiofar, CNPq and PRPq/UFMG
Keywords: Hypothetical proteins; peptides; vaccine

TB034 - FROM GENOMIC COMPARATIVE TO THE VALIDATION OF NEW MOLECULAR MARKERS FOR THE IDENTIFICATION OF SPECIES CAUSING CUTANEOUS LEISHMANIASIS IN COLOMBIA

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Cutaneous leishmaniasis (CL) is one of the main clinical manifestations of human infection caused by different species of Leishmania spp. All species of Leishmania that are pathogenic to humans besides other pathogens can cause cutaneous disease complicating the right diagnosis and consequently the treatment. Today there are not typing methods that have the ability to discriminate many different species in a clinical context in just one step. Thus, the validation of new molecular markers for the diagnosis and characterization of these species is a priority. In this work, the genomes of Leishmania panamensis, L. braziliensis and L. guyanensis were compared in order to obtain species-specific regions. A total of 25 pairs of primers were designed and the specificity was assessed by PCR using different trypanosomatids DNA species. Finally, a multiplex real time PCR was designed to diagnose and typing in one step. Of the selected pairs of primers for different species, only three showed specific amplification, while the rest showed cross amplification with some kind of Leishmania spp. or other trypanosomatids. The multiplex RT-PCR allowed the identification of the three species in only one reaction. In conclusion, the comparative genomic analysis identified species-specific regions that were validated as new molecular markers useful for the diagnosis and classification of Leishmania species causing cutaneous leishmaniasis in Colombia. **Supported by:**Colciencias, U de A
Keywords: Leishmaniasis; comparative genomics; diagnosis

TB035 - BALB/C MODEL CHALLENGED WITH DIFFERENT DOSES AND ROUTES OF INOCULATION OF LEISHMANIA INFANTUM CAN INDUCES DIFFERENT DEGREES OF INFECTION

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Experimental vaccines to protect against visceral leishmaniasis (VL) have been developed by using BALB/c mice infected with a large (107 to 108) inoculum of parasites. Remarkably, prior literature has reported that the poor protection observed is mainly due to the high susceptibility of this strain. To determine factors inherent to mice that might abrogate vaccine-induced efficacy, the present research sought to investigate the impact of the administration of different infective inoculums of Leishmania infantum in BALB/c mice, evaluating subcutaneous and intravenous routes of administration as well as parasitological and immunological parameters over different periods of time. This study shows that the injection of a highly infective inoculum in mice, through both subcutaneous and intravenous routes, results in a sustained infection. The mice developed a high parasite load in the liver; however, these values diminished over time. This result did not corroborate with the parasite load in the bone marrow and brain, and proved to be expressively different in the spleen and draining lymph nodes, where the values increased over time. Mice infected with a low dose of parasites (103) showed a certain resistance against infection, based mainly on the IFN-□and oxide nitric production. Considering all the elements, it could be concluded that the models employing high doses (107) of L. infantum in BALB/c mice can bring about an imbalance in the animals' immune response, thus allowing for the development of the disease at the expense of efficacy within the vaccine candidates. **Supported by:**FAPEMIG, CNPq, INCT-NanoBiofar, PRPq/UFMG
Keywords: Infection model; balb/c mice; leishmania infantum

TB036 - LEISHMANICIDAL ACTIVITY OF LAPACHOL DERIVATIVES IN *LEISHMANIA INFANTUM*

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Leishmaniasis is a disease caused by parasites of the genus *Leishmania*, and the main treatment still relies on toxic pentavalent antimonials. Therefore, the search for new compounds that may be used as a therapeutic alternative for treatment is very important. Naphthoquinones are secondary metabolites of many plants and have been studied for antiparasitic effect. Among the naphthoquinones, lapachol and several derivatives have been highlighted due their leishmanicidal activity. This study evaluated the in vitro activity of three derivatives of Lapachol in *Leishmania amazonensis*. The compounds were synthesized with structural changes and were named LQ-01 (C18H12O3), LQ-02 (C18H10O3) and LQ-03 (C18H10O3). LQ-01 and LQ-02 are para-naphthoquinones and LQ-03 is an ortho-naphthoquinone. Promastigotes of *L. infantum* were grown in medium Schneider and incubated with compounds for 72h and the parasite growth was assessed by resazurin. To evaluate the anti-amasâ€‹tigote activity, murine peritoneal macrophages were infected for 4 hours, treated and incubated for 72h. The activity was evaluated microscopically. For cytotoxicity assays, murine peritoneal macrophages were incubated with the compounds for 72h and cell viability was assessed by resazurin. LQ-01 and 02 have not shown antipromastigote activity, whereas LQ-03 exhibited potent leishmanicidal activity, with IC50 of 1.7 uM. Unfortunately, LQ-03 was also toxic to host cells and it was not tested for antiamastigote activity. LQ-01 showed LD50 of 110 uM and LQ-02 LD50 higher than 160 uM for murine macrophages. Interestingly, LQ1 and LQ2 were active on intracellular amastigotes, with IC50 of 40 µM and 2.5 µM, respectively. Thus, LQ-02 showed good anti-amastigote activity and a favorable selectivity index, higher than 64 suggesting that this compound may be a prodrug, since it showed no activity against promastigotes. These results suggest that LQ-02 may be a lead compound in the search for a new treatment for leishmaniasis. **Supported by:**CNPq , CAPES
Keywords: Leishmania infantum; lapachol; naphtoquinone

TB037 - LEISHMANICIDAL AND CYTOTOXIC ACTIVITIES OF *CITRUS SINENSIS* EXTRACTS

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American cutaneous leishmaniasis (ACL) is an infectious disease caused by parasites belonging to *Leishmania* genus. The disease causes skin and mucosal lesions, leading to significant tissue destruction and patient disfigurement. The current treatment for ACL is based on the use of highly toxic drugs such as pentavalent antimonials and amphotericin B. Previous reports demonstrated that crude extracts from the leaves and fruit peels of *Citrus sinensis*, popularly known as orange tree, have larvicidal, acaricide, anti-bacterial and anti-fungal activities. Thus, in this study, we aimed to evaluate the anti-*L. amazonensis* activity of different extracts obtained from *C. sinensis*. For this, *C. sinensis* leaves were dried, crushed and subjected to extraction with hexane (CH), ethyl acetate (CEA), dichloromethane/ethanol (CD/Et-1:1) or ethanol/water (CEt/W-7:3). Promastigote inhibition assays were performed using microplate serial dilution technique. Ultrastructural alterations were evaluated through transmission electron microscopy. In addition, the cytotoxic effects of extracts were assessed on J774.G8 cell lineage by MTT assay. The extracts that showed better activity leishmanicidal were the CH and CD/Et with IC_{50} of 25.91 and 54.23 µg/mL, respectively. These extracts also showed low toxicity against J774.G8, with the selectivity index of 1.4 and 2.3, respectively. Ultrastructural alterations observed on CD/Et-treated parasites included nucleus chromatin condensation, mitochondrial swelling, increased exocytic activity into the flagellar pocket and the presence of cytoplasmic vacuoles. Phytochemical analysis of CD/Et revealed the presence of amyrins as the main components. The results presented in this study show that *C. sinensis* may be a promising source of anti-*Leishmania* agents. Further investigation will be necessary in order to identify the substances responsible for the bioactivity. **Supported by:**CAPES e FAPERJ
Keywords: Leishmanicidal activity; citrus sinensis; leishmania amazonensis

TB038 - *IN VITRO* EVALUATION OF ANTI-*PLASMODIUM FALCIPARUM* ACTIVITY AND CYTOTOXICITY OF PYRAZOLOPYRIMIDINES DERIVATIVES

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The widespread of *Plasmodium falciparum* resistance to chloroquine (CQ), artemisinin derivatives, and other antimalarials is hampering the malaria control programs. The pyrazolopyrimidines (PYZP) molecules have been used in drug design against canine visceral Leishmaniasis and Chagas disease. Their mechanism of action is related to the inhibition of dihydroorotate dehydrogenase (DHODH) and is part of biosynthesis chain of pyrimidine nucleotides. Presently, six PYZP molecules were assessed for activity *in vitro* anti-*P. falciparum* (W2 clone chloroquine-resistant) using blood parasites and SYBR (a DNA intercalator). The cytotoxicities were tested against BGM cell lines from African Green Monkey with neutral red. The ratio between toxicity (MDL_{50}) and activity (IC_{50}) concentrations allowed to select the best drugs based on the selectivity index (SI) values. Five of the six molecules were active; three had SI higher than 200; thus, less activity then the control drugs amodiaquine (AMQN), pyronaridine (PYR) and CQ (SI >2500). These results confirm recent data in which hybrids of PYZP and 8-aminoquinolines were active, at nanomolar concentrations, against *P. falciparum* wild type (*Pf*_NF54) and the resistant strain (*Pf*_K1) (Kannan *et. al.*, 2015). Tests in mice infected with *P. berghei* showed that 5 mg/kg of AMQN and PYR reduced 100% and 99% of parasitemia, respectively, compared to untreated animals. The most promising PYZP will be tested *in vivo* to further clarify their potential as new antimalarial candidates.
Supported by:CNPq/FAPEMIG (fellowships to authors), MS, FIOCRUZ
Keywords: Malária; quimioterapia; plasmodium falciparum

TB039 - ANTI-*LEISHMANIA AMAZONENSIS* ACTIVITY OF *CURCUMA ZEDOARIA* EXTRACTS

ALVES, R.L.[*1]; GARCIA, A.R.[1]; AMARAL, A.C.F.[2]; VERMELHO, A.B.[1]; PINHEIRO, A.S.[1]; RODRIGUES, I.A.[1]
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The Leishmania genus consist in several species of flagellate protozoa that, when transmitted through the bite of the insect vector, can cause skin lesions or damage to bone marrow and internal organs. Pentavalent antimonials are the first choice drugs used for leishmaniasis treatment. However, besides having serious adverse effects, a significant portion of the population of many countries has become resistant to treatment with these drugs. Thus, new chemotherapeutic agents, safe and effective, become increasingly necessary. Plants from Curcuma genus are described in literature as source of compounds with different pharmacological properties, such as anti-inflammatory, antioxidant, antibacterial and antiprotozoal. The objective of this study was to evaluate the activity of *Curcuma zedoaria* extracts against *Leishmania amazonensis*. The rhizome of *C. zedoaria* was macerated with hexane (HEX) and dichloromethane (DCM), three time with each solvent.. Promastigote forms of *L. amazonensis* were treated with different concentrations of extracts and incubated in microplates for 120 hours at 26ºC in order to establish the inhibitory effect on parasite growth. DCM extract showed minimal inhibitory concentration (MIC) of promastigotes growth at 550 µg/mL. However, best results were obtained with HEX extract, where the MIC was found at 68.8 µg/mL. Concentrations below the MIC also proved to be effective in decreasing the number of parasites after 120 hours of treatment when compared to controls (untreated parasites). Thus, the results obtained in this study showed the potential antileishmanial activity of *C. zedoaria* extracts, especially HEX extract. However, further investigations will be necessary in order to identify and isolate the main components in the extracts and evaluate their bioactivity, as well as the citotoxic response of these substances for mammalian cells. **Supported by:**CAPES e FAPERJ
Keywords: Leishmania amazonensis; curcuma zedoaria; leishmanicidal activity

TB040 - LINEAR B-CELL EPITOPE MAPPING OF β-TUBULIN FROM LEISHMANIA BRAZILIENSIS: IMPLICATIONS FOR SERODIAGNOSIS OF HUMAN TEGUMENTARY LEISHMANIASIS

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Leishmaniasis is a disease caused by vector-borne parasites of Leishmania genus that affects approximately 12 million people around the world causing approximately 50,000 deaths each year. The correct and early identification of humans infected with Leishmania are key steps in the Leishmaniasis control and a method with high sensibility and specificity and low cost that allows screen of large number of samples is extremely valuable. In this study, initially, we identified antigenic L. braziliensis proteins that were revealed by an immunoproteomic approach, using a refined selection employing human TL and Chagas disease patient's sera. Additionally, we mapped linear B-cell epitopes in these proteins whose sequences are divergent in their orthologs in Homo sapiens and in Trypanosoma cruzi and compared their performance with the recombinant protein using ELISA assay. For this, we selected one protein identified as β-tubulin, and we carried out cloning, expression and purification of the recombinant protein to evaluate the potential as candidate to serodiagnosis of human tegumentary leishmaniasis. In the β-tubulin protein sequence were identified two predicted B-cell epitope (score: 1.680 and 1.482). Recombinant β-tubulin showed greater sensitivity, specificity and accuracy in immunodiagnostic test of human cutaneous or mucosal leishmaniasis (sensitivity = 100.00%, specificity = 82.50% and accuracy = 91.56%). Furthermore, the proposed pipeline to indentify new valuable antigens can be applied to diagnosis target discovery of others infectious diseases. **Supported by:**CNPq
Keywords: Tegumentary leishmaniasis; β

TB041 - PHAGE PARTICLES USED AS VACCINES TO PROTECT BALB/C MICE AGAINST LEISHMANIA AMAZONENSIS INFECTION

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Leishmaniasis is a disease with a wide spectrum of clinical manifestations caused by different species of protozoa belonging to the Leishmania genus. The treatment of disease is still based on the use of the parenteral administration of pentavalent antimonial compounds; however, several side effects reported by patients and increased parasite resistance have produced serious problems. Therefore, the development of new strategies to prevent leishmaniasis has become a high priority. In this study, polyclonal antibodies purified of sera from patients with cutaneous leishmaniasis (CL) were used to screening mimotopes in a peptide phage display library, in order to select those that could be recognized and evaluate them in vaccination protocols against Leishmania amazonensis infection. The selected peptide sequences were evaluated based on their frequencies and a bacteriophage clone, namely A5, was selected to test its ability to induce protection against challenge infection. Vaccinated mice with A5 clone plus saponin showed a high and specific production of IFN-□ IL-12, and GM-CSF after in vitro stimulation with the phage clone or L. amazonensis extract. In addition, a decrease in the parasite-mediated IL-4 and IL-10 response was also observed. Immunized and infected mice, as compared to the control groups (saline, saponin and wild-type phage clone plus saponin), showed significant reductions in the infected footpad swelling, as well as in the parasite burden in liver, spleen, bone marrow and in the paws' draining lymph nodes, in comparison to the control groups. The present study showed that a selected antigen by antibodies present in sera of CL patients by a phage display technique could be employ as a candidate to compose a vaccine against L. amazonensis infection. **Supported by:**FAPEMIG, INCT-NanoBiofar, and CNPq **Keywords:**Phage display; vaccine; leishmania amazonensis

TB042 - MODULATION OF EXPRESSION OF ESSENTIAL ENZYMES FROM TRYPANOTHIONE BIOSYNTHESIS CHANGE THE ANTIMONY-SUSCEPTIBILITY PHENOTYPE IN *L. (V.) GUYANENSIS*

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The antimony-resistance phenotype in *Leishmania* spp. is associated with increased levels of trypanothione and other intracellular thiols. This increase seems to be associated with higher expression of Ornithine Decarboxylase (ODC) and Gamma-glutamylcysteine synthetase (GCS) enzymes. In this study, to investigate the involvement of ODC and GCS in the antimony-resistance, we performed transfection assays with these genes in *L. guyanensis* and *L. infantum chagasi* species to generate overexpressors parasites. Pharmacological inhibition tests of ODC and GCS also was performed using the specific inhibitors DFMO and BSO, respectively. The trivalent antimony (SbIII) susceptibility test revealed that ODC and GCS overexpressors *L. guyanensis* clones had an increase of three and four-fold in the SbIII-resistance when compared to wild-type line, respectively. On the other hand, the transfection of *L. infantum* with odc or gcs, was not sufficient to alter the SbIII-susceptibility phenotype for this species. The pre-incubation of wild-type and overexpressors *L. guyanensis* lines with the DFMO or BSO inhibitors was able to sensitize all lines to SbIII. The wild-type *L. guyanensis* line was 60 and 21-fold more susceptible to SbIII when pre-incubated with DFMO and BSO, respectively, compared to the same line treated only with SbIII. The ODC and GCS overexpressors clones were 1.5 and 4.2-fold more susceptible to SbIII in the presence of DFMO and BSO, respectively. However, they still are more resistant to SbIII than wild-type line. Thus, our data revealed that the ODC and GCS enzymes are involved in the SbIII-resistance phenotype in *L. guyanensis*. In addition, the modulation of expression by increase of enzyme expression or by inhibition of enzymatic activity is sufficient to alter the SbIII-sensibility of parasites. These results support that ODC and GCS are important in the SbIII-resistance being a good molecular target to development of new chemotherapies. **Supported by:**FAPEMIG; CNPq; PROEP/CNPq/FIOCRUZ, Capes **Keywords:**Leishmania spp.; ornithine decarboxylase (odc); gamma-glutamylcysteine synthetase (gcs)

