TB017 - **EVALUATION OF THE GROWTH-INHIBITORY AND ULTRASTRUCTURAL EFFECTS OF QUINUCLIDINES ANALOGUES IN *LEISHMANIA AMAZONENSIS***

MACEDO-SILVA, S.T.[*1]; CÄMMERER, S.B.[2]; DE SOUZA, W.[1]; RODRIGUES, J.C.F.[3]
1.IBCCF, RJ, BRAZIL; 2.INSTITUTO DE QUÍMICA DA UNICAMP, SP, BRAZIL; 3.NÚCLEO MULTIDISCIPLINAR DE PESQUISA UFRJ-XERÉM (NUMPEX-BIO), RJ, BRAZIL.
e-mail:sara.teixeiracp2@gmail.com

Leishmaniasis is one of the most important neglected diseases caused by protozoan parasites of the *Leishmania* genus. Active *Leishmania* transmission is endemic in 98 countries. The available drugs have several side effects as toxicity and long-term treatment. Quinuclidines are known inhibitors of squalene synthase enzyme, a key enzyme in the sterol biosynthesis that has been extensively studied. Trypanosomatids have an essential requirement for ergosterol and other 24-alkyl sterols; thus, SQS enzyme is a promising target for drug development. Herein, new quinuclidines analogues (SB37-SB43) were evaluated against *Leishmania amazonensis.* The first results demonstrated a potent antiproliferative effect against both development stages of *L. amazonensis*. Cytotoxicity assay in macrophages resulted in CC_{50} values between 23.32 and 40.65 µM. The compounds SBC39 and SBC40 showed the higher selective index for intracellular amastigotes (117.7 and 180, respectively). For them, new techniques were used to understand the cellular mechanisms of action. Scanning electron microscopy showed significant alterations on the cell body of treated promastigotes that presented a rounded or distorted shape. Some images also suggested arrest of the cell cycle. Transmission electron microscopy revealed several alterations in the ultrastructure of promastigotes, such as mitochondrial swelling and disorganization of its membranes, appearance of many autophagosomes and lipid bodies, and presence of several vesicles inside the flagellar pocket. Significant projections of plasma membrane were also observed. Nile Red labeling was used to confirm the accumulation of lipid bodies. Besides, mitochondrial alterations were also confirmed by decrease of the electric transmembrane potential and increase of ROS after 48 h of treatment. Together, these results indicate that SBC39 and SBC40 have potent effect *in vitro* against *L. amazonensis* and should open possibilities for the chemotherapy of leishmaniasis.

Supported by:CAPES, CNPQ **Keywords:**Quinuclidines; sterol biosynthesis; leishmaniasis

TB018 - **A MODULAR METHODOLOGY TO INTEGRATE GENE EXPRESSION AND PROTEOMICS DATA INTO PROTEIN-PROTEIN INTERACTION (PPI) NETWORKS, USING PUBLIC DATABASES AND OPEN SOURCE SOFTWARES**

GUIMARÃES, F.G.[*1]; GONÇALVES, L.O.[2]; RESENDE, D.M.[2]; RUIZ, J.C.[1]
1.INSTITUTO RENÉ RACHOU - FIOCRUZ MINAS, BH, MG, BRAZIL; 2.INSTITUTO OSWALDO CRUZ, RJ, BRAZIL. e-mail:frederico@teia.bio.br

Nowadays there is a considerable amount of available biological data, but the odds in the process of obtaining information from them are overwhelming and growing. Differences between data formats, absence of a unified identifier for biological features and lack of integration between existing databases are some of the challenges researchers face in the task of biological information mining. With the main goal of integrate biological data from different sources for further analysis and interpretation, we developed a methodology that uses a series of *shell* and *Perl* scripts that extract, filter and format protein interactions data from *STRING* v.10.5 database and from high throughput genomic data (*RNASeq* and shotgun proteomics), integrating them into protein-protein interaction networks using *Cytoscape*. This methodology was modularly structured and can be adapted and/or integrated to different analytical protocols and organisms. Specifically on this study, the model organism used was *Trypanosoma cruzi*, the causative agent of Chagas disease. As results we generated a series of protein-protein interaction networks that emphasize, using *Cytoscape* visual styles, characteristics of biological interest, such as EC number, functional grouping, protein interaction types (binding, reaction, expression, activation, catalysis and post-translational modifications) and graph metrics. In these networks we could highlight a series of evidences of biological features, like topological associations between gene regulatory mechanisms (kinases, phosphatases and RNA polimerases) and clusters of proteins functionally associated. Finally, the developed methodological approach emphasize that is possible do mine new informations from data available in public databases and that genomic data integration into PPI networks could be valuable in converting high throughput data into biological knowledge.

Keywords: Bioinformatics; protein interaction network; trypanosoma cruzi

TB019 - **LOOKING FOR PROMISING TARGETS IN ANTICHAGASIC THERAPY: THE EFFECTS OF DISTINCT ENZYMES INHIBITION ON *TRYPANOSOMA CRUZI***

ZUMA, A.A.[*1]; DE CASTRO, L.S.[1]; LEITE, A.C.S.[1]; MANSSOUR, C.A.[1]; BRACHER, F.[2]; DE SOUZA, W.[1]
1.UFRJ, Rio de Janeiro, RJ, BRAZIL; 2.CENTER FOR DRUG RESEARCH, Munique, ALEMANHA.
e-mail:alinezuma@gmail.com

Chagas disease is caused by the protozoan *Trypanosoma cruzi* and affects about 8 million people around the world. The current available drugs cause several side effects, which discourages the continuity of the treatment. Thus, the development of new drugs is imperative. *T. cruzi* is a parasite that presents similar characteristics to other eukaryotic cells. However, its enzymes differ from human ones in some aspects and therefore they can be used as chemotherapeutic targets. In this work, we evaluated the effects of new compounds which target histone deacetylases (HDACs), histone acetyltransferases (HATs), topoisomerases and cysteine proteases. The effects of these drugs were evaluated on *T. cruzi* and LLC-MK_2 proliferation, viability and ultrastructure for up to 96 hours. Every 24 hours samples were collected for cell counting, viability assays and electron microscopy analyses. Our results show that the cysteine proteases inhibitors were the most effective drugs against *T. cruzi* epimastigote proliferation (IC_{50} from 0,5 µM to 8 µM), followed by topoisomerases (IC_{50} from 4 µM to 20 µM), HDAC (IC_{50} from 7 µM to 50 µM) and HAT inhibitors (IC_{50} of 40 µM). In general, most of the compounds did not affect protozoa viability. Regarding to parasite ultrastructure, different alterations were observed, such as disorganization of the flagellum, mitochondrial swelling, atypical kDNA topology and plasma membrane wrinkling caused by HDAC inhibitors; and cytoplasmic extraction and membrane blebbing promoted by topoisomerase inhibitors. Among the analyzes performed so far, cysteine proteases and HDAC inhibitors are the less toxic compounds to LLC-MK_2 (CC_{50} higher than 10 µM). The evaluation of anti-proliferative and lytic activity of these drugs on amastigote and trypomastigote forms, respectively, are under investigation. Hence, based on these data, these compounds can be explored as promising drugs in further analysis on chemotherapeutic studies against *T. cruzi*.
Supported by:CNPq e FAPERJ **Keywords:**Trypanosoma cruzi; chemotherapy; ultrastructure

TB020 - **DEVELOPMENT OF QUANTITATIVE REAL-TIME PCR (TAQMAN TRIPLEX SYSTEM) FOR THE DIAGNOSIS AND EVALUATION OF THERAPEUTIC EFFICACY IN CHAGAS DISEASE.**

SOUZA, L.R.Q.[*1]; DE BARROS, L.R.[1]; MONTEIRO, A.G.F.[1]; TAVARES COSTA, A.D.[2]; KRIEGER, M.A.[2]; MOREIRA, O.C.[1]; BRITTO, C.[1]
1.IOC/FIOCRUZ, Rio de Janeiro, RJ, BRAZIL; 2.IBMP/FIOCRUZ, Paraná, PR, BRAZIL.
e-mail:alicegf83@gmail.com

Chagas disease (CD) affects about 8 million individuals in endemic areas with 20 to 30% eventually developing chronic Chagas cardiomyopathy, the most severe manifestation of the disease in humans. Serology is the gold standard for the diagnosis of chronic CD, whereas conventional and quantitative Real Time PCR (qPCR) have been used as complementary tools for diagnosis in cases of congenital transmission, discordant serological results in chronic patients and for evaluating etiological treatment. Based on the proof of concept previously generated by our group for developing a prototype qPCR duplex diagnostic kit (T. cruzi nuclear satellite DNA [SAT] and a heterologous internal amplification control [IAC]), we propose here the addition of a second target [kDNA] for T. cruzi detection in a single reaction (TaqMan triplex qPCR) to improve the sensitivity of the test. The first steps for standardization consisted in defining the best concentration of primers and probes (SAT DNA: 900nM of each primer, 150nM probe; kDNA: 300nM of each primer, 50nM probe; IAC: 200nM of each primer, 50nM probe), and the adjustment of the standard curves with the use of synthetic DNA for the three targets, to reach better accuracy in quantifying parasite load. Our results demonstrated optimal identities for sequences obtained from the amplified products generated by each target (96% kDNA, 97% SAT DNA, 100% IAC), when compared to those described in the database, and these sequences were already synthesized. Preliminary tests with the synthesized sequences showed a linearity of 107 to 102 copies of DNA. Standardization will also involve the testing of linearity, inclusiveness, exclusivity, precision, limit of detection (LOD), limit of quantification (LOQ) and clinical sensitivity and specificity of the reaction. We have as perspectives the setting of the triplex reaction using reagents produced by IBMP/Fiocruz and validate the assay with a panel of blood samples from patients with chronic CD.
Keywords: Chagas disease; real time pcr; diagnosis

