TB17 - **MILTEFOSINE SUSCEPTIBILITY OF ISOLATES OF *LEISHMANIA (LEISHMANIA) INFANTUM* FROM DOGS OF THE MUNICIPALITY OF EMBU-GUAçU, BRAZIL**

FERREIRA, B.A.*[1]; YAMASHIRO-KANASHIRO, E.H.[2]; ROCHA, M.C.[2]; COTRIM, P.C.[2]; COELHO, A.C.[1]
1.UNICAMP, SP, Brazil; 2.USP, SP, Brazil.

Visceral leishmaniasis (VL) is a parasitic disease caused by the protozoan *L. (L.) infantum.* The disease is the most severe clinical form of leishmaniasis that can lead to death if it is not treated. In Brazil, about 3,000 new cases of the disease are reported annually, with an increasing number of cases in urban and peri-urban areas. Since VL is zoonotic in Brazil, domestic dogs constitute the main reservoir for the parasite, playing an essential role in transmission of disease to humans. The treatment of VL in Brazil consists in the use of pentavalent antimonials and amphotericin B, drugs that are considered expensive, toxic and that require parenteral administration. In canine visceral leishmaniasis (CVL), the only drug used for treatment in Brazil is miltefosine, a drug already used in the treatment of VL in Southeast Asia with effectiveness rate higher than 90%. In this study, we aim to evaluate the susceptibility to miltefosine *in vitro* of isolates of *L. (L.) infantum* from dogs of the municipality of Embu-Guaçu, located in the metropolitan region of the city of São Paulo. Isolates were previously typed by Instituto Oswaldo Cruz, FIOCRUZ, RJ, Brazil and then confirmed by polymerase chain reaction (PCR) of *hsp70* gene followed by digestion with the restriction enzyme *HaeIII* as previously described (Montalvo *et al*., 2012). The *in vitro* susceptibility of isolates to miltefosine in promastigote form were determined by calculating the EC_{50} and EC_{90} values. The EC_{50} values of miltefosine against promastigotes ranged from 6.5 ± 0.9 to 34.14 ± 1.56 µM. These findings suggest a moderate variation in miltefosine susceptibility of these isolates from dogs. Our next goal is to determine the EC_{50} values for the intracellular amastigotes. The results obtained in this study will contribute to evaluate the potential of miltefosine against isolates of *L. (L.) infantum* from domestic dogs, the most important reservoir of VL in urban areas in Brazil. **Supported by:** FAPESP. **Supported by:**FAPESP **Keywords:** Visceral leishmaniasis; leishmania infantum; miltefosine

TB18 - **MODIFIED LUCIFERASES AS A TOOL FOR PARASITE BURDEN DETERMINATION IN *LEISHMANIA AMAZONENSIS IN VITRO* AND *IN VIVO***

AGOSTINO, V.S.*[1]; TRINCONI, C.M.[1]; PRICE, H.P.[2]; ULIANA, S.R.B.[1]
1.ICB-USP, SP, Brazil; 2.KEELE UNIVERSITY, England.

Treatment of leishmaniasis relies on a few drugs presenting multiple shortcomings, and that makes the discovery of new alternatives a necessity. Drug efficacy in pre-clinical tests using animal models of leishmaniasis is classically based on the determination of parasite burden, measured through limiting dilution, a technique that requires animal euthanasia. Parasite expression of luciferases has been shown to be a reliable alternative for parasite burden determination, since it allows the detection of bioluminescence in a live animal. Modified enzymes have been developed in an attempt to improve light production. The aim of this work was to generate and characterize mutant lines of Leishmania amazonensis (La) expressing three different modified luciferases: NanoLuc (NL), NanoLuc-PEST (NLP) and RedLuc (RED) and compare those with the conventional firefly luciferase, Luc2. Mutants of L. amazonensis expressing those modified luciferases were previously obtained and their luminescence was evaluated based on serial dilutions spanning 1 to 106 promastigotes. Luminescence was 100 to 1000-fold higher in La-NL as compared with the other lines. The mutant lines retained the same susceptibility to amphotericin B as the parental line, as determined by the MTT assay and bioluminescence detection. Unexpectedly, La-NLP showed an unstable luminescence curve, making it impossible to determine the IC50. BALB/c mice were infected with La-NL and La-RL, in order to compare the bioluminescence of the mutants. Even though mice infected with La-NL showed at least 10-fold higher parasite burden than the ones infected with La-RL, luminescence detected from La-RL was 1000-fold higher. The data suggests that RL is the best option for in vivo imaging, while NL would be better when employed for in vitro experiments, since we were able to detect less than 10 La-NL parasites. In vivo comparisons with Luc2 are in progress. **Supported by:**FAPESP, CNPq **Keywords:** Leishmaniasis; luciferase; reporter gene

TB19 - **PAROMOMYCIN SUSCEPTIBILITY OF BRAZILIAN CLINICAL ISOLATES OF *LEISHMANIA* SPP. RESPONSIBLE FOR TEGUMENTARY DISEASE**

COSER, E.M.*[1]; YAMASHIRO-KANASHIRO, E.H.[2]; LINDOSO, J.A.L.[2]; COELHO, A.C.[1]
1.UNICAMP, SP, Brazil; 2.USP, SP, Brazil.

Leishmaniasis is a parasitic disease considered neglected by the World Health Organization, with restricted measures of control and treatment. In Brazil, the number of cases of disease has increased in recent years, especially in urban areas. The treatment of leishmaniasis in Brazil is limited to the use of parenteral drugs that are expensive and induce serious side effects. The drugs available to treatment are pentavalent antimonials and amphotericin B. Recently, two drugs were approved as alternatives to the treatment of visceral leishmaniasis in Asia: miltefosine and paromomycin, however these drugs have not yet been approved for use in Brazil. Here we aim to evaluate the susceptibility to paromomycin *in vitro* of clinical isolates of *Leishmania* spp. from patients with tegumentary leishmaniasis of the State of São Paulo, as well as the effectiveness of paromomycin *in vivo* in a murine experimental model of infection of two species that cause cutaneous disease in Brazil: *Leishmania braziliensis* and *L. amazonensis*. Typing of 11 clinical isolates by polymerase chain reaction (PCR) of *hsp*70 gene followed by digestion with the restriction enzyme *Hae*III indicated that species of these clinical isolates are *L. braziliensis* (4 isolates) and *L. amazonensis* (7 isolates). *In vitro* susceptibility of these isolates to paromomycin in promastigote form were determined by calculating the EC50 and EC90 values. The EC50 values of paromomycin against promastigotes ranged from 29.3 to 54.36 mM. The EC50 values for the reference strains of these two species were 145.23 and 27.79 mM for *L. amazonensis* and *L. braziliensis* respectively. Our next goal is to determine paromomycin susceptibility in the intracellular amastigote form of these clinical isolates and evaluate the effectiveness of this drug *in vivo* against the two main species responsible for the tegumentary leishmaniasis in Brazil. **Supported by:**FAPESP **Keywords:** Paromomycin; leishmania amazonensis ; leishmania braziliensis

TB20 - **PEPTIDES TARGETING *TRYPANOSOMA CRUZI* EPIMASTIGOTE SURFACE.**

SAENZ GARCIA, J.L.*[1]; YAMANAKA, I.B.[1]; PACHECO-LUGO, L.A.[1]; MIRANDA, J.S.[1]; BRANT, R.S.C.[1]; CAETANO, C.S.[2]; AVILA, R.A.M.[2]; DE MOURA, J.F.[1]; DAROCHA, W.D.[1]
1.UFPR, PR, Brazil; 2.UNESC, SC, Brazil.

Chagas disease is transmitted by insect vectors, approximately 6 to 8 million people are affected and another 20 million people are at risk of contracting the disease by residing in vulnerable areas. The lack of new medicines in the market, clinical resistance presented by some strains and the inability to develop a vaccine requires the search of new strategies to reduce the transmission and prevalence of the disease. To that end, molecules that bind to protozoon parasite surface have been developed, some based on antibodies such as scFv and nanobodies, which bind on the surface of the parasite and can be conjugated with drugs or lytic molecules. Another promising strategy is the peptide libraries based on phage display, recombinant bacteriophages expressing fused capsid surface peptides are exposed to cells, allowing to find binders, which can be used for diagnosis or treatment. Our research group used two libraries of bacteriophages, LX8CX8 (17 aa) and LX15 (15 aa). These libraries were incubated with epimastigote forms of *T. cruzi*, allowed to isolate bacteriophages targeting cell surface. The binding of one peptide from LX8CX8 was confirmed by ELISA, confocal microscopy and cytometry. From LX15, 30 clones were sequenced allowing the identification of 13 peptides, which also showed some degree of conservation. In silico analyzes to detect molecular mimicry identified one family of insect proteins that may bind to epimastigote surface. However, these results must be validated in vitro, and the peptides will be engineered for paratransgenesis assays. **Supported by:**CAPES/CNPQ **Keywords:** Peptides; phage display; trypanosoma cruzi

TB21 - **INVESTIGATION OF SUSCEPTIBILITY TO PAROMOMYCIN IN ISOLATES OF *LEISHMANIA (LEISHMANIA) INFANTUM***

OLIVEIRA, V.L.*[1]; YAMASHIRO-KANASHIRO, E.H.[2]; ROCHA, M.C.[2]; COTRIM, P.C.[2]; COELHO, A.C.[1]
1.UNICAMP, SP, Brazil; 2.USP, SP, Brazil.

Visceral leishmaniasis (VL) is a disease caused by the protozoan parasite *L. (L.) infantum* in South America and Europe. The disease may be lethal if the patient is not treated. In Brazil, about 3,000 new cases of the disease have been reported annually, with an increasing number of cases in urban and periurban areas. VL is zoonotic in Brazil and domestic dogs constitute the main reservoir for the parasite, playing an essential role in transmission of disease to humans. The treatment of VL in Brazil consists in the use of pentavalent antimonials and amphotericin B, drugs that are considered expensive, toxic and that require parenteral administration. Paromomycin is an aminoglycoside antibiotic extracted from cultures of *Streptomyces riomosus var.* This drug is highly effective against *L. (L.) donovani*, the parasite responsible for VL in Asia. It is urgent to investigate the potential of this drug against *L. (L.) infantum*, the species responsible for VL in the Mediterranean and Latin America. In this study, we aim to evaluate the susceptibility to paromomycin *in vitro* of isolates of *L. (L.) infantum* from dogs of the municipality of Embu-Guaçu, State of São Paulo, Brazil. Isolates of *L. (L.) infantum* were previously typed according to the protocol described by Cupolillo et al., 1994 and confirmed by the polymerase chain reaction (PCR) of the hsp70 gene followed by digestion with the restriction enzyme HaeIII . A total of 14 isolates were confirmed as *L. (L.) infantum* by this molecular typing method. Paromomycin susceptibility *in vitro* in promastigote form was determined by the (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (MTT) assay. The EC_{50} values of the isolates demonstrated a moderate variation in susceptibility to paromomycin, ranging from 70.68 µM to 125.6 µM. Our next goal is to determine the activity of paromomycin against the intracellular amastigote form of these isolates. Supported by FAPESP. **Supported by:**FAPESP **Keywords:** Visceral leishmaniasis; leishmania infantum; paromomycin

TB22 - **EFFECT OF NOVEL CARBA-ISOFLAVONONES DERIVATIVES AGAINST *LEISHMANIA INFANTUM*.**

PALMA, L.G.S.D.*[1]; FERNANDES, T.A.[2]; DE BRAGA, T.B.[2]; DOMINGOS, J.L.O.[2]; COSTA, P.R.R.[2]; CUNHA-JUNIOR, E.F.[1]; TORRES-SANTOS, E.C.[1]
1.FIOCRUZ, RJ, Brazil; 2.UFRJ, RJ, Brazil.

Visceral leishmaniasis is the second major cause of deaths by parasites atment faces the challenge of old and new concerns about toxicity, therap tance and few therapeutic options. Thus, the identification of antile al to fill the drug discovery pipeline for leishmaniasis. In the leishmanicidal activity of new 1-carba-isoflava on of their ADMET properties. The chosen compoun of isoflavonoids with antitumoral and leishmanicidal were incubated with 1-carba-isoflavanones (2-128µM by the resazurin assay. The derivatives can be divid pounds with methoxy groups were added in A and C second, five compounds with a hydroxy group was ad), while in the third, two compounds with Fluor is pres he most promising 1-carba-isoflavanones were 316, 47 12.1±1.8µM, 13.6±4.0µM and 14.7±2.2µM, respectively, .4µM). This result shows the importance of the substitutions in feature for leishmanicidal activity. Theoretical analyses suggested a fa arba-isoflavanones, because they do not infringe the Ro5 and the in silico an permeability in Caco2 cells and good absorption by human intestines. Toxicity pred ow that only compound 482 is expected to be mutagenic. This study reveals promising mole ies to proceed further studies of toxicity in host cells and antiamastigote activity. **Supported by:**CNPq e FAPERJ **Keywords:** Leishmania infantum; drug discovery; flavanones

TB23 - **MOLECULAR IDENTIFICATION OF TRYPANOSOMATID SMALL SUBUNIT RIBOSOMAL RNA (SSU/RRNA) SEQUENCES IN CLINICAL SAMPLES OF VISCERAL LEISHMANIASIS**

TAKAHASHI, T.Y.*[1]; TAKAMIYA, N.T.[1]; GONÇALVES, A.M.[1]; OLIVEIRA, C.A.[1]; SANTANA, A.K.M.[2]; MAGALHÃES, L.S.[2]; JESUS, A.[2]; ALMEIDA, R.P.[2]; SILVA, J.S.[3]; MARUYAMA, S.C.[1]
1.DEPARTMENT OF GENETICS AND EVOLUTION, FEDERAL UNIVERSITY OF SÃO CARLOS, SP, Brazil; 2.DEPARTMENT OF MEDICINE, FEDERAL UNIVERSITY OF SERGIPE, SE, Brazil; 3.FIOCRUZ- BI-INSTITUTIONAL TRANSLATIONAL MEDICINE PROJECT, RIBEIRAO PRETO MEDICAL SCHOOL/USP, SP, Brazil

Human Visceral Leishmaniasis (HVL) is a neglected tropical disease caused by protozoa *Leishmania infantum*, resulting in damage in organs such as the spleen, liver and bone marrow. It is the most lethal form of leishmaniasis. Clinical characterization of the disease and species involved are extremely important for better diagnosis, treatment and prognosis. The aim of this study was to identify sequences from small subunit of rRNA (SSU/rRNA) in samples from VL patients. Bone marrow (BM) aspirate samples were collected from 35 patients admitted at the University Hospital of the Federal University of Sergipe. The BM samples were used either to isolate parasites (35 clinical isolates) or to extract genomic DNA from patients (16 BM samples). DNA samples of peripheral blood from 16 patients were also analyzed. Cultures of promastigotes were established in supplemented Schneider medium for each BM sample and used to extract genomic DNA from parasites. Nested-PCR method using TRY927F/R and SSU561F/R primers was used to amplify SSU/rRNA sequences (about 561 bp) in DNA samples from patients and culture of clinical isolates. Positive PCR products were checked by electrophoresis in agarose gels and images documentated. Purified PCR products were sequenced with Sanger method using SSU561F primer. Sequence analysis evaluated by BLASTN searches in NCBI and TriTryDB databases showed that SSU/rRNA sequences from clinical isolates were more similar to other Leishmaniinae species, such as *Crithidia* and *Leptomonas*, than *Leishmania* genus. Phylogenetic analysis of these sequences also showed the same result. However, sequence analyses using SSU/rRNA amplicons detected in human DNA samples have presented heterogeneous results, with some sequences more related to Leishmania and others more related to Crithidia and Leptomonas. Ongoing analyses have been performed to select clinical isolates of HVL for whole-genome sequencing to the better characterization of these parasites.
Supported by: Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP, Grant 2016/20258-0); CAPES **Keywords:** Human visceral leishmaniasis ; small subunit rrna; leishmania

TB24 - **CASE EVALUATION OF CONGENITAL TRANSMISSION OF CHAGAS DISEASE: CASE REPORT**

TAKAMIYA, N.T.*[1]; COSTA, E.A.P.N.[1]; LUCHEIS, S.B.[1]; SANTOS, R.M.[1]
1.DEPARTMENT OF TROPICAL DISEASES AND IMAGING DIAGNOSIS - BOTUCATU MEDICAL SCHOOL/UNESP, SP, Brazil.