TB043 - A LEISHMANIA-SPECIFIC HYPOTHETICAL PROTEIN EMPLOYED AS A DIAGNOSTIC MARKER, AS WELL AS A VACCINE CANDIDATE, AGAINST VISCERAL LEISHMANIASIS

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The present study evaluated a *Leishmania*-specific hypothetical protein (LiHyD), in an attempt to select a new candidate antigen for the serodiagnosis of canine visceral leishmaniasis (CVL), as well as to investigated its immunogenicity in BALB/c mice, with the purpose to evaluate the protein in a future study as vaccine against visceral leishmaniasis (VL). The LiHyD protein (XP_001468360) was obtained as a recombinant protein, and its antigenicity was evaluated using a large sera panel, composed by sera samples of non-infected dogs living in endemic or non-endemic areas of leishmaniasis, from asymptomatic or symptomatic VL dogs, sera of Leish-Tec® vaccinated dogs, as well as samples of animals experimentally infected by *Trypanosoma cruzi* or *Ehrlichia canis.* The immunogenicity of rLiHyD plus saponin was evaluated in BALB/c mice, which were immunized three times and, one month after the last vaccination; their spleen cells were collected, cultured and in vitro stimulated with the recombinant protein; when the levels of IFN-γ, IL-12, GM-CSF, IL-4 and IL-10 were determined. ELISA experiments performed with the rLiHyD protein presented sensitivity and specificity values of 100%. The recombinant protein was recognizable by antibodies present in sera of asymptomatic and symptomatic VL dogs, but was not recognize by sera of dogs vaccinated with Leish-Tec® vaccine, or those infected with *T. cruzi* or *E. canis*. In addition, the protein was not recognize by antibodies of non-infected dogs living in endemic or non-endemic areas of disease. Spleen cells of BALB/c mice immunized with rLiHyD plus saponin showed a high production of IFN-γ, IL-12 and GM-CSF, when compared to the control groups, as well as low levels of IL-4 and IL-10. It is possible to conclude that the LiHyD protein could be considered as a promising tool for the improvement of serodiagnosis for CVL and, when combined with saponin, could be used as a vaccine to protect against VL. **Supported by:**FAPEMIG, INCT NanoBiofar, CNPq, and PRPq/UFMG. **Keywords:**Hypothetical protein; serodiagnosis; vaccine

TB044 - MIMOTOPES-EXPRESSING BACTERIOPHAGES APPLIED AS IMMUNOTHERAPIC AGENTS FOR THE TREATMENT OF TEGUMENTARY LEISHMANIASIS

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Leishmania amazonensis is one of the major etiologic agents of a broad spectrum of clinical forms of leishmaniasis and has a wide geographical distribution in the South America. The disease can induce high morbidity and mortality in populations of endemic and non-endemics areas. The purpose of this study was to evaluate the immunotherapeutic potential of a recombinant bacteriophages M13-based multiepitopes vaccine to protect mice against *L. amazonensis*. Five phage clones (using 5 x 10^{12} phages, of each) selected by phage display technique were used to treat mice (n=16, per group), which were infected subcutaneously with 1 x 10^{6} stationary promastigotes of *L. amazonensis*, and that presented lesions development about 2 to 3 mm. Five doses of the immunotherapy were administered, at 2-weeks intervals. As control, mice received 5 x 10^{12} wild-type phages or saline. Eight weeks after challenge, mice were euthanized and infected skin fragments, spleen and sera samples were collected to parasitological and immunological analysis. Mice treated with phages vaccine showed a significant reduction of the diameter of lesions, a high production of IFN-γ and low levels of IL-4 and IL-10 in the spleen cells cultures. A humoral response was predominantly of IgG2a isotype. The results indicate the immunotherapeutic potential of a recombinant bacteriophages M13-based multiepitopes vaccine, and its potential use for treat the disease caused by *L. amazonensis*. **Supported by:**FAPEMIG, INCT-NanoBiofar, CNPq and PRPq/UFMG
Keywords: Phage display; leishmaniasis; treatment

TB045 - IMPROVEMENT OF THE SENSITIVITY AND SPECIFIC OF SERODIAGNOSIS OF CANINE VISCERAL AND HUMAN TEGUMENTARY LEISHMANIASIS USING TWO RECOMBINANT PROTEINS DERIVED FROM *LEISHMANIA INFANTUM*

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A gold standard serological diagnostic test for leishmaniasis should be focused in markers that present a specific humoral response in the infected hosts. Canine and human leishmaniasis are endemic diseases in Brazil, but current serologic tests present limitations related to their sensitivity and/or specificity. Recently, an immunoproteomic approach in *Leishmania infantum* was performed using sera of visceral leishmaniasis (VL) dogs, and antigenic proteins of the parasite were identified. In the present study, the diagnostic properties of two of these proteins, cytochrome c oxidase (CcOx) (XP_001565615.1) and IgE-dependent histamine-releasing factor (HRF) (CAJ05086.1), were evaluated. Both antigens were obtained as recombinant proteins, and employed to test their antigenicity using canine VL (CVL) or tegumentary leishmaniasis (HTL) sera. For the CVL diagnosis, sera from non-infected dogs living in endemic or non-endemic areas of leishmaniasis, from asymptomatic or symptomatic VL dogs, sera from Leish-Tec® vaccinated dogs, and sera from animals experimentally infected by *Trypanosoma cruz*i or *Ehrlichia canis*, were used. For the HTL diagnosis, sera from non-infected subjects living in an endemic area of leishmaniasis, samples of cutaneous or mucocutaneous leishmaniasis patients, as well as sera from *T. cruzi*-infected patients, were used. ELISA experiments performed with the recombinant proteins presented sensitivity and specificity values of 100% for both forms of the disease, besides of a maximum Youden index (1.00), and high values of positive and negative predictive values for the serodiagnosis. The diagnostic capacities for both proteins were higher than these obtained by another *Leishmania* antigen assayed as a recombinant protein (rA2 protein) or with lysates of the parasites (Soluble Leishmanial Antigen, SLA). We conclude that the recombinant proteins could be considered promising for the improvement for the serodiagnosis of CVL and HTL. **Supported by:**FAPEMIG, INCT NanoBiofar, CNPq, and PRPq/UFMG
Keywords: Recombinant proteins; serodiagnosis; leishmaniasis

TB046 - THE USE OF DIFFERENT DOSES FROM A ANTIGENIC LEISHMANIA SPP. PREPARATION DEMONSTRATES THE VARIABILITY IN THE GENERATED IMMUNE RESPONSE AND IN THE PROTECTION OF BALB/C MICE AGAINST CHALLENGE INFECTION

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American Tegumentary Leishmaniasis (ATL) is a disease caused by different *Leishmania* species in Brazil and represents an important cause of morbidity and mortality to the populations. Numerous experimental vaccines employing different antigens have been developed to induce protection against disease; however, an effective vaccine still does not exist. We proposed to evaluate the protection elicited by immunization of BALB/c mice with different doses of soluble *Leishmania amazonensis* antigenic (SLA) extract associated or not with alum as adjuvant against challenge with *L. amazonensis*. Mice (n=8 per group) were immunized subcutaneously with 1, 50 or 100 µg of SLA plus alum (0.5, 25 or 50 µg, respectively), with SLA, alum or received saline. Three doses were administered at 2-week intervals. Four weeks after the final inoculation, mice (n=4) were sacrificed and spleen and sera samples were harvested for immunological analysis. The others animals (n=4) were infected subcutaneously into their right hind footpad with 10^6 stationary promastigotes of *L. amazonensis*. Eight weeks after challenge, mice were sacrificed and infected skin fragments were collected for parasitological analyses. Spleen and sera samples were also collected for immunological analysis. Mice vaccinated with 50 or 100 µg of SLA with or without alum showed significant reduction in the footpad swelling and parasite burden when compared to mice immunized with 1 µg of SLA, alum or saline. Protected mice presented a high production of IFN-γ and IL-12 and low levels of IL-4, IL-10 and TGF-β in the spleen cells cultures and a humoral response was predominantly of IgG2a isotype. Comparatively, the protection was better in the mice that not received alum. Results indicate that the immunization of BALB/c mice with 50 µg or 100 µg of SLA with or without alum can induce protect against *L. amazonensis* challenge infection. **Supported by:**FAPEMIG, CNPq and PRPq/UFMG
Keywords: Differential dose; vaccine; protection

TB047 - APICOPLAST FATTY ACID SYNTHESIS IS ESSENTIAL FOR TOXOPLASMA GONDII DIVISION

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Toxoplasma gondii is the protozoan responsible for causing toxoplasmosis, a disease with worldwide distribution. As member of the phylum Apicomplexa, T. gondii harbors a plastid-like organelle (apicoplast), which performs several essential metabolic functions. One of those is the type II fatty acid biosynthesis (FASII), whose function is essential for parasite survival and, therefore, has been focus of great interesting as a drug target. Although the molecular and biochemistry aspects of FAS II have been explored and characterized, analysis regarding the phenotypic consequences at structural level caused by the inhibition or disruption of apicoplast FASII is still missing. Such analysis undoubtedly could provide new insights of the role of FAS II products for the T. gondii lipid metabolism, and also in improving our knowledge in the context of the parasite biology. Thus, we evaluated the phenotype caused by the disruption of FASII both by chemical inhibition with triclosan, a well-known inhibitor of enoyl-ACP reductase enzyme, and by gene knockout utilizing a tet-inducible mutant of the acyl carrier protein. Our morphological analysis show that the interruption of FASII in tachyzoites of T. gondii led the formation of large parasites containing several tethered daughter cells, suggesting the division arrest late at cytokinesis. Parasites also presented apicoplast division deffect due to misspartition during cell division. Quantification analysis confirmed the division inhibition as the main phenotype caused by FASII disruption and supports that FAS II exerts an essential role in providing substrates that drives the final step of parasite division. **Supported by:**CAPES, FAPERJ and CNPq
Keywords: Cell division; fatty acid sythesis; toxoplasma

TB048 - OVEREXPRESSION OF ABCG4 ALTERS STEROL BIOSYNTHESIS AND SUSCEPTIBILITY TO REFERENCE DRUGS IN *LEISHMANIA AMAZONENSIS.*

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The transporter ABCG4 in mammalian cells is related with translocation of cholesterol and phospholipids. This transporter was characterized in *Leishmania infantum* and it is involved in transport of phosphatidylcholine analogues and resistance to alkyl-phospholipids (Castanys-Muñoz *et al*, 2007). Trypanosomes synthesize ergosterol-derived sterols, but a significant percentage of exogenous cholesterol is found in *Leishmania* spp. However, it is not clear how the cholesterol/ergosterol transport occurs in Leishmania spp. Then, we intend to study the function of ABCG4 transporter in the sterol content of *L. amazonensis* and its role in the resistance to ergosterol biosynthesis inhibitors and reference drugs. The ABCG4 transporter (LmxM15.0890) was obtained from genomic DNA of *L. amazonensis* by PCR. For homologous expression in the parasites, the gene was cloned into pGEM T easy vector expression using *E. coli* DH5α competent bacteria, subcloned into psp72αNEOα and transfected into *L. amazonensis* promastigotes (LaABCG4). Parasites were also transfected with the empty plasmid psp72αNEOα (LaPSP). Sensitivity of parasites to a set of drugs (ketoconazole, miconazole, terbinafine, simvastatin, miltefosine, amphotericin B and SbIII) was determined using resazurin. The ICs50 of ketoconazole, miconazole and simvastatin were similar in both strains, LaABCG4 and LaPSP.LaABCG4 was more sensitive to terbinafine, miltefosine and amphotericin B, with IC50 of 17.0, 8.1 and 0.3 µM versus 26.0, 13.5 and 0.9 µM in LaPSP, respectively. However, LaABCG4 was more resistant to SbIII, with IC50 of 77 µM, whereas LaPSP had an IC50 of 33 µM. The sterol composition was evaluated by thin-layer chromatography (TLC). LaABCG4 has an altered sterol profile in comparison to LaPSP strain, accumulating manly lanosterol and an unknown sterol. Taken together, these results suggest that ABCG4 plays a role in sterol homeostasis and susceptibility to reference drugs in *Leishmania amazonensis.***Supported by:**CNPq, CAPES, PAPES/FIOCRUZ, FAPERJ
Keywords: Abcg4; leishmania amazonensis; sterol biosynthesis

TB049 - DIFFERENT STRAINS OF *L. BRAZILIENSIS* ISOLATED FROM TYPICAL AND ATYPICAL LESIONS IN MINAS GERAIS STATE DISPLAY VARIABILITY IN DRUG RESISTANCE TO AMPHOTERICIN B

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Leishmaniases are one of the most important neglected diseases according to the World Health Organization. They exhibit a spectrum of clinical manifestation from self-healing benign cutaneous lesions to fatal visceral form. *Leishmania (Viannia) braziliensis* is the most common species incriminated as the agent of cutaneous and muco-cutaneous leishmaniasis in Latin America. An interesting aspect of this species is the appearance of atypical cutaneous lesions. Those may not be ulcerated and thus hinder the correct diagnosis. Although a correlation between genetically variant parasites and atypical manifestations occurs, no information on drug susceptibility is available compared to those causing typical lesions. In this work, *Leishmania* parasites isolates from typical and atypical lesions from endemic areas of Minas Gerais (São João das Missões and Belo Horizonte) were tested for their susceptibility to Amphotericin B. THP-1 cells were infected with one atypical strain (Mhayllor) and two typical strains (Francisco and Letícia). Typical *L. braziliensis* reference strain (MHOM/BR/75/M2903) was used as control. Amphotericin B was serially diluted (1; 0.5; 0.25; 0.12 and 0.06 ug/mL) and the classical microscopy in vitro method was performed during three consecutive days. Infected cells and intracellular amastigotes were stained with Panotico and counted. IC_{50} values were calculated using the Microcal sofware. The IC_{50} of the reference strain (M2903) was 0.13 ug/mL. Strains Letícia (typical) and Francisco (typical) exhibited higher IC_{50} values of 0.32 and 0.21 ug/mL, respectively. An interesting aspect was noticed in the Mhayllor strain, whose IC_{50} was very low (0.09 ug/mL). Although no clear correlation was observed while considering the type of lesion, our preliminary data indicated that there are differences in drug susceptibility among *L. braziliensis* strains (2-3-fold compared to the reference and Mhayllor strains).
Supported by:CNPq and FAPEMIG
Keywords: Leishmania braziliensis; chemotherapy; amphotericin b

TB050 - LEISHMANICIDAL ACTIVITY OF NITROHETEROCYCLIC COMPOUNDS ACTIVATED BY LEISHMANIA INFANTUM TYPE I NITROREDUCTASE

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The potential of nitro compounds in the treatment of leishmaniasis has been rediscovered due to a class of enzymes known as nitroreductases (NTRs). Type I NTRs are expressed only in prokaryotes and some lower eukaryotes. NTRs catalyze reduction of nitro compounds, generating more potent metabolites inside of the parasite. In this study, we evaluated the leishmanicidal activity of nitroheterocyclic compounds and their activation by *Leishmania infantum* type I NTRs. Promastigotes of *L. amazonensis* and *L. infantum* were cultured in the presence of 13 nitroheterocyclic compounds up to 100 µM for 72 hours. Five of them inhibited both parasites, with the highest effect on *L. amazonensis*. DS142 and LQB303 showed IC50 less than 12 µM for both species. The six most active compounds were assayed on *L. amazonensis* intracellular amastigotes. DS142, LQB303 and LQB304 showed to be the most effective in decreasing the infection of the macrophages. The LD50 values on uninfected macrophages were higher than 50 µM for LQB303 and DS142, indicating a selectivity index higher than 20-fold. In addition, *L. amazonensis* ROS levels were measured using H_2DCFDA. We observed a significant increase in ROS production for LQB303AL, when the promastigotes were treated with 14,34µM up to 4h. Finally, the NTR I gene of *L. infantum* was amplified, cloned and subcloned to obtain transfected promastigotes overexpressing the NTR I gene. This strain was then cultured with several concentrations of nitroheterocyclic compounds for 72h. The results suggest that most of the compounds were able of undergoing nitro reductions, highlighting LQB303, which was about 11-fold more potent on the overexpressing strain. These results suggest that the reduction of the nitro group in position 5 of the ring leads to activation and some modifications, such as replacing thiophene by furan ring, makes them more sensitive to enzymatic reduction and promising agents for the treatment of visceral and tegumentar leishmaniasis. **Supported by:**CNPq/ FIOCRUZ **Keywords:**Nitroheterocyclic compounds; nitroreductase activation; leishmanicidal activity