TB021 - **GENERATION OF RECOMBINANT POLYPEPTIDES TARGETING TRYPOMASTIGOTE SURFACE PROTEINS OF *TRYPANOSOMA CRUZI*.**

DEMEU, L.M.K.*[1]; DE OLIVEIRA, K.G.[1]; SOARES, R.J.[1]; PLAZA, C.[2]; YOSHIDA, N.[2]; DE MOURA, J.F.[1]; ALVARENGA, L.M.[1]; DAROCHA, W.D.[1]
1.UFPR, Curitiba, PR, BRAZIL; 2.UNIFESP, São Paulo, SP, BRAZIL.
e-mail:lara_demeu@hotmail.com

The Chagas disease treatment by available drugs needs some improvement, since it is inefficient or can cause severe side effects. As shown for *T. brucei*, nanobody engineering was used to improve sleeping sickness treatment and towards to block insect transmission. Similar to this approach, here we describe our initial efforts to develop specific molecules that bind 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-TcGP35/50) and mAb-3F6 (anti-TcGP82) were sequenced and assembled as scFvs. Synthetic gene of scFv-10D8 was cloned and expressed in *E. coli* as secreted polypeptide fused to 6xHis tag (pET22b). Mammalian cells invasion assay using periplasmic fraction of bacteria expressing scFv-10D8 or unrelated scFv was performed, and the preliminary results show a specific and dose dependent reduction of infected cells when metacyclic trypomastigotes were pre-incubated with scFv-10D8. To increment the expression levels of soluble scFv-10D8, it has been cloned in different systems such as fused to TrxA in pET32 vector, or in yeast expression vector (pGAP and pGAPZ). Currently, we are optimizing the scFv-10D8 expression conditions to purify functional molecules. Additionally to these efforts, we used metacyclics and tissue-culture derived trypomastigotes (MT and TCT) to screen phage display libraries (XCX4CX, XCX8CX, X8CX8, and X15) to identify peptides that bind to the parasite surface. After 3 rounds of panning, 7 clones that recognize TCTs and 3 that bind to MTs were sequenced. From this preliminary sequencing, 5 clones encode 2 distinct peptides. These identified peptides are being tested for TCT binding capacity. Once we confirm the binding capacity of each molecule above, we plan to test it to ablate *T. cruzi* infectivity.â€‹
Supported by:CAPES, CNPq e Fundação Araucária
Keywords: Anti-t.cruzi; scfv; recombinant antibodies

TB022 - **EVALUATION OF THE ANTI-*PLASMODIUM FALCIPARUM* ACTIVITY *IN VITRO* OF THE EXTRACTS OF RUBIACEAE FAMILY PLANTS**

CERAVOLO, I.P.*[1]; PEREIRA, P.O.[1]; SILVA, A.F.[2]; ALVES, T.M.A.[1]; ZANI, C.L.[1]; KRETTLI, A.U.[1]
1.FIOCRUZ-MG, Belo Horizonte, MG, BRAZIL; 2.EPAMIG, Belo Horizonte, MG, BRAZIL.
e-mail:ceravolo@cpqrr.fiocruz.br

A large number of Brazilian plant species used in traditional medicine to treat malaria and/or fever have been screened by our group, and several species showed to be active against *Plasmodium falciparum* in culture. In the present study, we investigated the antiplasmodial activity of fractions obtained from 355 extracts of Rubiaceae plant species, a family known to have species with activity against the malaria parasite. The activity *in vitro* was evaluated against blood forms of *P. falciparum* (chloroquine-resistant clone W2) using the SYBR test, by two different investigators. In all tests, the compounds activities were expressed by the inhibitory concentration of the parasite growth when compared to the drug-free controls. Thirteen samples (1.8%) that corresponded to the crude ethanolic extracts of *Remijia* sp (stalk, 88.5%, leaves, 59.6%), *Alibertia* sp (aerial parts, 84.7%), *Borreria* sp (stalk and leaves, 68.6% and 52.5%, respectively), *Pagamea* sp (stalk, 50.9%), *Guettarda* sp (stalk, 48.7%), and two different samples of *Psytrochia* sp (aerial parts, 48.3%; leaves, 96.4% and stalk, 96.8%) presented inhibition of growth >48% when tested at 20 ug/mL. The determination of the 50% inhibitory concentration of the parasite growth (IC_{50}), the cytotoxicity in monkey kidney cell line (BGM) by neutral red uptake incorporation assay to calculate the dose that kills 50% of the cells (MDL_{50}) for the active samples, and the determinations of the selectivity index are ongoing.
Supported by:CNPq, PAPES VII, IRR and FIOCRUZ
Keywords: Chemotherapy; plasmodium falciparum; ethnopharmacology

TB023 - **EFFECT OF APIGENIN AND AMPHOTERICIN B COMBINED THERAPY FOR IN VITRO INFECTION WITH LEISHMANMIA AMAZONENSIS.**

EMILIANO, Y.S.S.[*1]; ALMEIDA-AMARAL, E.E.[1]
1.FIOCRUZ, Rio de Janeiro, RJ, BRAZIL.
e-mail:yagoemiliano@hotmail.com

Caused by different parasites of genus Leishmania sp., Leishmaniasis has been reported in 98 countries and affects more than 12 million people around the world. Leishmaniasis current treatment is based on pentavalent Antimonials, Amphotericin B and Miltefosine. However, they are expensive, ineffective and can bring resistance and side effects. Therefore, it is necessary the search for alternative treatment. In this scenario, natural products, and combination therapy are alternatives to be explored. Apigenin is a flavonoid present in common fruits and vegetables and have several biological functions. In this present study, we evaluated the effect combination therapy with Apigenin and Amphotericin B in vitro in L.amazonensis intracellular amastigote. For investigate the interaction of apigenin with the Amphotericin B, L. amazonensis-infected THP-1 were incubated for 72h with different concentrations of apigenin combined with Amphotericin B. 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 apigenin with miltefosin was 1.9. This result demonstrates that combination apigenin with Amphotericin B has an effect additive, but experiments will also be conducted to demonstrate the in vivo effect this combination. **Supported by:**FAPERJ, CNPq, CAPES, PAPES, IOC/FIOCRUZ
Keywords: Leishmaniasis; association; apigenin

TB024 - **EVALUATION OF GENOMIC AND MITOCHONDRIAL DNA TARGETS FOR MOLECULAR TYPING OF TRYPANOSOMA CRUZI BY HIGH RESOLUTION MELTING**

PINHO, M.A.[*1]
1.IFRJ, Rio de Janeiro, RJ, BRAZIL.
e-mail:matheuspinhom@gmail.com

Chagas disease is an endemic neglected disease, affecting approximately 7 to 8 million people worldwide, mainly in Latin America. Trypanossoma cruzi is the flagellate protozoan responsible for the infection. The diversity found in the genome and the phenotype heterogeneity of this etiological agent are well recognized, presenting a set of populations called isolates or strains. This causes differences in their biological behavior and clinical manifestations, as variation in virulence levels and drug sensitivity. Thereby, some targets have been described in the T. cruzi genome with potential to differentiate into distinct genetic groups (Discrete Typing Units - DTUs from I to VI). Currently, multilocus conventional PCR is used as gold standard to T. cruzi genotyping. The characterization obtained by this methodology is limited, in some cases by the absence of amplified products or the need to perform several amplifications to obtain the parasite genotype. This work aims to develop and analyze the performance of real time PCR with High-Resolution Melting (HRM) assays to differentiate DNA sequences of T. cruzi, with primers designed to the following genomic and mitochondrial targets: 24S-α, COII, SL-IRac, SL-IR I and II and A10. Therefore, six reference strains/clones (from different genetic groups: DTU I to VI) were selected as reference for genotyping: DM28c, Y, 3663, 4167, BUG2149 and CL14. Our preliminary results, using COII as target, allowed differentiating T. cruzi isolates into three well defined variants. Despite the low sensitivity (DNA extracted from 104 parasites/mL), the gel electrophoresis with the real time PCR product confirmed the expected amplicon sizes for each DTU. In conjunction with COII, the perspective is that the other targets can also be capable of distinguishing between T. cruzi strains/clones, allowing the identification of the parasite at lower concentrations, compatible with Chagas disease chronic patient samples.
Supported by:CNPq e FAPERJ
Keywords:T. cruzi genotyping; high resolution melting; chagas disease

TB025 - **BIOASSAY-GUIDED FRACTIONS OF *AIOUEA TRINERVIS* MEISN. (LAURACEAE) AGAINST EPIMASTIGOTES FORMS OF *TRYPANOSOMA CRUZI***

ALMEIDA, J.M.[*1]; NUNES, F.O.[2]; PANIAGO, G.M.[1]; DORNELLES, C.F.[2]; GONÇALVES, G.[1]; PADOVANI, C.T.J.[1]; TOZETTI, I.A.[1]; GARCEZ, W.S.[2]; GARCEZ, F.R.[2]; FERREIRA, A.M.T.[1]
1.INBIO/UFMS, Campo Grande, MS, BRAZIL; 2.INQUI/UFMS, Campo Grande, MS, BRAZIL.
e-mail:jjuliomenta@hotmail.com

Introduction: The research on *Trypanosoma cruzi*, maintained with important multicentric efforts, facilitate the discoveries of new drugs with less toxicity and more effectiveness. In the last years several species of plants from Cerrado were studied and showed promising results against *T. cruzi*. Among those species, *Aiouea trinervis* has demonstrated high activity and promising lactones. Therefore, were selected for this study bioassay-guided fractions from subterraneous stem of *A. trinervis*. Objectives: Evaluate the activity of fractions of *A. trinervis* against *T. cruzi*. Material and Methods: The bioassay-guided fractions were obtained by partition liquid-liquid. Then the fraction hexane was first fractionated on silica gel 60 (70-230 mesh), and after by chromatographic column with Sephadex LH-20. The in vitro activity against epimastigotes of *T. cruzi* (strain Dm28) was evaluated through the MTS method and the statistic analysis was done with the software Graphpad prism. Results and Discussion: The fractions obtained ethyl acetate, dichloromethane and hexane display a IC_{50}/72 hours respective of 3.4 (R^2=0.89), 6.67 (R^2=0.85) and 1.83 (R^2=0.71). From the hexane, the only fractions active was H2 with 6.76 (R^2=0.9) and H3 with 9.24 (R^2=0.9). Descendant from H3 the subfractions display activities of 18.14 (R^2=0.81) to EAPH 3-5-1, 11.98 (R^2=0.72) to EAPH 3-5-2 and 24.6 µg/mL (R^2=0.79) to EAPH 3-5-4. Impaired with the processes of isolation, the biological assays demonstrate a reduction of the activity with the fractionation, which can assume the presence of others compounds with activity beyond lactones, and the presence of a phenomenon of synergism. Those hypotheses should be tested in future studies. Conclusions: Those results indicate a strong activity of fractions from *A. trinervis* against the protozoa *T. cruzi*. Which indicate the promising character from this plant to elucidate a range of compounds with activity on this parasite. **Supported by:**CAPES e Fundect
Keywords: Chagas disease; cerrado; bioassay-guided fractions

