TB1 - INHIBTION OF *TRYPANOSOMA CRUZI* HISTONE METHYLTRANSFERASES AND DEACETYLASES AFFECTS PARASITE PROLIFERATION, CELL CYCLE AND ULTRASTRUCTURE

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Histone methyltransferases (HMT) and deacetylases (HDAC) control chromatin remodeling and are essential enzymes for playing important roles in DNA replication, transcription, repair and in gene expression. Thereby, HMT and HDAC inhibitors have been used as chemotherapeutic agents, since they inhibit cell proliferation and promote cell cycle blockade. The aim of this study is to evaluate the role of Chaetocin (HMT inhibitor) and Trichostatin A (HDAC inhibitor) in *T. cruzi* proliferation, viability, ultrastructure and cell cycle. For this purpose, epimastigote forms were treated with 1, 5, 10 and 50 µM of both compounds until 96 hours of cultivation. Samples were collected after each 24 hours for counting on Neubauer's chamber, to MTS/PMS viability method, for processing to transmission (TEM) and scanning electron microscopy (SEM), and to flow cytometry. Our data showed that Chaetocin inhibited parasite proliferation (in 35%) and reduced cell viability (in 15%) resulting in IC50 of 2 µM for 48 hours of treatment. Flow cytometry analyses revealed that this compound led to cell cycle arrest after treatment with 5 µM for 72h. Trichostatin A was less efficient in diminishing cell growth, which was observed with 50 µM for 72h (IC50 of 50 µM). TEM analysis revealed that Chaetocin promoted an intense unpacking of nuclear heterochromatin and cytoplasmatic disorganization. Differently, Trichostatin A mainly affected reservosomes, which presented membrane rupture, although plasma membrane blebbing was also observed. SEM analysis showed that treatment with 50 µM Trichostatin A for 72h promoted cell roundness and size reduction, as well as the appearance of cell surface vesicles and cytoplasmatic projections at the posterior end of the cell body. Taking together, these data showed that the inhibition of HMT and HDAC alters different aspects of parasite cell biology, reinforcing the idea that such enzymes constitute potential targets for chemotherapy against *T. cruzi*. **Supported by:**CNPq e FAPERJ

TB2 - ANTI-*LEISHMANIA AMAZONENSIS* ACTIVITY OF α/β-AMYRIN DERIVATIVES

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Leishmaniasis is a public health problem in at least 98 countries, which therapy is based on pentavalent antimonials, pentamidine, amphotericin B and miltefosine. However, these drugs have limitations, such as toxicity, difficult administration and high cost. Important activities have been demonstrated for α/β-amyrin, such as anticancer, anti-inflammatory and bactericidal. Here we evaluated in vitro the anti-*Leishmania amazonensis* activity of α/β-amyrin derivatives with substitutions in the C3 and some with oxo radicals in C11 (8 derivatives were tested). We demonstrated that all derivatives inhibited promastigotes growth. Then we evaluated their toxicity for macrophages by mitochondrial activity and membrane integrity assays. Derivatives concentrations non-toxic for macrophages were selected for testing in intracellular amastigotes. Our results showed that 3,11-oxo-α/β-amyrone (OAMIRO) and 3-hydroxy-11-oxo-α/β-amyrone (OAMIRI), were the most active derivatives against amastigotes with IC_{50} of 54.6 and 38.2µM, respectively, after 24h treatment. To evaluate OAMIRO and OAMIRI capacity to modulate the microbicidal mechanisms of macrophages, nitric oxide (NO) and reactive oxygen species production were tested. Our data shows that at the IC_{50s} concentrations OAMIRO and OAMIRI decreased NO production in uninfected macrophages stimulated with IFN-γ however, the same treatment increased ROS production in infected macrophages stimulated with PMA. OAMIRO and OAMIRI alter the parasite cell cycle, increasing the percentage of cells in G0 phase. OAMIRO and OAMIRI affected the mitochondrial membrane potential, but only OAMIRI increase the number of annexin V^+ promastigotes, which suggests that the parasite is dying by incidental cellular death. Our results shows the anti-Leishmania effect of α/β-amyrin derivatives *in vitro*, which leads us to indicate OAMIRO and OAMIRI as promising candidates for future studies regarding leishmaniasis treatment. **Supported by:**CAPES, FAPERJ, CNPq.

TB3 - TRYPANOCIDAL AND LEISHMANICIDAL *IN VITRO* ACTIVITY OF DIAMINE DERIVATIVES

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The parasites *Trypanosoma cruzi* and *Leishmania* spp. cause Chagas disease and leishmaniasis, respectively. The treatment of both diseases are marked by low efficacy and several side effects. Diamines are amino-hydrocarbons that interfere in polyamines biosynthesis showing anti-parasitic activity. In this study we evaluate the anti-protozoal effect of 32 diamine derivatives. Culture forms of *L. amazonensis* (MHOM/BR/77/LOT0016) and *T. cruzi* (Y strain) were grown at 28^{o}C in Schneider and LIT + 10% FBS, respectively. Cells were harvested after three days of culture and 5 x 10^6 parasites/mL were incubated with different concentrations (1.25 - 100 µM) of diamines. For cytotoxicity assay 3T3 fibroblast cells (2 x 10^4 cells/well) were seeded on 96 well plates and incubated with 12.5 - 300 µM diamines in DMEM + 10% FBS. After 48h the toxic effect was evaluated by MTT. From 32 diamines evaluated 16 (50%) showed anti-protozoal activity. The IC_{50} anti-leishmanial effect varied from 4.91 to 95.53 µM. Compound GIB10 and GSC01 were at least four times more toxic to *L. amazonensis* than to mammalian cells. However, none of them were more active than amphotericin B (IC_{50}= 0,1 nM). Against *T. cruzi* epimastigotes six diamines (19%) displayed better results (IC_{50} < 10 µM) than benznidazole reference drug (IC_{50}= 12 µM). TAS08, GSC01 and GIB10 showed the highest selectivity index: 13.2, 11.6 and 9.3, respectively. Active diamines will be tested against intracellular amastigotes. Our data suggest that diamines derivatives may be promising molecules for new anti-leishmanial and trypanocidal drugs. **Supported by:**CNPq, UEPG, Fundação Araucária

TB4 - EFFICACY OF FUROSEMIDE ALONE OR IN COMBINATION WITH PENTOSTAM AGAINST MURINE VISCERAL LEISHMANIASIS

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Intramuscularly administered pentavalent antimonials such as Glucantime® and Pentostam® are the first line therapy against cutaneous leishmaniasis despite their toxicity and drug-resistance potential. In a previous study, we showed that Na^+-ATPase enzyme is a leishmanial putative drug target (De Almeida-Amaral, E.E et al., 2008). After determining that the Na^+-ATPase inhibitor furosemide, a long clinically approved diuretic drug, is orally effective against cutaneous leishmaniasis in mice, in this work we proposed to investigate if its efficacy is extended to visceral leishmaniasis and whether or not it can serve as a combination therapy with antimonials. *In vivo*, BALB/c mice were infected with *Leishmania infantum* (syn *L. chagasi*), and after 3 days of infection, they were i.p. treated with 20 or 7 mg/Kg of Pentostam® and/or orally treated with 50 mg/kg in a therapeutic regimen of 5 daily doses per week during 4 weeks. Controls received PBS. On day 32 day, the animals were sacrificed and the parasite loads were quantified in the spleens by LDA and in their imprinted livers by LDU units. The splenic amastigotes were expanded to promastigote forms in culture, and assessed for mRNA expression. For therapy toxicity, the sera were collected in the same day for alanine aminotransferase, aspartate aminotransferase and creatinine levels. The results showed that furosemide on its own effectively reduced both the spleen and liver parasite loads, and further enhanced Pentostam® efficacy. Parasites isolated from furosemide-treated mice expressed higher Na^+-ATPase than parasites from untreated (PBS) mice. No blood toxicity parameters were changed at the end of furosemide treatment. Combination therapy of oral furosemide and i.p. Pentostam® at sub-optimal doses was similarly effective as Pentostam® given in therapeutical doses. Together these results show that oral furosemide is potentially effective not only for the treatment of cutaneous but also visceral leishmaniasis. **Supported by:**CNPq

TB5 - COMPARATIVE EFFICACY OF AN AMASTIGOTE-SPECIFIC *LEISHMANIA INFANTUM* PROTEIN AND TWO SYNTHETIC PEPTIDES TO INDUCE PROTECTION AGAINST VISCERAL LEISHMANIASIS

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The present study aims to evaluate an amastigote-specific hypothetical *Leishmania* protein (LiHyp1) and two related synthetic peptides (LiPep1 and LiPep2) in an attempt to select new candidates for compose a vaccine against visceral leishmaniasis (VL). The immunogenicity and protective efficacy of each synthetic peptide or rLiHyp1 plus saponin was evaluated in BALB/c mice challenged with *Leishmania infantum*. Spleen cells of mice vaccinated with rLiHyp1, as well as LiPep1 and LiPep2 plus saponin, showed a high production of IFN-□, IL-12 and GM-CSF, in comparison to the control (saline and saponin) groups. The cellular response generated by immunogens was typically a Th1 response, with high levels of IFN-□, IL-12 and GM-CSF, besides of low levels of IL-4 and IL-10. Animals immunized 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, the protective efficaccy obtained in rLiHyp1 vaccinated mice was better than the observed in the LiPep1 and LiPep2 groups, evaluating the results obtained from parasite load. This study showed that an amastigote-specific *Leishmania* protein, LiHyp1, when combined with a Th1-type adjuvant, could be used as a protective agent to compose a vaccine against VL, and that the two immunogenic peptides obtained from this could also represent good vaccine candidates, since the vaccination protocols are improved. **Supported by:**FAPEMIG; CNPq

TB6 - PROSPECTIVE EVALUATION OF THE CELL ACTIVATION DEGREE AND IMMUNOSENESCENCE OF T LYMPHOCYTES IN VISCERAL LEISHMANIASIS/HIV-1 COINFECTED PATIENTS

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Chronic activation is a hallmark of HIV-1 infection and also of *Leishmania (Leishmania)infantum*. We have previously demonstrated that leishmaniasis is a cofactor to the heightened activation status in HIV-1 co-infected patients despite antiretroviral therapy (ART) and anti-*Leishmania* therapy. Such activation has been associated with an exhaustion of immune system and may contribute to the frequent relapses in visceral leishmaniasis (VL) patients. In this context, we evaluated the influence of *L. infantum* infection in the degree of quantitative and qualitative impairment of T lymphocytes through the analysis of the activation of immune system and immunosenescence status. Thirteen VL/HIV patients were followed from the active phase of VL up to 12 months post-treatment (mpt). VL/HIV were under ART and in use of secondary prophylaxis with B amphotericin (50 mg biweekly), since the end of VL therapy. Subjects with VL only (n=6), cases with HIV-1 only (n=17) and healthy subjects (n=12) were included. T-cells counts, *ex vivo* expression of molecules associated with cell activation (CD38/HLA-DR) and replicative senescence (CD57/CD27) were evaluated. CD4+T cells were under 350 cells/mm^3 in 9 out of 13 patients in all visits, and the number of cells was negatively correlated with the levels of $CD8^+$T cells activation (r=-0.40). The viral load remained low or undetectable up to 12mpt without influencing $CD38^+$/$HLA\text{-}DR^+$ levels. Finally, coinfected patients showed high percentages of senescent $CD4^+$T and $CD8^+$T cells, with a trend to higher levels at 12 mpt, especially in those who already presented previous episodes of VL. The results suggest that pathogenic mechanisms associated with VL, as chronic activation, can worsen the immunosuppression of aids. Also, the immunosenescence degree may reflect a chronically activated immune system in VL/HIV patients. Finally, the use of secondary prophylaxis for VL does not seem to improve the immune deficiency in part of the coinfected patients. **Supported by:**CNPq/FAPERJ/IOC-FIOCRUZ

TB7 - 4-AMINOPYRIDYL-BASED CYP51 INHIBITORS AS ANTI-*TRYPANOSOMA CRUZI* DRUG LEADS WITH IMPROVED PHARMACOKINETIC PROFILE AND IN VIVO POTENCY

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CYP51 is a P450 enzyme involved in the biosynthesis of the sterol components of eukaryotic cell membranes. CYP51 inhibitors have been developed to treat infections caused by fungi, and more recently the protozoan parasite *Trypanosoma cruzi*, the causative agent of Chagas disease. To specifically optimize drug candidates for *T. cruzi* CYP51 (TcCYP51), we explored the structure-activity relationship (SAR) of a N-indolyl-oxopyridinyl-4-aminopropanyl-based scaffold originally identified in a target-based screen. This scaffold evolved via medicinal chemistry to yield orally bioavailable leads with potent anti-*T. cruzi* activity in vivo. Using an animal model of infection with a transgenic *T. cruzi* Y luc strain expressing firefly luciferase, we ranked the biaryl and N-arylpiperazine analogs by oral bioavailability and potency. The drug-target complexes for both scaffold variants were characterized by x-ray structure analysis. Optimization of both binding mode and pharmacokinetic properties of these compounds led to potent inhibitors against experimental *T. cruzi* infection. **Supported by:**CNPq, FIOCRUZ, NIH

TB8 - *LEISHMANIA CHAGASI* KILLING BY INFECTED MACROPHAGES IS IMPROVE BY SOLUBLE CD40 LIGAND PRESENT IN SERA OF SUBJECTS EXPOSED TO *LEISHMANIA CHAGASI* INFECTION

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Introduction: CD40L is a type II membrane glycoprotein predominantly expressed by activated T cells, B cells, myeloid cells and platelets. Soluble derivatives of this molecule (sCD40L) are cleaved /shed from activated cells, and retain the ability to bind and activate CD40. We have reported that elevated levels of sCD40L are associated to clinical resolution of visceral leishmaniasis, showed by a negative correlation with spleen size and parasite loads, and with resistance to infection, since endemic at risk control subjects present high levels of this molecule. The objective this study is to investigate if this sCD40L present in sera of infected subjects is functional and is able to activate the microbicidal mechanisms of L.chagasi infected macrophages. Methods and Results: Monocytes-derived macrophages from normal human donors were infected with L. chagasi promastigotes and the infected cells were incubated for 72h in RPMI containg 20% human serum with high levels of sCD40L without or with 10µg/ml anti-CD40L, or IgG2b isotype control antibody. The supernatants were collected to cytokine measurement by Luminex assay, and cells were stained with Panotico fast to evaluate the infection levels.. The addition of serum sCD40L enhanced parasite killing and its neutralization with anti-CD40L increased infection. Additionally, we observed a decrease of IL-12, IL-15, IL-23, IL-27 and IL1β levels, compared with the sCD40L serum plus isotype treated cells. Negative associations were also observed between the levels of these cytokines in the supernatant of culture and the ratio of infection and number of intracellular amastigotes, conferred by serum sCD40L. Conclusion: sCD40L present in sera from L. chagasi infected subjects is functional and is able to improve the microbicidal activities of L.chagasi infected macrophages by inducing inflammatory cytokines. These findings suggest that sCD40L molecule is a potential molecule for immunotherapy in human visceral leishmaniasis. **Supported by:**CNPq UNIVERSAL Nº14/2011, PPP/ FAPITEC/CNPq Nº04/2011 PRONEX -FAPITEC/SE/ FUNTEC/ CNPq Nº14/2011

TB9 - NANOSTRUCTURED SYSTEMS OF QUERCETIN FOR ORAL TREATMENT OF MURINE VISCERAL LEISHMANIASIS

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Current treatment of leishmaniasis is based on drugs with severe side effects and/or invasive routes of administration, creating a need for alternatives. Antileishmanial activity of quercetin (Qc) has been previously demonstrated by our group using a cutaneous murine model of disease. However, the low water solubility and reduced bioavalability of Qc constitute a limitation to its use by the oral route. With the aim to potentiate Qc antileishmanial activity, it was formulated in three nanostructured systems: hydroxypropyl-β-cyclodextrin (HPβCD); lipid core nanocapsules of poly-epsilon-caprolactone (LNC); and nanospheres of poly-methacrylate (PMA). Oral efficacy of the formulations was evaluated using a murine visceral leishmaniasis (LVM) model, where BALB/c mice were infected with promastigotes of L. infantum in the caudal vein. At day 1 or 7 after infection, mice were treated daily with the formulations at 2mg/kg of Qc by gastric gavage, for 28 consecutive days. At the end of treatment, the animals were euthanized for quantification of spleen and liver parasite loads by limited dilution assay and dosage of splenic nitric oxide levels. ALT, AST and creatinine serum levels were dosed for evaluation of treatment toxicity. Treatment with Qc in HPβCD and LNC increased the drug oral efficacy, resulting in an activity similar to free Qc at a dosage 8x higher and without changing its toxicological safety. However, PMA particles did not potentiate the efficacy of Qc. In all the therapeutic regimes tested the spleen parasite load was shown to negatively correlate with local levels of NO. The LNC formulation of Qc behaved differently from the others, inducing lower levels of NO, which led to values similar to those found in non infected mice, showing a beneficial tendency to restore homeostasis. This study shows the optimization of Qc efficacy by complexation in HPβCD and LNC. **Supported by:**FCT/ CNPq / CAPES

TB10 - SCREENING OF NOVEL COMPOUNDS FOR CHAGAS DISEASE TREATMENT : *IN VITRO* AND *IN VIVO* ANTI- *TRYPANOSOMA CRUZI* ACTIVITY OF NATURAL PRODUCTS

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Chagas disease (CD) afflicts 8 to 11 million people in Latin America. The two major challenges for drug discovery and development to CD are the lack of appropriate in vitro and in vivo screening protocols and the little interest of the Big Pharmas. The Program of Technological Development for Health (PDTIS) of the Oswaldo Cruz Foundation (FIOCRUZ) has stimulated the building and consolidation of Technological Platforms (TP). With the aim to detect bioactive compounds against Trypanosoma cruzi, the Chagas Disease TP (PlaBio Tc) tested them on *T. cruzi* tissue culture forms, using the Tulahuen strain expressing the *Escherichia coli* beta-galactosidase as reporter gene. Briefly, trypomastigotes were left for 2h to infect L929 fibroblasts seeded in tissue culture micro plates. After 48h, the medium was discarded and replaced by fresh medium and test compounds. After 7 days, chlorophenol red beta-D-galactopyranoside was added to the plates, incubated overnight and the absorbance measured at 570 nm. Benznidazole at its IC50 (3.8 µM) was used as positive control. The results are expressed as percentage of *T. cruzi* growth inhibition. The PlaBio Tc investigated the trypanocidal activity of approximately 14,500 samples of natural products, compounds and drugs from different providers. In collaboration with the Bioprospecting TP – FIOCRUZ, 14,000 plant and fungi extracts were tested. Eleven active extracts were moderately selective (Selectivity Index - SI ranging from 6 to 40) and three were highly selective (SI > 50). The in vitro bioassay-guided fractionation of two moderately selective was performed. Unfortunately, the most selective fraction was inactive *in vivo* (extract with SI =18) or the fractions showed low selectivity (extract with SI = 40). The bioassay-guided fractionation of the three highly selective extracts is ongoing. The most active compounds will be tested *in vivo* assay. Altogether the results are encouraging to find a new lead compound for treatment of CD. **Supported by:**PDTIS/FIOCRUZ, FAPEMIG and CNPq

TB11 - GENERATION OF LUCIFERASE-EXPRESSING *LEISHMANIA INFANTUM CHAGASI*: ASSESSING MILTEFOSINE EFFICACY IN INFECTED HAMSTERS THROUGH INTRAVITAL MICROSCOPY

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Background: Luciferase has been validated as a quantitative tool for the determination of parasite burden in experimental leishmaniasis. However, there are no reports on luciferase detection in the model of progressive visceral leishmaniasis in hamsters. Objectives: To generate recombinant *Leishmania infantum chagasi* lines expressing the luciferase gene (Lc-LUC); to characterize Lc-LUC for biological properties *in vitro* as compared with the wild type line (Lc-WT) and to evaluate miltefosine (MTF) effectiveness in Lc-LUC infected hamsters. Methods: Mutants containing the *luc2P* gene integrated into the ribosomal DNA were obtained and integration was confirmed by PCR amplification. Lc-LUC and Lc-WT promastigotes' growth curves and susceptibility of intracellular amastigotes to Glucantime (Sb^5) and MTF were compared. The effectiveness of Sb^5 and MTF was determined in Lc-LUC infected hamsters through intravital microscopy and compared to the Leishman Donovan Units (LDU). Parasite load was quantified by bioluminescence before treatment initiation and animals were divided into homogeneous experimental groups. Treated groups were compared with untreated group and analyzed for statistical significance. Results: Bioluminescence generated by Lc-LUC was shown to correlate with the number of promastigotes. Lc-LUC and Lc-WT presented similar growth curves and both were equally sensitive to Sb^5 and MTF. LDU and bioluminescence results were comparable. MTF was effective in the treatment of Lc-LUC-infected hamsters, as demonstrated by the reduction in parasite burden in a dose-dependent manner and by prolongation of animal survival. Conclusions: The use of Lc-LUC is a reliable alternative for parasite burden quantification in hamsters. This tool has several advantages such as the possibility to estimate parasite load before drug treatment, allowing distribution of animals in homogeneous groups. Besides, we presented useful information about MTF efficacy against *L. infantum chagasi*. **Supported by:**FAPESP

