Tissue enzymes in blood are indicators of tissue lesions in Chagas'disease (Exp.Parasitol.40:411,1976; Comp.Biochem.Physiol.64B:11,1979). We analysed if creatino phosphokinase (CK) and its specific heart isoenzyme CKMB could indicate myocarditis in C57BL6 and Swiss *T. cruzi*-infected mice (Y strain), and compared them with animals treated with Benznidazole (Bz). Non-infected mice samples (n=282) allowed the definition of normal ranges. A cut-off was calculated with the mean+2sd, to which values of infected samples were referred. Plasma collected from mice infected with 10^4 parasites showed a CKMB increase at the 2nd week post-infection (wpi) in 41.6 and 15.4% of Swiss and B6 mice, respectively. CK levels increased as CKMB. Infection with 10^2 did not increase CKMB and allowed the survival of mice which could then be followed during the chronic phase. 80.9% and 3.7% chronic Swiss and B6 mice showed increased enzyme levels, respectively. Mice with high CKMB levels submitted to an activity test using running wheel showed low performance. Mice groups were named: infected & treated (Group **IBz**), infected & non treated (Group **I**), non-infected & non-treated (Group **N**). S*chemes* were: *(1)* infection with 10^4 parasites plus abortive treatment *per os* with 100mg/Kg Bz for 9 consecutive days; *(2)* infection with 10^2 parasites plus Bz treatment in drinking water (0.25 mg/ml) for 50 days; *(3)* infection with 10^4 plus Bz in drinking water at 0.10 and 0.25mg/ml for 15 days starting at the 2nd wpi. In *Schemes 1 and 2*, **IBz** mice presented no detectable parasitemia nor mortality. *Scheme 1* gave 100% cumulative mortality (%CM) in I mice, which dropped to 40% in *Scheme 2*. In *Scheme 3*, **IBz** showed 70 and 40 %CM respectively for 0.1 and 0.25mg/ml. Bz in *Scheme 1* avoided the splenomegaly and the increase of enzyme levels which were detected in **I** group at the 2nd wpi. However, in the 3rd week, when splenomegaly was reduced both in **I** and **IBz**, 26.3% of **IBz** mice had high CKMB levels. Thymic atrophy was clear in the third wpi in the **I** group and was reversed by **IBz**, but lymphoadenomegaly observed at the 2nd and 3rd wpi was not reversed by Bz. *Scheme 2* did not avoided CKMB increase in chronic mice, but frequency of mice with high CKMB levels from 80.9% to 51.3% in IBz. Heart histopathology results will be shown. The results suggest that CKMB can be used as a tracer of active chronic myocarditis and that Bz treatment reverts only partially heart damage.

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CH-29
LEISHMANIA AMAZONENSIS: IN VIVO EXPERIMENTS WITH DIARYLHEPTANOIDS FROM LEGUMINOSAE AND ZINGIBERACEAE PLANTS

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In a previous work we demonstrated that diarylheptanoids extracted from *Centrolobium sclerophyllum* (Leguminosae) are very active against *Leishmania amazonensis* promastigotes. In order to continue our studies with these class of compounds, we decided to evaluate the activity of curcumin, a phenolic diarylheptanoid derived from *Curcuma longa* (Zingiberaceae), against the extracellular forms of *L. amazonensis* Chemical modifications were done in the structure of the curcumin molecule, in order to increase the activity and we found that the most active compound was the methylated derivative. In this work we are now showing our *in vivo* results with the following dirylheptanoids derivatives: des-o-metilcentrolobine (LD50=57㎜), the most active compound extracted from *C. sclerophyllum* and the methylated curcumin (LD50=5㎜). In the *in vivo* experiments several groups of Balb/c mice were injected subcutaneously with $3x10^6$parasites/50㎖. The drugs were injected in a concentration of 20mg/Kg weigth, following different schedules, such as: a) only one injection of each drug, one week after the infection; b) two injections of each drug, being the second injection made after 15 days of the first dose; c) two injections of each drug, being the second dose given 45 days after the first dose. One month after the infection, we iniciated the measurements of the lesions up up to 75th day. The results showed that when only one dose of the compounds was applied, the mice treated either with the des-o-methyl centrolobine or methyl curcumin, at the 45th day of following up, showed a decrease of 34.5% and 55.5% in the lesion size, respectively, when compared with the mice inoculated with the parasites alone.