Chagas' disease (CD) is a neglected endemic disease caused by the protozoan *Trypanosoma cruzi*. Its classical form of transmission is through hematophagous triatomine insects. The disease reaches non-endemic regions through alternative transmissions (blood transfusion, oral, vertical and accidental). In this context there is the possibility that the congenital pathway is presenting new occurrences. The objective of the study is to report cases of maternal grandmother, their children serologically reactive for CD and their previously uninvolved granddaughter, all patients of the Hospital of the Clinics of the Faculty of Medicine of Botucatu - HC-FMB/UNESP evaluating them using diagnostic methods and clinical data analysis of these patients. Case report: natural family and resident in Taquarituba-SP. The maternal grandmother lived in a clay house in rural areas and reported contact with the triatomineum in childhood. Two children are also seroreagentes, both report that they had no contact with the insect. Serological tests for CD were performed by the clinical laboratory HC-FMB; in addition to a direct and indirect parasitological methods by the Laboratory of Infectious Diseases - FMB. The clinical history indicates that serology for CD was reactive in the matriarch, in her son and daughter. The serological tests of the child obtained a non-reactive result. The direct and indirect parasitological techniques demonstrate negative results for the patients. The results indicate that this child did not present contact with the protozoan. The investigated diagnosis patients with CD are in the chronic form of the disease, since no parasitemia was evidenced in the tests. Regarding the congenital transmission between the maternal grandmother and the offspring may have occurred, as well as the possibility of vector transmission can not be ruled out since the patients come from a municipality considered endemic to CD in the past.
Supported by: Keywords: Congenital chagas disease; trypanosoma cruzi; transmission mechanism

TB25 - **EVALUATION OF *TRYPANOSOMA CRUZI* CYCLOPHILIN 19 EXPRESSION IN MAMMALIAN CELLS**

ABUKAWA, F.M.*[1]; MCGWIRE, B.S.[2]; MORETTI, N.S.[1]; SANTOS, G.P.[1]; SCHENKMAN, S.[1]
1.UNIFESP, SP, Brazil; 2.OSU, USA

The cyclophilins belong to a family of enzymes conserved from prokaryotes to eukaryotes and have peptide-cis-trans-isomerase activity (Ppiase). The cyclophilins act as chaperones, stabilizing other proteins through the induction of conformational changes via distortion of the chain around proline residues. They also affect the function of proteins through exposure of groups that may act in cellular signaling or in the interaction with other proteins. Some cyclophilins are secreted and interact with receptors on the cellular surface. We have verified that the 19 kDa cyclophilin (Cyp19), the main cyclophilin of Trypanosoma cruzi, the parasite that causes Chagas's Disease, is expressed and secreted by all the T. cruzi life cycle stages found inside the mammal host. In intracellular form, the Cyp19 is released in the cytosol of host cells. The Cyp19 is homologous to human CypA, the cyclophilin that participates actively in inflammation processes when cells are exposed to oxidative stress such as hypoxia and ROS. Therefore, we hypothesized that the Cyp19 could have a role inside the infected cells. Therefore, we expressed Cyp19 in rat myoblast cells by using plasmid vectors containing the gene of the Cyp19 with or without the N-terminal of the protein, possible involved in the secretion and with a tag peptide of Influenza virus hemagglutinin protein (HA). In both cases, Cyp19 was overexpressed and detected in the cytosol, localizing preferably in the leading edges of the cells, similar to the localization of the endogenous CypA. These cells will allow us to test whether Cyp19 increased the resistance to oxidative, genotoxic agents and apoptosis inducers and whether it affects cellular migration, which could be relevant to understand the inflammatory process in the Chagas Disease. **Supported by:**FAPESP **Keywords:** Cyclophilin; inflammation; trypanosoma cruzi

TB26 - **DRUG REPURPOSING FOR THE TREATMENT OF CHAGAS DISEASE: STUDIES ON THE TOXIC EFFECTS OF THE NSAID NIMESULIDE AGAINST THE EVOLUTIVE FORMS OF *TRYPANOSOMA CRUZI***

TRINDADE, J.D.S.*[1]; VELLOZO, C.S.[2]; MARINHO GRAÇA, M.C.N.[2]; FREIRE-DE-LIMA, C.G.[2]; DECOTE-RICARDO, D.[1]; FREIRE DE LIMA, M.E.[1]
1.UFRRJ, RJ, Brazil; 2.UFRJ, RJ, Brazil

Chagas' disease (CD), the most prevalent parasitic infection in the American continent, is caused by *Trypanosoma cruzi*. This hemoflagellated protozoan gives rise to a life-threatening infectious disease in humans, resulting in severe cardiomyopathy and other serious clinical manifestations in ~30% of patients. Unfortunately, the drugs available for the treatment of *T. cruzi* infection, benznidazole (BZN) and nifurtimox, have important side effects. The role of non-steroidal anti-inflammatory drugs (NSAIDs) in inhibiting the COX pathway is well known. This pathway leads to the formation of prostaglandins and other end products, including PGE2, which plays an important role in *T. cruzi* infection. The aromatic nitro group present in the benznidazole structure also appears in nimesulide (NIM), a drug of the NSAID class. The structural similarity together with its mode of action has encouraged us to study the potential antiparasitic activity of NIM and may propose its repurposing to treat CD. As repurposed drugs are usually approved for clinical use, this discovery process can result in significant time and cost savings. This approach is particularly attractive for neglected diseases, since there is no significant investment in the development of new drugs in this field. Preliminary results indicated the trypanocidal activity of NIM (30 to 3μM) on epimastigotes of *T. cruzi*, Y strain (IC_{50} 12,9 μM). The reduction of the nitrous group present in the NIM to the respective aniline resulted in the loss of activity (IC_{50}> 100 μM), indicating the nitroaromatic moiety as a possible pharmacophore to the activity of NIM. Assays performed against other evolutionary forms of parasite revealed that NIM inhibited both amastigote replication (56%) and trypomastigote release (74%) compared to BZN (79% and 100%, respectively). The set of results pointed out the potential of NIM in the development of new antichagasic drugs. The likely mechanisms of action are now under investigation. **Supported by:**FAPERJ;CAPES;CNPq **Keywords:** Chagas disease; nimesulide; drug repurposing

TB27 - **HIT COMPOUND OPTIMIZATION: STRUCTURE-ACTIVITY RELATIONSHIP ANALYSIS AND BIOLOGICAL ACTIVITY AGAINST *TRYPANOSOMA CRUZI*.**

LARA, L.S.*[1]; MOREIRA, C.S.[2]; LECHUGA, G.C.[1]; CALVET, C.M.[1]; ROCHA, D.[2]; PEREIRA, M.C.S.[1]
1.FIOCRUZ, RJ, Brazil; 2.UNIVERSIDADE FEDERAL FLUMINENSE, RJ, Brazil

Chagas disease, a potentially life-threatening illness, is responsible for disability-adjusted life years. The current treatment has limited effectiveness and serious adverse effect. Therapeutic failure of promising candidates in phase 2 clinical trials highlights the urgent need to identify new effective and safe drugs. In this study, we seek to optimize a hit compound (2-{[(4-Chlorophenyl)sulfanyl](phenyl)methyl}-3-hydroxy-1,4-naphthotoquinone), with promising bioactive characteristics, through the design of a new series of analogues by addition of chlorobenzene groups (CL1-CL6) and electron donating aldehydes (CM1-CM13). A total of 19 derivatives was screened for their efficacy against trypomastigotes and also intracellular amastigotes. Cytotoxic effect of the compounds on Vero cells (CC_{50}) as well as the viability of trypomastigotes (IC_{50}) were evaluated by CellTiter-Glo® assay. The effect against intracellular amastigotes (IC_{50}) was determined by luciferase assay. All compounds of the CL series (IC_{50} < 10 M) and also compounds CM5 and CM9 (IC_{50} < 14 M) showed greater activity than BZ (IC_{50} = 22.3 M) against trypomastigotes. For intracellular amastigotes, the compounds CM5, CM7, CL1, CL2, CL5 and CL6 showed the highest activity among the derivatives analyzed (IC_{50} < 10 M) but still lower than Bz (IC_{50} = 1.4 M). Regarding cytotoxicity, only compounds CM4, CM5 and CM12 presented CC_{50} > 93 M. Furthermore, CM5 showed the highest value of selectivity index (SI) among the analyzed analogues, achieving a SI of 14 and 10, for amastigotes and trypomastigotes, respectively. *In silico* analysis of physicochemical parameters and ADMET properties show that compound CM5 does not violate Lipinski's rule of five, presents a high probability of intestinal absorption and is classified as non-mutagenic and non-inhibiting of hERG1 and hERG2. Further studies will be carried out to assess toxicity and efficacy of the compound CM5 in murine model of acute *T. cruzi*-infection. **Supported by:**Fiocruz, CNPq and PAPES VI **Keywords:** Trypanosoma cruzi; 1,4 naphthoquinines; chemotherapy

TB28 - **INVESTIGATION OF ARTEMISININ AND ITS DERIVATIVES ACTIVITY AGAINST LEISHMANIA (VIANNIA) BRAZILIENSIS**

GRAZZIA, N.*[1]; JUNIOR, S.B.[2]; GARCIA, V.L.[2]; MIGUEL, D.C.[1]
1.UNICAMP, SP, Brazil; 2.CPQBA - UNICAMP, SP, Brazil

Leishmaniasis is a protozoosis caused by Leishmania spp. affecting populations worldwide. Over the past five years, more than 1 million people have been affected by the cutaneous form of the disease, while about 20k deaths are recorded annually due to the visceral form. Several obstacles to control the disease are recognized, including: the failure of drug treatment; long-term regimens and parenteral administration for most drugs of choice; high toxicitycost; and reports of selection of resistant strains (Uliana et al., 2018; doi: 10.1017/S0031182016002523). Based on these drawbacks, the need to find new chemotherapeutic alternatives for leishmaniasis in its different clinical forms becomes indispensable. Therefore, in the present study, we aim at determining the leishmanicidal activity of artemisinin obtained from Artemisia annua L. leaf extracts and its derivatives: dihydroartemisinin, artemether, and artesunic acid. The cell viability was determined by the MTT method (Miguel et al., 2011; https://doi.org/10.1016/j.ijantimicag.2011.03.012). Leishmania (V.) braziliensis promastigotes were incubated in 96-well plates at 5x106 log-phase growth, followed by addition of the compounds in increasing concentrations and incubated at 26°C for 24h. After the incubation with the MTT reagent, absorbance was determined at 595 nm (Biotek SinergyHT). Our results show that artemisinin and its derivatives inhibit parasite viability with EC50 values of 280.7 (artemisinin), 62.2 (dihydroartemisinin), 312.5 (artemether), and 248.8 µM (artesunic acid). Our cytotoxic assay showed that the selectivity index of dihydroartemisinin is around 5.0 (CC50 for macrophage/EC50 for L. (V.) braziliensis). Preliminary data suggest that artemisinin and its derivatives, at doses ~EC20, may lead to parasite's mitochondrial dysfunction. Our results support the potential repositioning of these molecules towards experimental leishmaniasis.**Supported by:**CAPES E FAPESP **Keywords:** Artemisia annua l.; dihydroartemisinin; leishmania (v.) braziliensis

TB30 - **DRUG TRANSPOSITION, AN IMPORTANT TOOL FOR THE CHEMOTHERAPY OF LEISHMANIASIS: THE STUDY OF CLOTRIMAZOL, A KNOWN AZOLE, IN LEISHMANIA AMAZONENSIS.**

DE PAULA, T.G.*[1]; VERÇOZA, B.R.F.[1]; GODINHO, J.L.P.[1]; RODRIGUES, J.C.F.[1]
1.UFRJ, RJ, Brazil

Leishmaniasis is a neglected disease endemic in 98 countries that causes a range of clinical manifestation including the visceral, cutaneous and mucocutaneous forms. It is caused by protozoan parasites of the Leishmania genus. The current treatments available, although effective in c ating the disease, are very toxic and expensive; moreover, the number of cases of resist sed dramatically. Thus, the search for new chemotherapeutic targets, as w ew, more efficient and less toxic drugs is necessary. In trypan get is the difference in lipid composition of the mem on containing episterol, 5-dehydroepist 4-methylated sterols that ar ment is the drug trans ment of different disc known antifungal, against L ize research time. Antiproliferative assay clotrimazole is effective at inhibiting the growth at 10 µM. As observed for other azoles, clotrimazole causes mation of multivesicular bodies and increase the presence of lipids b sis promastigotes, probably induced by alterations in the lipid composition. C , new experiments are being carried out to better understand the effect of clotrimazole in Leishmania, as well to study the effects against intracellular amastigotes. In conclusion, clotrimazole is a potential candidate trying to identify new chemotherapeutic agents for the treatment of leishmaniasis, since its cream is already used to treat some mycoses.**Supported by:**CNPq
Keywords: Chemotherapy; ergosterol biosynthesis inhibitors; leishmania

