QT001 - Metalocomplexe FeHP(SO4) affects development of *Toxoplasma gondii* during host cell interaction

PORTES, J.A.[*1]; SOUZA, T.G.[2]; DOS SANTOS, T.A.T.[3]; DE LIMA, L.P.O.[4]; JUNIOR, A.H.[3]; DAMATTA, R.A.[3]; DE SOUZA, W.[1]; SEABRA, S.H.[2]
1.UFRJ, RIO DE JANEIRO, RJ, BRASIL; 2.UEZO, RIO DE JANEIRO, RJ, BRASIL; 3.UENF, CAMPOS DOS GOYTACAZES, RJ, BRASIL; 4.FIOCRUZ, RIO DE JANEIRO, RJ, BRASIL.
e-mail:julianaportes@yahoo.com.br

Toxoplasma gondii, the agent of Toxoplasmosis, is an obligate intracellular protozoan able to infect a wide range of vertebrate cells. Therefore, drugs to control this parasite must have an intracellular activity. The most common therapy for Toxoplasmosis is the combination of sulfadiazine and pyrimethamine, although this treatment is associated with adverse reactions. Because of this, the development of new drugs is necessary. In previous studies, drugs known as metalocomplexes have been shown several interesting biological activities. A new l-oxo di-iron complex is active against DNA of target cell, mimicking a nuclease and metallohydrolase enzymes. These new metalocomplexes also display antibacterial activity against Staphylococcus aureus. The metallo complex tested in this work comes from the reaction of FeSO4 _ 7H2O with the ligand HPClNOL (1-(bis-pyridin-2-ylmethyl-amino)-3-chloropropan-2-ol resulting in a complex [Fe(HPClNOL)(SO4)]2-l-oxo _ 6H2O 1 (Parrilha et al., 2008). Here, we report the outcomes of the cytotoxicity test with this drug during the interaction of T. gondii with LLC-MK2 cells. The drug did not arrest host cell growth, but was able to decrease the infection index of T. gondii with an IC_{50} of 2.5 µM in 48 hours of infection. Transmission electron microscopy analysis showed morphological changes of parasites including the appearance of inclusions at parasite cytoplasm, structures similar to amylopectin granules which are typically found in bradyzoites. The presence of cysts of bradyzoites was corroborated after 6 days of treatment by Scanning electron microscopy and staining with Dolichos biflorus lectin (specific to bradyzoites cyst wall). The derivative was also capable of to kill part of the parasite population by unknown mechanism. These results suggest that FeHP(SO4) is a compound potentially important for the killing and encystment of Toxoplasma gondii. **Supported by:**:CNPq, FAPERJ and PRONEX.

QT002 - In vitro and In vivo Investigation of the Efficacy of Arylimidamide DB1831 and its mesylated salt form - DB1965 - Against *Trypanosoma cruzi* Infection

SILVA, C.F.[*1]; BATISTA, D.G.J.[1]; OLIVEIRA, G.M.[1]; DE SOUZA, E.M.[1]; HAMMER, E.R.[1]; SILVA, P.B.[1]; DALIRY, A.[1]; ARAÚJO, J.S.[1]; BRITTO, C.[1]; RODRIGUES, A.C.M.[1]; LIU, Z.[2]; FARAHAT, A.A.[2]; KUMAR, A.[2]; BOYKIN, D.W.[2]; SOEIRO, M.N.C.[1]
1.FIOCRUZ, RIO DE JANEIRO, RJ, BRASIL; 2.GEORGIA STATE YNIVERSITY, ATLANTA, ESTADOS UNIDOS.
e-mail:franca@ioc.fiocruz.br

Chagas disease is caused by infection with the intracellular protozoan parasite *Trypanosoma cruzi*. At present, nifurtimox and benznidazole, both compounds developed empirically over four decades ago, represent the chemotherapeutic arsenal for treating this highly neglected disease. However, both drugs present variable efficacy depending on the geographical area and the occurrence of natural resistance, and are poorly effective against the later chronic stage. As a part of a search for new therapeutic opportunities to treat chagasic patients, pre-clinical studies were performed to characterize the activity of a novel arylimidamide (AIA--DB1831 (hydrochloride salt) and DB1965 (mesylate salt)) against T. cruzi. These AIAs displayed a high trypanocidal effect in vitro against both relevant forms in mammalian hosts, exhibiting a high selectivity index and a very high efficacy (IC(50) value/48 h of 5-40 nM) against intracellular parasites. DB1965 shows high activity in vivo in acute experimental models (mouse) of T. cruzi, showing a similar effect to benznidazole (Bz) when compared under a scheme of 10 daily consecutive doses with 12.5 mg/kg. Although no parasitological cure was observed after treating with 20 daily consecutive doses, a combined dosage of DB1965 (5 mg/kg) with Bz (50 mg/kg) resulted in parasitaemia clearance and 100% animal survival. In summary, our present data confirmed that aryimidamides represent promising new chemical entities against *T. cruzi* in therapeutic schemes using the AIA alone or in combination with other drugs, like benznidazole. **Supported by:**:**Supported by:**: FAPERJ, CNPq, DECIT/SCTIE/MS, MCT by CNPq, and PAPES/FIOCRUZ.

QT003 - In vitro effects of 2'-OH flavanone against promastigote and amastigote forms of *Leishmania* (*Leishmania*) amazonensis

GERVAZONI, L.F.O.*; INACIO, J.D.F.; CANTO-CAVALHEIRO, M.M.; ALMEIDA-AMARAL, E.E.
IOCFIOCRUZ, RIO DE JANEIRO, RJ, BRASIL.
e-mail:luizagervazoni@ig.com.br

Caused by different parasites of genus *Leishmania*, *Leishmania*sis is considered a neglected disease, which affects more than 12 million people around the world. Treatment of *Leishmania*sis is currently based on pentavalent antimonials and amphotericin B however, these drugs present serious problems regarding side-effects, variable efficacy and are expensive, leading to a new search of efficient compounds. Pure compounds have been reported to possess significant antiprotozoan activities with no side effects. 2'-OH flavanone is abundantly present in fruits and vegetables and has some biological functions including anti-inflammatory and anticancer. In this present study, we evaluated the effect of 2´-OH flavanone against *Leishmania* (*Leishmania*) amazonensis proliferation in promastigotes and intracellular amastigotes forms. 2'-OH Flavanone inhibited L. (L.) amazonensis promastigote growth in a dose-dependent manner with 24h of treatment reaching almost 80% of inhibition at the concentration of 96µM The IC50 for 2'-OH flavanone was 20.96µM. In addition, ROS levels were measured fluorimetrically using H_2DCFDA dye. We observed a significant increase in ROS production (176%) when the promastigotes were treated with 96µM of 2'-OH flavanone for 24h. Peritoneal macrophage infected with L. (L.) amazonensis and treated with different concentrations (3-12µM) for 24h were evaluated. The 2´-OH flavanone demonstrated a decrease on infected index in a dose-dependent manner with an IC50 of 2.56µM. We also evaluated the macrophage toxicity, treating cells with 2´-OH flavanone (3-96µM) without infection, achieving an IC50 of 79.3µM and a selectivity index of 30.95. Thus, our results suggested that 2´-OH flavonone is effective against both L. (L.) amazonensis forms promastigote and intracellular amastigote, without being toxic for macrophages, encouraging us to search the mechanism of action and point out the 2'-OH flavanone as a possible candidate for the chemotherapy of *Leishmania*sis. **Supported by:**:FAPERJ;IOC-FIOCRUZ

QT004 - Epigalocatechin-3-galate affects the course of cutaneus *Leishmania*sis by oral delivery and promotes reactive oxygen species production in *Leishmania* (Viannia) braziliensis

INACIO, J.D.F.*; GERVAZONI, L.F.O.; CANTO-CAVALHEIRO, M.M.; ALMEIDA-AMARAL, E.E.
IOCFIOCRUZ, RIO DE JANEIRO, RJ, BRASIL.
e-mail:job@ioc.fiocruz.br

*Leishmania*sis is considered as neglected tropical disease and its treatment is performed with pentavalent antimonials. However this chemotherapy induces many side effects and resistance to treatment. The epigallocatechin-3-gallate (EGCG) is a flavonoid that has anti-inflammatory, microbicidal and trypanocidal activities. In this study, we demonstrated the effect of EGCG in promastigotes and amastigotes forms of *Leishmania* (Viannia) braziliensis as well as in vivo effects on infected mices. In promastigotes, EGCG inhibited the growth in a time and dose dependent manner with 72h of treatment reaching 83.88% at 0.5mM (IC50=0.202mM). In amastigotes, the index of infection was inhibited 73.04% and 77.71% with 12µM of EGCG at 24 and 72h, demonstrating an IC50 4.45µM and 3.5µM respectively. Changes in levels of Reactive Oxygen Species (ROS) and H2O2 production were observed in promastigotes of L. (V.) braziliensis treated with increasing levels of EGCG. However, this production was reversed by pre-incubation with catalase and peg-catalase. EGCG also caused mitochondrial dysfunction, leading to a loss in mitochondrial membrane potential. As in promastigotes, EGCG was able to generate ROS in amastigotes, which were reversed by peg-catalase and catalase. The in vivo activity was evaluated in BALB/c mice infected with L. (V.) braziliensis and treated with EGCG by oral route. A significant difference was observed in lesion size of the group treated with EGCG compared to the control group. Biochemical analyzes demonstrated that EGCG was not toxic, whereas the group treated with meglumine antimoniate the levels of creatinine were altered. Taken together, our results suggest that EGCG has in vitro and in vivo *Leishmania*cidal activity, with high selectivity in murine macrophages and ROS production, as a part of its mechanism of action. These characteristics encouraging and suggests the EGCG as a possible prototype for the clinical treatment of cutaneous *Leishmania*sis. **Supported by:**:FAPERJ;IOC-FIOCRUZ;CNPQ

QT005 - In vitro antiproliferative synergism of ergosterol biosynthesis inhibitors acting at different steps of the pathway against *Leishmania amazonensis*

MACEDO-SILVA, S.T.[*1]; URBINA, J.A.[2]; DE SOUZA, W.[1]; RODRIGUES, J.C.F.[1]
1.IBCCF, RIO DE JANEIRO, RJ, BRASIL; 2.INSTITUTO VENEZOLANO DE INVESTIGACIONES CIENTÍFICAS, CARACAS, VENEZUELA.
e-mail:sara.teixeiracp2@gmail.com

*Leishmania*ses, caused by protozoan parasites of the *Leishmania* genus, are among the most prevalent neglected tropical diseases. These diseases are endemic in 98 countries. The first line treatments are pentavalent antimonials and, in cases of resistance, miltefosine, amphotericin B and its lipid formulation, but they unsatisfactory due to toxicity, limited efficacy, cost and difficult application. Thus, there is an urgent need to develop new drugs that are efficacious, safe, and more accessible to patients. Trypanosomatids have an essential requirement for ergosterol and other 24-alkyl sterols, which are absent in mammalian cells. Therefore, inhibition of ergosterol biosynthesis is increasingly recognized as a promising target for the development of new chemotherapeutic agents. The aim of this work was to investigate the effects of E5700, a squalene synthase inhibitor, in combination with itraconazole (ITZ) or posaconazole (POSA), two known azole antifungal agents which inhibit sterol C14*α*-demethylase (CYP51), against *Leishmania amazonensis* in vitro. E5700, ITZ and POSA alone produced a marked reduction in the viability of *L. amazonensis* promastigotes, with MICs of 30 nM, 1 µM, and 1 µM, respectively. Several combinations were tested and the most efficient was the combination of 1.25 nM E5700 with 40 nM ITZ or 10 nM POSA, which resulted in a FIC (fractional inhibitory concentration) values of 0.082 for the combination of E5700 with ITZ and 0.052 for E5700 with POSA, indicating a very potent synergistic action. The same concentrations were active against the clinically relevant intracellular amastigotes and the combinations were also synergistic, with similar values of FIC. In summary, our results indicate that combinations of ergosterol biosynthesis inhibitors acting at different steps of the pathway have synergistic activity against *L. amazonensis* and open up the possibility of a novel combination therapies for the treatment of *Leishmania*sis. **Supported by:**::**Supported by:** CNPq, CAPES, and FAPERJ.

QT006 - THE EFFECT OF MEMANTINE HYDROCHLORIDE ON THE LIFE CYCLE OF *Trypanosoma cruzi*

DAMASCENO, F.S.[*]; FURUSHO PRAL, E.M.; BARISON, M.J.; SILBER, A.M.
USP, SÃO PAULO, SP, BRASIL.
e-mail:flaviadamasceno@usp.br

Trypanosoma cruzi is the etiologic agent for Chagas' disease, which affects approximately 15 million people with about 40 million at risk of acquiring the infection. The available chemotherapy is limited to two drugs: nifurtimox and benznidazole. Both reduce the symptoms and mortality of people infected in the acute phase, but their application in the chronic phase is still controversial. Our group is exploring a "piggy-back" strategy. In this sense, we are looking for trypanocidal activity of drugs used for the treatment of other diseases. The aim of this study was to analyze the effect of Memantine, an antagonist of NMDA glutamate receptor in the life cycle of *T. cruzi* CL strain, clone 14. Epimastigote forms ($2.5x10^6$ cells ml_{-1}) were grown in LIT medium at pH 7.5 at 28°C, in 96-well plates, treated or not (negative control) with different concentrations. Cell growth was determined by following-up for 8 days the variation of cultures absorbencies at 620 nm. The absorbencies read at the 5th day of growth as a function of Memantine concentration were used to construct dose-response curves and calculate the IC_{50} values (172.6 µM). To evaluate the effect on infected cells, CHO-K1 cells ($5.0x10^4$ cells ml^{-1}) were infected with $2.5x10^6$ trypomastigote forms per well, and treated with different concentrations of Memantine or not (control). On the 5th day post-infection the bursted trypomastigotes were counted in a Neubauer chamber (IC_{50} 31 µM). The effect of Memantine was also evaluated on metacyclogenesis: epimastigote forms ($5.0x10^6$ cells ml^{-1}) were grown in LIT medium, transferred to Grace medium, and treated or not with the drug. On the 9th day the number of metacyclic forms was counted in a Neubauer chamber (33% inhibition compared to control). These results suggest that this molecule interferes with epimastigote replication and the host cell infection. Now, we are exploring the possible existence of a Memantine target, probably a glutamate receptor sensitive to NMDA. **Supported by:**:FAPESP

QT007 - Successful therapy with Pentoxifylline for *Trypanosoma cruzi* induced damage: from cardiomyocyte culture to reversion of chronic cardiomyopathy

PEREIRA, I.R.*; PEREIRA, G.V.; GIBALDI, D.; GARZONI, L.R.; LANNES-VIEIRA, J.
FIOCRUZ, RIO DE JANEIRO, RJ, BRASIL.
e-mail:pereirair@ioc.fiocruz.br

Pentoxifylline (PTX) is a phosphodiesterase inhibitor that also blocks cytokine expression and acts as an immunoregulator. In *Trypanosoma cruzi* acute infection, immune response associated with heart inflammation can lead to parasite control and regain of homeostasis, however in ~30% of patients myocarditis progresses resulting in chronic chagasic cardiomyopathy (CCC). In the present study, the action of PTX upon cardiomyocyte and the electrical function of the heart in murine models of T. cruzi-elicited CCC was investigated. Our results showed that PTX had a direct effect uppon primary cardiomyocyte cell culture infected with *T. cruzi* reducing the deposition of fibronectin (FN) and connexin 43 (Cx43) loss, markers of cardiomyocyte damage, without interfering in cell viability. Further, PTX does not act on blood trypomastigote forms of the parasite. In vivo, treatment with PTX in chronic phase of infection improved the electrical conduction of the heart, decreasing arrhythmias and atrio-ventricular block grades 1 and 2, significantly reducing the CK-MB activity levels in serum, Cx43 loss and decreasing deposition of FN in the heart tissue, without interfering in parasite load. Rather than hamper the progression of CCC, PTX was able to revert the cardiac alterations already installed. The mechanistic insights on PTX action in chronically T. cruzi-infected animals is under investigation. Altogether, our findings pointed that PTX may be a promising candidate drug to be further explored to treat T. cruzi-induced CCC alone or in association with trypanocide therapy. **Supported by:**:CNPq

QT008 - MELITTIN PEPTIDE GENERATES DIFFERENT DEATH PHENOTYPES ON TREATED *TRYPANOSOMA CRUZI* EPIMASTIGOTES AND TRYPOMASTIGOTES

OLIVEIRA, I.R.S.*; ADADE, C.M.; SOUTO-PADRÓN, T.C.
UFRJ, RIO DE JANEIRO, RJ, BRASIL.
e-mail:isabelle_rso@yahoo.com.br

Chagas disease, caused by the protozoan *Trypanosoma cruzi*, affects about 16-18 million people in the South and Central America, and its chemotherapy is based on that exhibit toxic effects and limited efficacy such as Benznidazole. Thus, new chemotherapeutic agents from natural sources, such as animal's venoms, are a line of research to be exploited. The *Apis mellifera* venom is a complex mixture of biological active molecules, among them melittin, object of this study, which corresponds to 40-50% of venom's dry weight. Previous studies reports the anti-tumor, anti-microbial and leishmanicidal melittin's properties, supporting our previous results which presented the peptide trypanocidal activity. Investigating the treated epimastigotes and trypomastigotes ultrastructure led us to believe the occurrence of different programmed cell death pathways. To investigate this hypothesis, we treated epimastigotes and trypomastigotes forms with their respective IC_{50} (2.44 µg/ml) and LD_{50} (0.14 µg/ml) for 24 h, and label them with monodansyl cadaverine (MDC), an autophagy biochemical marker; TUNEL reaction, a specific in situ marker of DNA fragments of apoptotic cells; propidium iodide (PI – viability indicator); and $DiOC_6$ (detects differences in the mitochondrial membrane). Treated epimastigotes and trypomastigotes exhibited PI-positive staining about 62-81% and 70-99%, respectively. Epimastigotes incubated with MDC showed more positive epimastigotes then trypomastigotes forms. The TUNEL method indicated high percent (ranging 47.8- 53.4%) of stained trypomastigotes contrary to epimastigote forms (6- 8.5%). The ultrastructure intensified the existence of different death pathways, where epimastigote form dies by autophagic death and trypomastigotes by apoptosis. This study presents that melittin is active against *T. cruzi* through the different cell death phenotypes among epimastigotes and trypomastigotes forms. **Supported by:**:CNPq, CAPES e FAPERJ

QT009 - Diamidine Transport in Bloodstream trypomastigotes of *Trypanosoma cruzi*

GOULART, E.N.[*1]; SOEIRO, M.N.C.[1]; DALIRY, A.[2]; BOYKIN, D.W.[3]
1.FIOCRUZ, RIO DE JANEIRO, RJ, BRASIL; 2.UFRJ, RIO DE JANEIRO, RJ, BRASIL; 3.GSU, ATLANTA, ESTADOS UNIDOS.
e-mail:evelyn_n_goulart@hotmail.com