TB12 - HOST CHOLESTEROL INFLUENCE THE ACTIVITY OF STEROL BIOSYNTHESIS INHIBITORS IN LEISHMANIA AMAZONENSIS

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Trypanosomes synthesize ergosterol-derived sterols, but a significant percentage of exogenous cholesterol is found in Leishmania spp, suggesting a biological role for this molecule. We previously demonstrated that the pharmacological inhibition of sterol biosynthesis in Leishmania amazonensis promastigotes leads to an increase in the uptake of LDL particles and in the content of cholesterol. We also noted that the parasites were rendered more susceptible to ergosterol inhibitors in the absence of exogenous cholesterol. This work aims to study the importance of cholesterol from the host cell to Leishmania amazonensis amastigotes, evaluating the potential of this system as a possible drug target. To assess the availability of LDL to intracellular amastigotes, murine peritoneal macrophages were infected with L. amazonensis-GFP and incubated with LDL-AlexaFluor 594 or infected with L. amazonensis and incubated with LDL-gold. We observed the presence of LDL particles inside of the parasitophorous vacuole, in close proximity to amastigotes, demonstrating that parasites have access to LDL and thus may be getting their cholesterol. The activity of inhibitors of ergosterol was evaluated in intracellular amastigotes in association with inhibitors of transport of LDL/cholesterol (LBqT01, progesterone and imipramine) or in medium cholesterol-free. Ketoconazole and miconazole showed to be more potent against intracellular amastigotes when combined with inhibitors of cholesterol transport or in the absence of exogenous cholesterol. Altogether, these results suggest that cholesterol may play an important role in counteracting the activity of inhibitors of ergosterol and the inhibition of this pathway could enable the clinical usage of azoles. **Supported by:**CAPES, PAPES/FIOCRUZ, FAPERJ

TB13 - EXPLOITING *TRYPANOSOMA CRUZI* P21 BIOLOGICAL ACTIVITIES

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Introduction: *Trypanosoma cruzi* is a protozoan that secretes in all developmental stages a protein called P21. Our previous studies using the recombinant form P21 of *T. cruzi* showed that this protein is involved in host cell invasion. Also, rP21 enhanced macrophage phagocytosis and actin polymerization by binding to CXCR4 receptor. This ability turned our attention to a possible activity of chemotaxis. In this regard, seen in the previous experiment rP21 attracts inflammatory cells in vitro and in vivo assays. Thus, in this paper we aim to explore the biological activities of p21 in chronic inflammation model. Methods: C57/BL6 mice were used for implantation of spongy matrix. The hard sponge was introduced in the interscapular region of dorsal midline incision. And every 72 hours were injected into the sponge different concentrations of rP21 (10, 40 and 100µg/mL). The animals were euthanized and the implants were removed, weighed and processed for histological and biochemical studies. Results: the inflammatory score observed on tissues from polystyrene-induced inflammation treated with rP21 was higher than that observed on the non-treated ones. Furthermore, we observed, high levels of N-acetylglucosaminidase in rP21 treated implants, indicating that rP21 not only recruit but also activates macrophages. On the other hand, only high dose of rP21 showed some impact on myeloperoxidase expression. Biochemical analysis showed that this protein does not have any impact on collagen deposit. However, we observed a significant decrease on the hemoglobin content, suggesting a potential anti-angiogenic role for rP21. In order to confirm this hypothesis, we will measure the number of blood vessels on histological preparations. Conclusion: Therefore, the rP21 protein showed chemotactic and anti-angiogenic activity and can activates macrophages. So this protein might be a potential target for the development of P21 antagonist compounds for treatment of Chagas's disease. **Supported by:**FAPEMIG/ CAPES/ CNPq

TB14 - EPITOPE MAPPING OF THE HSP83.1 PROTEIN OF *LEISHMANIA BRAZILIENSIS* DISCLOSES NOVEL TARGETS FOR THE IMMUNODIAGNOSIS OF TEGUMENTARY AND VISCERAL CLINICAL FORMS OF LEISHMANIASIS

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The gold standard serological diagnostic methods focus on antigens that elicit a strong humoral immune response that is specific to a certain pathogen. In this study, we used bioinformatics approaches to identify linear B-cell epitopes that are conserved among *Leishmania* species but are divergent from the host species *Homo sapiens* and *Canis familiaris* and from *Trypanosoma cruzi*, the parasite that causes Chagas disease, to select potential targets for the immunodiagnosis of leishmaniasis. Using these criteria, we selected heat shock protein 83.1 of *Leishmania braziliensis* for this study. We predicted three linear B-cell epitopes in its sequence. These peptides and the recombinant heat-shock protein 83.1 (rHSP83.1) were tested in ELISAs against sera from patients with tegumentary (TL) and visceral leishmaniasis (VL) and from dogs infected with *Leishmania infantum* (CVL). Our data showed that rHSP83.1 is a promising target for the diagnosis of TL. We also identified specific epitopes derived from HSP83.1 that could be used for the diagnosis of human TL (peptide-3), the diagnosis of both human and canine VL (peptides-1 and 3), and for the diagnosis of all TL, VL and CVL clinical manifestations (peptide-3). ROC curves confirmed the superior performance of rHSP83.1 and peptides 1 and 3 in comparison with the soluble *Leishmania braziliensis* antigen and the reference test kit for the diagnosis of CVL in Brazil (EIE-LVC Kit/Bio-Manguinhos/FIOCRUZ). Our study thus provides proof-of-principle evidence supporting the feasibility of using bioinformatics to identify novel targets for the immunodiagnosis of parasitic diseases using proteins that are highly conserved throughout evolution.**Supported by:**FAPEMIG, CNPq, INCTV, CAPES

TB15 - *IN VIVO* EFFECTS OF BENZNIDAZOLE IN COMBINATION WITH E1224 AGAINST *TRYPANOSOMA CRUZI* INFECTION

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Chagas disease remains a challenging infection due to the unavailability of safe and efficacious drugs. Combined therapy is envisioned as an adequate approach, since it may improve treatment efficacy whilst decreasing toxicity and the likelihood of resistance development. In this study, we evaluated the effect of treatment with benznidazole (Bz) when combined with E1224 (pro-drug of ravuconazole) in experimental acute murine infection. Female Swiss mice were infected with *T. cruzi* Colombian strain, highly resistant to Bz. Oral treatment of infected animals was started at the 4th day post-inoculation, at the doses of 37.5 or 50 mg/kg/day (mpk) of E1224 and 75 or 100 mpk of Bz administered individually or in combination. Cure assessment was based on fresh blood examination during and up to 60 days post-treatment followed by blood real-time PCR assay (30 and 180 days post-treatment). Our results demonstrated that Bz/E1224 combinations were well tolerated and all treatments, in monotherapy or combinations, prevented the death of infected animals, while in the control group, the mortality was 80%. The drugs were very effective in suppressing parasitemia during the treatment period. However, after the end of the treatment, parasitological and PCR assays indicated no cure among animals treated with different doses of E1224 or Bz individually. Interestingly, the combination therapy using E1224 at 50mpk plus Bz100 mpk and E1224 at 37.5mpk plus Bz 75mpk induced a 100% and 40% cure rate, respectively. Furthermore, the animals considered cured had levels of IgG anti- *T. cruzi* in serum significantly lower than those of the untreated animals, and similar to healthy mice. Our results demonstrated a positive interaction between E1224 and Bz in the treatment of T.cruzi murine infection. Additionally, this study expands the preclinical data on drug combinations and provides the basis for further studies, since anti-*T.cruzi* chemotherapy is moving towards multidrug treatment regimens. **Supported by:**FAPEMIG, CNPq, CAPES, DNDi

TB16 - MOLECULAR IDENTIFICATION OF A REGION ASSOCIATED WITH RESISTANCE IN PFOR GENE IN STRAINS OF AXENIC *GIARDIA LAMBLIA* EXPOSED TO NITROIMIDAZOLE

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Giardiasis is caused by an intestinal protozoan called *Giardia lamblia*. Nitroimidazoles, especially the metronidazole and secnidazol, are the principal drugs used for giardiasis treatment. Most cases progresses to cure but it is still estimated that 10% of infections present drug resistance. In vitro resistance of *G. lamblia* was already reported for metronidazole, but none is known with respect secnidazol and the genomic alterations caused by this family of drugs. Our objective was to identify whether secnidazol is able to induce resistance in strains of *G. lamblia* axenic, and which changes in the sites of nucleotide and amino acid could favor this resistance. To this end, genotype A of the *G. lamblia* trophozoites (clone C6 WB strain ATCC50803) were axenically cultured into seven experimental groups (G) as follows: G1control group, and groups 2, 3, 4, 5, 6, respectively exposed to 3, 6, 12, 24, 36 mg/L of secnidazol, and the group 7 exposed to increasing concentrations. The DNA was extracted with DNAzol, amplified and sequenced for the gene pfor, currently associated resistance since it envolved in the activation drug pathway, and the β-giardin gen was used as control. Dendrograms were constructed based on the nucleotide and amino acid sequences obtained, using WB strain of GenBank as reference. The analysis of the sequences showed that the number of changes in pfor gen was drug dose dependent. In accordance, pfor sequences from G1 clones, not exposed to the drug, were similar to the reference strain WB, while in the presence of higher doses of secnidazol, the clones were genetically more distant (G4 to G7 in this order). Cellular alterations such as impairment of the piriform shape, flagellar beating and adhesion ability were observed. Further, about 90% of the nucleotide and amino acid alterations were located in sites 3Fe-4S and 4Fe-4S binding, suggesting that these regions are propably more susceptible to changes due to drug pressure. **Supported by:**FAPERJ, CAPES, POM/IOC

TB17 - NEW NITRO COMPOUNDS AGAINST CHAGAS DISEASE: THE EFFECT OF THE PBN DERIVATES UPON *TRYPANOSOMA CRUZI*

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Chagas disease has become an increasingly globalized public health issue in the 21st century and the available drugs are not efficient against the chronic form of the disease. Previous studies showed that the nitrone (alpha-phenyl-N-tert-butyl nitrone-PBN) protected infected rats against chagasic cardiopathology. Therefore, we investigated the effects of two PBN derivates (LQB303 and LQB304) against infective and proliferative forms of *T. cruzi*. Initially, we tested the effects of the nitrones against metacyclic trypomastigotes and bloodstream trypomastigotes. MTT analysis demonstrated a great decrease in the viability of the parasites after 24h, indicating a great tripanocidal effect. Afterwards, peritoneal macrophages were infected with bloodstream trypomastigotes in the presence of LBQ 303 and the number of amastigotes was quantified after 48h. Indeed, during infection, the number of amastigotes was significantly reduced in infected cells treated with LQB 303. In order to assess the toxicity of LQB 303 on mammalian cells, peritoneal macrophages were incubated for 48h and the cell viability was evaluated by MTT. Interestingly, we observed that, even at high concentrations, LQB 303 did not reduce the macrophages viability. Additionally, epimastigotes were incubated with LQB303 or LQB304 and quantified by after 48h. We observed that even in low nitrones concentrations, epimastigotes showed a significant decrease in *in vitro* proliferation. Finally, epimastigotes and metacyclic trypomastigotes were exposed to LQB303 and the permeability of the plasma membrane to PI was assessed by flow cytometry. We observed a great increase in PI fluorescence and a decrease in cell size by FSC analysis, suggesting the loss of membrane integrity of both forms tested. In conclusion, taken together our results suggest that the PBN derivates, LQB303 and LQB304, presented considerable toxicity against the parasite, impairing the establishment of several developmental forms of *T. cruzi.* **Supported by:**INCT-EM, PIBIC-CNPq, FAPERJ

TB18 - ROLE OF DIETARY RETINOL ON THE LAAG ORAL VACCINE EFFICACY AGAINST *LEISHMANIA AMAZONENSIS* INFECTION

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Leishmaniases are diseases that have different clinical manifestations, depending on the parasite specie involved and the host immune status. Despite the intense effort in finding a safe and effective vaccine against leishmaniasis, none have yet been approved for human use. Our group has explored a new vaccination strategy through the mucosa to induce tolerance to parasite antigens related to the disease. We have already demonstrated that oral immunization with total antigen of *L. amazonensis* promastigotes (LaAg) induces protection in mice against cutaneous leishmaniasis. Retinoic acid, a metabolite of retinol (vitamin A) is a cofactor required for generation of regulatory T cells in the intestine, which have been associated in the oral tolerance. In this study, the influence of dietary retinol in *L. amazonensis* infection, as well as its role in the efficacy of the oral vaccine LaAg was evaluated. Thus, BALB/c mice subjected to dietary retinol restriction (Vit A-) or supplementation (Vit A+) were vaccinated with two doses of LaAg (100 µg protein/dose) with an interval of 7 days between them. One week after immunization, mice were subcutaneously infected in the footpad with $2x10^6$ *L. amazonensis* promastigotes and the lesion development was monitored for 60 days, when the parasite burden and cytokine profile in the infection site were evaluated. Vit A+ vaccinated group showed lower lesions and parasite load compared to the PBS group, which was not observed in the Vit A- animals. Moreover, Vit A+ animals vaccinated showed higher IL10 and IL12 levels and reduced IL4 levels in the lesions. These results suggest that dietary retinol is important for the LaAg oral vaccine efficacy. **Supported by:**CAPES, CNPq, FAPERJ

TB19 - PROTEIN INTERACTION WITHIN TRYPANOSOMA BRUCEI MAP-KINASE FAMILY

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Protein kinases are one of the largest protein families in eukaryotes, and have an important role in the intracellular control regulation and signal transduction. Among protein kinases, the mitogen activated protein kinase (MAPK) family participates in central cellular processes through phosphorylation cascades. These proteins coordinately regulate cell proliferation, differentiation, motility and survival. Despite the relevance of MAPKs for the cell, in trypanosomatids little is known about them. Therefore, our group has studied the MAPK signaling pathway in Trypanosoma brucei, a model organism that causes impact to human and animal health. We are studying protein-protein interactions among the forty members of MAPK signaling cascade, using yeast two hybrid system. The methodology consisted of gene sequence amplification by PCR, followed by recombination into pDONR 221® plasmid. The entry clones were recombined with the destination plasmids pAD (pDEST22) and pBD (pDEST32). The generated destination clones were transfected into both MAV103/203 and Y8800/8930 strains of Saccharomyces cerevisiae. After the mating and selection of diploids, cells were cultivated for ten days in YNB -URA (MAV), -HIS/100 mM 3 AT (MAV and Y), -HIS/1 mM 3 AT (Y) and -URA (Y) for phenotypic selection. Between the observed results, a hub was detected, comprising interactions between a MAP3K and members from three levels of the MAPK cascade. Interactions among MAPK and MAP2K, MAP3K and MAP4K, or between non adjacent levels, for example MAPK and MAP3K were also observed. Additionally, interactions described in Intact and Biogrid databases among MAPK ortologues and their partners - with ortologues in T. brucei - from eight model organisms were analyzed together. There was no overlap between the results here presented and interactions described in the analyzed databases. Altogether, results here described represent a contribution towards a better characterization of MAPK cascade in T. brucei. **Supported by:** Instituto Carlos Chagas/Fiocruz-PR, Araucaria Foundation, Program for Technological Development in

TB20 - ENCAPSULATION IN LIPID CORE NANOCAPSULES (LNC) IMPROVES THE TOPICAL EFFICACY OF CHALCONE CH8 IN CUTANEOUS LEISHMANIASIS

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Topical treatment of cutaneous leishmaniasis (CL) is desirable because it may be self-applied, promote higher drug concentration in the skin and prevent systemic side effects. However, the high lipophylicity of the antileishmanial chalcone CH8 hampers its topical use in the free form. Aiming at facilitating CH8 skin permeation, the drug was entrapped in lipid-core poly-€-caprolactone nanocapsules (LNC) before testing for topical efficacy in BALB/c mice infected with *L. amazonensis* GFP. For that, fluorescent lipid-core poly-€-caprolactone nanocapsules entrapping CH8 (LNC^F-CH8) were constructed so that their *in vitro* internalization by promastigotes and infected macrophages could be measured by fluorescence microscopy and flow cytometry. After that, we measured their *in vivo* skin absorption by intravital fluorescence microscopy. Finally, the *in vivo* antileishmanial efficacy of non-fluorescent LNC-CH8 was tested by topical route. The formulations were prepared by interfacial deposition of preformed polymers and in LNC^F-CH8 Rhodamine B was covalently bound to the polymer. *In vitro* studies of LNC^F-CH8 demonstrated that both promastigotes and macrophages increasingly internalized LNC^F-CH8 along with time. In infected macrophages LNC^F-CH8 localized throughout the cytoplasm and inside the parasitophorous vacuole. *In vivo*, LNC^F-CH8 were seen to permeate into the skin after topical application. Finally, therapy efficacy was evaluated in *L. amazonensis* GFP- infected ears of BALB/c mice after 15 doses from days 7 to 28 post infection. A significant reduction of parasite burden was observed in mice treated with LNC-CH8 compared to free CH8 and empty LNC. These results indicated that the encapsulation of CH8 in LNC promoted internalization by promastigotes, targeting to amastigotes, permeation into the skin and higher topical efficacy *in vivo* than free CH8. In conclusion, this study indicates the new formulation LNC-CH8 as a potential candidate for CL treatment by topical route. **Supported by:**CNPq

TB21 - THE EFFECT OF THE 4-AMINOQUINOLINE HYBRID AGAINST *LEISHMANIA AMAZONENSIS* ARE MEDIATED BY MITOCHONDRIAL DYSFUNCTION.

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The quinoline derivatives have been extensively used in the development of antiprotozoal drugs, typically as antiplasmodial and antileishmanial compounds. In this study, we evaluated the effect of the 4-aminoquinoline hybrid, named RMP112, against promastigote and amastigote forms of *Leishmania amazonensis* and its leishmanicidal action mechanism. Antipromastigote activity and cytotoxicity in peritoneal macrophages were determined using the tetrazolium-dye (MTT) colorimetric method after 72 h of treatment. The antiamastigote activity was evaluated in macrophages infected with *L. amazonensis* transfected with the gene of green fluorescent protein (GFP) and determined by fluorescence intensity after 72 h of treatment. The compound exhibited a strong leishmanicidal activity with an IC50 of 5.9 and 2.4 µg/mL (promastigotes and amastigotes, respectively), with low toxicity for macrophages (selectivity index = 16.7). To investigate mitochondrial function damage, promastigotes were treated with 24.0 µg/mL RMP112 for 24 h, incubated with Rhodamine 123 and we observed a marked decrease in mitochondrial membrane potential (ΔΨm) of 97.9%. Using the fluorescent probe JC-1, the results showed that 24.0 µg/mL RMP 112 induced a 74.0% decrease in membrane potential. Induction of oxidative stress was also observed by the increase of reactive oxygen species (ROS) levels by 66.9% in promastigotes treated with 31.0 µg/mL RMP112 and incubated with H_2DCFDA fluorescent dye. The compound did not induced alteration on promastigotes plasma membrane permeability demonstrated by propidium iodide (PI) labeling. Further studies are necessary to better understand if the mode action of RMP112 compound is related to apoptosis or another death of *Leishmania*. **Supported by:**CAPES, FAPEMIG, CNPq, UFJF.

