TB017 - **LEISHMANICIDAL ACTIVITY OF PRENYLATED NITROHETEROCYCLIC COMPOUNDS**

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Leishmaniasis is a group of neglected diseases with a high cost treatment, which presents difficulties such as toxicity and drug resistance cases besides their high morbidity rate and different clinical manifestations. The potential of the nitroheterocyclic compounds has been rediscovered in the last few years and literature provides some evidences that prenyl moieties increase lipophilicity and, consequently, improve the biological activity. In this study we evaluated the leishmanicidal activity of synthetic prenylated nitroheterocyclic compounds. Promastigotes of *Leishmania amazonensis* were cultured in the presence of several concentrations of six prenylated nitroheterocyclic compounds up to 100 µM for 72 h and quantified by MTT assay. Among them, only LQB-278 inhibited the parasite growth, in a dose-dependent manner with 15.7 µM IC50. LQB-278 was selected to antiamastigote activity on macrophages infected with *L. amazonensis.* LQB-278 showed to be effective in decreasing the infection of the macrophages with IC50 less than 12 µM. In order to determine the toxicity, LQB-278 was incubated with murine macrophages for 72 h. The effect on macrophage viability was quantified by resazurine and the LD50 value was 46 µM, indicating a selectivity index of 4.14. Further studies should be conducted to better understand the impact of the insertion of prenyl moieties for the leishmanicidal activity. **Supported by:**CNPq
Keywords: Nitroheterocyclic; antileishmanial; prenylation

TB018 - ***LEISHMANIA AMAZONENSIS* MUTANTS EXPRESSING MODIFIED LUCIFERASES**

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The leishmaniasis are a group of diseases, endemic worldwide, for which very limited therapeutic resources are available. The search for new drugs applicable to leishmaniasis treatment depends on pre-clinical tests where efficacy is measured through the determination of parasite burden, classically performed by limiting dilution. Reporter genes have been proposed as an alternative method for determining parasite burden. Luciferase has been demonstrated as quite efficient for that purpose, mainly on in vivo models. Modified luciferases, such as NanoLuc, NanoLuc-PEST and RedLuc, were produced in an attempt to improve the sensitivity of light detection. The aim of this work was to obtain and characterize *L. amazonensis* mutant lines expressing the modified luciferases NanoLuc (La-NL), NanoLuc-PEST (La-NLP) and RedLuc (La-RED). A reference strain of *L. amazonensis* was transfected with cassettes containing the genes encoding the three modified luciferases. Transfected lines were rescued and characterized. The mutant lines displayed growth curves indistinguishable from the wild-type parasites. Drug susceptibility of mutant lines was also unaltered, as judged by dose-response curves to amphotericin B. The luminescence of mutants was evaluated based on serial dilutions spanning 1 to 10^6 promastigotes. Luminescence was 100 to 1000-fold higher in La-NL parasites as compared with La-NLP, La-RED and lines expressing conventional luciferase (La-LUC2). Detection limits for La-NL, La-NLP and La-RED lines was 1, 10 and 10 parasites, respectively. The evaluation of the virulence and bioluminescence in vivo and in vitro of the mutants is in progress. *L. amazonensis* expressing NanoLuc, NanoLuc-PEST and RedLuc were obtained and may become instrumental in the evaluation of drugs applicable to leishmaniasis treatment. Luminescence produced by NanoLuc transformed promastigotes is greatly increased in comparison with conventional luciferase. Financial support: FAPESP, CNPq. **Supported by:**FAPESP, CNPq
Keywords: Leishmaniasis; luciferase; reporter gene

TB019 - **TOPICAL TAMOXIFEN FOR THE TREATMENT OF CUTANEOUS LEISHMANIASIS**

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Tamoxifen is an anticancer agent with *in vitro* activity against all Old and New World *Leishmania* species tested so far. Tamoxifen is also effective in experimental models of cutaneous and visceral leishmaniasis when administered systemically. The aim of this study was to test the efficacy of tamoxifen for the treatment of cutaneous leishmaniasis by the topical route. As the experimental model, we employed infection of BALB/c mice with a *Leishmania amazonensis* transgenic line expressing luciferase (LaLUC). Mice were inoculated with 10^6 LaLUC stationary-phase promastigotes at the base of the tail. Four weeks post-inoculation, animals were assigned into experimental groups (n = 5) and treated by the topical route with 1% tamoxifen in ethanol or 0,1% citrate tamoxifen in oil-free cream for 30 days. Control groups received the same vehicles used to formulate tamoxifen or 40 mg/kg/day meglumine antimoniate (Sb^V). Treatment efficacy was evaluated by lesion size and parasite burden, quantified through luminescence, at the end of treatment and four weeks later. Topical tamoxifen, formulated in ethanol or as a cream, was effective in reducing lesion size and parasite burden. Tamoxifen formulated as cream was superior to the ethanolic formulation. Treatment with topical tamoxifen in both formulations was superior to Sb^V therapy, although not achieving complete lesion resolution. In conclusion, topical use of tamoxifen showed superior and sustained effects in comparison with Sb^V monotherapy. Tamoxifen oil-free cream is a low-cost formulation, safe to humans and easily administered. Considering the additive properties previously observed for tamoxifen and antimonial combinations, topical use of tamoxifen represents a suitable alternative for combination treatment of human CL. **Supported by:**FAPESP, CNPq
Keywords: Leishmaniasis; topical; therapy

TB020 - **EFFECT OF SYNTHETIC HYDRAZONES IN PROMASTIGOTES AND AMASTIGOTES OF LEISHMANIA AMAZONENSIS.**

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Leishmaniasis is a protozoan disease caused by flagellate protozoan of the genus *Leishmania* and considered by the OMS as a neglected tropical disease. The first line treatment, based on pentavalent antimony, has some limitations, with regard to toxicity, safety and efficacy. So, there are an urgent need for more effective drugs. The present study evaluated the leishmanicidal activity of eleven synthetic hydrazones in *L. amazonensis*. The antipromastigote activity and cytotoxicity on peritoneal macrophages was determined by the MTT colorimetric method after 72 hours of treatment. Five compounds were selected for the antiamastigote assay. The antiamastigote activity was evaluated in macrophages infected with *L. amazonensis* transfected with red fluorescent protein (RFP) and determined by fluorescence intensity after 72 hours of treatment. All results were expressed as IC50 (concentration that inhibits 50% of the parasite growth). Among the eleven compounds evaluated, five showed activity against promastigotes (IC50 of 41.06, 50.84, 17.28, 17.36 and 18.22 µM). Regarding antiamastigota activity, among the five selected compounds, three compounds showed a promising leishmanicidal activity (IC50 de 24.93, 37.60 and 68.06 µM). All compounds not showed a toxic effect on murine macrophages. These results open perspectives for the design of new biologically active compounds and encourage to continue the studies these compounds in *L. amazonensis*, as evaluation of the action mechanism and the in vivo effect. **Supported by:** FAPEMIG, CNPq and UFJF.
Supported by:FAPEMIG/ CNPq / UFJF
Keywords: Leishmania; synthetic hydrazones ; antiamastigote assay

TB021 - **ACTIVITY OF A QUINOLINE DERIVATIVE IN *L. AMAZONENSIS* AND ITS EFFECT ON THE PARASITE MITOCHONDRIA**

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Introduction and Objectives: Leishmaniasis is an infectious parasitic disease, widely distributed around the world, which affects humans and animals. The drugs used in the treatment show toxicity, high cost, contraindications and increasing parasite resistance. Previous studies have shown that quinoline derivatives present antileishmanial activity. Thus, this study had as objective to analyze the leishmanicidal activity of a quinoline derivative compound, named NIC010, as well as its possible effect on the parasite mitochondria. Material and Methods: The antipromastigote activity in *L. amazonensis* and cytotoxicity on murine peritoneal macrophages were determined by colorimetric method (MTT) after 72 hours of treatment. The antiamastigote effect was evaluated in macrophages infected with *L. amazonensis* transfected with GFP and after 72 hours the relative fluorescence units were measured using a spectrofluorometer. To verify the effect of the compound in the mitochondria of the promastigotes, the mitochondrial membrane potential (ΔΨm) was analysed using the fluorescent probe JC-1 and Rhodamine 123 (Rho-123).The effect of the compound on the production of reactive oxygen species (ROS) was analysed using H2DCFDA. Results: The compound NIC010 showed antileishmanial activity, with IC50% of 43.25 µM and 5.48 µM for promastigote and amastigote forms, respectively, and exhibited a low toxicity to the host cell. Regarding the effect on mitochondria, the treatment of promastigotes with NIC010 induced a reduction in the ΔΨm of 34.5% by JC-1 and 25,5% by Rho-123, and a 61.67% increase in the ROS production. Conclusions: These results demonstrate that the compound NIC010 presents an antileishmanial activity and exerts effect on the mitochondria of the parasite, suggesting that this organelle is a potential target of this compound in *L. amazonensis*. **Supported by:** UFJF, CNPq and FAPEMIG.
Keywords:L. amazonensis; mitochondria; quinoline

TB022 - **EVALUATION THE LEISHMANICIDAL EFFECT OF ESTEROID DERIVATIVES IN *LEISHMANIA (LEISHMANIA) AMAZONENSIS***

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The search for new tools for the treatment of leishmaniasis is the main alternative towards the limitations of the therapies available currently, since these present toxicidade, high cost and commonly inefficient because the emergence of resistant strains. Several studies indicate a great variety of steroid derivatives with leishmanicidal activity. With this in mind, the objective of this work was to determine the leishmanicidal effect of ten steroid derivatives, as well, its cytotoxicity in mammalian cells. The tested compounds were named: (1) JAS 101R, (2) JAS 141, (3) JAS 142, (4) JAS 149R, (5) JAS 041C, (6) JAS 044 (7) JAS 049, (8) JAS 057, (9) JAS059, (10) JAS 064. The activity antipromastigote, and cytotoxicity in peritoneal macrophages were determined by colorimetric method (MTT) after 72h of incubation. The antiamastigote activity was evaluated using peritoneal macrophages infected with *L. amazonensis* transfected with the gene of the red fluorescent protein (RFP) and determined by fluorescence intensity after 72h. The treatments of promastigotes resulted in growth inhibition of parasites, and the most effectives were the compounds (8) and (9), with an IC50 32.81µM and 29.06 µM, respectively. Regarding the antiamastigote activity, highlight the compounds (3) and (4), with an IC50 of 15.34 µM e 2.05 µM, respectively. The majority of compounds did not show toxicity on murine macrophages, except the compound (8) (CC50of 53.13 µM). Taken together, these results indicate that steroid derivatives are a favorable prototype to the development of new drugs for the treatments of leishmaniasis and further studies to evaluate the action mechanism of these molecules will be done. **Supported by:** FAPEMIG, CNPq and UFJF.
Keywords: Leishmaniases; esteroid derivatives; leishmania amazonensis

TB023 - **ACTION MECHANISM OF A VANADIUM COMPLEX IN *LEISHMANIA AMAZONENSIS***

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Introduction and Objectives: Leishmaniasis is endemic in 98 countries and the chemotherapy exhibit limitations, thus is very important to search new compounds with leishmanicidal activity. Based on that, this work had as objective evaluate the possible action mechanism of a vanadium complex (named VanCo), already known to be effective in promastigotes and amastigotes of *Leishmania amazonensis*. Material and Methods: Promastigote forms of *L. amazonensis* were treated with 27 and 53 µM of the VanCo for 24 hours and the cells were labeled with annexin V-FITC or terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) or monodansilcadaverin (MDC) or propidium iodide (PI). Posteriorly, the stained cells were analyzed by flow cytometry or by fluorescence microscopy. Results: The treatment of promastigotes with VanCo caused translocation of phosphatidylserine to the outer leaflet of the plasma membrane, as measured by annexin V-FITC binding (from 12.42% in the untreated promastigotes to 82.52% and 87.05% in cells treated with 27 and 53 µM of the VanCo, respectively) and concomitant nuclear alterations that included in situ labeling of DNA fragments by TUNEL, and cell-cycle arrest at the sub-G0/G1 phase (from 8.90% in the untreated cells to 33.77% and 42.23% in cells treated with 27 and 53 µM of the VanCo, respectively). These data show that the promastigotes death was induced by an apoptotic-like mechanism. However, this compound also appears to induce autophagy in promastigotes of *L. amazonensis*, since the treatment with the VanCo compound increased the percentage of cells stained with MDC (from 57.6% in untreated promastigotes to 86.86% in cell treated with 53 µM of the VanCo). Conclusions: This results show that the compound VanCo appears to induce apoptosis-like and autophagy in promastigotes of *L. amazonensis*. **Supported by:**FAPEMIG, CNPq and UFJF.
Keywords: Leishmania amazonensis; vanadium complex; action mechanism

TB024 - **EFFECT OF RESVERATROL ANALOGUES IN *LEISHMANIA BRAZILIENSIS* IS RELATED TO OXIDATIVE STRESS**

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Introduction and Objectives: The search for new compounds with leishmanicidal activity is very important. So this work evaluated the antileishmanial effect of some resveratrol analogues in *Leishmania Braziliensis* and determined the possible occurrence of oxidative stress after treatment with the most effective compound. Material and Methods: Five compounds - named (1), (2), (3), (4) and (5) - were tested in promastigotes and amastigotes of *L. Braziliensis* and cytotoxicity on macrophages. The effect of the compounds in promastigote forms and in macrophages was determined by MTT colorimetric method, after 72 h of incubation period with the compounds. After 72 h of treatment of the *L. Braziliensis*-infected macrophages, the amastigotes viability was analyzed by counting intracellular parasites after Giemsa staining. The most effective compound against *L. Braziliensis* promastigotes has its action mechanism analyzed, using H_2DCFDA, to evaluate the occurrence of oxidative stress in treated promastigotes. Results: Among the five compounds tested, three compounds (1), (2) and (4) showed activity on promastigote forms with IC_{50} of 7.33, 10.27 and 24.04 µM, respectively. These compounds also were effectives against amastigotes, with IC_{50} of 26.87, 17.54 and 29.32 µM for the compounds (1), (2) and (4), respectively. All the compounds not showed toxicity in mammalian cells. The treatment of promastigotes with 14.66 µM of the compound (1) induced an increase of reactive oxygen species (ROS) production (56%, compared to the untreated control), indicating the occurrence of oxidative stress. Conclusions: These results show the potential effect of resveratrol analogues against *L. Braziliensis* and provide new perspectives for the synthesis of others molecules with leishmanicidal effect, however, more studies are need to elucidate the action mechanism of this compound. **Supported by:**FAPEMIG, CNPq and UFJF.
Keywords: Leishmania Braziliensis; resveratrol analogues; leismanicidal activity

TB025 - **DIFFERENCES IN THE DETECTION OF BRDU/EDU INCORPORATION ASSAYS ALTER THE CALCULATION FOR CELL CYCLE IN TRYPANOSOMATID PARASITES**

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The cell cycle can be considered, in general, as two distinct events: DNA replication (S phase), and mitosis (M phase), separated by two gap phases (G1 and G2). The knowledge about periods and timings of specific cell cycle phases for the trypanosomatids T. cruzi, T. brucei, and L. amazonensis is of paramount importance, since some of proteins and molecules can be used as potential target for drug development can act in a cell cycle phase dependent manner. Here, we show that the calculation of some cell cycles phases (G1, S, and G2) is dependent on DNA replication monitoring and, consequently, dependent on strategy used to do this in three analysed trypanosomatids. We used BrdU- and EdU-incorporated parasites (during different periods) with respective standard detection methods: IIF to detect BrdU with previous standard denaturation (2M HCl) and Click Chemistry to detect EdU. We found an enormous discrepancy between these two thymidine analogues in the trace of DNA replication to establish a percentual of parasites labelled. Furthermore, we showed different labelling patterns in the nucleus and kinetoplast of parasites analysed. After that, using Williams, and Woodward and Gull formulas, we compare period and times for each cell cycle phase for each trypanosomatid analysed (using EdU or BrdU). Finally, we used different concentrations of HCl (3, 4, and 5 M) to increase the exposition of incorporated BrdU providing a better detection and, consequently, leading percentual of BrdU-labelled to the same of EdU-labelled parasites. We conclude that differences between BrdU and EdU standard detection, commonly used to monitoring DNA replication, alters the percentage of cells labelled, the pattern of fluorescence observed, and consequently the calculation for cell cycle phases (G1, S, and G2). An increase of HCl concentration used to expose BrdU to detection can solve this discrepancy in these trypanosomatid parasites. **Supported by:**FAPESP
Keywords: Trypanosomatids; cell cycle phases; brdu/edu incorporation assay

TB026 - **BENZOFUROXAN DERIVATIVES AGAINST *TRYPANOSOMA CRUZI* AND ACTIVITY ON MITOCHONDRIAL TRYPAREDOXIN PEROXIDASE EXPRESSION**