The chemotherapy of Chagas disease (CD) is quite unsatisfactory mainly due to its poor efficacy, especially during the later chronic phase, and the considerable well-known side effects. In vitro and in vivo studies showed that aromatic diamidines (AD) and analogs are effective against *Trypanosoma cruzi*, the etiological agent of CD. Different transporters, such as P2, HAPT1 and LAPT1, have been identified as routes of entry of AD into African trypanosomes, while no studies have been conducted with T. cruzi. Thus, our aim was evaluate the uptake of AD in bloodstream forms (BF) of *T. cruzi* (Y strain). BF were harvested by heart puncture from *T. cruzi* infected Swiss mice at the peak of parasitemia and then treated at 37°C, with furamidine (DB75 at 32 µM), in the presence or not of adenosine, inosine and adenine, three well-known P2-P1, P1 and P2 transport inhibitors, respectively. After 15 min or 24 h of treatment, the percentage of live parasites was determined by light microscopy through direct quantification in Neubauer chamber. Our data showed that the incubation for 24h of BF with DB75 resulted in 64% of parasite death. The exposure of BF only with purine competitors (adenosine, adenine and inosine) did not result in considerable lack of parasite viability, reaching 3-7 % of parasite death. However, the addition of adenosine and adenine induced ≥70% of reduction in the rates of parasite death in the presence of DB75, contrasting to inosine incubation that resulted in no protection against DB75 effect. The results suggest that the uptake of diamines like DB75 in BF of *T. cruzi* could be mediated by P2 aminopurine transporter and thus it is unlikely to occur via P1-substrate. Further studies are underway to evaluate the internalization kinetics of each compound by T. cruzi. **Supported by:**:Fiocruz/CNPq, FAPERJ and CPDD

QT010 - LEISHMANICIDAL EFFECT OF 19-HYDROXYCORONARIDINE

FERREIRA, C.[*1]; SOARES, D.C.[1]; DELORENZI, J.C.[2]; VIEIRA, I.C.[3]; SARAIVA, E.M.[1]
1.UFRJ, RIO DE JANEIRO, RJ, BRASIL; 2.MACKENZIE, SÃO PAULO, SP, BRASIL; 3.UENF, CAMPOS, RJ, BRASIL.
e-mail:christian@tutopia.com.br

*Leishmania*sis is a neglected disease that affect millions of people worldwide, but few advances were made in its chemotherapy, except for the introduction of new treatment protocols or the reformulation of old drugs such as lipossomal amphotericin B. Excluding miltefosine, originally developed for breast cancer treatment, no new drug was introduced for the *Leishmania*sis treatment since the introduction of antimonials for over 80 years. The discovery of new drugs, synthetic or natural, that can be effective even against parasites resistant to the available drugs, with reduced or missing side effects, has been encouraged by initiatives such as DND*i*. The 19-hydroxycoronaridine (19-HC) is a rare natural alkaloid isolated from the bark of roots of the species *Tabernaemontana catharinensis*, analog of the coronaridine and 18-methoxycoronaridine, compounds with leishmanicidal activity. The aim of our study is to evaluate the effect of 19-HC in *Leishmania (Leishmania) amazonensis*. Our results demonstrated that 19-HC showed leishmanicidal effect for promastigotes and intracellular amastigotes of *L. amazonensis*, with IC_{50} of 35µg/mL and 34.8µg/mL, respectively. Evaluation of 19-HC cytotoxicity in murine peritoneal macrophages showed a CC_{50} (citotoxic concentration to 50%) of 87µg/mL when analyzed by XTT method, and no toxicity was observed in treated murine macrophages evaluated by Trypan blue dye assay. The treatment with 35µg/mL of 19-HC inhibited mitochondrial dehydrogenases activity of promastigotes in 35%. Our results also demonstrate that the compound does not induce the production of nitric oxide (NO) in macrophages, moreover, 19-HC decreased about 6 folds the NO production in IFN-γ activated macrophages, a result similar to that obtained with the 18-methoxycoronaridine. In conclusion, our results demonstrate the anti-*L. amazonensis* activity of 19-HC, indicating this compound as a promising candidate for future studies regarding treatment of *Leishmania*sis. **Supported by:**:CNPq, FAPERJ, CAPES, MACKPESQUISA

QT011 - CAMPTOTHECIN, A TOPOISOMERASE I INHIBITOR, AFFECTS *Trypanosoma cruzi* PROLIFERATION AND ULTRASTRUCTURAL ORGANIZATION

ZUMA, A.A.[*1]; MENDES, I.C.[2]; MACHADO, C.R.[2]; ELIAS, M.C.[3]; DE SOUZA, W.[1]; MOTTA, M.C.M.[1]
1.UFRJ, RIO DE JANEIRO, RJ, BRASIL; 2.UFMG, BELO HORIZONTE, MG, BRASIL; 3.INST BUTANTAN, SÃO PAULO, SP, BRASIL. e-mail:alinezuma@ig.com.br

The protozoa *Trypanosoma cruzi*, the aetiological agent of Chaga's disease, presents a single nucleus and a single mitochondrion with an enlarged portion named kinetoplast, that contains the mitochondrial DNA (kDNA). Topoisomerases are essential enzymes during replication, transcription and repair, since they modulate the topological state of DNA, thereby representing an important target in chemotherapeutic studies. In this work, we analyzed the effects of Camptothecin, a topoisomerase I inhibitor, on the proliferation, cell cycle, ultrastructure and mitochondrial function of the *T. cruzi* epimastigote form. For this purpose, cells were treated with different concentrations of the drug until 96 hours of cultivation. Samples were collected after each 24 hours for counting on Neubauer's chamber, for processing to transmission electron microscopy, to flow cytometry and to MTS/PMS viability method. Further assays include the qPCR technique and the incubation with the markers H_2DCFDA and JC-1. Our results showed that Camptothecin reduced cell viability and promoted high proliferation inhibition (IC_{50}=2,08 µM), in a dose-dependent manner, leading to cell cycle arrest in G2 phase and apoptosis. Transmission electron microscopy revealed ultrastructural alterations, such as mitochondrial swelling, accumulation of lipid bodies and remarkable unpacking of the perinuclear chromatin, although few DNA lesions were detected by qPCR. This compound also induced mitochondrial dysfunction, since treated cells presented higher levels of reactive oxygen species and loss of mitochondrial membrane potential. Taken together, our data suggest that the Camptothecin mechanism of action involves cell cycle blockage, followed by apoptosis of parasites. This work emphasizes the essential role of topoisomerase I on cell proliferation and ultrastructural organization, reinforcing the idea that this enzyme constitutes a promising target for antitrypanosomal chemotherapy. **Supported by:**:CNPq e FAPERJ

QT012 - In vitro anti*Leishmania*l activity of Morita-Baylis-Hillman adduct methyl 2-{2-[hydroxy(2-nitrophenyl)methyl])acryloyloxy} benzoate against different isolates of *Leishmania* (Viannia) braziliensis

AMORIM, F.M.[*]; RODRIGUES, Y.K.S.; BARBOSA, T.P.; NÉRIS, P.L.D.N.; CALDAS, J.P.A.; SOUSA, S.C.O.; LEITE, J.A.; RODRIGUES-MASCARENHAS, S.; VASCONCELLOS, M.L.A.A.; OLIVEIRA, M.R.
UFPB, JOÃO PESSOA, PB, BRASIL. e-mail:francianne.amorim@gmail.com

INTRODUCTION: Available treatments for *Leishmania*sis have a number of disadvantages such as high toxicity and the emergence of resistant parasites, which makes necessary the search for therapeutic alternatives. Morita-Baylis-Hillman adducts (MBHA) are molecules obtained by a reaction characterized by forming a carbon-carbon bond between the alpha position of an alkene and the carbonyl group of an aldehyde or ketone. This reaction has high yield and low financial cost. OBJECTIVE: To investigate the activity of MBHA methyl 2-{2-[hydroxy(2-nitrophenyl)methyl])acryloyloxy} benzoate (HNAB) on intracellular amastigotes of isolates of *L. (V.) braziliensis* obtained from patients with different clinical forms of tegumentary *Leishmania*sis. MATERIALS AND METHODS: Three isolates of *L. (V.) braziliensis* were used: MHOM/BR/2011/JMTS (localized cutaneous *Leishmania*sis), MHOM/BR/2010/JCNS (mucosal *Leishmania*sis) and MHOM/BR/2011/AF (disseminated cutaneous *Leishmania*sis). BALB/c peritoneal macrophages were distributed into 24-well plates (5 x 10^5 cells/mL) and incubated at 37°C at 5% CO2 for 2 hours, after this the promastigotes were added (5 x 10^6 cells). After 3 hours, the wells were washed and fresh medium with or without different concentrations of HNAB were added. After 24 and 72 hours, coverslips were collected and stained for analysis of the infection index and the supernatants were used to measure TNF-α, IL-6, IL-10 and nitric oxide. RESULTS: Using nontoxic concentrations to macrophages, the antiamastigote activity of HNAB was observed (EC50 ≤ 1.37 µg/mL). It was more active than meglumine antimoniate (EC50 ≤ 321.40 µg/mL). The activity of HNAB was associated with immunomodulatory properties, since it was able to decrease IL-6 and IL-10 production. However, it was independent of TNF-α and nitric oxide. CONCLUSION: This work reveals the anti*Leishmania*l activitiy of HNAB and suggests its potential for the treatment of human infections caused by *L. (V.) braziliensis*. **Supported by:**:Coordenação de Aperfeiçoamento de Pessoal de Nível Superior

QT013 - INCREASED BENZNIDAZOLE AND NIFURTIMOX RESISTANCE IN CL BRENER TRANSFECTED WITH TcI ABC TRANSPORTER GENES

NUNES, S.L.[*1]; ARAÚJO, R.G.A.[1]; SILVA, M.N.[1]; MACHADO, C.R.[2]; ZINGALES, B.[1]
1.USP, SÃO PAULO, SP, BRASIL; 2.UFMG, BELO HORIZONTE, MG, BRASIL.
e-mail:lessasol@usp.br

Clinical trials indicate regional differences in benznidazole (BZ) treatment outcome of Chagas disease, attributed in part to differences in the drug susceptibility of *T. cruzi* strains. Previous evidence from our group indicates that a *T. cruzi* ABC transporter single-copy gene (TcABCG1) is overexpressed in BZ-resistant strains. ABC transporters play an important role in drug resistance. Thus the goal of this study was to obtain additional evidence for the involvement of TcABCG1 in BZ resistance. The IC50 to BZ was determined in several strains that were classified into susceptible (S) and resistant (R). No differences in the TcABCG1 gene copy number were observed among S and R strains. Few non-synonymous amino acid substitutions were detected in the ATP binding domain of TcABCG1 of BZ-resistant TcI strains (Franco et al., this meeting). Accordingly, TcABCG1 genes of Silvio and YuYu (both TcI; BZ-R strains) were cloned in pROCKNeo and transfected in CL Brener (TcVI; BZ-S). As controls CL Brener gene and pROCKNeo were also transfected. Increased BZ resistance of 14; 26 and 32% was observed in transfectants carrying the CL Brener, Silvio and YuYu genes, respectively. Increment of nifurtimox resistance was also detected suggesting that the ABC transporter could confer cross-resistance to both drugs. A ~2.7-fold increment of the relative TcABCG1 transcript abundance was observed in the gene-transfected cultures. The integration of the recombinant pROCKNeo vector in CL Brener genome was confirmed. The relative abundance of TcABCG1 transcripts in parasites isolated from two patients with BZ-therapeutic failure was investigated. A direct correlation between BZ resistance and the ABC-transporter RNA relative abundance was observed. Taken together the data suggest that TcABCG1 may be one of the components involved in *T. cruzi* natural drug resistance. The role of this transporter in parasites in which BZ-resistance has been induced by drug pressure is under investigation. **Supported by:**:FAPESP and CNPq

QT014 - EVALUATION OF THE EFFECT, AS WELL AS THE THERAPEUTIC PROSPECTS OF MEMANTINE (1-AMINO-3, 5-DIMETHYL-ADAMANTANE), A NONCOMPETITIVE ANTAGONIST OF THE N-METHYL-D-ASPARTATE (NMDA) GLUTAMATE RECEPTOR IN ACUTE EXPERIMENTAL INFECTION BY *TRYPANOSOMA CRUZI*.

SOUZA, H.F.S.[*]; ROCHA, S.C.; DAMASCENO, F.S.; SILBER, A.M.
USP, SÃO PAULO, SP, BRASIL.
e-mail:higonando@usp.br

In mammals, the NMDA (N-methyl-D-aspartate) receptors are formed by ion channels such as sodium (Na^+), potassium (K^+) and calcium (Ca^{2+}) and are involved in the process of delayed memory. According to the literature, in epimastigotes of *Trypanosoma cruzi* there is an iNOS activity resembling that of mammals. This enzyme is possibly modulated by the activation of a NMDA receptor, as occurs in endothelial cells. In these cells the production of NO stimulates a guanylate cyclase, resulting in the formation of intracellular cyclic guanosine monophosphate (cGMP), which acts as a second messenger in several other cells. Based on this information, and on previous data from in vitro studies in our laboratory, in the present work, we evaluated the effect, as well as the therapeutic prospects of memantine (1-amino-3, 5-dimethyl-adamantane), a noncompetitive antagonist of a NMDA glutamate receptor in acute experimental infection by *T. cruzi*. Balb/C mice were inoculated with $1x10^3$ trypomastigotes of Y strain, which leads to an acute infection characterized by high parasitemia. The animals were treated with memantine (10mg/kg/day), showing a statistically significant reduction of the parasitemia of 43.6% ($p<0.05$) on the 9th day after infection (dpi) when compared to the control. Results *ex vivo* show that memantine did not have effect on the NO production by activated or not peritoneal macrophages. Moreover, by MTT assay it was shown that the viability of peritoneal macrophages was not affected by tested memantine concentrations. More experiments are being performed for consolidate the therapeutic prospects of memantine and its effect on immune system of mammalian host. **Supported by:**:CAPES, CNPq, INBEQMEDI and FAPESP

QT015 - Study of the effects of compounds coordinated with the metal core (metalocomplexes) on trophozoites of Trichomonas vaginalis

NETO, J.G.O.[*1]; SEABRA, S.H.[1]; DAMATTA, R.A.[2]; BENCHIMOL, M.[3]; JUNIOR, A.H.[2]
1.UEZO, RIO DE JANEIRO, RJ, BRASIL; 2.UENF, CAMPO DOS GOYTACAZES, RJ, BRASIL; 3.USU, RIO DE JANEIRO, RJ, BRASIL.
e-mail:jgeisebel@gmail.com

Trichomoniasis is caused by the flagellate protozoan *Trichomonas vaginalis*, which is an anaerobe facultative parasite that infects the human genital tract. It provokes the most commom nonviral sexually transmitted disease in the whole world.*T. vaginalis* infection is associated with problems such as low-birth-weight infants, infertility and cervical cancer in infected women, besides its association with transmission of the human immunodeficiency virus. Life cycle has two forms: an infective form, the trophozoite and another named pseudocyst or endoflagellar form, which the main feature is the rounded shape and internalized flagella, found in cells submitted to drugs or temperature stress. The Family of 5-nitroimidazoles is the only class of compound indicated for the treatment of tricomoniasis, specifically the metronidazole and tinidazole. Because resistance to this treatment is a concern, research for new drugs for *T. vaginalis* therapy is the main purpose of the present work. In previous studies, the family of compounds known as metalocomplexes has demonstrated biological activity acting as antifungi,antivirus and bactericida. Complex iron (III), cobalt (II), copper (II) and zinc (II) exhibited activity against *Staphylococcus aureus*. Because they are metal compounds, it allowed locate its site of action in the target cell by electron microscopy. In the present study, the activity of new compounds against *T. vaginalis* was tested in a period of up to 48 hours. The growth parasite curve was analyzed as well the parasite morphology after treatment. We show that metalocomplexes have antiproliferative effects on the trophozoitic form of *Trichomonas vaginalis*. By scanning electron microscopy we observed that these compounds induced pseudocysts formation. This work shows that metallocomplexes can be used as alternative drugs for the treatment of trichomoniasis.

QT016 - Therapeutic effect of Baccharis uncinella triterpene fraction on *Leishmania* (*Leishmania*) amazonensis-infected BALB/c mice

YAMAMOTO, E.S.[*1]; PASSERO, L.F.D.[2]; CAMPOS, B.L.S.[2]; LAURENTI, M.D.[2]; CORBETT, C.E.P.[2]; LAGO, J.H.[3]
1.UNESP, SÃO VICENTE, SP, BRASIL; 2.FMUSP, SÃO PAULO, SP, BRASIL; 3.UNIFESP, DIADEMA, SP, BRASIL. e-mail:seijieduardo@me.com

Leishmaniosis is a parasitic disease that affects tropical and subtropical regions of the World. The standard treatment is toxic and has numerous side effects to patients. For these reasons new leishmanicidal molecules should be developed. Recently, we showed that a fraction containing oleanolic and ursolic acids (triterpenes) eliminated promastigote and amastigote forms of *L.(L.)amazonensis* and *L.(V.)braziliensis*, without cause toxic effect to macrophages. The present work aimed to characterize the effect of triterpenes in *L.(L.)amazonensis*-infected BALB/c mice. The triterpenes were purified from *Baccharis uncinella* leaves by chromatographic methodologies. BALB/c mice were infected in the hind footpad with 10^6 *L.(L.)amazonensis* promastigotes; after 30 days 0.10 and 0.50 mg/kg of triterpenes were intraperitoneally administered in infected mice daily, during 5 days. Infected and healthy groups received only 100µl PBS. After 15 days of the last injection, groups were sacrificed, and the skin was collected to analyze parasitism. The draining-lymph node cells were cultured under specific stimulation for 48h, when the amounts of IL-4, IL-12 and IFN-g were quantified. The spleen, liver, lung, heart, kidney also were collected to analyze possible morphological changes. Compared with control group, the mice treated with 0.10 and 0.50mg/kg of triterpenes presented reductions of 55 and 83% in the lesion sizes, respectively. Similarly, the parasitism was reduced in 94 and 99%, respectively, compared to control mice. Concerning to the cellular immune response, the treatments induced increase on IL-12 and IFN-glevels. This fraction showed no toxic effects on visceral organs, but in spleen a significant increasing in white pulp has been verified. The present work suggests that triterpenes have leishmanicidal effects, and probably can have immunostimulatory effect in vivo, which can improve parasite elimination in BALB/c mice. Acknowledge: LIM50-HCFMUSP and FAPESP (2012/039903-9) **Supported by:**:FAPESP

QT017 - Leishmanicidal activity of sterol biosynthesis inhibitors associated with inhibitors of transport of LDL-cholesterol in *Leishmania amazonensis*

ANDRADE-NETO, V.V.[*1]; DE CICCO, N.N.T.[2]; CUNHA-JUNIOR, E.F.[1]; ATELLA, G.C.[2]; CANTO-CAVALHEIRO, M.M.[1]; TORRES-SANTOS, E.C.[1]
*1.INSTITUTO OSWALDO CRUZ, RJ,RJ, BRASIL; 2.UNIVERSIDADE FEDERAL DO RIO DE JANEIRO, RJ, RJ, BRASIL.*e-mail:valter_farma@hotmail.com

A significant percentage of exogenous cholesterol is found in all species of *Leishmania*, suggesting a biological role for this molecule. This work aims to study the importance for *L. amazonensis* of the use of cholesterol in various situations, assessing the potential of this system as a possible drug target. The activity of the sterol biosynthesis inhibitors (ketoconazole, miconazole, terbinafine and simvastatin) was evaluated in culture of promastigotes and intracellular amastigotes supplemented with complete or delipidated serum. It was observed that the deprivation of sources of cholesterol potentiates the effect of all evaluated inhibitors of sterol biosynthesis in both evolutionary forms. Furthermore, promastigotes treated with these inhibitors in presence of normal serum, showed an accumulation of cholesterol, suggesting a compensation mechanism, which could partially overcome ergosterol inhibition. Experiments with LDL-I125 indicated an increased uptake of LDL in ketoconazole and simvastatin-treated *L. amazonensis* promastigotes. The activity of the inhibitors of sterol biosynthesis associated with inhibitors of transport of LDL-derived cholesterol, progesterone, imipramine and LBqT01 (an experimental drug), was evaluated in promastigotes and intracellular amastigotes. We observed increased leishmanicidal activity in promastigotes in all associations. The association between LBqT01 and ketoconazole, miconazole or terbinafine showed synergy. The association between imipramine or progesterone and ketoconazole or terbinafine indicated an additive effect. Ketoconazole and miconazole showed a decrease by up to two times of the IC50 value in intracellular amastigotes when combined with inhibitors of cholesterol transport. Taken together, these results suggest that cholesterol plays an important role in the activity of sterol biosynthesis inhibitors and that the blockage of its use by *Leishmania* may be a possible drug target. **Supported by:**:CNPq, FAPERJ