TB22 - *IN VITRO* EFFECT OF AMAZONIAN LAURACEAE EXTRACTS AGAINST *TRYPANOSOMA CRUZI*

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Chagas disease, caused by protozoan *Trypanosoma cruzi*, affects 15 million people throughout the American continent. Chemotherapy for this illness is ineffective and side effects are commonly related. Also, development of drugs to treat Chagas is a difficult task and is still a great epidemiological problem. For this reason, search for new active compounds against protozoan parasites has increased; including research for verify the natural products activities. Lauraceae species produce substances with a huge variety of biological activities, such as antiviral, antibacterial, antioxidant and antitumor. Such as *Nectandra* species, that inhibit growth of *T. cruzi* epimatigote forms. At this study, antiparasitary activity of ethanolic extracts from leaves and stems obtained from twelve species of Amazonian Lauraceae were evaluated. *T. cruzi* trypomastigote were obtained from the supernatants of previously infected LLCMK2 cells monolayer and epimastigotes were cultivated in LIT medium. The Lauraceae extracts were diluted in DMSO and DMEM for final concentration (1, 5, 10, 50, 100, 500 and 1000 µg/mL), and incubated for 24 h at 37 °C, the viability was evaluated using the Pizzi-Brener method for trypomastigote. Epimastigote were incubated for 96 h at 28 ºC and IC_{50} calculated using Neubauer chamber. All extracts affect ability of *T. cruzi* at major concentrations (1000 and 500 µg/mL). The extracts with the highest activities against trypomastigote forms (EC_{50} < 10 µg/mL) were obtained from *Aniba panurensis* (stems and leaves), *Aniba parviflora* (leaves), *Mezilaurus duckei* (stems), *Rhodostemonodapne peneia* (stems), *Paraia bracteata* (stems and leaves), *Ocotea leucoxylon* (stems) and *Licaria martiniana* (stems). In addition, *A. panurensis* (stems), *A. parviflora* (leaves) and *P. bracteata* (leaves) showed the best results (IC50 <50 µg/mL) against epimastigote forms. These high activities of extracts could be helpful in the search of new active compounds against *T. cruzi.* **Supported by:**CNPq/FAPEAM/CAPEs

TB23 - THE POTENCIAL OF BENZNIDAZOLE TREATMENT ON DIASTOLIC DYSFUNCTION AND CARDIAC DAMAGE IN CHRONIC CANINE CHAGAS DISEASE

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This study was designated to evaluate the effect of benznidazole (Bz) treatment on myocardial damages and left ventricular diastolic dysfunction using dogs infected with Berenice-78 strain as experimental model. A total of 30 dogs were divided into three groups: 11 infected and treated with Bz during the chronic phase, 11 infected but untreated, and 8 non-infected/healthy. The trypanocidal efficacy was measured by parasite kDNA in blood and heart tissue. The effect of Bz in ameliorating the cardiac diastolic function and heart damages were evaluated by inflammation and collagen quantification (right atrium) and echodopplercardiogram. The potent suppression of parasitemia induced by Bz-treatment was associated with negative results in the blood and tissue PCR performed in the first month post-treatment in 82% of treated animals compared with 36% of untreated dogs. An increase in the frequency of positive results in blood and tissue-PCR was detected 12^{th} months post-treatment. Positive results were observed in 60% of blood and 80% of tissue samples taken from Bz-treated dogs. There were no significant differences among healthy and the infected animals, treated or not, with regards the intensity of cardiac lesions (inflammation and fibrosis) and diastolic function at the first month post-treatment. In contrast, significant alterations in the intensity of cardiac lesions and echocardiografic parameter related to diastolic function were detected in all infected dogs 12 months post-treatment. Infected dogs exhibited higher intensity of cardiac lesions and smaller values of early tissue septal velocity (E' SIV) than those observed in healthy dogs. Additionally, the treated animals showed a lifting of E/E' septal tissue filling pressure compared to their basal values and to those observed in healthy dogs. Thus, the temporary early suppression of *T. cruzi* induced by Bz treatment was not able to prevent myocardial lesions and diastolic dysfunction long time post-treatment. **Supported by:**CNPq, FAPEMIG, UFOP

TB24 - COMPARATIVE STUDY OF THE THIOSEMICARBAZONES AND SEMICARBAZONES ACTIVITIES IN THE EVOLUTIONARY FORMS OF *LEISHMANIA BRAZILIENSIS* AND *LEISHMANIA INFANTUM*

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Nowadays, the treatment of the leishmaniasis is primarily based in the use of pentavalent antimony; however they have exacerbated effects, demanding the development of new therapeutic drugs. The thiosemicarbazones and semicarbazones present a wide pharmacological profile including antibacterial activities, antitumor, antimalarial, among others. The objective of this work is to measure the in vitro activity of synthetic compounds of the class of thiosemicarbazones and semicarbazones, against promastigotes, axenic and intracellular amastigotes of *L.braziliensis* and *L. infantum (syn chagasi).* Promastigotes were maintained at 26ºC with weekly passages in Schneider's medium supplemented with 20% FCS. *L. braziliensis* axenic amastigotes were cultivated according to the described protocol for *L. amazonensis*, with pH 5,5 and temperature increased to 36ºC (Finkelstein and cols 1997). To obtain the *L. infantum* amastigotes, the protocol described by Sereno and collaborators (1997) was followed. To study the compound activity for all the evolutionary forms, a screening was done using a concentration range of 320 to 5µg/mL, where the lethal doses to 50% after 24h of incubation were established by reaction with MTT. Our preliminary results demonstrated that the 2MEOTIO and HTIO compounds are the ones which had the best leishmanicide activity on *L.braziliensis* and *L. infantum* promastigotes. In axenic amastigotes the 2MEOSEMI e HTIO compounds demonstrated better leishmanicide activity on *L. infantum*, while to *L. braziliensis* no activity of the studied compounds was noticed. Trials on the leishmanicide activity against intracellular amastigotes are being performed. As the reference drug was used the Pentamidine. We pretend to define new specific compounds against *Leishmania* sp., since it has been determined by our group that these compounds are not toxic to the host cell. **Supported by:**CAPES, FAPERJ, CNPq/PIBIC

TB25 - PHOSPHOPROTEOMIC STUDY OF WILD-TYPE AND ANTIMONY-RESISTANT *LEISHMANIA BRAZILIENSIS* LINES USING 2D-DIGE APPROACH

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Leishmania proteins undergo post-translational modifications, which are important for their functions and interactions. Among these, phosphorylation is one of the most studied and is involved in various cellular events. In this study, we performed a comparative phosphoproteomic study of antimony tartrate (SbIII)-resistant (SbR) and -susceptible (WTS) lines of *L. braziliensis*, using 2D-DIGE (two dimensional differential gel electrophoresis) approach followed by mass spectrometry (MS/MS). In order to investigate differential phosphoprotein abundance associated with the drug-induced parasite stress response and the SbIII-resistance phenotype, we used two samples from WTS line, one non-treated (LbWTS 0) and the other treated with SbIII 0.025 mg/ml (LbWTS 0.025), and two samples from SbR line treated with SbIII 0.025 and 2 mg/ml (LbSbR 0.025 and LbSbR 2, respectively). The results from comparative analysis were organized in four classes according to the SbIII treatment of each WTS and SbR *L. braziliensis* line. Subsequently, the 74 different phosphoproteins identified, which presented abundance differential, were grouped in the seven biological process categories following: protein folding/chaperones and stress response, antioxidant/detoxification, cytoskeletal proteins, metabolic process, RNA/DNA processing, protein biosynthesis and hypothetical proteins. Furthermore, we assessed the phosphorylation levels of six phosphoproteins by Western blot assays. The results showed differential levels of phosphorylation of these phosphoproteins analyzed, corroborating our phosphoproteomic findings. This study is the first report that suggests that the phosphorylation may activate important pathways in *L. braziliensis*. These pathways can help to reveal signaling events that are induced upon drug treatment and govern SbIII resistance phenotype in this parasite, contributing to the discovery of new chemotherapeutic targets against the leishmaniasis. **Supported by:**CNPq, FAPEMIG, UNICEF/UNDP/World Bank/WHO, PROEP/CNPq/FIOCRUZ, Convênio Instituto Pasteur/FIOCRUZ

TB26 - MOLECULAR ANALYSIS OF IRON SUPEROXIDE DISMUTASE (FESOD-A) IN WILD-TYPE AND ANTIMONY-RESISTANT *LEISHMANIA BRAZILIENSIS* AND *LEISHMANIA INFANTUM* LINES

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The metalloenzyme superoxide dismutase (SOD) is a central component in antioxidant defense in most organisms. It removes excess superoxide anion by converting it to oxygen and hydrogen peroxide. In this study, the gene encoding iron superoxide dismutase-A (FeSOD-A) has been characterized in wild-type (WTS) and antimony-resistant *Leishmania braziliensis* (LbSbR) and *L. infantum* (LiSbR) lines, previously in vitro selected. Southern blot results showed the presence of polymorphisms in the FeSOD-A gene sequence between both *Leishmania* species analyzed. The levels of FeSOD-A mRNA and protein expression were similar between WTS and SbR lines of both *Leishmania* species studied. However, specific enzyme activity assays revealed that both LbSbR and LiSbR lines showed a higher SOD activity than their respective LbWTS and LiWTS lines. In addition, these parasites also are more tolerant to oxidative stress generated by the herbicide paraquat. Functional analysis was performed to determine whether overexpression of the FeSOD-A gene in LbWTS/LbSbR and LiWTS/LiSbR lines would alter the antimony-resistance phenotype of transfected parasites. Western blot and enzyme activity assays showed that the protein expression level and activity of SOD were higher in the transfected parasites compared to non-transfected ones. Antimony susceptibility test (IC_{50} assay) showed that clones from both LbWTS and LiWTS lines, that overexpress the FeSOD-A enzyme, are 1.6 and 1.7-fold more resistant to SbIII compared to non-transfected lines, respectively. On the other hand, the overexpression of this enzyme in both LbSbR and LiSbR lines did not change the antimony-resistance phenotype. Interestingly, parasites from LbWTS line overexpressors of FeSOD-A are more tolerant to paraquat than those non-transfected parasites. In conclusion, our results indicate that FeSOD-A enzyme is involved in antimony-resistance phenotype in *L. braziliensis* and *L. infantum*. **Supported by:**CNPq, FAPEMIG, PDTIS/FIOCRUZ, UNICEF/UNDP/World Bank/WHO, PROEP/CNPq/FIOCRUZ

TB27 - EVALUATION OF PARASITE BURDEN AND MOLECULAR TYPING OF TRYPANOSOMA CRUZI IN BLOOD SAMPLES FROM PATIENTS WITH CHRONIC CHAGAS DISEASE FROM BRAZIL

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Chagas disease is a major public health problem in many Latin American countries. Currently, it is estimated that 7 to 8 million people are infected and 75 to 90 million are exposed to the disease. Trypanosoma cruzi, the etiological agent of disease, is represented by a set of strains or isolates that circulate in mammalian hosts and insect vectors, with large heterogeneity of biological behavior and different levels of virulence in humans and animal models, besides distinct levels of drug sensitivity and prognosis of the disease. Thus, one major challenge for the scientific community is to identify T. cruzi genetic markers capable to divide the isolates into discrete groups, searching for a surrogate marker for the pathogenesis of Chagas disease. In this work, we selected 144 patients from the National Institute of Infectious Diseases Evandro Chagas, 72 with positive serology and 72 with negative serology, from different regions of Brazil and presenting distinct clinical manifestations of the disease. For each patient, two blood samples were collected before the beginning of the treatment. To estimate parasitemia, DNA was extracted from blood samples using QIAamp DNA Mini Kit (Qiagen). The parasite load was estimated by TaqMan qPCR assay. Briefly, this multiplex assay comprises one target to T. cruzi nuclear satellite DNA and one target to human RNase P gene, as an internal control. So far, qPCR was performed for 278 samples, which 89 were positive for T. cruzi. Parasite load varied from 0.005 ± 0.003 to 336.09 ± 48.59 parasite equivalents/mL. In parallel, we are conducting the standardization of T. cruzi genotyping directly from blood samples, based on the methodologies based on conventional described by Burgos et al., (2010) and Ramirez et al., (2010), in order to investigate the correlation between parasite load, T. cruzi genotype and progression of Chagas disease. **Supported by:**CAPES/FAPERJ/IOC-FIOCRUZ

TB28 - EFFECT OF 1,2,3 TRIAZOLE DERIVATIVES AGAINST *LEISHMANIA* SPECIES ASSOCIATED TO CUTANEOUS LEISHMANIASIS.

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Leishmaniasis is a parasitic diseases caused by the flagellate protozoa of the genus *Leishmania*. The first-line treatment in Brazil is based on meglumine antimoniate (Glucantime). Other drugs used as second choice are amphotericin B and pentamidine. All these drugs have a large number of problems, including considerable toxicity, adverse effects, and high cost of production. So, is urgent the necessity of new drugs for chemotherapy of the leishmaniasis. The objective of this work was to evaluate the leishmanicidal activity of 1,2,3 triazole derivatives against promastigote of L. *amazonensis* and *L. major*. The anti-promastigote activity and cytotoxicity in peritoneal macrophages were evaluated by the MTT colorimetric method after 72 hours of treatment. Results were expressed as IC_{50} (molecular concentration that inhibits 50% of the parasite growth). Among the five compounds evaluated, four compounds exhibited a strong leishmanicidal activity (the IC_{50} < 1,0 µM). The compounds 1, 3, 4 and 5 exhibited a very significant leishmanicidal activity with IC_{50} of 0.16; 0.69; 0.10; 0.20 µM for *L. amazonensis*, respectively and IC_{50} of 0.25; 0.30; 0.13; 0,25 µM for *L. major,* respectively. Regarding the cytotoxicity in macrophages all compounds showed a toxic effect, which shows the low selectivity for the parasite. Modifications in the structure will be conducted to improve the selectivity of these compounds. **Supported by:**FAPEMIG; CNPq; UFJF

TB29 - LEISHMANICIDAL ACTIVITY *IN VITRO* OF STEROID DERIVATIVES

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Leishmaniasis is considered as neglected tropical disease caused by protozoa Leishmania. The discovery of new drugs are important for the chemotherapy of the leishmaniasis, since that all drugs fall down in limitations like efficacy, safety, or toxicity. The objective of this study was to evaluate the effect of steroid derivatives against L. amazonensis. The antipromastigote activity and cytotoxicity in peritoneal macrophages was determined by the MTT colorimetric method after 72 hours of treatment. The antiamastigote effect was evaluated in macrophages infected with L. amazonensis transfected with RFP (Red fluorescent protein) determined by the fluorescence intensity after 72 hours of treatment. The results were expressed as IC50 (concentration that inhibits 50% of parasite growth). Among the eight compounds tested, four compounds showed activity against promastigotes. Regarding antiamastigota activity, the compounds 1 and 8 showed a promising leishmanicidal activity (IC50 values < 10.0 µM). None of compounds showed an expressive toxicity on macrophages. The results show that the combination of the steroid derivatives is very interesting as leishmanicidal agents. **Supported by:**FAPEMIG, UFJF and CNPq

TB30 - INVESTIGATION OF BENZNIDAZOLE-RESISTANCE IN *TRYPANOSOMA CRUZI* REVEALS THAT DISTINCT MECHANISMS CAN ACT IN CONCERT

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Chagas Disease, caused by the protozoan *Trypanosoma cruzi,* affects millions of people worldwide, mostly in Latin America where it is endemic. The disease is also becoming a global public health issue due to human migration. Current chemotherapy options are limited to Benznidazole and Nifurtimox, which are compromised by toxic side effects that can lead to treatment discontinuation and low efficacy in the chronic stage. In addition, resistance to these compounds has been demonstrated in several *T. cruz*i strains in nature or after drug pressure selection and may be associated with treatment failures. To better understand potential resistance mechanisms against nitroheterocylic drugs, we examined three *T. cruzi* clones (Y strain) derived from a single population which had been selected for resistance by exposure to increasing concentrations of benznidazole. These clones exhibited differing levels of benznidazole-resistance (varying between 9 and 26-fold), and displayed cross-resistance to nifurtimox (2 to 4-fold). As type I nitroreductase (TcNTR) is the main activator of both benznidazole and nifurtimox, we examined the structure of the corresponding gene in each of the resistant clones. Karyotypic analysis failed to reveal any major changes in chromosome organisation in the resistant clones, although one clone had lost one copy of the chromosome containing *TcNTR*. The sequencing of the *TcNTR* genes in the drug-resistant clones revealed a C/T transition at position 568, generatiing a stop codon (TGA) in the middle of the gene. However, these processes alone are insufficient to account for the extent and diversity of benznidazole-resistance, indicating that additional mechanisms must operate. This intrinsic capacity of *T. cruzi* to rapidly acquire benznidazole-resistance by independent sequential steps, even within a single population may explain the reported treatment failures with this drug. This has important implications for drug development strategies. **Supported by:**Wellcome Trust, CAPES, CNPq

TB31 - ACTIVITY THERAPEUTIC OF SYNTHETIC ALDIMINES ON EXPERIMENTAL INFECTION BY TRYPANOSOMA CRUZI IN MURINE MODEL

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Several molecules have been synthesized and studied for treatment of Chagas diseases, but the ideal pharmaco was not still discovered. It is well known that the benznidazole (BZ) and nifurtimox (NF), usually employed for human treatment of this disease, present several limitations and low efficacy, especially on the chronic phase of the disease. Thus, this work aim the evaluation of the therapeutic activity of 11 synthesized aldimines never studied “in vivo”. Firstly, the knowledge of the action of these compounds on the experimental infection was studied using the rapid test of susceptibility. Swiss mice infected with Y T. cruzi strain were orally treated in the day of peak of parasitemia with a unique dose of 500mg/kg/day of each compound in parallel with mice not and treated with BZ and NF as reference drugs. For mice evaluation the impact of the compounds on parasitemia curve was compared as well as animals’ survival. Any aldimine reduced the parasitemia more than the usual drugs, but the compounds 3D7 and 3E8 showed similar survival than the animals treated with the reference drugs. The aldimine 3D7 was the most active to reduce the parasitemia. Following, all compounds were evaluated by the classic test used for chemotherapy evaluation in Chagas disease or long test. Then, mice infected with the same T. cruzi strain were treated at fourth day of infection, for 20 consecutive days, by oral route with 100mg/kg/day. Any aldimine showed better results than the reference drugs, and the compound 3D7 showed again significant reduction of the parasitemia than not treated animals and the higher survival than the other aldimines, associated yet to better results in the rapid test. Thus, new evaluations may be done with this aldimine involving structural changes. If the good results were confirmed posterior studies of new formulations are recommended. **Supported by:**FAPEMIG/Rede TOXIFAR, CAPES, CNPq and UFOP

TB32 - THERAPEUTIC COMBINATION OF TAMOXIFEN AND MILTEFOSINE FOR CUTANEOUS LEISHMANIASIS TREATMENT

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Background: The treatment of leishmaniasis presents many difficulties related to drug toxicity, high cost and parasite resistance. Among drugs approved for leishmaniasis chemotherapy, miltefosine (MTF) is the only oral agent available. MTF is being used to treat visceral leishmaniasis in India but an increase in the number of clinical relapses after MTF therapy has been noticed. In the search for new options for leishmaniasis therapy, we found that tamoxifen (TAM) is effective against Leishmania in vitro and in vivo. Objectives: The aim of this study was to evaluate the effect of TAM and MTF combinations against Leishmania in vitro and in vivo. Methods: The activity of TAM in combination with MTF was tested against promastigotes and intracellular amastigotes of a Leishmania amazonensis strain constitutively expressing luciferase (La-LUC) by a modified isobologram method. The effect of drug combination was evaluated in vivo in BALB/c mice infected with La-LUC. Mice were treated for 15 consecutive days with 2 or 4 mg/kg/day MTF and 6.5 or 13 mg/kg/day TAM in single or combined schemes. At the end of treatment and 30 days later, clinical status and parasite burden were evaluated through lesion size measurements and bioluminescence, respectively. Results: The average sum of fractional inhibitory concentration (x∑FIC) values was 1.3 and 0.7 for promastigotes and amastigotes, respectively. According to the adopted criteria for classification of drug interactions these x∑FIC indicate an addictive interaction. Mice treated with TAM and MTF in single or combined schemes displayed significant reduction in lesion size and parasite burden as compared with control groups. The efficacy of TAM plus MTF was similar to the response to MTF alone. Conclusions: The results of this study suggest that TAM in combination with MTF do not affect the activity of MTF. Ongoing studies will evaluate whether the combination reduces the chance of selecting MTF resistant parasites. **Supported by:**FAPESP