TB026 - ***POGONOPUS TUBULOSUS* INFLUENCE IN THE *TRYPANOSOMA CRUZI* PROTEIN PROFILE**

GONÇALVES, G.[*1]; GARCIA, C.S.[1]; ALMEIDA, J.M.[1]; PORTELA, A.[2]; PANIAGO, G.M.[1]; GARCEZ, F.R.[2]; GARCEZ, W.S.[2]; TOZETTI, I.A.[1]; FERREIRA, A.M.T.[1]
1.INBIO/UFMS, Campo Grande, MS, BRAZIL; 2.INQUI/UFMS, Campo Grande, MS, BRAZIL.
e-mail:jjuliomenta@hotmail.com

Trypanosoma cruzi is the etiological agent of Chagas disease. It is implicated in thousand deaths per year and can trigger serious disability to workers, thus require the allocation of substantial governmental recourses for patients' treatment. The treatments currently used are effective in 50% of the cases in the acute phase, but are not in the chronic stage. The bioassays is the simplest way to detect the biological activity of compounds and points to studies in search of new substances with therapeutic properties. The aim of this study was to evaluate the protein profile of epimastigote forms of *T. cruzi* Dm28c treated with biologically active plant extracts from *P. tubulosus*. For that, parasites in exponential growth was incubated at 28ºC (72h) with six plant extracts in IC_{50} concentrations pre determined from MTT assay. After the incubation the parasites were subject to a total protein extraction with β-mercaptoetanol and the resulting extract used as a sample to obtain the protein profile with Polyacrylamide electrophoresis (SDS-PAGE). Comparing the protein profile of the parasites treated or not with the plant extracts was observed a super expression of a band about 70 KDa. Among several peptides mentioned in the literature, present in different stress conditions, we can highlight the group of heatshocks proteins, as well as precursors of the cruzipain protein in *T. cruzi*, which may accumulate once protein synthesis is impaired. Since the mechanisms of protein expression represent highly complex processes dependent on many factors, complementary studies should be performed in order to better understanding of events that cause changes in the expression of some proteins of this parasite. It can be concluded that the protein extracts of *P. tubulosus* influence the protein profile of *T. cruzi*. Such factor, along with their reduced IC_{50} values indicates a strong biological activity of such plant extracts. **Supported by:**CAPES e Fundect
Keywords: Sds-page; bioassays; natural products

TB027 - **IDENTIFICATION OF NEW MOLECULAR MARKERS FOR THE GENOTYPING OF SPECIES OF THE *LEISHMANIA* GENUS**

BENTO, G.A.[*1]; CARDOSO, M.S.[1]; LUIZ, G.F.R.[1]; FUJIWARA, R.T.[1]; GONTIJO, C.M.F.[2]; BARTHOLOMEU, D.C.[1]
1.UFMG, Belo Horizonte, MG, BRAZIL; 2.CENTRO DE PESQUISAS RENÉ RACHOU, Belo Horizonte, MG, BRAZIL.
e-mail:gabyaria@hotmail.com

Leishmaniasis are parasitic diseases caused by obligate intracellular protozoa of the *Leishmania* genus and present a broad spectrum of clinical manifestations. Currently, there is no gold standard methodology for diagnosis and the available techniques result in false positive or false negative results, hampering the appropriate treatment of the patients and the efforts to control the disease.There is a great need for a fast and reliable species-specific diagnosis for leishmaniasis, since each species has its own characteristics and the correct identification of the etiological agent is critical for an adequate treatment. PCR-based diagnostic methods have been widely used for the identification of *Leishmania* species. Among them, the multiplex PCR is an attractive alternative since it is a fast technique, that present potentially high sensitivity and specificity, at low cost. Thus, the objective of this work is to generate a panel of primers optimized for multiplex PCR for the identification of different species of the *Leishmania* genus in a single PCR reaction. The markers were identified by using the online tool TipMT, developed by our group, which allows the design of species-specific primers based on genomic data. Non-specific alignments of primers with other trypanosomatids, and with human, dog and sandfly hosts were evaluated using Primer-Blast tool. It was possible to obtain species-specific markers for *L. amazonensis*, *L. braziliensis*, *L. donovani*, *L. infantum* and *L. mexicana*. The markers have on average a detection sensitivity of up to 0.1 ng of parasite DNA, and are further optimized for use at the same annealing temperature in the PCR. In addition, these primers were specific for their target DNA when tested against DNA from 11 species of *Leishmania*, as well as 6 other trypanosomatids and *Babesia* sp. The standardization of multiplex PCR is in progress. This study may contribute for a more efficient, fast and reliable molecular diagnosis of leishmaniasis. **Supported by:**FAPEMIG and CNPq
Keywords: Leishmania genotyping; molecular markers; multiplex pcr

TB028 - **ANTILEISHMANIAL ACTIVITY OF TRIAZOLIC DERIVATIVES**

ALMEIDA, A.C.[*1]; EVANGELISTA, A.C.E.[1]; MEINEL, R.S.[1]; DA SILVA, A.D.[1]; COIMBRA, E.S.[1]
1.UFJF, Juiz de Fora, MG, BRAZIL.
e-mail:ayladaschagas@hotmail.com

Leishmaniasis consists of a complex of vector-borne diseases with several clinical manifestations caused by different *Leishmania* protozoa. This disease is one of the major public health problems, which affect more than 12 million people worldwide, with 350 million people in considered risk areas. Currently, leishmaniasis chemotherapy has many limitations, such as toxic side effects, high cost, long course of treatment and drug-resistance, making necessary the development of new treatment strategies. In this study, we evaluated the action of triazolic derivatives against promastigote forms of *L. amazonensis*, *L. braziliensis* e *L. chagasi,* and also analyzed the toxicity of the compounds on murine macrophages. Antipromastigote activity and cytotoxicity in peritoneal macrophages were determined by MTT colorimetric assay after 72 h of treatment. Among the seven compounds evaluated, three exhibited significant activity against promastigotes of *L. amazonensis*, *L. braziliensis* and *L. chagasi*, with IC_{50} values ranging from 0.29 to 32.69 µM. The best compounds showed cytotoxic effects on macrophages, otherwise they were more toxic to parasites than to host cell. These preliminary results stimulate further investigations with this class of compounds against intracellular amastigote forms of *Leishmania* sp for the development of new chemotherapeutic agents.
Supported by:CAPES, CNPq, FAPEMIG and UFJF
Keywords: Antileishmanial activity; triazolic derivatives; chemotherapy

TB029 - **2'-HYDROXYFLAVANONE EFFECTS AGAINST *LEISHMANIA AMAZONENSIS* ANTIMONY RESISTANT**

GONÇALVES-OZÓRIO, G.[*1]; GERVAZONI, L.F.O.[1]; ALMEIDA-AMARAL, E.E.[1]
1.FIOCRUZ, Rio de Janeiro, RJ, BRAZIL.
e-mail:gabriella.ozorio@gmail.com

Leishmaniasis is a disease that deserves attention due to the wide variety of clinical manifestations and its high annual incidence. For over decades, Pentavalent antimony is known to be the first line treatment in many countries around the world, including Brazil. It is well known that leishmaniasis treatment presents serious problems as side effects, variable efficacy and are considered expensive. Besides, one of the major problems of leishmaniasis treatment is the generation of resistance, and antimony is the leading figure. Over the decades, the search for alternative compounds able to figure out chemotherapy problems, including resistance, has been increased. Natural compounds obtained from plants have it is significant antiprotozoal activity described. 2'-hydroxyflavanone is abundantly present in fruits and vegetables and has some biological functions including anti-inflammatory and anticancer. Previous work showed the ability of 2'-hydroxyflavanone to inhibit promastigotes of *L. amazonensis* resistant to potassium antimony tartrate (SbIII). This study evaluated the effects of 2'-hydroxyflavanone in vitro and in vivo against resistant to potassium antimony tartrate *L. amazonensis*. BALB/c mice peritoneal macrophages were infected with antimony resistant *L. amazonensis* promastigotes overnight and incubated 18 hours later with different concentrations of 2'-hydroxyflavanone or antimony for 72 hours. The resistance was confirmed and 2'-hydroxyflavanone was able to decrease the infection index in a dose dependent manner. In the in vivo study, BALB/c mice were infected with antimony resistant *L. amazonensis* promastigotes for 7 days and treated with 50 mg/kg/day of 2'-hydroxyflavanone, 100 mg/kg/day of Glucantime or the vehicle. 2'-hydroxyflavanone was able to control the lesion size compared to control and Glucantime groups. Taken together, these results demonstrate the effects of 2'-hydroxyflavanone against *L. amazonensis* antimony resistant *in vitro* and *in vivo*.