TB051 - *TRYPANOSOMA CRUZI* HIGH CONTENT SCREENING ASSAY: EXPERIMENTAL STANDARDIZATION AND TRIAGE VALIDATION

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The cell-based, high content screening (HCS) technology enables the triage of a large quantity of molecules within a relatively short period in an efficient and high throughput fashion. Other advantages of HCS assays are the simultaneous measurement of antichagasic activity and host cell toxicity, and higher sensitivity when compared to fluorescence-based assays. This phenotypic 384 well-plate format assay is projected to run in a fully automated screening platform, for liquid and compound handling, supported by a computerized microscopic system for image acquisition, processing and data analyzing of intracellular amastigote growth inhibition and host cell ratio readouts. In order to achieve assay standard feasibility, reproducibility and robustness, which are mandatory scaling-up features for large triage campaigns, experiment biological and analytical parameters must be accurately pre-defined. Two clinically relevant strains from distinct isolation (Sylvio X10/1 – TcI and Y – TcII) and three different mammalian cell lineages (U2-OS, LLC-MK2, THP-1) were tested in combination at various cell densities and tissue culture trypomastigotes multiplicity of infection (MOI) to result in a computer-aided high content analysis with statistically significant separation between positive and negative controls; also different parasites and host cells in vitro culture passage numbers were tested to verify if important variations occur between continuous, long-term and recent cell cultivation. Beznidazole and nifurtimox were used for assay validation as reference compounds in dose-response curves and as positive controls in all plates. In parallel, secondary assays were standardized and validated for T. cruzi strain panel testing; drug candidate killing-kinetics evaluation and compound washout assays for parasite recrudescence analysis. The best assay conditions will be used to screen a small commercial library for assay validation, followed by a 30,000 compound library triage. **Supported by:**CNPq/ Drugs for Neglected Diseases initiative (DNDi) **Keywords:**Trypanosoma cruzi; high content screening; drug discovery

TB052 - SI2, A POTENTIAL SIRTUIN INHIBITOR, DECREASE *T. BRUCEI* PROCYCLIC FORM (PCF) REPLICATION AND INDUCES APOPTOSIS-LIKE CELL DEATH MECHANISM

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Trypansoma brucei are the etiological agent of Human African Trypanosomiasis (HAT), an endemic Sub-Saharan disease, responsible of 10000 new cases per years. Current drug treatment presents several limitations in terms of: toxicity, administration and efficiency depending of the subspecies or phase of the disease. Sirtuin, an acetylase, essential in a number of biological processes, presents some atypical activities and unique functions in parasitic protozoa, making a potential drug target. Within this context, 45 new designed compounds based on known Sirtuin inhibitors were tested against *T. brucei* Lister 427 procyclic form (PCF). The Sirtuin Inhibitor 2 (SI2) exhibited a promising inhibition and dose-dependent profile (IC_{50} = 0.75 µM), so we investigate the mechanisms of action more in detail. Treated parasites (at IC_{50} and IC_{80}) were able to recover proliferation after removing the compound from the medium. Contrary to IC_{50}, IC_{80}-treated parasites exhibited an increase of annexin and annexin/propidium iodide labeling compared to control. Flow cytometry experiments indicate that SI2 decreases intracellular calcium levels and induces mitochondrial depolarization. Taking together, our results show that SI2 is a promising drug candidate against *T. brucei* and apparently triggers an apoptotic cell death mechanism. Although these results seem to be promissory, further study is necessary to investigate the SI2 mechanism of action. **Supported by:**CNPq, Fapesp, CAPES
Keywords: Sirtuin inhibitor; t. brucei; apoptosis

TB053 - DIFFERENCES IN SUSCEPTIBILITY TO MILTEFOSINE IN *LEISHMANIA (VIANNIA) BRAZILIENSIS* CLINICAL ISOLATES ARE NOT DUE TO POLIMORPHISMS IN THE MILTEFOSINE TRANSPORTER GENE.

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Approximately 26,000 new cases of cutaneous leishmaniasis are registered every year in Brazil and have *Leishmania (Viannia) braziliensis* as the main etiological agent. The arsenal available for leishmaniasis treatment is limited and challenging due to the need for parenteral administration, toxicity, high cost, and emergence of resistance. Miltefosine is the only oral drug available for leishmaniasis treatment and it is already in use for the treatment of visceral leishmaniasis in Asia and for cutaneous leishmaniasis in Colombia. The drug is not approved in Brazil, but recent clinical trials with cutaneous leishmaniasis patients have shown a higher efficacy of miltefosine when compared to meglumine antimoniate. In this work, we evaluated the susceptibility of 21 clinical isolates of *L. (V.) braziliensis* from different regions of Brazil to miltefosine in vitro. We determined the inhibitory concentrations of miltefosine for promastigotes and amastigotes and identified significantly decreased susceptibility in two out of 21 clinical isolates. Phosphocholine uptake studies showed a direct correlation between accumulation of the phospholipid and miltefosine susceptibility in the clinical isolates. Based on these findings, we investigated whether polymorphisms in the miltefosine transporter gene could explain the differential susceptibility phenotype. The miltefosine transporter gene (*LbrM 13.1380*) sequences from three isolates and from *L. braziliensis* M2903 reference strain were determined. We found 25 nucleotide polymorphisms among the isolates and the reference strain, but none of them could be correlated to the differential susceptibility of the isolates. Since *L. (V.) braziliensis* presents a second MT gene we are currently characterizing this MT paralogue (*LbrM 13.1400*) and investigating if there are differences in miltefosine transporter protein expression levels in these clinical isolates. **Supported by:** FAPESP, CAPES and CNPq
Keywords: Miltefosine; susceptibilty ; leishmania braziliensis

TB054 - IMPROVING SERODIAGNOSIS OF HUMAN LEISHMANIASIS WITH RECOMBINANT LEISHMANIA BRAZILIENSIS ENOLASE PROTEIN

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Serological tests have significant advantages for leishmaniasis diagnosis. These tests allow the early detection of infection before lesion formation, and they are non-invasive, quantitative and easily automated, allowing the concurrent analysis of a large number of samples. Leishmaniasis encompasses multiple clinical syndromes that are classified as cutaneous, mucosal, and visceral, and the development of each clinical form is associated with the genetic variability of both parasite and host. Cutaneous and mucosal leishmaniasis can cause substantial morbidity. In this study, we analyzed the potential of Enolase protein from Leishmania braziliensis to serve as antigen candidates for the serodiagnosis of human tegumentary leishmaniasis. For this, we evaluated sequence similarity between other phylogenetically related parasites and hosts. Additionally, we mapped linear B-cell epitopes in this protein with sequences that were divergent from their orthologs in Homo sapiens and Trypanosoma cruzi and evaluating their performance with recombinant protein using ELISA. Moreover, the predicted B-cell epitope in Enolase co-occurs with an intrinsically unstructured region, which suggests that this protein region has an unfolded structure and is therefore potentially accessible for antibody binding. In the Enolase protein sequence were identified two predicted B-cell epitope (score: 1.265).Here, we expressed this protein as His-tagged recombinant protein. ELISAs were performed to evaluate the reactivity of sera from patients with tegumentary leishmaniasis (TL) against recombinant Enolase and soluble Leishmania antigen (SLA). The sensibility, specificity and accuracy of recombinant Enolase (100.00%, 85.00% and 92.77%) was higher than SLbA (65.12%, 57.50 and 61.66%) resulting in improvement in leishmaniasis diagnosis. The performance of this serodiagnosis assay was improved using recombinant Enolase for TL diagnosis compared to SLA. **Supported by:**COLTEC/UFMG, FAPEMIG and CNPq
Keywords: Tegumentary leishmaniasis; enolase; b-cell epitope mapping

TB055 - RAVUCONAZOLE, A POTENT AZOLE AGAINST *LEISHMANIA AMAZONENSIS*

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Leishmaniasis is endemic in 98 countries and the treatments available have limited safety and efficacy, and difficult administration. Thus, there is an urgent need for safer and more effective therapies. Trypanosomatids have an essential requirement for ergosterol and other 24-alkyl sterols. Ravuconazole (RVZ) is a novel triazole agent that inhibits the cytochrome P450 dependent enzyme C14α-demethylase, essential for the conversion of lanosterol to ergosterol. Thus, we investigated the antiproliferative, ultrastructural and biochemical effects of RVZ in *L. amazonensis*. Our studies demonstrated that RVZ is able to reduce the cellular growth of *L. amazonensis* promastigotes (IC_{50} of 0.87 μM after 48h of treatment) and intracellular amastigotes (1.65 μM after 72h of treatment). The viability of promastigotes was also analyzed using the MTS assay, and the EC_{50} was 3.09 μM after 48h of treatment. Scanning electron microscopy and fluorescence microscopy using antibody against α-tubulin revealed several alterations on the shape of drug-treated promastigotes that appeared rounded, presenting significant alterations in the distribution of tubulin. Nile Red-labelled cells demonstrated an accumulation of lipid bodies after treatment, which was confirmed by transmission electron microscopy. Other alterations were observed as mitochondrial swelling, which was consistent with a reduction of the $\Delta\psi_m$, increase in the production of mitochondrial superoxide's, and a decrease of ATP content. An increase in the ROS production was also observed. Furthermore, promastigotes treated with RVZ displayed a reduction of endogenous sterols. Also, low concentration of RVZ produced an accumulation of 14α-methyl-ergosta-8,24-dien-3ß-ol (54.3%), which decreases to 27% with increasing levels of triazole, indicating that the primary target of RVZ is the cytochrome P450 dependent sterol C14α-demethylase. Taken together, these results indicate that RVZ is a promising compound against *Leishmania*. **Supported by:**CNPq, CAPES, PPSUS and FAPERJ
Keywords: Ergosterol; azoles; leishmaniasis

TB056 - EPITOPE MAPPING OF THE EUKARYOTIC INITIATION FACTOR 5A (EIF-5A) PROTEIN OF LEISHMANIA BRAZILIENSIS DISCLOSES NOVEL TARGETS FOR IMMUNODIAGNOSIS OF TEGUMENTARY FORMS OF LEISHMANIASIS

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At present, there is no gold standard test for Tegumentary Leishmaniasis (TL) diagnosis, and a combination of diagnostic methods is frequently needed to obtain more precise results. The search for more refined antigens of Leishmania spp. is necessary to identify molecules able to improve the sensitivity (Se) and specificity (Sp) values for the serodiagnosis of TL. In addition, the identification of proteins able to predict the success of a given treatment will help the follow-up of patients after treatment. In this study, we analyzed the potential of Eukaryotic initiation factor 5a (EIF-5a) protein from L. braziliensis for the serodiagnosis of human TL. For this, we evaluated sequence similarity between other phylogenetically related parasites (Trypanosoma cruzi) and hosts (Homo sapiens). Although EIF-5a is encoded in the genome of different parasites as well as in their hosts, differences among these protein sequences, in particular in the B-cell epitopes, may be sufficient to induce the production of Leishmania-specific antibodies. Moreover, in the EIF-5a protein sequence were identified two predicted B-cell epitope (epi-1: score 1.468; epi-2: score 1.464) that co-occurs with an intrinsically unstructured region (epi-1: score 0.657; epi-2: score 0.674), which suggests that these protein regions has an unfolded structure and is therefore potentially accessible for antibody binding. ELISAs were performed to evaluate the reactivity of sera from patients with TL against recombinant EIF-5a and soluble Leishmania antigen (SLA). The Se, Sp and accuracy of recombinant EIF-5a (100.00%, 92.50% and 96.38%) was higher than SLA (65.12%, 57.50 and 61.66%) resulting in improvement in leishmaniasis diagnosis. The performance of this serodiagnosis assay was improved using recombinant EIF-5a for TL diagnosis compared to SLA. **Supported by:**COLTEC/UFMG, FAPEMIG and CNPq **Keywords:**Tegumentary leishmaniasis; eif-5a; b-cell epitope mapping

TB057 - CHAETOCIN, A HISTONE METHYLTRANSFERASES INHIBITOR, AFFECTS IRREVERSIBLY *TRYPANOSOMA CRUZI* PROLIFERATION AND ULTRASTRUCTURE

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Histone methyltransferases (HMTs) are essential enzymes that control chromatin compactation and play important roles in DNA replication, transcription, repair and in gene expression. Since HMT inhibitors decrease cell proliferation and promote cell cycle arrest they have been used as chemotherapeutic agents in cancer treatments and parasite diseases. Importantly, trypanosomatid HMTs present particularities in relation to those described in human, thus becoming promising chemotherapeutic targets. In this work we evaluate the effects Chaetocin, an HMT inhibition, in *T. cruzi* proliferation, viability, ultrastructure and cell cycle. For this purpose, epimastigotes were treated with 1, 5, 10 and 50 µM of the inhibitor for 96 hours. Samples were collected after each 24 hours for counting on Neubauer's chamber, to viability assays, for processing to transmission (TEM) and scanning electron microscopy (SEM) and to flow cytometry. Obtained data showed that Chaetocin inhibited parasite proliferation in an irreversible manner and affected cell viability even after treatment with the lowest concentration (1 µM). This compound also interfered with *T. cruzi* cell cycle, as revealed by flow cytometry, which showed an increased number of protozoa in G2/M phase after treatment with 5 µM for only 1h. TEM analysis, including Phosphotungstic Acid technique (PTA), revealed that Chaetocin promoted intense unpacking of nuclear heterochromatin, disruption of the nucleolus and cytoplasmatic disorganization. These ultrastructural alterations were observed even after the removal of the drug from the medium. SEM analysis showed that treated parasites presented atypical morphology, as round shapes or the flattened of the posterior end of the cell body. Taking together, data showed that treatment with Chaetocin affects different aspects of parasite cell biology, reinforcing the idea that HMTs represent potential targets for chemotherapy against *T. cruzi.* **Supported by:**CNPq and FAPERJ **Keywords:**Trypanosoma cruzi; ultrastructure; chemotherapy

TB058 - ANTILEISHMANIAL ACTIVITY OF SYNTHETIC FLUORENONE DERIVATIVES

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Leishmaniasis can have different clinical manifestations depending on the parasite virulence factors and on the host immune response. The disease occurs in the cutaneous and visceral forms, with global incidence estimated about 1.2 and 0.4 million cases per year, respectively. In Brazil, the reference treatments are pentavalent antimony, pentamidine and amphotericin B. However, the current treatment has many side effects and cases of resistance have been reported, indicating how important the search for new antileishmanial drugs is. Fluorenones are fluorene oxygenated derivatives at C-9 position. The flourene is a member of polycyclic aromatic hydrocarbon class, which is produced during an incomplete burning process of products like coal, oil, gas and garbage. It has been shown that fluorenones have activity against fungus, cancer, virus and malaria. Thus, this study aims to evaluate the antileishmanial activity of fluorenone and their derivatives LCO-6 and LCO-7. For antipromastigote assays, Leishmania amazonensis promastigotes were cultured in the presence of varying concentrations (0.1, 1, 10, 100 or 1000 µM) of fluorenone, LCO-6 and LCO-7. After 72 hours of culture, the cellular viability was evaluated using Alamar Blue. To evaluate the anti-amastigote activity and cytotoxicity in mammalian cells, peritoneal macrophages from BALB/c mice were infected with L. amazonensis and incubated for 48 hours with different concentrations (0.1, 1, 10 or 100 µM) of fluorenone, LCO-6 and LCO-7. The results showed that fluorenone, LCO-6 and LCO-7 displayed IC50 for promastigotes of 6.5, 1.9 and 12.8 µM, respectively, and for amastigotes of 9.2, 4.9 and 9.4 µM respectively. Besides, fluorenone, LCO-6 and LCO-7 showed low cytotoxicity against macrophages with selectivity index of 4.4, 20 and 11 µM, respectively. These data suggest that LCO-6 is a promising compound for studies of mechanism of action and efficacy in cutaneous leishmaniasis treatment. **Supported by:**CNPq, CAPES, FAPERJ **Keywords:**Leihmania amazonensis; antileishmanial ; fluorenone