TB33 - IMMUNITY TO *LUTZOMYIA WHITMANI* SALIVA PROTECTS AGAINST EXPERIMENTAL *LEISHMANIA BRAZILIENSIS* INFECTION

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During bloodfeeding, infected sand fly injects saliva together with Leishmania parasites. Sand fly saliva contains molecules that modulate the host's hemostatic, inflammatory and immune responses. Immune response against *Phlebotomus papatasi*, *Phlebotomus duboscqi* or *Lutzomyia longipalpis* saliva is capable of protecting against *Leishmania major* infection. *Leishmania braziliensis* is responsible for the majority of American Cutaneous Leishmaniasis cases. It is transmitted by *Lutzomyia intermedia* or *Lutzomyia whitmani*, and both these sand fly species coexist at Corte de Pedra region (Bahia, Brazil). Although immunity generated by *L. intermedia* saliva does not protect against *L. braziliensis* in the mouse model, the outcome of *L. braziliensis* infection after immunization with *L. whitmani* saliva was not evaluated.
To investigate the effect of *L. whitmani* saliva exposure on *L. braziliensis* infection, Balb/c mice were immunized with *L. whitmani* salivary gland sonicate (SGS). Pre-exposure to *L. whitmani* SGS induced a specific cellular immune response at the inoculation site, followed by the production of IFN-γ and IL-10 in the lymph node and spleen. An increased expression of IFN-γ, but not IL-4, by CD4+ T cells was observed in the lymph node of immunized mice. After challenge with *L. braziliensis* plus saliva, mice immunized with *L. whitmani* saliva were able to control lesion development, exhibiting significant lower numbers of parasite in the ears, but not in the draining lymph node compared to controls. Protection correlated with a specific production of IFN-γ, but not IL-10 and IL-4 in the spleen of immunized mice. This study suggests a protective effect of immunization with *L. whitmani* saliva against *L. braziliensis* infection. These results reinforce the concept of using arthropod saliva in vaccine strategies against vector-borne diseases and the necessity to explore potential salivary gland components from sand flies coexisting in the same endemic area.
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TB34 - ACTIVITY OF THE SYNTHETIC NAPHTHOQUINONE LQB-166 AGAINST LEISHMANIA BRAZILIENSIS IN VITRO AND IN VIVO

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Naphthoquinones are bioactive molecules that can interfere with several cellular processes and have described anti-tumor and anti-protozoa activity. The aim of this study was to evaluate the direct and indirect antiparasitic effect of the lapachol analogue LQB-166 on intracellular amastigotes of L. braziliensis, modulation of the host cell and toxicity. Monolayers of peritoneal macrophages from SW mice were infected with L. braziliensis promastigotes (5:1), incubated with RPMI medium for 24h in order to assure the differentiation into amastigotes and treated with LQB-166 (0-800µM) for 48h at 37°C/5%CO2. 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 one week after the infection for 2 weeks (200µM/3x/week). After this period the parasite load was estimated by limiting dilution of the footpad. The naphthoquinone showed no toxicity on macrophages (LC50>1600 µM). On intracellular amastigotes forms all the concentrations tested decreased significantly ($p < 0.05$) the infection index and the IC50 was calculated in 205,8µM. 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 and lapachol in vivo using hamsters as experimental model for L. braziliensis. **Supported by:**CAPES

TB35 - EFFECT OF VANADIUM COMPLEXES IN *LEISHMANIA AMAZONENSIS* AND ACTION MECHANISM

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Introduction and Objectives: Leishmaniasis is a neglected tropical disease endemic in 98 countries. Chemotherapy exhibit limitations, including toxicity, high cost and resistance to the used drugs, which has compelled the search for new antileishmanial agents. Antiparasitic properties of vanadium complexes have been reported against malaria, amoebiasis and Chagas's disease. Thus, we analyzed the antileishmanial activity of vanadium complexes, referred as compound Van1, Van2 and Van3 Material and Methods: The compounds were assayed against *L. amazonensis* promastigote forms and for cytotoxic effects on mammalian cells, by MTT method, after 72h incubation period. The compounds also were tested against intracellular amastigote forms of *L. amazonensis* for 72 hours. The viability of the amastigotes was analyzed by the parasite counting. The most effective compound against *L. amazonensis* promastigote and amastigote forms had its action mechanism evaluated, using H_2DCFDA, rhodamine 123 and JC-1, to assess mitochondrial alterations and propidium iodide (PI), to evaluate the plasma membrane integrity of *L. amazonensis* promastigotes. Results: The Van2 and Van3 compounds were effective against *L. amazonensis* promastigotes (IC_{50} = 17.75 and 6.65µM, respectively) and only the Van3 showed activity in *L. amazonensis* amastigotes (IC_{50} = 3.51µM). All compounds showed toxicity in peritoneal macrophages, with CC_{50} below 60µM, where the Van3 showed CC_{50} = 24.32µM, ie, almost 7 times more selective for the intracellular parasite than macrophages. The treatment of *L. amazonensis* promastigotes with the Van3 compound caused mitochondrial damage and increase of ROS production, but did not alter the integrity of the plasma membrane of the parasite. Conclusions: These results show a good in vitro activity of the Van3 compound and this effect can be attributed to mitochondrial damage. **Supported by:**FAPEMIG, CNPq and UFJF

TB36 - *GUAREA GUIDONIA*: BIO-GUIDED SEARCH FOR LEISHMANICIDAL SUBSTANCES

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Leishmaniasis are diseases caused by parasites of the Leishmania genus. Leishmaniasis treatment is done using pentavalent antimonials, amphotericin B and miltefosine, which present toxicity, high cost and difficult administration. Natural products are source for substances with leishmanicidal potential, thus we performed a bio-guided assay to identify compounds with leishmanicidal activity in *Guarea guidonia*. Leishmanicidal effect of the fractions obtained was assayed in *Leishmania amazonensis* promastigotes. Our results demonstrated that hexane and dichloromethane fractions at 50 µg/mL inhibited 99 and 96% of the parasites growth, respectively. Hence the hexane fraction was fractionated, generating a hexane-methanolic fraction and a hexane-acetate fraction, which at 50 µg/mL, inhibited 99.9 and 71% of the promastigotes survival, respectively. Next, the hexane-methanolic fraction was partitioned into 6 new fractions and only F4, F5, F6 and F7 fractions at 50 µg/mL inhibited 90% of the promastigotes growth. The major constituent present in fractions F5, F6 and F7, a CPT1 terpene, was purified and tested for its potential leishmanicidal activity. Our results show that CPT1 was toxic for the intracellular amastigotes with IC_{50} of 6.2 µg/mL. CPT1 at 400 µg/mL was not toxic for the host macrophages as demonstrated by XTT and Trypan blue assays. Moreover, although CPT1 reduced nitric oxide production on LPS-activated macrophages (~25 times), it increased the production of reactive oxygen species in infected macrophages stimulated with PMA and inhibited the arginase activity in IL-4 stimulated-macrophages. Our data point CPT1 from *Guarea guidonia* as a promising substance for the development of a drug with leishmanicidal activity. **Supported by:**CAPES, FAPERJ and CNPq

TB37 - *IN VITRO* AND *IN VIVO* ANTILEISHMANIAL EFFECTS OF OLEANOLIC AND URSOLIC ACIDS

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Leishmaniasis is a neglected tropical disease, which includes a spectrum with different manifestations, ranging from skin lesions to a progressive fatal visceral infection. In 98 countries this disease can be found and the number of cases is estimated to be 12 million/year. Drugs used to treat patients are limited and toxics, justifying the development of new therapies. Thus this study aimed to evaluate the antileishmanial potential of oleanolic (OA) and ursolic acids (UA) against promastigote and amastigote forms of L. (L.) chagasi in vitro and in vivo. UA and OA were characterized by NMR (^{1}H and ^{13}C) as well as mass spectrometry. Promastigote forms were incubated with OA and UA (Cayman, USA) and after 24h promastigote viability was assessed by MTT. J774 macrophages were infected with L. (L.) chagasi promastigotes (MΦ:P-1:10) and co-cultures were treated with different concentrations of OA and UA; amphotericin B was used as standard drug. Hamsters were infected with $2x10^{7}$ promastigotes and treated daily, during 16 days with 1.0 and 2.0mg/kg of UA; 5.0mg/kg of amphotericin B was used as standard drug. After 15 days of last injection, the spleen parasitism and cell proliferation were quantified and histological alterations were analyzed. In vitro studies showed that UA eliminated L. (L) chagasi promastigotes with an effective concentration 50% of 1.8µg/mL. The infection index of macrophages decreased to 62% when treated with 10µg/mL of UA. OA did not present antileishmanial effects. In vivo studies showed that UA-treated animals showed lesser parasitism in spleen compared to control and histologically these animals presented preservation of white and red pulps, which was positively correlated with high rate of spleen mononuclear cell proliferation. These results indicate that the UA can be an interesting target for the development of new classes of drugs against LV, since their effects in vitro and in vivo were comparable with those induced by amphotericin B. **Supported by:**FAPESP

TB38 - PINAVERIUM BROMIDE IS AN EFFECTIVE IN VITRO DRUG AND AFFECTS THE MITOCHONDRIA AND PLASMA MEMBRANE OF *TRYPANOSOMA CRUZI*

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American trypanosomiasis or Chagas disease affects millions of people worldwide, with limited and toxic therapeutic alternatives. The find of new uses for approved drugs, also known as drug repurposing, has been a promising tool for Drug Discovery studies for neglected diseases. In this work, the lethal action of pinaverium bromide (PVB), a calcium channel blocker used to reduce gastrointestinal motility, was *in vitro* studied against *Trypanosoma cruzi*. The drug showed to be an effective compound, eliminating 100% of trypomastigotes, with a 50% effective concentration (EC50) of 3,7 µM. Benznidazole was used as standard drug and showed an EC50 of 440 µM. PVB also demonstrated cytotoxicity against mice conjunctive cells (NCTC) with an EC50 value of 18,7 µM. By using different fluorescent probes (Mitotracker® Red and SYTOX® Green), PVB showed a lethal effect in trypomastigotes, causing a rapid depolarization of the mitochondrial membrane potential and also, an alteration of plasma membrane permeability when compared with untreated cells. These data suggest that PVB affect the mitochondria and plasma membrane of *T. cruzi* and could be used for future experimental studies for Chagas disease. **Supported by:**CNPq 471458/2012-0 and CAPES.

TB39 - PROTECTIVE EFFICACY OF EPITOPES THAT MIMIC LEISHMANIA INFANTUM ANTIGENS SELECTED BY PHAGE DISPLAY AGAINST VISCERAL LEISHMANIASIS

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The development of new and cost-effective alternative therapeutic and prophylactic strategies to treat or prevent leishmaniasis has become a high-priority. In the present study, the phage display biotechnology was used for the identification of new antigens, represented by mimotopes expressed in the foreign surface of phage clones, which were specifically recognized by antibodies from sera of dogs with asymptomatic and symptomatic visceral leishmaniasis (VL).Twenty phage clones were selected after bio-pannings cycles, and they were evaluated in in vitro experiments of immune stimulation of splenocytes obtained from BALB/c mice naive and chronically infected with Leishmania infantum; in order to select those able to induce a polarized Th1 immune response, represented by high levels of IFN-gama and low levels of IL-4. Two phage clones (B10 and C01) were selected and employed in in vivo vaccination protocols in BALB/c mice. Vaccinated mice with B10 or C01 clones plus saponin showed a high and specific production of IFN-gama, IL-12, and GM-CSF after in vitro stimulation with the individual phages or Leishmania antigenic extract. Immunized and infected mice, as compared to the control groups (saline, saponin and wild-type phage clone plus saponin), showed significant reductions in the parasite burden in liver, spleen, bone marrow and in the paws' draining lymph nodes. Protection was associated with an IL-12-dependent production of IFN-gama, produced mainly by CD8 T cells. In these mice, a decrease in the parasite-mediated IL-4 and IL-10 response was also observed. The present study showed that two new antigens, selected by a yet non-described phage display technique, could be employ associated to a Th1-type adjuvant, as candidate to compose a vaccine against VL. **Supported by:**Capes

TB40 - ACTIVITY OF EXTRACTS OF *KALANCHOE PINNATA* AND ITS MAJOR FLAVONOID *IN VITRO* AGAINST *LEISHMANIA BRAZILIENSIS*

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The aim of this study was to evaluate in vitro the antiLeishmanial potential of substances extracted from plants against *Leishmania braziliensis* (Lb), the most important dermatropic *Leishmania* species on Brazil. We evaluated extratcts of *Kalanchoe pinnata* (Kp) grown in the habitat, under blue light, white light or ultraviolet light and its major flavonoid (MF). Promastigotes of Lb were cultured with 0-500µg/ml of Kp extracts for 96h/28°C and parasites were counted daily using a Neubauer chamber. For tests with amastigotes, monolayers of peritoneal macrophages of mice were infected with promastigotes of Lb (5 parasite/macrophage) and incubated with 0-500µg/ml of extracts or MF (0-100µg/ml) for 48 and 96h/37°C/5%CO2. Nitric oxide was measured by Griess reagent on macrophages supernatants. Kp extracts has little effect on promatigotes inhibiting 27,4% (Kp grown under blue light) and 17,35% (Kp grown in habitat). About amastigotes there was no improvement in the effect varying growing conditions of Kp, but the effect was time-dependent in Kp and MF. The treatment with Kp (Kp habitat and Kp branca) and MF (12,5; 25; 50 and 100µg/ml)show infection index reduction 40,4% and 69,3% (Kp habitat); 26,2% and 61,9% (Kp branca); 49% and 60,9% (12,5µg/ml); 59% and 61% (25µg/ml); 71,1% and 78% (50µg/ml); 75,7% and 79,5% (100µg/ml) in 48h and 96h respectively. The IC50 of the MF was 16,7µg/ml and 7,6µg/ml in 48h and 96h respectively. None of the extracts tested was able to stimulate nitric oxide production by macrophages. In macrophages pretreated for only 24h before infection, Kp extract cultivated habitat was able to reduce 69,3% ($p<0,05$) of infection index, suggesting effect on macrophage independent of nitric oxid production. Kp and its major flavonoid showed anti amastigote activity on Lb. The production of cytokines by macrophages and also the death of the parasite through apoptosis is being investigated. **Supported by:**CNPq

TB41 - EFFECTS OF A NOVEL SIRTUIN INHIBITOR ON *LEISHMANIA AMAZONENSIS*

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Treatment of leishmaniasis, a significant but often neglected tropical disease caused by protozoan parasites of the Leishmania genus, involves the use of antimonials, miltefosine, amphotericin B or pentamidine. However, the undesirable side effects of these drugs and the reports of drug-resistant parasites throughout the world demonstrate the need for new treatments that are safer and more efficacious and accessible. Sirtuins belong to the group of proteins histone deacetylases that are responsible for regulating many proteins involved in processes that are critical for cellular/organism survival, apoptosis, DNA repair, metabolism among others. Herein, we evaluated the effects of the KH-TFMDI, a novel histone deacetylase inhibitor, on *Leishmania amazonensis* promastigotes and intracellular amastigotes. The IC50 value was 3 µM for promastigotes and 1 µM for intracellular amastigotes after 48 h of treatment. Microscopic analyses revealed that promastigotes became elongated and thinner in response to KH-TFMDI, indicating changes in cytoskeleton organization. Immunofluorescence microscopy and western blotting using an anti-acetylated tubulin antibody revealed an increase in the expression of acetylated tubulin in response to KH-TFMDI. Furthermore, transmission electron microscopy revealed several ultrastructural changes, such as (a) mitochondrial swelling, followed by the formation of many vesicles inside the matrix; (b) the presence of many lipid bodies randomly distributed through the cytoplasm; (c) abnormal chromatin condensation; and (d) the formation of blebs on the plasma membrane. In addition, some images suggested that KH-TFMDI might induce parasite death by an apoptosis-like mechanism and/or autophagy. These observations indicate that histone deacetylases are promising targets for the development of new drugs to treat Leishmania, and KH-TFMDI is a promising drug candidate that should be tested in vivo. **Supported by:**CNPQ, CAPES e FAPER

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TB42 - MODULATION OF MACROPHAGE ACTIVITY BY GLUCANTIME®

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Glucantime® is a pentavalent antimonial used as first choice to treat leishmaniasis. However its mechanism is not fully understood. On intracellular amastigotes of Leishmania sp, Glucantime® induce damage to the DNA of the parasite. Studies suggest that Glucantime® enhances phagocytosis and TNF-α production by phagocytes. The aim of this study is to evaluate the modulation of macrophages by Glucantime®. Therefore monolayers of mice peritoneal macrophages were treated for 24 hours with Glucantime® (0,1, 1 and 10 mg/ml) before the infection with Leishmania braziliensis. After 48 hours of incubation with culture medium the infection index was evaluated by counting. Before and after the infection was evaluated nitric oxide (NO) production by Griess method, reactive oxygen species (ROS) by fluorimetry using the H2DCFDA dye and cytokines by Kit CBA. To evaluate if Glucantime® could modulate macrophages in vivo, Swiss Webster mice were treated for 5 consecutive days with 8 mg Glucantime® by intraperitoneal route. Peritoneal macrophages were evaluated about its capacity of control the in vitro infection with L. braziliensis and cytokines production. Results showed that the pre-treatment of macrophages with Glucantime® was able to reduce the infection index and increased NO and ROS production. The treatment with Glucantime® was able to significantly increase IL-12, TNF and IL-6 production, however the IL-10 production was not altered. There were no significant changes of these parameters for comparing to control after the L. braziliensis infection except for IL-6, IL-10 that decrease and TNF that increase. The macrophages from treated mice were capable of reduce the infection index by L. braziliensis and had the TNF production increased. These results suggest that Glucantime® is capable of activate macrophages in vitro and in vivo and this effect could contribute to the mechanism of action of this drug. **Supported by:**FAPERJ

TB43 - ACTIVATORS OF EIF2α KINASES PREVENT GROWTH OF *TRYPANOSOMA CRUZI*.

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Certain disubstituted urea compounds activate mammalian heme-regulated inhibitor (HRI) kinase, inhibiting translation initiation due to the phosphorylation of the eukaryotic translation initiation factor alpha (eIF2α) (Chen, T. *et al*., 2011, 2013). Here, we studied the effects of these activators in *Trypanosoma cruzi*, as eIF2α phosphorylation was shown to play a key role in the differentiation of this parasite (Tonelli, R.R. *et al.*, 2011). Three compounds BTdCPU (Chen, T. *et al.*, 2011), #I-m6, I-17 (Chen, T. *et al.*, 2013) were used, as well as an inactive diarylurea (NCdCPU, Chen, T. *et al.*, 2011).The three compounds inhibited epimastigotes (Y strain) multiplication, while no effect was observed with the inactive compound up to 10 µM. The dose-response curves indicated an IC50 of 3 µM for I-17, 5 µM for BTdCPU. Compound #I-m6 was active only at 10 µM. We then, tested the effect of these compounds on epimastigotes over-expressing wild-type eIF2α, or versions containing each one or both of the two possible phosphorylation sites (Serine 43 or Threonine 169) replaced by alanine sites. The parasites expressing double mutant were more resistant only to I-17, revealing the importance of eIF2α phosphorylation for the activity of this compound. We also evaluated the morphology of eIF2α overexpressing parasites treated with I-17. While wild type eIF2α expressing cells presented a higher globular morphology (over 30%), parasites containing the double mutated eIF2α have no morphological changes in the presence of I-17. Parasites knocked out for one (TcK2) among three putative eIF2α kinases found in *T. cruzi* were still affected by I-17. Therefore, we concluded that HRI-activators could be used to inhibit growth of *T. cruzi via* eIF2α phosphorylation. The possible target eIF2α kinases remains to be elucidated. **Supported by:**FAPESP and FAPERJ

TB44 - BIOCHEMICAL CHARACTERIZATION OF THE TRANSLATION INITIATION COMPLEX EIF3 IN *LEISHMANIA*

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The initiation of translation in eukaryotes is supported by the action of several eukaryotic Initiation Factors (eIFs). The largest of these is eIF3, comprising of up to thirteen polypeptides (eIF3a through eIF3m), involved in multiple stages of the initiation process. eIF3 has been better characterized from model organisms, but is poorly known from more diverged eukaryotes, including those belonging to the genus *Leishmania*. This study aimed to perform the biochemical characterization of the *Leishmania* eIF3 complex. To do this the sequence encoding the *L. major* EIF3E subunit, conserved in sequence and identified by bioinformatic approaches, was amplified, cloned and expressed as an N-terminally his-tagged protein in *E. coli*. The resultant recombinant protein was then used to immunize rabbits and produce a specific polyclonal anti-serum which was first used to confirm the subcelullar localization of its orthologue in *L. major* promastigotes. The *Leishmania* protein was found to strictly localize to the cellular cytoplasm, compatible with its role in translation initiation. The anti-serum was then used in immunoprecipitation assays, this time using total cell extracts from *L. infantum*, aiming to purify the whole eIF3 complex. An analysis of the precipitated samples through Western-blots with the same serum confirmed the efficiency of the procedure for the eIF3e orthologue, with none of it coming down in a control assay carried out with the pre-immune serum. These samples were then submitted to mass spectrometry analysis in order to confirm the subunit composition of the *Leishmania* eIF3. The mass spectrometry results were derived from the analysis of three sets of replicates comparing the anti-EIF3E antibodies with the pre-immune control. These results confirms the presence of 11 of the eIF3 subunits, many annotated as hypothetical proteins, and provides a basis for the study of eIF3 function, leading to a better understanding of translation initiation in this pathogen.