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Increasing expression of mitochondrial tryparedoxin peroxidase (mTcTXNPx), which has a peroxidase and peroxynitrite reductase activity, could be responsible to benznidazole (BZ) resistant strains of *Trypanosoma cruzi*. Benzofuroxan derivatives have antileukemic, antibacterial, antifungal, antiplasmodial and trypanocidal activities. Additionally, N-oxide-benzo[1,2-c]1,2,5- oxadiazole derivatives (BZTS and BZFS) showed cytotoxicity to amastigote form of *T. cruzi*, Tulahuen strain, and were not mutagenic in mice. The goals of this work were to evaluate the toxicity of benzofuroxan derivatives (BZTS and BZFS) on epimastigote forms of *T. cruzi*, Y strain, as well as the expression of mTcTXNPx. *In vitro* cytoxic assays were performed to measure the viability of *T. cruzi* and HepG2 cells, used as a mammalian cell model. The cytotoxic indexes (IC50) against *T. cruzi*, were 28.11 µM to BZTS and 31.11 µM to BZFS, whereas to BZ was 34.62 µM. Both benzofuroxan derivatives showed better activity than BZ, since reactive oxygen species were produced by aromatic nitro-group, related to the cytotoxic effects. Furthermore they were not toxic to HepG2 cells. Differences on mTcTXNPx expression, after treatment, were evaluated using purified-IgG antibody. The results show that no increase in the expression of 25.5 kDa mTcTXNPx although a polypeptide ranging from 46 to 58 kDa was visualized. This polypeptide could be the isoform of mTcTXNPx, due to the wide genetic diversity of *T. cruzi* population. It seems that some strains do not increase mTcTXNPx expression. Maybe other enzymes are involved in the resistance to oxidative stress by decreasing free radical and electrophilic metabolites. BZTS represents a potential trypanocidal agent that urges more studies to understand its molecular and cellular mechanisms of action.
Supported by:CAPES

Keywords: Trypanosoma cruzi; benzofuroxan derivatives; mitochondrial tryparedoxin peroxidase

TB027 - **THE PROTECTIVE AND TOLEROGENIC INTRANASAL LAAG VACCINATION PREVENTS THE DEVASTATING EFFECT OF SUBCUTANEOUS LAAG VACCINATION IN MURINE *LEISHMANIA AMAZONENSIS* INFECTION**

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There is so far no approved human vaccine for leishmaniasis. Most experimental vaccines are given by parenteral subcutaneous or intramuscular routes. Indeed, we and others have previously demonstrated that rather than protecting, adjuvant-free parenteral immunization with *Leishmania amazonensis* whole antigens (LaAg) increases mouse and monkey susceptibility to infection. On the other hand, mucosal oral or intranasal pre-immunization provides effective protection. We postulated that mucosal protection might be associated with immune tolerance induction and regulatory T cell (T_{reg}) generation. In this work, we tested the tolerogenic hypothesis by evaluating mucosal and peripheral immune responses of intranasally pre-vaccinated mice to peripheral challenge. BALB/c mice received 2 i.n. LaAg doses (10 µg each) or 2 s.c. LaAg doses (50 µg each) in a 7-day interval before *L. amazonensis* infection in the footpads. I.n. LaAg, but not s.c. LaAg, impaired normal hypersensitivity reaction upon infection challenge, suggesting a tolerogenic effect. Also, i.n. LaAg led to increased Th1 (Tbet and IL-12) and T_{reg} (Foxp3 and IL-10) transcription factor and cytokine expression in lesion-draining lymph nodes upon challenge. Prior i.n. LaAg prevented the disease-progressing effect of s.c. LaAg, promoting controlled lesion development and reduced parasite burden. I.n. LaAg did not alter the percentage of $CD4^+$ and $CD8^+$ T cell population in nasal-draining lymph nodes (CLN), but decreased $CD4^+$ $Foxp3^+$ T_{reg} population in a time dependent-manner, suggesting migration to peripheral circulation. Finally, adoptive transfer of i.n. LaAg-CLN cells to non-vaccinated recipient BALB/c mice protected the later against devastating infection challenge. Together, these data indicate that mucosal immunization is a useful strategy to induce immune tolerance and impair undesirable peripheral immune responses against *Leishmania*.**Supported by:**CAPES, CNPq, FAPERJ **Keywords:**Leishmania amazonensis; intranasal vaccine; immune tolerance

TB028 - ***IN VITRO* STUDIES TO DETERMINE THE EFFICACY OF AN ANALOGUE OF FENARIMOL AS A NEW CANDIDATE TO ANTILEISHMANIAL CHEMOTHERAPY**

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Leishmaniasis is a neglected disease that causes high socioeconomic impact. Over 350 million people reside in areas of active *Leishmania* transmission that is endemic in 98 countries. The available drugs are unsatisfactory due a high toxicity and long-term treatment that do not eliminate the parasites completely. Thus, development of new drug or/and more effective therapies are urgent needed. EPL-BS1246 is a derivative of the herbicide fenarimol and have been shown to be an inhibitor of *T. cruzi* CYP51 (sterol 14α-demethylase cytochrome P450), an essential enzyme for the conversion of lanosterol to ergosterol. Once trypanosomatids have an essential requirement for ergosterol and other 24-alkyl sterols, it is a promising target for drug development. Hence, the aim of this work was investigated the antiproliferative, ultrastructural and biochemical effects of EPL-BS1246 in *L. amazonensis*. Our studies demonstrated EPL-BS1246 presented a potent antiproliferative effect against both stages of *L. amazonensis* (IC_{50} of 2.71 µM after 48h of treatment in promastigotes; IC_{50} of 69.7 nM after 72h of treatment in intracellular amastigotes). Cell cytotoxicity assay in mammal cells resulted in a CC_{50} value of 41.74 µM after 72h of treatment, consequently in a high selective index of 599. Scanning electron microscopy showed significant alterations on the shape of drug-treated promastigotes that appeared rounded and swollen. Transmission electron microscopy revealed several alterations in the ultrastructure of intracellular amastigotes. Mitochondrion was the main organelle affected by treatment presented a significant swelling; this alteration was confirmed by decrease of the electric transmembrane potential ($\Delta\psi m$) and increase of the mitochondrial superoxide production. Finally, Nile Red labeling demonstrated an accumulation of lipid bodies induced by treatment. In summary, our results indicate that EPL-BS1246 is a potential

candidate against *Leishmania* sp., also by in vivo analysis. **Supported by:**CNPq, CAPES, PPSUS and FAPERJ **Keywords:**Leishmaniasis; ergosterol; sterol biosynthesis inhibitor

TB029 - **INTERACTIONS BETWEEN 4-AMINOQUINOLINE AND HEME: PROMISING MECHANISM AGAINST TRYPANOSOMA CRUZI**

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Chagas disease is a neglected tropical disease caused by the flagellated protozoan Trypanosoma cruzi. The current drugs used to treat this disease have limited efficacy and produce severe side effects. Quinolines, nitrogen heterocycle compounds that form complexes with heme, have a broad spectrum of antiprotozoal activity and are a promising class of new compounds for Chagas disease chemotherapy. In this study, we evaluated the activity of a series of 4-arylaminoquinoline-3-carbonitrile derivatives against all forms of Trypanosoma cruzi in vitro. Compound 1g showed promising activity against epimastigote forms when combined with hemin (IC50<1µM), with better performance than benznidazole, the reference drug. This compound also inhibited the viability of trypomastigotes and intracellular amastigotes. The potency of 1g in combination with heme was enhanced against epimastigotes and trypomastigotes, suggesting a similar mechanism of action that occurs in Plasmodium spp. The addition of hemin to the culture medium increased trypanocidal activity of analog 1g without changing the cytotoxicity of the host cell. The mechanism of action was demonstrated by the interaction of compound 1g with hemin in solution and prevention of heme peroxidation. Compound 1g and heme treatment induced alterations of the mitochondrion-kinetoplast complex in epimastigotes and trypomastigotes and also, accumulation of electron-dense deposits in amastigotes as visualized by transmission electron microscopy. The trypanocidal activity of 4-aminoquinolines and the elucidation of the mechanism involving interaction with heme is a neglected field of research, given the parasite's lack of heme biosynthetic pathway and the importance of this cofactor for parasite survival and growth. These results can improve and guide rational drug development and combination treatment strategies. **Supported by:**CNPq e FAPERJ **Keywords:**Quinoline; heme; trypanosoma cruzi

TB030 - **CHAETOCIN, A HISTONE METHYLTRANSFERASE INHIBITOR, AFFECTS *TRYPANOSOMA CRUZI* PROLIFERATION, CELL CYCLE AND NUCLEOLAR ORGANIZATION**

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The addition of methyl groups by histone methyltransferases (HMTs) modulates biological processes as DNA condensation, replication, transcription and gene expression. Trypanosomatid HMTs are not identical to those presented in human and, for this reason, they are promising chemotherapeutic targets. In this work, we evaluated the effects of Chaetocin, a HMT inhibitor, on *T. cruzi* proliferation, viability, ultrastructure and cell cycle. For this, epimastigotes were treated with 1, 5, 10 and 50 µM for 96 hours and submitted to counting on Neubauer's chamber. Viability assays, transmission (TEM) and scanning electron microscopy (SEM), flow cytometry and RNA quantification analyses were also performed. Our results show that Chaetocin inhibited irreversibly *T. cruzi* proliferation even when the drug was removed from the culture after 2 days of treatment. However, the percentage of viable parasites increased in the absence of this compound. Chaetocin also interfered with *T. cruzi* cell cycle, since the number of parasites increased in G2/M phase. On the other hand, after 8 days of drug removal, the number of pretreated parasites in G2/M decreased, suggesting that *T. cruzi* was able to recover cell cycle progression. TEM analysis revealed that Chaetocin promoted intense unpacking of nuclear heterochromatin, disassembly of the nucleolus and the formation of structures similar to autophagosome. These ultrastructural changes remained after drug removal. SEM showed that treated parasites were rounded and flattened. Moreover, the total rRNA levels were lower after treatment, even in cells submitted to the reversibility assays, which can be related to transcription impairment and consequently to nucleolar fragmentation. Taken together, the effects of Chaetocin on *T. cruzi* encourage the use of HMT inhibitors in chemotherapeutic studies against trypanosomiasis and also as a tool to further comprehend different aspects of the parasite cell biology. **Supported by:**CNPq e FAPERJ

Keywords: Trypanosoma cruzi; cell cycle; nucleus

TB031 - **SINGLE-DOSE LOCAL TREATMENT OF CUTANEOUS LEISHMANIASIS USING PLGA-BASED IMPLANT DELIVERY SYSTEMS LOADED WITH CHALCONE CH8**

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Current chemotherapy of cutaneous leishmaniasis (CL) uses systemic administration of toxic drugs that cause severe adverse reactions. Moreover, these require multiple painful injections that lead to high therapy evasion rate. Consequently, new drugs and appropriate delivery systems are required. We have previously shown that subcutaneous implants made with biodegradable microparticles promote sustained local drug release allowing an effective 3-dose treatment with a novel antileishmanial chalcone (CH8) in mice infected with Leishmania amazonensis. Here, we proposed to optimize PLGA-microparticles by increasing its drug (CH8) loading. For that, PLGA (poly lactide-co-glycolide acid) microparticles with varying polymeric matrixes were prepared by spray drying technique, as contrasting with the previous solvent evaporation technique that maximally incorporated 10% of CH8 (10%CH8-PLGA). Two formulations (18%CH8-PLGA and 18%CH8-PLGA-PVP) were produced with increased drug loading (18% CH8), round smooth surface, 8 µM mean diameter. These were shown to be packed with CH8 crystals in the inner core as seen by both SEM and RAMAN microscopy. 18%CH8-PLGA-PVP promoted the most effective and long-lasting parasite growth control when given subcutaneously to Leishmania amazonensis-infected mice, with only a transient local inflammatory reaction as seen by histopathological analysis. However, this inflammatory effect was not sufficiently to increase NO production by macrophages or induce skin sensitization. Together, these findings show that the PLGA-based implant was successfully optimized by using spray-drying technique, and addition of polyvinylpyrrolidone PVP onto the polymeric matrix allowing an effective and secure single-dose local treatment of CL.
Supported by:CNPq and CAPES
Keywords: Cutaneous leishmaniasis; chemotherapy; microparticles

TB032 - **FUROSEMIDE RESISTANCE INDUCES INFECTIVITY LOSS IN *LEISHMANIA AMAZONENSIS***

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Furosemide, a widely used loop diuretic, has been shown to critically inhibit parasite Na^+-ATPase. In this work, we proposed to generate furosemide-resistant parasites and to evaluate its effect on parasite infectivity loss as a way to obtain attenuated parasites. For *in vitro* resistance induction, *L. amazonensis* promastigotes were successively cultured at 26°C with increasing concentrations of furosemide. Parasites were then cloned in the presence of 2000 µM furosemide, yielding 4 resistant clones. Their growth profile in culture was compared with drug-sensitive (WT) parasites by daily counting in a Neubauer chamber and COULTER COUNTER® counting during 7 days. Parasites were analysed for size distribution by COULTER COUNTER® and topography by scanning electron microscopy (SEM). To assess *in vitro* infectivity, they were cultured with peritoneal macrophages at 10:1 cell ratio for 4h and 48h at 37°C, when macrophages were washed and stained with Giemsa. The results showed that all 4 clones and WT parasites had the same growth profile. However, clone LaRN looked stumpy, larger and had shorter flagelum than WT. LaRN was also non-infective for macrophages, not being able to penetrate or divide inside the cells. On the other hand, LaRM1 clone displayed intermediate infectivity, as they were able to penetrate (4h) but not multiply (48h) inside macrophages, unlike the other 2 clones that were both normally infective. In summary, these results show that despite their normal capacity to reproduce as promastigotes, furosemide-resistant parasites may change morphology and lose their capacity to infect macrophages. Ongoing experiments are evaluating the *in vivo* infectivity of LaRN and LaRM1 clones in mice, and the correlation between infectivity and Na^+-ATPase expression to establish the potential use of non-infective clones as live attenuated vaccines. **Supported by:**CNPq
Keywords: Furosemide; leishmania amazonesis; na+-atpase

TB033 - **CYTOTOXIC EFFECTS OF CLOTRIMAZOLE COMPLEXED WITH ZINC AGAINST *TRICHOMONAS VAGINALIS***

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Trichomonas vaginalis is a protozoan parasite agent of trichomoniasis in humans, which is the more prevalent non-viral sexually transmitted disease (STD). It has been shown that *T. vaginalis* could facilitate the entry of HIV, HPV, and contribute with cervical neoplasia and cancer. Imidazole compounds are the usual therapy for trichomoniasis and, among them, the the most used is metronidazole. However, it has been demonstrated drug resistance and unwanted side effects in humans. A search for an alternative chemotherapy is essential for the treatment of this pathogen. Clotrimazole (CTZ), a commercial drug already used against fungi, could be a potential drug against trichomonas. The aim of this work was analyze the effect of CTZ conjugated with zinc salts in *T. vaginalis*. The growth curve of the parasites was performed using two compounds: ZnAcCTZ and ZnClCTZ and the IC50 of both are 5,11 µM and 10,31 µM, respectively. We investigated the cytotoxic effects of ZnAcCTZ and ZnClCTZ, at IC50 concentrations in *T. vaginalis* by transmission (TEM) and scanning (SEM) electron microscopies, and immunofluorescence, using anti-pyruvate ferridoxin oxireductase (Pfor) antibody. An important cytotoxic effect was observed in the parasites growth when both ZnAcCTZ and ZnClCTZ were used when compared with CTZ data. By immunofluorescence we observed an alteration in hydrogenosomes fluorescence signal. By SEM, rounding parasites and cell lysis were seen, as well as membrane blebbing. These results were corroborated by TEM. In addition, after treatment with ZnClCTZ, parasites showed an abnormal number of flagella and cytoplasmatic cytoskeleton structures, as pelta and axostyle complex, but without cell division, indicating failure in cytokinesis. Thus, both ZnAcCTZ and ZnClCTZ exhibited a strong chemotherapeutic behavior against *T. vaginalis*.
Supported by:CNPq, FAPERJ, CAPES and UFRJ
Keywords: Trichomonas vaginalis; chemotherapy; electron microscopy