TB31 - **STUDY OF THE BIOLOGICAL ALTERATIONS INDUCED BY A NOVEL SIRTUIN INHIBITOR ON LEISHMANIA AMAZONENSIS.**

DA SILVA, G.S.*[1]; VERÇOZA, B.R.F.[1]; BRACHER, F.[2]; DE SOUZA, W.[1]; RODRIGUES, J.C.F.[1]
1.UFRJ, RJ, Brazil; 2.LUDWI G-MAXIMILIANS-UNIVERSITY, Germany

Leishmaniasis is a disease caused by protozoan parasites of Leishmania genus distributed worldwide. The clinical manifestations of leishmaniasis depend on the host immune system and tissue tropism of the parasite. Then, there are cutaneous, mucocutaneous and visceral leishmaniasis, which has important economic and social impact and should be fatal if not treated. However, there are some problems in chemotherapy, such as high toxicity rates, high cost and the forms of drug administration. Therefore, this disease needs attention for the identification of new chemotherapeutic targets, which are less toxic and more accessible to patients. In this scenario, class III deacetylase histones (sirtuins) are proteins present in cellular compartments, such as the nucleus, mitochondria and cytoplasm. The sirtuins can act in several cell signaling pathways, energetic metabolism, dynamics of cytoskeletal dynamics and gene transcription. In addition, there are 7 sirtuins (SIRT1-7) in humans and only one sirtuin, SIRT-2, was found in Leishmania with 37% similarity. The present study evaluated the effects of NIH119, a novel sirtuin inhibitor, against Leishmania amazonensis. We evaluated the effects of NIH119 in the growth, morphology and ultrastructure. Promastigote were significantly affected by the treatment, presenting IC50 of 1.25 µM. NIH119 was also active against intracellular amastigotes. Optical microscopy and scanning electron microscopy revealed changes in the cell body morphology of the promastigotes. Immunofluorescence microscopy indicated possible abnormal chromatin condensation and increase of lipid bodies, which was also observed by transmission electron microscopy. Furthermore, images also indicated presence of several small vesicles inside the flagellar pocket, indicating a possible increase in the secretory pathway. Currently, cell viability and cell cycle analyses are being performed to better understand the mechanisms of action of NIH119 in Leishmania.**Supported by:**CNPq **Keywords:** Chemotherapy; sirtuin inhibitors; leishmania

TB32 - **EPIGALLOCATECHIN-3-GALLATE AFFECTS THE PROLIFERATION OF *LEISHMANIA INFANTUM* PROMASTIGOTES BY THE INHIBITION OF TRYPANOTHIONE REDUCTASE AND PRODUCTION OF REACTIVE OXYGEN SPECIES**

FONSECA, M.S.*[1]; INACIO, J.D.F.[1]; ALMEIDA-AMARAL, E.E.[1]
1.FIOCRUZ, RJ, Brazil

Leishmaniasis is a disease caused by species of protozoan parasites of the genus Leishmania and its treatment has undesirable effects. Therefore, WHO proposes the research for new drugs for this disease. Epigallocatechin-3-gallate (EGCG) is a flavonoid that presents leishmanicide effect and capacity to generate reactive oxygen species (ROS). The objective of this study is demonstrate the effect of EGCG on *L. infantum* promastigotes proliferation and the possible mechanism of action. *L. infantum* promastigotes were incubated with EGCG (15.6µM-500µM) for 72h and cell viability was estimated by Alamar blue assay. The EGCG showed an inhibition of promastigote proliferation of 97% (500µM) presenting an IC_{50} of 192µM. To evaluate the production of H_2O_2, *L. infantum* promastigotes were treated with EGCG (125µM-500µM) for 72h and incubated with Amplex Red reagent. The promastigotes showed a dose-dependent production of H_2O_2, reaching 7-fold increase (500µM). The activity of trypanothione reductase (TR), essential enzyme for trypanosomatides survival, was evaluated by the Ellman method. EGCG was able to inhibit TR in a dose-dependent manner presenting a Ki of 509µM. To evaluate the mitochondrial membrane potential (ΔΨm), promastigotes of *L. infantum* were treated with EGCG (125-500µM) for 72h and incubated with JC-1. EGCG decreased the ΔΨm with a significant reduction of 85.6% (500µM). Determination of intracellular ATP levels was evaluated with CellTiter-Glo assay in *L. infantum* promastigotes incubated with EGCG (125µM-500µM). EGCG significantly decreased the intracellular ATP levels of these parasites in 60% (500µM). Taken together, these results demonstrate that EGCG is a promising compound for the treatment of visceral leishmaniasis, being able to inhibit the proliferation of *L. infantum* by the inhibition of TR, leading to increase in ROS levels, causing decrease in ΔΨm and consequent decrease in intracellular ATP levels.**Supported by:**CNPq, FAPERJ, CAPES, PAPES, FIOCRUZ **Keywords:** Egcg; trypanothione reductase; leishmania infantum

TB33 - **SYNTHESIS, CHARACTERIZATION AND ANTILEISHMANIAL ACTIVITY OF A NOVEL HYBRID PEPTIDE**

MENDES, B.*[1]; ALMEIDA, J.R.[2]; VALE, N.[3]; GOMES, P.[3]; SILVA, S.L.[2]; MIGUEL, D.C.[1]
1.UNIVERSIDADE ESTADUAL DE CAMPINAS, SP, Brazil; 2.UNIVERSIDAD REGIONAL AMAZÓNICA, Ecuador; 3.UNIVERSIDADE DO PORTO, Portugal

Current treatment for leishmaniasis is far from satisfactory. Therefore, efficient therapy strategies are urgently needed and should be a priority. Snake venoms are recognized sources of natural compounds, which can be a valuable tool in the search for new antimicrobial and antiprotozoal candidates [Izidoro et al., 2014; doi:10.1155/2014/196754]. Based on these properties, we synthetized and evaluated the leishmanicidal activity of a short peptide, which combines structural features of an Agkistrodon sp. C-terminal toxin domain and cell-penetrating peptides. The hybrid peptide was obtained using solid-phase peptide synthesis employing the Fmoc/tert-butyl strategy. Purity and molecular mass of the product were determined by high-pressure liquid chromatography and mass spectrometer, respectively. Inhibition of Leishmania (L.) amazonensis promastigotes growth curves was assessed by the MTT method [Zauli-Nascimento et al., 2010; doi: 10.1111/j.1365-3156.2009.02414.x]. Our hybrid peptide (1905.27 Da and 98.5% purity) presented good leishmanicidal activity, exhibiting 50% inhibitory concentration ~ 42 µM after 24h. Bone marrow derived macrophages were incubated with increasing concentrations of the hybrid peptide and presented cytotoxicity to 10% of macrophage population >200 µM. Based on these findings, additional experiments are ongoing in order to demonstrate the efficacy of this hybrid peptide against intracellular amastigotes of Leishmania sp. We expect that our results will provide clues for future development of more potent drug candidates against leishmaniasis.**Supported by:**CAPES and FAPESP **Keywords:** Leishmaniasis; peptide; drug candidate

TB34 - **COMPARISON OF SUBCUTANEOUS AND TATTOOING ROUTES FOR TREATMENT OF CUTANEOUS LEISHMANIASIS IN HAMSTERS**

VILLARIM, R.M.*[1]; SILVA, T.[1]; CUNHA, L.A.C.[1]; SILVA, A.J.M.[2]; SILVA, S.A.G.[1]
1.UERJ, RJ, Brazil; 2.UFRJ, RJ, Brazil

The current therapy for cutaneous leishmaniasis is long, toxic and mainly administered by parenteral route, which hinders the patient's adherence to treatment. Therefore new administration routes for antileishmanial drugs are been tested, such as subcutaneous injections. Tattoing has been used in medical research as drug delivery system in some skin conditions. The aim of this study was to compare two local administration routes, subcutaneous and tattoo, to treat hamsters with cutaneous leishmaniasis. We infected hamsters with Leishmania (V.) braziliensis (106 promastigotes in the paw) and after 7 days, the animals were treated with the synthetic molecule LQB-166 (3-phenyl-lawsone) or meglumine antimoniate (reference drug) by subcutaneous or tattooing routes during two weeks. The hamsters were treated by tattoo device twice a week with LQB-166 (2,5 µg/kg) or meglumine antimoniate (1mg/kg) or by subcutaneous route three times a week with LQB-166 (25 µg/kg) or meglumine antimoniate (10mg/kg). Control groups were untreated hamsters or treated with DMSO (tattoing or subcutaneous). We have observed a significant decrease of the parasite load in all treated groups, both subcutaneous injected and tattoed animals ($p<0,001$). However the lesion size decreased only in animals treated subcutaneously with meglumine antimoniate. We also observed a decrease in NO production in the lesion and draining lymph nodes in animals treated with subcutaneous meglumine antimoniate, which could be related to the less amount of tissue, since the lesion size in this group was much smaller. The dose administered by tattoo device is much lower than the subcutaneous route, and in addition there is a possible inflammation associated with the process of tattooing. This results show that tattoing is a possible drug delivery system to treat cutaneous leishmaniasis, with a low amount of drug required and a short treatment duration. We are currently performing the histological analysis of lesions. **Supported by:**CAPES **Keywords:** Cutaneous leishmaniasis; local treatment; naphtoquinones

TB35 - **LQB-118 ACTIVITY AGAINST *TRYPANOSOMA CRUZI*: AN *IN VITRO* AND *IN VIVO* STUDY**

BRITO, A.C.S.*[1]; CUNHA, L.A.C.[1]; NEVES, R.H.[1]; NETTO, C.D.[2]; COSTA, P.R.R.[2]; SILVA, S.A.G.[1]
1.UERJ, RJ, Brazil; 2.UFRJ, RJ, Brazil

Therapy of Chagas disease is very limited and not effective in the chronic phase. Previous studies from our group showed that LQB-118 has activity *in vitro* on trypomastigotes and intracellular amastigotes forms of *Trypanosoma cruzi* (clone Dm28c). The aim of this study was to evaluate the activity of LQB-118 *in vitro* and *in vivo* against experimental Chagas Disease. Epimastigotes of *T. cruzi* (Dm28c clone) were treated with LQB-118 (0-5µM) for 96h/27°C and quantified daily by Neubauer chamber. DNA fragmentation in epimastigotes forms was determined by TUNEL kit. Trypomastigotes (Y strain) were treated with LQB-118 (0-40µM) for 48h/27°C and analyzed daily by Neubauer chamber. *In vivo*, Swiss mice infected with 10^3 blood trypomastigotes intraperitoneally. Mice were treated orally with LQB-118 (40 mg/kg/day) for 12 days from 4º day post-infection. We evaluated the parasitemia, survival rates, organ weights, serum biochemical parameters and histopathology in the heart. *In vitro*, the LQB-118 was able to inhibit significantly the epimastigote growth at 96h the inhibition was 99,67% at 5 µM. LQB-118 IC_{50} in 48 hours was 2,34±0,20µM and 96h was 0,88±0,08µM and we did not observe DNA fragmentation. In trypomastigotes, LQB-118 was capable of significantly reduce parasite motility in 24h and 48h, with inhibition of 99,51% in 20µM. LQB-118 IC_{50} in 24hours was 5,88µM and 48h was 2,97µM. *In vivo*, the parasitemia in animals treated in LQB-118 decreased in both assays. The treatment reduced the parasitemia by 49,47% from the 11th dpi. Serum biochemical analysis showed that there was a creatine decrease in animals treated with LQB-118. Histological analysis did not show significantly reduction of amastigotes nests or inflammatory infiltrate. These results show that LQB-118 has activity on epimastigotes and blood trypomastigotes of *T. cruz*i, with no DNA fragmentation and protective action *in vivo* during the acute phase of the Chagas disease. **Supported by:**CAPES **Keywords:** Lqb-118; trypanosoma cruzi; chemotherapy

TB36 - **LEISHMANIA IDENTIFICATION AND DIAGNOSIS OF THE LEISHMANIASES BY HIGH RESOLUTION MELTING TARGETING THE AMINO ACID PERMEASE 3 CODING SEQUENCE**

MULLER, K.E.*[1]; ZAMPIERI, R.A.[2]; AOKI, J.I.[2]; MUXEL, S.M.[2]; NERLAND, A.H.[1]; FLOETER-WINTER, L.M.[2]

1.UNIVERSITY OF BERGEN, Norway; 2.UNIVERSITY OF SÃO PAULO, SP, Brazil

Over 20 different species of Leishmania are the causative agents of a large spectrum of clinical manifestations known as leishmaniases. These diseases are endemic in 98 countries and over 350 million people are at risk. An accurate diagnosis and species identification are paramount to guide treatment and follow-up procedures. However, there is no gold standard for species identification. High Resolution Melting (HRM) can detect differences in amplicons produced in real-time PCR, based on thermodynamic features of DNA, through specific signatures that reflect polymorphisms in nucleotide composition. The simultaneous HRM analyses of three amplicons targeting the amino acid permease 3 (aap3) gene, an exclusive target for trypanosomatids, allowed the discrimination of L. (Leishmania) donovani, L. (L.) infantum, L. (L.) major, L. (L.) tropica, L. (L.) mexicana, L. (L.) amazonensis, L. (Viannia) braziliensis, L. (V.) guyanensis, L. (V.) lainsoni, L. (V.) naiffi and L. (V.) shawi. The comparison among HRM profiles obtained with DNA from standard promastigote cultures and clinical and field samples led to the correct identification of parasites in human biopsies, naturally infected sand flies and experimentally infected mice. Also, T. brucei, T. cruzi, E. schaudinni, rat, mouse and human DNA were used as negative-controls and no amplification was observed. The approach proposed here is a relatively cheap, fast and robust strategy to detect and discriminate Leishmania species from all the endemic regions worldwide.**Supported by:**FAPESP, CNPq and UiB
Keywords: Pcr; hrm; leishmania discrimination