TB45 - DRUG REPURPOSING: ANTICANCER AGENTS AS ALTERNATIVE AGAINST LEISHMANIASIS

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Leishmaniasis are considered a complex of neglected tropical diseases caused by protozoan parasites belonging to the genus Leishmania, responsible for 20,000 to 40,000 estimated deaths per year. The lack of human suitable vaccines, associated with a restricted drug arsenal and the emergence of drug resistance, reveals the current scenario and the urgent need for alternatives to antileishmanial chemotherapy. Therefore, the aim of this research is to identify potential anticancer drugs against Leishmania parasites. For this purpose, sensitivity profiles of tamoxifen, letrozole, imatinib, pamidronate and zoledronate were obtained against promastigote forms of *Leishmania* (*Viannia*) *braziliensis* and *Leishmania* (*Leishmania*) *infantum*. The most active compound was tamoxifen, presenting half minimal inhibitory concentration (IC_{50}) of 2.5 ± 0.37 and 1.8 ± 0.28 µM for *L. infantum* and *L. braziliensis*, respectively; followed by imatinib where IC_{50} values reached 34.88 ± 1.56 µM for *L. infantum* and *L. braziliensis* presented 29.20 ± 1.24 µM. Curiously, letrozol was selectively active against *L. infantum*, presenting IC_{50} of 48.85 ± 0.4 while presented no antileishmanial activity against *L. braziliensis* at the highest tested concentration (500 µM). The susceptibility for pamidronate and zoledronate was not achieved due to drug precipitation. Positive control was performed using amphotericin B with IC_{50} of 0.01 µM. This drug screening confims the antileishmanial activity of tamoxifen and reveals the compound imatinib as candidate for further investigations aiming the search for alternative antileishmanial drug. Anti-amastigote assay, selective index and the selection of mutants resistant to these drugs are being performed. These findings will contribute to identify new drug targets as well as to better understand the mechanisms of action in Leishmania parasites and support the development of new chemotherapy strategies against leishmaniasis. Financial support: CNPq, FAPEMIG. **Supported by:**CNPq, FAPEMIG

TB46 - CELLULAR RECRUITMENT GENERATED IN MICE SKIN AFTER SENSITIZATION WITH DIFFERENT VACCINE ADJUVANTS

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An effective early innate immune response to vaccine adjuvants may significantly impact the overall vaccines immunogenicity and efficacy. In this context, adjuvants are important additives, since they enhance the antigen immunogenicity inducing an intense and prolonged immune response. The skin is considered an important route of immunization and the intradermal route has the advantage of delivering the antigen and maturation factors of dendritic cells in the compartment in which they reside, which makes the antigen presentation faster and, consequently, migration to draining lymph nodes. Herein, inflammatory cells influx in the skin induced by adjuvants were analyzed at 1, 12, 24, 48, 96, 168 and 336 hours after sensitization. The following adjuvants: Complete Freund's Adjuvant (CFA), Glucopyranosyl Lipid Adjuvant–stable emulsion (GLA-SE), Montanide Pet Gel A® (MPGA), Al(OH)3 and Resiquimod, were selected. Animals were inoculated with saline as control group. All adjuvants were able to induce an increasing in the inflammatory infiltrate in the early periods when compared to the control group. In differential analysis of cellular infiltrate we observed that GLA-SE, ACF and MPGA groups showed an increasing of neutrophils after 12h when compared to the control group. In the analysis of the macrophage population, MPGA and GLA-SE groups showed an increasing in the later times of 96h and 168h, respectively, when compared to the control group. Based on these results we can conclude that the cellular recruitment study promoted by the adjuvants is critical, since the innate immune response activation mediated by different cell types is extremely important to direct the adaptive response. Also, we believe that the differences between adjuvants are probably due to differences in their compositions and consequently their mechanisms of action. **Supported by:**CNPq, FAPEMIG, CAPES and UFOP

TB47 - (+)-PHYLANTIDINE ALKALOID WITH LEISHMANICIDAL ACTION AND INDUCER OF MACROPHAGE ACTIVATION

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Leishmaniasis is antropozoonotic disease caused by parasites of the genus Leishmania. These parasites proliferate primarily within mammalian macrophages and promote a diversity of clinical manifestations ranging from self-healing cutaneous lesions to fatal visceral leishmaniasis. The treatment available is chemotherapy, but is limited by toxicity and requires a long term treatment. The use of natural products from plants currently plays an important role and source as antileishmania agent. (+)-Phylantidine, is an alkaloid extracted from stem of Margaritaria nobilis of the family Phyllanthaceae. Thus, the aim of this study is evaluated the effects of (+)-phylantidine on promastigotes forms of Leishmania (Leishmania) amazonensis and host cell. Antiproliferative activity of promastigotes forms was observed when parasites were treated with 50 and 100 µg/mL, with reduction of 73.75% and 82.50% respectively when compared with non-treated parasites. In the period of 48 hours was observed an IC50 of 56.34 µg/mL. As the reference drug amphotericin B was used and observed reduction of 100% in parasites treated with 0.1 µg/mL for 96 hours. The treatment with 50 and 100 µg/ml of (+)-phylantidine promoted morphological alterations in the promastigote. Transmission Electron Microscopy analysis showed alterations in the membrane, flagellar pocket and increase in the number of structures like acidocalcisome. No citotoxic effect in mouse peritoneal macrophages was detected after treatment with phylantidine. However, ultrastructural analysis by Scanning Electron Microscopy revealed typical activated cell morphology in treated macrophages, with alteration in cell volume, an increase in cytoplasmic projections and spreading ability. These results demonstrated that alkaloid seems to be important not only for host cell but also to promastigotes growth inhibition. Thus, (+)-phylantidine could be an alternative source for Leishmaniasis treatment. **Supported by:**CAPES, CNPq/UFPa, UFPA, INBEB, FAPERJ, MCT/CNPq/FNDCT/PROCAD-NF CAPES/FAPERJ

TB48 - BIOLOGICAL AND ENZYMATIC CHARACTERIZATION OF PROTEASES FROM CRUDE VENOM OF THE ANT ODONTOMACHUS BAURI

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Hymenoptera venoms constitute an interesting source of natural toxins that may lead to the development of novel therapeutic agents. In that sense, the present study investigated the biological and enzymatic characteristics of the crude venom of the ant O. bauri. The electrophoretic profile of the crude venom revealed several protein bands, with higher staining for the bands from 29 to 160 kDa. The crude venom showed high proteolytic activity on azocasein at optimal pH 8.0 and 37oC. Also, gelatinolytic activity as determined by the zymogram method, showing six proteins with apparent molecular masses of 17, 20, 26, 29, 43 and 48 kDa. The crude venom degraded the fibrinogen α-chain faster than the β-chain, while the fibrinogen γ-chain remained unchanged. Additionally, venom stability was maintained at least for 20 days at 4oC. In the presence of protease inhibitors as aprotinin, leupeptin and EDTA, the azocaseinolytic activity was reduced by 45%, 29% and 9%, respectively, suggesting that the enzymes present in the crude venom belong to the three classes of proteases, with the serine proteases in greater intensity and metalloproteases in lower intensity. In biological assays, O. bauri venom showed hemolytic and coagulant activity in vitro, and defibrinating activity in vivo. Also, the venom showed antimicrobial activity against Staphylococcus aureus and Escherichia coli as well as antiparasitic activity on Toxoplasma gondii infection in vitro. In conclusion, the O. bauri crude venom consists predominantly of serine proteases with high proteolytic, fibrinogenolytic, hemolytic, coagulant, defibrinating, antimicrobial and antiparasitic activities. This study may open interesting perspectives for purification of enzymes of O. bauri crude venom with pharmacological interest for future studies related to therapeutics against physiological dysfunctions and infectious diseases. **Supported by:**CNPq; UFU e Fapemig

TB49 - INTRALESIONAL TREATMENTS WITH RETINOL OR RETINOIC ACID ARE EFFECTIVE AGAINST MURINE *LEISHMANIA AMAZONENSIS* INFECTION

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Retinol and its metabolite, retinoic acid (RA), are important immuneregulatory factors. In low concentrations, RA acts as a cofactor in the differentiation of naïve T cells into regulatory T cells, but in high concentrations it may induce Th17 differentiation. We have previously observed that dietary retinol-deficient mice are more resistant to *L. amazonensis* infection than mice on a retinol-supplemented diet. Based on these findings, in this study, the influence of retinol and RA in *in vitro* and *in vivo L. amazonensis* growth was investigated. Both retinol and RA inhibited promastigote growth *in vitro* (IC50 of 33±1,5 µM and 4±0,2 µM, respectively). Mor eover, neither retinol nor RA activated macrophage NO production irrespective of intracellular infection. As retinol and RA showed direct antileishmanial activity, their effect was investigated *in vivo*. Thus, BALB/c mice (n=5) were subcutaneously infected with $2x10^6$ *L. amazonensis*-GFP promastigotes in the ear. On day 9 post infection, they were given intralesional injections with retinol (5 mM, 15 µg/dose), RA (5 mM, 14 µg/dose) or PBS alone (10 µL/dose) twice a week for 4 weeks. Lesion development was monitored throughout treatment. Two days after the last dose (day 36 of infection) the local parasite loads and antigen recall responses were evaluated. Besides their faster lesion development as compared with untreated controls, retinol- and RA-treated mice showed decreased parasite loads. Moreover, lymph node cells from RA mice displayed higher proliferative response to parasite antigens. These results indicate that retinol and RA have an intrinsic antileishmanial activity, and at the doses given an immunostimulatory/pro-inflammatory and protective function against *L. amazonensis* murine infection. **Supported by:**CAPES, CNPq, FAPERJ

TB50 - ANTITRYPANOSOMATID ACTIVITY-GUIDED PHYTOCHEMICAL INVESTIGATION OF PIPER PELTATUM (PIPERACEAE)

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Trypanosomatids are kinetoplastid protozoa and are unique in containing one single mitochondria per cell, which is associated with the kinetoplast. Some trypanosomatids cause human illnesses, such as leishmaniasis and Chagas' disease, and constitutes a serious public health problem. The drugs used in the clinic are toxic and often ineffective. Thus, a systematic search for new therapeutic alternatives is necessary. Plant extracts constitute an important natural source of active compounds. Preparations from the Piperaceae family had their therapeutic proprieties investigated, presenting antiinflamatory, antitumor, antiviral, antibacterial and antimalarial activities. In this study, we investigated the activity of *Piper peltatum* against *L. amazonensis* (MHOM/BR/77/LTB0016), *L. braziliensis* (MCAN/BR/98/R619), *L. infantum* (MHOM/MA67ITNAB263) and *T. cruzi* (DM28c). The oily crude extract of *P. peltatum* was more active than the aqueous, with IC_{50} 5.6, 10.6, 7.6 µg/mL against promastigotes of *L. infantum*, *L. braziliensis* and *L. amazonensis*, respectively, and 2.2 µg/mL against epimastigotes of *T. cruzi*. This extract also showed high inhibitory activity against intracellular amastigotes, with IC_{50} 9.3, 1.7, 1.5 µg/mL in *L. infantum*, *L. braziliensis* and *L. amazonensis*, respectively, and presented low cytotoxicity on mammalian cells with LD_{50} of 30.7 µg/mL. From the crude extract, several fractions of different polarities were obtained. Using an activity-guided approach, we found that the dichloromethane-ethyl acetate fraction was the most active, and a pure compound was isolated from it. This compound presented IC_{50} 17.4, 44.6, 17.8 µg/mL against promastigotes of *L. infantum*, *L. braziliensis* and *L. amazonensis*, respectively and 15.8 µg/mL against epimastigotes of *T. cruzi*. Although the pure compound presented a good antileishmanial activity, these results suggest that this substance probably is not the only one active principle of the Piper peltatum extract. **Supported by:**CNPQ ; IOC

TB51 - SUBLINGUAL VACCINATION WITH LAAG AND LACK-DNA LEADS TO INCREASED EXPRESSION OF TOLEROGENIC CYTOKINES AND PROTECTION AGAINST CUTANEOUS LEISHMANIASIS IN BALB/C MICE

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We have previously demonstrated that intranasal immunization with *Leishmania amazonensis* promastigote total antigens (LaAg) or with DNA codifying the leishmanial LACK protein (LACK-DNA) protect BALB/c mice against cutaneous leishmaniasis, unlike intramuscular immunization, which exacerbates the disease. Based on the assumption that normally the mucosal immune system as a whole is specialized in inducing immune tolerance to exogenous antigens, in this study we evaluated whether the intranasal protection was extended to the sublingual mucosa. Thus, BALB/c mice were given two sublingual doses of LaAg (10 µg of protein/dose) or LACK-DNA (30 µg/dose). Seven days after the second boost, mice were challenged with $2x10^5$ *L. amazonensis* promastigotes in the footpad, and the lesion development was monitored for 70 days, when the parasite load was evaluated by limiting dilution assay. Unlike LaAg, LACK-DNA was not able to control the lesion growth. However, both sublingual vaccines prevented parasite growth in the lesions compared to the PBS group. Evaluation of cytokine expression by qRT-PCR in infection-draining lymph nodes 40 h after infection showed that sublingual LACK-DNA led to increased IL-10 and reduced IL-4 expression, while LaAg increased the TGF-β expression. These data indicate that LaAg and LACK-DNA vaccine are also effective when given by the sublingual route and suggest that the immune protective mechanism is involved with tolerance induction to disease-promoting leishmanial antigens. **Supported by:**CAPES, CNPq, FAPERJ

TB52 - LEISHMANICIDAL EFFECTS OF COMPOUNDS DERIVED FROM ISONIAZID AND PYRAZINECARBOXAMIDE

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Leishmaniasis treatment is high toxicity, elevated cost and limited effectivity. Then, the development of news drugs is necessary. Isoniazid and pyrazinecarboxamide (pyrazinamide) are used in chemotherapy of tuberculosis and some studies show that also activity against Leishmania spp. The aim of this study was to evaluate in vitro the leishmanicidal activity of compounds derived from isoniazid (G01, G02, G03, G05 and G06) and pirazinacarboxamida (R01, R02, R03, R05) on intracellular amastigotes forms of Leishmania braziliensis. Monolayer of peritoneal macrophages of SW mice were infected with promastigotes of L. braziliensis at a ratio of 5:1 and after treated with molecules (0-100µM) for 48h. The R01 was the most active derivatives of isoniazid inhibiting 58% and 83% of the infection index at 10 µM and 100µM, respectively. The G02 was the most active derivatives from pirazinacarboxamida inhibiting 88% at 10µM. The concentration that inhibit 50% (IC50%) of infection index was estimated at 13,17µM and 7,2µM for R01 and G02, respectively. R01 and G02 were not toxic to macrophages on the concentrations tested and their LC50 (concentration that is toxic to 50% of macrophages) was above 100µM. The results show that R01 and G02 promising activity against L. braziliensis. The mechanism action of R01 and G02 is being investigated. **Supported by:**CAPES

TB53 - KOJIC ACID INDUCES OXIDATIVE MECHANISM AND INCREASES PHAGOCYTOSIS OF LEISHMANIA (LEISHMANIA) AMAZONENSIS BY HUMAN NEUTROPHILS

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Neutrophils are phagocytes involved in the primary immune responses to eliminate pathogens and are first cells to migrate to the site of Leishmania infection. Leishmania is a protozoan responsible for the one of the most important infectious diseases in the world and chemotherapy is potentially useful, but besides the high cost, these drugs are toxic and require a long period of treatment. Bioproducts offer new perspectives and represent an important source of new anti-leishmanial agents. The kojic acid (KA) is a secondary metabolite synthesized by some species of fungi from Aspergillus, Penicillium and Acetobacter genera. KA has several applications, being used as a food additive, cosmetics, macrophage activator and anti-leishmanial agent. The present study was designed to determine the effects of KA- induced human neutrophils microbicidal mechanisms activation and during interaction with Leishmania (Leishmania) amazonensis. Human neutrophils were obtained from buffy coats donated from Hemocenter Fundation of Para State. Cells isolation were performed using HISTOPAQUE® 1077-density-gradient. Neutrophils were treated for 1 hour with 50 µg/mL of KA. No cytotoxicity effects were observed in treated neutrophils with by colorimetric MTT assays. Treated cells demonstrated increase microbicidal activity that occurred through reactive oxygen species (ROS) production, detected by Nitro Blue Tetrazolium (NBT) assay, CellROX® green dye and myeloperoxidase (MPO) activity. Subsequently, human neutrophils were assessed for their capacity to phagocytosis Leishmania parasites induced by KA and treated neutrophils had a higher capacity to phagocytosis. In addition, was observed decrease in the number of intracellular parasites 3 and 6 hours after infection. Thus, these results show that KA could be involved in neutrophil activation by ROS production suggesting a possible cell death mechanism in Leishmania. **Supported by:**CAPES, CNPq/UFPa and INBEB

TB54 - A TH1 RESPONSE ADJUVANT DERIVED FROM AGARICUS BLAZEI MUSHROOM IS EFFECTIVE IN INDUCING PROTECTION AGAINST VISCERAL LEISHMANIASIS WHEN ASSOCIATED TO A PROTECTIVE ANTIGEN

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The Visceral Leishmaniasis (VL) is incidence in about 98 countries, with about 50.000 deaths registered annually. The treatment of disease is difficult mainly due to the high toxicity of the drugs. Strategies employed for prevention have been ineffective, highlighting the need to prophylactic alternatives, such as vaccines. No less important than the choice of an antigen, the association of an adjuvant is important to success of a vaccine. *Agaricus blazei* is a mushroom found in Brazil and, recently, it was demonstrated an antileishmanial activity of this mushroom against different *Leishmania* species. Compounds as β-glucans and polysaccharides have been identified in this fungus, which have been shown to activate the hosts' immune response. In the present study, a purification process was performed using the *A. blazei*, in order to obtain rich-polysaccharides fractions to be evaluated like immune response adjuvants. Seven fractions were obtained and used for the *in vitro* immune stimulation of spleen cells derived from naive BALB/c mice; in an attempt to select those able to stimulate a higher production of IFN-gamma and lower levels of IL-4. Two fractions presenting the best results of specificity and selectivity were selected and evaluated in the immunization of BALB/c mice, in association with a protective protein of L. infantum, LiHyp1. Both fractions, namely F2 and F4, when associated with rLiHyp1, were able to induce an in vivo specific-Th1 immune response, primed by the high production of IFN-gamma, IL-12, and GM-CSF, as well as by low levels of IL-4 and IL-10 before infection, and both vaccines proved to be protective against L. infantum. The adjuvants fractions presented similar results in relation to the use of saponin, and they did not presented toxicity in the animals. In this context, these fractions could be considered as Th1 response inducers, and could be used in association with other antigens against Leishmania infection or in other disease models. **Supported by:**CAPES

TB55 - SYNTHESIS AND ANTILEISHMANIAL ACTIVITY OF NOVEL 1-ARYL-4-(4,6-DIHYDRO-1H-IMIDAZOL-2-YL)-1H-PYRAZOLE DERIVATIVE

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Leishmaniasis comprises a group of diseases with distinct clinical manifestations caused by different species of the protozoan parasite. The World Health Organization estimates that 350 million people are at risk of infection with leishmaniasis in endemic areas and that approximately 12 million are currently infected. Current chemotherapy of leishmaniasis is unsatisfactory. Efficacious and safe new drugs are needed. In the present work, the antileishmanial efficacy and cytotoxicity of novel chloro-1-aryl-4-(4,6-dihydro-1H-imidazol-2-yl)-1H-pyrazole and 5-amino-chloro-1-aryl-4-(4,6-dihydro-1H-imidazol-2-yl)-1H-pyrazole derivatives were evaluated. Many compounds containing pyrazole and imidazoline rings have been revealed as potential antileishmanial lead compounds.The novel chloro substituted imidazol-pyrazole derivatives and 5-amino-chloro-imidazol-pyrazole derivatives were obtained, in good yields and all the substances were fully characterized by usual methods (IR, 1H, 13C NMR). The antileishmanial efficacy these compounds were determined in vitro against L. amazonensis promastigotes. Parasites were cultured with and without the drugs in Schneider´s medium at 25°C, using Pentamidine as the standard drug. After 24 hours incubation, parasite viability was determined using the MTT (tetrazolium blue) assay. The results showed that among compounds assayed the substituted 3,5-dichloroaryl (IC50/24h =48.8±1.4 µM) and 5-amino-3,5-dichloroaryl (IC50/24h=66.3±2.4µM) were the most potent against L. amazonensis promastigotes. In addition, 3,5-dichloroaryl and 5-amino-3,5-dichloroaryl derivatives were less cytotoxic than Pentamidine. This study reinforces these novel derivatives as potential antileishmanial lead compounds for the design and synthesis of similar heterocycle derivatives.