TB059 - SYNTHESIS AND ANTILEISHMANIAL ACTIVITY OF NOVEL HALOGENS TETRAHYDROPYRIMIDINE DERIVATIVES

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Leishmaniasis is a protozoan disease that affects about 12 million people in the world,causing a serious public health problem. Current chemotherapy of leishmaniasis is unsatisfactory. Efficacious and safe new drugs are needed. In this work, the antileishmanial efficacy of novel halogens derivatives 2-(5-amino-1-aryl-1H-pyrazole-4-yl)-1,4,5,6-tetrahydropyrimidine and 2-(1-aryl-1H-pirazole-4-yl)-1,4,5,6- tetrahydropyrimidine were determined against Leishmania amazonensis , Leishmania braziliensis and Leishmania infantum. The cytotoxicity of these compounds was also evaluated on murine peritoneal macrophages. The pyrazole and tetrahydropyrimidine rings structure is already known to possess remarkable and significant biological and medicinal importance.The halogens derivatives were obtained, in good yields and all the substances were fully characterized by usual methods (IR, 1H, 13C NMR). The antileishmanial efficacy of six compounds of these 5-amino-aryl-pyrazole-tetrahydropyrimidine and aryl-pyrazole-tetrahydropyrimidine derivatives was determined in vitro against promastigotes forms of Leishmania sp. Parasites were cultured with and without the drugs in Schneider´s medium at 25°C, using Pentamidine as the standard drug. After 24 hours incubation, parasite viability was determined using the MTT(tetrazolium blue) assay. The results showed that among all the compounds tested substituted 5-amino-3,5-dicloroaryl (IC 50/24 = 48.8 ± 1.4 µM) was the most potent against promastigotes of L. Amazonensis. While in L. braziliensis and L. infantum, the derived 3,5-dicloroaryl was the most potent, with IC50/24h de 30,62 ± 5,64 e 200 ± 6,3 µM, respectively. In addition, these substituents 5-amino-3,5-dicloroaryl and 3,5- dicloroaryl were less toxic than pentamidine. This study reinforces these novel derivatives as potential antileishmanial lead compounds for the design and synthesis of similar heterocycle derivatives. **Supported by:**FIOCRUZ/
Keywords: Leishmania sp; pyrazole-tetrahydropyrimidine ; rings

TB060 - IMMUNOPROTEOMIC APPROACH USING SERA FROM CUTANEOUS AND MUCOCUTANEOUS LEISHMANIASIS PATIENTS TO IDENTIFY ANTIGENIC PROTEINS IN *LEISHMANIA (VIANNIA) BRAZILIENSIS*

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Introduction: The early and correct diagnosis of tegumentary leishmaniasis is essential for disease treatment .The serodiagnosis presents some problems, such as the low level of antileishmanial antibodies found in the majority of patients, as well as the cross-reactivity in patients infected by other trypanosomatides. In the present study, aiming to identify antigens in the total extracts of stationary-phase promastigote and amastigote-like forms of Leishmania (Viannia) braziliensis for their use in ELISA experiments, an immunoproteomic approach was performed employing sera from TL patients. Material and Methodes: Sera samples from CL and MCL patients with well-established diagnosis of the symptomatic disease, as well as sera of healthy and non-infected subjects living in endemic area of TL and sera of Chagas´ disease patients, were used in the immunoblotting assays. Results: In the results, protein spots recognized only by antibodies from CL and/or ML patients were identified; and a total of 20 proteins were revealed, being six, four and ten proteins expressed in the promastigote, amastigote-like or both parasite stages, respectively. From the identified 20 proteins, two of them are considered hypothetical proteins, and five were identified by both CL and ML sera classes. Previously known proteins as virulence factors, diagnosis markers and/or vaccine candidates, as well as drug targets; were identified. Conclusion: The present study represents a contribution in identifying antigenic L. (Viannia) braziliensis proteins, as well as in revealing the expression of two new hypothetical proteins in this parasite species. All antigens revealed in this study could be applied in future works for the improvement of the sensitivity and specificity for the serodiagnosis of TL. **Supported by:**Fapemig **Keywords:**Immunoproteomic; leishmania (viannia) braziliensis; , serological diagnosis

TB061 - PHOSPHOPROTEOMIC STUDY OF WILD-TYPE AND ANTIMONY-RESISTANT *LEISHMANIA BRAZILIENSIS* LINES USING THE QUANTITATIVE 2D-DIGE METHODOLOGY

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The post-translational regulation of a variety of intracellular events through the reversible phosphorylation of proteins plays a key role in the biology of trypanosomatids. In this study, we performed a comparative phosphoproteomics analysis of trivalent antimony (SbIII)-resistant and -susceptible lines of *L. braziliensis* using the quantitative 2D-DIGE (two dimensional differential gel electrophoresis) methodology followed by mass spectrometry. In order to investigate the differential phosphoprotein abundance associated with the drug-induced stress response and SbIII-resistance mechanisms, we compared non-treated and SbIII-treated samples of each line. Pair wise comparisons revealed a total of 116 spots that showed a statistically significant difference in phosphoprotein abundance, including 11 and 34 spots specifically correlated with drug treatment and resistance, respectively. We identified 48 different proteins distributed into seven biological process categories. The category “protein folding/chaperones and stress response” is mainly implicated in response to SbIII treatment, while the categories “antioxidant/detoxification”, “metabolic process”, “RNA/DNA processing” and “protein biosynthesis” are modulated in the case of antimony resistance. Multiple sequence alignments were performed to validate the conservation of phosphorylated residues in nine proteins identified here. Western blot assays were carried out to validate the quantitative phosphoproteome analysis. The results revealed differential expression level of three phosphoproteins in the lines analyzed. This novel study allowed us to profile the *L. braziliensis* phosphoproteome, identifying several potential candidates for biochemical or signaling networks associated with antimony resistance phenotype in this parasite, and contributing to understand the Leishmania biology and to drive drug-discovery efforts against the leishmaniasis. **Supported by:**FAPEMIG, CNPq, PROEP/CNPq/FIOCRUZ, convênio Instituto Pasteur/FIOCRUZ, UNICEF/UNDP/World Bank/WHO **Keywords:**Leishmania braziliensis; antimony resistance; phosphoproteomic analysis

TB062 - ANTI-*TRYPANOSOMA CRUZI* EFFECT *IN VITRO* OF NEW METALOCOMPLEXES COMPOUNDS

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Chagas disease, caused by *Trypanosoma cruzi*, is a major public health problem in the Americas. The disease affects 12 million people in endemic areas and at least 60 million still live under risk of contamination. The treatment of Chagas disease is based on Benznidazole, which is the single approved compound for therapy, and present several negative aspects, including resistance, inefficiency in the chronic phase of the disease and severe cytotoxic effects. Therefore, there is a need to develop new compounds able to treat more efficiently Chagas's disease. Some reports in the literature demonstrate that coordination compounds may be an interesting alternative for antiparasite therapy such as: Leishmania, Toxoplasma and Trypanosoma. Here we tested the *in vitro* effect of the iron compounds, I and II, on the growth of *T. cruzi* epimastigotes (Y strain). The parasites were treated with the compounds at concentrations ranging from 1 to 100 nM and were quantified by direct counting on a Neubauer chamber. The compound I presented the lowest IC50 values, that were of 4.14 nM, 4.65 nM and 4.81 nM, after 72, 96 and 120 h of treatment, respectively. The II compound presented IC50 values of 4.71 nM, 5.11 nM and 7.82 nM for the same treatment times. Ultrastructural analysis of the parasites after treatment with the compound II showed that the parasite mitochondria present changes in their cristae, with swelling and abnormal disposition around the kinetoplast. These results showed that the metalocomplexes compounds were active against *T. cruzi* epimastigotes presenting low IC50 values and affecting the mitochondria an essential organelle for the parasite survival. The next step will be to further analyze the effect of these compounds on the parasite ultrastructure in order to investigate the kind of cell death and the mode of action of the compounds. **Keywords:**Chagas disease; trypanosoma cruzi; metalocomplexes

TB063 - LEISHMANICIDAL ACTIVITY OF A BINUCLEAR CYCLOPALLADATED COMPLEX AGAINST *LEISHMANIA AMAZONENSIS*: *IN VITRO* AND *IN VIVO* ANALYSES AND POTENTIAL TOPOISOMERASE IB INHIBITOR

ARENAS VELÁSQUEZ, Á.M.[*1]; RIBEIRO, W.C.[1]; SANTORO, M.[2]; PASSALACQUA, T.G.[1]; RIBEIRO, A.R.[3]; DEL CISTIA, M.L.[1]; MAURO, A.E.[1]; DESIDERI, A.[2]; GRAMINHA, M.A.S.[1]
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Leishmaniasis is a neglected tropical disease caused by several species of *Leishmania*. Since the available drugs have shown to be highly toxic and cases of resistance have emerged, new therapeutic agents are urgently needed. Here we report the evaluation of a synthetic compound containing Pd(II) and *N,N*-dimethylbenzylamine (DMBA) against *Leishmania amazonensis*. The chemical cyclopalladated complex $[Pd(dmba)(\mu\text{-}N_3)]_2$ (CP1) exhibited leishmanicidal activity *in vitro* against promastigote (IC_{50}=13.15 ± 0.67 µM) and intracellular amastigote (IC_{50}= 10.14 ± 2.20 µM) forms of this parasite. The selectivity index to intracellular amastigote forms based on comparison with BALB/c macrophages was 49.90, indicating that CP1 presents high selectivity for *Leishmania* sp. *versus* mammalian cells. In *in vivo* assay, *L. amazonensis*-infected BALB/c in footpad treated with 0.35 mg/kg.day of CP1 during 30 days showed a decrease of foot lesion size and a reduction of 30% of parasite burdens when compared to the untreated control and similar reduction when compared to amphotericin B (2mg/kg.alternate days). Quantitation of tissue parasite burden was determined by *Leishman-Donovan units* (number of amastigotes per 1000 cell nuclei × tissue weight (g)) and real time qPCR. Additionally, CP1 did not cause exacerbated loss of weight as observed for mice treated with amphotericin B. The first step in drug discovery is to identify a suitable drug target. In preliminary results, CP1 showing *in vitro* a *L. donovani*-Topoisomerase IB Inhibitor at submicromolar concentrations which could explain its antileishmanial effect. The results herein presented suggest that the CP1 should be further considered as potential new hit in the search for the chemoteraphy of leishmaniasis. **Supported by:**CAPES **Keywords:**Leishmanicidal activity; cyclopalladated complex; topoisomerase

TB064 - A HYPOTHETICAL PROTEIN SELECTED IN *LEISHMANIA (VIANNIA) BRAZILIENSIS* BY AN IMMUNOPROTEOMIC APPROACH APPLIED WITH POTENTIAL SERODIAGNOSIS APPLICATION FOR TEGUMENTARY LEISHMANIASIS

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Introduction: Leishmaniasis comprises a spectrum of diseases found in tropical and subtropical countries of the world. The use of recombinant proteins is a valuable targets for serodiagnosis due to their increased sensitivity, specificity and potential for standardization. In the present study, a hypothetical protein identified by an immunoproteomic approach was cloned and evaluated as diagnostic markers for the serodiagnosis of TL .Material and Methods: ELISA experiments were performed using sera from patients with CL (n=23) and ML (n=20) and sera from non-infected individuals (n=30) and sera of Chagas' disease patients (n=10). Results: The hypothetical protein presented 95.35% of sensitivity and specificity value of 85.0 % and 90.36 % of accuracy; whereas the *L. braziliensis* antigenic preparation showed values of 65.12 and 57.50%, respectively and 61.44% of acuracy. Conclusion: In this context, this study represents a contribution in identifying new antigenic *L. braziliensis* proteins. The use of sera from CD and CS to previously exclude antigens could be considered a refinement in the assays, and the identified antigens could be applied in the improvement of the serodiagnosis of TL.
Supported by: Fapemig
Keywords: Immunoproteomic; leishmania (viannia) braziliensis; serological diagnosis

TB065 - DIETARY RETINOL DEFICIENCY IMPAIRS INTRANASAL LAAG VACCINE EFFICACY BUT INCREASES BALB/C MOUSE RESISTANCE TO *LEISHMANIA AMAZONENSIS* INFECTION

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Cutaneous leishmaniasis has clinical manifestations ranging from localized skin ulcer to disfiguring chronic lesions. Despite the intense effort in research using parenteral vaccination, no vaccine has yet been licensed for human use. Our group has explored a mucosal vaccination strategy to induce immune tolerance to disease-related parasite antigens. We have previously shown that intranasal vaccination with *Leishmania amazonensis* total antigens (LaAg) protects BALB/c mice against cutaneous leishmaniasis, unlike subcutaneous vaccination which exacerbates the infection. Dietary retinol (vitamin A) is a source of retinoic acid, which is involved in maintenance of mucosal immune tolerance. In this study, the role of dietary retinol in intranasal LaAg vaccine efficacy was investigated. Thus, BALB/c mice subjected for life to dietary retinol restriction ($Retinol^-$) or supplementation ($Retinol^+$) were given two doses of LaAg. After 24 h, cytokine and transcription factor expression in nasal mucosa draining cervical lymph nodes were evaluated by qRT-PCR. One week after immunization, mice were challenged with living parasites. Lesion development was monitored for 60 days, when the parasite burden and cytokine profile at the infection site were evaluated. We found that vaccinated $Retinol^+$ mice presented higher expression of Tbet, Foxp3, IL-12, IL-10 and TGF-β. Interestingly, non-vaccinated $Retinol^-$ mice developed smaller lesions and lower parasite burden than $Retinol^+$ mice. LaAg only conferred protection to $Retinol^+$ mice, which had increased IL-12 and reduced IL-4 at the infection site, compatible with local Th1 response. Moreover, macrophages from $Retinol^-$ mice were more resistant to *L. amazonensis* infection and produced higher levels of NO than $Retinol^+$ mice. Altogether, these results show that dietary retinol negatively influences macrophage leishmanicidal activity and protective peripheral immunity, but at the mucosal level it positively favors intranasal vaccine efficacy.
Supported by:CNPq, CAPES, FAPERJ
Keywords: Leishmania amazonensis; retinol; laag intranasal vaccine

TB066 - KINETIC OF THE *LEISHMANIA AMAZONENSIS* ARGINASE INHIBITION BY ROSMARINIC, TRANS-CAFFEIC AND CHLOROGENIC ACIDS.

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The polyamine biosynthesis is an interesting metabolic pathway in *Leishmania* because it is involved in antioxidant action against ROS and NO, produced by host macrophage. In *Leishmania*, arginase is the first enzyme of the polyamine biosynthesis that hydrolyses L-arginina into L-ornithine and urea. Recently, we have discovered that a catechol group in flavonoids is essential to inhibit arginase from *Leishmania amazonensis*. Searching for new compound with catechol group, we found three acids that are actives against several species of *Leishmania* promastigotes and amastigotes. One of them is the rosmarinic acid that have two catechol group in its structure. Rosmarinic acid is active against *L. major, L. donovani, L. guyanensis* and *L. amazonensis*. In this study, we have tested three catechol compounds against *L. amazonensis* arginase: rosmarinic, trans-caffeic and chlorogenic acids that showed an IC_{50} of the 1.5 ± 0.1 µM, 1.1 ± 0.1 µM and 3.5 ± 0.2 µM, respectively. The IC_{50} of the arginase inhibition is closed related to the IC_{50} obtained against amastigotes of *L. amazonensis*. All compounds showed competitive inhibition of arginase. These results provide new evidence that compounds containing catechol groups target arginase enzymes and can be used for developing novel compounds against *Leishmania* infection.**Supported by:**FAPESP
Keywords: Leishmania; arginase; rosmarinic acid

TB067 - LEISHMANICIDAL ACTIVITY OF TWO DITERPENES ISOLATED FROM COFFEE: CAFESTOL AND KAHWEOL.

