5. DISCUSSION

Visceral Leishmaniasis or Kala-azar is a disorder produced by a flagellated protozoan, *Leishmania donovani*. The disease is of major health importance leading to severe morbidity and mortality. The morbidity rate is reported around 85% without treatment in tropical and subtropical countries (Aikat *et al.*, 1979). The importance of Leishmanial infection has been fully realised only in recent years. In accordance with the goals of the W.H.O.'s Tropical Disease Research Programme, Visceral leishmaniasis has been given a high priority, being next to malaria. Although there is no quantitative evaluation at the moment, but rough estimates based on extrapolations of the research data available indicate that some 350 million people in the world are at risk of infection (WHO Tech Rep. Ser., 1990). But only a few experimental attempts have been made to analyse the biochemical, immunological and the diagnostic aspects of the disease. Till date no effective non-toxic therapy for *L. donovani* infection is available. Chemotherapeutic agents presently in use include several Amphotericin B, pentavalent antimonials (Pentostam) and Glucantime, a diamidine (Pentamidine) and a broad spectrum of antibiotics. All of these drugs when used as required may exert severe toxic reactions in treated individuals. Common side effects during treatment are vomiting and giddiness, jaundice, hematuria, pneumonia, ECG abnormalities and anaphylactic shock which may
further complicate the treatment. Furthermore, the above drugs often do not effect complete cure.

For drug treatment to be successful, the cooperation of the immune mechanism of the host is often required. In the case of Disseminated Leishmaniasis, the T cell-mediated arm of the immune-response is of paramount importance in containing and eventually eliminating the parasites from infected macrophages (Garnham & Hamphery, 1969).

Thus, one of the strategies which might be exploited in the treatment of Leishmaniasis is specific or non-specific immunostimulation with microbial products or synthetic polymers. Several recent studies have documented the effectiveness of immunostimulation in reducing the parasite burden in *L. donovani* infected mice.

The work done in this thesis is an extension of our previous observations on vaccination studies against *L. donovani* infection. This work further provides the first evidence that a combined treatment with lower concentrations of drug dose and adjuvant tre-halose - di mycolate (TDM) is equally or more effective than the currently employed standard procedure.

Tre-halose dimycolate (6-6'-diester's of trehalose), synthesized by *Mycobacteria*, *Corynaebacteria* and *Nacardiae* as a part of their cell wall has been shown to possess
several immunopotentiating properties (Lederer, 1979). The size of the esterifying fatty acids ranges from C28 in *corynebacteria* (cornye myco-colicacids) to C80 in *Mycobacteria* (mycolic acids) mycobacteria tre-halose dimycolate purified by centrifugal chemotherapy, has been named "P₃" by Ribi et al., (1976). Rapp et al., (1978) have introduced the term TDM (tre-halose dimycolate) for mycobacterial cord factor preparations to distinguish them from synthetic trehalose diesters.

Aqueous suspensions of TDM were first prepared by Masahiko Kato of Osaka (1967). Such emulsions are stable and less toxic, they are active against *Klebsiella pneumoniae* and *Listeria monocytogens* (Parant et al., 1977). Kumar et al., (1984), reported the absolute protection of mice following their immunization with *P. berghei* antigen - TDM (aqueous suspension) combination and Kierszenbaum et al., (1984) have reported that aqueous TDM suspension modulates mouse macrophage function, *in vitro*, by augmenting both internalization and intra cellular destruction of *Trypanosoma cruzi*.

The importance of macrophages is well documented for host protection against intracellular parasites. A parallelism exists between the cytotoxic activity of trehalose diester mediated macrophages and their ability to release H₂O₂ upon pharmacological triggering. This suggests
that H$_2$O$_2$ plays a role in the antitumor activity of these macrophages (Lepovire et al., 1982). A direct interaction of TDM with cell-surfaces is also indicated by experiments that 'an expansion' of selected population (macrophages) triggered by TDM, is involved in the manifestation of adjuvant activity and possibly other immunological properties of 'cord factor' (Kierszenbaum et al., 1984).

The biochemical studies and the immune enhancement/stimulation of the host was studied using a combined therapy of lower concentrations of drug and TDM. The drug treatment alone has also been assayed and characterized in detail. For these investigations *L. donovani* whole antigen was prepared from the promastigotes grown in BHMARB medium. The whole antigen preparation was further fractionated into soluble and particulate fractions. All such preparations were partially characterized prior to their use in immunological studies.

Sufficient evidence is available to indicate that infection of laboratory animals and humans with *L. donovani*, or related species, results in the accumulation of parasites in the liver, spleen, peritoneal exudate cells, lymph nodes and bone-marrow, producing pathological changes. Initially the number of amastigotes of *L. donovani* increases in the liver followed by a decrease during the later stages of infection. Whereas, in the spleen the amastigote appeared on day 14 post infection (PI) and their number increased till
the animal died. The results suggest that liver is the first sight of infection of *L. donovani* in Visceral Leishmaniasis.