TB034 - **LEISHMANICIDAL EFFECT OF 1.8 CINEOL, ALPHA-PINENE AND P-CYMENE OBTAINED FROM ESSENTIAL OIL OF BURSEACEA**

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Leishmaniasis are neglected diseases that affect more than 12 million people around the world. It is caused by protozoan of the genus Leishmania and present different clinical forms depending on their species. Leishmania amazonensis is the causative agent of cutaneous and diffuse cutaneous leishmaniasis in the Americas, causing ulcerations at the bite site, and may eventually spread to other parts of the body. The treatment used is highly toxic and expensive. Besides that, the severe side effects and the appearance of resistant parasites, stimulate the search for new substances with antileishmanial activity, including the prospecting of natural products. Previously, we showed that essential oil (EO) extracted from Burseacea were toxic for amastigotes and promastigotes forms and were not toxic for host cell in vitro. In this work, we evaluate the toxic effect of the three major components isolated (1.8 cineol, alpha-pinene and p-cymene) present in these EO. Amastigotes infected-macrophages were treated with different concentrations of these compounds for 24 hours. Our results showed that the three compounds were toxic for amastigotes in a dose-dependent manner. The highest concentration tested 75ug/mL of each one inhibited around 80% of amastigotes survival inside infected-macrophages and amastigotes killing assay showed 80% of amastigote death at 50ug/mL. Lipid analysis demonstrated that p–cymene-treated promastigotes present lipid metabolism alterations with a reduction on triacylglycerol, fatty acids and sterols. Electronic microscopy analysis on treated –promastigotes will be done to observe ultrastructural alterations. Nitric oxide (NO) production is an effective mechanism against the parasite, so we intend to dose the

NO of macrophages´s supernatants to observe if the compounds are able to modulate NO production by macrophages. **Supported by:**FAPERJ, CNPq e UFRRJ/PIBIC
Keywords: leishmaniasis; treatment; essentials oils

TB035 - **EARLY EFFECTS OF 2'-HYDROXYFLAVANONE AGAINST *LEISHMANIA AMAZONENSIS***

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Leishmaniasis is a disease that deserves attention due to the wide variety of clinical manifestations and its high annual incidence. Caused by different parasites of genus Leishmania, it has been considered a neglected disease, which affects more than 12 million people and has been reposted in 98 countries around the world. Currently, the treatment of leishmaniasis has presented serious problems as side-effects, variable efficacy, resistance and are expensive, leading to a new search of efficient compounds. Pure compounds obtained from plants have significant antiprotozoal activity. 2'-hydroxyflavanone is a flavanone, currently known to inhibit metastasis, vascularization and induce apoptosis in many types of cancer cells. Previously, we demonstrated the effect of 2'-hydroxyflavanone against Leishmania amazonensis promastigotes proliferation for 24, 48 and 72 hours. In order to determine when this 2'-hydroxyflavanone effects starts, 5 x 10^6 promastigotes of Leishmania amazonensis were incubated with different concentrations of 2'-hydroxiflavanone (12 – 96 µM). Viable cells were counted using Neubauer chamber every hour for 9 hours. 2'-hydroxyflavanone demonstrated a dose dependent inhibition profile from 3 hours of incubation with an IC_{50} of 21.59 µM, reaching 80% of inhibition at the highest concentration (96µM). ROS levels were measured using H2DCFDA, showing an increase of ROS levels, reaching 2.4 fold to control. Pre-incubation of L. amazonensis promastigotes with 300 µM of antioxidants N-acetyl cysteine and Glutathione did not protect the cells from the inhibition promoted by 2´-hydroxyflavanone. Taken together, these results demonstrate the early effects of 2'-hydroxyflavanone on promastigote forms of *Leishmania amazonensis*. **Supported by:**FAPERJ; CNPq; CAPES; PAPES; IOC/FIOCRUZ;
Keywords: Leishmaniasis; 2'-hydroxyflavanone; chemotherapy

TB036 - **EPIGALLOCATECHIN-3-GALLATE AFFECTS THE PROLIFERATION OF *LEISHMANIA INFANTUM* PROMASTIGOTES BY THE INHIBITION OF TRYPANOTHIONE REDUCTASE AND PRODUCTION OF REACTIVE OXYGEN SPECIES**

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Leishmaniasis is a disease caused by species of protozoan parasites of the genus Leishmania and its treatment has undesirable effects such as toxicity and drug resistance. Therefore, the WHO proposes the search for new drugs for this disease. The epigallocatechin-3-gallate (EGCG) is a flavonoid that has leishmanicide effect and capacity to generate reactive oxygen species. The objective of this study is demonstrate the effect of EGCG on *L. infantum* promastigotes proliferation, and if this effect is due to trypanothione reductase (TR) inhibition, essential enzyme for trypanosomatides survival. *L. infantum* promastigotes were incubated in the absence or presence of increasing concentrations of EGCG (15.6µM-500µM) for 72h and cell viability was estimated by AlamarBlue assay. The EGCG showed an inhibition of promastigote proliferation of 97% (500µM) presenting an IC_{50} of 192µM. To evaluate the production of H_2O_2, *L. infantum* promastigotes were incubated with increasing concentrations of EGCG (125µM-500µM) for 72h and H_2O_2 production was analyzed with Amplex Red reagent. The promastigotes showed a dose-dependent production of H_2O_2, reaching 9.5-fold increase compared to the control in a concentration of 500µM. The activity of TR was evaluated by the Ellman method. EGCG was able to inhibit TR in a dose-dependent manner presenting a Ki of 644µM. Linear correlations were observed between the percentage of inhibition of TR and the percentage inhibition of *L. infantum* promastigotes (R^2=0.96), the production of H_2O_2 and the percentage inhibition of *L. infantum* promastigotes (R^2=0.92). Furthermore, it was observed too, a linear correlation between production of H_2O_2 and percentage inhibition of promastigote (R^2=0.92). Taken together, these results may suggest that inhibition of TR by EGCG causes an increase in intracellular production of H_2O_2 leading to death of promastigotes of *L. infantum*, showing that EGCG can be a promising compound for treatment of visceral leishmaniasis.

Supported by:FAPERJ, CNPq, CAPES, PAPES, IOC/FIOCRUZ
Keywords: Leishmaniasis; epigallocatechin-3-gallate; trypanothione reductase

TB037 - **CELLULAR IMMUNE RESPONSE ANALYSIS IN DOGS WITH VISCERAL LEISHMANIASIS IN THE PRESENCE OF THE NEW RECOMBINANT ANTIGEN LCI10**

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The canine immune response against Visceral Leishmaniasis (VL) plays an important role in the control and treatment of the disease, as well as in resistance to re-infection. The Th1 response induces a resistance profile through macrophage activation, leading to parasitic destruction. A Th2 response favors parasite multiplication, through the inhibition of nitric oxide production. Proteins that stimulate a Th1 profile are being explored as vaccine candidates. The aim of this study is to evaluate the cellular immune response to new recombinant proteins in VL positive dogs, in order to support vaccine development studies. First, blood from VL positive dogs, from an endemic area of the Pernambuco state, were collected and used for separation of PBMCs. Cells from three dogs were pooled and then subjected to stimulation with the Lci10 antigen through a kinetics of concentration (2.5, 5, 10µg/mL) vs. time (24, 48 and 72 hours). For comparison, crude L. infantum soluble antigen (LSA) was used at 10 and 25µg/mL for the same time periods. After culturing, RNA extraction was performed, and the gene expression for cytokines of Th1 (IFN-γ, TNF-α and IL-2) and Th2 (IL-4, IL-10 and TGF-β) profiles was evaluated using the comparative CT method, after real time RT-PCR. The best results were generated using 5 µg/mL of the antigen. At 24h, expression for both Th1 and Th2 cytokines was lower than the one observed for the non-stimulated control and at 48h their levels were equivalent to the control. In contrast, after 72h of culturing, Lci10 was indeed able to induce a balanced and significant Th1/Th2 response. When compared with LSA in the same time point, the Lci10 antigen was able to decrease IL-10 expression and increase IFN-γ and TNF-α, leading to an increased Th1 profile. The recombinant Lci10, thus, induced a reversal of the deleterious (Th2) for a protective (Th1) immune response, proving to be a promising candidate to be used in immunomodulatory and vaccinology experiments.
Keywords: Canine visceral leishmaniasis; vaccinology; recombinant antigen

TB038 - **OVEREXPRESSION OF ABCG1 IN LEISHMANIA AMAZONENSIS ALTERS ITS STEROL HOMEOSTASIS AND SUSCEPTIBILITY TO AZOLES**

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Trypanosomatids have their own machinery for sterol synthesis (ergosterol). However, a high percentage of exogenous cholesterol is found in Leishmania spp. Transport and intracellular distribution of cholesterol and other sterols in their free form in Leishmania spp. is not clear. The ABCG1 transporter is implied with translocation of cholesterol and phospholipids in mammalian cells. The genome of Leishmania spp. has 42 genes belonging to ABC family. ABCG1 is in the genome of Leishmania spp., but its function has not been described. This project aims to study the role of ABCG1 in the maintenance of the sterol homeostasis in Leishmania amazonensis, correlating these findings to sensitivity to inhibitors of ergosterol biosynthesis. To generate overexpressing parasites, ABCG1 gene was cloned in the pGEMT vector using E. coli DH5α competent bacteria, subcloned inPSP72αNEOα vector and transfected in L. amazonensis. Gene sequencing and qPCR of gene expression were performed, confirming the process. No significant change in sterol profile of ABCG1 overexpressing parasites was observed in homeostasis conditions. Nevertheless, when the transfected parasites were exposed to ketoconazole, we observed a dose-dependent increase in cholesterol content in relation to the mock transfected parasites. This increase in cholesterol content was paralleled to an increase in the resistance to ketoconazole, with a raise of the IC50 from 4.5 uM in control parasites to 23.7

uM in ABCG1 overexpressing parasites. These results are suggestive of the involvement of ABCG1 in transport of exogenous cholesterol in Leishmania amazonensis.
Supported by:CNPq, CAPES, PAPES/FIOCRUZ, FAPERJ
Keywords: Abcg1; l. amazonensis; sterol biosynthesis

TB039 - **TREATMENT EVALUATION OF A TERPENE ISOLATED FROM LYCHNOPHORA PASSERINA IN MICE EXPERIMENTALLY INFECTED WITH TRYPANOSOMA CRUZI**

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Chagas disease (CD), originally present in Latin America, remains neglected and nowadays autochthone in other Continents. The only drug available to treat CD in BRA is benznidazole (BZ), which presents several limitations. This project aims the use of conventional formulations of LS Goyazensolida (Goya), previously active against Trypanosoma cruzi in vitro, isolated from L. passerina with the following objectives: to develop new formulations of Goya for oral administration, evaluate its toxicity in vitro and in vivo, and in addition its effectiveness against T. cruzi infection in murine model. For evaluation of cytotoxicity in “Vero” cells MTT was used, and for in vivo biochemical parameters were performed. Mice infected with T. cruzi Y strain were used for rapid test and treated with different doses of Goya, by oral route, for five consecutive days. The results obtained so far revealed toxicity above 350 ng/mL in "Vero" cells treated for 72h in the MTT test. There were no signs of nephrotoxic and hepatotoxic activity in the assayed doses of 50 and 100 mg/kg of Goya. Therefore a low toxicity "in vivo" was revealed by the low levels of creatinine, urea, AST and ALT parameters. Regarding the pilot analysis of the rapid test in mice treated for 5 days, it was observed a significant decrease of the parasitemia starting at 5th day of treatment with Goya at 0.5, 5.0 and 50.0 mg/kg/day compared to controls groups (INT, excipients and BZ treatments). The survival rates of mice 30 days after treatment with Goya were of 83% at doses 0.5 and 5.0 mg/kg/day and of 100% for Goya 50.0 mg/kg/day, as well for BZ. Control groups showed only 33% of survival rates. Treatments for 20 consecutive days with distinct doses of Goya are in progress. These findings represent a great perspective for treatment of CD and further new formulations involving nanocarriers will be explored in order to achieve better treatment efficacy. **Supported by:**FAPEMIG/REDE TOXIFAR-FAPEMIG, CAPES, CNPq and UFOP **Keywords:**Chagas disease; chemotherapy; terpene

TB040 - **EVALUATING THE EFFECTS OF DIFFERENT HISTONE DEACETYLASES INHIBITORS AGAINST *TRYPANOSOMA CRUZI***

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The protozoan *Trypanosoma cruzi* is the aetiological agent of Chagas disease, an endemic illness in Latin America. There are about 8 million people infected around the world, which encourages the development of new drugs against this parasite. In *T. cruzi* the level of chromatin condensation changes according to parasite’s evolutive stage, being more compacted in trypomastigotes than in the epimastigote form. The nuclear DNA organization is modulated by different enzymes, such as histone deacetylases (HDACs), and is directly related to replication, transcription, repair and gene expression. Based on that, HDACs have been used as chemotherapeutic targets. In this work, we evaluated the effects of new HDACs inhibitors (KV46, KV50 and KV30) in *T. cruzi* proliferation, viability and ultrastructure. For this purpose, parasites were treated with different concentrations of each compound and after 24 hours, samples were collected for counting on Neubauer’s chamber, for cellular viability assays, by MTS/PMS method and propidium iodide incorporation, and for transmission electron microscopy analyses. Our results showed that KV30 and KV46 were the most effective compounds against *T. cruzi* epimastigote proliferation, presenting an IC50 of 3 and 18 µM, respectively, after 48 hours of treatment. Whereas KV50 inhibited 50% of parasite proliferation only with 50 µM. Moreover, KV46 caused 50% reduction in the number of viable parasites after 72 hours of incubation. Regarding *T. cruzi* ultrastructure, KV46 caused mitochondrial swelling and intense cytoplasmic vacuolization with 5 µM for 24 hours. The compound KV50 promoted mainly mitochondrial swelling with 50 µM for the same time of treatment. The ultrastructural effects

caused by KV30 as well as the cellular viability of treated parasites are under experimental evaluation. Thus, our data obtained so far suggest that these HDACs inhibitors can be explored in further analysis in chemotherapeutic studies against *T. cruzi*. **Supported by:**CNPq e FAPERJ **Keywords:**Deacetylases; trypanosoma cruzi; chemotherapy

TB041 - **LEISHMANICIDAL ACTIVITY OF A SIRTUIN INHIBITORS-BASED COMPOUND SI2 AND SI38 IN LEISHMANIA AMAZONENSIS PROMASTIGOTE FORMS**

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Leishmaniasis are a group of neglected diseases affecting about 12 million people in the world, causing diverse cutaneous and visceral symptoms. The few leishmanicidal currently available have shown serious side effects and are giving rise to parasite resistance. In this context, it is relevant to identify new targets and boost the rational developing of new drugs. Sirtuins are broad group of NAD+ dependent protein lysine deacylases, which are involved in the epigenetic control of gene expression and the regulation of critical mitochondrial enzymes among others. Based on known sirtuin inhibitors, a collection of 45 substrate-related analogues (named as SI-1 to SI45) were designed and synthesized. The inhibitors were tested against Leishmania (L.) amazonensis promastigotes, and their effects were analyzed in terms of their ability to arrest the cell proliferation. The SI2 and SI38 exhibited promising dose-dependent profile with EC50 of 2 µM and 3.6 µM respectively. Both compounds have shown higher CC50 in mammalian cells (SI2: 15 µM and SI38: 121 µM) than the parasite, showing a promising selectivity index particularly in the case of SI38. The possible cell-death mechanism was also evaluated by incubating with Annexin V (AnV) - Propidium Iodide (PI) the cells previously treated with EC50 of each drug. Cytometry analysis showed that most of non-viable cells were stained with AnV but not with PI suggesting an apoptosis-like mechanism of death. Cytometry assays also showed that, when cells were treated with an EC50 of each drug, more than 70% of the cells remained unstained, indicating that a relevant population of treated cells were alive but unable to replicate. This fact led us to hypothesize that both, SI2 and SI38 are also able to alter the cell cycle. This possibility is currently being explored. Taking together, until now our results show that SI2 and SI38 are promising hits against L. amazonensis, and trigger an apoptosis-like cell death mechanism. **Supported by:**CNPq - FAPESP
Keywords: Leishmaniasis ; sirtuins ; epigenetic

TB042 - **THE MUTATION G133D ON *LEISHMANIA GUYANENSIS* AQUAGLYCEROPORIN 1 PROTEIN STRUCTURE IS HIGHLY DESTABILIZING AS REVEALED BY COMPUTATIONAL MOLECULAR MODELING AND HYPO-OSMOTIC SHOCK ASSAY**

TUNES, L.G.[*1]; ASCHER, D.B.[2]; PIRES, D.E.V.[1]; MONTE NETO, R.L.[1]