PASSOS, C.L.A.[*1]; FERREIRA, C.[1]; NOVAES, F.J.M.[1]; SOARES, D.C.[1]; REZENDE, C.M.[1]; SARAIVA, E.M.[1]
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Leishmaniasis is a public health problem in at least 98 countries and its therapy is based on pentavalent antimonials, pentamidine, amphotericin B and miltefosine. For all of them high toxicity, elevated cost and parasite resistance have been reported. All this, associated to the few advances made in relation to the development of new drugs and therapeutic approaches for this disease, stimulates the search of new alternatives for the treatment of leishmaniasis. Natural products are potential source of novel active molecules that may provide structural template for drug discovery. Studies have shown that coffee intake may be effective against heart disease, coronary disease, Parkinson's and Alzheimer's. Cafestol and Kahweol are two naturally occurring diterpenes in the lipid fraction of coffee and endowed with antitumoral, anti-inflammatory and antioxidant activities. The aim of this study was to evaluate the effect of Cafestol and Kahweol (C&K) against *Leishmania amazonensis*. In this study we first evaluated the cytotoxicity of C&K for host cells by the XTT method. Our results demonstrated a CC_{50} of 26.6 µg/mL. Besides, our results show that C&K presented an anti-*L. amazonensis* activity with an IC_{50} of 4.5 µg/mL for promastigote forms, while for intracellular amastigotes the IC_{50} was 15 µg/mL. The C&K selectivity index was 1.77. Our results showed that C&K do not induces nitric oxide (NO) production on macrophages infected with *L. amazonensis*, moreover, reduced 2.3-fold the NO production on IFN-γ and LPS-activated macrophages. To rule out a possible NO scavenger effect of C&K, a cell-free system was used with SNAP as a NO donor in the presence or not of C&K, and these drugs were not able to reduce NO levels. However, they increase 1.9-fold the production of reactive oxygen species in infected macrophages. Our data point the diterpenes C&K as promising substances for the development of a drug with leishmanicidal activity. **Supported by:**CAPES, FAPERJ, CNPq.
Keywords: Leishmania; cafestol; kahweol

TB068 - LEISHMANICIDAL ACTIVITY OF NEW AMIDOXIMES DERIVATIVES

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Leishmaniasis is caused by protozoa of the genus *Leishmania* and constitutes a serious public health problem. The drugs used in the clinic are toxic and often ineffective. Thus, a rational search for new therapeutic alternatives becomes necessary. In this study, we investigated the anti-*Leishmania amazonensis* (MHOM/BR/77/LTB0016) and anti-*L. infantum* (MHOM/MA67ITNAB263) activity of new amidoximes derivatives. We evaluated the activity against promastigotes treated with different concentrations of seventeen derivatives for 72 hours. Among all derivatives, the compound CLEM 6086 showed high activity with IC_{50} of 7.2 and 5.4 µM against *L. infantum* and *L. amazonensis* respectively. To evaluate the antiamastigote activity, peritoneal macrophages were infected with *L. amazonensis* or *L. infantum* for 4 hours and incubated with different concentrations of CLEM 6086 for 72 hours. CLEM 6086 presented excellent activity and demonstrated a decrease on the infection index in a dose-dependent manner, with IC_{50} of 14.7 and 6.2 µM against *L. infantum* and *L. amazonensis* respectively. Furthermore, this compound presented medium toxicity on murine peritoneal macrophages, with LD_{50} of 43.12 µM and selectivity index equal to 2.9 and 6.9 for *L. infantum* and *L. amazonensis* respectively. These results suggest that this is a promising hit for leishmaniasis treatment. However, it is necessary to improve the selectivity index and to elucidate its mechanism of action. **Supported by:**FIOCRUZ / CNPQ
Keywords: Amidoximes derivatives; leishmania ; chemotherapy

TB069 - 2'',3''-DIHYDROOCHNAFLAVONE STRONGLY REDUCES *TRYPANOSOMA CRUZI* Y AMASTIGOTE SURVIVAL INSIDE MICE MACROPHAGES.

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Trypanosoma cruzi is the causative agent of Chagas disease which affects 8 million people in Latin America. Due to parasite high capacity to evade host immune system, and low efficacy combined to serious side effects to patients caused by available drugs against Chagas disease, the identification of alternative anti- *T. cruzi* compounds is essential. Brazilian flora exhibits an immense diversity of metabolites that could fill these requirements. We had previously shown that 2'',3''-dihydroochnaflavone biflavonoid from *Luxemburgia nobilis* (Ochnaceae) interfered with *T. cruzi* Y strain epimastigote forms growth and led to morphological ultrastructural alterations, including lipid bodies accumulation (Florencio et al., 2013). In the present work, we investigated the lipid accumulation in *T. cruzi* Y epimastigotes treated with 7.5 µM of the biflavonoid for 4 days by Nile Red fluorescence labeling either by fluorometric quantification or fluorescence microscopy, but there was no significant difference compared to untreated parasites. We also observed that the tested drug affected *T. cruzi* Y intracellular amastigotes forms in Balb/c mice peritoneal macrophages infection assays. Treatment of infected macrophages with 2.5 and 5 µM of the biflavonoid for 120h led to association index (percentage of infected macrophages multiplied by the mean number of amastigotes per macrophage) reduction of 72% and 85%, respectively. Nonetheless, these drugs concentrations were harmless to host macrophages alone as demonstrated by XTT assays after 120h of treatment. Posteriorly, we intend to perform ultrastructural analyses of macrophages-amastigotes interactions to detect potential morphological alterations that 2'',3''-dihydroochnaflavone may cause to intracellular parasite forms. **Supported by:**CNPq e Faperj
Keywords: Chaga's disease; trypanosoma cruzi; natural products

TB070 - ANTIMALARIAL ACTIVITY OF SCHIFF BASES

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Introduction: Malaria is a parasitic disease caused by Plasmodium spp and is responsible for significant morbidity and mortality for humans, especially in the development countries where this disease is prevalent. In recent decades, the situation of malaria has worsened due to the resistance to standard treatment drug Chloroquine. This problem has required the development of safe, cheap and affordable compounds to treat malaria. Schiff bases have shown to be effective in treating virus, bacteria, fungal and plasmodial infections. This study evaluated antimalarial activity of Schiff bases against Plasmodium berghei in a murine model of infection. Material and Methods: Compounds JAS 070 (1) JAS 073C (2) and JAS 088 (3) were tested (10mg/Kg) by oral route, using the 4-day suppressive test in mice to evaluate the in vivo antimalarial activity. The inhibition of parasite multiplication was calculated using the formula: [(A-B)/A]x100, where A=average of parasitaemia in untreated group (H20 + DMSO 5%), B= average of parasitaemia in tested groups. At days 5, 7, 9 and 12 post infection, Giemsa stained blood smears were made. Citotoxicity of compounds (1), (2) and (3) were accessed using murine macrophages in a MTT colorimetric assay. Results: Compound (1) showed great antimalarial activity on day 9 post-infection, 74,1%, and on day 12 was noted a reduction in this activity, 44,13%. Compound (2) showed 62,38% of inhibition of parasite multiplication on day 9. Compound (3) showed a good antimalarial action on day 12 (62,07%). No compounds exhibit cytotoxicity against tested cells. Conclusion: According to the obtained results all the compounds tested showed promising results and deserve to be object of future investigations.
Supported by:CNPQ, UNIVERSIDADE FEDERAL DE JUIZ DE FORA
Keywords: Malaria; schiff bases; antimalarials

TB071 - STUDY OF EPIGALLOCATECHIN-3-GALLATE IN *LEISHMANIA INFANTUM* AND ASSOCIATION WITH REFERENCE DRUGS FOR TREATMENT OF LEISHMANIASIS

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Leishmaniasis is a neglected disease caused by parasites of the genus *Leishmania*. Current treatment for this disease still presents serious problems related to toxicity and drug resistance. The epigallocatechin-3-gallate (EGCG) has various pharmacological properties, such as antifungal, trypanocidal and antileishmanial. Researches in combination therapy in leishmaniasis demonstrate an increase on treatment efficacy and tolerance, reducing treatment duration and cost, and a delay the emergence of resistance. Therefore, the aims of this study were evaluate the mechanism of action of EGCG in *L. infantum* promastigotes and its association with the reference drugs for leishmaniasis treatment (meglumine antimoniate and amphotericin B) *in vitro*. Promastigotes of *L. infantum* were incubated in the absence or presence of increasing concentrations of EGCG (15.6µM, 31.2 µM, 62.2 µM, 125 µM, 250 µM, 500 µM and 1000µM) for 72h. EGCG decreases the number of viable cells in a dose-dependent manner, with an IC_{50} 196.8µM. The mitochondrial membrane potential (ΔΨm) was assessed and decreased significantly in a dose-dependent manner in promastigotes treated with EGCG, reaching 84.6% of inhibition. For investigate the interaction of EGCG with the reference drugs, promastigotes of *L. infantum* were incubated for 72h with different concentrations of EGCG combined with meglumine antimoniate or amphotericin B. The IC_{50} of the drugs alone, and IC_{50} of the combinations were used to generate an isobologram. The fractional inhibitory concentration (FIC) of the combination of EGCG with standard drugs was 1.4 and 0.9 for meglumine antimoniate and amphotericin B, respectively, showing an additive effect. Taken together, our results demonstrate an activity of EGCG in promastigotes of *L. infantum* with a decrease in ΔΨm as part of the mechanism of action. Combination of EGCG with reference drugs exhibited an additive effect, suggesting a new alternative for the treatment for leishmaniasis.**Supported by:**CNPQ, FAPERJ, CAPES, IOC/FIOCRUZ
Keywords: Chemotherapy; drug combination; leishmania infantum

TB072 - ACTIVITY OF HYDROXYETHYLAMINES AGAINST *LEISHMANIA* SPP

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In recent years, several reports have described the antileishmanial activity of hydroxyethylamines used in the highly active antiretroviral therapy (HAART). This project aims to evaluate the activity of 10 new synthetic hydroxyethylamines (HEAs), precursors in the synthesis of amprenavir, against *L. amazonensis*, *L. donovani, L. major* and *L. mexicana*. To evaluate the activity of HEAs in amastigotes, macrophages were infected, treated, and the infectivity index was determined. The results showed variations for each specie. Against *L. amazonensis*, the best HEAs were PMTB1, PMTB8, PMTB10, PLIP1, PLIP27, PLIP33, with IC_{50} of 5.5, 0.9, 3.5, 4.7, 16.9 and 4.9µM, respectively. Against *L. donovani*, only PLIP1 was active, with IC_{50} 10.3µM. Against *L. major*, PMTB1, PMTB10, PLIP27 showed IC_{50} of 10.3, 7.3 and 4.3µM, respectively. Against *L. mexicana*, PMTB10 and PLIP27 showed IC_{50} of 8.1 and 13.4µM. The best HEAs were PMTB10 and PLIP27, which were more potent against *L. major* than miltefosine. We evaluated the effect of the infection ratio in the antileishmanial activity of these compounds. Against *L. amazonensis*, IC_{50} of PMTB10 and PLIP27 were 8.0 and 8.6µM, respectively, to 1:1 infection ratio, and >8µM, to 3:1 ratio (3 promastigotes/macrophage). Against *L. major*, IC_{50} of PMTB10 and PLIP27 were 8.4 and 5.7µM, to 1:1 inoculum, and >8µM, to 3:1. Unexpectedly, the toxicity of the compounds observed by light microscopy increased when the inoculum was 1:3. Therefore, higher infection ratio leads to higher toxicity and lower antileishmanial activity. When individually combinated, both PMTB10 and PLIP27 reduced the IC_{50} of miltefosine against *L. major.* Taken together, our results suggest that PMTB10 and PLIP27 are good prototypes to proceed the search for a drug candidate more efficient and less toxic to treatment of leishmaniasis. **Supported by:**CAPES
Keywords: Leishmania spp; hydroxyethylamines; drug combination

TB073 - *IN VITRO* ACTIVITY OF COMPOUNDS DERIVATES OF ISONIAZID AND PYRAZINAMIDE IN *LEISHMANIA BRAZILIENSIS*

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In endemic areas with co-infections with *Leishmania spp* and *Mycobacterium tuberculosis* drug therapy for tuberculosis, such as Isoniazid (INZ) and pyrazinamide (PZA) have proven effective in the control of leishmaniasis. These data suggest that the INZ and PZA may also act on *Leishmania spp*. The aim of this study was to evaluate the in vitro activity of INZ and PZA, as well as synthetic molecules derived therefrom (series G and R, respectively) on *L. braziliensis*. Mice peritoneal macrophage toxicity was available by MTT method after treatment with various concentrations (0-20µM) of all molecules (48h) and cytotoxic concentration for 50% of cells (CC50) was estimated. Monolayers of macrophages were infected with *L. braziliensis* (5:1) and incubated with the molecules (0-100µM) for 48 hours. The selective index (SI) was calculated based from IC50 and CC50. The promastigotes viability was assayed after treatment with molecules (0-20µM) for 72h using MTT method. All molecules were active against intracellular amastigote and showed reduced infection index in a dose-dependent manner (*$p < 0.05$ as from 6,25µM) and SI upper 10. The R02 molecule showed the lowest IC50 (10,7µM), even less than the original molecule, PZA (IC50 33µM). On the promastigotes, the precursor molecule INZ did not show activity under the conditions tested, however, the G02 and G03 derivatives were active as from 2µM (** $p < 0.001$). The molecules derived from PZA, R01, R02 and R05 significantly decreased the viability of the parasite from 0,2µM (* $p < 0.05$ ** $p < 0.001$ *** $p < 0.0001$). Our data show that INZ and PZA have activity against intracellular amastigotes of *L. braziliensis*, which may in part explain the beneficial action of these drugs observed on leishmaniasis in areas where co-infections. The R02, derived from PZA, was more active, showing that the chemical modification performed was able to increase its action on the parasite. The mechanism of action of these molecules are being investigate. **Supported by:**FAPERJ
Keywords: Leishmania braziliensis; isoniazid; pyrazinamide

TB074 - DEVELOPMENT AND VALIDATION OF A HIGH CONTENT ASSAY FOR LEISHMANIA CHAGASI INFECTED THP-1 CELLS.

