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QT1 - INHIBITION OF THE OUABAIN-INSENSITIVE NA^+-ATPASE ACTIVITY FROM EPIMASTIGOTES OF *TRYPANOSOMA CRUZI* BY MILTEFOSINE

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Miltefosine, initially developed as an anti-tumor agent, is the newest drug for the treatment of visceral leishmaniasis. Several laboratories have shown that this ether-lipid analogue is also toxic against different forms of *T. cruzi*, but its mechanism(s) of action is(are) still unclear. In leukemic cells, Miltefosine seems to interfere with cellular signal transduction pathways by inhibition of protein kinase C (PKC) and Na^+, K^+-ATPase. In order to verify if Miltefosine is able to act in a similar way on *T. cruzi*, we initially tested its effects against on the ouabain-insensitive and furosemide-sensitive Na^+-ATPase activities of the parasites (1).

The Na^+-ATPase activitiy was measured as described previously (1-2) using an enriched plasma membrane fraction from epimastigotes obtained after sub-cellular fractionation by differential centrifugation in sucrose-containing buffer. The addition of increasing amounts of Miltefosine (0,003-0,3 mg/mL) resulted in a dose-dependent inhibition of the Na^+-ATPase activity. In a second stage, the inhibition of the Na^+-ATPase by Miltefosine was confirmed by performing activity measurements in the presence of increasing amounts of NaCl, containing or not 0,015mg/mL of Miltefosine. As expected, there was a Na^+-dependent increase of the Na^+-ATPase activity, with a $K_{0.5}$ and V_{max} of 13.7±1.9 mM Na^+ and 11.4±0.5 nmol Pi x mg^{-1} x min^1, respectively. In the presence of Miltefosine there was a change in the kinetic parameters, with the $K_{0.5}$ for Na^+ raising to 28.5±5.3 mM and the V_{max} dropping to 7.6±0.5 nmol Pi x mg^{-1} x min^1. When the membrane fractions were pre-incubated with Miltefosine (0,015 mg/mL) and diluted to a condition containing four times less drug before performing the assay, no inhibition was observed when compared to controls. Taken together, the results suggest that Miltefosine act as a reversible inhibitor of the *T. cruzi* Na^+-ATPase.

Supported by: CNPq, FAPERJ, TWAS and IFS.

References

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QT2 - ATIVIDADE ANTIPARASITÁRIA DA HEMOLINFA DE DUAS ESPÉCIES NATIVAS DE MOLUSCOS BIVALVES

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Peptídios ou proteínas antimicrobianas (PAM) são componentes do sistema imune inato, presentes na maioria dos seres vivos, que podem apresentar uma atividade microbicida potente contra um amplo espectro de microrganismos. Entretanto, dos PAM conhecidos atualmente, muito poucos têm sido avaliados quanto a sua atividade antiparasitária contra protozoários. Neste trabalho foi investigada a atividade antiparasitária da hemolinfa de duas espécies nativas de moluscos, o mexilhão *Perna perna* e a ostra do mangue *Crassostrea rhizophorae*, contra formas promastigotas de *Leishmania (V.) braziliensis* e formas epimastigotas de *Trypanosoma cruzi.* Foram utilizadas as duas frações hemolinfáticas: plasma (PL) e sobrenadante de lisado de hemócitos (SLH). Culturas de ambos parasitas (10^7 células/mL, concentração final) foram incubadas em microplacas de 96 poços, com um volume igual das frações hemolinfáticas de ambos bivalves em diferentes concentrações, por um período de até 48 h. A atividade antiparasitária foi determinada pelo ensaio colorimétrico do sal de tetrazolium (MTT). A inibição do crescimento dos parasitas foi avaliada a partir da concentração protéica mínima das frações hemolinfáticas capaz de inibir em 50% o crescimento normal do parasita (EC_{50}). Foi detectada uma atividade inibitória contra ambos parasitas, no PL tanto de *C. rhizophorae* (EC_{50} = 300mg/mL para *L. (V.) braziliensis* e EC_{50} = 150mg/mL para *T. cruzi*) como de *P. perna* (EC_{50} = 240 mg/mL para *L. (V.) braziliensis* e EC_{50} = 600 mg/mL para *T. cruzi*), após um período de incubação de 24h. O SLH de *C. rhizophorae* não apresentou atividade antiparasitária até 48 h, enquanto o de *P. perna* mostrou-se inibitório após 48h (EC_{50} = 45 mg/mL para *L. (V.) braziliensis* e EC_{50} = 150 mg/mL para *T. cruzi*). Controles adequados mostraram que a concentração salina da hemolinfa não interfere no crescimento de ambos protozoários. Os resultados obtidos apontam para ocorrência de uma atividade inibitória da hemolinfa de ambos bivalves sobre *L. (V.) braziliensis* e *T. cruzi.* Análises ulteriores são necessárias para melhor avaliar esta atividade e o interesse de se partir para uma purificação dos eventuais fatores antiparasitários.

QT3 - ATIVIDADE ANTIPARASITÁRIA DE PEPTÍDIOS DA HEMOLINFA DO CAMARÃO PENEÍDEO *LITOPENAEUS VANNAMEI*

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Peptídios antimicrobianos (PAM) são moléculas integrantes do sistema imune inato de vertebrados e de invertebrados que funcionam como antibióticos naturais e que apresentam geralmente uma atividade microbicida rápida e potente contra um amplo espectro de microrganismos. Dos PAM conhecidos atualmente, muito poucos têm sido avaliados quanto a sua atividade antiparasitária contra protozoários, sendo principalmente caracterizados em relação a sua atividade antibacteriana e antifúngica. Neste trabalho foi investigada a atividade antiparasitária do peptídio recombinante peneidina (Pen 3a), expresso em leveduras, e do fragmento C-terminal da hemocianina (C-ter), ambos isolados e caracterizados da hemolinfa de *L. vannamei*, espécie altamente cultivada em países da América Latina. A Pen 3a caracteriza-se principalmente pela sua atividade anti-bactérias Gram positivas, enquanto o C-ter pela sua atividade antifúngica. Em nossos ensaios, utilizamos formas promastigotas e epimastigotas de *Leishmania (V.) braziliensis* e *Trypanosoma cruzi*, respectivamente. Culturas de ambos parasitas (10^7 células/mL, concentração final) foram incubadas em microplacas de 96 poços com diluições seriadas (concentraçao inicial de 100 mM) de ambos peptídios. Os ensaios foram realizados em meio de cultura Schneider (*L. (V.) braziliensis*) ou TC-100 (*T. cruzi*) com suplementação de glicose (1%) e as placas foram incubadas por 20 h para avaliação do efeito inibitório sobre o crescimento (proliferação) dos parasitas. No caso da Pen 3a, foi também testada uma incubação de 5 h para verificar um possível efeito lítico/citotóxico. A atividade antiparasitária foi determinada pelo ensaio colorimétrico do sal de tetrazolium (MTT), quantificando a viabilidade celular das culturas de ambos parasitas. Foi detectada uma pequena atividade inibitória da Pen 3a e do C-ter contra ambos protozoários. O efeito mais significativo foi o da Pen 3a contra *T. cruzi*, causando uma redução de 30% na viabilidade dos parasitas a uma concentração de 12,5 mM, após 20h de incubação. Após esse mesmo período, a incubação com o C-Ter causou uma pequena redução (10%) na viabilidade celular a uma concentração de 100 mM contra ambos parasitas. A incubação por um período de 5 h com Pen 3a (ate 80 mM) não causou nenhum efeito sobre *T. cruzi* e uma pequena inibição sobre *L. (V.) braziliensis* (redução de 20% na viabilidade celular). Os resultados obtidos até o momento apontam

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para a ocorrência de uma fraca atividade antiparasitária dos peptídeos, sendo um pouco mais relevante a ação da Pen 3a sobre o *T. cruzi.* Um maior número de análises é ainda necessário para possibilitar conclusões mais definitivas.

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QT4 - ACTIVITY OF OLEANOLIC ACID AGAINST *LEISHMANIA (L.) MAJOR IN VITRO*

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Leishmaniasis is a major world wide health problem, with around 3 million people infected and 600 thousand new cases appearing each year. In Brazil, 30 thousand new cases appear annually only in the Northeast region. Since 30's pentavalent antimonals are the first line treatment for leishmaniasis. Disadvantages such as costs, long-term treatment, side effects and low efficacy against many strains are reported. Although great efforts had done along the last century to develop new drugs for leishmaniasis treatment, a drug with high efficacy and low side effects is still need. Furthermore, a drug for oral administration is desirable. All this, prompted the search for new chemotherapeutic agents. Among all strategies used to develop new agents against leishmaniasis, the research of natural products produced good results.

This work investigated the leishmanicidal activity of oleanolic acid (OA), a triterpene isolated from fruits of *Licania tomentosa* Benth, a Brasilian plant. Oleanolic acid was dissolved in DMSO then diluted in media for use. The leishmanicidal activity of oleanolic acid was investigated against the two evolutive forms of *Leishmania (L.) major* (LV39 strain). Promastigotes of *L. (L.) major* (10^6/well) was incubated in 96 well plate with different drug concentrations (1 to 50 µg/ml) or with DMSO at the same concentratrions carried by the drug. After 24 and 48hr, parasite survival was determined by MTT, a technique based on mitochondrial activity. Treatment with 20 µg/ml OA for 24h or 48h inhibited parasite survival in 88% and 95%, respectively. The activity on amastigotes was tested on *L. (L.) major* infected mouse peritoneal macrophages (10:1 parasite:macrophage ratio) by counting the number of intracellular parasites. Infected macrophages were treated with different drug concentrations and 24h later infection was evaluated by optical microcopy. Treatment with 20 µg/ml of OA resulted in 88% inhibition of parasite infection. The viability of mouse peritoneal macrophages was not affected by a 24h treatment with 20 µg/ml or lower doses of OA.

The results presented herein, showing that oleanolic acid is able to kill the two evolutive forms of *L. (L.) major* without affecting macrophages viability, points to this triterpene as a strong candidate for the development of a new leishmanicial drug.