QT018 - IMPACT OF BENZNIDAZOLE TREATMENT DURING THE ACUTE PHASE OF INFECTION ON THE EVOLUTION OF HISTOPATHOLOGICAL ALTERATIONS OF MICE INOCULATED WITH *TRYPANOSOMA CRUZI* FROM PARANÁ (TcII) AND AMAZONAS (TcIV)

GRUENDLING, A.P.[*1]; TESTON, A.P.M.[1]; MACEDO, L.C.[1]; KANESHIMA, E.N.[1]; MONTEIRO, W.M.[2]; GOMES, M.L.[1]; DE ARAÚJO, S.M.[1]; TOLEDO, M.J.O.[1]
1.UEM, MARINGÁ, PR, BRASIL; 2.UFAM, MANAUS, AM, BRASIL. e-mail:aninha_gru@bol.com.br

The clinical diversity of human Trypanosoma cruzi infection has been attributed to genetic heterogeneity of parasite populations and host's genetic background. There are few studies exploring the correlation of genetic diversity and biological characteristics of T.cruzi strains from Brazilian Amazon. Our goal was perform histopathological evaluations of mice inoculated with T.cruzi strains from states of Paraná (TcII) and Amazonas (TcIV) in acute and chronic phases. Were used three TcII strains (chronic patients –Paraná) and three TcIV strains (acute cases-Amazonas). For each strain, 13 Swiss mice, 21 to 28 days, were inoculated via IP with 10.000 blood trypomastigotes/animal. Parasitemia was evaluated daily from 3rd day after inoculation (dai). The animals were euthanized one day after the peak parasitemia (Pmax) in the recent acute phase (rAP), at 30° (late acute phase-lAP) and 100º (chronic phase – CP) dai. Fragments from heart, skeletal muscle,liver, spleen, brain, diaphragm, abdominal wall and large intestine were stained with HE. For each strain were obtained the pre-patent period (PPP), patent period (PP), Pmax, day of peak parasitemia (Dpmax), inflammatory process and tissue parasitism. Mice inoculated with TcII strains showed higher PP and Pmax, whereas that inoculated with TcIV strains had lower PPP, earlier Dpmax, and low levels of parasitemia. TcII displayed more organs with tissue parasitism in rAP and lAP in comparison with TcIV, which presented amastigotes nests only at rAP. No tissue parasitism was observed in CP. TcII had more organ's presenting inflammatory process than TcIV at all phases. The inflammatory process was more intense at lAP for both lineage and was observed more tissue damage for TcII.So, TcII from Paraná was considered more pathogenic to mice than TcIV from Amazonas in all stages of experimental infection, in agreement with lower severity of Chagas disease at Amazon region in relation to old endemic areas as the Paraná state. **Supported by:**:FUNDAÇÃO ARAUCÁRIA / CNPQ

QT019 - THE PROLINEMIA AS A VIRULENCE FACTOR IN THE EXPERIMENTAL *TRYPANOSOMA CRUZI* INFECTION

ROCHA, S.C.*; SOUZA, H.F.S.; SILBER, A.M.
ICB/USP, SAO PAULO, SP, BRASIL.
e-mail:sandracarla@usp.br

Trypanosoma cruzi is dependent on proline for a variety of processes such as energy metabolism, host-cell invasion, differentiation and resistance to osmotic, metabolic and oxidative stress. Recently we showed, *in vitro*, that a proline analogue interferes with proline uptake, diminishes the *T. cruzi* resistance to stress conditions and decreases the burst of trypomastigotes from host-cells. Based on these facts, we set up a “hyperprolinemia model” in Balb/C mice. The model was established by daily injections of proline, showing transient increases in the blood proline levels, leading to a complete normalization of the prolinemia within 2 hours after injection. We established that most of the applied proline was eliminated *via* the urine. The effect of transient hyperprolinemia on the virulence of *T. cruzi* was evaluated. We made six independents experiments and in three of them we observed an increased parasitemia. Histological analyzes and qPCR assays revealed no differences in the infection of several tissues. As it cannot be ruled out, an effect of hyperprolinemia on the immune system, we evaluated the effect of proline treatment on inflammatory infiltrate in histological sections using the Image J software. The treatment did not alter the number of inflammatory cells in the several analyzed tissues. Moreover, in *ex vivo* experiments, proline did not disturb the nitric oxide production and also did not affect the viability of peritoneal macrophages up to a concentration of 3 mM. Our results suggest a modest effect of the hyperprolinemia on the *T. cruzi* infection. This observation could be due to the fact that, in this experimental model, the hyperprolinemia is transient, not being enough to reach the intracellular medium (of the infected host-cells) to strongly influence the infection profile. To confirm the hypothesis that differences in prolinemia affect the infection, other hyperprolinemia models are currently being investigated. **Supported by:**:FAPESP, CNPq, INBEQMEDI

QT020 - Neolignan Licarin A promotes alterations in intracellular calcium levels and cell death by apoptosis and necrosis on *Leishmania amazonensis* promastigotes

NÉRIS, P.L.D.N.*; CALDAS, J.P.A.; AMORIM, F.M.; RODRIGUES, Y.K.S.; LEITE, J.A.; RODRIGUES-MASCARENHAS, S.; FILHO, J.M.B.; RODRIGUES, L.C.; OLIVEIRA, M.R.
UFPB, JOÃO PESSOA, PB, BRASIL.
e-mail:pattyneris@yahoo.com.br

Licarin A is a neolignan found in nature that has synthesis feasible chemical synthesis. Considering that the main drugs used for treating *Leishmania*sis exhibit high toxicity in humans, the aim of this study was to evaluate the anti*Leishmania*l activity of Licarin A on *Leishmania amazonensis*. Logarithmic-phase *L. amazonensis* promastigotes ($1x10^6$ cells/mL) were incubated with Licarin A for 72h at 25ºC, and the cultures were quantified in a neubauer chamber. Licarin A was incubated with *L. amazonensis* promastigotes ($1,5x10^6$ cells/mL) for one hour for intracellular calcium ($[Ca^{2+}]_i$) analysis with the probe Fluo 3AM (500nM), and for 3 hours for analysis of cell death with annexin V and propidium iodide. The results were evaluated by flow cytometry BD FACSCalibur. *L. amazonensis* promastigotes were exposed to Licarin A for 6 hours and overnight at 25°C, the genomic DNA was extract and was analyzed on agarose gel (1%). Murine macrophages (MØs) ($1x10^6$ cells/mL) were incubated with Licarin A and submitted to testing for cytotoxicity by the MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) and trypan blue methods. Licarin A presented antipromastigote activity against *L. amazonensis*, with IC_{50} value of 17.46 ± 2.06µg/mL. The neolignan increased the number of cells with detectable levels of $[Ca^{2+}]_i$, when compared to control, being this increase of 8% and 14% for the concentrations of IC_{50} and 2x IC_{50}, respectively. Exposure to 4x IC_{50} of Licarin A induced cell death by apoptosis (45.86%) and necrosis (47.69%) in *L. amazonensis* promastigotes, and also induced fragmentation of genomic DNA similar to cells in apoptosis, at the concentrations of 4x and 8x IC_{50}. Licarin A presents low cytotoxicity on MØs with a CC_{50} of 729.8µg/mL and 308.96µg/mL for the MTT and trypan blue assays, respectively. It can be concluded that the leishmanicidal activity of Licarin A occurs through apoptosis and necrosis death associated with changes in intracellular calcium levels. **Supported by:**:CNPq, CAPES

QT021 - Effects of linalool and eugenol on *Leishmania* chagasi.

DUTRA, F.L.*; OLIVEIRA, M.M.; LOPES, A.H.
UFRJ, RIO DE JANEIRO, RJ, BRASIL.
e-mail:dutralnando@gmail.com

The most commonly used drugs against visceral *Leishmania*sis are based on pentavalent antimonial compounds that play a fundamental role in its therapy for over 70 years. It has been shown that some compounds isolated from the Brazillian flora, such as linalool and eugenol, are able to kill some typanosomatids at low doses. In the present study, we show the effects of these compounds on *L. chagasi* (visceral *Leishmania*sis) and compared to the effects of glucantime. The MIC for eugenol on *L. chagasi* was 0.1 ng/ml and 50% inhibition of proliferation occurred between 100 ng/ml and 100 microg/ml. The MIC for linalool was 100 ng/ml and 50% inhibition of proliferation occurred between 100 ng/ml and 100 microg/ml. Regarding the ability to kill *L. chagasi*, the LD_{50} of linalool was between 350 and 450 microg/ml and eugenol was about 250 microg/ml. In the interaction of *L. chagasi* promastigotes with BALBc peritoneal mouse macrophages, when the macrophages were pretreated with linalool, eugenol or glucantime, there was no significant change in the profile, as compared to the untreated control group. In other set of experiments, *L. chagasi* was added to the peritoneal macrophages previous to any drug treatment and let to interact for 4 h with the macrophages. There was a significant decrease in the number of parasites present within the macrophages when these systems were challenged with the same concentrations of eugenol or linalool, suggesting that these drugs killed the parasites inside the macrophages. These compounds were able to modulate the activities of the *L. chagasi* protein kinases PKA and PKC, as well as the cystein proteases activities. Also, linalool promoted a decrease in *L. chagasi* oxygen consumption. In conclusion, both linalool and eugenol were able to inhibit the proliferation and to kill *L. chagasi*, but this effect was more prominent during the interaction assays, and can induce alterations in many aspects of parasite. **Supported by:**:CNPq, FAPERJ, CAPES, INCT – Entomologia Molecular

QT022 - Neolignan Licarin A presents anti*Leishmania*l activity against *Leishmania* major associated with decreased IL-6 and IL-10 production but independent of nitric oxide production

NÉRIS, P.L.D.N.*; AMORIM, F.M.; RODRIGUES, Y.K.S.; LEITE, J.A.; CALDAS, J.P.A.; RODRIGUES-MASCARENHAS, S.; FILHO, J.M.B.; RODRIGUES, L.C.; OLIVEIRA, M.R.
UFPB, JOÃO PESSOA, PB, BRASIL.
e-mail:pattyneris@yahoo.com.br

The treatment of *Leishmania*sis is based mostly on long-term administration of pentavalent antimonials or amphotericin B, expensive drugs associated with severe side effects. Considering that the search for alternatives treatments for *Leishmania*sis is necessary, the objectives of this study were to evaluate the anti*Leishmania*l activity of Licarin A on promastigotes and amastigotes forms of *Leishmania major*. Logarithmic-phase *L. major* promastigotes (1x106 cells/mL) were incubated with Licarin A for 72h at 25ºC, and quantified in a neubauer chamber under light microscopy. Licarin A was incubated with *L. major* promastigotes for 6 hours and overnight at 25°C, the genomic DNA was extract by chloroform/phenol method and was analyzed on agarose gel (1%). Murine macrophages (MØs) were infected with stationary-phase *L. major*, incubated with Licarin A at 37ºC in a 5% CO2 atmosphere. After 24 and 72h the infected macrophages were analyzed under light microscopy. Supernatants of these cultures were assayed for production of the interleukin (IL)-10 and IL-6, and nitric oxide (NO) by the Griess reaction. Licarin A presented leishmaniostatic activity on *L. major* promastigotes, with IC_{50} of 9.59 ± 0.94µg/mL, and leishmanicidal activity, since this neolignan induces fragmentation of the genomic DNA at the concentrations of 4x and 8x IC_{50}. Licarin A reduced the infection rate of MØs infected with *L. major* after 24 hours of treatment at the concentrations of 5µg/mL (77.44%) and 20µg/mL (96.92%), being this reduction greater after 72h treatment, for both concentrations of 5µg/mL (93.38%) and 20µg/mL (99.54%), when compared to control. This activity was associated with a decrease of IL-6 and IL-10, but was independent of NO production. It can be concluded that Licarin A presents antipromastigote and antiamastigote activity, as well as immunomodulatory property in a model of infection *in vitro* with *L. major*. **Supported by:**:CNPq, CAPES

QT023 - Activity of SQ-109, a squalene synthase inhibitor, and analogues against *Trypanosoma cruzi*

LAMEIRA, L.G.M.[*1]; VEIGA-SANTOS, P.[1]; LI, K.[2]; OLDFIELD, E.[2]; URBINA, J.A.[3]; DE SOUZA, W.[1]; CARVALHO, T.M.U.[1]
1.UFRJ, RIO DE JANEIRO, RJ, BRASIL; 2.UNIVERSITY OF ILLINOIS, CHAMPAIGN, ESTADOS UNIDOS; 3.IVIC, CARACAS, VENEZUELA.
e-mail:phercyles@biof.ufrj.br

Chagas' disease is caused by the protozoan *Trypanosoma cruzi*. Currently available treatments use compounds such as nifurtimox and benznidazole, which have a wide range of side effects and an unsatisfactory response during the chronic phase. Thereby, it is important to find new safer and more active drugs. Previous studies have validated the ergosterol biosynthesis pathway as a chemotherapeutic target in *T. cruzi* and *Leishmania* spp. In the present work we investigated for the first time the anti-*T. cruzi* activity of SQ-109 (N-[(2E)-3,7-dimethyl-2,6-octadienyl]-N'-tricyclo[3.3.1.13,7]dec-2-yl-1,2-ethanediamine), a compound in clinical development for the treatment of drug-resistant tuberculosis, which has also been shown to inhibit *T. cruzi* 's squalene synthase. We also evaluated the effects of SQ-109 and three analogs (1281, 1283 and 1304) against both proliferative stages of T. cruzi, cultured in vitro. All compounds inhibited the proliferation of epimastigote forms with IC50 values (144 h of exposure), of 4.2 µM for SQ-109 and 6.9 µM, 9.3 µM, and 6.1µM, respectively, for the analogs. The compounds also inhibited the proliferation of intracellular amastigotes in cultured murine peritoneal macrophages, with IC50 values (96 h of exposure) of 1.4 µM for SQ-109, and 1.6 µM, 1.9 µM and 1.7 µM, respectively, for the analogs. The selectivity index of SQ-109 activity was 12. Analyses by scanning electron microscopy revealed that SQ-109 caused depressions of the plasma membrane and rounding of the cell body. Thin sections of treated epimastigotes observed by transmission electron microscopy showed drastic changes in the Golgi complex, mitochondrial swelling, formation of myelin-like figures and cytoplasmic vesiculation. Taken together, our observations show that SQ-109 and analogs are promising selective inhibitors of *T. cruzi* proliferation. Further studies will evaluate their potential antiproliferative synergism with posaconazole. **Supported by:**:CNPq, CAPES, FINEP, FAPERJ, USPHS.

QT024 - In vitro activity of LQB118 on amastigote and trypomastigote forms of *Trypanosoma cruzi*

AZEVEDO, B.F.[*1]; SOUZA, L.C.[1]; SILVA, T.[1]; MEIRA, R.V.[1]; SIQUEIRA, L.M.[1]; COSTA, P.R.R.[2]; MENDES, M.A.P.[1]; SILVA, S.A.G.[1]
1.UERJ, RIO DE JANEIRO, RJ, BRASIL; 2.UFRJ, RIO DE JANEIRO, RJ, BRASIL.
e-mail:bruno_fazevedo@yahoo.com.br

Chagas disease is a neglected disease which treatment is restrict and unsatisfactory. Both Nifurtimox and Benznidazole act on trypomastigote and very little on intracellular amastigote form.Thus, the development of new drugs is required, mainly to act on intracellular amastigote forms. The synthetic pterocarpanoquinone LQB118 presents antitumoral activity and acts on L amazonensis and *L. braziliensis* in vitro and in vivo. The aim of this study was to evaluate in vitro, the anti-parasitic effect of LQB118 and their derivatives on amastigotes and trypomastigotes forms of *Trypanosoma cruzi* (Dm28c strain). For the evaluation of anti-parasitic effect on intracellular amastigotes, peritoneal macrophages from SW mice were infected with metacyclic trypomastigotes (obtained by metacyclogenesis of epimastigotes on TAU/TAUP culture media for about 4 days at 280 C) at a ratio of 5:1 and after were treated with LQB118 and their derivatives LQBs 168, 187, 182 and 236 at 20 µM for 72 h/37oC/5%CO2. Monolayer of infected macrophages was stained for counting of amastigotes and nitrite was determinate by Griess method in the supernatant. For the evaluation of IC50 infected macrophages were incubated at various concentrations of pterocarpanoquinones. For the evaluation on metacyclic trypomastigotes forms, parasites were incubated with 20µM of the LQBs118, 168, 187, 182 and 236 for 48 h at 28oC and after viability was evaluated by motility and MTT method. The results show that LQB118 inhibited the infection index into 73% ($p<0,05$) while the other derivatives had no effect in relation to control. The IC50 LQB118 on intracellular amastigotes was estimated at 7,5µM. On trypomastigotes forms, LQB118 at 20µM was the most active killing 74% of parasites($p<0,05$). The results show that LQB118 has an anti-parasitic effect against both amastigotes and trypomastigotes forms of T. cruzi. Studies about mechanism of action and therapeutic activity of LQB118 in infected mice have been initiated. **Supported by:**:CNPQ

QT025 - Biological activity and toxicological analyses of synthetic amidine compounds against *Trypanosoma cruzi* in vitro

ARAÚJO, J.S.[*1]; SILVA, C.F.[1]; SILVA, P.B.[1]; MEUSER, M.B.[1]; SILVA, M.F.V.[2]; AIUB, C.A.F.[2]; BOYKIN, D.W.[3]; FARAHAT, A.A.[3]; BANERJEE, M.[3]; SOEIRO, M.N.C.[1]
1.IOC/FIOCRUZ, RIO DE JANEIRO, RJ, BRASIL; 2.UNIRIO, RIO DE JANEIRO, RJ, BRASIL; 3.DEPARTMENT OF CHEMISTRY, GEORGIA STATE UNIVERSITY, ATLANTA, GEORGIA.
e-mail:ju.siciliano@gmail.com

More than one hundred years after its discovery by Carlos Chagas (1909), Chagas disease (CD) still exhibits lack of therapeutic options, as recent treatment is mostly based in benznidazole (BZ) and nifurtimox, which display limited efficacy, especially during the later chronic phase, and considerable side effects. On this basis, our group has evaluated some classes of synthetic heterocyclic compounds, especially aromatic diamidines and their analogs, such as arylimidamides (AIAs). Presently, the biological activity of dicationic (DB1979, DB1989 and DB1995) and monocationic (DB1996, DB1997, DB1980, DB2001, DB2002, DB2003, DB2004, DB2006 and DB2007) AIAs were investigated against bloodstream trypomastigotes (BT) and amastigotes forms of *Trypanosoma cruzi* at 4ºC and 37ºC in presence or not of whole mice blood, respectively, also aiming to evaluate the potential applicability in blood bank prophylaxis. Di-arylimidamides were the most active showing IC50 values ≤1±0.8 for BT and ≤1±1.4 µM for intracellular forms. However, most compounds lost their effect against BT incubated with blood, except for DB1989 (IC50=3.9±1.3 µM). Cytotoxic potential was investigated upon uninfected cardiomyocytes and revealed that mono-AIAs were the most toxic, showing loss of cellular viability at ≥1,18 µM after 48h of incubation. Toxicological analyses were also performed by AMES. A weak mutagenic potential profile of DB1989 and DB1994 was found on *Salmonella* Typhimurium reverse mutation test (up to 17.75 nM). Simultaneously, survival experiments showed high toxicity of both AIAs. New tests using metabolic activation are underway, according to the international guidelines, to further evaluate the toxicological profiles of these amidines. The sum of these results corroborate previous studies showing superior efficacy of AIAs as compared to classical diamidines like furamidine, being especially more effective than BZ, representing a great promise for future pre-clinical studies for CD. **Supported by:**:Fiocruz, FAPERJ, CNPq, DECIT/SCTIE/MS, MCT/CNPq, CPDD