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The Leishmania aquaglyceroporin 1 (AQP1) plays an important role in osmoregulation and antimony (Sb) uptake, being determinant for resistance to antimonials. We have previously demonstrated that G133D mutation on *L. guyanensis* AQP1 (LgAQP1) leads to reduced Sb uptake. Here we investigated the effects of G133D point mutation on LgAQP1 structure associated with Sb uptake and alterations in its osmoregulation abilities. High confidence molecular models of LgAQP1 were constructed by Modeller and MacroModel. A model of LgAQP1::G133D was optimized using Maestro. Glycine 133 is on transmembrane helix 3 and buried in both open and closed conformation. Using two computational methods mCSM and DUET we have evaluated the effects on protein stability. G133D mutation was predicted to be highly destabilizing, particularly in the open conformation (ΔΔG = -2.126 and -2.027 kcal/mol against closed conformation ΔΔG = -1.827 and -1.707 kcal/mol, respectively). Concerning protein-protein affinity, G133D is also highly destabilizing to the AQP1 tetramer formation for both open (-1.619 kcal/mol) and closed (-1.805 kcal/mol) pore models, as revealed by mCSM-PPI analysis. We have also constructed a model with glycerol bound in the pore. The shift in helices upon G133D mutation resulted in slightly fewer favorable contacts with glycerol molecule in the channel, which would explain the reduced affinity for similar drug like small molecules as SbO3. When challenged under hypo-osmotic condition, *L. guyanensis* harboring AQP1G133D presented increased cellular volume in 3 times and pronounced delay to recover osmosis homeostasis when compared to the wild-type, a profile that was enhanced in LgAQP1-/-

mutants. In conclusion, G133D is a high disruptive mutation that will greatly destabilize the monomer, compromise tetramer formation, alter pore conformation, leading to reduced Sb uptake and defects in osmoregulation. **Supported by:**CNPq; CAPES; FAPEMIG; Newton Fund; ISID
Keywords: Leishmania guyanensis; aquaglyceroporin 1; drug resistance

TB043 - **EFFECT OF DM01 AND MEM02 COMBINED THERAPY FOR IN VITRO AND IN VIVO INFECTION WITH L. AMAZONENSIS.**

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Caused by different parasites of genus Leishmania sp., Leishmaniasis has been reported in 98 countries and affects more than 12 million people around the world. Leishmaniasis current treatment is based on pentavalent Antimonials and Amphotericin B. However, they are expensive, ineffective and can bring resistance and side effects. The need for a cheaper and safer chemotherapy has been increasing researches for effective natural products and drug combination using natural products has been studied as a new alternative chemotherapy. In this present study, we evaluated the effect combination therapy with DM01, a natural product present in common fruits and vegetables and MEM02 in vitro in intracellular amastigote and in vivo in murine model of cutaneous leishmaniasis. To investigate the interaction of DM01 with the MEM02, L. amazonensis-infected THP-1 were incubated for 72h with different concentrations of DM01 combined with MEM02. The IC50 of the drugs alone, and IC50 of the combinations were used to generate an isobologram. The fractional inhibitory concentration (FIC) of the combination of DM01 with MEM02 was 1.6 showing an additive effect. For in vivo studies, DM01 (2mg/kg/day, every day), MEM02 (8mg/kg/day, every day) and the combination of DM01 with MEM02 (1mg/kg/day and 4mg/kg/day, every day) were administered orally. Combination of DM01 and MEM02, at doses corresponding to half the dose used in both monotherapy of DM01 or MEM02, was able to control the lesion size and reduce the parasitic load. Toxicological analysis demonstrated no changes in biochemical and hematological parameters. These characteristics are encouraging and suggest the combination of DM01 with MEM02, as a possible prototype for the clinical treatment of cutaneous leishmaniasis.
Supported by: Faperj, CNPq, PAPES, CAPES, IOC/Fiocruz
Keywords: Leishmaniasis; assosiation; apigenin

TB044 - **OVEREXPRESSION AND FUNCTIONAL ANALYSIS OF NUCLEOSIDE DIPHOSPHATE KINASE B AND ELONGATION FACTOR 2 IN *LEISHMANIA* *BRAZILIENSIS***

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Nucleoside diphosphate kinase b (NDKb) is a ubiquitous enzyme that catalyzes the transfer of phosphate group from a nucleoside triphosphate to a nucleotide diphosphate and it has key role in the purine metabolism in trypanosomatid protozoa. Elongation factor 2 (EF2) encodes a member of the GTP-binding translation elongation factor family and it is an important factor for synthesis of proteins. In this study, NDKb and EF2 genes were transfected in antimony (Sb^{III})-susceptible *Leishmania Braziliensis* line to determine whether overexpression of these proteins contributes to antimony resistance phenotype in this parasite. In addition, we investigated the effect of lamivudine and eukaryotic elongation factor 2 kinase (eEF2K) inhibitor on Sb^{III} susceptibility in parasites that overexpress the NDKb and EF2 genes, respectively. Western blot results showed that the expression levels of NDKb and EF2 proteins were 1.9 to 4.4-fold higher in the NDKb- or EF2-overexpressing *L. Braziliensis* lines than their respective wild-type (WTS) pair. Furthermore, these lines were also 1.5 to 2-fold more resistant to Sb^{III} in comparison to the untransfected WTS line. Susceptibility tests demonstrated that NDKb-overexpressing clones presented elevated resistance to lamivudine. However, this antiviral agent did not alter the leishmanicidal activity in association with Sb^{III}. EF2-overexpressing parasites were slightly more resistant to eEF2K inhibitor than the WTS line. Surprisingly, the combined treatment of Sb^{III} with this inhibitor enhanced the leishmanicidal activity against both *L. Braziliensis* lines compared to those incubated with Sb^{III} or eEF2K inhibitor alone. This result suggests that this association may be a valuable strategy for leishmaniasis chemotherapy. Our data represent the first study of NDKb and EF2 genes overexpression that shows an increase of Sb^{III} resistance in *L. Braziliensis* which can contribute to develop new strategies for leishmaniasis treatment.

Supported by:CNPq; FAPEMIG; UNICEF/UNDP/World Bank/WHO; PROEP/CNPq/FIOCRUZ
Keywords: Nucleoside diphosphate kinase b; elongation factor 2; antimony resistance

TB045 - **EFFECT OF N'N'-DIARYLUREAS IN LEISHMANIA AMAZONENSIS PARASITES.**

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Phosphorylation of the translation initiator factor eIF2a leads to an attenuation of translational process. eIF2a phosphorylation is mediated by four kinases, one of them is the protein kinase R-like kinase (PERK). It was reported that elF2a phosphorylation trough PERK activation inhibits the L. amazonensis differentiation from promastigotes to amastigotes forms. N'N'-Diarylureas are potent activators of the eIF2á kinases. Due to this ability, such compounds have been tested in the control of tumor cell proliferation by attenuating translation and inducing apoptosis. We have been studying the effects of one variation of N'N'-Diarylureas, the I-17 compound, in the viability of Leishmania parasites. Since these drugs were defined as important activators of eIF2α, this may result in attenuation of parasite translational process and may affects Leishmania differentiation. In infection index assays we observed a reduction of parasite proliferation in RAW 264.7 cells suggesting an effect of I-17 in the control of the infection. Puromycin incorporation assay in L.amazonensis and RAW 264.7 treated with I-17 followed by the treatment with puromycin (10 uM) showed a reduction of the puromycin incorporation. Puromycin inhibits the elongation of the translational process. The inhibition of the puromycin incorporation demonstrates that the compound may acts in the attenuation of protein synthesis, which may affects Leishmania infection. Additionally, in Griess assays we observed that RAW 264.7 cells treated with I-17 and infected with L. amazonensis showed an increase in nitric oxide (NO) production. The mechanism that leads to an increase in NO after I-17 treatment needs further investigations. As future perspectives, in vivo experiments with the I-17 compounds in mice treated with I-17 and infected with L. amazonensis will be proceed to evaluate the effects of this compound in the pathogenesis of L. amazonensis.

Supported by:cnpq
Keywords: Eif2alpha phosporilation; n,n-diarylureas; l. amazonensis

TB046 - **TRYPANOCIDAL EFFECT OF DIFFERENT SAMPLES EXTRACTED FROM *BACHARIS* SPP.**

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Trypanosoma cruzi causes Chagas disease and affects about 10 million people worldwide. Currently only two nitro-heterocyclic drugs are used to treat the disease, which have many side effects and are ineffective in the chronic phase of the disease. Therefore there is a search for new effective drugs, including obtained from plants. In this study we evaluated the trypanocidal and cytotoxic *in vitro* effects of 39 crude extracts, 9 essential oils, and 7 pure compounds obtained from *Baccharis* spp. *T. cruzi* (Y strain) epimastigotes were grown at 28oC in LIT + 10% FBS for three days and 5x106 parasites/mL were incubated with vegetal compounds (10 to 500 µM or µg/mL). For cytotoxicity assay 3T3 fibroblast cells were seeded on 96 well plates and incubated with 10-500 µM or µg/mL samples in DMEM + 10% FBS. After 48h the toxic effect was evaluated by MTT. Non cytotoxic trypanocidal samples were also evaluated against intracellular amastigotes. Screening showed IC50 from 49 to 438µg/ml for the extracts or essential oils, and 315 to 320µM for pure compounds. The essential oil extracted from *B. pentodonta* female inflorescence (PIF) showed the best trypanocidal activity (IC_{50} 49µg/mL). Essential oils extracted from leaves of *B. pentodonta* female (PLF) and PIF were at least four times more toxic to parasites than to 3T3 cell. Both essential oils (PIF and PLF) were also active against *T. cruzi* intracellular amastigotes, especially the essential oil of the leaves (PLF) that reduced 90% parasite load at 11.25µg/mL. These results show that essential oils obtained from leaves and flowers of *B. pentodonta* female plant have a potential trypanocidal effect. Isolation of the active compounds may bring new perspectives to combat *T. cruzi* infection.

Supported by:CNPq, Fundação Araucária, UEPG

Keywords: Trypanosoma cruzi; trypanocidal activity; baccharis spp

TB047 - **EFFECT OF A 7-CHLORO-4-QUINOLINYLHYDRAZONE DERIVATIVE AGAINST *LEISHMANIA AMAZONENSIS***

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Leshmaniasis chemotherapy has been hampered by significant toxicity, long-term therapy, high costs and drug resistance, making the development of new treatment strategies an urgent need. In this study, we evaluated the effect of a novel 7-chloro-4-quinolinylhydrazone derivative against promastigote and intracellular amastigote forms of L. amazonensis and a series of biological assays were performed to determine its feasible mechanism of action. Antipromastigote activity and cytotoxicity effects against murine macrophages were determined by MTT colorimetric assay. The hemolytic activity in human red blood cells was also evaluated and hemoglobin was quantified sprectrophotometrically. Antiamastigote assay was evaluated in macrophages infected with *L. amazonensis* transfected with green fluorescent protein (GFP). To evaluate the effects of the derivative on the mitochondrial function of promastigote, we measured the mitochondrial membrane potential using the fluorescent probes JC-1 and Mitotracker and the reactive oxygen species (ROS) production using a fluorescent probe, H_2DCFDA. This quinoline derivative showed a significant activity against promastigote and amastigote forms of L. amazonensis (IC_{50} values of 52.5 and 8.1 µM, respectively). The compound exhibited low cytotoxicity to murine macrophages and human erythrocytes and induced an oxidative imbalance in promastigote forms, reflected by an increase in the formation of ROS and a marked reduction of mitochondrial membrane potential. No alterations in the plasma membrane integrity of parasites were observed after propidium iodide (PI) labeling. Taken together, these results demonstrate that the compound is a selective antileishmanial agent, and preliminary observations suggest that its effects are mediated by mitochondrial dysfunction followed by ROS production. **Supported by:**CAPES, FAPEMIG, CNPq and UFJF
Keywords: Leishmania amazonensis; quinolinylhydrazone derivatives; mitochondrial dysfunction

TB048 - **TRYPANOCIDAL ACTIVITY OF 2-HYDROXY-3-PHENYLSULFANYLMETHYL- [1,4] -NAFTOQUINONE DERIVATIVES.**

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Globalization of Chagas disease draws attention to this neglected tropical disease that remains a major public health problem in Latin America. The current treatment available, introduced five decades ago, has limited effectiveness and serious adverse effect. Unsatisfactory results of benznidazole (Bz) efficacy in chronic patients and the therapeutic failure of azolederivatives in phase 2 clinical trials highlight the urgent need to identify new effective and safe drugs. In this study, we evaluated the trypanocidaleffect of the series 2-hydroxy-3-phenylsulfanylmethyl- [1,4] -naftoquinones against different *T. cruzi* genetic lineages. A total of 14 compounds was screenedfor their efficacy against both bloodstream and cell culture-derived trypomastigotes (2h and 24h of treatment) and also intracellular amastigotes (72h of treatment). Hepato- and cardiotoxic effect (CC50) as well as the viability of trypomastigotes (IC50) after compound treatment were evaluated by CellTiter-Glo® assay. The effect against intracellular amastigotes (IC50) was determined by counting after Giemsa staining and luciferase assay. Low cytotoxicity of the compounds was shown in mammalian cells, reaching CC50>100µM in most compounds analyzed. Among these synthetic compounds screened against trypomastigotes, four compounds (10, 11, 12 and 13) were effective in short-term treatment (2h; IC≤49.3µM) and 2 compounds (2 and 11) were more active than Bz (IC50=29.8µM) after 24 hours of treatment (IC50 ≤10µM). Ultrastructural analysis revealed drastic changes in the intracellular compartments of trypomastigotes treated with both promising candidate compounds (2 and 11). Compound 2 showed better activity against intracellular amastigotesand fits the criteria for a hit

compound against Chagas' disease. Further studies will be carried out to evaluate the efficacy of compound 2 in an experimental murine model of acute *T. cruzi* infection.
Supported by: Fiocruz, CNPq, PAPES FAPERJ
Keywords: Naphthoquinones; chagas disease; trypanosoma cruzi

TB049 - **QUINONES AS SPECIFIC INHIBITORS OF COMPLEX I, II OR III OF THE RESPIRATORY CHAIN AND REDOX METABOLISM IN LEISHMANIA INFANTUM**

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Quinones may induce redox cycle and oxidative stress or act as inhibitors of mitochondrial respiratory complexes. In this present study, we evaluated new naphthoquinones (LQBs) for their ability to induce mitochondrial changes in L. infantum and to inhibit complexes I, II or III of isolated mitochondria from murine liver. LQBs showed good leishmanicidal activity with IC_{50} less than 2.5 µM and IC_{90} less than 4 µM. All prototypes altered the parasite mitochondrial activity by accelerating reduction of resazurin and induced changes in mitochondrial membrane potential (ΔΨm). LQBs 149, 168, 182, 187, 222 and 236 also induced a significant increase in ROS production. In addition, we evaluated the respiratory activity on intact parasites in oxygraph. However, no significant changes were induced by any of the evaluated prototypes. The intracellular redox control in trypanosomatids is based on trypanothione reductase (TR), an important enzyme for the detoxification of ROS. LQBs also demonstrated the ability to inhibit the activity of TR. In an attempt to elucidate the selectivity of these substances, we assayed the activity of mitochondrial complexes isolated from murine liver. Our results suggest that LQBs 18, 32 and 168 may be inhibiting the complex I, LQBs 149 and 222 the complex II, the LQB 182 and 236 complexes III or IV and LQB 187 complex III in the highest concentrations. We conclude that all LQBs have the ability to induce mitochondrial changes in the parasite, though the mechanism of action on the parasite remains to be elucidated.
Keywords: Quinones; leishmania; mitochondria

TB050 - **EVALUATION OF THE POTENTIAL ANTILEISHMANIAL OF PLANT IN THE ANNONACEAE FAMILY**

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Leishmaniasis is a group of neglected tropical diseases, endemic in 88 countries that affect more than 12 million people. Current drugs are limited due to their toxicity, development of biological resistance. This study aims to evaluate the potential of leishmanicide of Annonaceae family plants (Annona sylvatica, A. crassiflora, A. muricata, A. coriacea, A. neolaurifoli , A. atemoya, Rollinia dolabripetala, and Xylopia aromatic). 50 mg of leaves or seeds of the these mentioned species were subjected to organic extraction with a combination of 1.0 mL of KH2PO4 buffer solution in Milli-Q H2 O (pH = 6.0) and 1.0 ml of methanol. Fifteen extracts were evaluated for their ability to prevent the proliferation of amastigotes of Leishmania infantum within DH82 canine macrophages. The inhibitory activity of these extracts was measured by ELISA. The cytotoxicity of the active compounds was assessed by analysis of metabolic activity by the MTT test. The extracts from the A. muricata and A. atemoya leaves inhibited the growth of intracellular amastigotes of L. infantum with the following values: IC 50 of 15 ug /ml and 19 ug / ml, respectively.
The IC 50 of the positive control, antimony III, was 97 mg/ml. The cytotoxicity values (LD 50) was 38.7 mg/ml for A. muricata and 4.2 mg/ml to A. atemoya and antimony III 68mg/ml. According to the literature, the Annonaceae family plants are rich in various metabolites, such as: acetogenins, trigoneline, quercetin and rutin, and has had some type of biological activity against protozoa, helminths and bacteria. The results presented here in this study demonstrate the potential of Annonaceae family plants in the development of drugs for treatment and control of Leishmaniasis, but more studies are underway for obtaining pure fractions, characterization of these extracts and identification of metabolites, contributing to the achievement of more effective doses and less cytotoxicity.
Keywords: Annonaceae ; leishmaniasis; drugs