TB56 - LEISHMANICIDAL ACTIVITY OF NITROHETEROCYCLIC COMPOUNDS

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Leishmaniasis is a group of neglected diseases that has been highlighted by presenting a high morbidity rate and different clinical manifestations. Their treatment presents difficulties such as high cost, toxicity and resistance cases. This study aimed to evaluate the leishmanicidal activity of nitroheterocyclic compounds. Promastigotes of *Leishmania amazonensis* were cultured in the presence of several concentrations of eighteen nitroheterocyclic compounds up to 100 µM for 72 hours and quantified colorimetrically by MTT assay. The compounds LQB-278, LQB-303 and LQB-304 showed IC50 less than 22 µM. Among them, LQB-303 and LQB-304 potently inhibited the parasitic growth, in a dose-dependent manner, with the following IC50: 9.36 µM and 9.73 µM. To evaluate the antiamastigote activity, nine selected compounds were assayed on macrophages infected with L. amazonensis. The infectivity index was determined by light microscopy. The same three compounds showed to be the most effective in decreasing the infection of the macrophages, with IC50 less than 14 µM. In addition, the compounds DS-26 and LQB-302 also showed IC50 less than 16 µM. In order to determine compounds toxicity, nine of them were selected and incubated with murine macrophages for 72 hours. The effect on macrophages viability was quantified by resazurin and the LD50 values were higher than 50 µM for LQB-303 and LQB-304, indicating a selectivity index more than 10-fold. The presence of the nitro group in position 5 of the furan ring is primarily responsible for its activity through nitro reductions. Thereby, it is possible to relate the leishmanicidal activity of these compounds with their molecular structures, since LQB-303 and LQB-304 have similar molecular structures with only a few modifications such as the presence of a nitro group or with the replacement of the furan ring by thiophene, making them promising agents for the treatment of leishmaniasis.**Supported by:**PAPES/CNPQ

TB57 - MELATONIN TREATMENT REDUCES *TOXOPLASMA GONDII* GROWTH IN HOST CELLS

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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 imune response of the vertebrate host during infection with *T. gondii* and *Trypanosoma cruzi*, helping to control parasite proliferation. In this study, LLC-MK2 cells infected with *T. gondii* were treated with different concentrations of melatonin. Growth of the parasite and cell morphology was assayed by light microscopy. Melatonin reduced the parasite growth by impressive 90%, when compared to untreated control, without causing significant damage to the host cells. Parasites were apparently dying by apoptosis. Further studies are necessary to confirm this death mechanism. **Supported by:**CAPES, UEZO, UENF, CNPq, FAPERJ

TB58 - 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 for this disease 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* activity of an iron(III) compound containing sulfadiazine coordinated to the iron center. The iron compound [Fe(H2BPClNOL)Cl2] was previously obtained as described in the literature and was reacted with sodium sulfadiazine given rise to the new compound. Here we describe the cytotoxic effects of this new iron compound on *T. gondii* infecting LLC-MK2 host cells at a treatment time of up to 48 hours. The infection index was quantified by optical microscopy. After 24h, the growth rate for *T. gondii* was reduced by 58% in the presence of the new compound, and the compound without sodium sulfadiazine was not active. After 48h, the compound without sodium sulfadiazine reduced the growth of the parasite by 66%, and the new compound reduced parasite growth by 82%, revealing an impressive biological effect by the addition of the sulfadiazine molecule. Further analysis of ultrastructure, cell death and host response will be performed in order to investigate the mode of action of this new compound on the parasite. **Supported by:**FAPERJ, CNPq, CAPES, UENF, UEZO

TB59 - SELECTIVE EFFECT OF PHYSALIS ANGULATA ON LEISHMANIA (LEISHMANIA) AMAZONENSIS DURING INTERACTION WITH MURINE BONE MARROW CELLS

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Leishmaniasis are neglected emerging diseases in 98 countries caused by several species of protozoa of the genus Leishmania. The most effective treatment for leishmaniasis is the chemotherapy and besides the high cost, these drugs are toxic and require a long period of treatment. Nowadays, some herbal products are considered an important alternative source of a new leishmanicidal agent, which includes the plant Physalis angulata widely used in popular medicine. Thus, this study aim to evaluate the leishmanicidal action of the aqueous extract obtained from Physalis angulata roots (AEPa). In the present study was observed an increased production of reactive oxygen species (ROS) in promastigotes of Leishmania (L.) amazonensis treated with 100 µg/mL of the AEPa in 48 (47.67%) and 72-hour (81.42%) period compared to controls (48h-26.01% and 72h-26.93%). Flow cytometric analysis showed that AEPa promoting the death by apoptosis of L. (L.) amazonensis promastigotes. Was observed an increase stained for annexin V of the treated promastigotes (59.19%) compared to the control (18.72%). No difference was observed in the stained for the propidium iodide (necrosis marker) and LC3b protein (autophagy marker) in promastigotes treated when compared to control. In addition, AEPa presented antiproliferative activity on intracellular forms of L. (L.) amazonensis. Murine bone marrow cells (BMCs) treated with the concentration of 100 µg/mL by 96 hours before interaction and 24 hours after internalization of the parasite showed a reduction of 23.8%. However, cells treated before interaction showed a slight reduction of 4.2%. Furthermore, ultrastructural analysis by Transmission Electron Microscopy demonstrated that was not possible to observe the presence of structures apoptotic in the BMCs treated with AEPa and no cytotoxic effects were observed in the cells treated with AEPa. These results demonstrate that AEPa has antileishmanial properties and has no cytotoxic effects on BMCs. **Supported by:**CNPq (Brazil), CAPES (Brazil), INBEB (Brazil), FAPERJ and FAPESPA

TB60 - CYTOKINE PROFILE AND NITRIC OXIDE PRODUCTION AFTER IMMUNIZATION OF BALB/C MICE WITH *L. CHAGASI* HEPARIN-BINDING PROTEIN (HBP*LC*)

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Visceral leishmaniasis (VL) is a disease caused by the intracellular protozoan *Leishmania infantum/chagasi*. This protozoa exhibit a strong tendency to invade the viscera causing severe lesions, which may result in patient death if not treated. Molecules in the parasite, considered as virulence factors, have been intensively researched. Among them are heparin-binding proteins (HBP), glycoproteins that are related in many works of the literature with the process of adhesion between cells. The historical use of surface molecules of pathogens as vaccine antigens and the participation of these molecules in adhesion processes in the host cell give us the basis for the use of this protein in immunization experiments to evaluate this immunogenicity. Cytokines (IFN-γ, IL-4) and NO production were evaluated in supernatants of splenocytes from BALB/c mice immunized with *L. chagasi* HBP (HBP*Lc*), associated or not with Incomplete Freund's Adjuvant (IFA). For this purpose, BALB/c mice were divided in four experimental groups: Control, IFA, HBP*Lc* and HBP*Lc* + IFA. Mice were intraperitoneally immunized and submitted two times to booster doses using the same protocol of the first immunization. Two weeks after the second booster, the animals were euthanized and the spleen was collected for NO and cytokine analysis by ELISA. Our results showed that HBP*Lc* and HBP*Lc* + AIF groups showed an increase in IFN-γ and NO levels, with higher production in the group from mice immunized only with the protein, if compared with the group HBP*Lc*+AIF. Furthermore, the HBP*Lc* + IFA group showed a higher production of IL-4. These results show that HBP*Lc* is a candidate antigen for use in vaccine formulations. Additional experiments testing other adjuvants and evaluating the performance of protection against parasite challenge should be conducted to evaluate the use of HBP in the control of LV. **Supported by:**UFV, CAPES, FAPEMIG, CASADINHO-PROCAD/CNPq

TB61 - LEISHMANICIDAL EFFECTS OF A PHOSPHOLIPASE A2 ISOLATED FROM *CROTALUS VIRIDIS VIRIDIS* SNAKE VENOM.

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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 and pharmacological tools for drug development. This work is based in *Crotalus viridis viridis* snake (Cvv) phospholipase A_2 (PLA_2) and its effects over *L. 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 PLA_2 was collected and its protein content measured. *L. amazonensis* promastigotes were incubated in Schneider medium, with 0.3125 to 10 µg/ml PLA2 at 26ºC, and the effect on cell's proliferation was evaluated by daily counting with a Neubauer chamber. Parasites viability was assessed by flow cytometry using propidium iodide (PI) labeling. Infected peritoneal macrophages were cultivated in RPMI medium, with 0.625 to 2.5 µg/ml PLA_2. The percentage of infected cells was evaluated. Morphological alterations were examined by light and electron microscopy. Treatment of promastigotes and intracellular amastigotes resulted in growth inhibition soon the first 24 h. The data obtained allowed us to estimate the IC_{50}/24 h for promastigotes of 2.50 ± 1.42 µg/ml and for intracellular amastigotes of 0.67 ± 1.2 µg/ml. Treated promastigotes presented unusual cell body shapes, with loss of membrane integrity, corroborated by the 96.6% of PI-positive labeling. No cytotoxicity was detected over uninfected macrophage treated with 10 µg/ml and evaluated by MTS assay. We presented the employee of Cvv PLA_2 against *L. amazonensis*, which inhibited the parasites in vitro growth. This work presented that Cvv PLA2 can be object of further research for a new leishmanicidal agent. **Supported by:**CAPES, CNPq, FAPERJ, Pronex

TB62 - STRYCHNOBIFLAVONE FLAVONOID PURIFIED FROM STRYCHNOS PSEUDOQUINA IS EFFECTIVE AGAINST LEISHMANIA AMAZONENSIS AND LEISHMANIA INFANTUM

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Leishmaniasis are diseases caused by protozoan parasites of the genus Leishmania and endemic in about 98 countries worldwide. The World Health Organization has classified the disease as one of the top six most important neglected diseases in the world, due to its high morbidity and/or mortality. Treatment of disease has been based on the administration of drugs that present high toxicity, such as pentavalent antimonials, amphotericin B and pentamidine, so that the abandonment of the patient and the increasing number of cases of parasites´ resistance are important problems to be solved. In this work, secondary metabolites obtained from the Brazilian Cerrado was investigated for its possible application in the treatment of leishmaniasis. Strychnos pseudoquina stem bark extract was evaluated for its antileishmanial activity against Leishmania amazonensis and L. infantum. Additionally, a bioactivity-guided fractionation was carried out using an ethyl acetate extract of the plant, and two flavonoids were isolated as the main responsible for its antileishmanial activity. The in vitro assays using flavonoids were performed and the result showed that the strychnobiflavone isolated was effective against promastigotes forms of L. amazonensis and L. infantum; presenting IC50 values of 3.16 µM and 3.4 µM, respectively; and a selectivity index of 39.6 and 36.7, respectively. The results showed that macrophages infected and later treated with strychnobiflavone flavonoid presented reductions in the parasite number in the order 87% and 82% against L. amazonensis and L. infantum, respectively; and the tested compound showed a low toxicity in murine macrophages. The data presented here demonstrate the potential of the strychnobiflavone to be used as an alternative treatment of leishmaniasis. **Supported by:**FAPEMIG

TB63 - HUMORAL RESPONSE AND PROLIFERATION OF SPLENOCYTES FROM BALB/C MICE IMMUNIZED WITH *L. CHAGASI* HEPARIN-BINDING PROTEIN (HBP*LC*)

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Visceral leishmaniasis (VL) is a neglected disease that affects vital organs and, if untreated, can lead to death. Molecules in the parasite, considered as virulence factors, have been intensively researched. Among them are heparin-binding proteins (HBP), glycoproteins that are related with the process of adhesion between cells. Their use as stimulator of the immune system, inducing a protective response against subsequent infections, is high value target for the control of VL in human or other animals like the dog, an important reservoir of the disease, especially in urban areas. The historical use of subunits of pathogens as vaccine antigens gives us the basis for the use of this protein in immunization experiments to evaluate a possible immunogenicity, aiming to the control of VL. Thus, we evaluate the cell proliferation and the profile of the humoral immune response in BALB/c mice immunized with *L. chagasi* HBP (HBP*Lc*). For this purpose, BALB/c mice were divided in four experimental groups: Control, IFA (Incomplete Freund's Adjuvant), HBP*Lc* and HBP*Lc* + IFA. The mice were intraperitoneally immunized and submitted two times to booster doses using the same protocol of the first immunization. Before each immunization, blood was collected to obtain serum for the dosage of the antibody isotypes IgG1 and IgG2a. Two weeks after the second booster, the animals were euthanized and the spleen was collected for lymphoproliferation assay. Our results show that HPB*Lc* and HBP*Lc* + IFA groups showed an increase in cell proliferation. We observed increased production of specific IgG2a in sera from mice immunized with HBP*Lc*, and lower production of IgG1. These results show that HBP*Lc* is a candidate antigen for use in vaccine formulations. Additional experiments testing other adjuvants and verifying the performance of protection against parasite challenge should be conducted to evaluate the use of HBP in the control of LV. **Supported by:**UFV, CAPES, FAPEMIG, CASADINHO-PROCAD/CNPq

TB64 - PHASE I AND II CLINICAL TRIAL EMPLOYING COMMERCIALLY AND NEW VACCINE CANDIDATE AGAINST CANINE VISCERAL LEISHMANIASIS

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Human visceral leishmaniasis and canine visceral leishmaniasis (CVL) are highly prevalent in Latin American countries, especially in Brazil. Some authors suggest the use of an anti-CVL vaccine, as an important control measure for both human and canine infection, since dogs are reservoirs of the parasite. This study aims to evaluate the immunogenicity of vaccine prototype LBSap, in comparison to the commercial vaccines Leishmune® and Leish-Tec®, in a vaccine clinical trial phase I and II. For this, twenty-eight dogs were classified into four groups: i) control; ii) Leish-Tec®; iii) Leishmune®; (iv) LBSap groups received 600 µg of *L. braziliensis* promastigotes protein and 1 mg of saponin adjuvant. Our results of the immunogenicity vaccine demonstrate increase in circulating population of T $CD8^+$ lymphocytes in the end of the immunization protocol (T1) in groups LBSap and Leish-Tec®, as long as six months after experimental challenge (T2) in LBSap group. It was also observed in T1, an increase of B lymphocytes (Leishmune® group) and monocytes $CD14^+$ (LBSap and Leishmune® groups), that reinforce the potential immunoprophylactic of these vaccines. In the in vitro analyzes aimed to evaluating the percentage of antigen-specific $CD4^+$ and $CD8^+$ lymphocytes producers of IFN-γ e IL-4, were observed an increase in both IFN-γ producing subpopulations in the group LBSap, and it also showed an increase in IFN-γ producers in $CD8^+$ lymphocytes in the Leish-Tec® group. Our immunogenicity results support the hypothesis of the vaccine process, with the LBSap vaccine prototype generating a protective immune response against the *L. infantum* parasite.**Supported by:**FAPEMIG, CNPq, CAPES

TB65 - FUNCTIONAL ANALYSIS OF CYTOSOLIC TRYPAREDOXIN PEROXIDASE IN ANTIMONY-RESISTANT AND –SUSCEPTIBLE *LEISHMANIA BRAZILIENSIS* AND *LEISHMANIA INFANTUM* LINES

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Tryparedoxin peroxidase (TXNPx) participates in defence against oxidative stress as an antioxidant by metabolizing hydrogen peroxide into water molecules. Reports suggest that drug-resistant parasites may increase the levels of TXNPx and other enzymes, thereby protecting them against oxidative stress. In this study, the gene encoding cytosolic TXNPx (cTXNPx) was characterized in lines of *Leishmania (Viannia) braziliensis* and *Leishmania (Leishmania) infantum* that are susceptible and resistant to potassium antimony tartrate (Sb(III)). Northern blot and real-time reverse transcriptase polymerase chain reaction analyses revealed that the level of TXNPx mRNA was approximately 2.5-fold higher in the Sb(III)-resistant *L. braziliensis* line than in the parental line. In contrast, no significant difference in cTXNPx mRNA levels between the *L. infantum* lines was observed. Southern blot analyses revealed that the cTXNPx gene is not amplified in the genome of the Sb(III)-resistant Leishmania lines analysed. Functional analysis of cTXNPx was performed to determine whether overexpression of the enzyme in *L. braziliensis* and *L. infantum* lines would change their susceptibility to Sb(III). Western blotting analysis showed that the level of cTXNPx was 2 to 4-fold higher in transfected clones compared to non-transfected cells. Antimony susceptibility test (EC50 assay) revealed that *L. brazileinsis* lines overexpressing cTXNPx had a 2-fold increase in resistance to Sb(III) when compared to the untransfected parental line. In addition, these clones are more tolerant to exogenous hydrogen peroxide (H2O2) than the untransfected parental line. In contrast, no difference in Sb(III) susceptibility and a moderate index of resistance to H_2O_2 was observed in *L. infantum* clones overexpressing cTXNPx. Our functional analysis revealed that the enzyme tryparedoxin peroxidase is involved in the antimony-resistance phenotype in *L. braziliensis*.**Supported by:**CAPES

TB66 - MOLECULAR AND FUNCTIONAL ANALYSIS OF MEMBRANE PROTEIN AQUAGLYCEROPORIN 1 IN *LEISHMANIA* SPP. LINES SENSITIVE AND RESISTANT TO ANTIMONY

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Aquaglyceroporins (AQP1s) are membrane channels that permit transport of small neutral solutes. Previous studies have shown that a down-regulation of AQP1 is correlated with lower potassium antimony tartrate (SbIII) uptake, decreasing the drug concentration within SbIII-resistant Leishmania lines. In this study, the gene encoding AQP-1 has been characterized in lines wild-type and SbIII-resistant from four New World Leishmania species. We investigated the levels of mRNA and protein. In addition, we transfected the *L. braziliensis* and *L. guyanensis* lines with AQP-1 gene and analyzed the susceptibility of transfected parasites to SbIII and silver nitrate. Real-time reverse transcriptase polymerase chain reaction analyses revealed that the level of AQP-1 mRNA was lower in the SbIII-resistant *L. guyanensis* and *L. amazonensis* lines than in their respective wild-type counterparts. On the other hand, no significant difference in AQP1 mRNA levels between susceptible and SbIII-resistant *L. braziliensis* and *L. infantum* lines was observed. In accordance with these results, western blot analysis showed a down-regulation of AQP1 protein in the SbIII-resistant *L. guyanensis* and *L. amazonensis* lines, contributing for decreasing of SbIII entry in these lines. Functional analysis showed that in comparison to non-transfected lines, wild-type and SbIII-resistant *L. braziliensis* clones overexpressing AQP-1 protein are 4- and 5-fold more susceptible to SbIII, respectively. Increased susceptibility was also observed in wild- type (2.5-fold) and SbIII-resistant (1.5-fold) *L. guyanensis* lines overexpressing AQP-1. On the other hand, when the wild-type *L. braziliensis* and *L. guyanensis* clones overexpressing AQP1 were pre-treated with silver nitrate, an AQP inhibitor, these parasites become 10- and 2-fold more resistant to SbIII compared to non-treated parasites. Our functional analysis revealed that AQP1 is involved in the antimony-resistance phenotype in *L. braziliensis* and *L. guyanensis*. **Supported by:**capes

TB67 - EFFECTS OF KOJIC ACID, A LEISHMANICIDAL BIOPRODUCT, ON MONONUCLEAR CELLS OBTAINED FROM MURINE BONE MARROW

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The Leishmaniasis is a group of diseases caused by various species of protozoan parasites of the genus Leishmania that infects cells of mononuclear phagocytic system. The monocytes proliferation generated in the bone marrow and the differentiation in macrophages plays a key role against this protozoa. Chemotherapy is one of the most effective treatments for this disease. Although a number of antileishmanial drugs are available, these drugs are toxic, expensive and require long-term treatment. In this context, the research for drugs that enhance the innate immune response is needed to restore the homeostasis and the immune response. Kojic acid (KA), a secondary metabolite, synthesized by some species of fungi from Aspergillus genera, has several applications as food additive, cosmetics, antitumor agent, macrophage activator and has a great potential for the topic treatment of cutaneous leishmaniasis. Thus, the aim of this study is to evaluate the immunomodulatory effects of kojic acid (KA) in the bone marrow cells of mice. These cells were obtained by flushing femurs, and maintained in cultures treated with KA at the concentration of 50 and 100µg/mL for 24-96h of culture. It was observed by optical microscopy that KA promoted increased cell adhesion, spreading ability and high number of cytoplasmatic projections and vacuoles in cytoplasm of the mononuclear cells from bone marrow. To confirm these results, cytometer analysis of surface markers showed that the KA induced cell differentiation in the bone marrow when these cells were treated with KA for 96h. In addition, Akt pathway was analyzed by western blot. KA seems to be able to activate the Akt signaling pathway that has a critical regulatory role in cellular development, homeostasis and differentiation. Furthermore, no cytotoxic effects were observed in cells treated with KA when compared to the untreated bone marrow cells. Thus, KA is able to promote the differentiation of bone marrow monocytes into macrophages. **Supported by:**CAPES, CNPq/UFPa, INBEB, FAPERJ, FAPESPA, MCT/CNPq/FNDCT/PROCAD-NF CAPES

TB68 - IMMUNOMODULATORY EFFECT OF KOJIC ACID, A LEISHMANICIDAL AGENT, ON HUMAN MONOCYTES

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Leishmaniasis is one of the most important infectious diseases in the world. The chemotherapy is the only effective treatment for the disease, but these drugs are high cost, toxics and require a long period of treatment. The kojic acid (KA) is a secondary metabolite synthesized by species of fungi from Aspergillus, Penicillium and Acetobacter genera. KA was effective in the topical treatment of experimental cutaneous leishmaniasis and no cytotoxicity effect on murine macrophage. Thus, this study evaluated the action of KA in the differentiation of human monocytes into macrophages, major host cells of the protozoan Leishmania. Monocytes were obtained from buffy coats donated from Hemocenter Fundation of Para State. Cells were incubated with 50 µg/mL of KA for 48 hours. We observed that the treatment of monocytes induced morphological alterations, such as an increase in cell size, numerous cellular projections and increased organelle number. Ultrastructural analysis by Transmission Electron Microscopy demonstrated that treated cells showed an a high number of autophagic vacuoles and analysis by flow cytometry showed high LC3b expression, suggesting an autophagic process. In addition, immunofluorescence and flow cytometry demonstrated the higher expression of EMR1-F4/80 on the cell surface. The viability of the monocytes was also maintained after KA treatment. These results demonstrate a new role for KA as an immunomodulator agent, inducing the differentiation of monocytes into macrophages. This novel function for KA could be an effective immunochemotherapeutic strategy in leishmaniasis treatment. **Supported by:**CNPq (Brazil), CAPES (Brazil), INBEB (Brazil), FAPERJ and FAPESPA.