QT026 - A new PBN derivate with trypanocidal activity against *T. cruzi* clinical relevant forms

CUPELLO, M.P.[*1]; SARAIVA, F.M.S.[1]; NOGUEIRA, N.P.A.[1]; IPPOLITO, P.[1]; MEUSER, M.B.[2]; LARANJA, G.A.T.[1]; MENNA BARRETO, R.F.S.[2]; DOMINGOS, J.[1]; COELHO, M.G.P.[1]; COSTA, P.R.R.[1]; DIAS, A.G.[1]; PAES, M.C.[1]
1.UERJ, RIO DE JANEIRO, RJ, BRASIL; 2.IOC, RIO DE JANEIRO, RJ, BRASIL.
e-mail:mauricio.cupello@yahoo.com.br

Chagas disease is a neglected illness caused by protozoan *Trypanosoma cruzi* and the deficiencies in the treatment justify the search for new chemotherapeutic options. Many studies have demonstrated that nitrones present a protective role against pathogens as seen in LPS-induced septic shock and in bacterial meningitis. Therefore, we evaluated the effect of the PBN derivate LQB 123 on *T. cruzi* forms relevant to mammalian infection. First of all, we analyzed the effect of LQB 123 during *T. cruzi* metacyclogenesis. Thus, Dm28c strain epimastigotes were maintained in TAU medium for 2 hours and after in TAU3AAG in the absence or in the presence of increasing concentrations of LQB 123. Our results showed a letal activity during metacyclogenesis. Additionally, bloodstream trypomastigotes, Y stain, were incubated with different concentrations of LQB 123 at 37ºC for 24h. Again, we observed a trypanocidal activity with an IC_{50} 264 µM. Then, we studied the effect of LQB 123 upon macrophage infection *in vitro*. For that, mice peritoneal macrophages were infected with culture trypomastigotes in a 1:10 ratio, for three hours, and incubated in DMEN with or without LQB 123 at 37ºC. This nitrone significantly decreased the macrophage infection *in vitro*. Finally, we tested the effect of various concentrations of this molecule on peritoneal macrophage survival and we did not observed any damage to the cells viability after 48h. Taken that, our data showed that LQB 123 presented a great trypanocidal effect in all parasite forms tested, impairing important forms of the parasite life cycle. Also, our results suggest LQB123 as new potential drug for additional *in vivo* tests, since it showed no harmful effect upon mammalian cells. In conclusion, we provide evidence of a new candidate drug for the therapy of Chagas disease. **Supported by:**:CNPq, FAPERJ, and INCT-EM

QT027 - EFFECTS OF Echinodorus grandiflorus ON *Leishmania* braziliensis

ALMEIDA, A.C.*; FREIRE, M.L.; ANTINARELLI, L.M.R.; RIBEIRO, C.J.; CHEDIER, L.M.; PIMENTA, D.S.; COIMBRA, E.S.
UFJF, JUIZ DE FORA, MG, BRASIL.
e-mail:ayladaschagas@hotmail.com

Introduction and Objectives: *Leishmania*sis is a complex of diseases caused by the protozoan *Leishmania*. Considered as neglected tropical disease, *Leishmania*sis affects about 12 million of people around the world. The treatment of this disease is restricted to a limited number of drugs that exhibit high toxicity, collateral effects and often costly. Research involving the use of natural products becomes a great alternative to the discovery of new drugs safer and more accessible. The aim of this study was to evaluate the leishmanicidal activity of *Echinodorus grandiflorus*, used for a long time in folk medicine and known as “chapéu de couro”. Materials and methods: We studied the leaf hexane extract and its ten fractions of the *E. grandiflorus*, obtained with solvents of increasing polarity (hexane, ethyl acetate and methanol) against promastigotes of *L. braziliensis.* Furthermore, the extract and fractions *E. grandiflorus* also were tested for cytotoxic effects on mammalian cells (murine macrophages). The viability of promastigotes of *Leishmania* and macrophages were assayed using the tetrazolium-dye (MTT) colorimetric method. The results were expressed as the concentrations inhibiting parasite or macrophages growth by 50 percent (IC_{50}) after three days incubation period. Results and Conclusions: The hexane extract and its fractions showed significant leishmanicidal activity against *L. braziliensis* promastigotes, with IC_{50} ranging from 14.4 µM to 50.2 µM. Most of the fractions tested showed no cytotoxicity against mammalian cells. These results demonstrate the leishmanicidal activity of the *E. grandiflorus* and further studies in intracellular amastigotes will be done. **Supported by:** FAPEMIG, CNPq and UFJF.

QT028 - LEISHMANICIDAL ACTIVITY OF MERCAPTOQUINOLINE DERIVATIVES

ALMEIDA, A.C.*; FREIRE, M.L.; ANTINARELLI, L.M.R.; DIAS, R.M.P.; DA SILVA, A.D.; COIMBRA, E.S.
UFJF, JUIZ DE FORA, MG, BRASIL.
e-mail:ayladaschagas@hotmail.com

Introduction and objectives: *Leishmania*sis is caused by species of the genus *Leishmania*, and is one of the major public health problems which affect more than 12 million people worldwide. For many years, pentavalent antimonials have been the first-line drugs used to treat *Leishmania*sis. However these drugs show variable efficacy, and require parenteral administration. The purpose of this study was to evaluate a series of mercaptoquinoline derivatives against promastigote and amastigote forms of *Leishmania* species. Materials and methods: Ten compounds were assayed against *L. amazonensis*, *L. braziliensis L. chagasi*, and *L. major* promastigote forms and were tested for cytotoxic effects on mammalian cells. These tests were determined by colorimetric method MTT, after 72 hours of treatment. The antiamastigote activity was determined by counting the number of intracellular parasites and the percentage of infected macrophages after 72 hours of treatment. The results in promastigotes and amastigotes were expressed as IC_{50} (concentration inhibiting parasite growth by 50%). Results and conclusions: Among ten compounds tested, two exhibited significant leishmanicidal activity. The compound 7-chloro-4-mercaptoquinoline (1) showed high activity against promastigotes of *L. amazonensis*, *L. braziliensis* and *L. chagasi* with IC_{50} values 12.8µM, 10.5µM and 6.0µM, respectively. The compound 7-chloro-4-(3-chloropropylmercapto) quinoline (2) showed activity against only promastigotes of *L. amazonensis* (IC_{50} value of 25.1 µM). The compound 1 showed significant activity against intracellular amastigotes of *L. braziliensis* (IC_{50} values of 6.3 µM) without cytotoxicity against macrophages. These results stimulate further investigations of this class of compounds for the development of new chemotherapeutic agents for *Leishmania*sis. **Supported by:** FAPEMIG, CNPq and UFJF.

QT029 - The anti-*Leishmania*l activity of pterocarpanoquinone LQB118 on *Leishmania* braziliensis using hamster as infection model

SOUZA, L.C.[*1]; SILVA, T.[1]; MEIRA, R.V.[1]; SIQUEIRA, L.M.[1]; AZEVEDO, B.F.[1]; MENDES, M.A.P.[1]; SILVA, A.J.M.[2]; COSTA, P.R.R.[2]; SILVA, S.A.G.[1]
1.UERJ, RIO DE JANEIRO, RJ, BRASIL; 2.UFRJ, RIO DE JANEIRO, RJ, BRASIL.
e-mail:liluicosta@yahoo.com.br

We have previously showed that the synthetic pterocarpanoquinone LQB118 has direct antiparasitic effect on intracellular amastigotes of *L. braziliensis*, being able to modulate the production of inflammatory cytokines in mice macrophages. Hamster (Mesocricetus auratus) is a good model for studies with *L. braziliensis*, since it does not resolve the infection spontaneously and represents better the human infection. The aim of this study was to evaluate the leishmanicidal activity of LQB118 in vitro and in vivo using golden hamster as experimental model. Peritoneal macrophages from hamsters were infected or not with *L. braziliensis* and incubated with LQB118 (0 to 20µM) for 24 and 48h. After staining with Giemsa, the number of amastigotes were counted under microscope. The survival of intracellular amastigotes was evaluated by the ability of the parasite differentiate into promastigotas after removal of LQB 118 and reincubation with Schneider's plus 20% at 28°C for more 5-7 days. Therapeutic activity of LQB118 was studied in groups of hamsters (5-6/group) infected in the footpad with *L. braziliensis* and treated with LQB118 by intralesional (26µg/kg/day) or oral (4,3mg/kg/day) routes after 7 days of infection for 8 weeks. LQB 118 showed a dose-dependent effect inhibiting intracellular amastigotes 7%, 27% ($p < 0.05$) and 42% ($p < 0.05$), respectively at 5, 10 and 20µM. Pre-treatment of macrophages with LQB118 (0-20µM) for 24h before infection was even more effective, inhibiting 80% the number of amastigotes/macrophage at 20 µM. Treatment of infected hamsters with LQB118 by intralesional or oral route was able to significantly control the lesions size in relation to untreated group ($p < 0.05$). These results indicate that besides the direct activity on the parasite, the LQB118 may modulate the macrophages, which may contribute to the therapeutic activity in hamsters. We are currently evaluating cytokine production in hamster macrophages by RT-PCR. Financial support CNPq. **Supported by:**:CNPq

QT030 - Antiparasitic activity of synthetic naphthoquinones against *Leishmania* braziliensis

MEIRA, R.V.[*1]; SILVA, T.[1]; SOUZA, L.C.[1]; MENDES, M.A.P.[1]; AZEVEDO, B.F.[1]; SILVA, A.J.M.[2]; COSTA, P.R.R.[2]; SILVA, S.A.G.[1]
1.UERJ, RIO DE JANEIRO, RJ, BRASIL; 2.UFRJ, RIO DE JANEIRO, RJ, BRASIL.
e-mail:rafaellamvm@gmail.com

Naphthoquinones are bioactive molecules that can interfere with several cellular processes and have described anti-tumor and anti-protozoa activity. The aim of this study was to evaluate the direct and indirect antiparasitic effect of the naphthoquinone lapachol and its synthetic analogues (LQB166, LQB180, LQB181, LQB209 and LQB219) on promastigotes and intracellular amastigotes of *L. braziliensis*, modulation of the host cell and toxicity. Promastigotes were incubated with naphthoquinones (200µM) for 96h/28°C and the parasites were counted daily. Monolayers of peritoneal macrophages from normal swiss mice were infected with *L. braziliensis* promastigotes (5:1) and incubated with naphthoquinones (200µM) for 72h at 37°C/5%CO2. The macrophages were stained and intracellular amastigotes counted under a microscope. Nitric oxide (NO) production was measured (Griess method) in the supernatant. Naphthoquinones toxicity was evaluated on non-infected macrophages by MTT assay. All naphthoquinones significantly inhibited the growth of promastigotes on the 3rd day of culture, compared with control ($p<0.001$). Lapachol inhibited 98% and LQBs 166, 181, 209 and 219 inhibited 76%, 63%, 68% and 100%, respectively. The naphthoquinones showed no toxicity on macrophages at the concentration tested (200 µM). On intracellular amastigotes forms all naphthoquinones tested decreased significantly ($p<0.001$) the number of parasites per macrophage. Lapachol decreased the number of amastigotes in 47.6% and LQBs 166, 180, 181, 209 and 219 decreased in 40.5%, 27.1%, 45.2%, 47.6% and 50% respectively. LQB180 increased NO production by infected macrophages ($p<0.005$). These data indicate that lapachol and its analogues inhibit promastigotes and intracellular amastigotes of *L. braziliensis*. Although not the most active on amastigotes, LQB180 increased the nitric oxide production by macrophages. We are currently investigating the modulation of macrophages by LQB180. Financial support PIBIC/UERJ **Supported by:**:PIBIC

QT031 - Activity of quercetin in *Leishmania* braziliensis using golden hamster as infection model

SANTOS, R.F.[*1]; BRITO, A.C.S.[1]; SIQUEIRA, L.M.[1]; SILVA, T.[1]; MEIRA, R.V.[1]; MENDES, M.A.P.[1]; COUTINHO, M.A.S.[2]; COSTA, S.S.[2]; SILVA, S.A.G.[1]
1.UERJ, RIO DE JANEIRO, RJ, BRASIL; 2.UFRJ, RIO DE JANEIRO, RJ, BRASIL.
e-mail:anne_bio@hotmail.com

Previous studies demonstrated therapeutic effect of quercetin flavonoid by oral route in mice infected with *L. amazonensis*. The aim of this study was to evaluate the activity of quercetin flavonoid in *Leishmania braziliensis in vitro* and *in vivo* using hamsters as experimental model. The antiparasitic effect of quercetin was evaluated *in vitro* on intracellular amastigotes using hamsters peritoneal macrophages infected with *L. braziliensis* (radio of 5 parasites/ macrophage) and treated for 48h. The effect on the modulation of macrophage activation was assessed by measuring levels of nitric oxide (NO) by Griess method and reactive oxygen species (ROS) by Kit H2DCFDA. *In vivo* therapeutic activity of quercetin was studied in groups of hamsters (5-6 animals) infected with *L.braziliensis* and treated with 2mg quercetin (5x/week) by oral route from the seventh day after infection for eight weeks. The therapeutic action was analyzed by the size of the lesion. The antiamastigote action of quercetin showed a dose dependent manner inhibiting 25 and 50 % at 50 and 100 µg/ml, respectively. The pre-treatment of macrophages with quercetin at 50 and 100 µg/ml for 24h before infection inibihited 50 e 60 % the growth of intracellular amastigotes, respectively. Nitric oxide production was unchanged by macrophages. However quercetin pre-treated macrophages before infection showed increase of ROS when compared to controls ($p<0,05$), while macrophages treated before and after infection showed a decrease of ROS production. This date was according to related pro and antioxidant effect of quercetin. *In vivo*, quercetin by oral route was able to control de size of lesion since the third week of treatment in relation untreated group ($p<0,05$) similarly to glucantime treated group. These dates show anti-*Leishmania*l action of quercetin is extended to *L. braziliensis* in hamster model, demonstrating its promising therapeutic effect in *Leishmania*sis. **Supported by:**:FAPERJ E CAPES

QT032 - LEISHMANICIDAL ACTIVITY OF NOVEL ANALOGUES OF LASSBio-1064

QUEIROZ, A.C.[*1]; ALVES, M.A.[2]; ARAÚJO, M.V.[1]; TENÓRIO, A.P.M.[1]; SANTOS, M.S.[1]; BARREIRO, E.J.L.[2]; LIMA, L.M.[2]; ALEXANDRE-MOREIRA, M.S.[1]
1.LAFI - UFAL, MACEIÓ, AL, BRASIL; 2.LASSBIO - UFRJ, RIO DE JANEIRO, RJ, BRASIL.
e-mail:allycq_farmacia@hotmail.com

*Leishmania*sis is a public health issue and is among the five parasitic diseases worldwide. The current treatment disease includes limitations of toxicity, variable efficacy, high costs and inconvenient doses and treatment schedules. These observations prompted us to investigate the leishmanicidal activity of novel synthetic derivatives, designed from molecular modification on the prototype LASSBio-1064. The cell line J774 viability was determined with MTT assay. Leishmanicidal effect against promastigotes and amastigotes of *Leishmania* major (MHOM/SU/1973/5-ASKH) was evaluated at concentration of 100 µM – 0,01 nM. Data obtained from experiments were expressed as the mean ± S.E.M. of triplicate cultures of representative assays. Statistical differences between the treated and the vehicle groups of in vitro experiments were evaluated by ANOVA and Dunnett hoc tests. Differences with a $p<0.05$ were considered significant. The compounds LASSBio-1700, 1701, 1704, 1705, 1706, 1708, 1709 e 1736 at concentration of 100 µM showed deleterious activity to the host cell evidenced by MTT assay. The prototype LASSBio1064 and the reference drug pentamidine not affected the viability of macrophages. Moreover, the derivatives LASSBio-1481, 1489, 1490, 1491, 1492, 1493, 1699, 1702, 1705, 1707, 1710 and 1736 exhibited high anti*Leishmania*l activity against promastigotes of *L. major*, with maximal effect greater than 65%. The reference drug pentamidine present maximal effect against promastigotes of *L. major* of 72.6 ± 2.0%. Compounds LASSBio-1483, 1485, 1489, 1491, 1492, 1699, 1705 and 1707, beyond of the standards LASSBio-1064 and pentamidine, also significantly diminished the number of intramacrophage amastigotes of *L. major*, presenting maximal effect greater than 50%. The derivatives LASSBio-1483, 1485, 1489, 1491, 1699 and 1707 can be considered lead-candidates for anti*Leishmania*l drugs since they presented pronounced leishmanicidal activity without cytotoxic effect to host cells. **Supported by:**:INCT-INOFAR (#573.564/2008-6), CNPq, FAPERJ and FAPEAL.

QT033 - Mitochondrial stress caused by the naphtopterocarpanquinone LQB-118 in *Leishmania amazonensis*.