Supported by:CNPq, CAPES, Faperj, IOC/Fiocruz
Keywords: Leishmania amazonensis; resistance; 2'-hydroxyflavanone

TB030 - **USE OF A NEW RECOMBINANT KINESIN-DERIVED ANTIGEN OF *LEISHMANIA* WITH POTENTIAL FOR THE DIAGNOSIS OF HUMAN AND CANINE VISCERAL LEISHMANIASIS**

SIQUEIRA, W.F.[*1]; CARDOSO, M.S.[1]; BUENO, L.L.[1]; BARTHOLOMEU, D.C.[1]; FUJIWARA, R.T.[1]
1.UFMG, Belo Horizonte, MG, BRAZIL.
e-mail:williane.f.siqueira@gmail.com

Visceral Leishmaniasis (VL), caused by protozoan *Leishmania infantum* and L. donovani, is the most severe form among the different types of leishmaniasis, which can lead to death if not diagnosed and treated. In Brazil, the VL is a zoonotic disease, being the dog its main reservoir. Among the measures of disease control, it is recommended the elimination of infected dogs, early diagnosis and treatment of human cases. However, the methods used to diagnosis of the disease are not sufficiently sensitive or specific. Aiming to contribute to the diagnosis and control of human VL (HVL) and canine VL (CVL), the objective of this study was to produce a new recombinant protein based on a highly repetitive sequence derived from the *Leishmania* kinesin and evaluate it in serological recognition tests. For the heterologous expression of the recombinant protein, the synthetic gene was assembled *in silico* and produced into the cloning vector pUC57. The gene was cloned and expressed in *Escherichia coli*, enabling the production of the recombinant antigen. The performance of the new recombinant protein was compared with other kinesin-derived antigens by ELISA assays for the diagnosis of HVL and CVL. Fifty samples of patients with HVL from endemic areas, 54 sera from *Trypanosoma cruzi* infected patients at chronic infection and 22 samples from negative patients from a non-endemic area were tested. The sensitivity and specificity for human detection using new recombinant protein were 98% and 100%, respectively, whereas for the antigens rKDDR was 98% and 96%; rK39, 98% and 71%; and rK26, 82% and 80%, respectively. ELISA tests using pool of sera from dogs with LVC and uninfected, against the recombinant protein, were able to distinguish between positive and negative cases. ELISA analyzes using individual sera from dogs are underway. The results suggest that the new antigen tested may contribute to the control of LV in humans and dogs through a more efficient and reliable diagnosis. **Supported by:**CAPES/FAPEMIG/CNPq **Keywords:**Leishmaniasis; immunodiagnosis; recombinant protein

TB031 - **PROOF OF CONCEPT FOR A CHIMERIC PROTEIN USED FOR VACCINATION AGAINST TRYPANOSOMATIDS**

FIGUEIREDO, L.A.[*1]; LUCAS, R.C.[1]; BARBOSA, F.S.[1]; MENDES, T.A.O.[2]; FUJIWARA, R.T.[1]
1.UFMG, Belo Horizonte, MG, BRAZIL; 2.UFV, Viçosa, MG, BRAZIL.
e-mail:luiza.almeida.figueiredo@gmail.com

The Family Trypanosomatidae comprises unicellular eukaryotic organisms of great medical and economic interest, since more than 1 billion people are infected worldwide by these protozoans. In this family are included the genera *Leishmania* and *Trypanosoma*, which are responsible for leishmaniasis and trypanosomiasis, respectively. Together these diseases generate approximately 4.27 million disability-adjusted life years (DALYs) due to the symptoms caused by parasitism. To date, there are no available human vaccines to protect against these diseases and the number of infected individuals continues to expand. Thus, in the present work we propose the development and use of a chimeric molecule formed by the fusion of DNA sequences that encode with immunogenic and conserved regions in the different parasites but absent in host species. The heterologous expression of a chimeric protein comprising 64 different peptides was produced in bacterial expression system, followed by subsequent protein purification and further use in the immunization of Balb/c mice. After 3 doses of vaccination with the chimeric protein formulated with MPLA or adjuvant alone at intervals of 15 days, animals were challenged with *L. infantum*, *L. mexicana* or *T. cruzi* strain Y. in vivo induction of specific immune responses induced by this vaccine was assessed by indirect ELISA. Parasite burden in specific organs was evaluated by quantitative PCR and a significant reduction of 68% in parasite load of animals challenged with *T. cruzi* was observed when compared to the control group. Moreover, we detected an 86% reduction in parasite burden on liver of vaccinated group, when challenged with *L. infantum*. For animals challenged with *L. mexicana* a significant reduction in the lesion diameter was detected after 12 weeks post infection in the vaccinated group. These preliminary results demonstrate the promising use of a potential vaccine against trypanosomatid infections. **Supported by:**CNPQ, FAPEMIG, CAPES
Keywords: Vaccine; trypanosomatids; chimeric protein

TB032 - **STUDIES ABOUT MECHANISM OF ACTION OF A QUINOLINE DERIVATIVE IN *LEISHMANIA AMAZONENSIS***

CALIXTO, S.L.[*1]; GRANATO, J.T.[1]; GLANZMANN, N.[1]; DA SILVA, A.D.[1]; COIMBRA, E.S.[1]
1.UFJF, Juiz de Fora, MG, BRAZIL.
e-mail:stephane.calixto@hotmail.com

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 of this disease present toxicity and high cost, as well as contraindications and increasing resistance. Based on that, this work had as objective evaluate the possible mechanism of action of a compound derived from quinoline (called NIC10), since it has been demonstrated in a previous work its antileishmanial activity, and its effect on the parasite's mitochondria. Material and Methods: Promastigote forms of *Leishmania amazonensis* were treated with 43 and 86 µM of the NIC10 for 24 hours, and the cells were labeled with annexin V-FITC, monodansilcadaverin (MDC), or propidiumiodide (PI). After, the stained cells were analyzed by flow cytometry or fluorimetry.To evaluate also changes in cell morphology and volume, giemsa staining and flow cytometry were used. Results: The treatment of promastigotes with NIC10 caused translocation of phosphatidylserine to the outer leaflet of the plasma membrane, as measured by annexin V-FITC binding (from 6.80% in the untreated promastigotes to 18.52% and 32.42% in cells treated with 43 and 86 µM of the NIC10, respectively), and concomitant alterations that included in cell-cycle arrest at the sub-G0/G1 phase (from 21.45% in the untreated cells to 31.82% in cells treated with 86 µM of the NIC10). Morphological changes, such as rounded bodies and reduction of cell volume were also observed in treated promastigotes, without rupture of the plasma membrane. The formation of autophagic vacuoles was not observed by labeling with MDC. Conclusions: These data suggest that treatment with the compound NIC10 induces some events related to apoptose-like in *Leishmania amazonensis*, with no death due to necrosis and autophagy processes. **Supported by:**CAPES, UFJF, CNPq and FAPEMIG.
Keywords: Leishmania amazonensis; quinoline; mechanism of action

TB033 - **ANTILEISHMANIAL EFFECT OF CHOLESTEROL DERIVATIVES IN LEISHMANIA SP**

GRANATO, J.T.[*1]; CALIXTO, S.L.[1]; DOS SANTOS, J.A.[1]; DA SILVA, A.D.[1]; COIMBRA, E.S.[1]
1.UFJF, Juiz de Fora, MG, BRAZIL.
e-mail:julianatgranato@hotmail.com

Leishmaniasis is a group of infectious diseases caused by intracellular protozoa of the genus Leishmania, responsible for a large global socioeconomic impact. The treatment for leishmaniasis is limited because the available drugs present high toxicity, parenteral administration, high cost and side effects. Cholesterol derivatives are a group of highly diversified compounds and many studies indicate the synthesis and application of them in pharmacology. However, studies involving the antileishmanial activity of this class of structures are scarce. With this in mind, the objective of this work was to determine the antileishmanial effect of cholesterol derivatives. In addition, the physicochemical and pharmacokinetic properties of the best compounds were also evaluated. Antipromastigote activity and cytotoxicity in peritoneal macrophages were determined by the MTT colorimetric method after 72 hours of treatment. The anti-amastigote effect was evaluated in macrophages infected with L. amazonensis after 72 hours of treatment. In general, the compounds showed varied effectiveness, with IC50 values ranging from 7.00 to 78.67 µM. The great majority of the compounds did not present toxic effect to mammalian macrophages until to the maximum concentration tested (150 µM). With regard to the anti-amastigote activity of L. amazonensis, the compounds 12, 13 and 16 (IC50 of 23.89, 23.33 and 15.34 µM, respectively) were the most effective for the intracellular form of the parasite, being more toxic to the parasite than the host cell. The in silico tests of physicochemical and pharmacokinetics parameters have shown that compounds 12, 13 and 16 exhibit important properties, such as passage through biological membranes and high gastrointestinal absorption with low toxicity prediction. Taken together, these results suggest that these molecules are drug candidates for oral administration.
Supported by:CAPES; FAPEMIG; CNPq; UFJF **Keywords:**Cholesterol; leishmania ; in silico

TB034 - **SEAWEED EXTRACT REDUCE THE GROWTH OF TOXOPLASMA GONDII ON HOST CELLS**

RODRIGUES, Y.M.[*1]; DAMATTA, R.A.[2]; SEABRA, S.H.[1]; MACHADO, N.I.[2]; DOS SANTOS, T.A.T.[2]; DE SOUZA, W.[3]; CINELLI, L.P.[4]
1.UEZO, Campo Grande, RJ, BRAZIL; 2.UENF, Campos dos Goytacazes, RJ, BRAZIL; 3.UFRJ, Rio de Janeiro, RJ, BRAZIL; 4.URFJ, Macaè, RJ, BRAZIL.
e-mail:ymr2@hotmail.com.br

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 of 5 different fractions (F9, F23, F44, F55 and F60). The crude extract was obtained after delipidation of the seaweed and proteolytic digestion with papain at 60°C. Fraction F9 was obtained by adding 10 % ethanol flowed by centrifugation. Through analysis by microscopy it was observed a reduction of the parasitic infection index with 500 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
Keywords: Toxoplasma gondii; dictyotacaribaea; sulfated polysaccharides

TB035 - ***IN VITRO* EVALUATION OF ANTI-*TRYPANOSOMA CRUZI* ACTIVITY OF SILIBININ**

SILVA, R.R.[*1]; TORCHELSEN, F.K.V.S.[1]; LIMA, A.P.B.[1]; ALMEIDA, T.C.[1]; MILAGRE, M.M.[1]; DA SILVA, G.N.[1]; DE LANA, M.[1]
1.CIPHARMA/EFAR/UFOP, Ouro Preto, MG, BRAZIL.
e-mail:rafaelrosi@gmail.com