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CH-30
L-AMINO ACID OXIDASE ACTIVITY FROM *BOTHROPS MOOJENI* VENOM ON *LEISHMANIA (LEISHMANIA) AMAZONENSIS* PROMASTIGOTES

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Cutaneous leishmaniasis is a tropical disease found mainly on endemic areas as Amazon and Northeastern of Brazil. It causes a disfiguring skin disease that is resistant to most treatments, except to those using toxic antimonial salts. The need of development of new drugs is eminent. Snake venoms have been reported as a strong inhibitors of

protozoas, but no attempts to define the active fraction were done. In this report, we analyzed the anti-*Leishmania* activity of the crude venom and the purified fraction (L-amino acid oxidase -LAO), from *Bothrops moojeni* venom, on *L.L.amazonensis* promastigotes, using an *in vitro* assay. The Efficient Concentration 50% ($EC_{50\%}$) of the crude venom was determinated, using the same method, resulting in about 2mg/mL. The purification procedure was followed by molecular exclusion chromatography and ion exchange chromatography, in a FPLC system. A fast procedure of detection of LAO activity, was developed by Venom Group-IPEN, using in a colorimetric assay, RPMI-PR$^-$ 1640 as substrate and peroxidase and OPD as a revealing reagents. *L.L.amazonensis* was grown as promastigotes in RPMI-PR$^-$ 1640 with 10% fetal bovine serum, at 25°C. The viability of promastigotes was detected by respiratory oxidative conversion of MTT in a colorimetric assay. This preliminary data, suggest that this venom had an active action against these parasites, and if adequately tested, could be used as an alternative therapy for cutaneous leishmaniasis.

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CH-31
LEISHMANICIDAL ACTIVITY AND ULTRASTRUCTURAL CHANGES INDUCED BY SULPHUR SYNTHETIC NEOLIGNAN ANALOGUES

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Leishmaniasis is a disfiguring and sometimes fatal protozoan disease affecting over 12 million people worldwide, and for which there is still no safe vaccine. Recently, a dramatic increase in the rate of *Leishmania* infections in HIV patients, together with the development of drug-resistance by the parasites has worsened this problem. Despite the tremendous progress made in the understanding of the biochemistry and molecular biology of the parasite, the first-choice treatment for the several forms of leishmaniasis still relies on daily intramuscular injections of pentavalent antimonials developed more than 50 years ago. Therefore, the search for novel, effective and safe therapeutic compounds has become a priority. We have previously demonstrated the strong activity of the synthetic sulphur neolignan analogue LS-SCl against *L. amazonensis* (IC_{50}=1mg/ml). In this study, the ultrastructural changes induced by LS-SCl in *Leishmania amazonensis* and the activity of its analogues on *L. donovani* was studied. Peritoneal macrophages were infected with *L. donovani* and cultivated for 48 h with 15 LS-SCl synthetic analogues at 80 mg/ml. At the end of culture, the number of intracellular parasites was determined. Out of all substances tested, only LS-SCl was active, decreasing the intracellular parasite load by 94 %. To investigate the ultrastructural changes produced by LS-SCl on the parasites, *L. amazonensis* promastigotes were cultivated with 50mg/ml LS-SCl for 48 h and then processed for electron microscopy. LS-SCl induced the development of a very large vacuole in the parasite and disruption of its kinetoplast. These results demonstrate that the antileishmanial activity of LS-SCl is not restricted to the cutaneous *L. amazonensis*, but is extended to other parasite species, such as the visceral *L. donovani*. The various molecular changes made on LS-SCl did not further improve its activity, indicating that its core sulphur structure is the most active and least toxic.

CH-32
MECHANISM OF ACTION OF N,N-DIMETHYL-2-PROPEN-1-AMINES ON *TRYPANOSOMA CRUZI* EPIMASTIGOTE FORM

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The ergosterol biosynthesis has been largely studied as a target in the development of new antifungal and antiprotozoal agents. A preliminary study showed that 3-(4'-Bromo[1,1'-biphenyl]-4-yl)-3-X-phenyl-N,N-dimethyl-2-propen-1-amines act on sterol synthesis from *T. cruzi* epimastigote form (De Conti et al. XXIII Reunião Anual da SBBq, Maio 1994, Abstr. O1 (1993)). The non-substituted derivative of this series demonstrated (Oliveira et al. Mem Inst. Oswaldo Cruz 91, 320 (1996)) also activity on *Trichophyton rubrum* (5507 CCT strain), inhibiting the ergosterol synthesis from this dermatophyte (Oliveira et al. Mem Inst. Oswaldo Cruz 92, Supl.1, 524 (1997)). More recently, the investigation of trypanocidal mode of action of this compound was continued using *T. cruzi* epimastigote form. The culture was maintained in LIT medium supplemented with 10% of FBS. When the density reach 10^7 epimastigotes/mL the parasites were exposed to the drug (12 mmol/L in DMSO) at two different periods (24 and 48 hours). After classical extraction procedure, the neutral lipids fraction (control and treated samples) was analyzed by HPLC and GC-MS. The chromatographic studies indicate reduction of ergosterol levels and increasing of squalene levels in the treated forms after 48 hours of exposition. Based on this preliminary results we can conclude that this new series of compounds act as inhibitors of ergosterol biosynthesis from *T. cruzi* epimastigotes.

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