CUNHA-JUNIOR, E.F.[*1]; CANTO-CAVALHEIRO, M.M.[1]; SILVA, A.J.M.[2]; COSTA, P.R.R.[2]; ALMEIDA-AMARAL, E.E.[1]; TORRES-SANTOS, E.C.[1]
1.FIOCRUZ, RIO DE JANEIRO, RJ, BRASIL; 2.UFRJ, RIO DE JANEIRO, RJ, BRASIL.
e-mail:edeziofcjr@yahoo.com.br

Aiming at the development of new drugs, the fusion of a synthetic quinone core with a pterocarpan portion produced the hybrid product LQB-118. This compound proved to be a promising prototype, due its efficacy in experimental cutaneous *Leishmania*sis (J Antimicrob Chemother 2011; 66: 1555-1559). In previous studies, we found evidences of selective LQB-118-induced apoptosis in *Leishmania amazonensis*, assessed by reactive oxygen species production, loss of mitochondrial membrane potential, phosphatidylserine exposure and DNA fragmentation (submitted). In continuation to that study, we aim to evaluate the early events involved in triggering the LQB-118- induced death in *L. amazonensis*. Promastigotes were incubated with LQB-118 and N-acetylcysteine or glutathione for 48 h. However, although the ROS production can be reversed by antioxidants, the cell viability and mitochondrial membrane potential cannot, suggesting that ROS production is not the primary cause of cell death. Interestingly, when promastigotes or intracelular amastigotes of *L. amazonensis* are incubated with LQB-118, an intense time and concentration-dependent mitochondrial stress is produced in the first hours, evidenced by stronger Alamar blue reduction. Inhibitors of complexes I (rotenone), II (TTFA) or III (antimycin A) failed reversing the stronger Alamar blue reduction in LQB-118-treated promastigotes. Only KCN (complex IV inhibitor) was able to reverse this phenomenon. Concomitant to this process, we also observed a time and concentration-dependent increase in ATP production by LQB-118-treated promastigotes in the early hours, measured by luciferine-luciferase reaction. After 48 h, both process end, with failure of mitochondrial activity and ATP production. These data suggest that LQB-118 induces a high activity of the respiratory chain in the early hours, inducing mitochondrial stress and ROS production, culminating with mitochondrial failure and parasite death. **Supported by:**:CAPES, FAPERJ, PAPES

QT034 - ANTI*LEISHMANIA*L ACTIVITY OF 18-ETHOXYCORONARIDINE HYDROCHLORIDE, A NOVEL IBOGA-TYPE ALKALOID, AGAINST *Leishmania* chagasi

DELORENZI, J.C.[*1]; MELO, T.S.[1]; FREMAN, S.[2]; BOULANGER, W.[3]; HURST, S.L.[2]
1.UPM, SAO PAULO, SP, BRASIL; 2.SAVANT HWP, SAN CARLOS/CA, ESTADOS UNIDOS; 3.OBITER, CHAMPAING/IL, ESTADOS UNIDOS. e-mail:jan.bertassoni@mackenzie.br

Leishmania parasites infections are still a wordwilde health problem affecting more than 12 million people around the world. Although the wide distribution in more than 88 countries few advances are observed in *Leishmania*sis treatment. We are still using as first-line choice the Antimonials, drugs used since 1937 with unconfortable administration way and severe adverse effects. The introduction of Miltefosine, originally developed for breast cancer treatment, and the development of new Anphotericin B formulations, were the two major advances in *Leishmania*sis treatment at last 20 years, but both of these drugs have issues with toxicity and efficacy. This situation includes *Leishmania*sis as an extremely neglected disease. The development of new drugs, natural or synthetic, is stimulated by organisms such as DNDi (Drugs for Neglected Disease iniciative). The 18-Ethoxycoronaridine Hydrochloride (18-EC, M.W. 418.96 and Formula of C23H31ClNO3) is a novel coronaridine congener alkaloid whose pharmacological capability is not explored yet. Its analogous alkaloids, Coronaridine and 18-Methoxycoronaridine have previously shown to have *Leishmania*cidal activity. The aim of this study is to evaluate the leishmancide activity of 18-EC against *Leishmania chagasi*, parasite that cause the visceral form of *Leishmania*sis, the most severe form of this infection. Our results showed a great inhibition of promastigote growth of 82, 83 and 90% when treated with 25, 50 and 100 micg/mL after 48 hours of treatment. The IC_{50} (Inhibitory Concentration of 50%) for promastigotes was 19.6micg/mL and for amastigotes the IC50 was 12.7micg/mL revealing a pronounced activity against *L. chagasi* in vitro. Evaluation of 18-EC cytotoxicity in murine peritoneal macrophages showed a CC50 (citotoxic concentration to 50%) of 72,4 micg/mL when analyzed by XTT method. Altogether these results suggest that 18-EC is a promising candidate for a new treatment development for Visceral *Leishmania*sis. **Supported by:**:MackPesquisa (Fundo Mackenzie de Pesquisa), CNPq, Hebron Farmacêutica, Savant HWP

QT035 - LEISHMANICIDE ACTIVITY OF 18-METHOXYCORONARIDINE HYDROCHLORIDE AGAINST *Leishmania* chagasi

DELORENZI, J.C.[*1]; MELO, T.S.[1]; FREMAN, S.[2]; BOULANGER, W.[3]; HURST, S.L.[2]
1.UPM, SAO PAULO, SP, BRASIL; 2.SAVANT HWP, SAN CARLOS/CA, ESTADOS UNIDOS; 3.OBITER, CHAMPAING/IL, ESTADOS UNIDOS.
e-mail:jan.bertassoni@mackenzie.br

Natural products are excellent source of new compounds to drug development. In part of the time it is necessary bioassay-guide structural changes to improve the pharmacological activities and/or reduce the possible toxic effects. Coronaridine is an iboga-type alkaloid isolated from some plants of Apocynaceae family. At beginning of 2000 years our group showed its leishmancide activity against *Leishmania amazonensis* (Delorenzi *et al*, 2001). We then screened a synthetic library of conronaridine congeners. The introduction of a Methoxy group (-OCH3) at C-18 produced the compound 18-Methoxycoronaridine Hydrochloride (18-MC, M.W. 404.93, Formula: $C_{22}H_{29}ClN_2O_3$). This compound revealed a greater anti*Leishmania*l activity against *L. amazonensis* when compared with Coronaridine and Glucantime (Delorenzi *et al*, 2002). In fact, 18-MC is now a lead compound in our efforts to develop a leishmanicide drug. *Leishmania*sis is still a wordwilde health problem affecting more than 12 million people around the world. Although the wide distribution in more than 88 countries few advances are observed in *Leishmania*sis treatment. The aim of this study is to evaluate the leishmancide activity of 18-MC against *Leishmania chagasi*, parasite that cause the visceral form of *Leishmania*sis, the most severe form of this infection. Our results showed a relevant inhibition of promastigote growth of 53, 64 and 75% when treated with 25, 50 and 100 micg/mL after 72 hours of treatment. The IC_{50} (Inhibitory Concentration of 50%) for promastigotes was 22.5 micg/mL and for amastigotes the IC_{50} was 16.1micg/mL revealing a pronounced activity against *L. chagasi in vitro*. In conclusion, our results demonstrate the anti-*Leishmania* chagasi activity of 18-MC, confirming its previously shown activity againts *L. amazonensis* and stimulating further studies for drug development for *Leishmania*sis treatment. **Supported by:**:MackPesquisa (Fundo Mackenzie de Pesquisa), CNPq, Hebron Farmacêutica, Savant HWP

QT036 - COMPARISON OF OLEANOLIC ACID (OA) ACTIVITY ON DIFFERENTS *LEISHMANIA* SPECIES.

MELO, T.S.[*1]; BALLOUK, L.M.[1]; ALMEIDA, R.L.[1]; FERNANDES, M.C.[2]; DELORENZI, J.C.[1]
1.UPM, SAO PAULO, SP, BRASIL; 2.UFRJ, RIO DE JANEIRO, RJ, BRASIL.
e-mail:tahirasmelo@hotmail.com

*Leishmania*sis is caused by protozoan parasites of the genus *Leishmania*, that affects more than 12 million people in 88 countries worldwide and has been estimated at more than 2 million new cases each year. For over eighty years, the treatment for *Leishmania*sis has been performed with pentavalent antimonials, which still the first choice treatment drugs and amphotericin B, the second choice. In general, these drugs are toxic, expensive and require a long treatment time, have adverse effects in the renal, cardiac and hepatic system. All these problems, together with the lack of a safe and effective vaccine emphasize the importance of developing new drugs against *Leishmania*sis. The Oleanolic Acid (OA) is one of two major metabolites of Eugenia caryophyllata. The aim of this study was to evaluate the leishmanicidal activity of OA against promastigotes and amastigotes of *Leishmania amazonensis*, *Leishmania* brasiliensis and *Leishmania* chagasi. Our results showed an inhibition of promastigote growth of 17.3, 70 and 71% after 24 hours when treated with 50µg/mL after for *L. amazonensis*, L. brasiliensis and L. chagasi, respectively. The IC50 (Inhibitory Concentration of 50%) for promastigotes were 15.7, 13.5 and 28.7 µg/ mL for *L. amazonensis*, L. brasiliensis and L. chagasi, respectively. On amastigotes we observed a reduction of 29, 46 and 58% in *L. amazonensis*-infected macrophages was observed when treated with 10, 20 and 40 µg/ mL, while a reduction of 19, 28.6 and 42% in L. brasiliensis-infected macrophages and a reduction of 22.5, 31 and 45.7% in L. chagasi-infected macrophages were observed at the same conditions. These results suggest a different sensibility on promastigotes and amastigotes of the three species treated. This could be due the biochemical differences of both forms of parasites and among the species tests. our results indicate that OA is a good candidate for future studies in the treatment of *Leishmania*sis.

QT037 - Antitrypanosomal activity of Fexinidazole metabolites, a new oral Nitroimidazole with potential for Chagas disease chemotherapy

MAZZETI, A.L.*; ARMACHE, N.N.; MARQUES, L.F.R.; NASCIMENTO, Á.F.S.; GONÇALVES, K.R.; MOTA, L.W.R.; DINIZ, L.F.; CALDAS, I.S.; DA SILVA PEDROSA, A.T.; BAHIA, M.T.
UFOP, OURO PRETO, MG, BRASIL. e-mail:anamazzeti@yahoo.com.br

Fexinidazole is a 5-nitroimidazole drug currently in clinical development for the treatment of human African Trypanosomiasis. The compound has been shown ability to cure both acute and chronic Chagas disease models in mouse. Fexinidazole acts as a pro-drug, which is oxidized in vivo to more therapeutically relevant species: sulfoxide and sulfone metabolites. In this study, we investigated the in vivo activity of Fexinidazole metabolites against the protozoan parasite *Trypanosoma cruzi*, the causative agent of Chagas disease, using mice as hosts. Female Swiss mice, 18-22g, (n=10), were infected with blood trypomastigotes of Y strain. Oral treatment of infected mice was administered at the detection of parasitemia, which occurs at the 4thday post-inoculation, at the doses of 10, 25, 50 and 100mg/kg/day (mpk) for 20 consecutive days. The results were compared to those achieved with the Bz-treatment using a standardized therapeutic scheme of 100mpk. A group of infected animals but receiving no treatment was used as control. Parasitological cure was determined based on two independent tests: fresh blood examination during and up to 60 days post-treatment followed by blood real-time PCR assay (30 and 180 days post-treatment). Fexinidazole metabolites showed a dose-dependent efficacy in the *T.cruz*i mouse infection. Oral treatment with both metabolites reduced the number of circulating parasites and protected against mortality compared with untreated mice, but without providing a parasitologic cure at the doses of 10 and 25mpk. Treatments at the doses of 50 or 100mpk of SFX or SFN provide 100% protection against mice mortality. Additionally both metabolites were effective in curing experimental Chagas disease in mouse at the doses of 50mpk (30 to 40%) and 100mpk (100%). The in vivo antitrypanosomal activity of sulfoxide and sulfone fexinidazole metabolites provide evidence that these compounds have the potential to be an effective oral treatment for human Chagas disease. **Supported by:**:DNDi, FAPEMIG, CNPq, Capes and UFOP

QT038 - Evaluation of the in vitro and in vivo anti*Leishmania*l activity of the novel aza-pterocarpan LQB-223

PINHEIRO, L.S.*[1]; CUNHA-JUNIOR, E.F.[1]; REMPEL, S.S.[1]; CANTO-CAVALHEIRO, M.M.[1]; BUARQUE, C.D.[2]; COSTA, P.R.R.[3]; TORRES-SANTOS, E.C.[1]
1.IOC, RIO DE JANEIRO, RJ, BRASIL; 2.UFRJ, PUC, RIO DE JANEIRO, RJ, BRASIL; 3.UFRJ, RIO DE JANEIRO, RJ, BRASIL. e-mail:liliane.senapinheiro@yahoo.com.br

Recently, the anti*Leishmania*l, antimalarial and antineoplasic activities of pterocarpanquinones have been demonstrated. These compound are designed by molecular hybridization of a naphtoquinone core with a pterocarpan moiety. Moreover, the antileukaemic activity of pterocarpan derivatives has been reported. In the present work, our purpose is to evaluate the *in vitro* and *in vivo* anti*Leishmania*l properties of a *N*-tosyl aza-pterocarpan (named LQB-223). *Leishmania amazonensis* promastigotes were incubated with LQB-223 and the parasite growth was evaluated after 72 h using MTT. LQB-223 exhibited strong antipromastigote activity (IC_{50} = 4.85µM). For intracellular amastigote assay, *L. amazonensis*-infected murine macrophages were treated with LQB-223 and after 72 h the cells were stained and counted by light microscopy. LQB-223 also was active against amastigote forms (IC_{50} = 2.15µM). To evaluate the toxicity of LQB-223, cell line macrophages J774 were incubated with the compound for 72 h and the effect on macrophage viability was quantified using MTT. LQB-223 showed low cytotoxicity (IC_{50} = 23 µM) and good selectivity index (10.7). For *in vivo* studies, LQB-223 was administered intraperitoneally or orally (8.2 mg LQB-223/Kg/day) five times a week to *L. amazonensis*-infected BALB/c mice throughout all experimental period lasting 45 days. Lesion development was measured with a dial caliper and the toxicity of the treatment was evaluated by body mass variation and serological analysis of renal and hepatic functions. At the end of the experiment, LQB-223 administered orally were able to inhibit the lesion development by 9,7% without altering body mass and serological markers of toxicity. LQB-223 administered intraperitoneally inhibited 8,9% but slightly impaired the hepatic function. Altogether, these results indicate that LQB-223 could be a potential prototype to development of new anti*Leishmania*l drugs and further experiments with LQB-223 administered orally will be done. **Supported by:**:INSTITUTO OSWALDO CRUZ (IOC)

QT039 - Activity of extracts of Kalanchoe pinnata and its clones in vitro against *Leishmania* braziliensis

*BRITO, A.C.S.[1]; SANTOS, R.F.[1]; COSTA, S.S.[2]; SILVA, S.A.G.[1].
1.UERJ, RIO DE JANEIRO - RJ - BRASIL; 2.UFRJ, RIO DE JANEIRO - RJ - BRASIL.
e-mail:carolinne_brito@hotmail.com

Plants have a rich variety of bioactive molecules and can be a source of new drugs. The aim of this study was to evaluate the effect of *Kalanchoe pinnata* (Kp) *in vitro* against *Leishmania braziliensis*, the most important dermatropic *Leishmania* species on Brazil. We tested Kp extracts cultivated the habitat, under blue, white and ultraviolet light. Promastigotes of *L. braziliensis* were grown with 0-500µg/ml of extracts for 96h/28°C. Parasites were count daily using a Neubauer chamber. For tests with amastigotes, monolayers of peritoneal macrophages of SW mice were infect with promastigotes of *L. braziliensis* (at a ratio of 1 parasite/macrophages) and incubated with 0-500µg/ml of extracts for 48 and 96h/37°C/5%CO2. In promastigotes Kp extract cultivated under blue light inhibited 27,4%; Kp cultivated in habitat inhibited 17,35%; Kp cultivated under white light inhibited 1,66%; Kp cultivated under ultraviolet light didn't have effect. In amastigotes incubated for 48h Kp cultivated in habitat inhibited 39,5% ($p<0,05$); Kp cultivated under white light inhibited 38,5% ($p<0,05$); Kp cultivated under blue light inhibited 24,8%; Kp cultivated under ultraviolet light inhibited 38,4%. In amastigotes incubated for 96h Kp cultivated under white light inhibited 56,4% ($p<0,05$); Kp cultivated habitat inhibited 50% ($p<0,05$); Kp cultivated under blue light inhibited 49,7% ($p<0,05$); Kp cultivated under ultraviolet light inhibited 30,5%. The extracts weren't able to stimulate nitric oxide production by macrophages, suggesting a direct action on the parasite. Extracts wasn't toxic in macrophage. These data demonstrate that the extract of Kp (blue) is the most active on promastigotes of parasites and Kp extract (white) is the most active on amastigotes. Changes in growth conditions seem to have diminished Kp activity on parasites, especially ultraviolet light. The production of cytokines by macrophages and also the death of the parasite through apoptosis are being invest

Supported by::CNPq/ FAPERJ

QT040 - Myocardial scars correlates with eletrocardiographic changes in dogs treated with Benznidazole in a *Trypanosoma cruzi* strain dependent manner.

NASCIMENTO, Á.F.S.[*1]; PEREIRA, D.E.[1]; DINIZ, L.F.[1]; GUEDES, P.M.M.[2]; MARTINS, T.A.F.[3]; LIMA, W.G.[1]; TORRES, R.M.[3]; DA SILVA PEDROSA, A.T.[1]; CALDAS, I.S.[1]; BAHIA, M.T.[1]
1.UFOP, OURO PRETO, MG, BRASIL; 2.UFRN, NATAL, RN, BRASIL; 3.UFMG, BELO HORIZONTE, MG, BRASIL.
e-mail:neto1988@gmail.com

The cardiac form of Chagas disease is evidenced by a progressive cardiac inflammation that leads to myocarditis, fibrosis and electrocardiographic (ECG) conduction abnormalities. Considering these characteristics, the aim of this study was prospectively evaluate the early ECG changes during experimental infection of dogs inoculated with Benznidazole (Bz)-susceptibly (Berenice-78) and Bz-resistant (VL-10, and AAS) *T. cruzi* strains and, later, evaluate the efficacy of Bz treatment to prevent these ECG alterations. ECG changes of treated and untreated animals were prospectively evaluated up to 270 days after infection, when collagen (right atrium) quantification was performed. All infected dogs presented high intensity of heart fibrosis and ECG alterations. Therapy with Bz reduced or prevented fibrosis in Bz-susceptible Berenice-78 and Bz-resistant AAS strains coincident with minor ECG alterations at 270 days. However, in those animals infected with a Bz-resistant VL-10 strain, specific treatment did not alter collagen deposition as well first atrioventricular block and chambers overload at 120 and 270 days after infection. These findings indicate that an effective antiparasitic treatment in the early stage of Chagas disease can lead to a significant reduction in the frequency and severity of the parasite-induced cardiac disease, even when parasites are not completely eliminated.