TB37 - **ACTIVITY OF SYNTHETIC NAPHTHOQUINONE LQB-166 ON DIFFERENT FORMS OF *TRYPANOSOMA CRUZI IN VITRO***

CUNHA, L.A.C.*[1]; BRITO, A.C.S.[1]; VILLARIM, R.M.[1]; SILVA, A.J.M.[2]; SILVA, S.A.G.[1]

1.UERJ, RJ, Brazil; 2.UFRJ, RJ, Brazil

Endemic in Latin America, Chagas is a disease caused by the protozoan *Trypanosoma cruzi*. The therapy of this disease is limited, toxic, and not effective in the infection chronic phase. The aim of this study was to evaluate the activity of the naphthoquinone LQB-166 (3-phenyl-lawsone) in vitro on *T. cruzi* and its toxicity on the mammalian cells (VERO and THP-1). *T. cruzi* epimastigotes (Dm28c) were incubated at different concentrations of LQB-166 (0-200µM) for 120h/27°C. Trypomastigotes (Y strain), were incubated with LQB-166 (0-3200µM) for 48h/37°C and 48h/4°C. Both parasite forms were counted daily using a Neubauer Chamber. To analyze the activity on intracellular amastigotes, peritoneal macrophages, from Swiss mice, were infected with trypomastigotes(Y strain) and treated with LQB-166 (0-800 µM) for 48h/37°C. After the treatment, we counted the number of uninfected and infected macrophages, and the number of intracellular amastigotes. To evaluate the toxicity in cell lines, VERO and THP-1 were incubated with LQB-166 (0-3200 µM) for 24h/37°C/5% CO2. As a result, LQB-166 was able to significantly inhibit the epimastigotes growth, from 50µM in 120h, with IC50 estimated in 100µM. LQB-166 showed no toxicity in the tested cell lines, with an IC50 estimated at 2103µM for VERO cells, 767µM and 2194µM for THP-1 monocytes and macrophages, respectively. Our results showed, that synthetic naphthoquinone LQB-166 exhibits a great activity in *T. cruzi* epimastigote forms and does not present toxicity in cell lines VERO and THP-1. However, in the concentrations tested the molecule was not effective against trypomastigotes (motility) and amastigotes (number) forms. We are currently analyzing the viability of amastigote form and the action of this naphthoquinone on cardiomyocytes (H9C2).**Supported by: Keywords:** T. cruzi; naphthoquinone; treatment

TB38 - **ANALYSIS OF TUBERCULOSTATIC DRUG IZONIAZID TO TREAT EXPERIMENTAL CUTANEOUS LEISHMANIASIS**

SILVA, T.*[1]; VILLARIM, R.M.[1]; SIQUEIRA, L.M.[1]; SANTOS, R.F.[1]; SILVA, S.A.G.[1]
1.UERJ, RJ, Brazil

Leishmaniasis is a complex of diseases with a wide spectrum of clinical manifestations caused by the genus Leishmania, which *Leishmania (Viannia.) braziliensis* is the most distributed species in the New World. The therapeutic arsenal is restricted, toxic and expensive and facing this panorama, the search for new treatments becomes necessary, including drug repositioning. This strategy reduces research time and costs. The aim of this study was to investigate if tuberculostatic drug isoniazid is effective in the treatment of hamsters infected with *L.(V.) braziliensis*. Treatment with isoniazid at dose corresponding to humans (10mg/kg/day/5x a week) by oral route was initiated 7 days after infection, associated or not with a subdose reference drug Glucantime (2mg /kg/day/5x a week) intraperitoneal route. Control groups were treated with Glucantime (8mg /kg/day/5x a week). The lesion course was evaluated twice a week during the 60 days of treatment, and after we analyzed parasite load and nitric oxide production in the lesions and draining lymph nodes. Our preliminary results showed that isoniazid alone at used dose was not effective in reducing lesion size or parasite load of treated animals. We did not observe the change in the production of nitric oxide in groups treated with isoniazid. We are currently testing higher doses of isoniazid to investigate the action profile of this drug in order to better know its potential as repositioning drug for leishmaniasis.**Supported by:**CAPES **Keywords:** Leishmania (viannia.) braziliensis; isoniazid; hamster gold

TB39 - **EFFECT OF BENZNIDAZOLE ON CEREBRAL MICROCIRCULATION DURING ACUTE TRYPANOSOMA CRUZI INFECTION IN MICE**

GONZAGA, B.M.S.*[1]; NISIMURA, L.M.[2]; BEGHINI, D.G.[1]; GOMES, F.[1]; ALMEIDA, V.E.F.[1]; TIBIRIÇÁ, E.V.[1]; HORITA, S.M.[1]; BARBIERI, I.[3]; GARZONI, L.R.[1]
1.FIOCRUZ/RJ, RJ, Brazil; 2.INSTITUTO CARLOS CHAGAS, PR, Brazil; 3.FIOCRUZ, GO, Brazil

Central nervous system alterations have been identified in Chagas disease in both human and experimental models, leading to meningoencephalitis and stroke. Recently our group demonstrated that Trypanossoma cruzi acute infection (Y strain) leads to cerebral microvascular Swiss Webster mice as consequence of endothelial dysfunction, capillary rarefaction, cytes rolling and adhesion. Only benznidazole (BZ) and nifurtimox are available these drugs present 80% efficiency in acute phase, and less then 20% cts that may lead to treatment interruption. In this context, is necessary. Since statins present pleiotropic effects endothelial function improvement, it could be an alternative pathy in Chagas disease. Our hypothesis is that lovastatin association with BZ, could decrease microcirculation damage perimental Chagas disease. Swiss Webster mice were forms of T. cruzi, and after 24 h, treatment was initiated statin, 50 or 100 mg/kg/day BZ or with the combination g/kg/day BZ. All treatment schemes, except lovastatin, mortality and prevented reduction of body weight. M capillary rarefaction and the increase in leukocyte rolling. monotherapy and using 50 mg/kg/day BZ in combination with L adhesion and improved the brain blood flow. VCAM-1 and MCP-1 exp ted by the infection. However, eNOS and ICAM-1 expression was increased in b animals. **Keywords:** Chagas disease; microcirculation; lovastatin

TB40 - **THERAPEUTIC AND IMMUNOMODULATORY EFFECT OF QUERCETIN IN RESISTANT AND SUSCEPTIBLE MODEL OF LEISHMANIA MAJOR INFECTION**

SANTOS, R.F.*[1]; BRITO, A.C.S.[1]; SILVA, T.[1]; VILLARIM, R.M.[1]; SILVA, S.A.G.[1]
1.UERJ, RJ, Brazil

The flavonoid quercetin has several biological activities, including anti-inflammatory, antioxidant, pro-oxidant, antiparasitic and immunomodulatory activities. In trypanososomatids, the action of quercetin, including in Leishmania species is reported. We had already demonstrated the therapeutic activity of quercetin in vitro and in vivo in Leishmania braziliensis. The aim of this study was to evaluate the therapeutic and immunomodulatory activity of quercetin in an experimental model of infection of L. major, using susceptible (BALB/c) and resistant mice (C57BL/6). The in vitro antiparasitic effect was evaluated on the growth of intracellular amastigotes of L. major. In vivo treatment of the animals with quercetin was perfomed orally (gavage) 5 times a week, with 20 and 40 mg/ kg. Treatments started after 7 days of infection during 7- 10 weeks. After the treatment, the animals were euthanized and the parasite loads were quantified in the paw and lymph nodes. The immunomodulatory effect was evaluated by cytokine production of treated mice splenic lymphocytes using cytometric bead array (CBA) method. In intracellular amastigotes, quercetin promoted a dose-dependent inhibition of infection index during 48 hours of treatment. In BALB/c mice, the treatment with 20 mg/kg of quercetin decreased parasite load and treatment with 40 mg/kg reduced lesion and parasite load. There was also increased production of IL-2, IL-4, and IL-6 cytokines by splenic lymphocytes. In C57BL/6 mice, treatment with quercetin at 40mg/kg resulted in a reduction in the parasite load and increased production of IL-10, IL-17, IFN-γ and TNF cytokines. These results shows that the flavonoid quercetin has antiparasitic and oral therapeutic activities, without signs of toxicity renal and hepatic in animals and the cytokine profile in each model was increased by oral treatment in L. major infected mice, showing it's immunomodulatory effect in this model of infection.**Supported by:**CAPES FAPERJ CNPQ **Keywords:** Leishmania major; quercetin; immunomodulation

TB41 - **FUNCTIONAL ANALYSIS OF MANNOSYLTRANSFERASE (GPI-14) IN *LEISHMANIA BRAZILIENSIS***

ROCHA, B.F.B.*[1]; RIBEIRO, C.V.[1]; MOREIRA, D.S.[1]; MAGALHAES, V.P.[1]; MURTA, S.M.F.[1]
1.FIOCRUZ-MINAS, MG, Brazil

The enzyme mannosyltransferase (GPI-14) is responsible for adding mannose residues to lipophosphoglycan (LPG), which is an important surface molecule for Leishmania-host interaction and survival of the parasite. Functional analysis was performed to determine whether overexpression of GPI-14 in Leishmania braziliensis would contribute to antimony (SbIII)-resistance phenotype of this parasite. Promastigote forms from L. braziliensis were transfected with the pIR1-BSD-GPI-14 construct and plated on semisolid M199 medium containing blasticidin (BSD). Clonal lines were recovered and PCR tests indicated the presence of the BSD marker, indicating the transfection efficiency. Levels of mRNA determined by real-time quantitative RT-PCR showed an increased expression of the GPI-14 gene in clones overexpressing this enzyme in comparison with the wild-type (LbWTS) line. In order to investigate the expression profile of the surface carbohydrates of these clones, we analyzed the fluorescence intensity of the parasites by flow cytometry. They were incubated with the Concanavalin-A lectin which binds to the terminal regions of α-D-mannosyl and α-D-glucosyl residues. The results showed that the clones transfected with GPI-14 gene express more mannose and glucose residues than the LbWTS line, showing the success of GPI-14 overexpression. Promastigotes forms of the parasites were submitted to antimony susceptibility tests to determine the effective concentration necessary to decrease growth by 50% (EC50). The data demonstrated that the clones overexpressing the GPI-14 enzyme were 4- and 44-fold more resistant to SbIII when compared to the untransfected parental line. Infection analysis using THP-1 macrophages showed that amastigote forms from both GPI-14 overexpressor clones were 3-fold more resistant to SbIII than the LbWTS line. These results suggest that the GPI-14 enzyme may be implicated in the SbIII-resistance phenotype in L. braziliensis. **Supported by:**CNPq, PIBIC/CNPq, FAPEMIG and IRR/Fiocruz **Keywords:** Leishmania braziliensis; mannosyltransferase; antimony-resistance

TB42 - **DEVELOPMENT OF A POLYVALENT CHIMERIC VACCINE FOR TRYPANOSOMIASIS**

FIGUEIREDO, L.A.*[1]; LUCAS, R.C.[1]; BARBOSA, F.S.[1]; ALMEIDA, R.M.[1]; OLIVEIRA, F.M.S.[1]; BUENO, L.L.[1]; BARTHOLOMEU, D.C.[1]; MENDES, T.A.O.[2]; FUJIWARA, R.T.[1]
1.UFMG, MG, Brazil; 2.UNIVERSIDADE FEDERAL DE VIÇOSA, MG, Brazil

The Family Trypanosomatidae comprises unicellular eukaryotic organisms of great medical and economic interest, since more than 1 billion people are infected worldwide by these protozoans. In this family are included the genera Leishmania and Trypanosoma, which are responsible for leishmaniasis and trypanosomiasis, respectively. Together these diseases generate approximately 4.27 million disability-adjusted life years due to the symptoms caused by parasitism. To date, there are no available human vaccines to protect against these diseases and the number of infected individuals continues to expand. Thus, in the present work we propose the development and use of a chimeric molecule formed by the fusion of DNA sequences that encode with immunogenic and conserved regions in the different parasites but absent in host species. The heterologous expression of a chimeric protein comprising 66 different peptides was produced in bacterial expression system, followed by subsequent protein purification and further use in the immunization of Balb/c mice. After 3 doses of vaccination with the chimeric protein formulated with MPLA or adjuvant alone at intervals of 15 days, animals were challenged with *L. amazonensis*, *L. mexicana* or *T. cruzi* strain Y. Parasite burden in specific organs was evaluated by quantitative PCR and a significant reduction of 68% in parasite load of animals challenged with T. cruzi was observed when compared to the control group. Moreover, we detected an 86% reduction in parasite burden on liver of vaccinated group, when challenged with *L. amazonensis*. For animals challenged with *L. mexicana* a significant reduction in the lesion diameter was detected after 12 weeks post infection in the vaccinated group. In the second experiment, animals were challenged with *L. infantum*, *L. mexicana* and *T. cruzi* Y and the results are still under processing and analysis. These preliminary results demonstrate the promising use of a potential vaccine against trypanosomiasis.**Supported by:**fapemig; CNPQ **Keywords:** Vaccine; trypanosomiasis; prophilaxis

TB43 - **IDENTIFICATION OF A CHALCONE MOLECULAR TARGET IN LEISHMANIA USING CUAAC CLICK REACTION**

OLIVEIRA, D.E.*[1]; STEEL, P.G.[2]; ROSSI-BERGMANN, B.[1]
1.UFRJ, RJ, Brazil; 2.DURHAM UNIVERSITY, England

The discovery of vital parasite targets are urgently needed for the rational design of novel inhibitors, once the current drugs are toxic and prone to resistance. Chalcones are a promising new class of antileishmanials whose target is still unknown. This project aims to identify the molecular target of chalcone CH8 and related analogues though the use of chemical probes. In this study, we used the nitro chalcone NAT22 as a model drug for its high selectivity and broad antileishmanial spectrum. We employed a biocompatible Huisgen-Sharpless cycloaddition CuAAC to identify the molecular target(s). As chemical tools, two chalcone probes were synthesized: 1) the NAT22 analogue pre-probe DEO37 containing an alkyne group; and 2) a trifunctional probe DEO53 containing biotin, rhodamine and an azide group for chemical linkage to DEO37. Insertion of alkyne group did not affect the broad spectrum of NAT22 antileishmanial activity, as DE037 also strongly inhibited (IC50 = 0.4 to 1.45 μM) Leishmania amazonensis, L. major, L. braziliensis, L. infantum and L. donovani promastigote growth. To identify chalcone targets on parasite proteome, proteins from DEO37-treated L.amazonensis promastigotes were linked to DEO53, and separated by SDS-PAGE gel electrophoresis yielding a single fluorescent band. Displacement assay with NAT22 confirmed a shared binding site with DEO37. The single fluorescent band was identified by Mass spectrometry-based proteomics as cytosolic tryparedoxin peroxidase (cTXNPx). cTXNPx identity was also confirmed by Western blotting. In summary, these results show that pre-probe / probe CuAAC click reaction proved to be an excellent tool for leishmanial drug target discovery that allowed the identification of cTXNPx, a critical Leishmania-specific enzyme involved in the parasite redox balance, as the NAT22 chalcone target. **Supported by:**CAPES, CNPq and The Royal Society. **Keywords:** Leishmania; molecular target; chalcone