TB051 - **THERAPEUTIC EFFECT OF EPIGALOCATECHIN-3-GALATE IN MURINE MODEL OF EXPERIMENTAL VISCERAL LEISMANIASIS BY ORAL ROUTE**

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Leishmaniasis is a disease caused by parasitic protozoans of the genus *Leishmania*. It is endemic in more than 98 countries and 350 million people live in a risk of infection. In this context, visceral leishmaniasisis the most severe form of leishmaniasis and is the second major cause of death by parasites.The control of this disease remains a problem,the current treatment is based on pentavalent antimonials, amphotericin B and miltefosine. However this chemotherapy induces many side effects and resistance to treatment.The epigallocatechin-3-gallate (EGCG) is a flavonoid, which has anti-inflammatory, microbicidal and trypanocidal activities. In this study, we demonstrated the therapeutic efficacy of EGCG in amastigotes forms of *L. infantum* as well its effects in experimental visceral leishmaniasis. Murine macrophage was infected with promastigote forms of *L. infantum* and after 5h, the *L. infantum*-infected macrophages were then incubated in the absence or in the presence of EGCG (0 – 48µM) for 72 h. Treatment with EGCG reduced the infection index in a dose-dependent manner with an $IC_{50}$of 3.6µM, reaching99.8% of inhibition at the highest dose tested (48µM). For evaluated the efficacy of EGCG in murine model of experimental visceral leismaniasis, female Balb/C were infected with promastigotes forms of *L. infantum*, after seven days, the treated was initiated with EGCG 25mg/kg twice for day by oral route for only five days and eighteen days after the end of treatment the animals were euthanized. EGCG inhibited 44% and 86% of the parasitic load in the liver and in the spleen, respectively. Taken together, our findings indicate that EGCG is effective against murine model of experimental visceral leismaniasis, suggesting that this compound could be a novel alternative for visceral leishmaniasis treatment. **Supported by:**FAPERJ; CNPq; CAPES; PAPES; IOC/FIOCRUZ
Keywords: Epigalocatechin-3-galate; visceral leishmaniasis; therapeutic effect

TB052 - **ESTABLISHMENT OF A NEW METHOD BASED ON FLOW CYTOMETRY WITH THE POTENTIAL APPLICATION IN SERODIAGNOSIS OF CHAGAS' DISEASE**

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Chagas disease is caused by the protozoan parasite Trypanosoma cruzi. The T. cruzi infection is usually controlled by a highly effective immune response, which does not eliminate the parasite from the vertebrate host, resulting in a low parasitemia and persistent tissue parasitism. In this work we proposed the validation of a new serodiagnosis methodology based on flow cytometry. This methodology has the potential to be used in the specific diagnosis of T. cruzi infection and could also be optimized to identify co-infections. To standardize this methodology, three recombinant proteins derived from the MASP family (mucin-associated surface protein), which is expressed in trypomastigotes of T. cruzi, were coupled to fluorescent microspheres. To confirm the coupling, anti-MASP sera produced in BALB/c mice were used. The analysis was performed using the MFI calculation and anti-MASP sera showed much higher values compared to the negative control, indicating that the coupling was successful. We have also performed two tests with these MASP recombinant proteins coupled to different fluorescent microspheres and with pools of sera from mice infected with T. cruzi CL Brener strain. In the first test, each individually coupled MASP was incubated with the sera. In the other one, all MASPs coupled to beads with different fluorescence, forming a mix, were incubated simultaneously with the sera. The profile of reactivity was very similar in both tests, indicating that both methods are effective and could potentially to be used in the serodiagnosis of Chagas disease. In addition, this result also suggests that this methodology could be optimized to identify co-infections by coupling antigens derived from different pathogens in distinct fluorescent beads. **Supported by:**CAPES, FAPEMIG, CNPq

Keywords: Diagnosis; beads; flow cytometry

TB053 - **TREATMENT WITH BENZNIDAZOLE, ITRACONAZOLE AND ITS ASSOCIATION OF THE EXPERIMENTAL TRYPANOSOMA CRUZI INFECTION IN DOG MODEL.**

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Chagas disease (CD) is a serious public health problem in Latin America and its treatment remains neglected. The benznidazole (BZ), the only drug available in BRA presents serious side effects and low therapeutic efficacy. After clinic assays have demonstrated that the first generation of azole compounds was not able to promote parasitology cure and block CD progression, the study of these compounds associated to BZ and nifurtimox have been stimulated. This study evaluated the parasitological profile of dogs infected with VL10 *T. cruzi* strain, resistant to BZ, treated during acute phase, and the therapeutic action of the isolated drugs BZ and itraconazole (ITRA) and its association BZ+ITRA. Thus, 20 young mongrel dogs of both sexes, with15-20 kg/body weight, born and maintained at Animal Science Center/UFOP were used. The dogs were inoculated with 2.0 x 103 blood trypomastigotes/kg body weight, via IP and divided in 4 groups [treated with BZ: 7mg/kg in 2 daily doses; treated with ITRA: 18 mg/kg/day; treated with BZ+ITRA at the same posologies of the monotherapy; infected not treated control group (INT)]. The treatment started on the first day of patent parasitemia for 60 days. For post-treatment evaluation the animals were submitted to fresh blood exam, hemoculture (HC), PCR and conventional serology (ELISA). Clinical aspects and mortality were recorded daily and the laboratorial tests at 1, 6, 12 and 18 months after treatment. The comparison of parasitemia between the groups treated with BZ and BZ+ITRA showed significant decrease in relation to INT, and that the dog B7 of BZ+ITRA group and the dog B4 of ITRA group were negative in all parasitological exams, but positive in ELISA. These findings suggest that these animals are in process of cure or were cured, and that ITRA alone and/or in association with BZ lead to better therapeutic efficacy than BZ. After necropsy heart tissue qPCR highly sensitive will be performed in order to better evaluate all animals.
Supported by:CAPES
Keywords: Chagas disease; treatment; dog model

TB054 - **ORAL TREATMENT OF EXPERIMENTAL CUTANEOUS LEISHMANIASIS WITH A SELECTIVELY ACTIVE SEMISYNTHETIC EUGENOL DERIVATIVE.**

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The conventional treatment of cutaneous leishmaniasis is based on multiple and painful injections with toxic drugs. In this study, we propose to develop an oral treatment for this disease using a semisynthetic eugenol derivative named PG-R33 (4-(3- (4-allyl- 2-methoxyphenoxy)propyl)-1- (4-methylbenzyl)- 1H-1,2,3- triazole). In vitro, PG-R33 activity tested against Leishmania amazonensis promastigotes for 72h at 26 ^{o}C showed IC 50 = 7.5 µM. Activity against intracellular amastigotes was IC 50 = 1.6 µM. The selectivity index (anti-amastigote IC 50 / anti-macrophage CC 50) for PG-R33 and the reference drugs Pentamidine and Glucantime was 132.5, 4.7 and 1.6, respectively. ADMET analysis satisfied Lipinski's five rules and showed 99% probability of human intestinal absorption. For in vivo studies, BALB/c mice were infected in the ear with *L. amazonensis*-GFP and on day 7 received PG-R33 orally (40 mg/kg, 5x/week) or intralesionally (i.l.) (1,5mg/kg, 1x/week). Controls received Glucantime (1,5mg/kg, i.l. 1x/week). The lesion sizes and parasite loads were measured with a dial calliper and fluorimetry, respectively. Toxicological parameters (AST, ALT and creatinine) were measured in the serum using commercial kits. On day 50 of infection, oral PG-R33 was as effective as i.l. Glucantime in preventing lesion development, and reduction in the parasite burden. No toxicological changes were observed at the end of the experiment. Together, these results showed that PG-R33 is more selective than the reference drugs, and orally active against cutaneous leishmaniasis.**Supported by:**CNPq
Keywords: Antisleishmanial; eugenol; clove

TB055 - **IDENTIFICATION OF POTENTIAL NEW TARGETS FOR LEISHMANIASIS AND CHAGAS DISEASE**

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Leishmania spp. and Trypanosoma cruzi are etiologic agents of human diseases of worldwide distribution and impact. Researches focused on the development of new treatments for these diseases suggest cytochrome P450 complex enzymes (CYP450) as promising therapeutic targets. These enzymes are involved in several metabolic pathways, such as metabolism of sterols and xenobiotics. CYP51 of Trypanosoma spp. and Leishmania spp., for instance, has been pointed as a target for chemotherapy. The availability of several complete genomes of trypanosomatids allows robust comparative, evolutionary, and functional analyses, offering new opportunities to better understand important biological processes of these organisms. Here, we aim to perform a comprehensive identification and characterization of CYP450 enzymes encoded in the genomes of Trypanosoma and Leishmania species, in order to disclose new putative targets for leishmaniasis and Chagas treatment. Shortly, the set of CYP450 enzymes cataloged in the CYPED database (approx. 41,000 sequences) was compared to a genomic dataset comprising 7 Leishmania species and 7 Trypanosoma species. In a different experiment, the set of human CYP450 enzymes retrieved from the Swiss-Prot database (53 sequences) was compared to 6 species of Leishmania (included in the previous dataset). In both cases the genomic data were obtained from the TriTrypDB. All comparisons were performed with the BLAST algorithm. In the first experiment, 116 statistically significant hits ($E \leq 0.001$), covering all trypanosomatid species in our dataset, and comprising 9 different families of CYP450, were selected for further analyzes. The second experiment revealed at least 4 putative homologs of human CYP450 enzymes encoded in each Leishmania genome analyzed.
Supported by:FAPERJ, CAPES, CNPq e PLATAFORMA DE BIOINFORMÁTICA DA FIOCRUZ RPT04A
Keywords: Cyp450; trypanosomatids; therapeutic target

TB056 - **INTERACTION OF THE TRYPANOSOMA BRUCEI CAP-BINDING PROTEIN TBEIF4E2 WITH A HISTONE-MRNA BINDING PROTEIN HOMOLOG**

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The control of gene expression in Trypanosomatids is particularly unusual and mediated by post-transcriptional events such mRNA processing, half-life control and selection for translation. In higher eukaryotes, the initiation of mRNA translation is promoted by proteins known as eukaryotic Initiation Factors (eIFs). One of these is the eIF4F complex, which enhances the recognition of mRNAs by the ribosome for their translation. eIF4F is formed by the subunits: eIF4E, the cap binding protein; eIF4G, a scaffolding protein; and eIF4A, a RNA helicase. The eIF4E subunit binds to the cap structure found in mRNAs and is essential for mRNA stabilization and selection for translation initiation. Curiously, six eIF4Es are found in Trypanosomatids (named EIF4E1 to 6), with different molecular properties and participating in distinct eIF4F-like complexes, but with their role in mRNA selection for translation still unclear. To date EIF4E3 and EIF4E4 seem to be the most suitable for functioning in general translation, while EIF4E1 seems to have a role in translation repression EIF4E5 and EIF4E6 may be involved in mRNA localization and metabolism in the stress response. EIF4E2 is less well known, but our results indicate that its expression is controlled during growth culture and that it binds to mature mRNAs. Here we also report the description of a new partner for TbEIF4E2, identified as one of two putative Histone mRNA binding protein (HBP) whose genes are found in the genome of different Trypanosomatids. This protein, named here TbHBP2, is a 47.90 kDa protein with a conserved mRNA binding domain found in typical HBPs but apparently with no evolutionary relationship with the HBP2 found in metazoans. This new complex points to the direction of possible selective mRNA recognition mediated through the binding of the new eIF4E partner to a subpopulation of mRNAs which would mark them for translation activation or repression and which may be important for cell differentiation. **Supported by:**CNPq, Fiocruz

Keywords: Eif4e; hbp; translation initiation

TB057 - **EVALUATION OF IMMUNE RESPONSE *IN VITRO* AND THERAPEUTIC EFFICACY IN MICE INFECTED WITH *LEISHMANIA INFANTUM* TREATED WITH A MIXED FORMULATION OF CONVENTIONAL AND PEGYLATED LIPOSOMES CONTAINING MEGLUMINE ANTIMONIATE (GLUCANTIME®)**

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Pentavalent antimonial has been the first choice treatment for visceral leishmaniasis in BRA. However, it has several side effects that leads to low adherence to treatment. In this context, the use of liposome-encapsulated meglumine antimoniate (MA) arise as an important strategy for chemotherapy enhancement. The aim of this study was evaluate the cell-immunity and therapeutic efficacy of a mixed formulation of a conventional and pegylated liposome containing MA in BALB/c mice. The mice were infected intravenously with 1x107 promastigotes of L. infantum and a single dose treatment regimen was performed. The experimental groups (n = 12) were divided as follows: 1) Control [PBS]; 2) Empty Liposomes [Empty Lipo]; 3) Free MA, 20 mg/kg [MA]; 4) Liposomal formulation of MA (20 mg/kg) consisting of mixed conventional and pegylated liposomes [Lipo MA]. The treatment regimens were administered 14 days post-infection (d.p.i) and the euthanasia was performed 28 d.p.i. The in vitro stimulation of splenocytes with soluble antigens of L. infantum (SLA) showed a significant increase of IFN-γ-produced by CD8+ T-cells and a decreased of IL-10-producing by CD4+ and CD8+ T-cells in the Lipo MA compared to the PBS group. Moreover, Lipo MA animals showed an increase in the IFN-γ/IL-10 in CD4+ and CD8+ T-cells when compared to the PBS group. The therapeutic efficacy was evaluated by qPCR in spleen and we observed a positivity of 41.7%, 50.0%, 25.0%, and 0.0% in the PBS, Empty Lipo, MA and Lipo MA groups, respectively. In the liver, the percentages were 83.3%, 100.0%, 83.3%, and 41.4%, respectively to the PBS, Empty Lipo, MA and Lipo MA groups. Thus, Lipo MA group had a significant decrease of parasite burden in the spleen and liver compared to the other groups. Our results demonstrated that the Lipo MA is a promising antileishmanial formulation that induces a type 1 immune response with an important reducing in the parasite burden in the spleen and liver of treated mice.â€‹**Supported by:**FAPEMIG, PPSUS, CNPq, CAPES, UFOP, INCT-DT.
Keywords: Leishmania infantum; liposome; pentavalent antimonial

TB058 - **LIVE ATTENUATED *LEISHMANIA INFANTUM* AS PROMISING VACCINE CANDIDATE AGAINST LEISHMANIAIS**

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Vaccination is the most suitable approach to prevent leishmania infection and there is no safe human vaccine against leishmaniasis available. Live attenuated *Leishmania* strains have been used as strategy for immunization. In order to obtain an attenuated Leishmania parasite for vaccine purposes we selected gene candidates essential for the amastigote stage to be disrupted by gene knockout (KO). We performed KO of LiVCG3 – stands for *L. infatum* vaccine candidate gene 3, a target that was previously associated with growth defects in amastigote stage – by two rounds of conventional homologous gene replacement. Neomycin and hygromycin were used as resistance markers on KO cassettes constructed by fusion PCR approach. LiVCG3$^{-/-}$ mutants were confirmed by PCR, and LiVCG3 was present only in *L. infantum* wild type and not in LiVCG3$^{-/-}$. KO cassette integration was confirmed by PCR, using primers that anneals within the cassette sequence and in a chromosomal flanking sequence. All six mutant clones presented the fragment with expected length, suggesting that both cassettes were correctly integrated. Gene copy number was quantified by RT-qPCR and results showed that there is no copy of the target gene in LiVCG3$^{-/-}$ mutants, suggesting that VCG3 was successfully deleted. We also evaluate the fitness of LiVCG3$^{-/-}$. Although there is no difference in promastigotes growth, LiVCG3$^{-/-}$ amastigotes exhibit a cytokinesis defect and wasn't able to sustain the infection in THP1 macrophages, forming multinucleate cells. After 7-days of incubation we observed a reduction of 38% on LiVCG3$^{-/-}$-infected macrophages and a decreased of amastigotes/macrophage ratio of 91%. Despite VCG3 gene being related with glucose uptake in Leishmania, LiVCG3$^{-/-}$ mutants were equally sensitivity to antimony when compared to the wt counterpart. LiVCG3$^{-/-}$ mutants are promising vaccine candidates and we are performing *in vivo*

experiments to evaluate if immunizations resulted in significant protection. **Supported by:**CNPq; CAPES; FAPEMIG; ISID
Keywords: Leishmania; vaccine; live attenuated