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Leishmaniasis is a complex group of diseases that is subdivided into cutaneous, muco-cutaneous and visceral. The currently available drugs are associated with many limitations and therefore it is essential the discovery of new effective drugs, tolerable and economically feasible. In this context, the High Content Screening represents a potential alternative for antileishmanial drug discovery, since it is possible to test from hundreds to thousands compounds in a short period of time and thus lowering assay costs. Although image-based assays seen to be able to identify compounds against Leishmania, the HCS system still need to be optimized for more reliable, sensitive and robust results. The present assay was based on the use of stationary- phase Leishmania promastigotes to infect PMA-differentiated THP-1 cells and the automated quantification of the infection ratio two days after drug exposure. For assay development and optimization, we established cells density and PMA treatment in combination with the best condition for Leishmania parasite, such as culture medium, initial density of parasite, the days in culture for highest proportion of metacyclic forms and the multiplicity of infection (MOI). In order to validate the assay, we performed five experiments in different days, including tests of inter and intra experiment variability, Z´-factor calculation and DRCs determination. In summary, we developed and validated a new high content screening assay for triaging compounds against Leishmania chagasi that will enable the identification of new starting points for drug discovery for leishmaniasis. **Supported by:**CNPq
Keywords: High content screening; leishmania chagasi; visceral leishmaniasis

TB075 - EFFECT OF LOVASTATIN ON CEREBRAL MICROCIRCULATION DURING ACUTE TRYPANOSOMA CRUZI INFECTION IN MURINE MODEL

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Central nervous system alterations occur in Chagas Disease (CD), leading to meningoencephalitis, stroke and cognitive impairment. Microvascular alterations have been implicated in the pathogenesis of Chagas cardiomyopathy. Our group recently demonstrated that mice acutely infected by T. cruzi presented cerebral microcirculatory alterations, such as, endothelial dysfunction, reduction on functional capillary density and increase on leukocyte rolling and adhesion. Here we investigated if the treatment with lovastatin 20 mg/kg/day (starting 24h post infection for 15 days) would improve this cerebral alterations caused by T.cruzi infection. We utilized Swiss Webster mice acutely infected by Y strain of T. cruzi. Our preliminary data show that when compared to non-infected group, lovastatin treatment was not able to alter parasitemia and weight loss in infected mice. Cerebral histopathological studies reveled that acutely infected mice presented parasites nest at 15° and 22°dpi and nodular response at 22° dpi. Western Blot analysis revealed that the infection decreased Ve-cadherin expression, which was recovered with lovastatin treatment. Compared to non-infected group, acute CD significantly induced cerebral functional microvascular alterations, including functional capillary rarefaction and increased leukocyte rolling and adhesion at 15° dpi as previously demonstrated. Our preliminary data showed that lovastatin was not able to reverse these alterations. We just observed a tendency of reversion on cerebral microvasculopathy after the treatment.
Keywords: Chagas disease; capillaries alterations; leukocyte rolling and adhesion

TB076 - CHARACTERIZATION OF THE DISULFIRAM EFFECT IN *TRYPANOSOMA CRUZI*

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Chagas disease is caused by the hemoflagellate *T. cruzi*. Chemotherapy against this parasite is constrained to Benznidazole (Bz) and Nifurtimox treatment. Those drugs have toxic side effects, variable efficacy and the prevention of heart injury in chronic phase is unresolved. Therefore, alternative therapeutics are needed and several drug targets are under investigation. Disulfiram (DSF) has been widely used for alcohol dependency, and also its efficacy to treat tumor progression or different type of infections has been reported. In this work, we aimed to determine the efficacy of DSF and its main products of metabolic degradation against *T. cruzi*. We observed that diethyldithiocarbamate (DDC), S-methyl-N,N-diethylthiocarbamate sulfoxide (MeDTC-SO) and S-methyl-N,N-diethylthiocarbamate sulfone (MeDTC-SO2) inhibit proliferation of epimastigotes (epi) derived from the six DTUs of T. cruzi. The IC_{50} values were determined in all the strains, varying between 0.6-1.5 µM (DSF), 1.5-2.2 µM (DDC), 1-1.7 µM (MeDTC-SO), 0.1 – 0.87 µM (MeDTC-SO_2). All of them were compared against Bz, revealing IC_{50}s between 3,4 - 7,8 µM. Cytotoxic effects in LLC-MK_2 cells were also evaluated, showing CC_{50} values of: 3,6 ± 0,03 (DSF), > 10 µM (DDC), 2,1 ± 0,6 (MeDTC-SO) and 0,1 ± 0,06 µM (MeDTC-SO_2). The effect of DSF on *T. cruzi*-infected LLC-MK_2 cells revealed lower IC_{50} for trypomastigotes (Ty) bursting than those determined in the epi stage. Those were: 38 ± 4,3 nM and 19,5 ± 0,43 nM for DSF in the TcI and TcII lineages, respectively. Selectivity index was determined as 95, 185:1 (for two strains) based on the CC_{50} and IC_{50} values determined for Ty stage. DSF also reduced (55%) the infection rates during the amastigote proliferation. Molecular docking, enzymatic rates, and parasites over-expressing the mitochondrial enzyme TcP5CDH, second enzyme of proline catabolic path, revealed a putative target for DSF. Our data postulate proline degradative pathway as druggable target in *T. cruzi*. **Supported by:**FAPESP
Keywords: Proline catabolism; mitochondrion; drug repositioning

TB077 - PROGRAMMED CELL DEATH IN *LEISHMANIA AMAZONENSIS* INDUCED BY 4-AMINOQUINOLINE DERIVATIVE

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Chemoterapy for leishmaniasis has been unsatisfactory by significant toxicity, long-term terapy, high costs and drug resistance, making the development of new treatment strategies as urgent need. In a previous study, we noticed leishmanicidal effect of 4-aminoquinoline hybrid, named AMQ-j on promastigote of *Leishmania amazonensis* was related to mitochondrial stress. In this study, we evaluated the effect of this compund against promastigote and amastigote forms of *L. amazonensis* and a serious of biological assays were performed to detemine its feasible mechanism of action. Antipromastigote activity was determined by MTT colorimetric assay and antiamastigote assay was evaluated in macrophages infected with *L. amazonensis* transfected with GFP. Compound AMQ-j exhibited a strong activity against promastigote and amastigote forms of *L. amazonensis* (IC_{50} values of 5.9 and 2.4 ug/mL, respectively) and low cytotoxic effect in macrophages. To evaluate the effects of AMQ-j on the mitochondrial function of *L. amazonensis*-infected macrophages, we measured the mitochondrial membrane potential using the fluorescent probe JC-1. We also evaluated the effects of reactive oxygen species (ROS) production in *L. amazonensis*-infected macrophages teated with AMQ-j using a fluorescent probe, H_2DCFDA. Treatment of *L. amazonensis*-macrophages with the compound at 7.0, 13.5 and 27.0 ug/mL for 24 hours led to a significant reduction in the mitochondrial membrane potential and increased in ROS production at all concentrations evaluated. Programmed cell death induced by AMQ-j in *L. amazonensis* promastigotes was analysed using an annexin V-FITC/PI assay and DNA content analysis by flow cytometer. AMQ-j induced promastigote sub G0/G1 phase cell cycle arrest accompanied by phosphatidylserine externalization. In conclusion, we have demonstrated that AMQ-j exhibits promising leishmanicidal activity and the compound was able to induce some events related to programmed cell death. **Supported by:**CAPES, FAPEMIG, CNPq and UFJF
Keywords: Leishmania amazonensis; 4-aminoquinoline; programmed cell death

TB078 - HIGH-THROUGHPUT SCREENING OF NATURAL COMPOUNDS AGAINST *TRYPANOSOMA BRUCEI*

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The flagellated parasite *Trypanosoma brucei* is the causative agent of Human African Trypanosomiasis (HAT), also known as sleeping sickness. This vector-borne disease affects around 20,000 people annually and threatens more than 65 million of people located in 36 sub-

technology employed to screen 11,000samples of theMycoDiverseTMlibrary (Hypha DiscoveryLtd.) whichiscomposedofextracts and fractions from fermentations of higher fungi. The assay relies on indirect determination of parasite population viability by quantification of total DNA present in the well by the Sybr Green I DNA fluorescent dye. Based in this technology we were able to select 287 compounds (2.8% hit rate) with a relative activity ≥ 50% against the parasite and citotoxicity ≤ 50% based on previously available information of the compound library. Each selected compound was further tested in dose-response curves to determine theIC50 and the selectivity towards the parasite. **Supported by:**Fapesp
Keywords: Drug discovery; high-throughput screening; trypanosoma brucei

TB079 - LEISHMANICIDAL EFFECTS OF A PHOSPHOLIPASE A2 ISOLATED FROM *CROTALUS VIRIDIS VIRIDIS* SNAKE VENOM ARE CHARACTERIZED BY AUTOPHAGIC DEATH PROFILE.

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Treatment of Leishmaniasis is based on drugs which exhibit toxic effects and limited efficacy. Therefore the search for new drugs is a lining research to be exploited. Several toxins have been used as therapeutic agents. This study is based on *Crotalus viridis viridis* (Cvv) phospholipase A2 (PLA2) and its effects over *Leishmania amazonensis* parasites. The crude venom extract was loaded onto to a reverse phase analytical (C8) column using a high performance liquid chromatographer. A linear gradient of water/acetonitrile with 0.1% trifluoroacetic acid was used. The peak contained the PLA2 was collected and its protein content measured. The promastigotes were incubated in Schneider's medium, with 0.3125 to 10 µg/ml PLA2 at 26ºC, and the effect on cell proliferation was evaluated by daily counting. Infected peritoneal macrophages were cultivated in RPMI medium, with 0.625 to 2.5 µg/ml PLA2. The percentage of infected cells was evaluated. The data obtained allowed us to estimate the IC50/24 h for promastigotes of 2.45 ± 1.42 µg/ml and for intracellular amastigotes of 0.67 ± 1.2 µg/ml. Promastigotes viability was assessed by flow cytometry using propidium iodide labeling where 96.6% of treated parasites were stained. Morphological alterations were examined by electron microscopy and an autophagic cell death profile was suggestive. To confirm this hypothesis, we treated the promastigotes with 2.45 µg/ml for 24 h, and label them with monodansyl cadaverine and anti-LC3B antibody, where positive staining was detected with both markers. To investigate the mitochondria damage, treated parasites were stained with DiOC6 and MitoSOX Red mitochondrial superoxide indicator. The reduction in DiOC6 labelling and the increased fluorescence of MitoSOX staining, confirmed the damage. This study shows that Cvv PLA2 is active against *L. amazonensis* and treated promastigotes exhibit a profile of autophagic cell death.**Supported by:**FAPERJ, CAPES, CNPq
Keywords: Leishmania amazonensis; phospholipase a2; chemotherapy

TB080 - LIPID BODY FORMATION INDUCED BY CROTOXIN CAN LEAD TO A MACROPHAGE INFLAMMATORY PATHWAY DURING LEISHMANIA (LEISHMANIA) AMAZONENSIS INFECTION

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American Tegumentary Leishmaniasis is a disease caused by several species of Leishmania genus, among them *L. amazonensis*. They are obligatory intracellular parasite and it targets macrophage as its host. Macrophages have structures known as lipid bodies that store lipids used for the production of eicosanoids such as prostaglandin E2 (PGE2), a pro -inflammatory cytokine to combat protozoan infection. *Leishmania* parasite presents mechanisms to evade these defenses. Thereby reducing the production of prostaglandin E2, reduces pro- inflammatory response, which favors *Leishmania* proliferation. Some natural substances are studied to observe if they are able to prevent parasite immune system evasion. Crotoxin (CTX), isolated from snake venom of *Crotalus durissus terrificus*, is a strong candidate to be use as alternative to leishmaniasis treatment. This study had the objective to evaluate CTX activity during macrophage – *Leishmania* infection. It was observed by Sudan Black staining, that infected macrophages treated with 2.4 µg/mL of CTX presented significantly increase the number of lipid bodies compared to untreated infected cells. Ultrasctructural analysis evidenced high amount of electron dense lipid droplets in treated macrophages. Elisa assays showed a PGE2 level increase in infected macrophages and treated with 4.8 µg/mL of CTX by 48 hours. In addition, cytometry analysis showed that CTX also induces an increase of lipid bodies in promastigotes of *L. (L.) amazonensis* after 24 hours of treatment with 2.4 µg/mL. In conclusion, the results obtained in this study show for the first time that the CTX induces the formation of lipid bodies in infected macrophages and promastigotes of *Leishmania (L.) amazonensis*. However, to our knowledge, this is the first report correlating formation of lipid bodies during macrophage –*Leishmania* interaction after treatment with crotoxin. Therefore open new discussion about the role of snake venom during *Leishmania* infection.**Supported by:**Fapespa; UFPa; CAPES; Instituto Evandro Chagas; Instituto Butantan.
Keywords: Crotoxin; leishmaniasis; chemotherapy

TB081 - ESSENTIAL OILS OF *MYRCIA OVATA* CAMBESS. (MYRTACEAE) AND *EREMANTHUS ERYTROPAPPUS* MACLEISH. (ASTERACEAE) AFFECT *LEISHMANIA AMAZONENSIS* INTRACELLULAR AMASTIGOTES SURVIVAL.

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Leishmania amazonensis is an important etiological agent of cutaneous leishmaniasis in America continent, eventually causing diffuse cutaneous clinical form of the disease, which is characterized by deficient cellular immune response and low responsiveness to routine chemotherapy treatments. The first choices of treatment against leishmaniases are pentavalent antimonials, however with the costs of presenting toxicity and collateral effects to mammal hosts. New alternatives as promising chemotherapy agents are natural products whose effects have shown to be satisfactory. We had previously shown that essential oils OELM from *Myrcia ovata* Cambess (Myrtaceae) and OEC from *Eremanthus erytropappus* (DC.) Macleish. (Asteraceae) interfered with *L. amazonensis* procyclic promastigotes growth in vitro. We also observed that treatment with OELM and OEC induced ultrastructure alterations in the parasites, but there was no harm to mice peritoneal macrophages after 24h of treatment with these essential oils (Cardoso *et al.*, 2014). In the present work, we investigated the effect of OELM and OEC against *L. amazonensis* amastigote forms in mice peritoneal macrophages infection assays. Treatment of infected macrophages with a range from 0.1 to 15 µg/mL with both oils induced association index (percentage of infected macrophages multiplied by the mean number of amastigotes per macrophage) reduction of around 70% for both OELM and OEC in the highest concentration after 96h. Therefore, we performed viability investigations of mice macrophages exposed to the oils for longer periods of time (96h), based on XTT assay, and observed that the drugs still were not harmful to macrophages in the aforementioned doses. Currently, we are analyzing potential alterations in *L. amazonensis* lipid metabolism caused by these essential oils by thin layer chromatography. **Supported by:**FAPERJ & CNpQ
Keywords: Leishmania amazonensis; chemotherapy; natural products

TB082 - IN VIVO EVALUATION OF LEISHMANICIDAL ACTIVITY OF BENZOPHENONES DERIVATIVES BY QPCR

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Outside of India, the drug list for the treatment and control of leishmaniasis is based on pentavalent antimony, which has many limitations like high toxicity, long-term regimen and contraindication in cases of pregnancy. This study was designed to allow the detection and quantification of *L. (L). infantum chagasi* amastigotes in hamsters after treatment using some compounds derived from benzophenones, which have shown significant leishmanicidal activity in vitro. For this purpose, real time PCR (qPCR) was used for the evaluation and quantification of the parasites per gram of tissue. In this assay, the efficiency of 2-Hydroxy-4-O-(3,3-dimethyl)-allylbenzophenone (HDAB), 4-O-(3,3-Dimethyl)-allylbenzophenone (DAB) and 4,4′-Di-methoxybenzophenone (DMB) compounds was evaluated after ten days of treatment using the dose of 50 mg/kg/day in suspension, orally administered. The treatment began 50 days after inoculation, allowing the establishment of the infection. Glucantime was the most effective in the treatment of infected animals (1.51 x 104 and 2.25 x 103 amastigotes from spleen and liver, respectively). After treatment, the compound HDAB was able to reduce the number of amastigotes in the spleen (3.63 x 105 amastigotes) and DAB in the liver (1.63 x 105 amastigotes) of infected hamsters. The number of amastigotes after treatment with both compounds was statistically significant ($p < 0.05$) compared to the negative control group (4.64 x 106 and 3.29 x 105 parasites per gram of spleen and liver, respectively). The higher activity from DAB and HDAB compounds in the in vivo context could be explained by their in vitro leishmanicidal activity and lipophilicity. **Supported by:**PNPD CAPES, UNIFAL-MG, FAPEMIG
Keywords: Visceral leishmaniasis; in vivo activity; benzophenones derivatives

TB083 - ANTI-*TOXOPLASMA* ACTIVITY OF A NEW IRON(III) COMPOUND COORDINATED WITH SULFADIAZINE

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Toxoplasmosis is one of the most widespread diseases affecting the human population in the world and it is caused by the protozoan *Toxoplasma gondii*. The current treatment is based on the combination of sulfadiazine and pyrimethamine and presents several side effects. In order to develop new compounds to treat toxoplasmosis, the main objective of this work was to evaluate the in vitro anti-*Toxoplasma* activities of a iron(III) compound containing sulfadiazine coordinated to the iron center. The iron compound [Fe(H2BPClNOL)Cl2] was obtained as described previously in the literature and then it was coordianted with sodium sulfadiazine giving rise to the new compound tested here. This new iron compound was used to treat *T. gondii* infected LLC-MK2 host cells up to 48 hours. Cell infection index was performed by optical microscopy. Previous data show that after 24 hours, the growth rate for *T. gondii* was reduced by 58% in the presence of this new compound (10 µM), but the compound without sodium sulfadiazine (10 µM) was not active. After 48 hours, the compound without sodium sulfadiazine (10 µM) reduced the growth of the parasite by 66%, the new compound (10 µM) reduced the growth of the parasite by 82%, revealing a biological effect by the addition of the sulfadiazine molecule. Scanning electron microscopy showed parasites grouped in a structure similar to a bradizoyte tissue cysts after 48 hours of treatment with the new coordinated compound. The ultrastructural analysis of the parasites after this new compound treatment showed damages on the rhoptry and an unusual disposal of this organelle in the parasite cytoplasm. Further work includes the investigation of the kind of death suffered by the parasites after compound tretament. **Supported by:**FAPERJ, CNPq, CAPES, UENF, UEZO
Keywords: Toxoplasma gondii; sulfadiazine; iron(iii) compound