TB44 - **THE IN VITRO ACTIVITY OF HYBRID COMPOUNDS OF PYRIDINE PROTOTYPES WITH THE 1,3,4-TIADIAZOLIC SUBUNITY OF MEGAZOL ON TRYPANOSOMA CRUZI**

BARBOSA, J.M.*[1]; DE CASTRO, S.L.[1]; FREITAS, R.[2]; FERREIRA, E.[3]; MANSSOUR, C.A.[2]; SALOMÃO, K.[1]
1.FIOCRUZ, RJ, Brazil; 2.UFRJ, RJ, Brazil; 3.FARMANGUINHOS, RJ, Brazil

There is an intense effort to find new drugs of Chagas disease, since the available ones, benznidazole (Bz) and nifurtimox (Nif) are not satisfactory, showing poor activity in the late chronic phase, severe collateral effects and limited efficacy against different parasitic isolates. In this study we determined the in vitro effect of hybrid compounds of a prototype pyridine, which are sterol biosynthesis inhibitors (SBIs), with megazol on two different strains of T. cruzi. On trypomastigotes of the Y strain, 8 compounds were more active than ketoconazole and posaconazole (standard SBIs). Besides, the compounds 2033 and 2035 tested on intracellular amastigotes (Tulahuen strain) were also more active than both standards. Compounds 2035 and 2035HCl were the most effective against trypomastigotes (Y strain), but the most selective was 1947HCl, presenting low toxicity to macrophages and cardiomyocytes, was selected for the subsequent assays. Treatment of intracellular amastigotes with 1947HCl, for 24 and 48 h, led to a decrease in the endocytic index. Trypomastigotes (Y strain) treated with 1947HCl presented blebs on the body and flagellum membranes, changes in kinetoplast and retraction of the parasite body. When assayed by flow cytometry on trypomastigotes treated with 1947HCl presented no alterations on ROS generation, the integrity of the plasma membrane and the mitochondrial membrane potential, indicating that its mode of action is not directly related to inhibition of sterol biosynthesis or generation of ROS. **Supported by:**Faperj, CNPq and FIOCRUZ **Keywords:** Trypanosoma cruzi; inhibitors of ergosterol biosynthesis ; nitroimidazoles

TB45 - **MITOCHONDRIAL DISFUNCTION AND ROS PRODUCTION ARE ESSENTIAL FOR ANTI-*TRYPANOSOMA CRUZI* ACTIVITY OF β-LAPACHONE-DERIVED NAPHTHOIMIDAZOLES**

BOMBAÇA, A.C.S.*[1]; VIANA, P.G.[1]; SANTOS, A.C.C.[2]; SILVA, T.L.[3]; RODRIGUES, A.B.M.[1]; GUIMARÃES, A.C.R.[1]; GOULART, M.O.F.[3]; SILVA JR, E.N.[2]; MENNA BARRETO, R.F.S.[1]
1.FIOCRUZ, RJ, Brazil; 2.UFMG, MG, Brazil; 3.UFAL, AL, Brazil

Chagas disease is caused by hemoflagellate protozoa *Trypanosoma cruzi* and comprehends one of the most important neglected tropical disease, especially in Latin America countries, showing an association between low-income populations and mortality. The nitroderivatives used in current chemotherapy present severe limitations, justifying the continuous search for alternative drugs. Since 1990s, our group have been investigating the trypanocidal activity of natural naphthoquinones and derivatives, and three naphthoimidazoles (N1, N2 and N3) derived from β-lapachone were most effective *in vitro*. Analysis of their mechanism of action, employing cellular, molecular and proteomic approaches, point the parasite mitochondrion as the primary target of these compounds, being trypanothione synthetase overexpressed after the treatment. We evaluated the mitochondrial and reactive oxygen species (ROS) participation for naphthoimidazoles anti-*T. cruzi* action. The pre-incubation of the parasite forms with antioxidants (urate and α-tocopherol) strongly protected the cells from trypanocidal effect of naphthoimidazoles, decreasing ROS levels produced, and also reverted mitochondrial swelling phenotype. The addition of pro-oxidants (menadione and H_2O_2) before the treatment also induced an increase in parasite lysis. Despite the oxygen uptake being strongly reduced by N1, N2 and N3, only in N1-treated parasites urate partially restored mitochondrial respiration. In parallel, trypanothione reductase activity was remarkably increased after the treatment with N1 and N3, and the molecular docking demonstrated that these two compounds positioned in pockets of this enzyme. Based on our findings, the direct impairment of mitochondrial electron transport chain by N1 led to oxidative misbalance, exacerbating ROS generation and subsequent parasite death. Although other mechanistic proposals can not be discarded, mainly in N2- and N3-treated parasites, requiring further investigations. **Supported by:**FAPERJ, CNPq and FIOCRUZ **Keywords:** Trypanosoma cruzi; naphthoimidazoles; antioxidant defenses

TB46 - **PRESENCE OF SODIUM DEOXYCHOLATE IN AMPHOTERICIN B FORMULATION LEADS TO SEVERE TOXICITY DURING INTRALESIONAL TREATMENT OF CUTANEOUS LEISHMANIASIS**

GONDIM, F.C.*[1]; ROSSI-BERGMANN, B.[1]; SOUSA-BATISTA, A.J.[1]
1.UFRJ-IBCCF, RJ, Brazil

Conventional leishmaniasis treatment is given by systemic routes, resulting in severe generalized toxicity which is particularly unacceptable in the case of localized cutaneous leishmaniasis (LCL). Intralesional treatment with antimonials has appeared as an appropriate strategy to avoid systemic toxicity in humans. In the present study, we evaluated the intralesional efficacy and toxicity of Amphotericin B (AmB), currently the most effective available antileishmanial on the market. For that, we used both the AmB formulation Anforicin (Cristalia), which contains sodium deoxycholate, and AmB powder (–Cristalia) combined with dimethyl sulfoxide (DMSO). These had their efficacy tested against CL in L. amazonensis GFP-infected BALB/c mice. After 7 days of ear infection the AmB formulations were locally injected 2 times a week for 2 weeks at 10, 50 and 150 µg / dose. Controls received PBS and PBS + DMSO. After 34 days of infection, the ears were grinded and the parasite loads were determined by both fluorimetry and limiting dilution assay. The amounts of AmB in the homogenized tissue were dosed by HPLC. Results showed that AmB was detectable in the ears for as long as 19 days of treatment termination, irrespective of its formulation. AmB+DMSO was as effective as Anforicin, in a dose-dependent fashion. However, AmB+DMSO was much safer than Anforicin, which produced extensive tissue necrosis. These data indicate that intralesional treatment with AmB is an effective and safe strategy to avoid systemic toxicity in CL, as long as the drug is administered without deoxycholate. **Supported by:** **Keywords:** Leishmania; deoxycholate; amphotericin b

TB47 - **A PROMISING INTRANASAL VACCINE AGAINST CANINE VISCERAL LEISHMANIASIS CONFERS EARLY PROTECTION IN MICE AGAINST *LEISHMANIA INFANTUM* INFECTION**

BEZERRA, I.P.S.*[1]; ROSSI-BERGMANN, B.[1]
1.INSTITUTO DE BIOFÍSICA CARLOS CHAGAS FILHO, UNIVERSIDADE FEDERAL DO RIO DE JANEIRO, RJ, Brazil

Visceral leishmaniasis (VL) is caused in America and in Europe by *Leishmania infantum* and is lethal if not treated. Although dogs are the main domestic reservoirs, available canine vaccines are given only after 4 months of age. Our group has developed a tolerogenic strategy based on mucosa vaccination to prevent the early Th2 response elicited by the infecting parasite. Indeed, intranasal (i.n.) vaccination with whole *L. amazonensis* antigens (LaAg) has proven to protect mice and hamsters against both cutaneous and VL, showing a broad spectrum of action. Moreover, retinoic acid (a vitamin A metabolite) encapsulated in nanoparticles (RA-NP) acts as an adjuvant for i.n. LaAg, increasing protection in BALB/c mice against *L. amazonensis* and in hamsters against *L. braziliensis* infection by enhancing regulatory T cell population in nasal mucosa. Besides being needle-free, an i.n. vaccine could be administered in dogs from 3 weeks of age, conferring earlier protection. Based on these findings, we proposed to evaluate LaAg/RA-NP i.n. vaccine efficacy against *L. infantum* infection in mice, aiming at the development of an innovative vaccine against canine VL. For comparative evaluation, BALB/c mice were either immunized with 2 i.n. doses of LaAg/RA-NP or 3 s.c. doses of the marketed Leish-Tec® (10 µg protein+50 µg saponin/dose). Controls were not vaccinated. After immunization, animals were challenged with *L. infantum* promastigotes. We found that LaAg/RA-NP is more effective than Leish-Tec®, reducing 94% of the parasite load in the spleen and 91% in the liver compared to the non-immunized group against 54% and 82% reduction promoted by the latter. In addition, LaAg/RA-NP proved to be effective in newborn mice (10 days-old), reducing the parasite load in the spleen (75%) and liver (82%) compared to non-immunized animals. Therefore, results demonstrate that i.n. LaAg/RA-NP is a promising vaccine to be tested against canine VL particularly aimed at newborn animals. **Supported by:**CNPq **Keywords:** Visceral leishmaniasis; leishmania infantum; intranasal vaccine

TB48 - **LQB 303 IMPAIRS IN THE MITOCHONDRIAL PHYSIOLOGY OF THE PROLIFERATIVE FORMS OF *TRYPANOSOMA CRUZI***

MACEDO, C.M.*[1]; SARAIVA, F.M.S.[1]; PAULA, J.I.O.[1]; COSTA, D.S.S.[1]; DIAS, A.G.[1]; PAES, M.C.[1]; NOGUEIRA, N.P.A.[1]
1.UERJ, RJ, Brazil

Chagas disease, a neglected illness affecting 7 million people worldwide. This disease is caused by *Trypanosoma cruzi* and the chemotherapy is very limited, not effective in the chronic phase, thus, more effective alternatives are mandatory. Previous data showed that α-phenyl-tert-butyl-nitrone (PBN) decreased mitochondrial reactive oxygen species levels and preserved respiratory system efficiency and energy status, but failed to decrease parasite persistence in chronically infected mice hearts. We have been studying the trypanocidal effect of the modified molecule, derived from PBN, LQB 303, against different forms of *T. cruzi in vitro*, but its action mechanisms are still unknown. Here we investigated the effect of LQB 303 on the mitochondrial physiology of the proliferative forms of *T. cruzi*, using high resolution respirometry and flow cytometry to determine the mitochondrial physiology of axenic amastigotes and epimastigotes forms treated with LQB 303 for 24h and 48h, respectively. After challenging the parasites with LQB 303 we observed that both amastigotes and epimastigotes reduced the rate of oxygen consumption in ROUTINE respiration (basal oxygen consumption using endogenous substrates in intact cells), as well as reduction in "PROTON LEAK" and "ETS" (maximal oxygen consumption after uncoupling), but increased "ROX" (residual mitochondrial oxygen consumption) by two and three times, respectively. We also evaluated the effect of LQB 303 on the mitochondrial membrane potential of T. cruzi amastigote forms by flow cytometry and observed that these parasites have a lower mitochondrial membrane potential, once there was a great decrease in TMRM labelling. Our results suggest that the induction of changes in mitochondrial physiology leads to a deficiency in oxygen consumption by these parasites, a fact that may negatively impact the production of mitochondrial ATP and contribute to an impairment in the proliferation of both amastigotes and epimastigotes forms. **Supported by:**FAPERJ, CNPq e INCT-EM **Keywords:** Mitochondrial physiology; chemotherapy; trypanosoma cruzi

TB49 - **EVALUATION OF THE LEISHMANICIDAL ACTIVITY OF EUSIDERIN A AGAINST *LEISHMANIA AMAZONENSIS***

FELISBERTO, V.C.*[1]; GOMES, J.S.C.[1]; DE PAULA, L.R.[1]; NOGUEIRA DOS SANTOS, C.E.[1]; SOARES, D.C.[1]; CARBONEZI, L.H.[1]; SARAIVA, E.M.[1]
1.UFRJ, RJ, Brazil

Leishmaniasis is an important public health problem that affects millions of people around the world, and few advances have occurred in relation to its chemotherapy. Leishmaniasis treatment still relies on pentavalent antimonials, amphotericin B and miltefosine, which have high toxicity and several side effects leading to patients withdrawal and to increased incidence of drug-resistant cases. All this point heartens the discovery of new drugs for the treatment of this pathology, and natural products constitute an important source of substances with leishmanicidal activity.