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QT5 - "IN VITRO" AND "IN VIVO" TRYPANOCIDAL POTENTIAL OF *VERNONIA* SPECIES (ASTERACEAE)

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The protozoan parasite *Trypanosoma cruzi* is responsible to determinate Chagas' disease, a serious debilitating disease that affects millions of people in Latin America. Tripomastigotes are capable of invading and replicating in different types of cells causing a chronic and multifocal disease. Benznidazole is only drug employed to treatment of this infection in Brazil but its acts are dubious still. The efficacy depends on strain of *T. cruzi.* In the present work we evaluated the trypanocidal potential of different plant extracts of the genus *Vernonia* "in vitro" and "in vivo" assays, against two strains of *T. cruzi* (Y and Bolivia). The "in vitro" assay was carried out using blood with 10^5 trypomastigotes forms/mL and it was added in extract solutions (*V. cognata, V. fruticulosa, V. lacunosa* and *V. rupestris*) prepared in dimethyl sulfoxide 5% (DMSO): saline (1:20) to provide different concentrations 2.000, 1.000 and 500 mg/mL. The bioassays were performed in triplicate on microtitle plate (96 wells); negative and positive controls containing either DMSO or gentian violet respectively were run in parallel. The plate was incubated at 4°C during 24 hours and the number of parasites determined according to Brener method. The better results found by the "in vitro" assay were evaluated "in vivo" assay. The better crude extract analyzed were ethyl acetate and hexanic of *Vernonia fruticulosa* and *V. rupestris*, extracted of leaves. Male, healthy and young mice (BALB/C) were inoculated intraperitoneally with 4 x 10^3 tripomastigotes forms and 48 hours after that the extracts were administrated. The extracts were prepared in and administered by oral way at 8 mg/kg/day for 20 days. Positive control (benznidazole) and negative control (DMSO 5%) groups were done. The count of parasites was performed according to Brener method. We observed that the strains showed different behaviors. "In vitro" assay there were a difference between Y and Bolivia strains and *V. fruticulosa* showed the lowest value of IC_{50} by the two strains. To "in vivo" assay with extract of *V. fruticulosa* in Y strain reduced the parasitemia but the mice had precocious death while compared with positive control. Bolivia strain showed a high parasitemia curve and survival period even the controls the same occurred with *V. rupestris* to Y strain. To Bolivia strain this crude extract showed lower parasitemia and reduced the survival of animals if compared with positive and negative controls. We concluded that the genus *Vernonia* is not capable to treat the parasite infection by the Y and Bolivia strains. To prevent the Chagas's disease *V. fruticulosa* need more studies to know that one fraction can be capable to it.

Supported: CNPq

QT6 - PIPERACEAE AND SOLANACEAE SPECIES AGAINST *LEISHMANIA (L.) AMAZONENSIS*

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The number of new cases of leishmaniasis has increased in the last years in Brazil. Vassouras is a small town situated in an endemic region in the interior of the Rio de Janeiro state, where there is a high number of infected people in rural areas. The present work is part of a project that aims to research the therapeutical potential of the Rio de Janeiro state flora. Plant extracts of several polarities from different organs of the families Piperaceae, Solanaceae, Cucurbitaceae and Rubiaceae have been evaluated. Promastigotes of *Leishmania (L.) amazonensis* were cultivated with extracts in several concentrations for 96h at 26°C. To evaluate the antiamastigote activity, murine peritoneal macrophages were infected with *L. (L.) amazonensis* and treated with extracts for 48h at 37°C. Leaf extracts of *Aureliana angustifolia* (Solanaceae), *Piper mallacophylum* and *Peperomia scandens* (Piperaceae) at 100 mg/ml inhibited in more than 90% the growth of both forms of the parasite. The solvents used in the extration have also influenced the result, being the polarity directly proportional to the leishmanicidal activity. The microscopical analysis of the macrophages treated with extracts indicated variable degree of cytotoxicity in concentrations higher than 100mg/ml. These

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results indicate that the phytochemical study guided by leishmanicidal assays of the active plants can result in the isolation and identification of new phytomedicines for leishmaniasis treatment. FUSVE.

QT7 - ANTI-LEISHMANIAL ACTIVITY FROM *TITHONIA DIVERSIFOLIA* LEAVES EXTRACT

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Leishmania is a protozoan parasite responsible for a group of diseases whose symptoms range from mild cutaneous lesions to fatal visceral involvement. The unavailability of appropriate drugs to treat the disease is a result of a combination of factors including the lack of ongoing "research and development" into neglected diseases, such as leishmaniasis for which there is virtually no market. With that in mind, we decided to search for anti-protozoan activity in crude extract from the leaves of a plant, *Tithonia diversifolia,* known in Brazil as "margaridão". Dicloromethane leaves extract (DLE) was prepared and tested for its leishmanicidal activity against *Leishmania (L.) major* axenic promastigotes. The DLE was shown to be cytotoxic, acting on promastigotes and the IC_{50} value lies between 1,5-2 mg/mL. Furthermore, scanning electron microscopy experiments demonstrate drastic morphological alterations in *L. (L.) major* promastigotes. We are currently testing the DLE effect on mammalian cells and on parasites' invasion kinetics.

Supported by FAPESP.

QT8 - PRELIMINARY ANALYSIS OF THE TRYPANOCIDAL AND LEISHMANICIDAL PROPERTIES OF XANTHONES DERIVATIVES.

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Xanthones have a broad spectrum of biological activities including cytotoxic and anticancer, anti-inflammatory and analgesic, antifungal, antiprotozoal and antidepressant activity. The present study was designed to determine the *in vitro* leishmanicidal and trypanocidal activity of the xanthone (C1) and six substituted xanthones, including 4-methyl-xanthone (C2), 1-carboxy-xanthone (C3), 2-carboxy-xanthone (C4), 3-carboxy-xanthone (C5); 4-carboxy-xanthone (C6) and 3-methoxy-xanthone (C7). Epimastigotes forms of *Trypanosoma cruzi* (Y strain) and promastigotes of *Leishmania (L.) amazonensis* (579 strain) were washed twice in cold PBS and their concentration was adjusted to $5x10^6$ cells/mL in LIT medium. Different concentrations of each compound (500, 300, 100, 30, 10, 3 and 1mg/mL) were incubated in the presence of 200?L of the parasites suspension in 96 microwell plates at 26°C for 72 hours. As controls, parasites were incubated in the absence of any drug and in the presence of benznidazole (50mg/mL) or amphotericin B (50ng/mL). Three to four experiments were carried out in triplicate and the number of surviving parasites was determined in Neubauer chambers. The substituted xanthones showed a concentration-dependent inhibitory effect on the growth of *L. amazonensis* promastigotes. Among the seven xanthones tested, C1, C5 and C10 revealed a major inhibitory effect on the growth of *L. (L.) amazonensis*, with IC50 values of 317.6mg/mL, 235.9mg/mL and 180.2mg/mL and inhibition rates of 87±2%, 90±1% and 85±4%, respectively. Except for C5, which revealed IC50=319.1mg/mL and 70±8% of growth inhibition, all other xanthones presented no effect against *T. cruzi.* Along with xanthone C5, which has a methyl substituent group in position 4 and revealed both leishmanicidal and trypanocidal activities, our results also showed promising leishmanicidal activity in some substituted xanthones.

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QT9 - ANTI-*LEISHMANIA (L.) AMAZONENSIS* ACTIVITY OF SUPERCRITICAL CO_2+ETHANOL EXTRACTS FROM *TABERNAEMONTANA CATHARINENSIS.*

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Leishmaniasis is a worldwide disease still treated with expensive compounds that present severe side effects, and are frequently ineffective, emphasizing the importance to search new compounds against this disease. *Tabernaemontana catharinensis* is a species belonging to the *Apocynaceae* family that is rich in indole alkaloids, used in folk medicine as antimicrobial, anti-inflammatory, antitumor and analgesic. Coronaridine, an indole alkaloid isolated from *T. catharinensis*, exhibited a potent leishmanicidal effect (Delorenzi et *al,* 1998 and 2002). In this work we used supercritical fluid extraction [SFE] to obtain *T. catharinensis* extracts. The easy removal of solvent from the final extract, the high selectivity and the use of moderate temperatures in the process, are the most significant advantages of this methodology.

Alkaloids, coronaridine and voacangine, were quantified in both extracts by gas chromatography [GC-FID]. The leishmanicidal effect of the two extracts, E2 and E4, was evaluated, as well as, their cytotoxicity to macrophages. Mouse murine macrophages infected with *L. (L.) amazonensis* stationary phase promastigotes for 24 hs were treated with E2 and E4 during 24 hs more. Our results showed that E2 (10 and 100 µg/ml) inhibited 20 and 80%, respectively, of amastigotes survival, while E4 (10 and 100 µg/ml) inhibited 26 and 100%, respectively, of parasite growth. In order to test the safety of this extracts, viability of mouse macrophages treated with both extracts was checked by Trypan dye exclusion and XTT assays. Both tests showed that E2 and E4 were not toxic for macrophages. These results point to the use of this methodology as a possibility to easily produce compounds to treat leishmaniasis.

Supported by: CNPq, FAPESP.