TB059 - **EVALUATION OF CYTOKINE PROFILE PROMPTED BY DIFFERENT ADJUVANT SYSTEMS IN THREE DIFERENT DOSES**

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An effective early innate immune response to vaccine adjuvants may significantly impact the overall vaccines immunogenicity and efficacy. In this context, adjuvants are important additives, since they enhance the antigen immunogenicity inducing an intense and prolonged immune response. Nowadays, another approach in adjuvants field has been tested, using systems of 2 or more adjuvants associations, looking for a better response than with the adjuvants alone using a lower dose of adjuvants and antigens. The skin is considered an important route of immunization and the intradermal route has the advantage of delivering the antigen and maturation factors of dendritic cells in the compartment in which they reside, which makes the antigen presentation faster and, consequently, migration to draining lymph nodes. Herein, the objective of this work was evaluated the cytokine levels (IL-17, IL-2, IFN-□, TNF-□, IL-4 and IL-10) in the skin induced by adjuvants associations at 48 hours after sensitization. The following adjuvant systems: Saponin + Monophosphoryl Lipid A (SM), Saponin + Resiquimod (SR), Monophosphosryl Lipid A + Resiquimod (MR) and Saponin + Monophosphoryl Lipid A + Resiquimod (SMR) in three different concentration ratios. Animals inoculated with saline as a control group and with adjuvants alone as an internal control were included. All adjuvants systems were able to induce increased levels in the pro-inflammatory cytokines (IL-2, IFN-□ and TNF-□□ at 48h after sensitization when compared to the control group. In addition, the triple association (SMR) showed a little production of IL-4. Based on these results we can conclude that the cytokine profile production by these associations induce preferably a type 1 than a type 2 cytokine profile, except SMR association that resulted mix response. Taken together our results, we can conclude that the choice of the correct adjuvant systems with the right dose can be more efficient than adjuvant alone. **Supported by:**CNPq, FAPEMIG, CAPES, UFOP **Keywords:**Adjuvant systems; vaccine; cytokines

TB060 - **SEAWEED EXTRACT REDUCE THE GROWTH OF TOXOPLASMA GONDII ON HOST CELLS**

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Studies with seaweed have been the focus of many researchers because they are sources of sulfated polysaccharides. This substance is currently one of the most used for production of anticoagulants. The structures of these compounds can diversify with the type and location of the plant. They are complex compounds because they show distinct combinations of monosaccharides and sulfate group distribution. Studies show that this sulfated compound is active on growth of T. gondii, the agent of toxoplasmosis, reducing the same. Here, experiments were conducted in order to verify the activity of a fraction of the extract obtained from the algae Dictyota caribaea against T. gondii. For this purpose, interactions between parasites in its tachyzoite form with LLC-MK2 cells were treated with Dictyota caribaea extract, in many concentrations. The crude extract was obtained after delipidation of the seaweed and proteolytic digestion with papain at 60°C. Fraction was obtained by adding 10 % ethanol flowed by centrifugation. Through analysis by microscopy it was observed a reduction of the parasitic infection index with 30 µg/ml of the extract when compared to the untreated control. This result suggests that this ethanolic fraction may contain substances with activity against Toxoplasma gondii. **Supported by:**Faperj, CAPES, cnpq
Keywords: Toxoplasma gondii; dictyota caribaea; sulfated polysaccharides

TB061 - ***TRYPANOSOMA CRUZI* HAS TWO PHOSPHORYLATION SITES IN EIF2α REQUIRED TO INFECTION AND DIFFERENTIATION INTO TRYPOMASTIGOTES**

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We previously demonstrated that phosphorylation at threonine 169 (Thr169) in the alpha subunit of translation initiation factor 2 (eIF2alpha) controls initiation and decreases the differentiation of insect proliferative forms into infective metacyclic forms of *T. cruzi* (Tonelli et al. 2011). Differently from other eukaryotes, trypanosomes have an extra N-terminus domain in eIF2alpha. Therefore, we investigated the role of this extra domain and we found that in addition to the Thr169 present in a conserved position of eIF2alpha, a second phosphorylation at Ser43 was also occurring in response to starvation or oxidative stresses, and after the incubation with the recombinant protein corresponding to the catalytic domain of the recently characterized eIF2alpha kinase 2 of *T. cruzi* (Augusto et al. 2015). Furthermore, the relative levels of both phosphorylated forms were found increased when the dividing intracellular amastigotes transforms into trypomastigotes. To demonstrate that these phosphorylations are responsible for the control of differentiation, parasites overexpressing wild type, or eIF2alpha mutated in each or both sites to a non-phosphorylatable residue (Ala) were prepared. All mutated parasites showed an impaired shutdown of translation during nutritional stress and reduced differentiation into trypomastigotes. The double mutated overexpressors completely prevented formation of trypomastigotes inside mammalian cells. The infected cells only released partially differentiated parasites. These results demonstrate a unique feature for eIF2alpha in *T. cruzi* and indicate that the differentiation of the parasite into non-proliferative trypomastigote stages requires changes in translation under the control of the translation initiation. **Supported by:**FAPESP; CNPq
Keywords: Eif2; translational control; trypomastigogenesis

TB062 - **ORNITHINE DECARBOXYLASE OR GAMMA-GLUTAMYLCYSTEINE SYNTHETASE OVEREXPRESSION PROTECTS *LEISHMANIA* SPECIES FROM SUBGENUS *VIANNA* AGAINST ANTIMONY**

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Ornithine decarboxylase (ODC) and gamma-glutamylcysteine synthetase (GSH1) are step-limiting enzymes of polyamines and gluthatione biosynthetic pathways, respectively. These pathways provide precursors for biosynthesis of trypanothione, an essential and unique thiol of trypanossomatids. The antimony-resistance phenotype in *Leishmania* spp. is associated with increased levels of trypanothione and other intracellular thiols. This increase seems to be associated with higher expression of ODC and GSH1 enzymes. However, the potential role of these enzymes in trivalent antimony (Sb^{III})-resistance for the New World *Leishmania* species has not yet been addressed. In this study we overexepressed ODC and GSH1 genes, separated, in four New World *Leishmania* species (*L. (Viannia) guyanensis; L. (V.) Braziliensis; L. (Leishmania) amazonensis and L. (L.) infantum*) to investigate the contribution of these genes to Sb^{III}-resistance phenotype. Western blot analysis using specific antibodies against these enzymes showed that the level of ODC was 1.7 to 12.6-fold and of GSH1 was 1.5 to 70.3-fold higher in transfected clones compared to non-transfected cells. Susceptibility test to Sb^{III} showed that *L. (V.) Braziliensis* and *L. (V.) guyanensis* lines overexpressing ODC or GSH1 were 2.1 to 4.3-fold more resistant to Sb^{III} compared to their non-transfected lines, respectively. In contrast, the overexpression these enzymes in the *L. (L.) amazonensis* and *L. (L.) infantum* lines did not change the Sb^{III}-resistance phenotype. Interestingly, our data shows that enhanced levels of ODC and GSH1 are sufficient to affect susceptibility to Sb^{III} in *Leishmania* species from subgenus *Viannia*, but not in species from subgenus *Leishmania*. Together, these results show that ODC and GSH1 enzymes are implicated in Sb^{III}-resistance in *L. (V.) Braziliensis* and *L. (V.) guyanensis* lines and they suggest that the mechanism of antimony resistance in *Leishmania* spp. may differ between the different *Leishmania* subgenus. **Supported by:** FAPEMIG; CNPq; PROEP/CNPq/FIOCRUZ, CAPES

Keywords: Ornithine decarboxylase; gamma-glutamylcysteine synthetase; leishmania spp

TB063 - **"EFFECT OF NEW IRON METALOCOMPLEXES AGAINST THE GROWTH OF *TRYPANOSOMA CRUZI* EPIMASTIGOTES *IN VITRO***

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Chagas disease is a neglected tropical disease caused by *Trypanosoma cruzi*, a major public health problem in Latin America affecting approximately 8 million individuals and about 60 million live under risk of contamination. The available therapy for this disease is based on two nitroheterocycles, Nifurtimox and Benznidazole, that exhibit severe side effects, including resistance, inefficiency in the chronic phase of the disease, severe cytotoxic effects and variable efficacy. Therefore, new drugs are urgently needed. Some reports in the literature demonstrate that coordination compounds may be an interesting alternative for antiparasite therapy against *Leishmania* spp., *Toxoplasma gondii* and *Trypanosoma cruzi*. Here we tested the *in vitro* effect of the iron compounds, Fe-alfa- naftol-BMPA and Fe-beta- naftol-BMPA, on the growth of *T. cruzi* epimastigotes (Y strain). Epimastigotes were treated with the compounds at concentrations ranging from 1 to 100 nM and their number quantified. The Fe-alfa- naftol-BMPA presented an IC 50 value of 4.14 nM and 4.81 nM, after 72 and 120 h of treatment, respectively. The Fe-beta-naftol- BMPA presented an IC 50 value of 4.71 nM and 7.82 nM for the same treatment times. Ultrastructural analysis of the parasites after treatment with the Fe-beta- naftol-BMPA showed that the parasite mitochondria present changes in their cristae, with swelling and abnormal disposition around the kinetoplast. Confocal images with JC-1 marker showed that after treatment with both compounds the parasite lost the mitochondrial membrane potential. The next step will be to further analyze the effect of these compounds, specially of Fe-alfa-naftol-BMPA, on the parasite ultrastructure in order to investigate the kind of cell death and the mode of action of the compounds. **Supported by:**FAPERJ, CNPq, CAPES.
Keywords: Chagas disease; trypanosoma cruzi; metalocomplexes

TB064 - **EVALUATION OF THE LOXY ANTIGEN FROM LEISHMANIA INFANTUM EXPRESSED IN THE NON-PATHOGENIC CLONE TRYPANOSSOMA CRUZI CL-14 AS A VACCINE AGAINST VISCERAL LEISHMANIASIS.**

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The Leishmania infantum LOxy antigen, previously identified in an immunoproteomic analysis, was shown to protect mice against L. infantum in association to saponin adjuvant. In this work, two strategies were followed to further explore this aspect. First, the nonpathogenic clone Trypanosoma cruzi CL-14 was transfected with the LOxy gene and evaluated as a live vaccine. Additionally, the LOxy antigen was tested in a formulation containg aluminum salt and the B-class-344 CpG ODN. The successful transfection of the T. cruzi CL-14 LOxy parasites and the LOxy protein expression were confirmed by RT-PCR, Western Blott and Immunofluorescence. BALB/c mice were vaccinated in a homologous prime-boost protocol, at 30-day interval with 107 T. cruzi CL-14 LOxy parasites by intaperitoneal route or with the recombinant protein formulation subcutaneously. Sera were used for detection of anti-rLOxy antibodies and splenocyte cultures were prepared to measure cytokine levels. The animals were challenged with 107 L. infantum promastigotes and the parasite load in spleen and liver was measured by qPCR. Results showed that low specific antibody titers were obtained when mice were immunized with the recombinant CL14. Interesting, mice immunized with control parasite CL-14 was also able to induce a significant increasing in IFN-γ production. This cytokine production could be due the antigen similarities founded between T. cruzi and L. infantum. The same pattern were observed when parasite burden were analyzed, where groups immunized with control and transgenic parasite as well as the one immunized with LOxy+Alumen/CpG showed a decrease in the parasite burden although no statistic differences was achieved due the high variability observed in the PBS control group. Our results confirm the protective effect induced by vaccination with LOxy and suggest that the CL-14 strain maybe used not only as a vaccine vector but also as a promising

cross-species vaccine candidate against trypanossomatids. **Supported by:**INCTv, CNPq, CAPES
Keywords: Visceral leishmaniasis; vaccine; t. cruzi cl-14

TB065 - **ASSOCIATION OF METHYLENE BLUE AND PYRIMETHAMINE ON IN VITRO AND IN VIVO *NEOSPORA CANINUM* INFECTION**

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Neospora caninum is an apicomplexan parasite strongly related to reproductive problems in cattle. The combat of neosporosis is not well established and several fronts are under development, which are predominantly based on immune protection, immunomodulation and chemotherapy. As an Apicomplexa, *N. caninum* is potentially sensitive to administration of anti-malarial drugs as therapeutic sources. Drugs such as methylene blue (MB) and pyrimethamine (Pyr) are the therapeutic options for malaria; thus, their use for neosporosis should be assessed. In this work, we tested the effects of MB and Pyr on *N. caninum* proliferation and invasion, using LacZ-tagged tachyzoites. Additionally, the drug effects were evaluated in a dexamethasone animal model and the innate immune response was analysed. The drugs inhibited the parasite proliferation at nanomolar dosages (0.35 and 0.31 µM for MB and Pyr, respectively). The association of these drugs produced an antagonistic interaction in vitro (CI = 1.8) and drug/immune synergism in vivo, according to Chou and Talalay method for drug combination index. MB, Pyr and MB/Pyr association decreased 22, 28 and 54 %, respectively, the in vivo parasite burden in the peritoneal exudate, evaluated by CPRG assay. The association MB/Pyr also improved the innate immune response (oxide nitric, antigen-presenting cells and active macrophages), a key factor for the host fight against *N. caninum*. The repositioning of well-established drugs opens a short-term strategy to obtain low cost therapeutic approaches against neosporosis. **Supported by:**CAPES
Keywords: Neospora caninum; methylene blue; pyrimethamine

TB066 - **ANTI-*TOXOPLASMA GONDII* ACTIVITY OF INORGANIC COMPOUNDS COORDINATED OR NOT WITH SULFADIAZINE**

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Toxoplasma gondii, the agent of toxoplasmosis, is an obligate intracellular protozoan parasite able to infect a wide range of vertebrate cells. Toxoplasmosis is a disease that causes severe damage to immunocompromised hosts and the most used therapy is the combination of pyrimethamine and sulfadiazine, which causes side effects. Thus, it is important to search new compounds against *T. gondii*. Metalcomplexes are inorganic compounds that present interesting biologic activities such as fungicidal, bactericidal and antiviral. In this work new metalcomplexes compounds coordinated or not with sulfadiazine were tested against *T. gondii.* The compounds with the best performance were the copper compounds (B2310 and B10109) and the iron core compound N5414 that is coordinated to sulfadiazine. The compounds B2310 and B10109 irreversibly controlled the in vitro growth of the parasites from the RH strain with an IC50 after 48 h of 3.57 µM and 0.78 µM, respectively. Although treatment leads to death of part of parasite population, another one resisted the compounds action and converted to bradyzoites. Parasite cell death was similar to apoptosis including membrane blebs, cytoplasmic vesicles and apoptotic bodies. The B2310 compound also interfered with the correct disposition of the inner membrane complex, affecting the parasite division. N5414 was active against T. gondii, but the cell death type after in vitro treatment was not determined. This compound was also able to reduce the number and size of brain cysts after in vivo treatment of Swiss mice orally infected with cysts of the ME-49 strain. In conclusion, the selected compounds were effective against T. gondii at the micromolar range, inducing parasite death by different mechanisms and causing the conversion of tachyzoites into bradyzoites. Further

studies are needed to determinate the mode of action of these compounds and their in vivo activity. **Supported by:**Faperj CNPq CAPES
Keywords: Chemotherapy; toxoplasma gondii; metalocomplexes

TB067 - **IN VITRO SCREENING OF ANTI-LEISHMANIAL ACTIVITY OF HEDERAGENIN DERIVATIVES**

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Leishmaniasis is a neglected tropical disease endemic in 88 countries that affect thousands of people every year. The drugs currently available for Leishmania treatment are almost the same as the ones used 50 years ago. These drugs are limited due to their toxicity, the presence of biological resistance in several Leishmania species, the length of treatment and their high cost. Thus, it is important to continue the search for a new, cheaper, effective and less toxic treatment. In this study, we evaluated the potential of 60 hederagenin derivatives to inhibit the intracellular growth of Leishmania infantum amastigotes parasitizing macrophages DH82, in vitro. The ELISA test was employed to measure the inhibitory activity of these compounds on intracellular parasites. The cytotoxicity of the active compounds were evaluated by metabolic activity, using MTT assay. Hederagenin and eight derivatives: compounds 3, 4, 22, 32, 34, 44, 49 and 52 were able to inhibit the intracellular amastigote proliferation with the respective IC50 values: 61, 9.7, 12, 18, 38, 32 11, 2 and 6 µM. These compounds showed better results than the positive control, salt antimony III (80 µM) and also low toxicity against Macrophage DH82, BGM and HepG2 cells. Moreover, hederagenin derivatives 3, 4 and 49 demonstrated excellent anti-leishmanial activity at micromolar level and high selectivity indexes: 6.4, 6.9 and 22.5, respectively. These results denotes the potential of hederagenin derivatives, in special the 3, 4 and 41 compounds as promising candidates for the treatment of leishmaniasis. More studies are necessary to investigate the efficiency and safety of these compounds in vivo.
Supported by:FAPEMIG, CAPES e CNPq
Keywords: Hederagenin derivatives; leishmania infantum; anti-leishmanial activity

TB068 - **EVALUATING THE EFFECTS OF CHEMOTHERAPY WITH METALLOCOMPLEXES IN *LEISHMANIA AMAZONENSIS***

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

TB069 - **ANTILEISHMANIAL ACTIVITY OF CRESCENTIA SP. AND SCHWARTZIA SP. EXTRACTS**

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Leishmaniasis is a group of endemic and neglected diseases caused by Leishmania spp. parasites. The first choice treatment for leishmaniasis is based on the use of pentavalent antimonials. Even though antimonials have been successfully used in the treatment of so many patients for long time, they have high toxicity, treatment failures and resistance of parasites, increasing the need to search for new drugs. The objective of this study was to evaluate the leishmanicidal activity of extracts, partitions and pure substances from different plant species. The samples studied were obtained from Crescentia sp. (CCFEH, CCFED, CCFEAc, CCFEAq and CCFEBu), Schwartzia sp. (SBFE3, SBFE3D, SBFE3H, SBFE3Ac, SBFE3Aq and SBFE3Bu) and the pure substances AG and GM. L. amazonensis promastigotes were incubated in Schneider's medium at 26°C for 72 h with extracts/partitions (0-128 µg/ml) or pure substances (0-128µM). The parasite growth was evaluated using resazurin by fluorimetry 560/590nm. The extracts of Crescentia sp. showed IC50 greater than 128 µg/mL. Among Schwartzia sp. samples, only SBFE3Bu extract showed leishmanicidal activity, with IC50 55.30 ± 6.14 µg/ml. The pure compound GM showed IC50 equal to 44.33 ± 7,88 µM. Based on these results, we can conclude that SBFE3Bu and the pure compound GM have promising leishmanicidal activity. Thereby, the next step will be to confirm the leishmanicidal activity in intracellular amastigotes. **Supported by:**CNPq, PAPES/FIOCRUZ, FAPERJ
Keywords: Leishmania amazonensis; crescentia sp.; schwartzia sp.