Supported by::CNPq, FAPEMIG e UFOP

QT041 - Evaluation of in vitro activity of new quinone derivatives of lapachol

REMPEL, S.S.[*1]; CUNHA-JUNIOR, E.F.[1]; CANTO-CAVALHEIRO, M.M.[1]; GOMES, S.[2]; SILVA, A.J.M.[2]; COSTA, P.R.R.[2]; TORRES-SANTOS, E.C.[1]
1.FIOCRUZ, RIO DE JANEIRO, RJ, BRASIL; 2.UFRJ, RIO DE JANEIRO, RJ, BRASIL.
e-mail:ste_soares_rempel@hotmail.com

Considered by World Health Organization (WHO) a neglected disease, *Leishmania*sis affects over 12 million people in the world per year. Treatment receives low investment from the pharmaceutical industries, requires long periods of administration and presents low efficacy, resistance, toxicity and high cost. Quinones represent a very important class of molecules which is directly related to many metabolic processes in the cell and presents trypanosomicidal effect. The purpose of this study is to evaluate the activity of 12 derivatives from lapachol (LQBs) in promastigotes and intracellular amastigotes of *L. amazonensis* and their toxicity in murine macrophages. Promastigotes (1x106/ml) were incubated with different concentrations of LQBs or none by 72 h and the parasite viability was analyzed by MTT assay. The activity on intracellular amastigotes was evaluated by light microscopy in *L. amazonensis*-infected murine macrophages after incubation with several concentrations of LQBs for 72 h. Toxicity was evaluated in murine macrophages by MTT assay after 72 h of incubation. The differences on the structure of each substance were related to some aspects of the drug action to the cells. The closing of the ring in "3" rendered low toxicity and antiamastigote activities, while the addition of a diethylformamide in "2" gives higher activity to intracellular amastigotes but also higher toxicity. On the other hand, when both modifications took place in the same compound, a series with selective antiamastigote activity was originated, from which LQB-270 presented the lowest IC50 (1.5 µM) on intracellular amastigotes and selectivity index higher than 10. This structure-activity study pointed LQB-270 as the most promising candidate to be evaluated in experimental cutaneous *Leishmania*sis. **Supported by:**:CNPq, FAPERJ, PAPES

QT042 - Leishmanicidal activity of extracts from Calophyllum brasiliense (Clusiaceae) leaves

VASCONCELOS, M.F.[*1]; BRENZAN, M.A.[2]; CORTEZ, D.A.G.[2]; CANTO-CAVALHEIRO, M.M.[1]; TORRES-SANTOS, E.C.[1]
1.INSTITUTO OSWALDO CRUZ, RIO DE JANEIRO, RJ, BRASIL; 2.UEM, MARINGÁ, PR, BRASIL.
e-mail:marielavasconcelos@hotmail.com

*Leishmania*sis is a group of neglected diseases that affect approximately 12 million people in 98 countries. The drugs used to control the disease are associated with low efficacy, high toxicity, difficulties of administration, high costs and increasing resistance. The search for new alternatives of treatment is a priority. Calophyllum brasiliense (Clusiaceae), popularly known as “guanadi-cedro” and “pau-de-azeite”, is a large tree that grows mainly in the Brazilian Atlantic Rainforest. Xanthones, sitosterols, terpenes, cumarines, flavonoids and triterpenes with analgesic antimicrobial, antiprotozoan and antiviral activities have already been isolated from C. brasiliense. This study aimed to evaluate the leishmanicidal activity of C. brasiliense. The leaves were collected, dried, macerated and subjected to different extraction procedures. *Leishmania amazonensis* promastigotes were cultured in the presence of several concentrations of the extracts up to 100 µg/mL for 72 hours and quantified colorimetrically by MTT assay. Under these conditions, 8 extracts were active, with IC50 less than 20 µg/mL. The activity was also evaluated in intracellular amastigotes. The infectivity index was determined by light microscopy and the extracts showed a significant antiamastigote activity, with IC50 less than 8 µg/mL. The hexane extract and the dichloromethane extract, both Soxhlet extracted, showed IC50 of 3.3 µg/mL, approximately. To evaluate the toxicity, macrophages were incubated with the extracts for 72 hours. The effect on the viability of the macrophages was quantified by MTT and the LD50 values were more than 80 µg/mL, indicating a selectivity index more than 20-fold. In addition to above results, further procedures will be made to elucidate the structures of the compounds involved. Thus, C. brasiliense, extremely abundant in Brazil, has a therapeutic potential and may become a new phytotherapeutic anti*Leishmania*l agent. **Supported by:**:CAPES, FIOCRUZ

QT043 - Anti*Leishmania*l activity and evaluation of the mechanism of action of new tetrazole derivatives

FAIÕES, V.S.[*1]; DOS SANTOS, M.S.[2]; BERNARDINO, A.M.R.[3]; CUNHA-JUNIOR, E.F.[1]; ANDRADE-NETO, V.V.[1]; ALMEIDA-AMARAL, E.E.[1]; LEON, L.L.[1]; CANTO-CAVALHEIRO, M.M.[1]; TORRES-SANTOS, E.C.[1]

1.FIOCRUZ, RIO DE JANEIRO, RJ, BRASIL; 2.UNIFEI, ITAJUBÁ, MG, BRASIL; 3.UFF, RIO DE JANEIRO, RJ, BRASIL.

e-mail:vsfaioes@ioc.fiocruz.br

*Leishmania*sis are diseases caused by protozoan parasites of the genus *Leishmania*, ranging from mild skin infections until the disfiguring mucocutaneous and the potentially fatal visceral form, constituting a serious public health problem. The drugs currently used are toxic and often ineffective. Thus, a rational search for new therapies is necessary. In this study we evaluated the anti-*Leishmania amazonensis* activity of nine tetrazole derivatives. The compound 5 - [5-amino-1-(4'-methoxyphenyl)-1H-pyrazol-4-yl]-1H-tetrazole (MSN20) presented IC50 of 13.5 µM on intracellular amastigotes and low toxicity to murine peritoneal macrophages (LD50 > 250µM), indicating selective activity. Quantification of nitrite in the supernatant of infected macrophages treated with MSN20 revealed that this substance has not been able to increase nitric oxide production, suggesting that this prototype does not modulate the response of the host cell, acting selectively on the parasite. As azole antifungal agents are well known inhibitors of the sterol biosynthesis, the sterol composition of promastigotes treated with MSN20 was analyzed by thin layer chromatography (TLC). No changes were observed in the sterol profile, even at concentrations above the IC50. Several tetrazoles are involved in redox reactions in mitochondria. Thus, oxidative metabolism was also evaluated in treated parasites. The prototype did not significantly alter the production of reactive oxygen species (ROS) or mitochondrial membrane potential (ΔΨm) in promastigotes of *L. amazonensis*. Thus, the present results indicate MSN20 as a new seletive anti*Leishmania*l compound, and the study of other parasite targets can reveal its mechanism of action. **Supported by:**:CNPq

QT044 - Modulation of cellular growth and protein expression in *Leishmania* (Viannia) braziliensis promastigotes by depletion of iron

RODRIGUES, C.M.[*1]; MENNA BARRETO, R.F.S.[1]; SABÓIA VAHIA, L.[1]; DE SOUZA, E.M.[1]; WAGHABI, M.C.[1]; CUERVO, P.[1]; DE JESUS, J.B.[2]

1.FIOCRUZ, RIO DE JANEIRO, RJ, BRASIL; 2.UFSJ, MINAS GERAIS, MG, BRASIL.

e-mail:camilarodrigues87@yahoo.com.br

Iron is an essential element for the *in vitro* and *in vivo* survival of microorganisms, acting as a cofactor of several enzymes and playing a critical role in host-parasite relationship. *Leishmania (Viannia) braziliensis* is a parasite widespread in the new world and considered to be the major etiological agent of American Tegumentary *Leishmania*sis. In this study, incubation of promastigote forms of *L. (V.) braziliensis* with the iron chelator 2,2-dipyridyl affected proliferation in a dose and time-dependent manner. In subsequent cytotoxicity assays, the ratio between the fluorescence emitted by the reduction of resazurin and the concentration of the iron chelator was also dose-dependent. The IC(50) values obtained after 24 and 48 hours of treatment were, respectively, 100 and 82,3 µM. Ultrastructural analysis of the treated promastigotes revealed a remarkable mitochondrial swelling with loss of cristae and matrix and presence of concentric membranar structures inside the organelle. The treatment also induced Golgi disruption and intense cytoplasmic vacuolization. Fluorescence-activated cell sorting analysis of tetramethylrhodamine stained parasites showed that 2,2-dipyridyl collapsed the mitochondrial membrane potential. However, incubation of parasites with propidium iodide demonstrated that this collapse was not associated with plasma membrane permeabilization. TUNEL assays indicated no DNA fragmentation in the chelator-treated promastigotes. Finally, two dimensional electrophoresis of parasites showed that proteins involved with metabolism of nucleic acids and coordination of post-translational modifications suffered up or down-regulation after treatment with the chelator. Thus, our results show that iron depletion affects growth, ultrastructure and protein expression of *L. (V.) braziliensis* and suggest that induction of cell death does not occur by classical apoptosis. **Supported by:**:CAPES

QT045 - Benznidazole decreases cytokine expression of inflammation cell in dogs treated and cured in acute phase of experimental Chagas disease

DUZ, A.L.C.[*1]; VIEIRA, P.M.A.[1]; GUEDES, P.M.M.[2]; VELOSO, V.M.[1]; CARDOSO, J.M.O.[1]; OLIVERI, L.M.R.[1]; DE LANA, M.[1]; TAFURI, W.L.[1]; BAHIA, M.T.[1]; CARNEIRO, C.M.[1]
1.UFOP, OURO PRETO, MG, BRASIL; 2.UFRN, NATAL, RN, BRASIL.
e-mail:anacd07@yahoo.com.br

Benznidazole (Bz) is the only drug available for Chagas disease treatment in Brazil. Despite the side effects, it is still indicated in the treatment of chagasic patients due its effectiveness during the acute phase of infection. In this study, we evaluated during the chronic phase the cytokines expressed by inflammatory cells in the heart of dogs infected with strains susceptible or partially resistant to treatment with Bz, classified in mice experiments. To this, 18 dogs were infected with 2000 blood trypomastigotes/kg of body weight of Be-78 (n=11) or Y (n=7) strains, and treated with 7 mg of Bz/kg, divided into two daily doses during 45 days. The oral treatment was started immediately after the appearance of parasitemia. The cure was evaluated by PCR, hemoculture and serological tests. The negativation of these tests was observed in 85% and 100% of animals infected with Be-78 or Y strain, respectively. Six months after treatment, in the chronic phase, the groups untreated, treated cured or treated not cured were euthanized and the right atrium and interventricular septum were collected. After this, these fragments were routinely processed for obtaining serial sections that were immunohistochemical staining for cytokines (IFN-γ, TNF-α, IL-10 and IL-4) for quantification of inflammatory cells expressing each of these cytokines. We observed that the right atrium of the dogs infected with Be-78 strain treated/cured (n=6/11) exhibited a significant reduction of the expression of IFN-γ, TNF-α, IL-4 and IL-10 in relation to the infected/untreated (n=4/11) group, otherwise the animals treated/not-cured showed a profile similar to untreated (n=1/11) group. All dogs infected with the Y strain treated/cured (n=4/7) exhibited a significant reduction of IL-10 expression in right atrium in relation to the infected/untreated (n=3/7). Therefore, when the Bz treatment promotes healing, the cardiac damage caused by inflammation is minimized. Suported by CNPq, CAPES and FAPEMIG. **Supported by:**:CNPq, CAPES and FAPEMIG

QT046 - TC95, an efficient hybrid molecule on *Leishmania amazonensis* in vitro treatment

GODINHO, J.L.P.[*1]; GEORGIKOPOULOU, K.[2]; CALOGEROPOULOU, T.[2]; DE SOUZA, W.[1]; RODRIGUES, J.C.F.[1]
1.UFRJ, RIO DE JANEIRO, RJ, BRASIL; 2.NATIONAL HELLENIC RESEARCH FOUNDATION, INSTITUTE OF ORGANIC AND PHARMACEUTICAL CHEMISTRY, ATHENS, GRÉCIA.
e-mail:joseanegodinho@yahoo.com.br

*Leishmania*sis are a parasitosis caused by organisms of the *Leishmania* genus which are associated with significant rates of morbidity and mortality throughout the world. Nowadays, the current chemotherapy is based in antimonials, amphotericin B and pentamidine. In India, Germany and Colombia, miltefosine is the main choice to treatment. However, there is an urgent need for safer and more efficacious drugs with new parasite targets. An interesting approach in drug development is the combination of different inhibitors with known cell effects on parasite forms. Both, trifluralin, a dinitroaniline herbicide, and miltefosine have activity against protozoan parasites, but with high IC50 and high cytotoxicity for host cells. In this work, we investigate the cellular alterations caused by of TC95 treatment, a novel compound that combines trifluralin and miltefosine molecules , against *Leishmania amazonensis* promastigotes during 12 h of treatment. After treatment, the viability of promastigotes tretated with 2,3,4,5,8 and 10 µM of TC95 were intensily reduced. When observed by electron microscopy, cells displayed profound shape alterations appearing rounded and swollen. The mitochondrion is the main organelle affected, presenting an intense swelling with loss of the matrix content and disorganization of the mitochondrial membranes. Other cellular components were affected such as, Golgi complex, and nuclear chromatin. Lipid bodies increased in number and structures typically found in autophagic process have appeared. Taken together, these results indicate that TC95 affect essential organelles which are important targets during the treatment with new and promising compounds against *Leishmania* sp. **Supported by:**:CNPq, FAPERJ, CAPES and PRONEX.

QT047 - Investigation of the effect of azido and triazole chalcones in *Trypanosoma cruzi*

FERREIRA, J.C.[*1]; DA SILVA, G.D.[2]; BARBOSA, L.A.[2]; VILLAR, J.A.F.P.[2]; GARZONI, L.R.[3]; MOREIRA, O.C.[4]

1.IFRJ, RIO DE JANEIRO, RJ, BRASIL; 2.UFSJ, DIVINÓPOLIS, MG, BRASIL; 3.FIOCRUZ, RIO DE JANEIRO, RJ, BRASIL; 4.FIOCRUZ/ IFRJ, RIO DE JANEIRO, RJ, BRASIL.
e-mail:otacilio@ioc.fiocruz.br

Chagas Disease, which etiologic agent is the flagellate protozoan *Trypanosoma cruzi*, represents a major public health issue, affecting millions of people in Latin America. The treatment currently relies on the use of nifurtimox and benzonidazole, which present several side effects. Moreover, the effectiveness is confirmed only in the acute phase of the disease. The chalcones are defined as a class of ketone α, β-unsaturated (–CO-CH=CH-) presenting aromatic rings at their ends, and acting as biosynthetic precursors to a variety of natural products. Because of this, there are several pharmacological studies to investigate the properties of these compounds as antifungal, antitumor, anti-malaria, anti-*Leishmania*, anti-*Trypanosoma cruzi*, anti-HIV, among others. In this study, we investigated the cytotoxic effect of 20 azido or triazole chalcones in *Trypanosoma cruzi*. The experiments were performed incubating $1x10^7$ epimastigotes/mL (Y strain) in BHI medium supplemented with 10% Fetal Bovine Serum, at 28°C, in a total volume of 1 mL. The cell number was monitored by counting in a Neubauer chamber, every 24 hours for 6 days. All chalcones were freshly dissolved in DMSO and added to the cultivation medium before the cells. In parallel, a control sample with the same DMSO concentration (1% v/v), but in the absence of chalcones, was performed in all experiments. A screening of the cytotoxic effects was performed in the presence of the chalcones, at the concentration of 10 µM or 100 µM. We observed that 14 compounds showed a statistical significant cytotoxic effect to *T. cruzi* at 100 µM. The three chalcones that presented higher effects, C3NAG, CPBRTG and CBTG, were selected to investigate the IC50 values. At 72 hours of cultivation, the IC50 were, respectively, 32,96±14,92, 34,16±5,74 and 14,24±6,99 µM. So far, these chalcones have presented a potential to be further evaluated as candidate for Chagas disease chemotherapy. **Supported by:**:CNPq/ FAPERJ/ FIOCRUZ

QT048 - ANTIMALARIAL ACTIVITY OF RESVERATROL ANALOGS AND DERIVATIVE METAL COMPLEXES

LOPES, F.V.[*]; SOARES, R.R.; SILVA, D.A.C.; DOMINGOS, M.O.; DA SILVA, A.D.; ABRAMO, C.
UFJF, JUIZ DE FORA, MG, BRASIL.
e-mail:fernandavaleriolopes@hotmail.com

Introduction: Malaria is the most important parasitic disease of humans and is responsible for significant morbidity and mortality in many parts of the world. The situation is aggravated by the spread of drug-resistant parasite strains specifically *Plasmodium falciparum.* Parasite erythrocyte invasion triggers an oxidative stress in infected cells, causing increased levels of intracellular Ca^{2+} and a change in plasma membrane chemical composition and phosphatidylserine exposure, allowing its recognition by macrophages, which remove these cells, further aggravating the anemia that occurs during plasmodial infection. Resveratrol is a polyphenol with several activities such as antitumor, anti-inflammatory and inhibits eryptosis causing anemia reduction. The aim of this study is to evaluate the antimalarial activity of resveratrol analog and their copper compound. Material and Methods: Antimalarial activity was evaluated *in vivo* in a murine model infected with *P. berghei* strain NK65 using the 4-day suppressive test. Both compounds, p-carboxi-o-hidroxi-N-(o'-hidroxibenzilideno) *(1)* and $[Cu(C_{14}H_{11}NO_4)_2(H_2O)_2]$ *(2)*, were tested in a dose of 10mg/Kg. Giemsa stained blood smears were made on days 5, 7, 9 and 12 after inoculation. The antimalarial activity was expressed as percentage inhibition of the parasite multiplication. Results: The compound *(1)* showed enhanced activity at 9 day post-infection, with 68% suppression, similar to the result observed in positive control chloroquine (70%). The compounds *(2)* showed increased activity at days 9 (50%) and 12 (71%) post-infection, over again similar to positive control chloroquine (70% and 68%, respectively). The average survival of mice treated with compounds (1) and (2) was 18 ± 2 and 20 ± 4 days post-infection, respectively. This result was considered similar to chloroquine (20 ± 8). Conclusion: Given its suppression values, these compounds are promising antimalarials and therefore may be objects of future investigations.

QT049 - ANTIMALARIAL ACTIVITY OF RESVERATROL ANALOGS COMPLEXED WITH COBALT, NICKEL AND ZINC

LOPES, F.V.*; SOARES, R.R.; SILVA, D.A.C.; DOMINGOS, M.O.; DA SILVA, A.D.; ABRAMO, C.
UFJF, JUIZ DE FORA, MG, BRASIL.
e-mail:fernandavaleriolopes@hotmail.com

Introduction: Malaria has remained one of the most devastating diseases in tropical and subtropical regions of the world despite the global fight against the disease. Among the symptoms of malaria is anemia, which occurs in the later stages of the disease. The anemia is associated with more serious cause of malaria, which contributes to increased mortality and morbidity. Resveratrol is a polyphenol with several activities such as antitumor, anti-inflammatory and inhibits eryptosis causing anemia reduction. Metal components have been shown to be suitable for the treatment of parasitic diseases due to the action on the parasite biomolecules, thus interfering in its metabolism. This study evaluated the antimalarial activity of resveratrol analogs complexed with cobalt, nickel and zinc. Material and Methods: Antimalarial activity was evaluated *in vivo* in a murine model infected with *Plasmodium berghei* strain NK65, using the 4-day suppressive test. The compounds, $[Co(C_{14}H_{11}NO_4)_2(H_2O)_4]_{1/2}(H_2O)$ *(1)*, $[Zn(C_{14}H_{11}NO_4)_2(H_2O)]$ *(2)*, and $[Ni(C_{14}H_{11}NO_4)_2(H_2O)_2]$ *(3)*, were tested in a dose of 10mg/Kg. Giemsa stained blood smears were made on days 5, 7, 9 and 12 after inoculation. The antimalarial activity was expressed as percentage inhibition of the parasite multiplication. Results: The compound *(1)* and *(3)* showed the highest activity on day 7 post-infection, with 56% and 71% of parasitemia suppression, respectively, similar to positive control chloroquine (68%). On day 9 post-infection, the compound *(2)* showed suppression of 61%, similar to positive control chloroquine (70%). The average survival of mice treated with compounds (1), (2) and (3) was 20 ± 2, 18 ± 2 and 20 ± 6 days post-infection, respectively. This result was considered similar to chloroquine (20 ± 8). Conclusion: The results of this study suggest that the compounds complexed with metals like cobalt, nickel and zinc may have considerable antimalarial activity and therefore may be objects of future investigations.