QT10 - INHIBITION OF *TRYPANOSOMA CRUZI* GROWTH BY *PTERODON PUBESCENS* OILY EXTRACT

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Trypanosoma cruzi is the etiologic agent of Chagas´ disease, an endemic disease in Central and South America. Chemotherapy of this disease is still very unsatisfactory, being based in nitrofurans and nitroimidazoles. These compounds are inadequate due to frequent toxic side effects and limited efficacy having little or no activity in the chronic phase of the disease. These facts show us the

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urgency for the development of new drugs more effective and less toxic for Chagas´ disease. *Pterodon pubescens* Benth (leguminosae), known as Sucupira branca is a native tree specie of Brazil and its seeds are used as hydroalcoholic infusion presenting anti-rheumatic, analgesic, anti-inflammatory and cercaricide properties. Toxicological studies demonstrated that the *P. pubescens* seeds extracts did not present acute or sub-acute toxicity. Geranylgeraniol and related substances (14,15-epoxygeranylgeraniol and 14,15-dihydro-14,15-dihydroxygeranylgeraniol) have been associated to the cercaricide activity of Pterodon pubescens oil. Isoprenoids are involved in cell proliferation and differentiation, and much work is being done nowadays to study these compounds, especially farnesol and geranylgeraniol, that are also involved in post-translational prenylation of proteins, facilitating protein-protein interactions and membrane-associated protein trafficking. In this work we study the effects of oleaginous extract of *P. pubescens* seeds (Ppoe), the hexanic fraction (Hex) and geranilgeraniol in the growth of *T. cruzi* epimastigotes from Y strain. Ppoe was obtained by maceration of *Pterodon pubescens* seeds in ethanol at room temperature for 15 days. A hexanic extract (Hex) from Ppoe was obtained by liquid-liquid extraction. Geranilgeraniol was further obtained from Hex by HPLC in a C8 column and characterized by GC-MS and NMR. The epimastigotes were grown in BHI, with 10% SFB, 10mg l^{-1} hemin and 20mg l^{-1} folic acid for 25ºC for 7 days in the presence and in the absence of Poep or H2. The EOPp, Hex and H2 presented a dose-dependent inhibition of epimastigotes growth with an IC50 of 12.31; 13.64 and 31.84 mg/ml respectively. Studies to the mechanism of action of the geranilgeraniol in the cellular division of the *T. cruzi* are in course in our laboratory.

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QT11 - TRYPANOCIDAL ACTIVITY OF THE GALLIC ACID DERIVATIVES

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Gallic acid (3,4,5-trihydroxybenzoic acid) is a phenolic compound commonly found in plant species as an ester or hydrolyzable tannins, presenting some important biological activities such as inhibition of tumoral cells proliferation and anti-parasite activity against *Trypanosoma brucei* and *Leishmania* spp. In this work we have evaluated the "in vitro" trypanocidal activity of 25 derivatives of the gallic acid which were synthetized by the Department of Chemistry (UFSC). The assays were performed in triplicate in 96 well microplates by incubating 180mL of each parasite form suspension with 20mL of the testing compounds in distinct concentrations (100, 50, 10, 5 e 1mM). Assays were performed with *Trypanosoma cruzi* Y strain culture epimastigotes and blood trypomastigotes. Epimastigotes harvested in LIT medium were washed in PBS and had their concentration adjusted to $5x10^6$ parasites/mL. Trypanocidal assays with these forms were carried out for 72h at 27ºC using benznidazole (100mM) as positive control. Blood trypomastigotes obtained from experimentally infected mice had their concentration adjusted to $1x10^6$ parasites/mL and were incubated with each compound concentration for 48h at 4ºC using crystal violet as positive control. As negative controls, parasites were incubated in the absence of any drug and in the presence of DMSO 2%. The trypanocidal activity was carried out by determining the number of live parasites in Neubauer chambers for epimastigotes and according to Brener method (1962) for trypomastigotes. Four out of 25 assayed compounds showed trypanocidal activity against epimastigotes: eptyl gallate IC_{50}= 4.1 mM (2.8 - 6.1), octyl gallate IC_{50}=33.9 (28.1 - 40.9), dodecyl gallate IC_{50}=2.3 (1.7 - 2.9) and undecyl gallate IC_{50}=4.24 (3.0 - 5.8). None of these active compounds showed activity against *T. cruzi* blood trypomastigotes and no erithrocyte lysis was observed. The absence of activity against these forms may be attributed to hydrolysis of the alkyl chain by blood components. Further studies are in progress in order to modify the active compounds in order to avoid the action of blood components in the molecule structure.

Supported by CNPq and UFSC.

QT12 - TRYPANOCIDAL ACTIVITY OF *EUGENIA JAMBOLANA* LAM.

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Chagas' disease is an important endemic illness in Latin America, caused by the protozoan parasite *Trypanosoma cruzi*. Although transmission has been reduced, an effective treatment for the infected population is lacking. Chemotherapy of Chagas disease is limited to the drugs benznidazole and nifurtimox, which present low efficacy and several side effects. Moreover, the increased resistance of the parasite to these drugs is one of major problems for the successful of the treatment. Myrtaceae is a family belonging to the superorder Myrtiflorae, order Myrtales (*sensu* Dahlgren, 1982). This family consists of 130 genera and about 3000 species, many of which are used in popular medicine as diuretic, anti-inflammatory and anti-hipertensive. *Eugenia jambolana* was collected at FIOCRUZ campus, Rio de Janeiro State, Brazil. Essential oil of *E. jambolana* was extracted and tested *in vitro* against trypomastigotes forms from Y strain of *T. cruzi*, showing a dose depended trypanocidal effects in 24h of incubation (IC50 0,8mg/ml). When tested on release of trypomastigotes forms by infected macrophages, and development of amastigotes inside these cells, the essential oil also demonstrate a dose dependent effect. Expression of NO and TNF-a production by infected and noninfected macrophages treated with jambolana essential oil, indicates that the toxicity against the parasite is independent of cellular activation. These results suggest *E. jambolana* essential oils as potentially useful in the Chagas disease therapy.

QT13 - EVALUATION OF THE BIOLOGICAL ACTIVITY *IN VITRO* OF PROPOLIS EXTRACT OVER PROMASTIGOTES AND AMASTIGOTE FORMS OF LEISHMANIA (VIANNIA) BRAZILIENSIS.

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Leishmaniosis is a parasitic infection caused by a protozoan of *Leishmania* gender. It causes a social and economic impact, being considered the second infection in incidence after malaria. Antimonials are nowadays the first choice drugs, although display several side effects and parasistic resistance. For these reasons many other substances are being tested among them propolis, an apitherapic product that has been used recently with susccessfull results against protozoan. In this work, it was evaluated the biologic activity of propolis extract through *in vitro* assays over the promastigotes and amastigote forms of *Leishmania (Viannia)*

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braziliensis. The assays were done in triplicate, being used for promastigote forms the following propolis concentrations: 1, 10, 30, 50, 100, 250, 500, 750 mg/mL, in axenic media (LIT modificado) with aproximatelly 10^6 parasites/mL. For amastigotes forms, concentrations of propolis were 10, 100, 250 mg/mL using a macrophage culture infected with $1.5x10^6$ parasites/mL. Biological activity was seen for promastigotes forms, demonstrating a noteworthy leishmanicyd role for propolis. Although it was not observed any statistical difference concerning to positive control (Anfotericin B). Any biological activity was noticed for amastigote forms in none of the used concentrations.

SUPPORTED - CAPES

QT14 - TRYPANOCIDAL ACTIVITY OF DIFFERENT PROPOLIS SAMPLES FROM BRAZIL

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Propolis is a resinous substance that honeybees collect from different plant exudates and is used to block cracks, seal their honeycombs and also to prevent growth of microorganisms and protect the hive entrance against intruders [1]. Our laboratory is involved for some years on the investigation of propolis activity against *Trypanosoma cruzi* [2-4], inserted in a more wide multidisciplinary study, which aims to perform a systematic analysis about the chemical composition and biological activity of Brazilian propolis. In temperate zones the main sources of propolis are different poplar buds and the bioactivity of this bee product has been associated mainly to flavonoids and phenolic acids and esters. The samples from tropical zones, such as Brazil with its vast biodiversity, have become a subject of increasing scientific and economic attention. In Brazilian samples, several new compounds with microbicidal and cytotoxic activities have been already identified [5].

In this context we analyzed the chemical composition of 26 ethanolics extracts from different localization of Brazil by high temperature high resolution gas chromatography coupled to mass spectrometry [6] and high performance liquid chromatograpy [4] and assayed their activity against bloodstream trypomastigotes. To monitor such activity the parameter used was the value of ED_{50}/24 h that correspond to the concentration of the extract that lysis 50% of the parasites after 24 h of treatment. The values these the ED50/24 h varied between 186 e 2200 mg/mL. Multivariate analysis will be applied to correlate the trypanocidal activity with the chemical composition of each extract.

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[1] De Castro (2001) *Ann Rev Biol Sci 3*:49-83; [2] Higashi & De Castro, (1994) *J. Ethnopharmacol* 43*:*49; [3] De Castro & Higashi (1995) *J. Ethnopharmacol* 46:55; [4] Marcucci et al (2001) *J. Ethnopharmacol* 74:105; [5] Bankova, De Castro, Marcucci (2000) *Apidologie* 31:3; [6] Pereira & Aquino Neto (1999) Trends Anal Chem 18:126.

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QT15 - CHEMICAL COMPOSITION AND MICROBICIDAL ACTIVITY OF EXTRACTS FROM BRAZILIAN AND BULGARIAN PROPOLIS

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The chemical composition of ethanol extracts from a Brazilian (Et-Bra) and a Bulgarian (Et-Blg) was determined by high temperature high resolution gas chromatography coupled to mass spectrometry and assayed *in vitro* against against *T. cruzi*, several fungi and bacteria species were determined. *Candida albicans, Sporothrix schenckii* and *Paracoccidioides brasiliensis* were selected due to their importance as etiologic agents of mycosis in Brazil and *Neisseria meningitidis, Streptococcus pneumoniae* and *Staphylococcus aureus* are three common inhabitants of the human nasopharynx that has the potential of causing fatal diseases. The agar cup method was used for analysis of fungicidal activity and diameters of less than 15 mm were considered as lack of activity. The minimal bactericidal concentration was determined by means of the broth microdilution method and corresponds to the lowest concentration of the extract that yields negatives subcultures.

Et-Blg presents a predominance of flavonoids (42%), aromatic acids and esters (12%) and fatty acids (7%) (Prytzyk *et al.* (2003), J. Ethnopharmacol, in press), while in Et-Bra it was determined only one flavonoid, pinostrobin (1%), aromatic acids and amyrins. Both extracts showed a similar content of aromatic acids and derivatives, but different individual compounds were detected.