Introduction: Chagas' disease (CD), caused by the protozoan *Trypanosoma cruzi*, is originally an endemic disease in Latin America that nowadays affects countries of several continents due to human migrations. Around 30-40% of the affected patients evolves for the chronic phase of the CD and can develop cardiomyopathy, megaesophagus and/or megacolon. Benznidazole (BZ), the only available drug to treat patients in Brazil, has important limitations such as low efficacy at the chronic phase and adverse reactions. Thus, the discovery of new drugs and therapies are needed. In this work, we evaluated the anti-*T. cruzi* activity of Silibinin (SLB), a natural flavanone derived from the milk thistle (*Silybum marianum*) plant, which has several medicinal uses, such as hepatoprotection, antioxidant, anti-cancer, anti-inflammatory, antiviral and anti-*Leishmania* protection, among others. Methods and Results: For trypanocidal evaluation, blood trypomastigotes forms of *T. cruzi* Y strain were treated with different concentrations (6.25, 12.5, 25, 50, 100 and 200 µg/mL) of SLB and, after 24 h in culture, the number of live parasites was counted in Neubauer's chamber in parallel to BZ used as control of parasite's death. For the cytotoxicity assay, VERO cells were seeded in 96-well plates and incubated with the effective concentrations (100 and 200 µg/mL) of SLB. The toxic effect was assessed after 24 h by MTT. The results showed that SLB: i) was able to significantly reduce the number of parasites in 54.22% and 69.88% at concentrations of 100 and 200 µg/mL, respectively; ii) showed IC50=80,57µg/mL, what demonstrates its trypanocidal activity similar to BZ at usual in vitro assay concentration (6µg/mL) and that SLB at concentration of 200µg/mL is significantly more active than BZ; iii) was not toxic to the VERO cells at these concentrations with trypanocidal effect. Conclusion: Our data indicate that SLB presents trypanocidal activity and it opens new perspectives for CD treatment. **Supported by:**CAPES
Keywords: Trypanosoma cruzi; chagas disease; silibinin

TB036 - **ACTIVITY OF LQB-166 (2-HYDROXY-3-PHENYL-1,4-NAPHTHOQUINONE) AGAINST LEISHMANIA (VIANNIA) BRAZILIENSIS IN VIVO**

VILLARIM, R.M.[*1]; SILVA, T.[1]; SOUZA, T.P.[1]; SILVA, A.J.M.[2]; SILVA, S.A.G.[1]
1.UERJ, Rio de Janeiro, RJ, BRAZIL; 2.UFRJ, Rio de Janeiro, RJ, BRAZIL.
e-mail:rafaellamvm@gmail.com

Leishmaniasis are neglected diseases with different clinical outcomes and are caused by protozoa of the genus Leishmania. In Brazil, the main agent of cutaneous and mucosal leishmaniasis is Leishmania (Viannia) braziliensis. The current therapeutic arsenals available for these diseases are toxic, expensive and parasite resistance is a growing problem. Given this scenario it is necessary to expand the alternatives for treatment of leishmaniasis. Naphthoquinones are bioactive molecules with antitumor and antiprotozoal activities. The aim of this study was to evaluate the effect of LQB-166 (2-hydroxy-3-phenyl-1,4-naphthoquinone), a lapachol analogue, on L. (V.) braziliensis in experimental models of infection. Infected BALB/c mice were treated after onw week of infection by intralesional route with 200µM of LQB-166 3 times/week for 2 weeks and infected hamsters Golden were treated after lesion establishment by intralesional route with 200µM of LQB-166 3 times/week for 5 weeks. This synthetic naphthoquinone was capable of controlling the lesion size and significantly decrease the parasite load ($p<0,001$) on BALB/c mice. On infected and treated golden hamsters the reduction of the lesion was small, but the parasite load in the infected footpad and draining lymph node was significantly reduced ($p<0,001$). These data show that 2-hydroxy-3-phenyl-1,4-naphthoquinone has activity against L. (V.) braziliensis, therefore it is a promising molecule to continued preclinical studies. **Supported by:**CAPES
Keywords: Leishmaniasis; hamster; balb c

TB037 - **GENERATION AND CHARACTERIZATION OF IRON SUPEROXIDE DISMUTASE-DEFICIENT *LEISHMANIA INFANTUM* LINES**

DA SILVA, P.A.[*1]; SANTI, A.M.M.[1]; MURTA, S.M.F.[1]
1.INSTITUTO RENÉ RACHOU, Belo Horizonte, MG, BRAZIL.
e-mail:anamariamurtasanti@yahoo.com.br

Pentavalent antimonial-containing compounds remain first-line therapies against leishmaniasis. As antimony-resistant parasites are being reported and several adverse reactions are related to these drugs, searching out new therapies is necessary. The iron superoxide dismutase A (*FeSODA*) is a metalloenzyme involved in the antioxidant defense by converting superoxide radicals to oxygen and hydrogen peroxide. This enzyme is found in trypanosomatids and it is absent in humans, which must be exploited as a potential target for leishmaniasis therapy. To investigate the role of *FeSODA* in *Leishmania infantum*, parasites lacking one *FeSODA* allele (*LiFeSODA*$^{+/-}$) were created by conventional homologous gene replacement. Knockout cassettes containing geneticin or hygromycin as resistance markers flanked by 3' and 5' UTR regions of this gene were constructed by fusion PCR approach and their correct sequence were confirmed by sequencing reaction. Then, the constructions were separately electroporated into wild-type (WT) *L. infantum* and clonal lines of *LiFeSODA*$^{+/-}$ were selected. The screening of the *LiFeSODA*$^{+/-}$ mutants was made by PCR and both resistance markers genes were amplified from genomic DNA of the mutants. Allelic integration in *LiFeSODA*$^{+/-}$ mutants was also accessed by PCR, using primers that anneals within the cassette sequence and in a chromosomal flanking sequence. The PCR showed fragments with expected length, suggesting the correct chromosomal integration of each cassette in several clones. Thereafter, the phenotype of *LiFeSODA*$^{+/-}$ mutants was evaluated. The growth curves of *LiFeSODA*$^{+/-}$ mutant lines were very similar to *LiWT* parasites. In addition, no difference in antimony-susceptibility phenotype was observed between *LiFeSODA*$^{+/-}$ mutant and parental *L. infantum* lines. Now, we are obtaining *FeSODA* null mutants in order to access the impact of the lack of this gene in *L. infantum* phenotype, especially in what concerns susceptibility to antimony and oxidative stress.
Supported by:FAPEMIG; CNPq; CAPES; UGA/FAPEMIG
Keywords: Leishmania infantum; antioxidant defense; iron superoxide dismutase

TB038 - **ANTILEISHMANIAL ACTIVITY OF TUBASTRAEA COCCíNEA EXTRACT IN VITRO**

SOUZA, T.P.[*1]; VILLARIM, R.M.[1]; CARPES, R.M.[1]; FELZENSZWALB, I.[1]; SILVA, S.A.G.[1]
1.UERJ, Rio de Janeiro, RJ, BRAZIL.
e-mail:thainasouza2@gmail.com

Leishmaniasis are a group of neglect tropical diseases. In Brazil, the most common tegumentary form of this disease is caused mainly by Leishmania (Viannia) braziliensis. The current therapeutic arsenal is limited, with pentavalent antimonials as first choice of treatment. These drugs present a lot of serious collateral effects and are not always effective, therefore it has been studied alternative treatments especially from natural products. This study aimed to test the in vitro antileishmanial potential of Tubastraea coccínea extract. This species has known pharmacological activities such as antiinflamatory and antioxidant action. Based on this knowlodge we decided to study the possible antileishmanial activity of T. coccínea extract. The experiments were realized on promastigotes forms of L. (V). braziliensis. Stationary phase parasites were trated with extract (0-500µM) for 48h and 72h. The number of viable promastigotes were counted under a microscope using Neubauer chamber. Citotoxic activity was tested on human monocytes from THP-1 cell line. The monocytes were incubated with extract (0-500µM) for 18h and cell viability evaluated by MTT assay. Our results have shown that T. coccínea exract has antiparasitic activity on promastigotes forms of L. (V.) braziliensis with low toxicity for human monocytes. IC50 = 58 µg/m and CC50 > 500 µg/mL. We are currently testing this extract on intracellular amastigotes forms of L. (V.) braziliensis and its possible modulation of imune response in macrophages. **Supported by:**CAPES
Keywords: Leishmaniasis; coral extract; treatment

TB039 - **TREATMENT WITH MELATONIN INDUCES A REDUCTION OF PARASITAEMIA IN LLC-MK2 CELLS INFECTED WITH TOXOPLASMA GONDII**

MACHADO, N.I.[*1]; PORTES, J.A.[2]; DOS SANTOS, T.A.T.[1]; DE SOUZA, W.[2]; DAMATTA, R.A.[1]; SEABRA, S.H.[3]
1.UNIVERSIDADE ESTADUAL DO NORTE FLUMINENSE DARCY RIBEIRO, RJ, BRAZIL; 2.UNIVERSIDADE FEDERAL DO RIO DE JANEIRO, RJ, BRAZIL; 3.CENTRO UNIVERSITÁRIO ESTADUAL DA ZONA OESTE, RJ, BRAZIL.
e-mail:nayinocencio90@gmail.com

It is known that the current treatment used for toxoplasmosis has side effects, being essential the development of new therapies with the capacity to reduce side effects, while maintaining broad coverage and prophylactic therapy. Melatonin is a hormone that participates in the circadian cycle in vertebrates and has antioxidant, immunomodulatory and antitumoral functions. It has been shown that melatonin is able to modulate the immune response and parasite development during infection by Toxoplasma gondii, Trypanosoma cruzi and Leishmania infantum. The aim of this study was to analyze the effects of melatonin treatment in LLC-MK2 epithelial cells infected by T. gondii. Hence, infected cells treated or not with melatonin were examined by optical microscopy and the infection rate quantified, showing reduction in the parasite proliferation after treatment, especially after 6 days. The possible type of death of the parasite caused by melatonin treatment was analyzed. Positive staining for apoptosis in the population of the parasite was observed after treatment. Furthermore, analysis by electron microscopy was performed to assess the morphology of the host cell and the parasite. Scanning electron microscopy results indicate reduction in parasitic proliferation, corroborating the infection rate data. Analysis by transmission electron microscopy indicated the parasite death by necrosis, with plasma membrane rupture and cytoplasm leakage. In vivo experiments were performed to analyze the survival of the host after treatment with melatonin or sulfadiazine (compound currently used) and similar efficacy was obtained with both treatments. These results suggest that the use of melatonin may be an alternative treatment for toxoplasmosis, reducing the parasitic proliferation in the host organism. **Supported by:**FAPERJ **Keywords:**Chemotherapy; synergistic treatment; toxoplasma gondii