Piperaceae family contains plants that demonstrated antimicrobial activity, such as leishmanicidal, anti-*Trypanosoma* and anti-*Plasmodium*. Here we evaluated the leishmanicidal activity of eusiderin A, a neolignan isolated of leaves extracts from *Piper abutiloides* on *Leishmania amazonensis*. Our results have shown that eusiderin A treatment of *L. amazonensis* promastigotes and amastigotes forms presented a concentration-dependent leishmanicidal effect with IC_{50s} of 2.3 µg/mL for promastigotes, 19 µg/mL for axenic amastigotes and 53 µg/mL for intracellular amastigotes. Moreover, eusiderin A presented a low toxicity for host macrophages. The anti-leishmanial activity of eusiderin A was independent of reactive oxygen species (ROS), nitric oxide (NO) and TNF-α production by macrophages, and it did not affect arginase activity in these cells. Eusiderin A did not disturb the permeability of the parasite plasma membrane assayed by Sytox green or induce the exposure of annexin-V ligands, as well. Our data have shown that eusiderin A affects promastigotes' mitochondrial membrane potential and accumulation of neutral lipid bodies, suggesting that this neolignan causes incidental death. **Supported by:**CNPq, FAPERJ, CAPES **Keywords:** Leishmania amazonensis; eusiderin a; antileishmanial activity

TB50 - **BENZNIDAZOLE THERAPEUTIC IN SWISS MICE EXPERIMENTALLY INFECTED WITH *TRYPANOSOMA CRUZI* IV STRAINS FROM THE WESTERN BRAZILIAN AMAZON**

TESTON, A.P.M.[*1]; FERNANDES, N.S.[2]; ABREU, A.P.[2]; ZANUSSO JUNIOR, G.[1]; GOMES, M.L.[2]; TOLEDO, M.J.O.[2]

1.UNINGÁ, PR, Brazil; 2.UNIVERSIDADE ESTADUAL DE MARINGÁ, PR, Brazil

Trypanosoma cruzi discrete typing units could presents distinct infection dynamics and response to treatment with benznidazole (BZ). We demonstrated higher levels of parasitemia in mice inoculated with T. cruzi IV strains by oral route compared to intraperitoneal route. Our goal was to evaluate and compare the efficacy of BZ in mice orally (OR) and intraperitoneally (IP) inoculated with *T. cruzi* IV strains from the Western Brazilian Amazon. Swiss mice were inoculated by OR or IP routes with 2x106 metacyclics trypomastigotes of the strains AM14, AM16, AM64, and AM69 obtained from two outbreaks of acute Chagas disease orally acquired in the Amazonas, Brazil. The mice were treated with BZ (100 mg/kg/day-20X) from 10 days after inoculation and fresh blood examination, hemoculture, conventional and qualitative real-time polymerase chain reactions were used to monitor the cure. The strains exhibited different infection rates and responses to BZ treatment. Infectivity varied from 84 to 100% between strains, with AM16 exhibiting lower rates and AM14 and AM64 with 100% of positive animals in all groups. BZ treatment showed beneficial effects, despite the infection route. However, therapeutic failure was observed in a greater proportion in OR animals and the strains exhibited variation in susceptibility. Cure rates obtained for the OR and IP animals were, respectively: 18.2% (1/11) and 58.3% (7/12) for AM14 strain; 53.8% (7/13) and 84.6% (11/13) for AM16; 50% (6/12) and 66.7% (8/12) for AM64; and 75% (9/12) and 91.7% (11/12) for AM69. AM14 and AM16 exhibited different susceptibility based on the infection route. The AM69 was the only strain considered sensitive to BZ and AM64 was partially resistant, regardless of the infection route. The results provided evidence of the infection dynamic and biology of *T. cruzi* IV strains after different routes of inoculation. Furthermore, suggest that the efficacy of BZ treatment may depend on the infection route and the strain of *T. cruzi.* **Supported by:**Conselho Nacional de Desenvolvimento Científico e Tecnológico, Fundação Auracária, CAPES **Keywords:** Chagas' disease; oral infection; benznidazole

TB51 - **TRYPANOCIDAL ACTIVITY OF COMPOUNDS ISOLATED FROM *TRIXIS VAUTHIERI (ASTERACEAE)***

SALES-JUNIOR, P.A.[*1]; ALVES, T.M.A.[2]; COTA, B.B.[2]; SIQUEIRA FILHO, E.P.[2]; CALDEIRA, A.S.P.[2]; KOHLHOFF, M.[2]; MARQUES, F.R.[2]; ROCHA, F.S.[1]; MURTA, S.M.F.[1]; ZANI, C.L.[2]

1.LABORATÓRIO DE PARASITOLOGIA CELULAR E MOLECULAR - CPQRR/ FIOCRUZ MINAS, MG, Brazil; 2.LABORATÓRIO DE QUÍMICA DE PRODUTOS NATURAIS BIOATIVOS - CPQRR/ FIOCRUZ MINAS, MG, Brazil

Chagas' disease is extremely prevalent in Latin America and has low rates of cure, mainly in its chronic phase. Thus, new therapies are urgently needed. The major challenges for drug discovery and development for Chagas disease are the lack of effective in vitro and in vivo screening protocols and interest of the pharmaceutical industry. Fiocruz implemented a Technological Platform for the research of anti-Trypanosoma cruzi drugs (PlaBio Tc) which abides to steps, requirements and decision gates for the determination of the efficacy of novel drugs for *T. cruzi* proposed by Fiocruz and Drugs for Neglected Diseases initiative (DNDi). The PlaBio Tc and the Bioprospecting platform from Fiocruz identified the trypanocidal activity of the crude extract prepared from the aerial parts of *Trixis vauthieri (Asteraceae)*, a plant from the Asteraceae family. A bioguided-chemical fractionation afforded fractions that killed more than 90% of the parasites at concentration of 1 µg/mL. After other purification steps an inseparable mixture of trixikingolides 1 and 2 in 4:1 proportion were obtained. This mix showed an IC_{50} value of 0.46 µM against *T. cruzi* and 15.1 µM on L929 (selectivity Index = 32.8). The IC_{50} of the mix over the trypomastigotes and intracellular amastigotes forms of *T. cruzi* is eight times lower than that of benznidazole (IC_{50} = 3.8 µM), the reference drug used at the experiments. This is the first report on the trypanocidal activity of trixikingolides. The next steps will involve the isolation of these compounds in sufficient amount for in vivo assays using partially- and drug-resistant *T. cruzi* strains. **Supported by:**PDTIS/Fiocruz, FAPEMIG, CNPq and Fiocruz Minas (CAPES/PNPD) **Keywords:** Chagas disease; chemotherapy; trixis vauthieri

TB52 - **UNVEILING SIX HIGHLY SELECTIVE ANTILEISHMANIAL AGENTS VIA THE OPEN SOURCE COMPOUND COLLECTION 'PATHOGEN BOX'**

SILVA, J.A.S.[*1]; TUNES, L.G.[1]; COIMBRA, R.S.[1]; PIRES, D.E.V.[1]; MONTE NETO, R.L.[1]
1.INSTITUTO RENÉ RACHOU - FIOCRUZ MINAS, MG, Brazil

Chemotherapy is the main strategy to treat all forms of leishmaniasis, despite the limited drug arsenal, emergence of resistant parasites and toxicity. In order to identify new antileishmanial agents, here we performed screening of the 'Pathogen box' - an open access drug discovery resource that provides 400 curated chemical compounds active against neglected diseases causative agents. Twenty-seven out of 400 small molecules were able to inhibit the growth of intracellular *Leishmania* (*Viannia*) *braziliensis* by, at least, 80% at 5 µM. They were originally described as antikinetoplastids, antimalarial, antituberculosis and antitoxoplasmosis agents, revealing the potential for drug repurposing. Three out of 27 were the reference drugs: amphotericin B, buparvaquone and pentamidine. The 24 compounds were then evaluated for toxicity against THP-1 macrophages and the IC_{50} established for L. braziliensis intramacrophagic amastigotes. Pharmacokinectics properties were also computationally predicted using the pkCSM software (biosig.unimelb.edu.au/pkcsm/). Six out of 24 compounds were highly selective against the parasite, presenting selective indexes ranging from 104 to 745 folds. The IC_{50}s are at nanomolar range varying from 470 to 40 nM. Indeed, they were also active against antimony-resistant (SbR) clinical isolates or laboratory-selected resistant parasites, revealing the potential to treat SbR infections. Among the 6 selected small molecules, 4 were already described as antikinetoplastids while 2 were previously reported to be active against *Toxoplasma gondii*, *Plasmodium*, *Mycobacterium tuberculosis* and *Neospora caninum*. The target(s) is(are) yet to be described for *Leishmania* parasites. We are currently performing molecular docking analysis to have clues of their mechanism of action. In this work we highlight the importance of the 'Pathogen Box' to be used as a high potential source of antileishmanial drugs. **Supported by:**CNPq; CAPES; Fapemig; MMV **Keywords:** Leishmania; drug discovery; pathogen box

TB53 - **EFFECT OF INHIBITORS OF CASEIN KINASE 2 (CK2) IN THE INTERACTION BETWEEN *LEISHMANIA BRAZILIENSIS* AND MACROPHAGES**

GOMESA, S.M.R.[*1]; LIMA, A.K.C.[1]; ALBUQUERQUE, R.A.A.C.[1]; DUTRA, P.M.L.[1]
1.UERJ, RJ, Brazil

The genus Leishmania has a life cycle in which they differ from avirulent promastigotes to metacyclic promastigotes in the gut of the vector, and from promastigotes to amastigotes into vertebrate host macrophages. The CK2 protein kinase is an enzyme related to processes of developmental regulation, including cell cycle and differentiation. The present work aims to study the role of the CK2 enzyme in Leishmania braziliensis cell growth and the interaction of this parasite with mammalian cells. The L. braziliensis promastigotes grown in Schneider medium, added 10% FCS at 27ºC, until reach 5 passages (infective strain). Growth curves were carried out in the presence or in the absence of CK2 inhibitors TBB (1µM) and K137E4 (1.25; 2.5; 5 and 10 µM). The mouse peritoneal macrophages were incubated with the inhibitors and their viability was assessed by MTT method. The interaction assays were carried out in the absence (control) or in the presence of the inhibitors TBB (1µM) and K137E4 (1.25; 2.5; 5 and 10 µM). The results are expressed as a mean ± standard error of the mean (mean ± SEM). In the present work, we demonstrated that inhibitors of CK2, TBB, and K137E4, were able to inhibit significantly the growth of infective L. braziliensis promastigotes. These inhibitors added during the course of the interaction between L. braziliensis and macrophages promoted a significant reduction in the number of amastigotes by macrophages and, consequently, in the infection index. We can conclude that the protein CK2 is important for the growth of L. braziliensis and for L. braziliensis-macrophages interaction. TBB and K137E4 were not toxic to peritoneal macrophages of BALB/c mice in the concentrations used in this work. **Supported by:**CNPQ FAPERJ **Keywords:** Ck2 ; protein kinase; leishmania braziliensis

TB54 - *IN SILICO* PREDICTION AND *IN VITRO* IDENTIFICATION OF HUMAN *VS*. MOUSE LIVER METABOLITES OF A PROMISING ANTILEISHMANIAL CHALCONE

BARRETO, A.R.R.*[1]; DE SOUZA, A.M.T.[1]; VIEIRA, B.A.A.[1]; VALENTE, A.P.C.[1]; STEEL, P.G.[1]; BERGMANN, B.R.[1]
1.UFRJ, RJ, Brazil

Considering treatment limitations such as toxicity, high cost, parenteral administration, and emerging drug resistence, new drugs are urgently needed for leishmaniasis treatment, especially by the oral route. Previously, we observed that a synthetic nitro chalcone (NAT22) is more active than the intralesional route, suggesting a critical liver metabolism. The present work aimed at investigating the generation of chalcone liver metabolites as sources of new drugs using NAT22 as model chalcone. Also, the study will allow to validate mice as suitable models for antileishmanial chalcone therapy. NAT22 human liver metabolites were predicted in silico using Predictor™ software. For in vitro metabolism, mouse and human isolated liver microsomes were used in the presence of a NADPH-regenerating system for 90 min. *In silico*, NAT22 was predicted to be substrate for CYP 1A2, 2A6, 3A4 and 2C8 liver enzymes. The predicted human metabolites are two demethylated and a hydroxylated compounds, but only one demethylated compound is known. NMR analysis also identified CH8 as one of the major microsome-generated metabolites. No substantial NMR spectrum differences were seen between mouse and human NAT22 metabolites, suggesting that mice are appropriate models for antileishmanial chemotherapy with chalcones. **Supported by:**CAPES **Keywords:** Leishmania; chalcone; in silico and in vitro metabolism

TB55 - *IN VITRO* EFFECT OF NEW IRON METALOCOMPLEXES COMPOUNDS AGAINST THE GROWTH OF *TRYPANOSOMA CRUZI*

MOREIRA, F.F.*[1]; PORTES, J.A.[2]; AZEREDO, N.F.B.[1]; FERNANDES, C.[1]; JUNIOR, A.H.[1]; DE SOUZA, W.[2]; DAMATTA, R.A.[1]; SEABRA, S.H.[3]
1.UENF, RJ, Brazil; 2.UFRJ, RJ, Brazil; 3.UEZO, RJ, Brazil

Chagas disease is a neglected tropical disease, caused by *Trypanosoma cruzi*, and is a major public health problem in Latin America affecting approximately 18 million individuals and about 60 million lives are at risk of contamination. The available therapy for this disease is based on two drugs, Nifurtimox and Benznidazole, which exhibit severe cytotoxic effects, including resistance, inefficiency in the chronic phase of the disease, and variable efficacy. Therefore, new compounds against this parasite are urgently needed. Coordination compounds like metalocomplexes have already been tested against *Leishmania* spp. and *Toxoplasma gondii*, possibly being an interesting alternative for antiparasite therapy against *T. cruzi*. Metalocomplexes are metal core compounds that can be coordinated with organic binders. Transition metals offer more advantages when compared to other ordinary drugs based on organic compounds. Here we tested the *in vitro* effect of two iron compounds on the growth of epimastigotes of *T. cruzi* (Y strain). The parasites were treated with the compounds ranging from 1 to 100 nM and their number quantified. Compound I presented an IC_{50} value of 4.14 nM and 4.18 nM, after 3 and 5 days of treatment, respectively. Compound II presented an IC_{50} value of 4.71 nM and 7.82 nM for the same treatment times. Parasites treated with the lowest IC_{50} value compound presented mitochondrial with altered cristae, and swelling and abnormal disposition around the kinetoplast. In addition, treatment with both compounds caused loss of the mitochondrial membrane potential of the parasites. These compounds were active against epimastigotes of *T. cruzi* presenting an exceptional low IC_{50} values and affecting the mitochondria, which is an essential organelle for parasite survival. The next step will be to test the compound on intracellular amastigotes and analyze parasite cell death type and the possible mechanism of action of the compounds. **Supported by:**FAPERJ, CNPq, CAPES, UENF, UFRJ, UEZO **Keywords:** Chagas disease; trypanosoma cruzi; metalocomplexes

TB56 - **INVESTIGATION OF IRON SUPEROXIDE DISMUTASE ROLE IN *LEISHMANIA INFANTUM* THROUGH GENERATING KNOCKOUT LINES BY DIFFERENT APPROACHES**