In assays with bloodstream trypomastigotes the ED_{50}/24 h for Et-Bra were 66.2±3.7, 380.6±54.5 and 452.6±53.9 mg/mL in assays performed in the presence of 0%, 5% and 100% blood, and the corresponding values for Et-Blg were 143.0±15.0, 187.4±10.4 and 534.9±52.4 mg/mL. Both extracts showed a similar activity against trypomastigotes and a decrease in activity due to presence of blood. Et-Blg and Et-Bra showed a strong and similar activity against *C. albicans*, *S. schenckii* and *P. brasiliensis* Although both extracts present striking differences in chemical composition they showed similar effect against *T. cruzi* and fungi, indicating that other compounds besides flavonoids are responsible for these microbicidal effects, such as the high content of aromatic acids, and possibly the presence of amyrins. In the assays with bacteria Et-Blg was more effective, particularly *N. meningitidis* and *S. pneumoniae*. It is possible that the bactericidal activity is directly associated with flavonoids, since the main difference between the two extracts is the high level of these bioactive compounds in the Bulgarian extract.

Due to the complexity and diversity of propolis, correlation between chemical composition and biological activity is an approach that can lead to the standardization and categorization of different samples, allowing considerations for a more ample therapeutic use of this bee product

Supported by CNPq and Fiocruz.

QT16 - THE IMMUNOLOGICAL PROFILE OF *T. CRUZI*-INFECTED MICE TREATED WITH PROPOLIS

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In vivo models have been employed in studies with propolis, usually by administration by oral route of ethanolic or aqueous extracts. In murine models propolis showed a potent activity as anti-inflammatory, hepatoprotective and tissue regenerative agents, effects associated with its anti-oxidative (1). Our laboratory is involved on the investigation of the activity of propolis against *Trypanosoma cruzi* (2-4). Treatment of infected mice with 50 mg/kg of propolis led to lower parasitemia in relation to control group, without interfering with the survival, and also leading to no toxicity effect up to 700 mg/kg.

In this study we determine the immunological profile of 4 groups of mice:

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non-infected non-treated (G1), non-infected treated (G2), infected non-treated (G3) and infected treated (G4). Propolis (50 mg/kg) was administered daily by gavage for 7 and 14 days (corresponding to 8 and 15 dpi for the infected groups). Differential counting of leukocytes was determined after blood staining with hematoxilin-eosin and spleens (3 animals/day) from each group were collected and weighted to determine the absolute mass and for flow cytometry analysis using a panel anti-mouse monoclonal antibodies (5). The distribution of leukocytes and the main lymphocyte subsets in the spleen of the four groups after 7 days of treatment (dt) showed that in G3 the number of lymphocytes increased and neutrophils decreased, in relation to G1 and G2, maintaining this profile at 14 dt. Comparison of G3 and G4 showed that the cellular immune response, detected at 8 dpi (7 dt) in non treated mice, occurred only later in infected and treated mice and that such treatment led to a delay in the switching from innate to adaptative immune response. At 14 dt, despite showing increased spleen mass, the splenocyte number of G3 was similar to G1, and the frequency of $CD8^+$ cells of G4 was higher than in G3 in spite of $CD4^+$ cells present no difference between both groups. Also at 14 dt, the percentage of expression of $CD69^+$, in $CD4^+$ and $CD8^+$ subsets, in G4 was lower than in G3, indicating that cell activation due to infection was partially inhibited by propolis treatment.

Based on these results, we suggest that the treatment of mice with propolis rich in flavonoids (6) seems to modulate the immune response during *T. cruzi* infection, leading to a decrease of parasitemia levels and encouraging us to continue this research of alternative natural products for Chagas disease.

(1) De Castro SL (2001) Ann Rev Biomedical Sci 3:49-83; (2) De Castro SL, Higashi KO (1995) J Ethnopharmacol 46: 55-58; (3) Bankova VS et al. (2000) Apidologie 31:3-15; (4) Marcucci MC et al. (2001) J Ethnopharmacol 74: 105-112; (5) Olivieri BP et al. (2002) Antimicrob Agents Chemother 46: 3790-3796; (6) Prytzyk E et al. (2003) J. Ethnopharmacol, in press.

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QT17 - EFFECT OF ATORVASTIN TREATMENT ON *LEISHMANIA (L.) MAJOR* INFECTION

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Statins, inhibitors of HMG-CoA Reductase, are used to control cholesterol in humans. It has been shown that they can modulate inflammatory diseases and interfere with immune response diminishing Th1 and augmenting Th2 responses. *Leishmania (L.) major* is a well-studied parasite that induces type 1 immune response in resistant models (C57BL/6 mice) and type 2 immune response in susceptible mouse strains (BALB/c mice). In the present study the effect of atorvastatin (AT) on *L. (L.) major* infection was investigated. C57BL/6 mice were treated with atorvastatin (10 mg/Kg/day, *per os*) beginning at –2 days (ATLm) or 14 days (LmAT) of infection with *L. (L.) major* ($1x10^6$ stationary forms in hind footpads). Control groups were infected and treated with vehicle (PBS). The evolution of the lesions was accessed weekly for 10 weeks and no differences between groups were found. At the end of experiment, the animals were sacrificed and the parasitism was quantified. The treated groups presented a significant higher parasitism (more than 2 log fold increase per mg of lesion). The IFN-γ production at draining lymph node was also accessed by ELISA. Interestingly, only the ATLm group presented statistically higher IFN-γ levels whereas LmAT presented a tendency of higher IFN-γ production when compared to the PBS-treated group. These results may be associated to a higher parasitism presented in ATLm and LmAT groups. The presented data suggest that long-term treatment with atorvastatin may alter the resistance to *Leishmania* infection.

Support: CNPq

QT18 - MESIONIC DERIVATIVES DECREASES THE NITRIC OXIDE PRODUCTION BY *LEISHMANIA (L.) AMAZONENSIS* PROMASTIGOTES

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Leishmania is a parasitic protozoa (Kinetoplastida: Trypanosomatidae), which causes different diseases in human including: cutaneous, mucocutaneous and visceral leishmaniasis (TDR, 1999). In previous work, our group demonstrated that the nitric oxide (NO) pathway is involved on *Leishmania (L.) amazonensis*-macrophage interaction (Genestra *et al*., 2003a). Besides, NO production by *L. (L.) amazonensis* promastigotes and axenic amastigotes already was measured and a constitutive nitric oxide synthase (cNOS) was purified and characterized by immunofluorescence and affinity chromatography (Fonseca-Geigel, 2000; Genestra *et al*., 2003b). The significance of the occurrence of NOS in *Leishmania* is not known at the present, but the data up to now showed that NOS is prominent in promastigotes containing a high number of metacyclic forms, suggesting an association with differentiation and infectivity of the parasite. As a part of our research program of chemotherapy against leishmaniasis, we assayed the effect of two salts of mesoionic derivatives. The compounds have an interesting structure feature and presented useful and wide-ranging biological activities. In these work, parasites (MHOM/BR/77/LTB0016 strain) were cultured in Schneider's medium supplemented with 10% of fetal calf serum and pH 7.2/26ºC. The group tests were assayed with 4-phenyl-5-(4'-methoxy or 3'-methoxy cinnamoyl)-1,3,4-thiadiazolium-2-phenylamine chlorides, the most active compounds against *L. (L.) amazonensis* (Silva *et al*., 2002). The IC_{50} used was: 4'OCH_3=0.17 mmol/L, 3'OCH_3=0.04 mmol/L and 0.46 mmol/L for Pentamidine (reference drug). After 24 hours, the parasites were counted, centrifuged and the supernatants were used for the measurement of NO production by the Griess method (Green *et al*., 1982). The results demonstrated that the two compounds tested inhibit significantly (about 60 to 70%/ $p<0.001$) the NO production by the parasites, while Pentamidine inhibited only about 25%. If cNOS-*L. (L.) amazonensis* participates in the establishment of infection within the macrophage and survive in its "adverse" environment, including resistance to the toxic products from the host inducible NOS biosynthetic pathway, the data of the active mesionic class on NO pathway suggest it as possible target within the parasite. Further experiments will be done in order to evaluate the effect of 1,3,4-thiadiazolium-2-amidine mesoionic on NADPH consumption by cNOS purified from *L. (L.) amazonensis* and in NO production by axenic amastigotes of these parasites.

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QT19 - BIOCHEMICAL, ANTIPROLIFERATIVE AND ULTRASTRUCTURAL STUDIES WITH BPQ-OH, A SPECIFIC INHIBITOR OF SQUALENE SYNTHASE, ON PROMASTIGOTE AND AMASTIGOTE FORMS OF *LEISHMANIA (L.) AMAZONENSIS* AND *LEISHMANIA (L.) MAJOR*

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Parasites of the genus *Leishmania* are responsible for leishmaniasis, which affect million of people around the world. Squalene synthase (SQS) is an important enzyme that catalyzes the first committed step in the sterol biosynthesis. Some SQS inhibitors have been studied in mammalian cells with the objective of developing new drugs for the treatment of high cholesterol rates. *Leishmania* is a parasite that require an amount of endogenous sterols for growth and viability and SQS is an essential enzyme for the ergosterol biosynthesis (Urbina *et al.*, Mol. Biochem. Parasitol., 125, 35-45, 2002). In this work we decided to investigate the effect of BPQ-OH, a specific inhibitor of SQS, on promastigote and amastigote forms of *Leishmania (L.) amazonensis* and *Leishmania (L.) major*. The IC_{50} for the promastigote forms of *L. (L.) amazonensis* was 1.03mM. *L. (L.) major* was more resistant, presenting an IC_{50} of 9mM. Total growth arrest and cell lysis was observed both species incubated with 10mM of BPQ. Intracellular amastigote forms of *L. (L.) amazonensis* were 8-fold more sensible than promastigote presenting an IC_{50} of 0.106mM. The presence of BPQ-OH in the culture of both species led to an increase in the expression of the SQS enzyme when compared with the control without treatment. Densitometry results of the Western-Blot showed that *L. (L.) major* presented an increase in the expression when compared with *L. (L.) amazonensis*. This result indicates that the overexpression of SQS in the presence of BPQ-OH is probably related with the resistance presented by *L. (L.) major*. Ultrastructural analysis of the treated parasites revealed intense alterations in the morphology of *L. (L.) amazonensis*. The main ultrastructural change was observed in the plasma membrane, that presented an intense rupture and formation of elaborated structures. Alterations in the mitochondrion-kinetoplast complex was observed, as intense mitochondrial swelling, rupture of their membranes and an abnormal compaction of the kinetoplast. Other alterations include the appearance of multivesicular bodies, myelin-like figures, some changes in the flagellar membrane and the presence of parasites with two or more nuclei and kinetoplast. We conclude that BPQ-OH is a potent inhibitor of the SQS of *L. (L.) amazonensis* and *L. (L.) major*, showing a good IC_{50}, and that SQS is an important enzyme to the control of the ergosterol biosynthesis in members of the Trypanosomatidae family.