TB040 - **EGCG IS EFFECTIVE IN LEISHMANIA INFANTUM PROMASTIGOTES BY TRYPANOTHIONE REDUCTASE INHIBITION**

INACIO, J.D.F.[*1]; FONSECA, M.S.[1]; LIMAVERDE-SOUSA, G.[1]; ALMEIDA-AMARAL, E.E.[1]
1.IOC - FIOCRUZ, Rio de Janeiro, RJ, BRAZIL. e-mail:job@ioc.fiocruz.br

The redox balance has several important functions for the protozoan parasite, and enzymes present in this system play a vital role for this parasite. Leishmania survives in a hostile environment caused by the production of ROS and reactive nitrogen species by the host's immune system macrophages. Trypanothione reductase (TR) is present only in trypanosomatids and participates in the redox system, which makes this enzyme an excellent target for the development of antileishmanial drugs. The epigallocatechin-3-gallate (EGCG), is a flavonoid that has pharmacological properties such as anti-inflammatory, microbicidal and trypanocidal activities. Recently, our group demonstrated the in vitro and in vivo effect of EGCG in L. amazonensis and L. braziliensis. In the present study, we demonstrated the effect of EGCG in L. infantum promastigotes and TR inhibition in vitro and shed light on the molecular mechanism of interaction between EGCG and TR. For this, L. infantum promastigotes were incubated in increasing concentrations of EGCG (15.6-500µM) for 72 hours and the cell viability was estimated by alamar blue assay. EGCG showed an inhibition of promastigotes proliferation of 97% (500µM) and an IC50 of 192µM. The activity of TR was evaluated by Ellman method. EGCG was able to inhibit TR activity in a dose-dependent manner presenting a Ki of 644.5µM. Using molecular docking calculations, we showed that the EGCG could effectively dock into the substrate binding site together with the substrate molecule, with the estimated free energy of biding (using Autodock 4.2 force field) of -7.96 kcal/mol. On the other hand, in the absence of substrate EGCG docks into NADPH site, with estimated free energy of binding of -8.18 kcal/mol. Taken together, our results suggest that EGCG inhibit the L. infantum promastigotes proliferation by TR inhibition. Given TR as a molecular target, it is important to continue the study of EGCG as part of a drug discovery campaign against Leishmaniasis.
Supported by:IOC/FIOCRUZ, CAPES, FAPERJ, CNPq and PAPES **Keywords:**Leishmania infantum; trypanothione reductase; egcg

TB041 - ***PIPER ABUTILOIDES*** **SUBSTANCES FOR DEVELOPMENT OF LESHMANICIDAL AGENT**

FELISBERTO, V.C.[*1]; DE PAULA, L.R.[1]; NOGUEIRA DOS SANTOS, C.E.[1]; SOARES, D.C.[1]; CARBONEZI, L.H.[1]; SARAIVA, E.M.[1]
1.UFRJ, Sao Goncalo, RJ, BRAZIL.
e-mail:valfelisberto2009@hotmail.com

Treatment used for leishmaniasis are pentavalent antimonials, amphotericin B and miltefosine, which have high toxicity and several side effects leading to patients withdrawal and to increased incidence of drug-resistant cases. The search for new substances, which are efficient against the parasite with low toxicity to host cells, is important to improve leishmaniasis therapy.
The Piperaceae family contains plants that demonstrated antimicrobial activity, such as leishmanicidal, anti-*Trypanosoma* and anti-*Plasmodium*. Here we evaluated the leishmanicidal activity of Eusiderin A isolated of leaves from *Piper abutiloides* after a bioassay-guided fractionation.
The previous bioassay-guided was used to evaluate the anti-promastigote activity of *Piper abutiloides* leaf extract. Our results showed that *Piper abutiloides* extract (AL1730F) demonstrated a dose-dependent activity with IC_{50} of 20µg/mL, while hexane fraction (Frhex) and dichloromethane fraction (Frdic) demonstrated IC_{50} of 4 µg/mL and 7 µg/mL, respectively. Subsequently, successive fractions of Frhex were performed until identification of the substance Eusiderin A. After we verify the toxicity of Eusiderin A on BALB/c macrophages. Our data showed low toxicity with IC_{50} >200 µg/mL. On the other hand, Eusiderin A exhibits IC_{50} of 2.3 µg/mL for promastigotes, 10 folds greater than activity of AL1730F, and IC_{50} of 26 µg/mL for amastigotes. The anti-amastigote activity of Eusiderin A was independent of micobicidal activity of Reactive Oxygen Species (ROS), since Eusiderin A decreased ROS levels in macrophages stimulated with Phorbol 12-myristate 13-acetate (PMA) infected or not with *Leishmania amazonensis*.
Our results stimulate us to study its mechanism of action Eusiderin A, as well as the leishmanicidal potential of its derivatives for development of new leishmanicidal substances.
Supported by:CAPES, FAPERJ and CNPq **Keywords:**Leishmania; piper abutiloides; eusiderin a

TB042 - **EFFECTS OF ANTI-TOXOPLASMA GONDII OF METALLOCOMPLEXE COMPOUNDS COORDINATED WITH SULFADIAZINE**

DUARTE, A.G.P.[*1]; MOREIRA DE CARVALHO, G.O.A.[2]; DAMATTA, R.A.[3]; FORTES, F.S.A.[1]; SEABRA, S.H.[1]
1.UEZO, Rio de Janeiro, RJ, BRAZIL; 2.UFRRJ, Rio de Janeiro, RJ, BRAZIL; 3.UENF, Rio de Janeiro, RJ, BRAZIL.
e-mail:arygpduarte@gmail.com

Toxoplasma gondii, toxoplasmosis agent, is a obligate intracellular protozoan that is able of infecting a broad spectrum of vertebrate cells. Toxoplasmosis is a pathology related to severe damages to immunocompromised hosts and its current chemotherapy is quite restricted, being more used the combination of sulfadiazine and pyrimethamine, which is a therapy associated with adverse reactions. This fact highlights the importance of the study of new drugs against *Toxoplasma gondii*. Our group has been studied the biological effect of new metallocomplexe compounds, which are inorganic compounds that present promising biological activity as fungicide, bactericide and antiviral. The metallocomplexes, ferric compounds dinuclear N0414 (Fe alfanaftol BMPA) and N5814 (Fe beta-naphthol BMPA) showed activity against *Toxoplasma gondii in vitro* and it was nontoxic to LLC-MK2 cells, being able to reduce the activity of crucial antioxidant enzymes for the defense of the parasite. In this study, it was investigated the activities of metallocomplexes family compounds coordinated to sulfadiazine as the nucleus compound of ferric N0414 and N5814.The last ones showed anti-Toxoplasma activities and were able to eliminate the infection in almost all host cells. **Supported by:**FAPERJ **Keywords:**Toxoplasma gondii; toxoplasmosis; chemotherapy

TB043 - *IN VITRO* BIOLOGICAL ACTION OF THYMOL ACETATE AGAINST *LEISHMANIA AMAZONENSIS*

ROMEIRO, B.[*1]; FLORENCIO, M.[1]; PALMEIRA, D.[1]; RAMUZ, J.[1]; GOMES, G.A.[2]; PINTO SILVA, L.H.[1]; DE CARVALHO, M.G.[1]; FAMPA, P.[1]
1.UFRRJ, Seropedica, RJ, BRAZIL; 2.UNIVERSIDADE ESTADUAL VALE DO ACARAÚ, Sobral, CE, BRAZIL.
e-mail:florencio.mel@gmail.com

The leishmaniases are a group of diseases caused by protozoan parasites from more than 20 Leishmania species, and that affect around 12 million people worldwide. The parasites are transmitted by insects of *Phlebotomus* or *Lutzomyia* genera during female blood meal. *Leishmania amazonensis* is one of the species responsible for the cutaneous form of the disease in the New World. Currently, drugs used for the treatment of leishmaniases are limited and still based on toxic and expensive pentavalent antimonials as first choice and, in case of therapeutic failure, amphotericin B and pentamidine are used as alternatives. Natural products from plants are a wide source of possible active compounds for the development of new drugs for control and prevention of leishmaniases. Thymol acetate is a thymol (usually found in plants essential oils composition) derivate with described action against *L. infantum* (de Morais, 2014). In the present work, we tested thymol acetate against *L. amazonensis* promastigote and amastigote forms. Promastigotes were treated with 5, 10, 15 and 20 µM for 96h, which led to growth inhibitory effect of 20, 99.1, 99.2 and 99.25%, respectively. In order to verify the cytotoxicity to vertebrate host cells, murine peritoneal macrophages were treated or not with 1, 5 and 10 µM of the compound for 48h. XTT assays were performed and at least 60% of cell viability was maintained. Finally, we treated amastigotes-infected macrophages with different thymol acetate concentrations for 24h, and after counting at least 600 hundred cells, we observed a reduction of 40% in the number of infected macrophages treated with 5 µM of thymol acetate when compared to non-treated control macrophages. Results obtained so far show the importance of thymol acetate in therapeutics against *L. amazonensis*.
Supported by:FAPERJ **Keywords:**Chemotherapy; natural products; neglected diseases

TB044 - *IN VITRO* MODULATION OF *LEISHMANIA AMAZONENSIS* RESPONSE TO DIFFERENT NUTRACEUTICALS IDENTIFIED BY COMPUTATIONAL BIOLOGY