TB084 - ANTI-TRYPANOSOMA CRUZI ACTIVITY OF NEW 3-PHENYLTHIO-NOR-β-LAPACHONE DERIVATIVES

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Chagas disease is caused by the protozoan Trypanosoma cruzi and affects approximately eight million individuals in Latin America, of whom 30–40% either have or will develop cardiomyopathy, digestive megasyndromes or both. The available chemotherapy for this diseaseis effective against acute infections but show poor activity in the late chronic phase, with severe collateral effects and limited efficacy against different parasitic isolates. Therefore there is an intense effort to find new drugs for treatment of this disease. Naphthoquinones are present in several families of higher plants, being considered privileged structures in medicinal chemistry. They are involved in a variety of biological activities, including virucidal, anticancer, acting as a topoisomerase inhibitor and apoptosis inductor, and trypanocidal activity, being able to generate ROS by a redox cyclingwhich have stimulated the study of these bioactive compounds against T. cruzi. Our aim is the evaluation of 3-phenylthio-nor-β-lapachone derivatives against bloodstream trypomastigote forms of T. cruzi. The new compounds possess a broad range of activity, with IC50/24h from 9.2 to 182.7μM, higher than the original quinone (391.5μM) and four of them higher than standard drug benznidazole (103.6μM). The most active was compound 13b (IC50/24 h= 9.2μM), being 11 times active than benznidazole and the less toxic derivative to heart muscle cells. **Supported by:**CNPq, faperj e fiocruz
Keywords: Naphthoquinone; trypanosoma cruzi; chagas disease

TB085 - THE EFFECT OF CARVACRYL ACETATE AGAINST *LEISHMANIA AMAZONENSIS* PROLIFERATION AND INFECTION IN VITRO

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Leishmaniases form a disease complex caused by species of protozoa belonging to *Leishmania* genus. *Leishmania amazonensis* is an important cutaneous leishmaniasis etiological agent in America, eventually causing diffuse cutaneous clinical form of the disease, which is characterized by deficient cellular immune response and low responsiveness to routine chemotherapy treatments. Pentavalent antimonials are the first choice drugs against leishmaniases, however presenting toxicity and collateral effects to patients. Natural products of vegetal origin exhibit great potential to be explored as chemotherapy against different pathogens. Carvacrol has already been efficiently utilized against *L. amazonensis* (Pastor et al., 2015). Here, we investigate anti- *L. amazonensis* action of carvacrol chemically modified molecule carvacryl acetate. We tested the concentrations of 0.5, 1, 3 and 5 μM against procyclic promastigotes until four days of growth in vitro. The IC_{50} was approximately 0.5 μM at four days after treatment, while the parasites treated with the highest concentration of 5 μM, presents no growth since day zero of drug exposure. Posteriorly, we observed that the tested drug affected *L. amazonensis* intracellular amastigotes in Balb/c mice peritoneal macrophages infection assays. Treatment of infected macrophages with 5, 10 and 15 μM of the compound for 48h led to association index (percentage of infected macrophages multiplied by the mean number of amastigotes per macrophage) reduction around 50% in these cases. Nonetheless, these drugs concentrations were harmless to host macrophages alone as demonstrated by XTT assays after 48h of treatment. Currently, we are testing higher concentrations of carvacryl acetate in the interaction assays. We also intend to perform ultrastructure analyses to investigate if the drug causes morphological alterations in the parasite and other assays to determine if oxidative stress is triggered in *L. amazonensis* promastigotes after treatment. **Supported by:**FAPERJ & CNpQ
Keywords: Leishmania amazonensis; carcacryl acetate; cutaneous leishmaniais

TB086 - TREATMENT WITH MELATONIN INDUCES A REDUCTION OF PARASITEMIA IN LLC-MK2 CELLS INFECTED WITH *TOXOPLASMA GONDII*

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Toxoplasmosis is an infection caused by *Toxoplasma gondii*, an obligate intracellular protozoan that is distributed worldwide, affecting about one third of the world population. However, most infections are asymptomatic but can lead to severe manifestations in immunocompromised individuals. Melatonin is a highly conserved molecule found in almost all species which regulates the circadian cycle in vertebrates. It has been shown that melatonin is able to modulate the immune response of the vertebrate host during infection with *T. gondii* and *Trypanosoma cruzi*. During malarial infections, melatonin showed a reduction in liver damage. In this study, LLC-MK2 cells infected with *T. gondii* were treated with different concentrations of melatonin. The parasite growth and cell morphology was analyzed by light microscopy and obtaining the infection rate. In addition, marking by TUNEL was made to display the possible death of the parasite by apoptosis, as well as in vivo experiments to evaluate the survival of the host during treatment, and scanning electron microscopy to analyze the morphological changes before and after the treatment. We observed that melatonin reduced parasite growth by impressive 90%, when compared with the untreated control, without causing significant damage to the host cells. By TUNEL assays demonstrated that a part of the parasites die by this mechanism. Additional studies are being developed to elucidate another mechanism *T. gondii* possible death. In in vivo experiments, animals treated with melatonin resemble animals treated with sulfadiazine, drug currently used, succumbing from the ninth day of infection. Analysis of scanning electron microscopy showed that melatonin treatment leads to a reduction of the parasitemia and modifies the morphology of the parasite without harming the host cell. These results suggests that toxoplasmosis treatment with melatonin can be effective, becoming a promising therapeutic alternative. **Supported by:**CNPq, CAPES, Faperj
Keywords: Apoptosis; melatonin; toxoplasma gondii

TB087 - 2'-HYDROXYFLAVANONE: A STUDY OF MECHANISM OF ACTION IN *LEISHMANIA AMAZONENSIS*

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Caused by different parasites of genus Leishmania, Leishmaniasis is considered a neglected disease, which affects more than 12 million people around the world. Treatment of Leishmaniasis is currently based on pentavalent antimonials and amphotericin B however, these drugs present serious problems regarding side-effects, variable efficacy and are expensive, leading to a new search of efficient compounds. Pure compounds have been reported to possess significant antiprotozoan activities with no side effects. 2'-hydroxyflavanone is abundantly present in fruits and vegetables and has some biological functions including anti-inflammatory and anticancer. In this present study, we evaluated the effect of 2´-hydroxyflavanone against *Leishmania amazonensis* proliferation in promastigotes and its possible mechanism of action. Promastigotes were treated with different concentrations of 2'-hydroxyflavanone (3-96 µM) for 24, 48 and 72 hours, demonstrating a dose-dependent manner inhibition with an IC_{50} 20.96 µM (24h), 21.17 µM (48 h) and 9.8µM (72h). To investigate mitochondrial damage, promastigotes were treated with 2'-hidroxyflavanone (12-96 µM) for 24 hours, incubated with JC-1, and measured fluorimetrically. 2'-hydroxyflavanone showed a marked decrease in mitochondrial membrane potential with a dose dependent profile and 68% depolarization at the highest concentration (96 µM). It has been demonstrated that mitochondrial damage increases reactive oxygen species (ROS). ROS levels were measured in promastigotes treated with 2'-hydroxyflavanone (12-96 µM) for 24 hours. ROS levels increased in a dose-dependent manner, reaching 1.8 fold compared to the control with the highest concentration (96 µM). Taken together, these results demonstrate the effect of 2'-hydroxyflavanone on promastigote forms of *L. amazonensis* and suggest the mitochondrial damage followed by ROS production as mechanism of action. **Supported by:**CNPq; CAPES; FAPERJ; IOC/FIOCRUZ **Keywords:**Leishmaniasis; 2'hydroxyflavanone; mechanism of action

TB088 - ULTRASTRUCTURAL ALTERATIONS IN CLINICAL ISOLATES OF LEISHMANIA (L.) INFANTUM INDUCED BY ANTIMONIAL

MAGALHÃES, L.S.[*1]; CUNHA, L.C.S.[1]; BONFIM, L.G.S.[1]; MOTA, S.G.[1]; CAMPOS, R.N.S.[1]; FIGUEIRA, C.P.[2]; JESUS, A.[1]; ALMEIDA, R.P.[1]; BORGES, V.M.[2]; DE MOURA, T.R.[1]
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Leishmaniasis chemotherapy is still the main strategy to control the disease and derivatives of pentavalent antimony have been used as first-line drugs for more than 70 years. Drug resistance is a major concern, as few treatment alternatives are available. The efficacy of different treatments is a consequence of immunological profile of the patient, the pharmacokinetic properties of the drug and the intrinsic differences of each strain of Leishmania. A few studies examining the drug sensitivity of clinical isolates have shown concordance with molecular patterns identified within in vitro-selected mutants. The aim of this study was to evaluate in vitro effects of antimony in clinical isolates using electron microscopy. Methods and results: four isolates of Leishamania (L.) infantum chagasi obtained from antimony-refractory patients and two isolates from antimony-responsive patients were exposed in vitro to the trivalent antimony (active form of the drug) and submitted the essay to transmission electron microscopy. Unexposed condition was used how control. We observe that the isolates obtained from antimony-refractory patients showed a smaller number of ultrastructural changes compare to isolates from antimony-responsive patients. In isolated from responsive patients, antimony exposure changed organelle structures, with a large presence of cells almost without cytoplasmic content, increased eletrodensity with cytoplasmic vacuolization and compression, and disorganization of the kinetoplast, whose indicate unviability and cell death. Interestingly, in the isolates from antimony-refractory patients was observed general cell conservation. The main change was the cytoplasmic content compression and preservation of morphology and other organelles. Conclusion: isolates from antimony-refractory patients show different ultrastructural characteristics after exposure to antimony in vitro, suggesting that refractory treatment may be associated to parasites resistance mechanisms.
Supported by:CNPq; FAPITEC/SE; CAPES **Keywords:**Leishmaniasis; resistance; chemoterapy

TB089 - ANTILEISHMANIAL ACTIVITY OF NEW THIOUREAS DERIVATIVES

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Drugs used for leishmaniasis therapy are pentavalent antimonials, amphotericin B and miltefosine, which have high toxicity and several side effects leading to patients withdrawal and to increased incidence of drug-resistant cases. The search for new substances, which are efficient against the parasite with low toxicity to host cells, is important to improve leishmaniasis therapy. In this perspective, molecules that possess a thiourea group as pharmacophore are promising prototypes, since they present a broad spectrum of biological activities including: anti-HIV (inhibitors of HIV capsid assembly), anticancer, anticonvulsant, anti-mycobacterial, anti-HCV and antimicrobial properties, evidencing their importance for the drug research area. Here we tested the leishmanicidal activity of ten thioureas derivatives, which are identified as LabTIFs 09, 11, 13, 15, 18, 34, 48, 55, 66 and 68. We demonstrated low citotoxicity for macrophages treated with all thioureas derivatives until 200 µM, with exception of LabTIF 55 (49.2 µM CC_{50}). We also evaluated the antipromastigote effect of LabTIFs showing that all were active against promastigotes of *Leishmania amazonensis*, although LabTIFs 11, 13, 15, 66 and 68 were more effective, with IC_{50} of 80, 54, 46, 22 and 21 µM, respectively. LabTIFs 11 and 68 altered the promastigotes cell cycle, increasing 12 and 18 folds the number of promastigotes in G0 phase, suggesting a parasite incidental death. IC_{50} of 150, 70, 145, 110 e 81 µM were obtained respectively, after intracellular amastigotes treatment with LabTIFs 11, 13, 15, 66 and 68. Preliminary results demonstrated that anti-amastigotes activity of LabTIFs is independent of nitric oxide (NO) production, once LabTIFs inhibited NO production by infected macrophages. Our results show thioureas derivatives as promising candidates for future studies regarding treatment of leishmaniasis. **Supported by:**FAPERJ, CNPq, CAPES
Keywords: Leishmanicidal activity; thioureas; leishmaniasis

TB090 - TREATMENT WITH SEAWEED EXTRACT OF *DICTYOTA CARIBAEA* REDUCES THE PARASITE LOAD IN THE INTERACTION BETWEEN *TOXOPLASMA GONDII* AND HOST CELLS.

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Studies with seaweed have been the focus of many researchers because they are sources of sulfated polysaccharides, this substance is currently one of the most used for production of anticoagulants. These compounds have different ways of connection between monosaccharides and distribution of the sulfate group. The structures of these compounds varies according to the kind and location in the plant. Thus, each polysaccharide may have different behavior. Experiments were conducted in order to verify the activity of an extract fraction obtained from seaweed *Dictyota caribaea* against *Toxoplasma gondii*, the protozoan parasite that causes toxoplasmosis. Currently, chemotherapy against toxoplasmosis is performed with the use of sulfadiazine, an antibiotic that is effective against the parasite, due to its susceptibility to sulpha. For this purpose, interactions between tachyzoites of *Toxoplasma gondii* and LLC-MK2 cells were treated with different concentrations of *Dictyota caribaea* extract. Analysis by microscopy shown a reduction of the parasitic load present in the interaction after treatment with the extract, compared to the untreated control. This result suggests that this fraction of the extract obtained from *Dictyota caribaea* will may contain substances with activity against *Toxoplasma*. **Supported by:**CAPES, CNPq, FAPERJ
Keywords: Toxoplasma gondii; dictyota caribaea; sulfated polysaccharides

TB091 - EVALUATION OF THE *IN VITRO* LEISHMANICIDAL EFFECT OF AROMATIC SCHIFF BASES AGAINST *LEISHMANIA AMAZONENSIS*

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Introduction and Objectives: Leishmaniasis is a tropical disease considered by OMS as neglected and endemic in 98 countries. Treatment involves toxic and inefficient drugs, therefore is very important to search new compounds with leishmanicidal activity. In this study, leishmanicidal effect of several compounds derived from aromatic Schiff bases was tested. Material and Methods: Compounds were assayed against *Leishmania amazonensis* promastigote forms and murine peritoneal macrophages by MTT method after 72 hours of treatment. Effect of the compounds in intracellular amastigotes was evaluated by using RFP-transfected *L. amazonensis* and the relative fluorescence units (RFUs) were measured using a spectrofluorometer after 72 hours of treatment. The most effective compound against *L. amazonensis* promastigote and amastigote forms had its action mechanism evaluated, using H_2DCFDA and JC-1, to assess mitochondrial alterations and propidium iodide (PI), to evaluate the plasma membrane integrity of *L. amazonensis* promastigotes. Results: Three of the five compounds were not effective against *L. amazonensis* promastigotes. Compound Jas163B was toxic only for promastigotes (IC_{50} = 55.27µM) and compound Lar052x was effective against promastigotes (IC_{50} = 30.62µM) and intracellular amastigotes (IC_{50} = 28.04µM) of *L. amazonensis*. All compounds were not toxic to mammalian cells (CC_{50}>150µM). Treatment of *L. amazonensis* promastigotes with Lar052x caused mitochondrial damage and increase of ROS production, but did not alter the integrity of the plasma membrane of the parasite. Conclusions: These results indicate that Lar052x showed a promising *in vitro* leishmanicidal activity and further studies need to be conducted to know the mechanism of action against the protozoan.
Supported by:FAPEMIG, CNPq e UFJF
Keywords: Schiff bases; leishmania amazonensis; leishmanicidal effect

TB092 - PRECLINICAL PHARMACOKINETICS, SUBACUTE TOXICITY AND EFFICACY OF PTEROCARPANQUINONE LQB-118 AGAINST EXPERIMENTAL VISCERAL LEISHMANIASIS.