SANTI, A.M.M.[*1]; DA SILVA, P.A.[1]; MURTA, S.M.F.[1]
1.RENÉ RACHOU INSTITUTE, MG, Brazil

Leishmaniasis is a neglected tropical disease caused by protozoan parasites of the genus *Leishmania*. Their antioxidant defense presents some differences when compared to the host, which must be exploited as a potential targets for leishmaniasis chemotherapy. The iron superoxide dismutase A (FeSODA) is a metalloenzyme involved in the antioxidant defense by converting superoxide radicals to oxygen and hydrogen peroxide inside the mitochondria. Despite the fact that SOD is also found in humans, the catalytic site of this enzyme containing Cu/Zn/Mn instead the Fe, which is found in *Leishmania*. Since FeSOD is absent in the human host, this enzyme is considered as a potential target for chemotherapy against Leishmaniasis. To investigate the role of FeSODA in *Leishmania infantum*, we attempt to generate FeSODA null mutants by conventional homologous gene replacement. Although we were able to replace both alleles of the gene, a third copy of the gene appeared in the genome, due to the genomic plasticity of those parasites. Despite the presence of the third copy, the Western blot analyses have shown that the FeSODA expression is reduced in the parasites with double gene replacement. Phenotypic analysis also has shown that those parasites were more susceptible to oxidative stress generated by menadiona and they present lower ability to maintain the infection in THP-1 macrophages when compared to the wild-type line. In order to investigate whether FeSODA is actually an essential gene, we attempt to generate FeSODA knockout line using CRISPR/Cas9 system. Parasites expressing spCas9 were submitted to three rounds of transfection using three different sgRNA guides and a specific donor DNA containing stop codons. We are able to obtain edited parasites in which at least one FeSODA copy was deleted. We are currently screening the FeSODA knockouts clones to evaluate if we are able to generate parasites with the complete ablation of FeSODA in *L. infantum* by using CRISPR. **Supported by:**FAPEMIG; CNPq; CAPES; UGA/FAPEMIG **Keywords:** Leishmania infantum; iron superoxide dismutase; crispr/cas9

TB57 - **ASCORBATE PEROXIDASE ENZYME IS INVOLVED IN *LEISHMANIA BRAZILIENSIS* ANTIMONY-RESISTANCE PHENOTYPE**

MOREIRA, D.S.[*1]; MURTA, S.M.F.[1]
1.FIOCRUZ-MINAS, MG, Brazil

Ascorbate peroxidase (APX) is a redox enzyme of the trypanothione pathway that converts hydrogen peroxide (H2O2) into water molecules, regulating the oxidative stress in Leishmania and avoiding the cell damage for the parasite. In this study, we overexpressed the APX gene in L. braziliensis to investigate the contribution of this enzyme in the trivalent antimony (SbIII)-resistance phenotype of this parasite. Western blot assays revealed that the APX protein level was 5-fold higher in the transfected clones from L. braziliensis than in the wild-type (LbWTS) line or transfected with empty vector. APX-overexpressor clones were approximately 8-fold more resistant to SbIII in comparison with the untransfected control (LbWTS). This result demonstrates that greater amounts of APX enzyme are necessary to reduce the toxic effects produced by SbIII and to prevent the death of the parasite due the perturbations caused in its redox potential. In addition, our results indicated that APX-overexpressing L. braziliensis lines were about 1.8-fold more tolerant to exogenous H2O2 than LbWTS line, suggesting that APX enzyme plays an important role in the defense against oxidative stress. Susceptibility results showed that the transfected clones were more resistant to isoniazid, an antibacterial agent that interacts with APX. Surprisingly, this compound raised the leishmanicidal SbIII effect, indicating that this combination might be a good strategy for leishmaniasis treatment. This study is the first of the literature to demonstrate that isoniazid has effect against L. braziliensis. Therefore, our data indicate that APX enzyme is an attractive therapeutic target which is implicated in the SbIII-resistance mechanism of L. braziliensis, contributing to direct the development of new strategies for leishmaniasis chemotherapy.**Supported by:**CNPq; IRR/FIOCRUZ; FAPEMIG; CAPES; PDTIS/FIOCRUZ. **Keywords:** Leishmania braziliensis; ascorbate peroxidase; chemotherapy

TB58 - **VALIDATION OF CRISPR/CAS9 TOOLKIT IN *LEISHMANIA INFANTUM* USING THE PARALYZED FLAGELLA PROTEIN 16**

DA SILVA, P.A.*[1]; MURTA, S.M.F.[1]
1.RENÉ RACHOU INSTITUTE, MG, Brazil

The toxic effects related to pentavalent antimonial-containing compounds, which are fist-line therapies against leishmaniasis and the existence of antimony-resistant parasites emphasize the importance of identifying new targets for leishmaniasis chemotherapy. To identify these targets, loss of function is frequently analyzed. However, their classical methodologies such as homologous gene replacement are time-consuming and laborious. The CRISPR/Cas9 gene editing tool has been applied successfully in *Leishmania* parasites and has been shown to be faster and more efficient. Hence, our aim is to use a CRISPR/Cas9 high-throughput genome editing toolkit for performing functional studies of genes involved in important metabolic pathways in *L. Infantum* by both tagging and disrupting these genes. Firstly, we attempt to validate this toolkit in *L. infantum* RPV strain by tagging the gene paralyzed flagella protein 16 (PF16) which encode central pair projections of axoneme with a fluorescent protein to produce a fluorescent flagellum. Thus, promastigotes of *L. infantum* RPV strain were transfected with the pTB007 plasmid encoding the T7 promoter and the spCas9 gene. Western blot analysis confirmed the Cas9 expression in the transfected parasites. Then, these parasites were transfected with a donor DNA containing the green fluorescent protein mNeonGreen followed by a blasticidin (BSD) resistance gene and the DNA templates for *in vivo* production of the sgRNAs. BSD-resistant parasites were selected and confocal microscopic analysis showed presence of green fluorescence at the flagellum, as expected. Currently, we are disrupting the PF16 gene, to validate this CRISPR/Cas9 toolkit in *L. infantum* RPV strain before editing promising targets for leishmaniasis chemotherapy. **Supported by:**FAPEMIG; CNPq; CAPES; UGA/FAPEMIG **Keywords:** Leishmania infantum; pf16; crispr/cas9

TB59 - **IN VITRO ANTILEISHMANIAL ACTIVITY AND CYTOTOXICITY OF TETRAOXANES ANALOGUES LEAD TO IDENTIFICATION OF NEW PROMISING COMPOUNDS TO TREAT LEISHMANIASIS**

ALMEIDA, R.M.*[1]; ANTOLINEZ, I.V.[1]; BARBOSA, L.C.A.[1]; FERREIRA, S.R.[2]; FUJIWARA, R.T.[1]
1.UFMG, MG, Brazil; 2.UFSB, BA, Brazil

Leishmaniasis is a neglected tropical disease endemic in 98 countries, posing as a risk to 350 million people, with an incidence of 1.5 million new cases every year. Although this disease was discovered more than 100 years ago, the currently available drugs for treatment of leishmaniasis are almost the same as the ones used 50 years ago. The efficacy of the treatment based on these drugs is limited due to their toxicity, the presence of resistant strains in several *Leishmania* species, the length of treatment and its high cost. Thus, the continuous search for a new, cheaper, effective and less toxic treatment for this disease is still necessary. Tetraoxanes are compounds characterized by the presence of peroxidic bonds within its structure. The synthesis of these compounds has been widely studied because of its great antimalarial potential. In this study, we evaluated the potential of 4 asymmetrical 1,2,4,5-tetraoxane compounds to inhibit the intracellular growth of *Leishmania amazonensis* amastigotes parasitizing canine macrophages (DH82), in vitro. The ELISA test was employed to measure the inhibitory activity of these compounds on intracellular parasites. The cytotoxicity of the active compounds was evaluated by metabolic activity using MTT assay. The tested compounds RC3, RC29, RC47 and RC73 were able to inhibit the intracellular amastigote proliferation with the respective IC50 values: 0.6, 19.7, 99.5 and 22.6 μM. With the exception of compound RC47, all compounds presented better results than the positive control antimony salt III (80 μM) and low toxicity against canine macrophage (DH82), African green monkey kidney cells (BGM) and Human hepatocytes-like cells (HepG2). Moreover, the compound RC3 showed excellent antileishmanial activity at micromolar level and high selectivity index of 157. Here, the present results denote the potential of tetraoxane analogues, in special the compound RC3, as promising candidate for the treatment against leishmaniasis. **Supported by:**FAPEMIG **Keywords:** Antileishmanial; tetraoxane; leishmania amazonensis

TB60 - **LGO TOXICOLOGICAL AND PHARMACOKINETICS STUDY: DEVELOPMENT OF A NEW EXPERIMENTAL THERAPEUTIC SCHEME FOR LEISHMANIASIS**

GERVAZONI, L.F.O.*[1]; GONÇALVES-OZÓRIO, G.[1]; SILVA, A.C.A.[1]; SILVEIRA, G.P.E.[1]; PEREIRA, H.M.[1]; PINTO, D.P.[1]; FONSECA, L.B.[1]; CUNHA-JUNIOR, E.F.[1]; ALMEIDA-AMARAL, E.E.[1]
1.FIOCRUZ, RJ, Brazil.

Leishmaniasis is a disease that deserves attention due to the wide variety of clinical manifestations and its high annual incidence. Although there are many drugs available as alternatives for leishmaniasis treatment, they remain mostly ineffective, toxic, expensive and longstanding. LGO was previously tested in vitro and in vivo by our group, which was observed that 50mg/kg/day administrated by oral route was able to reduce lesion size and parasitic load in L. amazonensis in vivo infection. Considering LGO promising results and drug development process, this study evaluated the toxicological and pharmacokinetics parameters of oral LGO administration. Swiss mice, acute and 28 days-repeated doses oral toxicity were performed according to OECD guidelines (N° 425 and 407, respectively). In both toxicological models, LGO did not present any mortality, considering doses of 175, 550 and 1750mg/kg in acute up and down model and 5, 50, 500mg/kg in subacute model. Weight gain, food/water consumption, clinical, hematological and biochemical parameters had minor alterations or did not significantly altered in both toxicological models. Pharmacokinetic study was performed in female Balb/c mice plasma, after administration of 10mg/kg of LGO orally, with stablished times (2.5min - 360min). The quantification method was performed by LC-MS/MS and pharmacokinetics parameters were calculated using WinNonlin software. Preliminary pharmacokinetic results demonstrates that LGO is orally absorbed, with a Tmax of 2.5min and a Cmax of 120.11ng/mL. LGO T1/2β estimated was 66.07min and ASC0-∞ was 4002.63ng/mL. Taken together, these results demonstrates the safety of LGO up to a highest dose such as 1750mg/kg and corroborates previous in silico analysis of a good oral absorption, with satisfying pharmacokinetics parameters. Once validated, pharmacokinetic parameters will be used to calculate a new therapeutic scheme to be tested against leishmaniasis cutaneous and visceral models**Supported by:**FAPERJ; CNPq; CAPES; PAPES; IOC/FIOCRUZ **Keywords:** Leishmaniasis; toxicology; pharmacokinetics

TB61 - **NANOMILLING OF CHALCONE NAT22 IMPROVES ITS ORAL EFFICACY AGAINST CUTANEOUS LEISHMANIASIS**

BORSODI, M.P.G.*[1]; PACIENZA-LIMA, W.[1]; SOUSA-BATISTA, A.J.[1]; MENEZES, J.C.V.[1]; OLIVEIRA, D.E.[1]; ARRUDA-COSTA, N.[1]; SILVA, A.J.M.[1]; CABRAL, L.M.[1]; ROSSI-BERGMANN, B.[1]
1.UFRJ, RJ, Brazil

Conventional treatment of cutaneous leishmaniasis (CL) is based on multiple and painful injections with toxic drugs, urging safer and more active drugs especially for non-invasive use. We have been studying a synthetic trimethoxylated chalcone (NAT22), highly active against *L. amazonensis* promastigotes (IC50 0.6 µM) and intracellular amastigotes (IC50 0.5 µM). However, NAT22 is highly lipophilic, hampering its oral. This work aims to improve its bioavailability by nanocrystalization. For that, NAT22 crystals (~400 µm) were submitted to dry milling followed by wet milling, yielding nanocrystals (250 nm, herein nanoNAT22). Promastigotes were incubated with different concentrations of NAT22 or nanoNAT22 for 72 h at 26 °C, when cell viability was assayed by MTS. For anti-amastigote activity, bone marrow-derived macrophages were infected with promastigotes (1:10) for 24 h at 37 °C and then treated for further 48 h with nanoNAT22 or NAT22. After staining, amastigotes/ 100 macrophages were counted under optical microscopy. Cytotoxicity was assessed by LDH release in supernatants. For *in vivo* studies, BALB/c mice were infected in the ear with *L. amazonensis*-GFP. Seven days later, were orally treated daily (40 mg/kg) with nanoNAT 22 or NAT22 for 5 weeks. Controls received intralesional Glucantime (1.5 mg/kg, 1x/week). Lesion sizes were measured 2x/week. On day 52 of infection, ear parasites were measured by fluorimetric and limiting dilution assays. Results showed that nanoNAT22 in PBS were as active against promastigotes and amastigotes as NAT22 in DMSO. NanoNAT22/PBS cytotoxicity against macrophages was much lower than nanoNAT22/ DMSO (CC50 30 µM and 7.5 µM, respectively). In vivo, oral nanoNAT22 was 1.4-fold more effective than NAT22 regarding lesion growth and parasite loads. Thus, nanomilling significantly improves solubility, anti-parasitic selectivity, oral bioavailability and oral efficacy of NAT22 chalcone against CL. **Supported by:**CNPq **Keywords:** Chalcone; nanomilling; leishmania

TB62 - **REPURPOSING AS A STRATEGY FOR THE DISCOVERY OF A NEW ANTILEISHMANIAL**

CHARLTON, R.*[1]; STEEL, P.G.[1]; DENNY, P.W.[1]; BERGMANN, B.R.[2]
1.DURHAM UNIVERSITY, United Kingdom; 2.UNIVERSIDADE FEDERAL DO RIO DE JANEIRO, RJ, Brazil