TB69 - COMBINATION THERAPY WITH 2'-HYDROXYFLAVANONE AND AMPHOTERCIN B, MILTEFOSINE AND POTASSIUM ANTIMONY TARTRATE AGAINST *LEISHMANIA AMAZONENSIS* PROMASTIGOTES.

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Caused by different parasites of genus Leishmania sp., Leishmaniasis has been reported in 98 countries and affects more than 12 million people around the world. Leishmaniasis current treatment is based on pentavalent antimonials, amphotericin B and miltefosine. However, they are expensive, ineffective and can bring resistance and side effects. The need for a cheaper and safer chemotherapy has been increasing researches for effective natural products. On the other hand, drug combination has been studying as a new alternative chemotherapy. 2'-hydroxyflavanone belongs to a class of flavonoids, the flavanones, known for their antitumor and anti-inflammatory properties. Previously, we demonstrated the effect of 2'-hydroxyflavanone against amastigotes and SbIII -sensitive and -resistant promastigotes of L. amazonensis with an IC50 of 21µM. The mechanism of action seems to involve mitochondrial damage and increase of ROS production. In this study we evaluate the combination of 2'-hydroxyflavanone and potassium antimony tartrate (SbIII), miltefosine and amphotericin B, reference drugs in leishmaniasis chemotherapy, against L. amazonensis promastigotes. At first, the IC50 of these drugs was determined. The IC50 value for amphotericin B, miltefosine and SbIII was 0.3µM, 30.7µM and 30µM respectively. Different concentrations of these drugs were tested in combination with 3, 6 and 12µM of 2'-hydroxyflavanone. Association with 3µM demonstrated an IC50 of 0.29, 16.85 and 35,8µM for amphotericin B, miltefosine and SbIII, respectively. With 6µM the IC50 was reduced for 0.24, 16.3 and 20.2µM. The 12µM concentration was able to reduce the IC50 for 0.07, 8,9 and 14,8µM. Together, these results suggest that combination with 2'-hydroxyflavanone can be promising in Leishmaniasis chemotherapy decreasing side effects and resistance. However, future experiments to determine the type of association, indicating synergism, antagonism or additive effect in vitro and in vivo should be performed. **Supported by:**FAPERJ; CNPQ; CAPES; IOC/FIOCRUZ

TB70 - NEW INSIGHTS INTO CUTANEOUS LEISHMANIASIS DUE TO *LEISHMANIA (VIANNIA) NAIFFI* IN MANAUS, AMAZONAS

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In the State of Amazonas, American tegumentary leishmaniasis (ATL) is endemic and presents a broad spectrum of clinical manifestations due, in part, to the large diversity of Leishmania species circulating in this region. The main species associated with human disease is *L. (Viannia) guyanensis*, followed by *L. (V.) braziliensis*. Occurrence of human infections associated to *L. (V.) naiffi* is considered rare and presenting benign clinical course, as well as good response to antimonial treatment. In this study we are reporting cutaneous leishmaniasis (CL) cases caused by *L. naiffi*. The patients were from the city of Manaus, and were attended at the FMTHVD outpatient clinic from 2011 to 2013. *Leishmania spp* isolated from skin lesion tissue fragments from 30 CL patients (aged 8-68 years) were characterized by multilocus enzyme electrophoresis. As expected, the most common species was *L. guyanensis* 66.7% (20/30). However, the frequency of infections caused by *L. naiffi* was 26.7% (8/30), showing that this specie is highly associated with CL in the studied region. The mean time of disease evolution of *L. naiffi* cases was 54 days. The majority of patients (66.6%) had only one skin-ulcerated lesion. No case associated to *L. naiffi* infection evolved to spontaneous cure, although self-cure was expect considering the literature. Furthermore, two patients experienced treatment failure after both antimonial and pentamidine therapeutic scheme. Our results is describing an increasing of *L. naiffi*-associated CL cases in Manaus, highlighting this expansion could be associated with a new scenario where this species may not have a self limiting nature as described previously and therapeutic failure can be expected. The present results reinforce the importance of well-conducted Leishmania spp characterization to infer on relevant epidemiological aspects. Further research in Amazonian region will provide more information on human ACL caused by *L. naiffi.* **Supported by:**CNPq, FAPERJ, IOC/FIOCRUZ, FAPEAM.

TB71 - TARGETED AND NON-TARGETED CATIONIC NANOPARTICLES LOADED WITH PLASMIDS EFFICIENTLY TRANSFECT CELLS AND ELICIT STRONG HUMORAL RESPONSES AGAINST BLOOD AND HEPATIC STAGE MALARIAL ANTIGENS

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Cationic liposomal nanoparticles are a new strategy for the delivery of antigens to avoid the use of recombinant viral vehicles, and DNA vaccines elegantly avoid hurdles in the production of difficult vaccine antigens. Herein, we conjugated both approaches encapsulating eucaryotic expression vectors encoding different plasmodial genes fused or not to the sequence encoding the major surface antigen of the hepatitis B virus (HBs). We then delivered them as unguided or guided (containing a specific antibody at its surface) DNA loaded nanoparticles to cells or used them for immunization in Balb/C mice. The genes encoding $Pvmsp1_{19}$, Pvcs and Pfrh5 fused to the HBs gene or unfused gfp and Pfrh5 were used. In some cases, targeting of nanoparticles was achieved by inserting antiCD4 and antiICAM1 antibodies post encapsulation. As a control, we encapsulated also a vector containing *Photinus* luciferase. After i.p. administration and using intravital microscopy, we observed that unguided or guided particles accumulated in Peyer´s patches and afterwards caused significant proliferation in inguinal lymph nodes. All imunizations generated very reproducibly significant antibody titers against the antigens against which they were administered (titers of up to $1{:}10^4$). Also, sera were able to recognize proteins in native parasite extracts in Western blots (PfRH5). Additionally, the purified IgG fraction of PfRH5-immunized mice showed 50% reinvasion inhibition at as low as 5 µg/ml IgG when tested in *Plasmodium falciparum in vitro* invasion assays which is comparable to results from other groups using adenoviral delivery systems. These results underline the potential of nanoparticle encapsulated DNA vaccines. **Supported by:**FAPESP, CNPq

TB72 - ANTI-LEISHMANIA ACTIVITY OF AN ENDOPHYTIC FUNGUS EXTRACT ISOLATED FROM HUMIRIA BALSAMIFERA.

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Endophytic fungi have been of great interest to the researches and pharmaceutical industry in recent years, including those with anti-protozoal action in species of the genus Leishmania. With the goal of using resources of the Restinga de Jurubatiba/Macaé in search of a new anti-cutaneous leishmaniasis drug, extracts of endophytic fungi isolated from regional plants were tested in anti-L. amazonensis promastigotes assay (0, 5, 25 and 50 µg/mL). The endophytic fungus extract isolated from the specie Humiria balsamifera (HB12b2) was the most active (IC50 4,6 µg/mL). Control vehicles did not have any activity. To analyze the antiamastigote activity, after 24 h of infection, infected macrophages were treated with HB12b2 (2,5; 5; 10 and 20 µg/mL) during 48 h at 37oC/5% CO2, and the number of amastigotes was valued in optical microscope (IC50 1,8 µg/mL). Significant cell toxicity occured in higher concentrations (10 and 20 µg/mL). Although nitric oxide is an important leishmanicidal molecule, HB12b2 did not induce its production. These results suggest that, although HB12b2 extract presented toxicity in higher concentrations tested, it has a good anti-leishmania activity and a low IC50 value. Also, its anti-parasite action works in a different way than nitric oxide production. So future fractionations of HB12b2 will be done aiming to maintain its anti-parasite activity and increase its therapeutic index.

TB73 - PREVALENCE AND GENOTYPING OF *GIARDIA LAMBLIA* IN CHILDREN AND DOGS OF RIO DE JANEIRO, BRAZIL

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Giardiasis is a zoonosis caused by an intestinal protozoa denominated *Giardia lamblia*. This disease affects mainly children with rates of 2-7% in developed countries and higher than 50% in developing countries. *G. lamblia* genotypes are classified to A to G, according to host that it infected. Genotype A infects humans and domestic animals (such as dogs and cats). Thus, this work focuses on *G. lamblia* genotype adapted to mammals in Rio de Janeiro. The aim was to determine the rates of *G. lamblia* prevalence and the circulating genotype in two populations: a daycare center and a canine population. It was collected 62 and 77 fecal samples of children that attend the Macacos' community (C1) and Salgueiro's community (C2) respectively. We also collected 60 samples from dogs in different neighborhoods. All samples were submitted to a fecal parasitological exam. DNA was extracted from positive samples for *G. lamblia* by using QIAamp DNA Stool mini kit and, subsequently, amplified by the β-giardina (β-GIA) coding gene. PCR products were purified and sequenced by using BigDye® Terminator Cycle Sequencing Kit. The chromatograms were analyzed and compared with existent nucleotide sequences from GenBank. The percentages of *G. lamblia* infection were: C1 - 21% (13/62), C2 – 49% (38/77) and dogs - 20% (12/60). All sequenced samples were grouped in the A1 genotype. In this study, the rate of prevalence in Rio de Janeiro varied between 20% and 50%. No *G. lamblia* dog-specific genotypes (C or D) were found. Our prelimary results suggest that genotype A is circulating in Rio de Janeiro, rising strong evidences for antropozoonotic cycles. The samples from C2 will be further sequenced. **Supported by:**FAPERJ, CNPq, POM/IOC

TB74 - *IN VITRO* AND *IN VIVO* EFFICACY OF QUERCETIN AGAINST *LEISHMANIA*

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We had already demonstrated that quercetin inibihited the growth of intracellular amastigotes of *Leishmania braziliensis* and its therapeutic activity *in vivo*. The aim of this work was investigate quercetin effect on ROS production by hamsters peritoneal macrophages infected with *L. braziliensis* and also its effect in Balb/c infected macrophages with *L. major*. Macrophage ROS production was measured by H2DCFDA and Amplex Red. The antiamastigote activity was perfomed using BALB/c peritoneal macrophages to *L. major* infection model. ROS production was increased when hamsters macrophages were treated with 100 and 50µg/ml of quercetin in a dose- and time dependent manner, without endangering the cell viability. ROS production on treated macrophages before L. braziliensis infection was increased when compared to controls, while macrophages treated before and after infection showed a decrease of ROS production. The increase of H2O2/ROS production was associated to the inhibition of *L. braziliensis* intracellular amastigotes, that seems be more effective when macrophages were treated before infection. Quercetin inhibited infection index macrophages infected with L. major s in 79% 53 and 44% at 100, 50 and 25 µg/ml, respectively. Cytokines were measured in the supernatants of Balb/c macrophages by cytometric bead array (CBA), showing a increase of IL-10 and a decrease of TNF-α, IL-6. In vivo Balb/c mice treated by oral route with quercetin was able to control de lesion size and parasite burden in relation to untreated controls ($p<0,05$). Taken together, our results suggest that quercetin can induce the production of ROS in hamsters peritoneal macrophages infected with *L. braziliensis*, and that this action mechanism can be also against *L. majo*r, belongs another subgenus of Leishmania. These dates suggesting that quercetin as promising candidate for Leishmaniasis chemotherapy. **Supported by:**CAPES E FAPERJ

TB75 - EVALUATION OF ACTIVITY OF QUINOXALINE 1,4-DI-*N*-OXIDE AND OXAZOLES DERIVATIVES ON *LEISHMANIA (LEISHMANIA) AMAZONENSIS* PROMASTIGOTES

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Leishmaniasis is a disease caused by parasitic protozoans of the genus *Leishmania*. It is endemic in more than 98 countries and 350 million people live in a risk of infection. In Brazil, *Leishmania (Leishmania) amazonensis* is responsible for most cases reported and is considered a species of epidemiologic importance. The control of this disease remains a problem, the current treatment is based on pentavalent antimonials, amphotericin B and miltefosine. However, they are expensive, ineffective and can bring resistance and side effects. Therefore, effective and non-toxic pharmacotherapy against this disease is necessary. Extensive studies of new molecules with leishmanicidal activity, including synthetic compounds, persists as a challenge in find new classes of effective molecules. It has been described that quinoxaline and oxazoles derivatives has a range of reported biological effects, including anti-protozoa activity. In the present study we reports the in vitro activity against *L. (L.) amazonensis* promastigotes of two series of derivatives of quinoxaline 1,4-di-*N*-oxide (series T) and oxazoles (series S). Promastigotes were incubated by 72 hours in the absence or in the presence of compounds and the cellular viability was determinate using Alamar Blue assay. The derivatives of quinoxaline 1,4-di-*N*-oxide, T72, T85, T73, T69 and T70 were more effective, presenting an IC50 value of 0.8µM, 0.83µM, 0.85µM, 0.9µM and 0.92µM, respectively . In oxazoles derivatives, the compounds S65, S73 and S74, showing an inhibition of 97% in the higher concentration (20µM) and an IC50 value of 2.9µM, 3.1µM and 5.67µM, respectively. In summary, we observed that of the 19 derivatives tested, 8 compounds have showed great activity in *L. (L.) amazonensis* promastigotes, encouraging us to performed new tests for the search of a candidate compound for the treatment of leishmaniasis. **Supported by:**FAPERJ; CNPQ; CAPES; IOC/FIOCRUZ

TB76 - EFFICACY THE CYCLOBENZAPRINE AGAINST EXPERIMENTAL LEISHMANIASIS: A TRANSLATIONAL STUDY

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The development of effective new drugs that target parasitic protozoa in humans is challenging. Differences in the clinical forms, pharmacokinetic requirements and pre-existing drug resistance increase the complexity of the problem. The emergence of cheaper, safer and orally available drugs is urgently needed. One strategy to accelerate this process is the well-known approach of "old drug, new use research". Tricyclic antidepressants have been reported to be active against Trypanosomatids. Based on this information, our group evaluated the effectiveness of cyclobenzaprine, a muscle relaxant structurally similar to tricyclic antidepressants, on experimental cutaneous and visceral leishmaniasis. Cyclobenzaprine showed activity against intracellular amastigotes of *L. infantum* and *L. amazonensis*, with IC_{50}=12.6 and 12.2µM, respectively. Then, BALB/c mice were infected with *L. infantum* and orally treated for five consecutive days (D21-25) with vehicle only, 6.16 or 12.32mg/kg of cyclobenzaprine. Miltefosine 20.55mg/kg was used as a positive control. The doses were calculated from the human posology, using the formula for dose translation based on body surface area. The animals were euthanatized 28 days post-infection. Cyclobenzaprine was active, resulting in a dose-dependent reduction of the parasitic load in the liver and spleen. From the parasitic load was possible to estimate the ED_{50} and ED_{90} in mg/kg in the target organs (liver ED_{50}=6.37 and ED_{90}=12.51; spleen ED_{50}=6.6 and ED_{90}=14.2). However, preliminary results on experimental cutaneous leishmaniasis with a dose of 3.08mg/kg/day showed no efficacy. This setback probably occurred due the specific pharmacokinetic requirements needed to treat this clinical form of leishmaniasis. Altogether, these results point out cyclobenzaprine as a promising drug for the treatment of visceral leishmaniasis and a future candidate for drug repurposing. **Supported by:**CAPES, PAPES/CNPq

TB77 - ESSENTIAL OILS OF MYRCIA OVATA CAMBESS. (MYRTACEAE) AND EREMANTHUS ERYTROPAPPUS (DC.) MACLEISH. (ASTERACEAE) EFFECTS IN LEISHMANIA AMAZONENSIS.