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The pterocarpanquinone LQB-118 was designed and synthesized based on molecular hybridization and exhibited antiprotozoal and anti-leukemic cell line activity. Our previous work demonstrated that LQB-118 was an effective treatment of experimental cutaneous leishmaniasis and that the mechanism of action involves induction of oxidative stress with characteristic events of cell death via apoptosis in *Leishmania amazonensis*. In this study, we developed a precise and accurate method, involving high-performance liquid chromatography coupled with a UV detector (HPLC-UV), for quantification of LQB-118 in the plasma of mice. The pharmacokinetic profile of LQB-118 showed rapid absorption after oral administration of 100 mg/kg, with the peak of maximum concentration between one and two hours, half-life of 4.5 hours, AUC_{0-t} 7898 ng/mL*h and MRT_{0-t} 6.2 h. The *in vivo* subacute toxicological analysis showed no change in clinical, biochemical or hematological parameters. Histologically, all of the analyzed organs were normal with the exception of liver, in which a few focal points of necrosis with leukocytic infiltration were observed at the dose five times higher than the therapeutic. These changes were not accompanied by increase in transaminases. Corroborating the previous results for CL, oral treatment with LQB-118 was effective in the VL model, reducing the parasitic load in the liver (ED_{90}=3.4mg/kg) and spleen (ED_{90}=5.7mg/kg) in a dose-dependent manner. The response to 10 mg/kg/23day orally inhibited the development of hepatosplenomegaly with a 99% reduction in parasite load. Our findings indicate that LQB-118 is effective at treating different clinical forms of leishmaniasis and presents no relevant signs of toxicity at therapeutic doses, demonstrating that this framework is suitable to develop promising drug candidates for oral treatment of leishmaniasis. **Supported by:**FAPERJ, PAPES VI/FIOCRUZ, CAPES and CNPq **Keywords:**Pterocarpanquinone; visceral leishmaniasis; pharmacokinetics

TB093 - STUDY OF NATURAL MILTEFOSINE-RESISTANCE MECHANISM IN *LEISHMANIA* (*L*.) *CHAGASI* ISOLATES FROM VISCERAL LEISHMANIASIS PATIENTS WITH DIFFERENT TREATMENT OUTCOMES

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Visceral leishmaniasis (VL) treatment relies on a few chemotherapeutic drugs including Sb(V), amphotericin B and miltefosine. Miltefosine has been highly active against VL in India. However, susceptibility differences to miltefosine have been observed in clinically relevant *Leishmania* species. Miltefosine resistance mechanisms are being elucidated in laboratory *Leishmania* spp. strains but are less clear in clinical isolates. In this study, we used comparative proteomics (2D-DIGE/MS) and genomics (Illumina) approaches to highlight molecular differences between *L*. (*L*.) *chagasi* (= *L*. (*L*.) *infantum*) isolates from VL patients in Brazil with different miltefosine treatment outcomes. The high-resolution proteomics showed 46 proteins that exhibited significantly different abundances ($p<0.05$) between the isolates from one cured and one relapsed patient. Most of the proteins up-regulated in the proteome of the isolate from the relapsed patient were associated with redox homeostasis, stress response, and drug translocation. Whole genome sequencing was carried out with isolates from cured (n=14) and relapsed (n=12) patients. 93 orthologs groups exhibited a significant difference ($p<0.01$) in gene dosage between the two groups, including a deletion of a locus on chromosome 31 (containing four genes; miltefosine sensitivity locus, MSL) which was associated with isolates from relapsed patients. It was inferred that this deletion process occurs by homologous recombination using repetitive sequence flanking the MSL, and apparently is not induced by miltefosine pressure. Re-expression of individual MSL genes in a miltefosine resistant promastigote line did not restore the in vitro miltefosine susceptibility phenotype. These data suggest that miltefosine-resistance mechanisms in *Leishmania* spp. are complex and multifactorial. **Supported by:**CNPq; CAPES; FAPES **Keywords:**Leishmania (l.) chagasi; miltefosine resistance; proteome - genome

TB094 - THE USE OF MICRONEEDLES ASSOCIATED WITH AMPHTOTERICIN B FORMULATIONS FOR TOPICAL TREATMENT OF CUTANEOUS LEISHMANIASIS

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Despite its high toxicity, amphotericin B (AnfoB) is the most potent drug approved for human use in the treatment of cutaneous leishmaniosis (CL). However, its administration is by systemic routes and still there is not an approved formulation for topical use due to its high molecular weight. In order to increase transdermal transport, various transdermal enhancement technologies have been developed, as, for example, microneedles. So, the aim of this work was to evaluate the use of microneedles of 500 µM length as a physical system able to facilitate the passage of the solid lipid nanocapsules entrapping the AmB (SLN-AnfoB) to dermis. The solid lipid nanoparticles have been prepared by the hot microemulsion method and were characterized physicochemically. After this, for in vivo experiments, we use two formulations of AnfoB, one using 5% of free AnfoB in Lanette cream and 3% Azone (chemical permeant agent) and the other of SLN-AnfoB incorporated in Lanette cream. For in vivo assay, BALB/c mice were infected in the ears with L. amazonensis GFP and treated from day 13 until day 37 after infection, twice a week during four weeks with the two AnfoB formulations, preceded by microneedles roller application. The free AnfoB formulation was evaluated also without microneedles application. The SLN-AnfoB had a mean size of 143 nm, a polydispersity index of 0.34 and the Amphotericin B concentration was 0,2%/g of powder. In vivo studies demonstrated that animals treated with SLN-AnfoB associated with microneedles had a smaller lesion and parasite burden than untreated animals. In addition, the animals which received free AnfoB in Lanette more microneedles had a lower parasite burden than the group without microneedles. Lanette cream associated with microneedles had no effect. Thus, we conclude that the microneedles are an interesting tool to promote the effectiveness of topical amphotericin B and SLN-AnfoB are a potential candidate for topical treatment of CL.
Keywords: Leishmaniasis; nanoparticles; microneedles

TB095 - FROM SCHIZODEMES TO DTUS: KDNA AS A MOLECULAR MARKER TO GENOTYPE *T. CRUZI* BY HIGH RESOLUTION MELTING.

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Chagas disease (CD) is an endemic and still neglected disease, caused by the flagellate protozoan *T.cruzi*. It presents distinct clinical manifestations, but there still unknown how the genetic heterogeneity of parasite and host contributes to the disease course. In 1980, Morel et al. demonstrated that kinetoplast DNA (kDNA) restriction endonuclease products could be used to characterize *T. cruzi* strains and clones. Currently, methods used to genotype *T. cruzi* are mostly based on multilocus conventional PCR; however, this methodology presents some limitations. Thus, this work aims to evaluate kDNA as a single molecular marker to the genotyping of *T. cruzi* by High Resolution Melting (HRM). To amplify a fragment from the conserved region of kDNA minicircles, we designed primers based on the alignment of 131 sequences from 19 strains representing four DTUs (I, II, V and VI), available on the GenBank (NCBI). It was observed a polymorphic region of 11 bp between two highly conserved regions that have demonstrated potential to differentiate subpopulations of the parasite. Also, to amplify the variable region of kDNA minicircles, 121/122 primers were used. The Real Time PCR assays were conducted with 9 reference strains/clones: Colombian, Dm28c, Y, 3663, 222, 4167, CAM III, SO3 and CL. The HRM assays targeting kDNA conserved region could distinguish *T.cruzi* isolates in 3 variants. Remarkably, reference strains isolated from sylvatic regions (from DTUs III and IV) presented the most distinguishable profile of the HRM curves. In contrast, assays targeting kDNA variable region could distinguish parasite isolates up to 6 variants. In order to simulate the application of this assay in patient blood samples, we performed HRM assays using blood spiked with *T. cruzi*, and similar genotyping profile was observed. So far, our results suggest the high potential of the HRM targeting kDNA to genotype *T.cruzi* from cultivation or Chagas disease patient samples.
Supported by:FAPERJ
Keywords: Chagas disease; genotyping; high resolution melting

TB096 - DEVELOPMENT OF TRIAZOLES AS POTENT INHIBITORS OF TRYPANOSOMA CRUZI

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Chagas disease is caused by the kinetoplastid Trypanosoma cruzi and is one of the seventeen Neglected Tropical Diseases, which together affect more than 1 billion people worldwide and are endemic in 149 countries.The available therapy for this diseaseis unsatisfactory, therefore there is an intense effort to find new drugs for treatment of this disease. The research on ergosterol biosynthesis inhibitors (EBI) stands out in the search for new active drugs against T. cruzi, and several enzymes associated with sterol biosynthesis have been investigated, especially C14α-sterol demethylase (CYP51), which catalyses the removal of the C14 methyl group of lanosterol. Two CYP51 inhibitors - posaconazole and ravuconazole - were submitted to Phase II clinical trials for the treatment of chronic patients with disappointing results when employed is monotherapy schemes. To address concerns regarding the use of azoles, new derivatives designed and synthetized bearing the pharmacophore of EBI with optimized pharmacokinetic parameters and relative low synthetic cost. These derivatives were obtained by a simple four-step synthetic route with overall good yields. So far, all the evaluated derivatives showed excellent anti-T. cruzi activity when evaluated on tulahuen strain, on trypomastigotes and amastigotes forms. One of which being more than 200-fold more potent than fluconazole, an EBI with anti-T. cruzi activity, and 17-fold more potent than benznidazole, presenting a selectivity index greater than 1500. Experiments are underway analyzing the trypanocidal activity of other T. cruzi strains. The more active compounds will be subjected to in vivo evaluation. **Supported by:**cnpq, faperj e fiocruz
Keywords: Ergosterol biosynthesis inhibitors; trypanosoma cruzi; chagas disease

TB097 - EFFICACY OF PLGA-PVP MICROPARTICLES LOADED CHALCONE NAT22 AGAINST CUTANEOUS LEISHMANIASIS

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Cutaneous leishmaniasis treatment is very painful and toxic. Aiming at a localized and single dose therapy, we have used biodegradable systems for sustained antileishmanial drug release in the skin. In the present study, the novel chalcone NAT22 whose chemical synthesis proved to be simpler than the former analogue chalcone CH8 was used as the active compound. The formulation comprised of poly-(lactide-co-glycolide) PLGA and poly-vinil pirrolidone (PVP) polymeric microparticles loaded with 10% of NAT22 (MpNAT22) was prepared by emulsion and solvent evaporation method. Empty microparticles (Mp) were prepared in the same way in the absence of NAT22. Both Mp and MpNAT22 were spherical measured a mean 13 µm diameter and with a smooth surface seen by FEG-SEM. *In vitro*, *L. amazonensis*-infected macrophages were treated with (0; 0,1; 1 and 10 µM) of NAT22 in the free or microparticulated forms for 96 h, when the nitric oxide and lactate dehydrogenase were measured in the supernatants and the cells stained for intracellular parasite quantitation. For *in vivo* studies, BALB/c mice were infected in the ear with *L. amazonensis.* After 7 days, mice were given a single subcutaneous injection with MpNAT22, free NAT22, empty microparticles or PBS alone. Alternatively, they were given 8 bi-weekly doses of free NAT22. On day 32 of infection, the animals were sacrificed and the parasite loads were quantified in the ear by Limiting Dilution assay. The results showed that MpNAT22 was effective in killing intracellular parasites, in a manner independent of macrophage NO, and led to no macrophage cytotoxicity. Likewise, a single dose with MpNAT22 was more effective than 8 doses of free NAT22 in reducing the parasite loads in the lesion. These findings show that formulation in PLGA-PVP microparticles increased chalcone NAT22 antileishmanial activity, and may have promoted sustained drug release in the lesion site, supporting its use for single localized treatment of cutaneous leishmaniasis. **Supported by:**CNPq
Keywords: Leishmania amazonensis; clalcone; localized treatment

TB098 - ANTILEISHMANIAL ACTIVITY OF C-3 FUNCTIONALIZED PHTALIDES.

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Leishmaniasis are parasitic diseases caused by different species of protozoa the genus *Leishmania*. Clinically, the disease range from cutaneous forms to visceral form, with the latter form can be lethal. The drugs currently used to treat these diseases have serious side effects associated. In the search for new drugs more active and safe, we evaluates the antileishmanial activity of a series isobenzofuran-1(3H)-ones (phtalides), class of heterocycles presenting a benzene ring fused to γ-lactone one, and which have activity against DNA topoisomerase type II, essential for KDNA replication trypanosomatids. This way, 31 phtalides containing alicyclic and aromatic groups as well as 2-aryl-2-oxoethyl groups attached to C-3 position of the nucleus isobenzofuranone were synthesized (compounds 1-31) and tested at different concentrations in *Leishmania amazonensis* and *L. infantum chagasi* promastigotes. At the end 72 hours treatment at 26°C, viability the parasites was determined fluorometrically by reduction of resazurin in resorufin of the alamarBlue, using wavelengths of 555 nm excitation and 585 nm emission. Compounds 1, 7, 10, 11, 12 and 19 were most promising with values of IC50 <13 µM. The most active compound, fitalida 12, has C-3 position functionalized with the group 2- (ortho-methylphenyl) -2-oxoethyl and showed IC 50 values of ~ 2.5 µM for both species. The antileishmanial activity exhibited by the compounds shows they can be considered attractive candidates for the treatment of leishmaniasis. The encouraging results indicate the continuity of evaluation studies this class of substances. **Supported by:**CNPq
Keywords: Antisleishmanial; isobenzofuran-1(3h)-ones; phtalides

TB099 - DIFFERENTIAL GEL ELECTROPHORESIS (DIGE) ANALYSIS OF THE ACTIVITY OF NAPHTHOIMIDAZOLES IN *TRYPANOSOMA CRUZI* BLOODSTREAM TRYPOMASTIGOTES

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Chagas' disease is an endemic illness in Latin America, caused by the protozoan *Trypanosoma cruzi*. Effects of available drugs are not satisfactory, therefore we are studying the activity of naphthoimidazoles (N1, N2 and N3) on all T. cruzi life stages. The action mechanisms of these compounds have been previously analyzed in epimastigotes by proteomic approaches, confirming the mitochondrion as the main target of the treatment. In this study, we investigated the effect of these compounds on bloodstream trypomastigotes using 2D-DIGE (two-dimensional difference gel electrophoresis). Differential protein spots were identified by MALDI TOF/TOF, being all MS/MS searched against Kinetoplastida and Mus musculus NCBI (RefSeq) database using Mascot and statistically validated by Scaffold (FDR 0.05). Proteotypic peptides of candidate proteins were loaded in Skyline and Selected Reaction Monitoring (SRM) method was build/refined after analysis in XEVO-TQ-S. Mass spectral libraries were built from MS/MS spectra of nLC-LTQ XL/Orbitrap analysis of tryptic peptides obtained from a control sample after in solution digestion. Preliminary data analyzing 2 transitions/peptide and 2 peptides/protein showed that label free SRM successfully generated quantification data for three identified DIGE spots: major paraflagellar rod protein and elongation factor 2 were found more abundant in N1 treatment group than control and a hypothetical protein was found less abundant in N1 and N2. SRM method parameters (RT window, collision energy and chromatography gradient) are being improved for further accurate quantification data acquisition. From 25 distinct proteins identified from DIGE spots, spectral library was successfully built for 16 of them, with at least two peptides/protein (80% of proteins with ≥ 6 peptides) and 5 transitions/peptide. Such additional information about T. cruzi cell biology could be crucial for the development of alternative drugs for this neglected tropical disease. **Supported by:**FAPERJ, CNPq and FIOCRUZ
Keywords: Trypanosoma cruzi; naphthoimidazoles; proteomics

TB100 - IN VITRO EVALUATION OF FARNESYL PYROPHOSPHATE SYNTHASE INHIBITORS IN PROTOZOAN.

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Sterols impart significant importance to the properties and structure of membrane. Protozoa such as Leishmania spp. synthesize ergosterol instead of cholesterol, while Giardia lamblia has to get cholesterol from the host. G. lamblia has only a few enzymes of sterol metabolism, such as the enzyme farnesyl diphosphate synthase (FPPS), the enzyme it is expressed in protozoa and in the mammalian. It is inhibited by biphosphanates and these compounds were recently shown to be active against some protozoan, but were not tested on L. infantum and G. lamblia. Thus, in this work we evaluated the activity of alendronate and its analogs in the proliferation of both organisms, performed the IC50, and evaluated the ultrastructural alterations. Our results showed that alendronate has better cytotoxicity on promastigotes of L. infantum, IC50 of 55 µM, when compared with trophozoites of G. lamblia which showed IC50 in the range of 294 µM. We also observed that risedronate presented higher anti proliferative activity than alendronate in L. infantum, IC50 in the range of 7.1 µM. Ibandronate, also displayed strong anti proliferative activity. The 5 fold increased in the IC50 for alendronate in Leishmania, compared to Giardia, can be related to structural differences in the catalytic site of the enzyme expressed in different organisms. It was shown, by X-ray diffraction, that the catalytic site of FPPS expressed in Leishmania differ from the enzyme expressed in mammalian host, which can affect the affinity of the enzyme for the substrate. The ultrastructural alterations found in promastigotes treated with alendronate were consistent with alterations in the ergosterol biosynthesis pathway. As evaluated by electron microscopy, it was observed disorganization of the protozoan membranes, displaying myelin figures and mitochondrial alteration. FPPP is a good metabolic target, and new analogs of bisphosphanate more specific to the enzyme expressed in Leishmania can be produced. **Supported by:**FUNDECT AND PIBIC FIOCRUZ
Keywords: Leishmania; giardia; bisphosphanates