CANCELADO

Leishmaniasis is a vector-borne Neglected Tropical Disease, caused by protozoan parasites of the genus Leishmania for which there is a shortage of effective viable non-toxic drugs. T[illegible]ere are approximately 1.3 million new cases of leishmaniasis each year with the great[illegible]n the poorest communities. This means that desperately needed new antileishm[illegible]ve to be both affordable and accessible. Established medicines with cheaper [illegible]mes may hold the cure for this neglected disease. The repositioning [illegible]ew concept but, with the ambitious target of controlling or [illegible]is strategy is still an important one.
Previous work in our group identified Lei[illegible]e synthase (LmjIPCS) as a potential drug targ[illegible]e involved in the biosynthesis of sphingolipids [illegible] mammalian orthologue (sphingomyelin synthase[illegible]ctive antileishmanials. Using a microtiter plate co[illegible] active compounds were screened for activity aga[illegible]n over-the-counter antihistamine, showed subm[illegible]es of leishmania (L. major, L. amazonensis, L. [illegible]tive against against L. amazonensis intramacrophage a[illegible]ccessful in vitro results and a well-studied pharmacokinetic and m[illegible]astine was chosen as an ideal candidate to progress into a mouse mo[illegible]nificant reduction in parasite burden when clemastine was given via the IL rout[illegible]g its potential as a localised antileishmanial therapy.
This [illegible]tation will share these findings together with details of synthetic studies towards new active and more accessible analogues of clemastine. **Supported by: Keywords:** Leishmania; drug discovery; sphingolipids

TB63 - **SEAWEED DICTYOTA CARIBEAE EXTRACT RICH IN SULPHATED POLYSACARIDES CONTROL IN VITRO GROWTH OF TOXOPLASMA GONDII**

RODRIGUES, Y.M.*[1]; SEABRA, S.H.[2]; DAMATTA, R.A.[1]; DOS SANTOS, T.A.T.[1]; CINELLI, L.P.[3]
1.UENF, RJ, Brazil; 2.UEZO, RJ, Brazil; 3.UFRJ, RJ, Brazil

Some seaweed extracts are rich in sulphated polysaccharides and have been the focus of much research. Sulphated polysaccharides are composed of a disorder combination of monosaccharides and sulfate groups and are currently one of the most used compounds for anticoagulant, antioxidant, and anti-inflammatory testing among other applications. Drugs with sulfa groups are currently used for the treatment of toxoplasmosis, a disease caused by Toxoplasma gondii. The aim of this work was to verify if the Dictyota caribaea seaweed ethanolic extract rich in sulphated polysaccharides control T. gondii growth. LLC-MK2 epithelial host cell was infected with tachyzoites and treated with 5 different extracts(total, F9, F23, F44, F55, and F60) of the D. caribeae at different concentrations. The total extract was obtained after algae delipidation and proteolytic digestion with papain. The fractions were obtained by addition of different ethanol volumes: F9 (10%), F23 (30%), F44 (80%), F55 (150%) and F60 (300%). Dilution of the extract in culture medium caused no significant change in the osmolarity of the solution. Optical microscopy analysis showed a significant reduction in parasite growth after treatment with extract F9 when compared to untreated control. F9 treatment also caused a morphological shift from the parasite arc shape to a round shape. The ethanolic fraction may be a potential compound with anti-T. gondii activity. **Supported by: Keywords:** Toxoplasma gondii; dyctiota caribeae; sulphated polysaccharides

TB64 - **PHASE I AND II CLINICAL TRIAL EMPLOYING KMP-11 VACCINE AGAINST CANINE VISCERAL LEISHMANIASIS**

AGUIAR-SOARES, R.D.O.*[1]; ROATT, B.M.[1]; CARDOSO, J.M.O.[1]; MATHIAS, F.A.S.[1]; REIS, L.E.S.[1]; PEREIRA, J.F.[1]; FORTES DE BRITO, R.C.[1]; OSTOLIN, T.L.V.D.P.[1]; KANOMATA, P.Y.[1]; VASQUES, B.V.[1]; NAGAOKA, M.Y.D.[1]; CARMO, T.N.D.[1]; COSTA, A.F.P.[1]; GIUNCHETTI, R.C.[2]; REIS, A.B.[1]

1.UFOP, MG, Brazil; 2.UFMG, MG, Brazil

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 KMP-11 after complete immunization protocol in a vaccine clinical trial phases I and II. For this, fourteen dogs were classified into two groups: i) control group (n=7) received 1 mL of sterile 0.9% saline solution; ii) KMP-11 group (n=7) received 100 µg of recombinant KMP-11 protein plus 1 mg of saponin adjuvant. Analysis of the humoral immune response by anti-Leishmania total IgG antibodies with EIE® (Bio-Manguinhos®) in the serum of dogs, fifteen days after third immunization, showed an increase in mean optical density of KMP-11 group above threshold of positivity. Our results of serological reactivity using TR DPP® (Bio-Manguinhos®) showed that all dogs of different groups were negative, demonstrating the ability of the test to discriminate vaccinated dogs. Our results of the immunogenicity vaccine demonstrated increase in circulating population of monocytes $CD14^+$ in the end of the immunization protocol (T1) in KMP-11 group. The approach of in vitro assay aimed to evaluate the percentage of antigen-specific $CD4^+$ and $CD8^+$ lymphocytes producers of IFN-γ e IL-4, where an increase in both IFN-γ producing subpopulations in the group KMP-11 was observed. Our immunogenicity results support the hypothesis that the vaccine process with the KMP-11 vaccine is be able to generate a protective immune response against the *L. infantum* parasite. Future studies will be carried out to evaluate the potency of the vaccine, measuring the parasitic load by qPCR in different tissues affected by the parasite after the experimental challenge with *L. infantum*. **Supported by:**FAPEMIG, CNPq, CAPES, FINEP, FIOCRUZ, UFOP and UFMG **Keywords:** Leishmania infantum; canine visceral leishmaniasis; kmp-11 vaccine

TB65 - **TERNARY THERAPY FOR TREATMENT OF VISCERAL LEISHMANIASIS**

SANTOS-PINTO, L.A.*[1]; SANTOS-SILVA, K.E.[1]; ANDRADE-NETO, V.V.[1]; TORRES-SANTOS, E.C.[1]

1.IOC/FIOCRUZ, RJ, Brazil

Translational studies, involving repositioning and polychemotherapy, are interesting approaches that can lead to a rapid coping with this serious public health problem, which is leishmaniasis. We propose the evaluation of an association of three drugs: miltefosine, ezetimibe and an azole. Azoles are inhibitors of ergosterol biosynthesis with potent leishmanicidal activity in vitro, but with inconsistent in vivo activity. Ezetimibe is an inhibitor of the cholesterol transporter NCP1L1, with synergistic leishmanicidal activity in vitro in association with azoles and efficacy in murine leishmaniasis model. Miltefosine is the only available drug active by oral route, but several reports suggest a risk of developing resistance, a hallmark of monotherapy. Leishmanicidal activity of the drugs ketoconazole, posaconazole, itraconazole, ezetimibe and miltefosine alone or associated was evaluated in intracellular amastigotes of L. infantum. Synergism was analyzed by the Fractional Inhibitory Concentration Index (FICI). The FICI values were used to analyze the type of effect obtained in the association: FICI ≤ 0.5 - synergism; 0.5 < FICI < 4 - additive; FICI ≥ 4 - antagonism. The ICs50 of the drugs were 7.1 ±1.0 µM, 0.4 ± 0.1 µM, 0.8 ± 0.1 µM, 9.5 ±1.0 µM e 0.5 ±0.4 µM, to ketoconazole, itraconazole, posaconazole, ezetimibe e miltefosine, respectively. Different combinations of posaconazole + ezetimibe + miltefosine (3:2:3 and 1:4:1) and itraconazole + ezetimibe + miltefosine (3:2:2 and 1:4:1) had FICI values below than 0.5, suggesting synergic effect. The results pointed these ternary combinations as promising protocols to be evaluated for the treatment of visceral leishmaniasis. **Supported by:**FIOCRUZ **Keywords:** Azoles; miltefosine; ezetimibe

TB66 - **EFFECT OF SCHINUS MOLLE L. ESSENCIAL OIL AND ITS DERIVATE TERPINEN-4-OL ON LEISHMANIA AMAZONENSIS**

PALMEIRA, D.[*1]; FLORENCIO, M.[1]; RAMUZ, J.[2]; ROMEIRO, B.[3]; CHAVES, D.S.A.[4]; FAMPA, P.[3]
1.PPGCV- IV/UFRRJ, RJ, Brazil; 2.PPGCV-IV/UFRRJ, RJ, Brazil; 3.DCFAR - ICBS/UFRRJ, RJ, Brazil; 4.DCFAR-ICBS/UFRRJ, RJ, Brazil

Leishmaniases are a group of diseases caused by protozoans that belong to Leishmania genus (Trypanosomatidade family). They are classified as neglected diseases for occurring predominantly at underdeveloped countries. The parasites present heteroxenous transmission cycle exhibiting two main life forms along their development: promastigotes at digestive tract of the insect vector (Psychodidae family sandflies) and amastigotes that infect mammal host macrophages. There are four distinct clinical manifestations of leishmaniases which are associated to specific species of the parasite: cutaneous; diffuse cutaneous, mucocutaneous and visceral. The available therapy against these diseases is not completely effective and present high costs and important side effects. The identification of new drugs against Leishmania is necessary. Natural products present great potential as effective drugs. We investigated the effect of Schinus molle L. (Anacardicaceae) essential oil (A2M2) and of its derivate terpinen-4-ol against Leishmania (Leishmania) amazonensis promastigotes as well as against intracellular amastigotes infecting Balb/c mice peritoneal macrophages. Cytotoxicity of the compounds for the host cells was also evaluated. A2M2 presented IC50/24h of approximately 0.03 µg/mL and terpinen-4-ol with IC50/24h of 1.5 mM. Both compounds inhibited promastigote forms of the parasite in a dose-dependent manner. They were not cytotoxic to macrophages at different doses. The association index (percentage of infected macrophages multiplied by the mean number of amastigotes per macrophages) reached a decrease of 45 and 60% after treatment with A2M2 and terpinen-4-ol at the higher tested doses. These results suggest that Schinus molle essential oil and terpinen-4-ol present satisfactory effectiveness against Leishmania amazonesis. Posteriorly, we intend to analyze alterations at the morphology of the parasite by ultrastructural analyses after treatment with A2M2 and terpinen-4-ol. **Supported by:**CAPES, FAPERJ
Keywords: Leishmania amazonensis; schinus molle l; natural products

TB67 - **STUDY OF LEISHMANICIDAL ACTIVITY OF DIFFERENT SUBSTANCES ISOLATED FROM *TETRADANIA RIPARIA* LEAVES EXTRACT**

DE PAULA, L.R.[*1]; SOARES, D.C.[1]; LOPES, A.G.[1]; DIAS REZENDE, V.L.[2]; SILVA, R.S.F.[1]; LEITÃO, G.G.[1]; SARAIVA, E.M.[1]
1.UFRJ, RJ, Brazil; 2.IFRJ, RJ, Brazil

Leishmaniasis are neglected diseases, which affects millions of people in 98 countries in tropical and subtropical regions. Leishmaniasis is caused by parasites of the genus Leishmania and displays three major clinical forms: cutaneous, mucocutaneous and visceral. Pentavalent antimonials, amphotericin B and miltefosine are the main drugs currently available for leishmaniasis treatment. All of them present problems that limit their use, such as, adverse side-effects, induction of parasite resistance and high costs. Thus, the discovery of new drugs, synthetic or natural, which are effective against parasites with reduced or absent side effects for the treatment of this pathology is important. Previously, it was demonstrated that the Diterpene quinone 7α-hydroxyleanone (7α) extracted from Tetradania riparia leaves was active against Trypanosoma cruzi, a parasite that causes Chagas' disease. Here we evaluated the leishmanicidal activity of 7α isolated from the same source and the product of its reaction (alr003p) as well. Our data demonstrated that 7α compound at 15 µg/mL inhibits approximately 84% of promastigotes growth and exhibits a dose-dependent activity with IC50 of 2.96 µg/mL. The alr003p also showed dose-dependent leishmanicidal activity and inhibited approximately 91% of promastigotes growth at 50 µg/mL with an IC50 of 8 µg/mL. The evaluation of cytotoxicity through dehydrogenase enzymes viability assay has shown that 7α and alr003p compounds have similar toxicity to the host cells with CC50 greater than 10 µg/mL. Evaluating anti-leishmanial activity against intracellular amastigotes forms showed a dose-dependent effect of 7α with an IC50 of 1.36 µg/mL, an activity well above that demonstrated by alr003p that showed a significant inhibition only at 10 µg/mL. These results point 7α for further studies to determine its mechanism of action. **Supported by:**CNPQ/FAPERJ/CAPES **Keywords:** Leishmania; 7alfa; alr003p

TB68 - **LEISHMANIA AMAZONENSIS INFECTION CONTROL USING METALLOCOMPLEXES AS CHEMOTHERAPY AGENTS: NEW POSSIBILITIES.**

LORETE, A.R.M.[*1]; TOSTES, J.M.C.[1]; FERNANDES, C.[2]; JUNIOR, A.H.[2]; DE SOUZA, W.[3]; FORTES, F.S.A.[1]; DAMATTA, R.A.[2]; SEABRA, S.H.[1]

1.UEZO, RJ, Brazil; 2.UENF, RJ, Brazil; 3.UFRJ, RJ, Brazil

Leishmaniasis is a complex disease that is considered a serious public health problem. It is caused by protozoa of the genus Leishmania and is transmitted by vectors of the phlebotominae subfamily. The species Leishmania amazonensis cause diffuse cutaneous leishmaniasis, for which therapy causes several side effects and leads to drug-resistant strains. This situation has stimulated the search for alternative treatments, such as the use of metallocomplexes. The last ones are coordinated metal compounds with a metallic core. The action of such compounds hve been studied in several parasite species of the Trypanosomatidae family, including species of L. amazonensis. In this study, we check the effect of the A3910 compound, which has a cobalt core, on promastigotes of L. amazonensis of the WHOM/BR/75/Josefa strain. In vitro toxicity assays, viability analysis and electron microscopy were performed. Preliminary results indicate that A3910 inhibits their growth and induces morphological changes of the parasite, such as alterations in the cell body format, shortening of scourge, invaginations and degranulation in the parasite. Furthermore, the drug was not toxic to mice peritoneal macrophages. Future studies will be performed to evaluate its mechanism of action and the in vivo efficacy. **Supported by:**FAPERJ/CAPES/CNPq **Keywords:** Leishmania amazonensis; a3910; metallocomplexes