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QT20 - PIPERINE AND ITS DERIVATES: POSSIBLE MECHANISMS OF ACTION AGAINST *TRYPANOSOMA CRUZI*. A COMPARATIVE STUDY INVOLVING CHEMICAL STRUCTURE AND BIOLOGICAL ACTIVITY.

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Piperine is a natural alkaloid isolated from *Piper nigrum* and has a wide range of biological activities such as inhibitor of hepatic metabolism and antiinflamatory, analgesic, larvicidal and leishmanicidal effects. Ten (10) derivates have been prepared from the natural product through simple chemical modifications and their toxic effects against *T. cruzi* were evaluated. The results allowed us to get information about the importance of the amide function as well as the length and saturation degree of the central carbon chain for the biological activity. The results assessed in our laboratory with epimastigotes and amastigotes forms have demonstrated the promising activity of the natural product, when compared with its derivatives. The toxic effect of piperine and its derivates on macrophages was evidenced through the phagocytic and trypan blue exclusion test, showing the selectivity of the observed toxicity against the parasites. When epimastigotes were submitted to the treatment with piperine, they showed significant morphologic alterations in the length of the cellular body and flagellum when compared with the control. Treatment of the parasites with intermediate concentrations of the drug interfered with the parasite's citokinesis, promoting the accumulation of cells with 2 nucleus and 2 kinetoplastes but not completely duplicated. Preliminary results have demonstrated that piperine and some derivates were able to inhibit, in a dose dependent way, malate dehydrogenase and glucose 6-phosphate dehydrogenase, respectively NAD^+ and $NADP^+$ dependent enzymes. These data suggest that one of the possible mechanisms of action of piperine is the perturbation of the oxido-reductive balance through a generalized inhibition of dehydrogenases that affects the entire energy metabolism of the parasite.

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QT21 - SYNERGISM BETWEEN STEROL BIOSYNTHESIS INHIBITOR WITH THE CURRENT TREATMENT FOR HUMAN TOXOPLASMOSIS

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Toxoplasma gondii is responsible for chronically infecting approximately 30% of the global human population (1), causing congenital disease, including ocular demages in infants worldwide and is involved in fatal encephalitis in immunocompromised individuals. In human toxoplasmosis, pyrimethamine associated with other folate inhibitors such as sulfadiazine is the most usual treatment, with high efficacy in toxoplasmic encephalitis and chorioretinitis (2). However, this combination is associated with frequent and severe adverse reactions, such as hematologic toxicity due to pyrimethamine and cutaneous rash, leukopenia and thrombocitopenia due to the sulfonamide (3). The antiproliferative effects of these folate inhibitors were potentiated by the simultaneous incubation of tachyzoites of the RH strain with the inhibitor of $D^{24(25)}$ sterol methyltransferase, 22,26-azasterol, as indicated by concave isobolograms and fractional inhibitory concentrations. Monolayers of the epithelial cell LLCMK2 in a 24-well plate were allowed to interact with tachyzoites for 6 hours before the addition of the drugs. After the incubation with the drugs, coverslips were fixed in Bouin's fixative stained with Giemsa and observed in a light microscope. The percentage of infected cells and the parasite proliferation index were determined by examination of at least 400 cells of two different coverslips. Our results using pyrimethamine (0,1mg/ml) and sulphadiazine (25mg/ml) in infected cells for 24 hours of treatment were quite similar to those previously reported (4 and 5) reaching an inhibitory rate of 74% of parasite proliferation. The association of the same concentration of these drugs with 10mM 22,26-azasterol for 24 hours resulted in complete growth arrest of *T. gondii* tachyzoites. Reducing the concentration of folate inhibitors in ten times in combination with 10mM of azasterol produced 80% of inhibition of parasite proliferation, indicating a synergistic effect. The ultrastructural analysis of this material reveled dramatically cytotoxic effect on parasites mitochondria and endodiogeny process.

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QT22 - THERAPEUTIC ACTION OF ATP ON CUTANEOUS LEISHMANIASIS

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The current available therapy of leishmaniasis is painful and presents severe side effects. In this context, our group has been studying the action of ATP in infection. Since the ATP receptor $P2X_7$ is highly expressed on macrophages, and its activation leads to pore formation on the cell membrane, we explored the possibility that $P2X_7$ could be used to favour drug entrance. Peritoneal macrophages were infected and cultured for 48 hours. The cells then were treated with ATP (0, 500, 1000, 2000 and 4000 mM) plus Lucifer Yellow dye, for 10 min at 37ºC, and read at fluorimeter. The infection itself increased cellular permeability, but in presence of ATP, permeabilization was much more notable, sugesting that *L. amazonensis* infection increased $P2X_7$ receptor expression *in vitro*. *In vivo*, infected BALB/C mice were treated for three weeks, two times a week, with PBS (20 ml), Glucantime (3mg in 20ml), Glucantime (3 mg) plus ATP (50 mM, association volume 20 ml), and ATP (20ml at 50 mM) in the foot lesions. The analysis of parasite load showed that ATP did not increase the therapeutic effect of Glucantime, but showed itself an important anti-leishmanial action, reducing the numbers of parasites by 65% (ATP alone), and 71% (ATP plus Glucantime) in relation to PBS controls. To indirectly evaluation of $P2X_7$ expression *in vivo*, infected and uninfected feet were cut off and homogenized in PBS using a tissue grinder. The homogenate was treated with ATP (500mM and 5mM) plus Topro 3 dye, and analyzed by FACS. These preliminary results suggest that the therapeutic effect of ATP *in vivo* may be associated with the increased cell permeability and that ATP may serve as a good adjuvant for the treatment of leishmaniasis.

Acknowledgment CNPq

QT23 - SYNTHESIS OF NOVEL QUINOLINE DERIVATIVES WITH A POTENTIAL ANTILEISHMANIAL ACTIVITY

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Visceral leishmaniasis (VL) is a severe disease associated with infection of the reticuloendothelial system by *Leishmania* species. Recent large scale epidemics of VL in east Africa, India and in a small proportion, in Brazil, and the emergence of a HIV epidemic make VL a priority for the World Health Organization. Pentavalent antimonials have been cornerstone of treatment for the last six decades, but antimonial-resistance strains have been reported. Within the past five years, miltefosine has been demonstrated as the first effective and safe oral treatment against VL, but besides the teratogenic effects, the price of miltefosine is yet to be determined. Based in these circunstances, the development of new drugs is imperious.

In this report, we studied the *in vitro* activity of four novel synthetized quinoline compounds against *L. (L.) chagasi* promastigotes and intracellular amastigotes, and determined the *in vitro* toxicity against mammalian cells by the MTT assay. The 50% Effective Concentration (EC_{50}) showed that compound HGV02 was the most effective against promastigotes, with an EC_{50} value of 0.10 mg/mL. Compunds HGV01, HGV03 and HGV04, showed intermediate values of 5.66 mg/mL, 7.48 mg/mL and 4.87 mg/mL, respectively. Pentamidine was used as standard drug and showed an EC_{50} of 1.15 mg/mL. The intracellular amastigote assay by using compounds at 10 mg/mL, showed lack of antileishmanial activity for compounds HGV 01, HGV 03, HGV 04, but compound HGV02 reduced more than 99% the parasite burden of the infected macrophages. Glucantime was used as control and showed 100% reduction in parasite burden. All compounds demonstrated citotoxicity for peritoneal macrophages, despite of the high Selectivity Index (toxicity for mammalian cells/EC_{50} in promastigotes) of 314 obtained with HGV02. These results confirmed the potential antileishmanial activity of these novel quinoline derivatives, and allow the use of these molecules as models for the design of new synthetic compounds.

This work was supported by FAPESP (Proj. 99/08491-4) and LIMHCFMUSP-49.

QT24 - LETHAL EFFECT OF NEW DERIVED-NAPTHOQUINONE DRUGS AGAINST *CRITHIDIA* AND *HERPETOMONAS*.