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Leishmaniases form a disease complex caused by species of protozoa belonging to Leishmania genus, affecting more than 12 million people worldwide, and for this reason areconsidered some of the major neglected diseases. Pentavalent antimonials are the first choice drugs to treat leishmaniases, however with the costs of presenting toxicity and collateral effects to mammal hosts. New bets as promising chemotherapy agents are natural products whose effects have shown to be satisfactory. In this study we tested essential oils OELM from Myrciaovata Cambess (Myrtaceae) and OEC from Eremanthus erytropappus (DC.) Macleish. (Asteraceae) against Leishmania amazonensis in vitro. We observed the effect of the oils activity against promastigote forms growth at 5, 10, 20 and 30 µg/ml concentrations in a dose-dependent manner. The IC50 after 4 days of treatment was 8.69 µg/ml and 9.53 µg/ml for each oil respectively. Ultrastructure analyses indicate morphological changes with mitochondria swelling and disorganized nucleolus as well as lipid accumulation or lipid disorganization in parasite promastigotes treated with both oils. At OELM treated parasites, we also observed the presence of suggestive autophagosomes. In order to further investigate the effects in parasite lipid metabolism, we submitted OEC and OELM treated promastigotes to gas chromatography-mass spectrometry. The results are being analyzed. Additionally, we evaluated the citotoxicity effect of the oils to mice peritoneal macrophages analyzed by XTT mitochondrial functionassay. The CC50 in this assay was 29.2 µg/ml and 16.5 µg/ml for OEC and OELM, respectively. Currently, we are evaluating the capacity of OELM and OEC to interfere with L. amazonensis amastigote intracellular forms internalized by mice macrophages. **Supported by:**CNPq/FAPERJ

TB78 - A COMMON EIF4E BINDING MOTIF IS PRESENT IN TWO TRYPANOSOMATIDS HOMOLOGUES OF THE TRANSLATION INITIATION FACTOR EIF4G

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A checkpoint in the gene expression in eukaryotes is the initiation stage of protein synthesis, whereas the complex eIF4F (formed by the subunits eIF4A, eIF4E and eIF4G) performs a crucial role by facilitating the recognition of the mRNA by the ribosomes. eIF4G is a scaffolding protein which binds to eIF4A and eIF4E. Previously, two eIF4G homologues identified in trypanosomatids, EIF4G3 and EIF4G4, were seen to have different eIF4E binding partners. EIF4G3 binds to EIF4E4, whilst EIF4G4 binds to EIF4E3 and these interactions are mediated by the short N-termini of the two eIF4G homologues. Here, the two interactions between EIF4G/EIF4E were further investigated. First, a sequence comparison of several trypanosomatid EIF4G3 and EIF4G4 orthologues identified conserved residues in their N-terminal regions. Site-directed mutagenesis was then used to generate recombinant Leishmania major EIF4G3 and EIF4G4 carrying mutations targeting likely binding sites for their eIF4E partners. Binding interactions were then tested through pull-down assays. For EIF4G3, single mutations in two neighbouring residues, I8A and R9A, impaired its interaction with EIF4E4. In contrast, single mutations in the equivalent positions in EIF4G4 (I25A and L26A) did not interfere with its binding to EIF4E3, but this interaction was abolished when both residues were simultaneously mutated (IL25-26AA). The equivalent double mutations in EIF4G3 and EIF4G4 were analysed through overexpression in Trypanosoma brucei procyclic cell lines. Upon transgene induction, cultures expressing the mutants showed only a minor reduction on cell growth. As expected, mutations in residues IL9-10AA of T. brucei EIF4G3 prevented its interaction with EIF4E4 and the equivalent mutations in EIF4G4 (LL27-28AA) impaired EIF4E3 binding. The identified residues from both eIF4G homologues reflect a common eIF4E binding mode with a consensus similar, but distinct, to the consensus described for higher eukaryotes. **Supported by:**CAPES, CNPq, FIOCRUZ and FACEPE

TB79 - IN VITRO LEISHMANICIDAL ACTIVITY OF SYNTHETIC DERIVATES OF ALDIMINES AND 1,4-DIHIDROPIRIDINE AGAINST L. INFANTUM

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Visceral leishmaniasis (VL) is a fatal infectious disease if non treated. Ninety percent of VL cases occur in India, Bangladesh, Sudan, Nepal, and Brazil. The conventional drugs for treatment of VL have limitations including unresponsiveness, relapse, specific toxicities and parenteral administration lasting for long durations. Moreover, they are less effective in HIV–VL-coinfected patients. These issues also come with the isolation of resistant parasites to all drugs available, what makes so important the drug screening studies for new alternatives for leishmaniasis treatment. In this study, we synthesized 7 aldimines derivatives and 5 1,4-dihidropiridine derivates and analyzed these compounds about the leishmanicidal activity against L. infantum promastigotes, cytotoxity against DH8 canine cells and THP1 human cells. These activities were performed using MTT assay and calculating the IC50% (ug/mL), CC50% (ug/mL) and expressing the SI for each chemical compound. Our results showed that, with the exception of drug 3H9, all tested synthetic compounds showed moderate leishmanicidal activity. The aldimines 3H8 (IC50%: 4.46) and 3H7 (IC50%: 4.08) and the 8B5 1,4-dihidropiridine derivate demonstrated (IC50%: 3.44) to be highly active against Leishmania infantum promastigotes. The compounds 8B6 (IC50%: 0.47) and 8B7 (IC50%: 0.03), both 1,4-dihidropiridine derivates, very high activity against these parasites, similar to the hight effective drug Amphotericin B (IC50%: 0.02). **Supported by:**UFOP, FAPEMIG, CNPq, PPSUS/MS and DECIT/MS

TB80 - PTEROCARPANQUINONE LQB-118 INDUCES APOPTOSIS IN *LEISHMANIA (VIANNIA) BRAZILIENSIS* AND CONTROLS LESIONS IN INFECTED HAMSTERS

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Previous results demonstrate that the hybrid synthetic pterocarpanquinone LQB-118 presents antileishmanial activity against L.amazonensis in a mouse model. The aim of the present study was to use a hamster model to investigate whether LQB-118 presents antileishmanial activity against *Leishmania (Viannia) braziliensis*, which are the main species related to American tegumentary leishmaniasis. Promastigotes were treated with LQB-118 for 48h at 5-20µM/28°C and the number of parasites were counted in a Neubauer chamber. Peritoneal macrophages of hamsters were infected and incubated with LQB-118 for 48h/37ºC/5%CO2. The survival of intracellular amastigotes was evaluated by the ability of the parasite differentiate into promastigotas after removal of LQB-118 and reincubation with Schneider's. The cell death induced by LQB-118 in the *L.braziliensis* promastigotes was analyzed using an annexin V-FITC/PI kit and the in situ labeling of DNA fragments by TUNEL was used to investigate apoptosis in the intracellular amastigotes. Infected hamsters were treated starting seven days after infection with intralesionally administered LQB-118 (26µg/kg/day,three times a week) or orally (4,3mg/kg/day,five times a week) for eight weeks. LQB-118 was active against intracellular amastigotes and promastigotes *L.braziliensis*, resulting in IC50 values of 3.4±0.1 and 7.5±0.8µM,respectively. LQB-118 induced promastigote phosphatidylserine externalization and intracellular amastigote DNA fragmentation without affecting the viability of macrophages. The treatment of *L.braziliensis*-infected hamsters with LQB-118,either orally or intralesionally,was effective in the control of lesion size,parasite load and increase intradermal reaction to parasite antigen. Taken together,these results show that the antileishmanial effect of LQB-118 extends to *L.braziliensis* in the hamster model,involves the induction of parasite apoptosis and shows promising therapeutic option by oral or local routes in leishmaniasis. **Supported by:**CAPES

TB81 - SELENIUM METABOLISM IN TRYPANOSOMATIDS

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Selenoproteins are characterized by the incorporation of at least one amino acid selenocysteine (Sec-U) encoded by in-frame UGA stop codons. Although not a ubiquitous pathway in all organisms, it was also identified in several protozoa, including the Kinetoplastida. Previous reports demonstrated that Trypanosoma brucei selenophosphate synthetase (SPS), central enzyme in the selenocysteine synthesis, is required under sub-optimal growth conditions, suggesting probable role in oxidative stress protection of the parasite and its absence severely hampers the parasite survival in the presence of an oxidizing environment. The presenting work demonstrated growth defects for the both forms of T. brucei under normal growth conditions and treatment with stressors of endoplasmic reticulum, tunicamycin and DTT, reduced the viability of tetracycline-induced RNAi lineages, although no increase of BiP expression was observed. Complex purification using SPS fused to PTP-tag demonstrated no stable interaction with other proteins and Indirect Immunofluorescence assay showed cytoplasmic localization, but is possible to observe not conventional pattern, with presence of dots along of the cytoplasm. Selenoprotein T (SelT) is not required to procyclic growth and it lack in bloodstream form presented slight growth reduction. Treatment with ER stressors also reduced SelT RNAi lineage and no increase of BiP expression was observed. The data suggest which selenoproteins participation in the ER defense, but not direct activation of the Unfolded Protein Response (UPR). SelT single knock-out in Leishmania amazonensis no presented decrease of promastigote viability nor increase of cell sensibility of stressors of ER. Small reduction of amastigote growth inside of macrophages was observed. New experiments are being planned to evaluate the participation of selenoproteins in other steps of endoplasmic stress response, such as Ca+2 homeostasis. **Supported by:**FAPESP

TB82 - ANTI-LEISHMANIA ACTIVITY OF *VERNONIA CRETONOIDES* EXTRACT.

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Leishmaniasis is a major public health problem, classified as a neglected disease according to WHO. Although its high mortality, the current clinical treatment is invasive, toxic and has a high cost. Our group have been working in find new bioactive molecules extracted from plants of Restinga de Jurubatiba / Macaé-RJ against cutaneous leishmaniasis. Nine extracts were tested in an anti-*L. amazonensis* promastigotes assay (0, 5, 25 and 50 µg/mL). The extract from the specie *Vernonia cretonoides* was the most active (IC_{50} 9 µg/mL). To analyze the antiamastigote activity, after 24 h of infection, infected macrophages were treated with *V. crotonoides* extract (2,5; 5; 10 and 20 µg/mL) during 48 h at 37°C/5% CO_2, and the number of amastigotes was valued in optical microscope (IC_{50} 2,6 µg/mL). Significant cell toxicity occured in the higher concentration (20 µg/mL). Although nitric oxide (NO) is an important leishmanicidal molecule, the *V. crotonoides* extract treatment did not induce its production. These results indicate that *Vernonia crotonoides* extract may be a potential anti-cutaneous leishmaniasis drug, killing promastigotes and amastigotes without significant cell toxicity, and that its effect seems to occur in a way other than NO production.

TB83 - BIOLOGICAL CHARACTERIZATION OF *LEISHMANIA AMAZONENSIS* TRANSFECTED WITH GFP AND RFP FLUORESCENT PROTEINS.

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The development of new therapies for leishmaniasis requires faster and more sensitive methodologies for in vitro and in vivo drug and vaccine testings. In that sense, parasites expressing reporter genes have served as a valuable tool. Our group has been using a green-fluorescence L. amazonensis expressing GFP (La-GFP), which has been successfully used for rapid assessment of parasite loads, but not for intravital imaging due to the autofluorescence in the green spectrum displayed by several murine tissues. For this end, a red-fluorescent L. amazonensis expressing mCherry (La-RFP) was produced by our group. This work aims to study the La-RFP potential as a tool for biological in vitro and in vivo tests, including intravital imaging, compared to the La-GFP. In vitro results showed that in the absence of geneticin (G418) antibiotic there was no difference between the promastigote growth rate for La-GFP and La-RFP. Both La-GFP and La-RFP standed G418 concentrations as high as 2000 µg/mL, but selected La-GFP parasites displayed fluorescence intensity 10 times greater than La-RFP. When tested for plasmid stability, after 20 days of culture in G418-free medium, the percentage of fluorescent parasites was 60% for La-GFP versus 5% for La-RFP. Although both La-GFP and La-RFP parasites were similarly infective to macrophages, they were slightly less infective than parental La-WT. As for in vivo infectivity to BALB/c mice, again they were similarly infective to each other, nevertheless both were less infective than La-WT. We conclude that in the conditions tested, the generated La-RFP is not suitable for in vivo imaging, once its fluorescent is not stable and less bright than La-GFP. Moreover, the La-GFP present in the laboratory is still the best tool for our methods applied, since its fluorescence is more intense and stable than the La-RFP. **Supported by:**CNPq, FAPERJ

TB84 - LOADING OF AN ANTILEISHMANIAL CHALCONE IN PLGA MICROPARTICLES DOES NOT LEAD TO INCREASED DRUG UPTAKE BY INFECTED MACROPHAGES IN VITRO

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We have shown that loading of an antileishmanial chalcone (CH8) in biodegradable PLGA microparticles (PLGA-CH8) at 10% ratio increases its efficacy in the subcutaneous treatment of murine cutaneous leishmaniasis caused by *Leishmania amazonensis* (accompanying abstract). In the present study, we investigated in vitro whether or not the increased activity was due to increased microparticle uptake by macrophages. Thus, *L. amazonensis* - infected bone marrow-derived macrophages were incubated during 48 h with different concentrations of CH8 either in the free form or as PLGA-CH8, and were then assayed for intracellular parasite growth. We found that parasite growth was inhibited more with free CH8, when compared to untreated cells or cells treated with PLGA-CH8. To compare drug uptake, at the end of incubation time the cell monolayers were extensively washed with PBS, and lysed in acetonitrile for CH8 quantification by HPLC. We found that despite the significant phagocytosis of PLGA-CH8 microparticles, a lower intracellular concentration of CH8 was found in macrophages treated with PLGA-CH8 when compared with free CH8, in agreement with its lower antileishmanial activity. To compare the capacity of free CH8 and PLGA-CH8 to activate macrophage microbicidal mechanisms, the production of NO was measured in the cell supernatants by the Griess method and ROS by H_2DCFDA dye. We found that both CH8 forms reduced NO production by uninfected macrophages, while they were of no effect in infected cells. Similarly, none CH8 forms altered ROS production, regardless of the macrophages being infected or not. These results indicate that in the conditions tested, encapsulation of CH8 in PLGA microparticles does not lead to increased antileishmanial activity *in vitro*. Therefore, the observed increased *in vivo* efficacy of PLGA-CH8 may be due to sustained drug release in the dermis rather than to increased phagocytosis of the particulated drug by infected macrophages. **Supported by:**CNPq / CAPES / GlaxoSmithKline

TB85 - EVALUATION OF ACTIVITY OF *N*-TOSIL AZA-PTEROCARPAN DERIVATIVES AGAINST *LEISHMANIA INFANTUM*: STRUCTURE-ACTIVITY RELATIONSHIP STUDY

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Treatment of leishmaniasis receives low investment from the pharmaceutical industries, requires long periods of administration and presents low efficacy, resistance, toxicity and high cost. Previously, our group showed the leishmanicidal activity of pterocarpanquinones and aza-pterocarpanquinones. In particular, LQB-223, an *N*-tosyl-aza-pterocarpan, represented an interesting scaffold for the development of new antineoplasic and antiparasite compounds. The purpose of this study is to evaluate *N*-tosyl-aza-pterocarpan derivatives based on LQB-223, against promastigotes and amastigotes of Leishmania infantum and their toxicity in murine macrophages. Promastigotes were incubated with LQBs by 72h and the parasite viability was analyzed by MTT. The activity on intracellular amastigotes was evaluated by light microscopy in *L. infantum* infected murine macrophages after incubation with LQBs for 72h. Toxicity was evaluated in murine macrophages by MTT assay after 72h of incubation. The new derivatives differ from LQB-223 in two major characteristics: the substitution of the oxygen by a nitrogen in the ring 3 and the replacement of the last aromatic ring by a methylic ester group. In tests of activity, the addition of a *p*-toluenesulfonic group caused the molecule LQB-331 to be less effective on promastigotes (144.6µM) compared with LQB-330 (43.5µM). However, LQB-331 was better than LQB-330 in inhibiting intracellular amastigotes (20.5µM x 47.6µM). The same happened with the addition of a methyl group on LQB-333, that shown IC_{50} of 70.8µM and 21.5µM, against promastigotes and amastigotes, respectively. LQB-330 presented a good toxicity profile, with LD_{50} of 203.4µM in murine macrophages. LQB-331 and LQB-333 were even less toxic to macrophages, with LD_{50} of 359.0µM and 323.5µM. The prototypes with better selectivity index (SI) were LQB-331 and LQB-333, with SI equal to 17.5 and 15.0, respectively. This SAR study reveals two promising molecules to perform further in vivo studies. **Supported by:**FAPERJ; CNPq; CAPES; IOC/FIOCRUZ

TB86 - LOCALIZED TREATMENT OF CUTANEOUS LEISHMANIASIS WITH PLGA MICROPARTICLES LOADED WITH CHALCONE CH8

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Despite its skin localization, conventional therapy of cutaneous leishmaniasis (CL) is based on multiple parenteral injections with systemically toxic drugs. Aiming at a localized therapy, we have used biodegradable systems for sustained antileishmanial drug release in the skin dermis. For that, poly-(lactide-co-glycolide) PLGA microparticles loaded with 10 % of the lipophylic chalcone CH8 were prepared by multiple emulsion and solvent evaporation methods, and measured 6.2 µm of diameter and had a zeta potential of -12.5 mV. When tested in vitro on Leishmania amazonensis-infected macrophages, CH8/PLGA promoted higher parasite killing than free CH8 in a manner independent of NO and ROS activation and macrophage cytotoxicity. In vivo, their efficacy was tested in BALB/c mice infected in the ear with L. amazonensis-GFP. On days 9, 16 and 23 of infection the animals received at the infection site a subcutaneous implant of CH8/PLGA containing 30 µg of CH8. Controls received free CH8, empty PLGA, 30 µg of Glucantime, or 10 µl of PBS alone. Treatment efficacy was monitored by measuring the ear tickeness throughout infection and parasite loads by LDA and fluorometry on days 30 or 90 of infection. Systemic toxicity was evaluated by AST, ALT and creatinine serum levels. Tissue inflammation and implant degradation were monitored by histopathology kinetics. We found that CH8/PLGA treatment was more effective and durable than free CH8 or Glucantime in controlling lesion and parasite growth. No signs of toxicity were detected. Moreover, a single dose with CH8/PLGA on day 9 was as effective as 3 doses with free CH8. Although empty PLGA had no antileishmanial efficacy, PLGA implants caused a transient ear inflammation that was resolved. These findings show that PLGA microparticles promoted a sustained chalcone CH8 release at the lesion site, with a durable and safe therapeutic effect, supporting its use for localized treatment of CL. **Supported by:**CNPq

TB87 - SYSTEMIC RESPONSE OF IMMUNOTHERAPY WITH LBMPL VACCINE FOR VISCERAL LEISHMANIASIS – A PRECLINICAL STUDY USING SYMPTOMATIC DOGS AS A MODEL FOR HUMAN DISEASE

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Human visceral leishmaniasis (HVL), caused by *L. infantum*, is the most fatal form of leishmaniasis. Dogs are extremely susceptible to infection presenting clinical, pathological and immunological alterations very similar to human disease and are considered the most important model for evaluation of new treatment strategies (immunotherapy and immunochemotherapy). Recently our group aimed to characterize the immunotherapeutic effect of the vaccine composed by *L. braziliensis* antigens plus monophosphoryl lipid A (LBMPL) on HVL using dogs naturally infected as experimental model. Herein, it was proposed to analyze the clinical, hematological, biochemical and the immune response (*ex vivo*) in symptomatic dogs submitted to three series of immunotheraphy with LBMPL. Our major results showed that after immunotherapy with LBMPL the dogs presented a strong and sustained improvement of clinical signs/symptoms with significant gain of body weight. When assessed the complete blood count it was observed that all parameters, emerged normal at the end of immunotherapy (especially erythrogram). In addition, after immunotherapy dogs showed normalization of main biochemical parameters such as urea, creatinine, AST and bilirubin. When we evaluated the immune response, ex vivo, we observed increased counts of T-$CD3^+$ circulating lymphocytes synchronous with T-$CD4^+$ and T-$CD8^+$ subsets. On the other hand, we observed a decrease of $CD21^+$ B cells after completed the immunotherapeutic protocol. Furthermore, increased counts of NK cells ($CD5^-CD16^+$) and $CD14^+$ monocytes were observed in animals after immunotherapy. When we evaluated the ratio of T-cells/B-cells, it could be observed an increase at the end of experiment in this ratio. On the other hand, decrease in the T-$CD4^+$/T-$CD8^+$ ratio after completed the immunotherapy was observed. Taking together, our findings support a therapeutic potential of the LBMPL vaccine against visceral leishmaniasis using dogs as experimental model of immunotherapy. **Supported by:**FAPEMIG, CNPq, UFOP, Rede Mineira de Bioterismo, Rede TOXIFAR.

TB88 - EVALUATION OF PARASITEMIA AND PERIPHERAL BLOOD OF MICE INFECTED BY TRYPANOSOMA CRUZI Y STRAIN UNDERGOING TREATMENT WITH BENZNIDAZOLE (BZ) AND/OR PROTEASE INHIBITOR BOWMAN BIRK TYPE (BBI) DURING THE ACUTE PHASE OF CHAGAS DISEASE

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The benznidazole (N - benzyl- 2 – nitroimidazole acetamide BZ) is the unique drug used in human Chagas disease treatment in Brazil. Knowing that the immune system mobilization in Chagas' disease is important in reducing the parasite load, but contributes to the onset of clinical manifestations, it is necessary to seek a compound able not only to eliminate the parasite, but also to reduce inflammation caused by it. Proteases have been selected as a target in the development of new drugs and anti chagasic studies have demonstrated that certain protease inhibitors have trypanocidal action and reduce inflammation and tissue injury. In this way, 120 Swiss mice were distributed into five groups: non-infected and untreated, infected and untreated, infected and treated with BBI, infected and treated with BZ, infected and treated with BBI and BZ, and then parasitemia and the phenotypic profile of the peripheral blood was evaluated. By evaluating the parasitemia curve was observed that BBI group showed the highest peak of parasitaemia, compared to the groups CI, BZ and BZ/BBI. The pre-patent period was 6 days for all groups, and the patent period was significantly higher for animals of BBI and CI group compared to groups BZ and BZ/BBI. Regarding the haematological profile, it was observed that treatment with BBI induced an increase of neutrophils in animals of this group compared to the others on day 20 after infection. Finally, to perform the immunophenotype of peripheral blood cells was observed that on day 30 there was an increase in CD4+ cells in animals treated with BBI and associated BZ when compared to animals treated with BZ. Thus, despite treatment with BBI have not reduced parasitemia, induced changes in the immune response consistent with a possible improvement of cardiac histopathology, so that evaluations of the inflammatory process is needed. **Supported by:**Fapemig; CNPq; CAPES