TB070 - **STUDY OF THE ACTIVITY OF COMPOUNDS ERGOSTEROL SYNTHESIS INHIBITORS OF *TRYPANOSOMA CRUZI***

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Chagas disease caused by the protozoan Trypanosoma cruzi, is one of the neglected tropical diseases affecting 5-7 million people and its treatment is restricted to benznidazole (Bz) and nifurtimox. The major limitations of these drugs are their limited and variable curative activity in the established chronic form of the disease and their toxic effectsIn this context, an intensive research program has been focused upon the search for alternative drugs. Because T. cruzi has a strict requirement for specific endogenous ergosterol, enzymes involved in this biosynthetic pathway are potential targets beingthe most studied enzyme C14α-sterol demethylase (CYP51) which catalyses the removal of the C14 methyl group of lanosterol. In this study we determined the in vitro effect of two different series of sterol biosynthesis inhibitors (SBIs): 1,2,4-triazoles and hybrid compounds of a prototype pyridine with megazol. The effect of these compounds was evaluated on bloodstream trypomastigotes, intracellular amastigotes and epimastigotes. Among the 1,2,4-triazoles derivatives tested on trypomastigotes (Y strain) 25q was more active than Bz; and 25g, 25h, 25l were the most active against intracellular parasites (Tulahuen strain).The treatment in cultures infected with amastigotes (Y strain), with these derivatives, led to a reduction in infection, and also the number of parasites per infected cell after the first 48 hours of treatment. In relation to the hybrid compounds, only one (1947) showed good activity against trypomastigotes and epimastigotes of the Y strain.
Keywords: Doença de chagas; quimioterapia; inibidores da biossíntese de ergosterol

TB071 - **NOVEL SBV-PORPHYRIN IS HIGHLY ACTIVE AGAINST SB-RESISTANT *LEISHMANIA* SPP. BY TARGETING ERGOSTEROL BIOSYNTHESIS PATHWAY**

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Leishmaniasis chemotherapy has several drawbacks including cost, side effects and resistance development by parasites, requiring urgent research for alternative molecules to treat these diseases. We have previously synthesized and characterized a series of metalloporphyrins with antileishmanial activity. Here we investigate the antileishmanial activity of an antimony(V) porphyrin (Sb^VP) against promastigote and amastigote forms of *L. major*, *L. infantum*, *L. amazonensis*, *L. guyanensis* and *L. Braziliensis*, Sb-sensitive (SbS) and Sb-resistant (SbR) lines and its toxicity against THP-1 macrophages and L929 fibroblasts. The IC_{50}/72 h of SbVP amongst all Leishmania spp. evolutive forms ranged from 0.016 – 0.94 µM, being Sb^VP 28-125 times more selective towards *L. infantum* amastigotes when compared to THP-1 and L929 toxicities, respectively. This prompted us to evaluate the sterol profiling of promastigotes upon Sb^VP exposure by GC/MS. Indeed, there is an accumulation of intermediate lipids on ergosterol biosynthesis pathway (such as methylated sterol) and a suppression of ergosterol signal. A similar profile was observed when cells were treated with miconazol (positive control). No changes were detected upon Bismuth-porphyrin exposure nor porphyrinic ligand alone. This indicates a specific antileishmanial activity of Sb^VP through ergosterol biosynthesis inhibition. We then validate this observation by exposing promastigote forms to concomitant treatment with Sb^VP and amphotericin B (AmB), which targets ergosterol molecule. The combined treatment increases antileishmanial activity, corroborating the idea of ergosterol biosynthesis pathway as a major target. The Sb resensitization of SbR mutants together with the high antileishmanial activity in vitro, even against promastigote forms (less sensitive to Sb^V), made Sb^VP a promising drug candidate for in vivo efficacy evaluation. **Supported by:**CNPq; CAPES; FAPEMIG; ISID **Keywords:**Leishmania; antimony porphyrin; ergosterol

TB072 - **PROBE DEVELOPMENT FOR THE IDENTIFICATION OF CHALCONE MOLECULAR TARGETS IN LEISHMANIA.**

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Chalcones are a promising class of new antileishmanials whose target is still unknown. In previous work, we have reported the selective activity of the chalcone (CH8) against Leishmania spp., suggesting a conserved drug target. We synthesised some CH8 analogues with alkyne groups for cycloaddition coupling (Huisgen-Sharpless reaction), without affecting the molecule activity. The aim is to discover the possible protein targets, we then proposed the production of trifunctional probes containing the azide group to be a link between them and the analogues. The probes will also have a fluorescent group and a biotin to enable target enrichment (DEO53). Some probes will have just fluorophore (PGS14.4) or biotin (DEO56) as different alternatives to identify the targets. We synthesised by aldol condensation, the CH8 analogue called NAT22, and the molecule with alkyne group afforded the intermediate DEO37. The probes were produced by adding all needed groups in an amino acid core. We tested these compounds against L.amazonensis and L.major promastigotes, incubating it at 26°C with NAT22, DEO37, DEO53 or PGS14.4, for 72 hours and then cell viability was assayed by AlamarBlue. NAT22 and DE37 showed excellent activity for both species, with IC50 between 0.3µM to 2.2µM. Nonetheless, the PGS14.4, DEO53 and DEO56 probes showed significantly decreased activity against all species, likely due to its large molecular size. To prove that these molecules work to find chalcone targets, L.amazonensis promastigotes were permeated (digitonin), incubated with PGS14.4 and some organelle dyes and imaging with confocal microscope. It was possible to visualize the probe in some organelles, mainly on mitochondria. Promastigotes

pre-incubation with DEO37 before cell lysis by sonication and further click reaction allows the purification of some proteins by SDS-PAGE. In summary, all probes appear to be a useful tool for the identification of the chalcone target in Leishmania. **Supported by:**CNPq, CAPES **Keywords:**Leishmania; chalcone; trifunctional probe

TB073 - **EFFECT OF PROTEASE INHIBITOR *BOWMAN-BIRK* TYPE ASSOCIATED WITH BENZNIDAZOLE IN EXPERIMENTAL *TRYPANOSOMA CRUZI* INFECTION**

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The inflammatory process can trigger the fibrosis and loss of neurons, fundamental in the pathogenesis of the cardiac and digestive forms of Chagas disease. Therefore, it is important to search for a treatment capable of eliminate the parasite and reduce the associated inflammation. Thus, the aim of this study was to evaluate the effect of treatment with the protease inhibitor *Bowman-Birk type* (BBI), a potent inhibitor of inflammation associated with benznidazole (BZ) along the *Trypanosoma cruzi* infection, on the parasitism and inflammatory process. To this end, 240 Swiss mice were divided into five groups: non-infected and untreated (CNI); infected and untreated (CI); infected and treated with BBI (BBI); infected and treated with BZ (BZ); infected and treated with BZ and BBI (BZ/BBI). For the treatment was employed 100 mg/kg body weight of BZ and/or 3mg of BBI via gavage for 20 days. On the 10th, 20th, 30th and 120th day after infection (DAI), six animals from each group were necropsied to collect heart and colon, that were subjected to evaluation of parasitic burden and histology. In evaluation of the tissue parasitism was observed that the animals of the group treated only with BBI were unable to control parasitism unlike the animals treated with BZ. The animals treated with BZ/BBI controlled tissue parasitism in the acute phase of infection, however it was observed in 120th DAI the presence of parasites in some animals from this group. Regarding the histopathological evaluation, there was a reduction in the inflammatory process in BZ/BBI group on the 20th DAI, while the animals of the BZ group the inflammatory process had been reduced only at 30th DAI. Thus, it is concluded that BBI, despite its immunomodulatory effect, did not prove beneficial in the parasitological treatment of DCh in this therapeutic regimen, probably due to non-establishment of an effective immune response against *T. cruzi*. **Supported by:**Fapemig, CNPq, Capes **Keywords:**Trypanosoma cruzi; quimioterapia; inibidor de protease

TB074 - **IMMUNOLOGICAL EVALUATION OF THE ACCELERATED CLEARANCE BLOOD PHENOMENON FOR THE LIPOSSOMAL TREATMENT OF LEISHMANIA INFANTUM INFECTED MICE**

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Introduction: WHO encourages the study of new drugs or alternative forms of administration for the treatment of visceral leishmaniasis, among them the liposomal formulations. These formulations could lead to reuse of tartar emetic(TE),a potent anti Leishmania drug with limited use due to serious toxic effects. Objectives: This study evaluated the effect of the accelerated blood clearance(ABC)phenomenon in Leishmania infantum infected BALB/c mice treated with pegylated liposomes containing TE. This effect is responsible by the rapid clearance of a second dose of pegylated liposomes from the circulation due to the removal by macrophages. Methods: conventional and pegylated liposomal formulations were prepared by the hydration of a thin film of lipids with a solution of the drug. The effect of TE formulations on nitric oxide (NO) production by LPS/IFN-γ stimulated J774-A1 cells was evaluated by Griess method.In order to evaluate the effect of ABC phenomenon on BALB/c treatment with TE pegylated liposomes, mice were initially treated with empty pegylated liposomes. One week later, they were treated with pegylated liposomes containing 12 mg SbIII/kg. Parasite load was evaluated by limiting dilution analysis.Cytokines production by spleen cells was also evaluated by ELISA. Results: TE formulations had no effect on NO production by J774-A1 cells. Pegylated formulation of TE significantly reduced the parasite load in liver, spleen and bone marrow of L. infantum treated mice. However, ABC phenomenon had no effect on treatment with pegylated formulation of TE. Free drug and conventional formulation were able to reduce parasite load only in liver. Furthermore, it was observed that the treatment with free TE or TE formulations caused

significant decrease in IL-10 production by splenic cells. Conclusion: Pegylated liposomes containing TE were more effective in reducing parasite load than conventional liposomes. ABC phenomenon had no effect on treatment with pegylated liposomes. **Supported by:**CAPES / CNPq / UFOP **Keywords:**Visceral leishmaniasis; liposomal treatment; abc phenomenon

TB075 - **USE OF RECOMBINANT PROTEINS CYT AND CNI TO DEVELOP A RAPID TEST FOR THE DIAGNOSIS OF CANINE AND HUMAN VISCERAL LEISHMANIASIS.**

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Rapid and accurate diagnosis and treatment are the mainstay measures for control of visceral leishmaniasis (VL). Different serological methods are used, such as ELISA, IFA, Western Blot, agglutination test and immunoassay applying recombinant antigens. However, these various methods show limitations, such as the cross-reactivity, lab work and timing consuming, and especially the failure in the identification of asymptomatic dogs. In this context, rapid tests have come up as an excellent platform for the diagnosis of canine (CVL) and human (HLV) visceral leishmaniasis. Here, we have developed a quick test for the diagnosis of CVL, using two new recombinant proteins (named Cyt and Cin) from Leishmania (Leishmania) infantum chagasi. Initially, the test standardization for each protein was performed, separately. Different parameters were evaluated, including: the size of colloidal gold particles, the concentration of each antigen in the test line, the setting of control line, the membrane type, the running buffer and the reading time. To evaluate the potential of Cyt protein sera from 39 dogs, 19 dogs with LVC and 20 healthy dogs, were tested. For CNI protein, sera from 57 dogs, 24 dogs with CLV and 33 healthy dogs, they were tested. For evaluation in the diagnostic of HLV 34 sera, 16 from patients with HVL and 18 from healthy controls were tested. Finally, sensitivity and specificity were calculated for each rapid test. The rapid test using Cyt protein showed sensitivity of 89.5% and 70% of specificity and with the CNI protein 92% sensitivity and 73% specificity in the diagnosis of CVL. Using the LVH sera, the CNI protein showed 86% sensitivity and 94% specificity. The tests with Cyt and human sera are being performed. The proteins Cyt and CNI may be useful for improving the diagnosis da LVC e LVH, in a lateral flow chromatography assay for fast diagnosis of VL. **Supported by:**FAPEMIG, CNPq, MS/DECIT
Keywords: Visceral leishmaniasis; recombinant protein; ateral flow immunochromatography

TB076 - **ULTRASTRUCTURAL EVALUATION OF FARNESYL PYROPHOSPHATE SYNTHASE (FPPS) INHIBITORS IN PROTOZOAN.**

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The enzyme farnesyl pyrophosphate synthase (FPPS) is a key enzyme involved in the biosynthesis of sterols and in the synthesis of farnesylpyrophosphate, a metabolite essential for isoprenoid biosynthesis. Protein prenylation is ubiquitous in eukaryotes, but along evolution some eukaryotes, such as *Giardia*, have lost the sterol biosynthetic enzymes. In contrast, *Leishmania* and other trypanosomatids are able to synthesize ergosterol instead of cholesterol, which is synthesizes by the mammalian host. The differences in the sterol metabolism make FPPS a good candidate for drug target. Thus, we aim to evaluate and compare the activity of biphosphonates, FPPS inhibitors, in the proliferation/viability and ultrastructure of *Leishmania infantum* and *Giardia duodenalis*. Our results demonstrated that risedronate (IC_{50}= 12.3 µM ± 6.0) followed by ibandronate (IC_{50}= 83.8 µM ± 24.8) displayed higher anti proliferative activity than alendronate (IC_{50}= 112.2 µM ± 68.3) and neridronate (IC_{50}= 137.0 µM ± 32.3), in *L. infantum*. In *G. duodenalis*, only risedronate, ibandronate and alendronate displayed anti proliferative activity (IC_{50}= 311 µM ± 120.2; 271 µM ± 62.2; 260 µM), as evaluated by the viability method MTS/PMS. The ultrastructure evaluation of *L. infantum* and *G. duodenalis* treated with bisphosphonates was performed by electron microscopy. In Leishmania, it was observed blebbing of the plasma membrane, mitochondria swelling, myelin figures, double membrane vesicles and vacuoles in the cytoplasm. In *G. duodenalis* it was observed concentric membranes nearby the nucleo, nuclear pyknosis and myelin figures with engulfment of cytoplasmic and nucleo. This ultrastructural alterations are compatible with programmed cell death, autophagy

and apoptosis. The increased anti proliferative effect of bisphosphonates in Leishmania, compared to *Giardia*, can be related to differences in the metabolism and in the catalytic site of the FPPS enzyme, as observed by X-ray diffraction. **Supported by:**FUNDECT, CNPQ and PIBIC Fiocruz
Keywords: Leishmania; giardia; bisphophonates