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Flagellate protozoa of the Trypanosomatidae family, which includes agents of important parasitic diseases such as leishmaniosis and Chagas' disease, infect a wide range of hosts including animals and plants(Vickerman, 1994). Trypanosomatids belonging to the *Herpetomonas* genus are commonly found in the digestive tract of houseflies and blowflies, usually under the elongated promastigote and opisthomastigote forms. Monoxenous trypanosomatids are usually found in insect hosts and are considered to be not capable to cause parasitic diseases in vertebrates (Wallace, 1966). However, recent data suggested that monoxenous trypanosomatids could be implicated in human infections (Jimenez et al.,1996, Pacheco et al. 1998, Silva JS et al, 2001). This way, we decided to study the effect of new drugs against these trypanosomatids. The biological activities of the naphthoquinones and derivatives a and β-lapachone, extracted from trees of the genus *Tabebuia*, have been greatly studied. The diversity of microbicidal effects, the easily to achieve the quinones in Brazilian forest and the synthetic alternative pathway of obtaining it, led us to consider a-lapachol and β-lapachone as starting points for chemotherapy studies. In the present study, we display the effect of the new semi synthetic substances, napthoquinone derivatives, on *Crithidia* and *Herpetomonas*. Materials and Methods 1- Parasite- *Crithida fasciculate* (CI-IOC-048) and *Herpetomonas samuelpessoai* (CI-IOC-067) were obtained from Dr. M. Auxiliadora Sousa (Trypanosomatids Collection IOC, RJ Brazil) and were kept in liver infusion tryptose (LIT) or BHI-medium. 2-Trypanocidal Assay and substances- A stock solution of substance - α-lapachone (α-lap), β-lapachone (β-lap), Diazo of β-lapachone (6-diazo-β-lap), epoxide of nor-α-lapachone, fenilidrazone-β-lapachone, etil-furano-nor-β-lapachone, lapachol, LAPAc2O (lapachol acetate), bacetone and epoxide of lawsone - was prepared in dimethyl sulfoxide (DMSO), with the final concentration in the experiments of the 0.1%. 3-Analyze of the drugs effect was done after quantification of active parasites on the 1st till the 7th day of the culturing, by counting in a optical microscopy (Olympus BX41). The final concentration of all the drugs tested was 50mM (group control was treated with DMSO 0.1%). Results- The substances α-lap, 6-diazo-β-lap, epoxide of nor-α-lap, 6-fenilidrazone-β-lapachone, etil-furano-nor-β-lapachone, lapachol and bacetone partially inhibited the growth of both, *C. fasciculate* and *H. samuelpessoai,* parasites. However, the substances Alil nor-b-lapachone and epoxilau, were lethal these parasites. These substances were able to kill the

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parasites in a period of 72 hours. Here, we are showing the trypanosomicidal potencial of these drugs on the different kinds of monoxenous trypanosomatids.

Supported by UFF/ FAPERJ.

QT25 - EFFECTS OF SYNTHETIC PEPTIDETEMPORIN A ON *TRYPANOSOMA CRUZI* EPIMASTIGOTES FORMS

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Antimicrobial peptides have been found in animal (vertebrate and invertebrate) and plants kingdom but most of the reports have focused on bacterial than eukariotics cells. They have a considerable structural diversity and consequente aminoacid heterogeneity. Many of them present a potential terapeutic application while others may be useful tools to probe and define important structural function of proteins segments. In this work, flow cytometry, MTT activity and parasites mobility were used to study the effect of one Synthetic peptide from *Rana temporaria* skin (Temporin A) on *T. Cruzi* epimastigotes Cells forms. For flow cytometry, *T. cruzi* epimastigotes forms were grown for 2h with different concentrations of Temporin A (1mM - 1000mM) and after washing ethidium bromide and fluorescein diacetate were addedies and the optical density measured at 600nm and 410nm. *T. cruzi* epimastigote mixture cells containg 700mM/10^7cells of the Temporin A presented 100% uptake of the dyes. These results with the low activity of the mitochondrial desigenasys MTT mensured demonstrated that the *T. cruzi* membranes is a target for lysis induced pore formation. The analysis of other antimicrobial peptides from natural sure is in progress.

Supported by FIOCRUZ and CNPq

QT26 - PROTEASOME AS TARGET OF ANTIMALARIAL DRUGS

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The development of new antimalarial drugs is an urgent priority considering the increasing prevalence of drug-resistant *Plasmodium falciparum* parasites and the absence of effective vaccines or vector control measures. The existence of a *Plasmodium* proteasome has been shown indirectly by using inhibitors. Lactacystin inhibits the *in vitro* development of erythrocytic stages of *P. falciparum* and its inhibitory effect is cell cycle stage-especific and, despite the low therapeutic index of lactacystin that reduced parasitemia in rats, the parasite proteasome remains as a prominsing candidate drug target. Lactacystin was found to inhibit the growth of three different lines of *P. falciparum* at similar molar concentrations, and was consistently more effective against chloroquine-resistent than chloroquine-sensitive-parasite (Li *et al*., 2000, International Journal for Parasitology 30, 729-733). A gene encondig a 20S proteasome b-subunit has been cloned in *P. falciparum*. During erythrocytic stages the parasite undergoes radical morphological changes and many rounds of replication, events that likely require proteasome activity. Proteasomes are major components of the eukaryotic cellular machinery mediating the normal turnover of proteins and the degradation of proteins that have been improperly folded or denatured (Gantt *et al*., 1998, Antimicrobial Agents Chemotherapy 42, 2731-2738). In our laboratory, preliminary results demonstrated that proteasome activity was presented in schizont stage, suggesting that activity is probably relationated with erythrocytic schizogony where occurs highest turnover of proteins and nucleic acid synthesis. We are testing the limonene, nerolidol and lovastatin in cultures of *P. falciparum* (isolated NF 54, clone 3 D7). The values of IC_{50} were: a) nerolidol 760 nM, b) limonene 1,22 mM and c) lovastatin 50 mM. In studies reported in the laboratory have been observated that nerolidol could be interfering in the elongation involved in the biosynthesis of the isoprenic chain attached to benzoquinone ring of coenzyme Q (Macedo *et al*., 2002, FEMS Microbiology Letters 207, 13-20) and, limonene arrested parasite development and inhibits isoprenylation of proteins in *P. falciparum* (Moura *et al.,* 2001, Antimicrobial Agents Chemotherapy 45, 2553-2558). Therefore, we will study how inhibition of isoprenylation of proteins and interference in the elongation of the isoprenic chain attached to benzoquinone ring of coenzyme Q in cultures of the *P. falciparum* could be interfering in the proteasome activity. In human cancer, pro-drug form of lovastatin have been demonstrated to inhibit the proteasome activity (Sharmila Rao *et al*.,1999, Biochemistry 96, 7797-7802) suggesting that lovastatin could be inhibiting this activity in *P. falciparum*.

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QT27 - ORAL DELIVERY OF MEGLUMINE ANTIMONIATE USING β-CYCLODEXTRIN FOR THE TREATMENT OF LEISHMANIASIS

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The need for daily parenteral administration represents one of the most serious limitations in the clinical use of pentavalent antimonials against leishmaniasis. In this work, we investigated the ability of β-cyclodextrin (CD) to enhance the oral absorption of antimony and to promote the oral efficacy of meglumine antimoniate (MA) against experimental cutaneous leishmaniasis. The occurrence of interactions between CD and MA was demonstrated through the changes induced in the spin-lattice relaxation times of both MA and CD protons. When MA was given orally to Swiss mice in either free or CD-complexed forms, antimony plasma levels after MA/CD were found to be about three times higher than after free MA. Anti-leishmanial efficacy was evaluated in BALB/c mice experimentally infected with *Leishmania (L.) amazonensis*. Animals daily treated with oral MA/CD (32 mg Sb/Kg) developed significantly smaller lesions when compared to animals treated with MA (120 mg Sb/Kg) and control animals (treated with saline). The effectiveness of oral MA/CD was equivalent to that of MA given intraperitoneally at a 2-fold higher antimony dose. The anti-leishmanial efficacy of MA/CD was confirmed by the significantly lower parasite load in the lesions of treated animals, when compared to saline controls. This work reports for the first time the effectiveness of an oral formulation for pentavalent antimonials.

This work was supported by grants from CNPq (521010/97-7 and Brazilian Nanobiotechnology Network), CAPES and FAPEMIG.

QT28 - TARGETING *LEISHMANIA (L.) CHAGASI* AMASTIGOTES INTO MACROPHAGES: THE USE OF DRUGS ENTRAPPED INTO LIPOSOMES CONTAINING PHOSPHATIDYLSERINE

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Macrophages, the target cells in the therapy of Leishmaniasis, express a range of surface glycoproteins, named scavenger receptors, able to bind different classes of molecules, including phosphatidylserine, a negatively charged lipid that could be easily included in liposomal formulations, increasing the targeting ability of drugs. In this study, we devised liposome-entrapped antimony (Sb-LP) with the negatively charged lipid phosphatidylserine, in order to improve their targeting to infected macrophages.

By determining the 50% Effective Concentration in *Leishmania (L.) chagasi*-infected macrophages, Sb-LP was 16-fold more effective (EC_{50} = 14.11 mM) than the free drug (EC_{50} = 225.9 mM). Confocal microscopy analysis showed the delivery of DIL-labeled PS-liposomes and ethidium bromide-labeled PS-liposomes to the *L. (L.) chagasi* parasitophorous vacuole (PV), suggesting that fusion between the PV and the liposomal formulation migth have occured, since intracellular amastigotes appeared fluorescent after shorth time incubation. Experimental studies, using BALB/c mice infected with *L. chagasi* amastigotes, showed that pentavalent antimony entrapped into PS-liposomes was at least 133-fold more effective than free antimony, since 100% reduction in the liver parasite burden was only achieved with 100 mg SbV/Kg using free drug, compared to 0,75 mg SbV/Kg by using the PS-liposome entrapped antimony. These results suggest that PS-liposomes could improve the efficacy of old drugs, such as pentavalent antimony, via targeted delivery to *Leishmania*-infected cells.

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QT29 - NATURAL ALTERATION IN THE SUSCEPTIBILITY/ RESISTANCE PATTERN TO BENZNIDAZOLE IN *TRYPANOSOMA CRUZI* POPULATIONS MAINTAINED IN ACUTE OR LONG TERM CHRONIC INFECTIONS IN MICE OR DOGS

Caldas S, Santos FM, Cáu SBA, Caldas IS, Veloso VM, Lana M, Tafuri WL, Bahia MT

Núcleo de Pesquisa em Ciências Biológicas – UFOP

The susceptibility to drugs used in etiologic treatment of Chagas disease is defined by the genetic characteristics of *T. cruzi* strains (Toledo *et al*., *AAC* 47:223, 2003). However, some reports have been published concerning the in vivo induction to drug resistance by pressure of the benznidazole (Bz) in *T. cruzi* strain with partially resistance to this drug (Murta *et al.*, *Parasitology* 116:165, 1998). On the other hand, the natural induction of resistance to Bz in *T. cruzi* populations during the long term chronic phase in dogs was demonstrated recently by Veloso *et al., Mem Inst Oswaldo Cruz*, 96:1005, 2001.

