The present drugs of choice for visceral leishmaniasis, such as Pentostam®, are expensive, toxic and cases of drug resistance have been reported. An urgent need therefore exists for new, safer and cheaper leishmanicidal agents. Ethylenediamine tetraacetic acid (EDTA) has previously been shown to inhibit the growth of *Leishmania donovani in vitro*. The mode of action of EDTA is thought to be chelation of manganese ions from phosphoenolpyruvate (PEP carboxykinase, which is a vital enzyme in the metabolism of the *Leishmania* parasites. The following experiments discuss the effect of ethyleneglycol-bis-(□-aminoethyl ether) N,N,N^1,N^1,-tetraacetic acid (EGTA) on *L. donovani in vitro*; and the effect of the chelating agents EDTA, EGTA, N-(2-hydroxyethl)-ethylenediamine triacetic acid (HEEDTA), and Pentostam on *L. donovani*-infected Syrian hamsters and BALB/c mice. Pentostam, which was used as a reference drug, is a registered trade mark for a sodium stibogluconate formulation manufactured by the Wellcome Foundation (London), and is a standard regimen for leishmaniases. A comparison on Giemsa and acridine orange stains in the diagnosis of *L. donovani*-infected biopsies is also described.

Exposure of *L. donovani* culture promastigotes to EGTA concentrations of between 0.2 to 1.6 mg/ml significantly inhibited their growth. This effect was, however, not dose dependent in cell free media. *In vitro* treatment of mouse peritoneal macrophages with EGTA concentrations above 0.1 mg/ml significantly affected cell viability. No toxic effects on macrophages were recorded at concentrations $\leq$ 0.1 mg/ml. Treatment of *L. donovani*- infected macrophages with EGTA at concentrations of 0.05, 0.1 and 0.2 mg/ml contributed significantly to a decline in amastigote parasite-loads in the macrophases. The higher the chelator concentration within the acceptable toxic levels for macrophages, the greater was the rate at which parasites were cleared from the macrophages. Data from *in vitro* studies showed that EGTA substantially reduced parasite-loads of *L. donovani* promastigotes in cell free media, and of amastigotes in *L. donovani*-infected macrophages. A need therefore arose to investigate the antileishmanial potential of these chelators *in vivo*.

A challenge inoculum of $3x10^6$ *L. donovani* promastigotes derived from a primary culture and given ip was found to be the lowest dose that could effect a viceral infection in both hamsters and BALB/c mice. Such as infection can only be detected by culture, but not on smear. Similarly, an inoculum of $1x10^8$ promastigotes resulted in a grade 5 parasite-load in both laboratory animal rodent models. A grade 5 parasite load results in a visceral infection in which the identification and quantification of amastigotes on smears can be easily done. Toxicity experiments established that an EDTA concentration of 0.23 mmoles/kg/day administered 5 days a week, for four weeks, was non-toxic to both hamsters and BALB/c mice. Though Pentostam was potentially toxic to both rodent models, a concentration of 100 mg/kg/day was found to be the most appropriate in treating either rodent model when experimentally infected with *L. donovani*.

Hamsters were challenged ip with 1×10^8 *L. donovani* promastigotes and later subjected to treatment. Bone marrow and liver culture results from *L. donovani*-infected Pentostam-treated hamsters were all negative for parasites, while 5 out of 6 spleen cultures (83.33%) from these hamsters were negative. Except for one negative HEEDTA-treated bone marrow aspirate, all the vicera-derived culture biopsies from PBS and chelator (EDTA, EGTA, HEEDTA)-treated animals yield parasites in culture. These results indicate that Pentostam had cleared parasites in 5 out 6 (83.33%) hamsters, while the chelators did not clear parasites from any single animal.

Parasite-loads calculated from Giemsa-stained spleen smears indicated that 3 out of 6 (50%) of the Pentostam-treated animals had cleared the infection from their spleens, whilst none of the chelator or PBS-treated animals had cleared the parasites. Smears prepared from the liver of Pentostam-treated animals had significantly lower parasite-loads than the chelator or PBS-treated animals. Parasite-load results obtained from the smears of bone marrow aspirates indicated that 6/6 (100%); 4/6 (66.67%); 3/5 (60%); 2/6 (33.33%) and 0/4 (0%) hamsters had cleared parasites in this organ for the Pentostam,

HEEDTA, EDTA, EGTA and PBS-treated animals respectively. These results suggest that the activity of chelators against *L. donovani*-infected hamsters is weak.

BALB/c were challenged ip with a parasite dose of $3x10^6$ *L. donovani* promastigotes and later subjected to treatment. Because of the small parasite inoculum used, the activity of chelators against *L. donovani*-infected mice was assessed by culture technique only. Cultures of spleen biopsies from Pentostam and chelator-treated mice yielded parasites significantly later than the PBS-treated control group. This is an indication that chelators may have a leishmanicidal activity comparable to Pentostam when a small parasite inoculum is used to challenge experimental animals. However, it was also observed that significantly more cultures (7/28 = 25%) from spleen biopsies of Pentostam-treated mice did not yield parasites, while only 0-3.7% of the chelator-treated animals were negative for parasites.

A possible explanation for the leishmanicidal action of sodium stibogluconate is that the antimony (Sb^5) content of the drub competes with manganese ions of the parasite, leading to inactivation of manganese-dependent enzymes such as PEP carboxykinase which are vital for the parasites. It is thought that EGTA chelates manganese from PEP carboxykinase, a TCA-rate limiting enzyme in the metabolism of *Leishmania* parasites. Chelation of manganese would destablilize PEP carboxykinase, and therefore severely interfere with the parasites metabolism. A manganese-complex is also probably used by these parasites as a defense mechanism against oxygen-derived radicals. All these factors would render the *Leishmania* parasite susceptible to digestion in the lysosomal vacuoles of the macrophage, hence the observed significant reduction in parasite-loads of *L. donovani*-infected EGTA-treated macrophages *in vitro*. The low activity of chelators on *L. donovani*-infected laboratory animal rodent models may suggest that chelators do not reach the parasites which are in lysosomal vacuoles in sufficient quantities to result in a significant leishmanicidal effect. Other possible reasons for the low activity of chelators on *L. donovani in vivo*, and probable innovative approaches that are required to enhance the leishmanicidal potential of chelators in animal models, are described in the thesis.

Parasite-loads calculated from identical biopsy materials from the viscera of *L. donovani*-infected hamsters were compared after staining with Giemsa and acridine orange. Parasite-loads of identical biopsy materials from either the liver or bone marrow were not significantly different from each other when either stain was used. However, the parasite-loads obtained from the two stains were significantly different when used for the diagnosis of identical spleen biopsies. Both Giemsa and acridine orange were found to be suitable in staining *L. donovani* promastigotes. Being a quick and simple technique, acridine orange has a potential in the diagnosis of kala-azar.