LIMA, D.A.[*1]; CARVALHO, L.R.S.[1]; CRUZ, R.M.S.[1]; BORGES, F.S.[1]; PASSOS, J.C.[1]; LIMA, S.Y.V.[1]; LIARTE, A.S.C.[1]; MURTA, S.M.F.[2]; LIARTE, D.B.[1]
1.UFPI, Teresina, PI, BRAZIL; 2.FIOCRUZ - CPQRR, Belo Horizonte, MG, BRAZIL.
e-mail:davi.brago@gmail.com

Protozoan parasites belonging to Leishmania genus are the causative agents of leishmaniasis that produces a wide spectrum of clinical disease in humans. Nutraceuticals are food supplements that modulate the parasitic metabolism and may optimize the conventional treatment. In a previous study, DNA microarray and bioinformatics analysis identified potential modulating nutraceuticals of Leishmania response to antimonials. Here, the in vitro effect of 7 nutraceuticals (3 vitamins V1-3, 3 amino acids A1-3 and a mineral salt S1) was evaluated in L. amazonensis promastigote forms in M199 medium (5% fetal bovine serum). The drug susceptibility was evaluated by measuring the inhibitory concentration (IC50 and IC90) determined using a colorimetric AlamarBlue dye reduction assay. The results showed that the trivalent antimony (SbIII) IC50 and IC90 was 100µg/ml and 200µg/ml, respectively. In the absence of SbIII, the supplementation of cells with nutraceuticals did not affect the parasite growth, except A1, which tested in 100X concentration in M199 medium caused significative parasite growth reduction. In the presence of SbIII, 2 nutraceuticals (S1 and A1) at 100X concentration in M199 medium modulated the parasite growth. Parasites incubated with S1 and SbIII, the IC50 and IC90 values were 1.5- and 7-fold higher than the culture parasites non-supplemented, respectively. In contrast, using A1 and SbIII the IC50 and IC90 were 2.2- and 1.3-fold reduced, respectively. S1 did not modulate the parasitic growth in the absence of SbIII, possibly indicating a nutraceutical-drug interaction. Nutraceuticals are food products, its ability of interfering on experimental chemotherapy of leishmaniasis can contribute to the development of new therapeutic schemes, taking advantage of the potential of nutrients and minimizing the drugs necessity. **Supported by:**CNPq; FAPEMIG; FIOCRUZ
Keywords: Nutraceutical; leishmania amazonensis; antimonials

TB045 - **EFFECT OF RESVERATROL ON *LEISHMANIA BRAZILIENSIS***

MACHADO, P.A.[*1]; CALIXTO, S.L.[1]; GRANATO, J.T.[1]; COIMBRA, E.S.[1]
1.UFJF, Juiz de Fora, MG, BRAZIL.
e-mail:stephane.calixto@hotmail.com

Introduction and Objectives: American cutaneous leishmaniasis (ACL) is a complex disease with clinical features caused by different Leishmania species. In Brazil, *Leishmania braziliensis* is the main etiological agent of ACL. The drugs used for the treatment of ACL are administered parenterally and are associated with a high frequency of adverse effects. Resveratrol is a polyphenol found in black grapes and redwine, and this compound achieved renown in the scientific literature when it was postulated as responsible for the cardiac protective effects of wine. Regarding leishmaniasis, resveratrol has shown effect on *L. major* and *L. amazonensis*, but has not been previously tested against *L. braziliensis*. Thus, this study aims to analyze the antileishmanial activity of resveratrol against promastigote and amastigote forms of *L. braziliensis*. In addition, to compare the effect of resveratrol against different Leishmania species. Material and Methods: Three Leishmania species were used: *L. amazonensis, L. major and L. braziliensis*. The antipromastigota activity and cytotoxicity on murine peritoneal macrophages were determined by colorimetric method (MTT) after 72 hours of treatment. The antiamastigote assay was evaluated in infected macrophages y of the amastigotes was analyzed by the parasite counting. Results: Resveratrol showed a moderate effect against promastigotes and amastigotes of *L. braziliensis*, with IC50 values of 75.13 and 124.48 µM, respectively. As expected, resveratrol inhibited the growth of promastigotes and amastigotes of *L. amazonensis* (IC50 values of 22.23 and 35.85 µM, respectively) and *L. major* (IC50 values of 54.38 and 52.40 µM, respectively). Conclusions: *Leishmania braziliensis* was less sensitive to the effect of resveratrol when compared to other *Leishmania* tested in this work. These results confirm the differences of sensitivity of each Leishmania species, and it reinforces the difficulties of treatment of ACL. **Supported by:**CAPES, FAPEMIG, CNPq and UFJF
Keywords: Leishmania braziliensis; resveratrol; american cutaneous leishmaniasis

TB046 - **THE ANTILEISHMANIAL EFFECT OF A HYDRAZONE COMPOUND IS RELATED TO MITOCHONDRIAL DISFUNCTION**

CRISPI, M.A.[*1]; GRANATO, J.T.[1]; CALIXTO, S.L.[1]; MACHADO, P.A.[1]; CORREIA, N.L.[2]; SOUZA, M.V.N.[3]; COIMBRA, E.S.[1]
1.UFJF, Juiz de Fora, MG, BRAZIL; 2.FIOCRUZ-FAR MANGUINHOS, Rio de Janeiro, RJ, BRAZIL; 3.FIOCRUZ- FAR MANGUINHOS, Rio de Janeiro, MG, BRAZIL.
e-mail:julianatgranato@hotmail.com

Leishmaniases is a tropical disease caused by flagellate protozoan of Leishmania genus, affecting more than 12 million people worldwide. The treatment available exhibit limitations, including toxicity, high cost, contraindications and resistance. The search by new compound with antileishmanial activity is very important. In a previous work, we showed a promising antileishmanial effect of some hydrazone compounds. In this work, we investigate if the antileishmanial activity of the best hydrazone compound (named PNAT20) is related to mitochondrial dysfunction. Leishmania, like other trypanosomatids, presents single mitochondria that could act as an important drug target. To analyze the effect of the compound PNAT20 (17.28 and 34.58µM, corresponding to 1 and 2 times IC50 value) in the parasite mitochondria, the mitochondrial membrane potential and the production of reactive oxygen species (ROS) were analyzed by using H2DCFDA and Mitotracker® Red CM-H2XROS, respectively. The treatment of promastigote forms of L. amazonensis with PNAT20 at 17.28 and 34.58µM induced a significant reduction in the mitochondrial membrane potential (38.87% and 32.72%, respectively) and increase of production of ROS (20.0% and 37.65% , respectively), compared to untreated control. These results demonstrate that the compound exerts effect on mitochondria of the parasite, leading to mitochondrial dysfunction, contributing to the parasite death. **Supported by:**CNPq; FAPEMIG; CAPES and UFJF
Keywords: Hydrazone; leishmania ; mitochondrial dysfunction

TB047 - **IN VITRO SCREENING FOR ANTILEISHMANIAL ACTIVITY OF ASYMMETRICAL 1,2,4,5-TETRAOXANES LEAD TO IDENTIFICATION OF NEW PROMISING COMPOUNDS TO TREAT LEISHMANIASIS.**

ALMEIDA, R.M.[*1]; ANTOLINEZ, I.V.[1]; BARBOSA, L.C.[1]; FERREIRA, S.R.[2]; FUJIWARA, R.T.[1]
1.UFMG, Belo Horizonte, MG, BRAZIL; 2.UFRR, Boa Vista, RR, BRAZIL.
e-mail:raquel_m_almeida@hotmail.com

Leishmaniasis is a neglected tropical disease endemic in 98 countries, posing as a risk to 350 million people, with an incidence of 1.5 million new cases every year. Although this disease was discovered more than 100 years ago, the currently available drugs for treatment of leishmaniasis are almost the same as the ones used 50 years ago. The efficacy of the treatment based on these drugs is limited due to their toxicity, the presence of resistant strains in several Leishmania species, the length of treatment and its high cost. Thus, the continuous search for a new, cheaper, effective and less toxic treatment for this disease is still necessary. In this study, we evaluated the potential of 11 asymmetrical 1,2,4,5-tetraoxane compounds characterized by the presence of peroxidic bonds within its structure, to inhibit the intracellular growth of Leishmania amazonensis 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 was evaluated by metabolic activity using MTT assay. The tested compounds RC21, RC56, RC60, RC65 e RC83 were able to inhibit the intracellular amastigote proliferation with the respective IC50 values: 133, 88, 16, 18 and 227μM. The RC60 and RC65 compounds presented better results than the positive control antimony salt III (80 μM) and the compound RC65 presented low toxicity against macrophage DH82, BGM and HepG2 cells. Moreover, the compound RC65 showed excellent antileishmanial activity at micromolar level and high selectivity index of 55. Here, the present results denote the potential of 1,2,4,5-tetraoxane, in special the compound RC65, as promising candidate for the treatment against leishmaniasis. **Supported by:**FAPEMIG, CAPES, CNPq
Keywords: Antileishmanial; leishmania amazonensis; tetraoxane

TB048 - **ANTI-*TRYPANOSOMA CRUZI* EFFECT OF NEW METALOCOMPLEXES COMPOUNDS AGAINST THE GROWTH OF EPIMASTIGOTES *IN VITRO***

MOREIRA, F.F.[*1]; PORTES, J.A.[2]; AZEREDO, N.F.B.[1]; FERNANDES, C.[1]; JUNIOR, A.H.[1]; DAMATTA, R.A.[1]; DE SOUZA, W.[2]; SEABRA, S.H.[3]
1.UENF, Campos dos Goytacazes, RJ, BRAZIL; 2.UFRJ, Rio de Janeiro, RJ, BRAZIL; 3.UEZO, Rio de Janeiro, RJ, BRAZIL.
e-mail:felipefmoreira91@gmail.com

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 treatment of Chagas 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, there is a need to develop new compounds able to treat more efficiently Chagas's disease. Some reports in the literature demonstrate that coordination compounds may be an interesting alternative for antiparasite therapy against Leishmania spp., *Toxoplasma gondii* and *Trypanosoma cruzi.* Here we tested the *in vitro* effect of the iron compounds, I and II, on the growth of *T. cruzi* epimastigotes (Y strain). The parasites were treated with the compounds at concentrations ranging from 1 to 100 nM and their number quantified. The compound I presented an IC_{50} value of 4.14 nM and 4.81 nM, after 72 and 120 h of treatment, respectively. The II compound 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 compound II 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. These results showed that the metalocomplexes compounds were active against *T. cruzi* epimastigotes presenting low IC_{50} values and affecting the mitochondria an essential organelle for the parasite survival. The next step will be to further analyze the effect of these compounds, specially of compound I, 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