In this study new *T. cruzi* populations of dogs with chronic Chagas disease (2 to 10 years) were isolated. These dogs were previously inoculated with the Berenice-62 and Berenice-78 parental *T. cruzi* strains, that presented 100% of susceptibility to Bz. After isolation, all *T. cruzi* populations that showed resistance to Bz were maintained in Swiss outbreed mice, through successive cycles of treatment (SCT) with Bz. Mice were treated with oral doses of 100 mg of Bz/kg, for 20 consecutive days. Animals were considered cured when parasitological (fresh blood examination, hemoculture), molecular (PCR), and serological tests were negative. After treatment the animals were submitted to immunosuppressive therapy with cyclophosphamide. After reactivation of infection the trypomastigotes were inoculated in mice that were treated again. These SCT were repeated in order observe the stability of Bz resistance/susceptibility phenotype. In this study five *T. cruzi* populations (Be-62 A and B, Be-78 C, D and E) isolated of dogs infected with Be-78 and Be-62 *T. cruzi* parental strains were evaluated. In general, the parasites maintained in treated mice through of SCT showed an altered Bz-resistance: (1) 0% in 1º SCT for Be-62A; (2) 20% (1º SCT) to 100% after the 5º SCT for Be-62B (3) 90% (1º SCT) to 100% in 3º CST, with maintenance of 100% of Bz-resistance until the13º SCT for Be-78C; (4) 90% (1º SCT) to 100% in 6º SCT for Be-78D; (5) 80% (1º SCT) to 14,3% in 4º SCT for Be 78E;. All animals inoculated with Be-78C showed natural reactivation of the parasitemia post-treatment after the 9º treatment cycle.

In this work we demonstrate that *T. cruzi* populations isolated with dogs inoculated with the same *T. cruzi* parental strain present different Bz resistance levels. Besides, the maintenance of these *T. cruzi* populations in successive blood passages in treated mice altered in several ways the Bz resistance level, exemplified by the greater Bz resistance observed in Be-78C and for the decrease of the Bz resistance during the SCT in Be-78E.

Financial support: UFOP, FAPEMIG

QT30 - NATURAL ALTERATION IN THE SUSCEPTIBILITY/ RESISTANCE PATTERN TO BENZNIDAZOL IN *TRYPANOSOMA CRUZI* POPULATIONS MAINTAINED IN ACUTE OR LONG TERM CHRONIC INFECTIONS IN MICE OR DOGS.

Caldas S, Santos FM, Caldas IS, Veloso VM, Martins HR, Lana M, Tafuri WL, Bahia ML

Núcleo de Pesquisa em Ciências Biológicas, UFOP

Natural induction of resistance to Benznidazol (Bz), in *T. cruzi* populations, during the chronic infection of dogs was demonstrated previously by Veloso *et al.* (*Mem Inst Oswaldo Cruz*, 96:1005, 2001).

In this work new *T. cruzi* populations were isolated from dogs with chronic infections (inoculated with Bz-susceptible strains), and susceptibility to Bz was determined after the isolation and during the maintenance these populations through successive blood passages in mice (SBP). Swiss outbreed mice were infected by intraperitoneal route with the Berenice-62 A and B and Berenice-78 C, D, E isolated from different dogs after 2 to 10 years of infection with Be-62 and Be-78 parental *T. cruzi* strains, respectively. The Bz susceptibility was determined in the first blood passage in mice, and to follow the *T. cruzi* populations were maintained in SBP (in the absence of specific treatment). Sixteen mice were inoculated at each five SBP for the evaluation of the infectivity, parasitaemia curves, mortality and susceptibility to Bz. The parasitological cure was determined by fresh blood examination, hemoculture, serological test, PCR and reactivation of the disease by immunosuppressive therapy with cyclophosphamide. After only one blood passage in mice we observed 0%, 20%, 80%, 90% and 80% of resistance to Bz amongst the animals inoculated with isolate Be-62 A and B and Be-78C, D, E respectively, showing a natural induction of Bz resistance during long term chronic infection in dogs. On the other hand, a new change in Bz-susceptibilities was found during the maintenance of the parasite in BSP (acute phase). Diverse variation patterns were observed such as: (1) stability in the Bz resistance in Be-62 A (0 to 20% in 10 SBP), Be-62 B (20 to 40% in 15 SBP) and Be-78 C (80 to 100% in 55 SBP); (2) reduction in the Bz resistance in Be-78 D (90 to 22% in 40 SBP), and Be-78 E (80 to 30% in 10 SBP). The alteration of the Bz resistance degree during the SBP was not accompanied by significant variation in other biological parameters such as the parasitaemia curves pattern, infectivity and mortality

These results are in agreement with the hypothesis that the maintenance form of the *T. cruzi* (long term chronic infections or in successive blood passages -acute phase), can influence the susceptibility/resistance phenotype of benznidazole in the *T. cruzi* populations studied.

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QT31 - EVALUATION OF BENZNIDAZOLE TREATMENT IN MIXED INFECTIONS OF BALB/C MICE WITH CLONES OF *TRYPANOSOMA CRUZI* FROM DIFFERENT GENOTYPES.

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The use of biochemical and molecular markers is useful on the demonstration of *T. cruzi* natural mixed infection in humans as well as vectors and reservoirs. Mixed infections were experimentally demonstrated in vectors (Pinto et al, Parasitol. Res. 84: 348, 1998) and mice (Lana et al, Exp. Parasitol, 96:61, 2000). It was showed that there is not just a juxtaposition of the parasites of the mixture suggesting a stimulation or inhibition from one clone to another, sometimes reciprocal. The goal of this work is to characterize the mixed infection with clones from different genotypes comparing their biological properties and susceptibility to benznidazole with results provided from monoclonal infections obtained for Toledo et al, AAC 47: 223 (2003). Until now the clones used into the mixtures were: two clones from genotype 19, two from genotype 20 (*T. cruzi I*) and two from genotype 39 (*T. cruzi II) (*Tybayrenc & Ayala, Evolution 42: 277, 1988), one more susceptible and other more resistant to BZ (Toledo e cols, AAC 47:223, 2003).Groups with 16 BALB/c mice were inoculated via IP, with 5000 blood trypomastigotes of each clone. After the pre-patent period, 8 animals were treated during the acute phase by oral doses of 100mg/kg weight for 20 consecutives days and evaluated in parallel with 8 not treated control mice. The mortality rates were daily registered until the 120º day and the hemoculture performed 30 days post treatment.

Preliminary results indicate that the infectivity was always 100%, similar or higher than the observed in monoclonal infections when compared with the most infective one. The mortality rates are similar to the observed in monoclonal infections. The analysis of parasitemia showed an apparent stimulator effect between the clones (6/9 = 66% from the experiments) suggesting more than a juxtaposition of individual clones, since the parasitemia level were higher than the theoretical media from the mixture (arithmetic media from monoclonal infections). Only one experiment (Gambacl1 + Cuicacl1), showed parasitemia lower than the theoretical mixture, which suggest an inhibitory effect between the clones. The chemotherapic treatment strongly reduced the parasitemia of all mixtures that remained subpatent after the beginning of the treatment. These results corroborate data of monoclonal infections. However in some cases the reactivation of parasitemia was observed after the end of treatment although with lower level in relation to the control group. The results of hemoculture showed that mixtures with clones partially susceptible to BZ (Gamba + SO3cl5) were 100% positive indicating the permanency of the infection in all animals. Serological results (ELISA and anti-trypomastigotes antibodies –AATV by Flow-Citometry) and PCR that will be used as criterion of cure were not evaluated yet.

Grants: FAPEMIG, CNPq, CAPES e UFOP.

QT31 - BENZNIDAZOLE TREATMENT FOLLOWING ACUTE *TRYPANOSOMA CRUZI* INFECTION TRIGGERS CD8$^+$ T CELL EXPANSION AND PROMOTES RESISTANCE TO REINFECTION

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Many studies have shed light on the mechanisms underlying both immunoprotection and immune dysregulations arising after *Trypanosoma cruzi* infection. However, little is known about the impact of benznidazole (N-benzyl-2-nitroimidazole acetamide), the available drug for the clinical treatment in Brazil, upon the immune response in the infected host. Here we investigate the effect of benznidazole therapy on the lymphoid compartment during the course of experimental *T. cruzi*-infection. Although amelioration of a variety of clinical and parasitological signs was observed in treated mice, recovery from splenocyte expansion was not detected. Interestingly, this sustained splenomegaly observed in benznidazole-treated mice was due to a preferential expansion of CD8$^+$ T lymphocytes. Moreover, although blocking the expansion of recently activated CD4$^+$ and CD8$^+$ T cells seen in infected hosts, benznidazole treatment led to a selective expansion of effector/memory CD8$^+$ T lymphocytes, associated with lower rate of apoptosis. Besides, surviving benznidazole-treated infected animals were protected from death after reinfection, performed in late phases after the primary infection. Together, these data suggest that, in addition to its well-known direct role in blocking parasite replication *in vivo*, benznidazole appears to directly affect immune regulation in *T. cruzi* infected hosts.

Supported by CAPES, IOC and CNPq

QT33 - TARGET ORGANELLES OF BETA-LAPACHONE DERIVATIVES IN *TRYPANOSOMA CRUZI*

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Plants containing naphtoquinones are commonly employed in folk medicine and studies about the anti-tumoral activity of beta-lapachone, inducing apoptosis [1-2] and inhibiting topoisomerases, led to the suggestion of it potential use in the clinics as adjuvant for treatment of certain types of cancer [3]. The effect of this quinone against *Trypanosoma cruzi* is associated to free radicals generation [4]. Naphthoquinones derivatives, isolated from *Tabebuia* ("ipês"), and semi-synthetic derivatives totalizing 60 compounds have been assayed against trypomastigotes of *T. cruzi*, and three with the highest activity against were naphthoimidazoles derived from beta-lapachone, with the aromatic moieties phenyl (N1), 3-indolyl (N2) and *para*-phenyl (N3) linked to the imidazole ring [5-7].