TB077 - **BENZNIDAZOL AND NIFURTIMOX SIGNALS AN STRESS RESPONSE THROUGH EIF2α PHOSPHORYLATION IN *TRYPANOSOMA CRUZI***

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The eukaryotic initiation factor 2 (eIF2) containing GTP delivers the methionyl-transfer RNA (tRNA-Met) for the initiation of protein synthesis. Once the initiation codon is found, translation elongation starts and the eIF2 associated with GDP is released. For a new round of initiation, the GDP is exchanged by GTP. The phosphorylation of the alpha subunit of eIF2 (eIF2α), induced by specific protein kinases activated by stress conditions, prevents this exchange and is therefore a key step in regulating translational initiation. Here we found that benznidazol and nifurtimox, the available drugs for the treatment of Chagas disease, used at suboptimal concentrations, caused eIF2α phosphorylation of *Trypanosoma cruzi* eIF2α as detected by using specific antibodies against the phosphorylated threonine 169 of the protein. This effect started 30 min after addition of the compounds, and it was increased with drug concentration, suggesting that translation arrest was induced in the parasite. In parallel, we found that the levels of enzymes that detoxify reactive oxygen species (ROS) such as superoxide dismutases and peroxiredoxins are increased in the presence of the drugs. As the toxicity of both compounds is related to induction of ROS, we speculate that general translation arrest caused by eIF2α phosphorylation resulted in the increased expression of the detoxifying enzymes. Therefore, we concluded that the stress created in *T. cruzi* by benznidazol and nifurtimox treatment could generate a survival response in the parasite, which could be target to improve the treatment of Chagas disease. **Supported by:**FAPESP E CNPQ
Keywords: Eif2α drug treatment

TB078 - **ENROFLOXACIN AND TOLTRAZURIL ARE ABLE TO CONTROL INFECTION BY *TOXOPLASMA GONDII* IN BEWO CELLS AND HUMAN VILLOUS EXPLANTS FROM THIRD TRIMESTER**

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Congenital toxoplasmosis occurs due to transplacental passage of tachyzoites during pregnancy, reaching the circulation and fetal tissues. Classical treatment is based on combination of sulfadiazine and pyrimethamine plus folinic acid. Due to teratogenic effects and bone marrow suppression caused by pyrimethamine, the establishment of new therapeutic targets is indispensable to minimize the side effects and improve the control of the infection. Previous studies demonstrated that enrofloxacin and toltrazuril were able to control the infection by *Neospora caninum* and *Toxoplasma gondii*. Therefore, the aim of this present study was to evaluate the efficacy of enrofloxacin and toltrazuril in the control of *T. gondii* proliferation in human trophoblast cells (BeWo lineage) and in human villous explants from third trimester. BeWo cells and villous were treated with enrofloxacin, toltrazuril, sulfadiazine, pyrimethamine or association (sulfadiazine + pyrimethamine) in other to verify their viability by MTT or morphology analysis, respectively. Next, they were infected with *T. gondii*, and treated or not with the same drugs for analyses to *T. gondii* intracellular proliferation by beta-galactosidase assay. ELISA was performed in the supernatant to evaluate the cytokine profile. Enrofloxacin and toltrazuril did not change the viability in cells and villous. Furthermore, the drugs decreased intracellular proliferation of *T. gondii* in BeWo cells and villous explants when compared to infected and untreated conditions. In infected BeWo cells, enrofloxacin induced high levels of IL-6 and low levels of MIF, whereas, toltrazuril induced high levels of MIF. In villous, enrofloxacin induced high MIF production. Thus, enrofloxacin and toltrazuril were able to control the parasitism in BeWo cells and villous explants, and probably due to modulation of immune response, and

direct action on parasite can not be excluded. Future experiments are necessary to verify this hypothesis. **Supported by:**FAPEMIG, CAPES e CNPq
Keywords: Congenital toxoplasmosis; enrofloxacin; toltrazuril

TB079 - **GENERATION OF RECOMBINANT PEPTIDES AGAINST TRYPOMASTIGOTE SURFACE PROTEINS OF *TRYPANOSOMA CRUZI*: NEW TOOLS TO IMPROVE DRUG DESIGN**

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The treatment of Chagas disease patients using conventional drugs need some improvement, since it can be inefficient or can cause severe side effects. As shown for *Trypanosoma brucei*, nanobody engineering can be used to improve sleeping sickness treatment. Similar to this approach, here we describe our initial efforts to develop specific molecules that binds to *T. cruzi* cell surface. We have created scFvs based on sequences obtained from previously described monoclonal antibodies or identified peptides by using phage display technology. Thus, variable regions IgG light and heavy chains of hybridomas expressing mAb-10D8 (anti-TcGP35/50), mAb-2B10 (anti-GP35/50), and mAb-3F6 (anti-TcGP82) were sequenced and assembled as scFvs. Synthetic gene of scFv-10D8 was cloned and expressed in *E. coli* as 6xHis fusion protein. Currently, we are optimizing the scFv-10D8 expression conditions to purify functional molecules. In parallel to these efforts, we used fixed metacyclics and tissue culture derived trypomastigotes (MT and TCT respectively) to screen phage display libraries (XCX4CX, XCX8CX, X8CX8 and X15) to identify peptides that binds to the parasite surface. After three rounds of panning, seven clones that recognize TCTs and 3 that bind to MTs were sequenced. From this preliminary sequencing, 5 out of 7 clones encode two distinct peptides. These identified are being tested for TCT binding capacity. Once we confirm the binding capacity of each molecule described here, we plan to test it to ablate *T. cruzi* infectivity
Supported by:CAPES CNPq **Keywords:**Anti-t.cruzi; peptide ligands; recombinant antibodies

TB080 - **PHASE I AND II CLINICAL TRIAL EMPLOYING LEISH-TEC®, LEISHMUNE® AND LBSAP VACCINES AGAINST CANINE VISCERAL LEISHMANIASIS.**

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Human visceral leishmaniasis and canine visceral leishmaniasis (CVL) are highly prevalent in Latin American countries, especially in BRA. Some authors suggest the use of an anti-CVL vaccine, as an important control measure for both human and canine infection, since dogs are reservoirs of the parasite. This study aims to evaluate the immunogenicity of vaccine prototype LBSap in comparison to the commercial vaccines Leishmune® and Leish-Tec®, after complete immunization protocol in a vaccine clinical trial phase I and II. For this, twenty-eight dogs were classified into four groups: i) control; ii) Leish-Tec®; iii) Leishmune®; (iv) LBSap groups received 600 µg of *L. Braziliensis* promastigotes protein plus 1 mg of saponin adjuvant. Analysis of the humoral immune response by anti-*Leishmania* total IgG antibodies with EIE® (Bio-Manguinhos®) in the serum of dogs, fifteen days after three immunizations, showed an increase in mean optical density of LBSap and Leishmune groups above threshold of positivity. Our results of serological reactivity using TR DPP® (Bio-Manguinhos®) showed that all dogs of different groups were negatives, demonstrate the ability of the test to discriminate vaccinated dogs. In the *in vitro* analyzes of immunogenicity an increase in total lymphoproliferative activity in groups LBSap and Leishmune were observed, occurring in LBSap group an antigen-specific increase of CD4+ and CD8+ T-lymphocytes. Our immunogenicity results support the hypothesis of the vaccine process with the LBSap vaccine prototype is also able to generate a protective immune response against the *L. infantum* parasite. **Supported by:**FAPEMIG, CNPq, CAPES, FIOCRUZ, UFOP and UFMG. **Keywords:**Leishmania infantum; canine visceral leishmaniasis; vaccines

TB081 - **IMPROVEMENT OF CANINE VISCERAL LEISHMANIASIS (CVL) DIAGNOSIS BY INDIRECT ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA) USING RECOMBINANT ECTO-NUCLEOSIDE TRIPHOSPHATE DIPHOSPHOHYDROLASE (NTPDASE-2) FROM *LEISHMANIA INFANTUM CHAGASI* AS A NEW ANTIGEN**

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Canine visceral leishmaniasis (CVL) is a relevant public health problem. In BRA, the euthanasia of infected dogs is one of the methods used to control the disease spread. In this context the diagnosis are based ELISA and IFA techniques using complex antigens (MS, 2006). But these techniques have limitations in relation to specificity and sensitivity. Our research group has published a new recombinant antigen, Ecto-Nucleoside Triphosphate Diphosphohydrolase NTPDase-2 of L. infantum chagasi, with potential for application in diagnosis of CVL (De Souza et al., 2013). In this work, we aimed to improve this diagnosis by ELISA. Immunoassays were performed varying the form of antigen purification (FPLC purification and manual purification) and the influence of antigen conformation using it in native or denatured condition. In the native state protein was diluted at different concentrations in refolding buffer for 24 and 48 hours and evaluated for enzymatic activity. In denatured state, the protein was kept in 8M urea. The purified antigens were evaluated for purity and sensitivity in ELISA assays. The results showed a lower purity (77.8%) and lower sensitivity in the ELISA assay for the protein purified by FPLC, while manually purified protein had a greater purity (100%) and increased sensitivity in the ELISA test. The best enzyme activity was obtained by diluting the 40x or 50x protein in refolding buffer for 24 hours. However, diagnosis by ELISA using the refold protein was not more effective than denatured protein, indicating that recognition of the antigen: antibody in this case is preferably not dependent of antigen conformation. Our data demonstrate that higher purity of antigen increase the sensibility the ELISA test, increased the efficiency of epidemiological survey. Furthermore, our data confirm the potential of this antigen for the diagnosis of CVL demonstrating that it is possible to improve the detection of antibodies levels varying test conditions. **Supported by:**UFV, CAPES, CNPq, FAPEMIG
Keywords: Leishmaniasis; diagnosis; elisa

TB082 - **EFFECT OF NOVEL α-ARYL-α-TETRALONE DERIVATIVES AGAINST *LEISHMANIA INFANTUM*: PHENOTYPIC SCREENING AND *IN SILICO* ADMET ANALYSIS.**

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Visceral leishmaniasis is the second major cause of deaths by parasites after malaria. The drug arsenal against leishmaniasis is small and has disadvantages in terms of toxicity, efficacy, price or treatment regimen. Here, we evaluated the leishmanicidal activity of α-aryl-α-tetralones (1-carba-isoflavanones) and performed in silico estimation of their ADMET properties. The chosen compounds are isosters of isoflavanones, a group of isoflavonoids with antitumoral and leishmanicidal properties. Promastigotes of *L. infantum* were incubated with 1-carba-isoflavanones (2-128µM) for 72 h and cell viability was evaluated by the resazurin assay. The derivatives can be divided in three groups: in the first, methoxy groups were added in A and C rings (LQB 309, 310, 312, 314 and 316), in the second, a hydroxy group was added in A ring (LQB 394, 448, 479, 480 and 482), while in the third, Fluor is present at 5-position in ring A (LQB 501 and 502). The most promising 1-carba-isoflavanones were LQB 316, 479, 501 and 502, with IC50 8.2±2.3µM, 12.1±1.8µM, 13.6±4.0µM and 14.7±2.2µM, respectively, similar to miltefosine (IC50 = 10.9±2.4µM). This result shows the importance of the substitutions in ring A, which apparently is a key feature for leishmanicidal activity. Theoretical analyses suggested a favorable profile for 1-carba-isoflavanones, because they do not infringe the Ro5 and the in silico analysis suggests permeability in Caco2 cells and good absorption by human intestines.

Toxicity predictors show that only LQB 482 is expected to be mutagenic. This study reveals promising molecules to proceed further studies of toxicity in host cells and antiamastigote activity. **Supported by:**FAPERJ, CNPq
Keywords:L. infantum; carba-isoflavonones; admet

TB083 - **RETINOL INCREASES *LEISHMANIA AMAZONENSIS* INFECTION *IN VIVO* AND *IN VITRO***

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Leishmaniases are diseases caused by Leishmania parasites and are divided into tegumentary and visceral forms. They occur mainly in poor countries, where alimentary deficiencies are common. Retinol (vitamin A) deficiency is the most prevalent, affecting about one third of the world population in pre-school age, causing immunosupression. *Leishmania amazonensis* infection has great medical importance in BRA as it can evolve into severe diffuse clinical manifestation. In this study, we evaluated the importance of retinol in L. amazonensis infection in vivo and *in vitro*. Thus, BALB/c mice susceptible to infection were subjected to dietary retinol restriction (Retinol-) or supplementation (Retinol+) and afterwards subcutaneously infected in the footpad with L. amazonensis promastigotas. Lesion development was monitored for 60 days, when the parasite burden and cytokine profile in the infection site were evaluated. Retinol– mice were more resistant to infection then Retinol+ mice, developing lower lesions and reduced parasite burden. Moreover, Retinol- mice presented higher levels of IFN-y in the lesions and less IL-4 and TGF-B compared to Retinol+ mice, indicating Th1 profile. Corroborating these data, macrophages from Retinol- mice presented reduced parasite load upon *in vitro* infection with L. amazonensis promastigotes and higher NO production then Retinol+ macrophages. In addition, pretreatment of macrophages with retinol or retinoic acid (RA, a retinol metabolite) *in vitro* increased susceptibility to infection. Together, these data suggest that retinol and RA are immunomodulatory agents that deactivate macrophages, turning them more susceptible to infection, or that these molecules are important for complete macrophage maturation and establishment of Leishmania infection.
Keywords: Leishmania amazonenis; retinol; retinoic acid

TB084 - **NANOCRYSTALS OF CHALCONE NAT22 EFFICACY IN ORAL TREATMENT OF EXPERIMENTAL CUTANEOUS LEISHMANIASIS**

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The conventional cutaneous leishmaniasis treatment is based on multiple and painful injections with toxic drugs. In this study, we proposed to develop an oral treatment for this disease using nanocrystals of a novel nitrogenated chalcone NAT22 (NanoNAT22) which chemical synthesis proved to be simpler than the former chalcone CH8 analogue. Among the strategies to improve the solubility of insoluble drugs and increase *in vivo* bioavailability, the nanocrystals development appears with great potential for application due to its easy scaling up and possibility of using in several pharmaceutical forms. The resulting increased surface area due to the particle size reduction improves drug solubility and bioavailability. NanoNAT22 measuring 250nm diameter was tested against *Leishmania amazonensis* promastigotes for 72h at 26oC and showed IC50=0.3µM. Activity against intracellular amastigotes was IC50=0.6µM. The selectivity index (anti-amastigote IC50/anti-macrophage CC50) for NanoNAT22, NAT22 and the reference drugs Pentamidine and Glucantime (GLU) was 74, 15, 4.7 and 1.6, respectively. ADMET analysis satisfied Lipinski's five rules and showed 100% probability for human intestinal absorption. For *in vivo* studies, BALB/c mice were infected in the ear with L. amazonensis-GFP and on day 7 they were treated with 40 mg/kg of oral NanoNAT22 in therapeutic regimen of 5 daily doses per week during 4 weeks. Controls received 1,5mg/kg of i.p. GLU, once per week. Lesion sizes and parasite loads were measured with dial calliper and fluorimetry, respectively. Toxicological parameters (AST, ALT and creatinine) were measured in the serum using commercial kits. On day 50 of infection, oral NanoNAT22 was as effective as i.l GLU in preventing lesion development and reducing parasite

burden. No toxicological changes were observed at the end of the experiment. Together, these results showed NanoNAT22 is more selective than the reference drugs, and orally active against cutaneous leishmaniasis. **Keywords:**Antisleishmania; chalcone; nanocrystals

TB085 - **AN IRON METALCOMPLEXE COMPOUND IMPROVE THE ANTI-*TOXOPLASMA GONDII* ACTIVITY OF SULFADIAZINE *IN VITRO***

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Toxoplasma gondii, is the toxoplasmosis agent and a protozoa able to infect and replicate in any nucleated cell. Toxoplasmosis is a disease related to severe damages to immunocompromised hosts and the current therapy is limited, and cause side effects. This fact highlights the importance of studies to create new drugs actives against T. gondii. The literature presents many studies of effects of compounds based in metals, known as metalcomplexes, being actives against Toxoplasma gondii. Beside the activity against this apicomplexan, the inorganic compounds showed antifungal, antiviral and antibacterial activities. The dinuclear iron compound [Fe(HPClNOL)(SO4)]2-µ-oxo, controled the activity of antioxidant enzymes of Toxoplasma gondii and was secure to the host cells LLCMK2.The present work investigated the activity of new metalcomplexes that have in its structure an iron core coordinated to sulfadiazine against tachyzoites of Toxoplasma gondii in vitro maintained in LLC-MK2 cells. The analysis of infected and treated cells showed that the compound N4013.1, reduced significantly the infection rate compared to infected cells that were treated only with sulfadiazine, with metalcomplexes or untreated cells. In addition, the host cell viability was high even after treatment with high concentrations of compound. Electron microscopy analysis showed that treated parasites presented cytoplasmic inclusions similar to amylopectin granules commonly found in bradyzoites. The next step includes the search of the kind of dead suffered by the treated parasites and investigation of induction of cystogenesis through ultraestructural analysis and use of specific markers by fluorescence microscopy. Thus, the new coordinated compound presented a better performance than sulfadiazine alone with selective activity against the parasite and the advantage of safety, being non-toxic to the host cells.**Supported by:**FAPERJ, CNPq e CAPES **Keywords:**Toxoplasma gondii; metalcomplexes; toxoplasmosis