QT050 - Herbal extracts: alternative approaches to control *Trypanosoma cruzi* intracellular multiplication

TEIXEIRA, T.L.*; SILVA, C.V.; SOUZA, M.A.; TEIXEIRA, S.C.
UFU, UBERLÂNDIA, MG, BRASIL.
e-mail:thaiselara@yahoo.com.br

Chagas disease, or American trypanosomiasis, is caused by the kinetoplastid protozoan parasite *Trypanosoma cruzi*. According to the World Health Organization 7.7 to 10 million people are chronically infected with *T. cruzi*, and 10,000 to 14,000 deaths per year. The chemotherapy used in treatment of Chagas disease is considered ineffective and has serious side effects. In previous studies we observed some effect of extracts derived from plants belonging to the genus *Ruta*, *Ageratum* and *Tabebuia* on *T. cruzi* tripomastigotes in axenic parasite culture. However, we had not observed the effect of these extracts during amastigote forms multiplication inside mammalian cells. Thus, the aim of this study was to determine the effect of plant extracts from *Ruta graveolens*, *Tabebuia impetiginosa* and *Ageratum conyzoides* on amastigotes intracellular multiplication . Methods: peritoneal macrophages (BALB/c) were plated in 24 well plates, after 24 hours parasites were added to invade cells during five hours. Then, cells were treated with extracts in predetermined concentrations. After 72 hours, coverslips were fixed and observed under fluorescence microscope after cell staining with polyclonal antibody against the parasite. Results: Two hundred infected cells were counted and the number of amastigotes in each infected cell was determined. A significant decrease ($P<0.05$) in the number of amastigotes in cells treated with the extracts was observed compared to control cells. Treatment with extracts from *A. conyzoides*, *T. impetiginosa* and *R. graveolens* generated a reduction in the number of amastigotes up to 40%, 50% and 50% respectively. Conclusion: The plant extracts showed toxicity against *T. cruzi* amastigotes and impared parasite intracellular multiplication. **Supported by:**:CNPq / FAPEMIG

QT051 - In vivo activity of E1224, a pro-drug of Ravuconazole, in acute murine model of *Trypanosoma cruzi* infection

DINIZ, L.F.[*]; FERREIRA, H.C.; MOTA, L.W.R.; MARQUES, L.F.R.; ARMACHE, N.N.; CALDAS, I.S.; MAZZETI, A.L.; NASCIMENTO, Á.F.S.; DA SILVA PEDROSA, A.T.; BAHIA, M.T.
UFOP, OURO PRETO, MG, BRASIL.
e-mail:liviafcd@yahoo.com.br

Ravuconazole is a potent and specific anti-*T.cruzi* agent *in vitro* but its *in vivo* activity in mice and dog experimental models is limited, probably due the unfavorable pharmacokinetics properties in these models. The E1224 is a water soluble pro-drug which converts to Ravuconazole leading to the drug's improved absorption and bioavailability. The current study evaluates the *in vivo* anti-*T. cruzi* activity of E1224 using mice as hosts. Female Swiss mice, 18-22g, (7/group), were infected with blood trypomastigotes of Y strain. Oral treatment of infected animals was administered at the detection of parasitemia (4thday post-inoculation) at the doses of 10, 20, 30, 40 and 50mg of E1224 per kilogram of bodyweight (mpk) for 20 days. The results were compared with those achieved with the Benznidazole-treatment using a standardized therapeutic protocol. A group of infected animals but receiving no treatment was used as control. Parasitological cure was determined based on two independent tests: fresh blood examination during and up to 60 days post-treatment (naturally or induced by Cyclophosphamide immunossupression) followed by blood real-time PCR assay (30 and 180 days post-treatment). Our results demonstrated that E1224 is very effective in suppressing the proliferation of the parasite and preventing the death of infected animals. Treatments at the doses of 10 to 50mpk of E1224 prevented the mortality in all infected mice, while in the control group the mortality was 100% within 18 days. Furthermore, parasitological and PCR assays indicated cure indices of 71.42% to 100% among animals treated with different doses of E1224. Interesting, the treatment with 10mpk induced 100% of cure. In conclusion, our data demonstrated that E1224 is a promising candidate to Chagas disease treatment, which can be used in low doses and not induce detectable adverse reactions. **Supported by:**:DNDi, FAPEMIG, CNPq, Capes and UFOP

QT052 - INHIBITORY ACTIVITY OF ESSENTIAL OILS FROM Burceracea AGAINST *Leishmania amazonensis*

DA SILVA MATEUS, M.H.[*1]; DE FREITAS GARCIA, M.C.[1]; SOUZA, B.M.[1]; SOARES, D.C.[2]; SIANI, A.C.[3]; RAMOS, M.F.S.[3]; SARAIVA, E.M.[2]; PINTO SILVA, L.H.[1]
1.UFRRJ, SEROPÉDICA, RJ, BRASIL; 2.UFRJ, RIO DE JANEIRO, RJ, BRASIL; 3.FOC, RIO DE JANEIRO, RJ, BRASIL. e-mail:michellemedvet@gmail.com

*Leishmania*sis is a neglected tropical disease with high prevalence on the word and represents great challenge for public health. According to WHO, around 600000cases are reported annually. Current treatment rely mainly on antimonials and amphotericin B, unsatisfactory due to their toxicity, high costs, and increasing problems with drug resistance. Different approaches have been applied to identify novel hits against *Leishmania* sp., and one of them is the analysis of naturally occurring plant-derived compounds. In this study, the effects of two essential oils extracted from species of the *Burceracea* family, *Protium altsonii*, identified as oil 7 (O7) and *Protium hebetatum* identified as (O8) on *Leishmania amazonensis in vitro*. These oils are both composed by the same active principles (1,8- cineol, p-cimene and α-pinene) in different ratios. Anti*Leishmania*l activity of O7 and O8 were evaluated on promastigotes culture as well as on amastigotes-infected macrophages. Besides that, we analysed their cytotoxicity for peritoneal macrophages through XTT assay. Results showed that O7 and O8 inhibited promastigotes' growth in a dose-dependent manner. The maximal inhibition observed was 36,75%; 47,97% and 55,5% of promastigotes growth inhibition for 1µg/mL, 10µg/ml and 25µg/mL of O7, respectively, and 41,36%; 62,45% and 71,98% for 0,1µg/mL, 1µg/ml and 10µg/mL of O8. Moreover, both extracts were able to reduce amastigotes' survival inside macrophages in a dose-dependent manner. At 25µg/mL O7 inhibited 61,96% of amastigotes survival, while O8 50µg/mL inhibited around 53,42%. At 25µg/mL, neither O7 nor O8 were cytotoxic to peritoneal macrophages as evaluated by the XTT assay as well as the isolated active principles themselves, after 24h of treatment. Further experiments will be performed in order to iddentify their mechanisms of action. Therefore, results point both oils as effient compounds against *L.amazonensis* and provide new perspectives for *Leishmania*sis' treatment. **Supported by:**:CNPQ

QT053 - Analysis of autohemotherapy as alternative approach for novel parasitic treatment

PAVÃO, B.P.*; DE SOUZA, E.M.; SILVA, C.F.; OLIVEIRA, G.M.; BATISTA, M.M.; BARCINSKI, M.A.; SOEIRO, M.N.C.
FIOCRUZ, RIO DE JANEIRO, RJ, BRASIL.
e-mail:beatriz.pavao@ioc.fiocruz.br

Chagas disease (CD) caused by *Trypanosoma cruzi*, is a serious public health problem in many endemic areas of Latin America affecting more than 10 million people. Current therapy for CD is not satisfactory as exhibits limited efficacy and causes undesirable side effects. Autohemotherapy previously called serum therapy consists in immediate intramuscular or subcutaneous reinjection in the thigh of freshly drawn autologous blood and that has been employed in a wide range of disease conditions. Although all over the world this medical practice has been reported as effective in the elimination of clinical symptoms caused by bacteria, fungi, parasitic and virus infection as well as controlling autoimmune diseases, yet few scientific studies have been done proving its efficiency, and it has been considered as an illegal practice requiring further rigorous pre-clinical and clinical investigations. Presently, our aim is evaluate pre-clinical studies on Swiss Webster mice injected with autologous blood (via intramuscular in right leg) exploring also its potential effect on in vitro T.cruzi infection of peritoneal macrophages obtained from treated mice. Mice groups (male, n=6) consisted of those animals: (i) that did not received any treatment (control group), (ii) that were only drawn, (iii) received 10µl of autologous blood, (iv) received 20µl of autologous blood and (v) received 20µl of NaCl. Our preliminary data showed that all groups presented similar values for body weight follow up and animal behavior tests. Hematological analysis also showed similar values for all analysis except for leukocytes and platelets that presented reduced levels in mice group that received 20µl autologous blood. Preliminary analysis suggests a slight decrease in in vitro infection of macrophages collected from blood-treated animals as compared to those obtained from untreated mice. In vitro and in vivo studies are underway aiming to further explore biological aspects of this therapy. **Supported by:**:FIOCRUZ

QT054 - Biological Effect of aromatic diamidines against bloodstream trypomastigotes of *Trypanosoma cruzi* in vitro

SILVA, F.H.G.*[1]; BATISTA, M.M.[1]; BATISTA, D.G.J.[1]; PAVÃO, B.P.[1]; PATRICK,[2]; BAKUNOV, S.A.[2]; BAKUNOVA, S.M.[2]; TIDWELL, R.[2]; SOEIRO, M.N.C.[1]
1.IOC/FIOCRUZ, RIO DE JANEIRO, RJ, BRASIL; 2.UNIVERSITY OF NORTH CAROLINA, CHAPEL HILL, ESTADOS UNIDOS.
e-mail:hildemagnaguedes@hotmail.com

Chagas disease (CD) caused by the obligatory intracellular protozoan *Trypanosoma cruzi* is a relevant public health problem in Latin America where about 10 million people are infected. This neglected disease also affects Europe, North America and Asia due to migration of infected people. The current therapy performed by two nitroderivatives (benznidazole and Nifurtimox), introduced in clinical used more than 04 decades ago, has serious limitations such as adverse effects, long-term treatment, natural resistance of some parasite strains, and low effect in later chronic phase of the disease. Classical aromatic amidines like pentamidine and furamidine represent an important class of DNA ligands presenting high anti-parasitic activity against many pathogens, including *Trypanosoma cruzi*. This study focused on the *in vitro* activity of five aromatic amidine compounds - 19SAB003, 19SAB005, 23SMB046, 27SMB005 and 28SMB008 against bloodstream trypomastigote forms of *T. cruzi* (Y strain) incubated at 37ºC for 24 hours. Our preliminary data show that all tested compounds showed IC_{50} >32 µM, being less effective as compared to Bz (13±2 µM). Our results suggest stimulate further investigations of this class of compounds for the rational design of new chemotherapy agents for Chagas disease. **Supported by:**:Capes; Cnpq; Faperj; Fiocruz

QT055 - Efficacy of a sesquiterpene lactone in controlled releasing systems for experimental Chagas disease

ALMEIDA, T.C.*; BRANQUINHO, R.T.; SILVA, M.R.S.; MOSQUEIRA, V.C.F.; SAÚDE-GUIMARÃES, D.A.; E SILVA, J.C.V.O.; DE LANA, M.
UFOP, OURO PRETO, MG, BRASIL.
e-mail:tamires-cunha@hotmail.com

RM is a sesquiterpene lactone in vitro active against *T. cruzi* isolated from *Lychnophoras*(Arnica). The development of phytochemicals in nanostructured(NS) formulations enables to increase bioavailability of substances,improve pharmacokinetics and reduce the toxicity.The only drug available to treat Chagas disease is benznidazole(BZ),therapeutically limited.This work evaluates the activity of RM compared to BZ on the treatment of the acute phase of the infection with the *T.cruzi* CL strain in murine model.The *L. trichocarpha* was collected at Ouro Preto,MG.RM was isolated and physic-chemical characterized by Branquinho(2010),as well as its NS formulation produced with a conventional polymer.Swiss female mice weighting 20g were IP inoculated with 10000 blood tripomastigotes of the CL strain.They were divided in groups:NS-RM(2mg/Kg/day),free RM(2mg/Kg/day), BZ(50mg/Kg/day) and negative controls.The intravenous treatment was carried out in 2 programs:1st starting 24h post-infection for 10 days,2nd starting on the 7thday post-infection for 20 days.Parasitemia was evaluated daily by fresh blood exam for 30 days and survival was registered.Parasitological and serological tests were applied to evaluate treatment efficacy.Subpatent parasitemia was registered in:all the mice treated with NS-RM and BZ(2nd program);62.5% of the mice treated with NS-RM and all treated with BZ(1st program);12.5% of the mice treated with free RM.The negative controls survived for 20 days.All animals treated with NS-RM and BZ,75%(1st program) and 50%(2nd program) of the ones treated with free RM survived the acute phase.They were necropsied 6 months after for histological evaluation.Cured animals:all treated with NS-RM and BZ(2nd program);62.5% treated with NS-RM and all treated with BZ(1st program).RM shows a dose 25 times lower than BZ.The 2nd program is better than the 1st one in curing the mice.RM is a promising candidate as an alternate treatment for Chagas disease,but requiring more studies. **Supported by:**:FAPEMIG, UFOP, CAPES

QT056 - EVALUATION OF CROSS-RESISTANCE OF ANTIMONY AND FUROSEMIDE IN *LEISHMANIA AMAZONENSIS* AND L. DONOVANI

ARRUDA-COSTA, N.*; MEYER-FERNANDES, J.R.; ROSSI-BERGMANN, B.
UFRJ, MAGE, RJ, BRASIL.
e-mail:nataliarruda22@hotmail.com

Pentavalent antimonials such as Glucantime® and Pentostam® are the first line therapy against cutaneous *Leishmania*sis despite their toxicity and drug-resistance potential. We recently described the anti*Leishmania*l activity of furosemide (Lasix, an Na^+-ATPase inhibitor) *in vitro* and in *L.amazonensis*-infected mice[1,2]. In this work, we evaluated the furosemide responsiveness of antimony-resistant promastigotes of *L. amazonensis* and *L. donovani* to furosemide. For this, Sb-resistance was induced in promastigotes by successive expansion of surviving parasites with increasing concentrations of antimony tartrate (SbIII). The so-obtained Sb-resistant *L. amazonensis* and *L. donovani* promastigotes were then cultivated for 72h at 26°C in the presence of various concentrations of furosemide. Their growth rate was colorimetrically determined using MTS reagents, and their IC50 was compared with wild-type Sb-sensitive promastigotes. The results showed that contrary to *L. amazonensis* in which Sb-resistance induced cross-resistance to furosemide, Sb-resistant *L. donovani* was rendered more sensitive to furosemide, indicating the potential use of furosemide in the treatment of antimony-resistant *L. donovani* visceral *Leishmania*sis.

[1]De Almeida-Amaral, E.E; Caruso-Neves, C; Pires, V.M.P.; Meyer-Fernandes, J.R. (2008) *Leishmania amazonensis*: characterization of an ouabain-insensitive Na+-ATPase activity. Experimental Parasitology, 118(2):165-171.

[2]ARRUDA-COSTA, N. Ação *in vitro* e *in vivo* do inibidor da Na^+-ATPase (furosemida) na leishmaniose cutânea. Dissertação (Mestrado em Ciências Biológicas (Biofísica)). Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, Rio de Janeiro, 2012.

Supported by::CNPq

QT057 - STUDY OF LEISHMANICIDAL ACTIVITY OF DERIVATIVES FROM COMBRETASTATIN A-4

ARAÚJO, M.V.[*1]; DO AMARAL, D.N.[2]; QUEIROZ, A.C.[1]; BRITO DA MATTA, C.B.[1]; DA SILVA, A.E.[1]; BARREIRO, E.J.L.[2]; LIMA, L.M.[2]; ALEXANDRE-MOREIRA, M.S.[1]
1.UFAL, MACEIÓ, AL, BRASIL; 2.UFRJ, RIO DE JANEIRO, RJ, BRASIL.
e-mail:morgana_vital@hotmail.com

*Leishmania*sis is considered a public health problem, affecting millions of people around the world.Considering that so far there is no ideal therapeutic for *Leishmania*sis, this study aims investigate the leishmanicidal activity of derivatives from combretastatin A-4. The cell line J774 viability was determined with MTT assay. Leishmanicidal effects against promastigotes of *Leishmania* major (MHOM/SU/1973/5-ASKH) were evaluated. Data obtained from experiments were expressed as the mean ± S.E.M. of triplicate cultures of representative assays. Statistical differences between the treated and the vehicle groups of in vitro experiments were evaluated by ANOVA and Dunnett hoc tests and considered significant when $p < 0.05$. The derivatives LASSBio 1591, 1593, 1594, 1596 and pentamidine at concentration of 100 µM showed activity deleterious to the host cell evidenced by MTT assay. However, the derivatives LASSBio 1586, 1587, 1588, 1589, 1590, 1592 and 1595 not affected the viability of macrophages at concentration of 100 µM. At concentration de 10 µM all derivatives and pentamidine not affected the viability of macrophages, except LASSBio 1596 showed activity deleterious to the host cell. Moreover, all derivatives exhibited high anti*Leishmania*l activity against promastigotes of *L. major*, with maximal effect greater than 77,7%. The derivatives from combretastatin A-4 LASSBio 1586, 1587, 1588, 1589, 1590, 1592 and 1595 are strong candidates for anti*Leishmania*l prototypes drug because since they exhibit presented pronounced leishmanicidal activity against promastigotes of *L. major* without citotoxity effect against host cells.

QT058 - EFFICACY OF CHALCONE CH8 AND QUERCETIN IN THE ORAL TREATMENT OF VISCERAL *LEISHMANIA*SIS

SILVA, C.I.M.[*]; FALCÃO, C.A.B.; ROSSI-BERGMANN, B.
UFRJ, RIO DE JANEIRO, RJ, BRASIL.
e-mail:cintia.iana@gmail.com

In previous studies our group has shown the efficacy of the synthetic chalcone CH8 (Boeck P., *et al* 2005) and the flavonoid quercetin (Muzitano, M., *et al* 2008) in the oral treatment of murine cutaneous *Leishmania*sis. This study aims to investigate if such leishmanicidal activity is extended to the visceral form of the disease. For this, BALB/c mice were infected in the caudal vein with $5x10^6$ promastigotes of *Leishmania chagasi,* and treatment with quercetin, CH8 or miltefosine was initiated 2 days after the infection. Treatment was carried out orally, on a daily basis, during 28 consecutive days. At day 30 post infection the animals were sacrificed, the individual spleens collected and the parasite load of the organs quantified by limited dilution assay. Results showed that treatment with either quercetin or CH8 resulted in a significant decrease of the spleen parasite burden, while treatment with miltefosine led to a complete reduction of the parasite load. To investigate if the drugs induced hepatic, cardiac or renal toxicity, the blood of individual mice was collected at the end of the treatment and the serum concentration of the enzymes alanine aminotransferase (TGP), aspartate aminotransferase (TGO) and creatinine was quantified, respectively. None of the drugs induced toxicity, supporting the safe use of CH8 and quercetin in the oral treatment of visceral *Leishmania*sis, with the advantage of chemotherapy being performed through a non-invasive route. **Supported by:** FCT.