In the present work, we observed effect of these three derivatives against amastigotes of *T. cruzi* interiorised in peritoneal macrophages and heart muscle cells primary cultures, and host cell damage occurred only at concentrations about 20-fold higher than those needed to block intracellular parasite proliferation.

Flow cytometry and ultrastructural studies of epimastigotes and trypomastigotes treated with N1, N2 or N3, showed swelling of the mitochondrion in both parasite forms. It were also observed disorganization of the reservosome morphology in epimastigotes, and damage of the kinetoplast in trypomastigotes. The mitochondrion alterations were associated with decrease of the membrane potential of this organelle, in relation to untreated parasites, detected by rhodamine 123 [8], despite the maintenance of the integrity of the plasma membrane monitored by propidium iodide [9]. Experiments using acridine orange as marker of acidic compartments, such as reservosomes and acidocalcisomes in epimastigotes [10], and acidocalcisomes in trypomastigotes [11] showed a decrease in fluorescence in treated parasites.

Taken together, these data suggest that the mitochondrion and acidic compartments are the first targets of the action of naphthoimidazoles against *T. cruzi*. The potent activity of the naphthoimidazoles on intracellular amastigotes and bloodstream trypomastigotes together with their low cytotoxicity to the

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mammalian cells encourage us to performed *in vivo* experiments with this derivatives of beta-lapachone.

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QT34 - TRYPANOSOMICIDAL EFFECT OF NEW DERIVATIVES, AGAINST *T. CRUZI*, SYNTHESIZED FROM α AND β-LAPACHONE.

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Chagas' disease, caused by the parasite *Trypanosoma cruzi*, is endemic in Latin America. It is a very serious public health problem in several countries, with about 17 million people known to be infected with the parasite, and a further 100 million at risk of infection, either through contact with an insect vector or via blood transfusion. At present, the only available therapeutic agent for Chagas' disease in Latin America is benzanidazole. In this context, an intensive research program has been focused on the search for alternative natural and synthetic drugs. The biological activities of the naphthoquinones and derivatives a and β-lapachone, extracted from trees of the genus *Tabebuia*, have been intensively studied. The diversity of microbicidal effects, the easy access to natural sources of these quinones in Brazilian rain forest and the synthetic alternative routes led us to consider α-lapachol and β-lapachone as starting points for chemotherapy studies. In the present work we describe the effect of the new semi synthetic substances, napthoquinone derivatives, on epimastigote form of *T. cruzi*. Materials and Methods 1- Parasite- *T. cruzi* Dm28c epimastigotes was raised in liver infusion tryptose (LIT) or BHI-medium. 2-Trypanocidal Assay and substances- A stock solution of substance - α-lapachone (α-lap), β-lapachone (β-lap), Diazo of β-lapachone, epoxide of nor-α-lapachone, fenilidrazone, Alil nor-β-lapachone, lapachol, lapachol with zinc and acetic anidrid, bacetone and epoxide of lawsone - was prepared in dimethyl sulfoxide (DMSO), with the final concentration in the experiments of the 0.1%. 3-Analyze of the drugs action- was realized by number of active (alive) parasites on the 1st, 2nd, 3rd, 4th, 5th and 7th days of the culture, using a Neubauer chamber. The final concentration of all drugs was 50mM. and the group control was treated with DMSO 0.1%. Results- The substances α-lap, diazo of β-lap, epoxide of nor-α-lap, fenilidrazone, alil nor-β-lapachone, lapachol and bacetone inhibited the growth partially but they were not lethal for *T. cruzi*. However, the substances β-lap, alil nor-β-lapachone and epoxilau, were lethal for *T. cruzi.* These substances killed the parasites in a period of 72 hours. These results revealed these substances to have good trypanosomicidal activities.

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QT35 - TRYPANOCIDAL EFFECT OF NEW DERIVATIVES, AGAINST *T.CRUZI*, SYNTHESIZED FROM α AND β-LAPACHONE.

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Chagas' disease, caused by the parasite *Trypanosoma cruzi*, is endemic in Latin America. It is a very serious public health problem in several countries, with about 17 million people known to be infected with the parasite, and a further 100 million at risk of infection, either through contact with an insect vector or via blood transfusion. At present, the only available therapeutic agent for Chagas' disease in Latin America is benzanidazole. In this context, an intensive research program has been focused on the search for alternative natural and synthetic drugs. The biological activities of the naphthoquinones and derivatives a and β-lapachone, extracted from trees of the genus *Tabebuia*, have been intensively studied. The diversity of microbicidal effects, the easy access to natural sources of these quinones in Brazilian rain forest and the synthetic alternative routes led us to consider lapachol and β-lapachone as starting points for chemotherapy studies. In the present work we describe the effect of the new semi synthetic substances, napthoquinone derivatives, on epimastigote form of *T. cruzi*. Materials and Methods 1- Parasite- *T. cruzi* Dm28c epimastigotes was raised in liver infusion tryptose (LIT) or BHI-medium. 2-Trypanocidal Assay and substances- A stock solution of substance - α-lapachone (α-lap), β-lapachone (b-lap), Diazo of β-lapachone (6-diazo-β-lap), epoxide of nor-α-lapachone, fenilidrazone-β-lapachone, etil-furano-nor-β-lapachone, lapachol, LAPAc2O (lapachol acetate), bacetone and epoxide of lawsone - was prepared in dimethyl sulfoxide (DMSO), with the final concentration in the experiments of the 0.1%. 3-Analyze of the drugs action- was realized by number of active (alive) parasites on the 1st, 2nd, 3rd, 4th, 5th and 7th days of the culture, using a Neubauer chamber. The final concentration of all drugs was 50mM. and the group control was treated with DMSO 0.1%. Results- The substances α-lap, 6-diazo-β-lap, epoxide of nor-α-lap, 6-fenilidrazone-β-lapachone, etil-furano-nor-β-lapachone, lapachol and bacetone inhibited the growth partially but they were not lethal for *T.cruzi*. However, the substances β-lap, etil-furano-nor-β-lapachone and epoxilau, were lethal for *T.cruzi.* These substances killed the parasites in a period of 72 hours. These results revealed these substances to have good trypanosomicidal activities.

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QT36 - A NEW SYNTHETIC CHALCONE WITH INCREASED ACTIVITY AND SELECTIVITY AGAINST MURINE CUTANEOUS LEISHMANIASIS.

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We showed previously the therapeutic effectiveness of the chalcone 2'-6'-dihydroxy-4'-methoxychalcone (DMC), isolated from the plant *Piper aduncum* against murine leishmaniasis (Antim. Ag. Chemot. 43:1234-41,1999). Nineteen analogous were synthesized adding different substitute groups on the original plant molecule to optimize its efficiency. The chalcones were incubated at differents concentrations with fluorescent promastigotes and intracellular amastigotes of *Leishmania (L.) amazonensis*-GFP. The leishmanicidal activity was expressed as fluorescent intensity of the samples. For cytotoxic activity against mammalian cells, the mouse lymphocytes were incubated for 48 hours with different concentrations of the chalcones in the presence of 2.5 mg/ml Con A with and inhibition of cell proliferation measured by incorporation of 3H-thymidine in the last 6 hours. Alternatively, mouse macrophages were incubated for 48 hours with the chalcones and cell viability measured by incorporation of

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propidium iodide. Four chalcones exhibited high leishmanicidal activity and low citotoxicity to mammalian cells. Presence of Cl and Br, as well as the NO_2 group increased the leishmanicidal activity of the original molecule. However, Cl also increases the citotoxicity to lyphocytes and macrophages. Para substitutions in the second ring drastically reduced its antileishmanicidal activity. The NO2-containing CH8 chalcone was the most selectively active molecule, and this was selected for *in vivo* assays. BALB/c mice were infected with $2x10^6$ promastigotes of fluorescent *Leishmania (L.) amazonensis* in the ear and afer 7 days treated s.c. in the lesion with CH8 (3,3 mg) or Pentostan (200 mg) in a volume of 10 ml of saline, twice a week, for four weeks. The CH8 chalcone-treated animals developed significantly smaller lesions as compared with saline controls. The parasite burden was also effectively controlled, and was as low as obtained in animals treated with much higher Pentostan doses.

These resullts show that structure/activity analysis of the synthetic chalcones led to a potential lead compound (CH8) with in vitro and in vivo antileishmanial selective activity.

QT37 - EFFECT OF THE ANTIMALARIAL DRUGS QUINIDINE AND CLOTRIMAZOLE ON HEMOZOIN FORMATION IN THE BLOOD FEEDING INSECT *RHODNIUS PROLIXUS*

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The blood-feeding organisms use hemoglobin as the major food source and its digestion result in heme release. Free heme is a powerful generator of reactive oxygen species which can damage a diversity of macromolecules. A diversity of protective mechanisms have been described in order to avoid the toxic effects of free heme. One of these mechanisms relies in the aggregation of heme into a harmless crystal named hemozoin (Hz), in different hematophagous organisms such as *Plasmodium*, *Schistosoma* and the triatomine insect *Rhodnius prolixus*. Quinoline-derived drugs, such as chloroquine, form complexes with heme blocking Hz production in *Plasmodium* and in *R. prolixus.* In this present work, we investigated the effects of inhibition of heme aggregation by two antimalarial drugs, quinidine and clotrimazole, in *Rhodnius prolixus* midgut. When adult insects were fed with different concentrations of quinidine, there was a dose-dependent inhibition of Hz production *in vivo*. At 500 mM, quinidine inhibited Hz production in more than 99% in the midgut. The azole derivative clotrimazole, also inhibited Hz formation *in vivo* when insects were fed with 50mM of this drug. Moreover, the inhibition of heme aggregation by quinidine results in a dose-dependent increase of heme levels in the hemolymph. Furthermore, a slight reduction in protein content in the hemolymph of quinidine fed insects, was observed. Taken together these results suggest that heme detoxification into Hz is a target of the quinoline and others antimalarial drugs and that this process has an important physiological role to this insect.

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