TB049 - **EFFECT OF STOMOXYN, AN ANTIMICROBIAL PEPTIDE FROM *STOMOXYS CALCITRANS*, AGAINST *LEISHMANIA AMAZONENSIS* AND *TRYPANOSOMA CRUZI***

RAMUZ, J.[*1]; PALMEIRA, D.[1]; ROMEIRO, B.[1]; FLORENCIO, M.[1]; FAMPA, P.[1]
1.UFRRJ, Seropédica, RJ, BRAZIL.
e-mail:jramuz@gmail.com

Antimicrobial peptides (AMPs) are low molecular weight molecules that are part of the innate immunity. AMPs are produced by a large variety of prokaryotic and eukaryotic organisms and they show activity against bacteria, viruses, fungi and protozoa. Stomoxyn is an AMP found in the intestinal epithelium and in the saliva from the stable fly *Stomoxys calcitrans*, important vector of several pathogens. It has already been shown that stomoxyn presents action against the protozoan parasite *Trypanosoma brucei rhodesiensis* (Boulanger et al, 2002) besides antifungal and antibacterial activity. In this work, we tested the effect of stomoxyn (provided by Bulet, P. from CNRS, FRA) against the protozoan *Leishmania amazonensis*, causative agent of cutaneous leishmaniasis in the Americas and against the protozoan *Trypanosoma cruzi*, causative agent of Chagas disease. We tested the efficacy of stomoxyn against epimastigote forms of *T. cruzi* at concentrations ranging from 0.1 to 100 µM for 96 hours. In order to test the interference of Fetal Bovine Serum (FBS) in the effectiveness of the AMP, we tested concentrations ranging from 0.1 to 1 µM with 1% of FBS. We did not register any growth inhibitory effect. For *L. amazonensis*, the promastigote forms were incubated with 1 to 400 µM of stomoxyn for 96 hours. The results showed growth inhibition of 51, 34, 40, 51 and 29% at 1, 10, 25, 50 and 100 µM, respectively. The influence of FBS concentration was also assayed: parasites were incubated with 1% of FBS at concentrations ranging from 0.1 to 1 µM of stomoxyn for 96 hours with no effect. We also tested the cytotoxicity of the AMP to murine macrophages incubated with 1 to 100 µM of stomoxyn, using the XTT assay. The results after 48 hours showed that the peptide does not cause cell damage. These results show that stomoxyn has no effect against *T. cruzi* in those concentrations however the effect against L. amazonensis shall further be explored due to the results that showed potential effect.
Supported by:CNPq e FAPERJ
Keywords: Antimicrobial peptides; stomoxyn; neglected diseases

TB050 - **ANTI-TRYPANOSOMA CRUZI ACTIVITY OF HALOGEN- AND SELENIUM-CONTAINING QUINONES**

BARBOSA, J.M.[*1]; DE CASTRO, S.L.[2]; SILVA JR, E.N.[3]; SALOMÃO, K.[2]
1.UNIRIO, Rio de Janeiro, RJ, BRAZIL; 2.FIOCRUZ, Rio de Janeiro, RJ, BRAZIL; 3.UFMG, Belo Horizonte, RJ, BRAZIL.
e-mail:julianabmunirio@gmail.com

Chagas disease, caused by the protozoan Trypanosoma cruzi, affects approximately six million individuals, who can develop cardiomyopathy, digestive megasyndromes or both. At the present, the only available drugs used in the treatment for this disease are benznidazole (Bz) and nifurtimox (Nif). Both of them are effective against acute infection however, show poor activity in the late chronic phase, with severe collateral effects and limited efficacy against different parasitic isolates. In this context, over the past 20 years, our group has been working on experimental chemotherapy for Chagas disease in collaboration with medicinal chemistry research group, with special emphasis on quinoidal compounds. Quinones are considered privileged structures in medicinal chemistry due to their biological activities and structural properties, it can be found in various plant families or as synthetic substances and are the focus of research aimed at the identication of derivatives with efficacy and selectivity toward T. cruzi. In this work thirty four halogen- and selenium-containing quinones were synthesized and were evaluated against bloodstream forms of T. cruzi (Y strain) and mammalian cells (macrophage). From these compounds, 22 were more active than benznidazole, the reference drug, with 2, 3, 13, 14, 19, 20, 21, 22, 25, 28, 51, 55, 56, 57 and 59 exhibiting IC50/24h values lower than 2 µM, with selectivity index (SI) between 7.5 and 62.5. The most active compound (E)-5-styryl-1,4-naphthoquinone (59) (IC50/24h = 0.19 ± 0.08 µM), is fifty fold more active than benznidazole (IC50/24 h = 9.68 ± 2.35 µM), with an SI of 62.5. Although the studies described here are preliminary, it is worth noting that the combination of a practical synthetic route, high in vitro trypanocidal activity and low toxicity to mammalian cells, makes 59 a very attractive hybrid quinone in the search for better trypanocides. **Supported by:**cnpq e FAPERJ
Keywords: Trypanosoma cruzi; chemotherapy; quinones

TB051 - **IDENTIFICATION OF POTENTIAL NEW MOLECULAR TARGETS FOR THE TREATMENT OF LEISHMANIASIS**

SILVA, R.L.[*1]; ANDRADE-NETO, V.V.[1]; PEREIRA, L.O.R.[2]; CUERVO, P.[2]; GUIMARÃES, A.C.R.[3]; TORRES-SANTOS, E.C.[1]; CATANHO, M.[3]
1.LABORATÓRIO DE BIOQUÍMICA DE TRIPANOSOMATÍDEOS - IOC - FIOCRUZ, RJ, BRAZIL; 2.LABORATÓRIO DE PESQUISA EM LEISHMANIOSE - IOC - FIOCRUZ, RJ, BRAZIL; 3.LABORATÓRIO DE GENÔMICA FUNCIONAL E BIOINFORMÁTICA – IOC - FIOCRUZ, RJ, BRAZIL.
e-mail:raphalopesdasilva@gmail.com

Hemeproteins belonging to cytochrome P450 complex (CYP450) are enzymes well known for their role in drug metabolism. But, beyond that, they are involved in several other metabolic pathways, such as sterol metabolism. Several authors have suggested inhibition of CYP51 (sterol C14-demethylase) from *Leishmania* spp. as a possible target for chemotherapy of leishmaniasis. The sequencing and annotation of the genomes of several *Leishmania* species contributed to the development of robust comparative and functional analyzes, allowing a better understanding of important biological processes, or even revealing other biological processes still unknown in these organisms. In this work, the identification and characterization of CYP450 enzymes encoded in the genome of six species of the genus *Leishmania* was performed to reveal new putative targets for the repositioning of drugs for leishmaniasis. The set of human CYP450 enzymes deposited and annotated in the SwissProt database, totalizing 53 sequences, was compared with the set of proteins of the species of the genus *Leishmania* predicted and annotated in the online database TrytripDB. The comparisons were made through a search for local similarity with the use of the blastp program. Four statistically significant hits (E-value ≤ 0.001) were found in each species of the genus, representing putative homologues to distinct sequences belonging to different known families of human CYP450. All sequences have characteristic motifs founded in the CYP450 family. Functional annotation analyzes demonstrated the presence of characteristic sites of the CYP450 family. Secondary structure and proteomic analyzes are being performed with the sequences to evaluate their potential as drug targets. **Supported by:**FAPERJ/CAPES/CNPq **Keywords:**Cyp450; leishmania sp. ; therapeutic target

TB052 - **IDENTIFICATION OF BINDING PEPTIDES TO *TRYPANOSOMA CRUZI* CELL SURFACE USING A PHAGE DISPLAY APPROACH**

SAENZ GARCIA, J.L.[*1]; PACHECO-LUGO, L.A.[1]; CAETANO, C.S.[2]; AVILA, R.A.M.[2]; DE MOURA, J.F.[3]; DAROCHA, W.D.[1]
1.LABORATORIO DE GENÔMICA FUNCIONAL DE PARASITOS, PR, BRAZIL; 2.LABORATÓRIO DE BIOLOGIA CELULAR E MOLECULAR, SC, BRAZIL; 3.LABORATORIO DE IMUNOQUIMICA, DEPARTAMENTO DE MICROBIOLOGIA E PARASITOLOGIA, PR, BRAZIL.
e-mail:jose711garcia@hotmail.com

Chagas disease, also known as American trypanosomiasis, is a potentially life-threatening illness caused by the protozoan parasite *Trypanosoma cruzi*, which is transmitted by insects from Reduviidae family. Since the conventional treatment with nitroheterocycles drugs shows serious adverse reactions and questionable efficiency, different research groups have tested polypeptide based approaches to interfere with parasite cell cycle in other trypanosomatids. For example, Nanobodies against *T. brucei* VSGs are being expressed in endosymbiont bacteria aiming to block parasite transmission or used to improve drug delivery by combining them to nanoparticles or lytic peptides. These strategies are supported by the fact that surface players are candidates to develop surface ligand to impair their function since they may act as virulence factors. In this work, we used a phage display approach to identify peptides derivate of two peptide libraries, LX15 (15 aa) and X8CX8 (17 aa) (where X corresponds to any amino acid). After testing different biopanning conditions using live or fixed epimastigotes, we sequenced 10 clones encoding the same polypeptide, named here as EPI18. The bacteriophage encoding EPI18 binds to epimastigotes from distinct strains. To confirm these results this polypeptide was synthesized and biotinylated to perform flow cytometry and confocal microscopy analyses. Our preliminary assays show its specificity and binding capacity towards to epimastigotes surface compared to an unrelated peptide. These results suggest that EPI18 may have potential biotechnological applications such as peptide based strategies to control parasite transmission.
Supported by:FUNDAÇÃO ARAUCARIA, CAPES, CNPQ
Keywords: Phage display ; t. cruzi ; peptide