Boeck P., Falcão C.A.B., Leal P.C., Yunes R.A., Filho V.C., Torres-Santos E.C., & Rossi-Bergmann B. (2006) Synthesis of chalcone analogues with increased anti*Leishmania*l activity. Bioorg & Med Chemistry 14, 1538-1545

Muzitano, M.F., Falcão, C.A.B., Cruz, E.A., Bergonzi, M.C., Bilia, A.R., Vincieri, F.F., Rossi-Bergmann, B., Costa, S.S. (2008) Oral Metabolism and Efficacy of *Kalanchoe pinnata* Flavonoides in a Murine Model of Cutaneous *Leishmania*sis. Planta Med, 74, 1-5

Supported by::Fundação Para a Ciência e Tecnologia - Portugal/União Europeia

QT059 - ENCAPSULATION IN POLY(EPSILON-CAPROLACTONE) NANOCAPSULES INCREASES THE ORAL EFFICACY OF QUERCETIN AGAINST CUTANEOUS *LEISHMANIA*SIS

BATISTA, A.J.S.[*1]; POLETTO, F.[2]; POHLMANN, A.R.[2]; ROSSI-BERGMANN, B.[1]
1.UFRJ, RIO DE JANEIRO, RJ, BRASIL; 2.UFRGS, PORTO ALEGRE, RS, BRASIL.
e-mail:ariane@biof.ufrj.br

Our group has previously described the in vivo activity of quercetin against *Leishmania amazonensis* when administered by the oral route (Muzitano, M.F., et. al., 2008). With the purpose to improve this efficacy we encapsulated quercetin (Q) and its pentacetylated derivative (P) in poly(epsoiln-caprolactone) nanocapsules (LNC), prepared by interfacial deposition of preformed polymer. To test these formulations, BALB/c mice were infected in the ear with $2x10^6$ *L. amazonensis* GFP-promastigotes. After 7 days of infection the oral treatment was initiated daily for 52 days with either the free drugs (Q and P) or their LNC formulations (LNC-Q e LNC-P, respectively). The ear lesion sizes were measured throughout the infection with a dial calliper. On day 59 of infection, the animals were sacrificed and the parasite load of the lesions was quantified by Limiting Dilution Assay and by fluorimetry. We observed that nanoencapsulation increased drug effectiveness by 40-fold in relation to free drugs. To evaluate if the formulations induced cardiac, liver or kidney toxicity, the serum concentrations of the enzymes aspartate aminotransferase (TGO), alanine aminotransferase (TGP) and creatinine were dosed respectively. None of the formulations tested showed detectable toxicity. These results show that the nanocapsules raised the efficacy of free Q and P in the treatment of cutaneous *Leishmania*sis through a non-invasive oral administration. **Supported by:**:CNPq

QT060 - Manganese(II) complexes with N4-methyl-4-nitrobenzaldehyde, N4-methyl-4-nitroacetofenone, and N4-methyl-4-nitrobenzophenone thiosemicarbazone: investigation of in vitro activity against *Trypanosoma cruzi*

BATISTA, D.G.J.[*1]; SILVA, P.B.[2]; LACHTER, D.R.[3]; SILVA, R.S.[3]; LOURO, S.R.W.[3]; SOEIRO, M.N.C.[4]; TEIXEIRA, L.R.[1]
1.IOC/FIOCRUZ, RIO DE JANEIRO, RJ, BRASIL; 2.ICO/FIOCRUZ, RIO DE JANEIRO, RJ, BRASIL; 3.PUC, RIO DE JANEIRO, RJ, BRASIL; 4.IOC/FICORUZ, RIO DE JANEIRO, RJ, BRASIL.
e-mail:denisegama@ioc.fiocruz.br

Thiosemicarbazones and their metal complexes represent an interesting class of compounds with a wide range of pharmacological applications. Many examples of this class of small molecules have been evaluated as having antitumor, antibacterial, antifungal, antiviral and antiprotozoal activities. Chagas' disease, caused by the *Trypanosoma cruzi*, is a tropical neglected illness and still represents a serious public health problem in these affected areas claiming for care and resolution of its current challenges, including the imperative need to sustain public policies related to the transmission control and the requirement for new chemotherapic agents. Then, in the present study, the Mn(II) complexes $Mn(H4NO2Fo4M)_2Cl_2$, $Mn(H4NO_2Ac4M)_2Cl_2$ and $Mn(H4NO_2Bz4M)_2Cl_2$ of $H4NO_2Fo4M$, $H4NO_2Ac4M$ and $H4NO_2Bz4M$ were synthesized and their cytotoxicity, trypanocidal efficacy and selectivity evaluated in vitro against both bloodstream trypomastigotes and intracellular amastigotes of *T. cruzi*. Our results showed that $H4HO_2Fo4M$, $H4NO_2Bz4M$, and metal complexes $Mn(H4NO_2Fo4M)_2Cl_2$ and $Mn(H4NO_2Ac4M)Cl_2$ were not active against bloodstream trypomastigotes, showing IC_{50} values higher that 142µM, while $H4NO_2Ac4M$ was moderately active (IC_{50}68µM). However, complex $Mn(H4NO_2Bz4M)_2Cl_2$ presented interesting trypanocidal activity, with IC_{50}19µM and reaching 95% of parasite death at dose of 50µM. The toxicity of compounds demonstrated that none of these compounds reduced the cellular viability when higher doses-200µM and longer periods of incubation-72h were performed. Following this, we evaluated in T.cruzi-infected cardiac culture assays aiming to analyze their activity against the intracellular forms. However, the thiosemicarbazones and their Mn(II) complexes exhibiting IC_{50} values between 111 and >200µM. Thus, our results suggest further investigations of this class of compounds for the rational design of new chemotherapy agents for Chagas disease. **Supported by:**:Capes, Faper, Cnpq, Fiocruz

QT061 - Evaluation of the therapeutical efficacy of a sesquiterpene lactone in a nanostructured formulation on experimental Chagas disease

SILVA, M.R.S.*; BRANQUINHO, R.T.; SAÚDE-GUIMARÃES, D.A.; MOSQUEIRA, V.C.F.; E SILVA, J.C.V.O.; DE LANA, M.
UFOP, OURO PRETO, MG, BRASIL.
e-mail:mariannerocha@uol.com.br

RM is a lipophilic substance in vitro active against *T. cruzi* isolated from *Lychnophoras* (Arnica). The use of nanostructured (NS) formulations as a carrier for lipophilic compounds improves its therapeutic efficacy in vivo and reduces toxicity. The only drug available to treat Chagas disease is benznidazole (BZ). This work evaluates the activity of RM compared to BZ on the treatment of the acute phase of the infection with *T. cruzi* Y strain (partially resistant to treatment) in murine model. RM was isolated and characterized by Branquinho (2010), as well as its NS polymeric formulations produced, one with conventional polymer (NSRM1), other with stealth polymer (NSRM2). Young Swiss female mice weighting 18-20g were intraperitoneally inoculated with 10000 blood tripomastigotes of Y strain. The intravenous treatment started on the 4th day of infection and lasted 20 days. The mice were divided in groups: NSRM1 (2mg/Kg/day), NSRM2 (2mg/Kg/day), free RM (2mg/Kg/day), BZ (50mg/Kg/day) and negative controls. Parasitemia was evaluated daily by fresh blood exam for 30 days and survival was registered. Parasitological (hemoculture and PCR) and serological (ELISA) tests assessed treatment efficacy. All (100%), 75%, 87.5% and 12.5% of the mice treated with NSRM2, NSRM1, BZ and free RM presented subpatent parasitemia. All treated with NSRM1, NSRM2, BZ and 50% treated with free RM survived the acute phase and were necropsied after 6 months. The negative controls survived for 20 days. Parasitological and serological tests showed that NSRM2 was able to cure 100% of the animals, while BZ cured 75%. NSRM1 cured 62.5% of the mice at a dose 25 times lower than BZ. The polymeric constitution of the formulations improved the pharmacokinetic parameters of RM, maintained its high concentration in plasma and increased its efficacy. This work evidences RM's efficacy in NS formulations for the treatment of experimental Chagas disease thus requiring further studies. **Supported by:**:FAPEMIG, CNPq, UFOP

QT062 - Biological activity against *Trypanosoma cruzi* and *Leishmania amazonensis* of essential oils from Vernonia brasiliana Less.

MACHADO, F.C.*; MARTINS, M.M.; BORGES, M.S.; DE OLIVEIRA, A.; CHANG, R.; DE MORAIS, S.A.L.; DE MELO, G.B.; SILVA, C.V.
UFU, UBERLÂNDIA, MG, BRASIL.
e-mail:fabriciomachado@live.com

Vernonia brasiliana Less. is widely used in folk medicine. In this context, we produced essential oils from the leaf, flower and root of *Vernonia brasiliana* and evaluate its antimicrobial, antiprotozoal and cytotoxicity activity. *Staphylococcus aureus* and *Escherichia coli* were used with minimum inhibitory concentration (MIC) ranging from 2048 to 4µg/mL. For the antiprotozoal activity, we investigate those essential oils against trypomastigotes of *Trypanosoma cruzi* and promastigotes of *Leishmania amazonensis* using a microdilution plate and concentrantions ranging from 1024 to 8µg/mL. Vero and Macrophages RAW 264.7 cells were also tested in those same conditions. All the three oils showed no antimicrobial but with a considerable activity against *Trypanosoma cruzi* (half maximal effective concentration [EC_{50}] of leaf essential oil at 211µg/mL; flower essential oil at 174µg/mL and root essential oil at 101µg/mL) and *Leishmania amazonensis* (EC_{50} of leaf essential oil at 210µg/mL; flower essential oil at 191µg/mL and root essential oil at 239µg/mL). No toxicity in Vero or RAW was found at those concentrations with cytotoxicity found only above concentrations of 321µg/mL.These results opens an opportunity to future studies trying to identify potential compoundsof *Vernonia brasiliana* Less. on the treatment of Chagas' disease and *Leishmania*sis. Financial Support: FAPEMIG, CAPES and CNPq. **Supported by:**:FAPEMIG / CAPES / CNPq

QT063 - LEISHMANICIDAL ACTIVITY OF MTB2 IN *Leishmania amazonensis*-INFECTED MURINE MACROPHAGES

GUEDES, C.E.S.*; DIAS, B.R.S.; PETERSEN, A.L.O.A.; CRUZ, K.P.; ALMEIDA, N.J.; BORGES, V.M.; VERAS, P.S.T.
CPQGM - FIOCRUZ, SALVADOR, BA, BRASIL. e-mail:cesgbio@yahoo.com.br

*Leishmania*ses are emerging diseases with a broad spectrum of clinical presentations. The treatment usually involves pentavalent antimonials, a long-term therapy with the emergence of resistant strains. The search for new anti-*Leishmania*l drugs has certain urgency. The present study aimed to evaluate the efficacy of MTB2, to control *L. amazonensis* (L.a) infection in CBA murine macrophages (MØ). The direct effect of this compound on L.a was evaluated in parasite cultures exposed to increasing concentrations of MTB2 for 24, 48 and 72h, followed by direct parasite counts. MTB2 caused a direct inhibitory effect on parasite growth, inducing a 90% promastigote kill rate after 48h. (IC50=41.13µM). The effect of this compound on parasite load was evaluated after 6, 24 and 48h of treatment, by determining the n° of L.a per infected MΦ and the percentage(%) of infected MØ. Treatment with 50, 75 and 100µM of MTB2 resulted in a reduction in the % of infected MΦ of 75%, 98% and 99%. A drastic reduction in the n° of L.a per infected MΦ was also observed following MTB2 treatment: 51% at 50µM, 70% at 75µM, and 77% at 100µM. These effects occurred independent of NO and $O2^{\cdot}$-production. Interestingly, treatment with 50µM MTB2 reduced MCP-1 production, yet IL-12, TNF-α and IL-6 remained unaffected. The ultra-structural alterations induced by MTB2 in *L. amazonensis*-infected MØ were evaluated by electronic microscopy observations. As soon as 6h of treatment, increasing number of lipid bodies and enhancement of eletrondensity in parasite cytosol were observed. After 24h, “empty” vacuoles, multivesicular bodies and vesicles suggestive of autophagy were seen in parasite cytosol. After 48h, only debris was found inside MΦ parasitophorous vacuoles. Further efforts involving this compound may lead to the development of a new generation of anti-*Leishmania*l drugs. In vivo and proteomic studies will be performed to expose the underlying mechanism involved in MTB2 control of *L.a* infection. **Supported by:**::CNPq - 306672/2008-1

QT064 - SKIN ABSORPTION OF CHALCONE-ENTRAPPED FLUORESCENT NANOCAPSULES IN *LEISHMANIA AMAZONENSIS*-INFECTED AND NON-INFECTED MICE USING BIOLUMINESCENT IMAGING.

LOPES, M.V.*[1]; ROSSI-BERGMANN, B.[1]; POHLMANN, A.R.[2]; MEDEI, E.H.[1]; GUTERRES, S.S.[2]; GUERRA, B.[1]; POLETTO, F.[2]; GOMES, A.M.O.[1]
1.UFRJ, RIO DE JANEIRO, RJ, BRASIL; 2.UFRGS, PORTO ALEGRE, RS, BRASIL.
e-mail:milevaleria@yahoo.com.br

Though topical treatment would be the most appropriate way to treat cutaneous *Leishmania*sis, no effective topical treatment exists for this disease in Brazil, mainly due to the difficulty of the active drug to cross the skin barrier. Over the last years, new drug delivery systems have been considered appropriate to carry drugs across the skin. Therefore, with the aim to develop an efficient system to deliver the lipophilic anti*Leishmania*l chalcone CH8 across the skin to the infected dermis, we monitored skin absorption and macrophage internalization of rhodamine-labeled lipid-core poly-epsilon-caprolactone nanocapsules entrapping the chalcone CH8 (LNCF-CH8). LNCF-CH8 measuring 210 nm was prepared by interfacial deposition of rhodamine-coupled preformed polymer. To evaluate topical absorption, LNCF-CH8 was applied onto the shaved skin of mice. At different times after application, bioluminescent images were taken with IVIS LUMINA. To evaluate the effect of *Leishmania* infection in LNCF-CH8 subcutaneous absorption, mice were infected in one ear with *L. amazonensis* and after 15 days LNCF-CH8 particles were injected into both ears and images were taken as above. For in vitro macrophage uptake, normal and green fluorescent (GFP-transfected) *L. amazonensis*-infected macrophages were incubated for 2 hours with LNCF-CH8. Images of fluorescent particles and parasites were taken and analyzed by intravital multiphoton microscopy. We observed a decreased fluorescence after 120 min of LNCF-CH8 topical application. Subcutaneously injected LNCF-CH8 produced a faster decrease of fluorescence in infected than non-infected skin. In vitro, LNCF-CH8 was shown to co-localize with the parasites inside macrophages. These results demonstrated that lipid-core LNCF-CH8 nanocapsules can deliver the lipophilic drug CH8 across the skin and in to infected macrophages, showing their great potential for use in the topical treatment of cutaneous *Leishmania*sis. **Supported by:**::CAPEs

QT065 - The anti-*Leishmania*l activity of derivates from pterocarpanoquinone LQB118 against *Leishmania* braziliensis

SILVA, T.[*1]; MEIRA, R.V.[1]; SOUZA, L.C.[1]; SIQUEIRA, L.M.[1]; AZEVEDO, B.F.[1]; MENDES, M.A.P.[1]; DA SILVA, A.J.M.[2]; COSTA, P.R.R.[2]; SILVA, S.A.G.[1]
1.UERJ, RIO DE JANEIRO, RJ, BRASIL; 2.UFRJ, RIO DE JANEIRO, RJ, BRASIL.
e-mail:thatasorrizo@hotmail.com

The synthetic pterocarpanoquinone LQB118 have showed direct and indirect antiparasitic effect on promastigotes and intracellular amastigotes of *Leishmania amazonensis* and L.braziliensis, related with apoptosis and modulating the macrophages in vitro. The aim of this study was to evaluate the antiparasitic activity of pterocarpanoquinones derivates from LQB118, named LQB168, LQB182, LQB187 and LQB236, on promastigotes and intracellular amastigotes forms of *L. braziliensis*. We also evaluated the possible modulation of the host cell and its toxicity on macrophages. Promastigotes were incubated with pterocarpanoquinones at 20µM for 72h/28ºC and the cell viability was estimated by MTT assay. For the test with intracellular amastigotes, monolayers of peritoneal macrophages from normal swiss mice were infected with *L. braziliensis* promastigotes (5:1) and incubated with pterocarpanoquinones (20µM) for 48h at 37°C/5%CO2. The macrophages were stained and intracellular amastigotes counted under a microscope. In the supernatant nitric oxide production was measured by Griess method. Pterocarpanoquinones toxicity was evaluated on non-infected macrophages by MTT assay. On promastigotes forms LQBs 168, 182 and 236 significantly ($p>0,05$, $p<0,001$ and $p<0,05$, respectively) decresead the cell viability in 22,32%, 37,16% and 25,62% respectively, compared with control. The pterocarpanoquinones showed no toxicity on macrophages at the concentration tested (20µM). On intracellular amastigote form only LQB182 decreased significantly ($p <0.05$) the number of parasites per macrophage in 74%, but no change nitric oxide production by macrophages. These results indicate anti-*Leishmania*l activity of a new pterocarpanoquinone LQB182. We are currently evaluating IC50 and the antiparasite mechanism of LQB182 on *L. braziliensis*. **Supported by:**:cnpq/faperj

QT066 - Activity of the chalcone CH8 encapsulated in PLGA microparticles against Cutaneous *Leishmania*sis

OLIVEIRA, D.E.[*]; BATISTA, A.J.S.; ROSSI-BERGMANN, B.
UFRJ, RIO DE JANEIRO, RJ, BRASIL.
e-mail:douglas@biof.ufrj.br

The activity of the chalcone CH8 has previously been shown by our group against murine cutaneous *Leishmania*sis. With the purpose to develop a topical therapy for this disease, improve cutaneous penetration and efficacy of this drug, we encapsulated the CH8 in poly-lactic-co-glycolic acid (PLGA) microparticles. The particle efficacy in vitro was assessed by infecting peritoneal macrophages with promastigotes of *Leishmania amazonensis* and incubating the cells with either the free or the encapsulated CH8 (PLGA-CH8). After 48 h at 37 ºC, the number of amastigotes was quantified using optical microscopy. We observed that PLGA-CH8 reduced the parasite load (IC50 14,72 uM) when compared to the untreated control with no cytotoxicity associated. No effect and no toxicity were observed when using PLGA without CH8. Then, to investigate if PLGA-CH8 induced cell microbicide mechanisms, we analyzed the production of nitric oxide (NO) through Griess reagents, and reactive oxygen species (ROS) using plate fluorimeter and H2DCFDA dye. We observed that the free CH8 increased ROS and NO release, but the PLGA-CH8 did not led to an increase in these parameters. These results suggest that the drug encapsulation may interfere with the macrophage microbicide mechanism when compared to the free CH8. Further experiments will be carried out to investigate the mechanisms of action of the encapsulated drug. **Supported by:**:PIBIC/CNPq

QT067 - TREATMENT OF CUTANEOUS *LEISHMANIA*SIS USING A SUBCUTANEOUS IMPLANT OF PLGA MICROPARTICLES LOADED WITH AMPHOTERICIN B

PACIENZA-LIMA, W.[*1]; GONDIM, F.C.[1]; FALCÃO, C.A.B.[1]; CERIZE, N.N.P.[2]; RÉ, M.I.[3]; ROSSI-BERGMANN, B.[1]

1.IBCCF-UFRJ, RIO DE JANEIRO, RJ, BRASIL; 2.IPT, SÃO PAULO, SP, BRASIL; 3.CENTRE RAPSODEE, ÉCOLE DES MINES D'ALBI, LILE, FRANÇA.

e-mail:wallaceplima@bol.com.br

The conventional treatment of cutaneous *Leishmania*sis (CL) is based on multiple injections with antimonial or amphotericin B (AmB) which produce systemic toxicity. In this study, we aimed at developing a single-dose therapy consisting of a subcutaneous implant of poly-(lactide-co-glycolide) PLGA microparticles (mps) loaded with amphotericin B (AmB/PLGA) for sustained drug release system. *In vitro*, *L. amazonensis-GFP*-infected macrophages were treated for 72 h with AmB/PLGA, or AmB and PLGA controls, and the parasite load and NO production was measured by fluorimetry and Griess method. *In vivo*, BALB/c mice were infected in the ear with *L. amazonensis-GFP*, and on day 26 of they received an intralesional injection with AmB/PLGA (50µg /500µg). Controls were liposomal AmB (Ambisome®,50µg), free AmB (50µg), empty PLGA (500µg) and 10µl of PBS. Lesion sizes were measured throughout infection with a dial calliper and the parasite loads on day 69 by fluorimetry. Chronic toxicity was assessed on day 69 by elevation of AST, ALT and creatinin serum levels. Local skin sensitivity was assessed by the Mouse Ear Swelling Test. Histopathological studies were done at various times up to day 120 after implant to monitor tissue inflammation and polymer degradation. The *in vitro* results showed that AmB/PLGA had higher anti-amastigote and NO activities than free AmB. *In vivo*, a single dose of AmB/PLGA implant, but not PLGA alone or liposomal AmB, effectively reduced the lesion growth and the parasite burden. No indicators of heart, liver and kidney toxicity were detectable in the serum. Likewise, the AmB/PLGA implant led to undetectable MEST response. A transient cellular infiltration peaking on day 7 was seen. These findings show that the subcutaneous implant of AmB/PLGA promoted a sustained drug release at the lesion site, with a durable and safe therapeutic effect, supporting its use for a single-dose localized treatment of CL. **Supported by:**:CAPES; CNPq; GlaxoSmithKline