It is sufficiently clear from the results of drug dose pattern experiments that 10 mg drug dose decreases the parasite load. But a remarkable decline in parasite burden was achieved when the drug was combined with TDM. Total leukocyte count was increased in the infected animal group and in the drug (lower concentration) treated animal groups. TLC was around 6357 when drug and TDM were combined at a parasite count of 5076. The haemoglobin concentration was also found increased in the infected animal groups. The combined use of drug and TDM more effectively reduces the anaemic condition than the lower drug alone. Haemoglobin was found increased from 9.45 to 15.14 with a higher therapy of 15.9 gm/100 ml concentration.

The differential leukocyte count was shown in Table VII. In the infected animal group, the polymorph was reduced from 32.4 ± 2.07 to 17.2 ± 1.40, lymphocyte increased from 63 ± 2.12 to 75.6 ± 2.19 monocyte increased from 2.4 ± 0.55 to 3.6 ± 0.89 and eosonophil from 1.4 ± 0.55 to 2 ± 1.73. The lower concentrations of drug were not found as effective as in combined therapy using drug and TDM. In Kala-azar there is a marked reduction in the number of all forms of granulocytes leading to leucopenia, with relative lymphocytosis (Aikat *et al.*, 1979, Srivastava *et al.*, 1984).
In spite of the enormous amount of literature available on leishmaniasis, very limited information exists on the physiological changes produced in the host by *L. donovani*. This deficiency can be described primarily due to the fact that biochemical studies on liver and serum of human subjects naturally infected with leishmaniasis are often complicated by the presence of concurrent infections and nutritional deficiencies (Desowitz, 1987).

In view of the above considerations, studies were set up to determine some of the detectable biochemical changes occurring in infected and treated hamsters. Liver has been shown to be involved both directly and indirectly in *L. donovani* infection. Phenomenon of fatty acid degeneration in addition to centrilobular necrosis was mainly observed in liver during leishmanial infection, this was later confirmed by some biochemical studies. In our finding total liver fats were found to have increased in the infected animal. The lower drug treatment did not significantly reduce the increased level of total lipid, while the same amount of drug when combined with TDM caused significant drop in the increased level of total lipid and the level was more or less equal to the normal.

A similar pattern of increase in hepatic lipid content was also observed in rats, monkeys, and humans infected with *P. berghei, P. knowlesi* and *P. falciparum*, respectively (Rao
et al., 1969; Angus et al., 1971; Fletcher et al., 1987 and Bajpai and Dutta, 1990). Accumulation of lipid in liver of animal was also detected by electron microscopy (Fletcher et al., 1987) and by histochemistry (Angus et al., 1971 and Mercado and Von Brand; 1958). So the increase in the level of total lipids in the tissue mainly causes hyperlipidaemia in heavy infections. There are several reasons for accumulation of fat in liver which causes fatty liver in animals and humans during leishmanial infection. Liver plays a decisive role in metabolism and transport of lipids, as well as in the maintenance of lipid levels in the liver and in blood circulation.

Similarly, fall in phospholipid levels in the liver has been observed during the course of leishmanial infection in hamsters. Sharma et al. (1979) also reported decrease in phospholipid contents in the infected rat, but the decrease was insignificant. The combined treatment of drug and TDM more effectively normalized the level as compared to the lower drug treatment alone.

More significantly our results indicate a remarkable alterations in the rate of lipid peroxidation during leishmaniasis. These results get further support from the observations given by previous workers on other protozoal diseases (Saxena et al., 1981, Chauhan, et al., 1981) who have also shown an increased level of lipid peroxidation in
the liver of mice during malarial infection. Enhancement in the rate of lipid peroxidation following malarial infection is similar to the observations of Bajpai and Dutta (1987).

Recently, a significant increase in the rate of lipid peroxidation in cerebellum and brain system of mice following *P. berghei* infection was shown by Mahdi et al. (1989). Such raised levels of lipid peroxidation are perhaps available due to an increased susceptibility of liver to an oxidative damage under the stress of leishmanial infection (Sharma et al., 1979). The drug treatment repaired this oxidative damage by reducing the parasite load in the liver. Combined therapy of drug and TDM more effectively controlled leishmanial infection and more or less normalized the level.

A close correlation exists between the cellular injury, peroxidation of membrane lipids and oxidative damage to cells. In consonance with the observations on the effect of toxic chemicals or in pathological conditions, the liver homogenates of golden hamsters infected with *L. donovani* are shown to produce higher amounts of lipid peroxides. Such an increase is directly proportional to the number of amastigotes in the liver of golden hamsters. This increase in lipid peroxides may be due to increased susceptibility of hepatic tissues to oxidative damage under the stress of *L. donovani* infection.
The decrease in the level of cholesterol in the infected animals might be due to an increased uptake by the infecting parasites. In biochemical studies, fatty acid infiltration in the liver was not observed, though a state of hypoglycaemia was seen. Decrease in hepatic glycogen is rather a common feature during protozoan or helminthic infections. A similar decline in the polysaccharide reserves has also been observed by many workers (White et al., 1983). Homewood and Neama (1980) and Saxena et al., (1981) have observed depletion in total carbohydrate contents of liver in mice and monkeys infected with *P. berghei* and *P. knowlesi* respectively. Recently, Phillips et al., (1989) and Kawo et al., (1990) have also reported a state of hypoglycaemia in humans during *P. falciparum* infection and in golden hamsters infected with *Ancylostoma ceylanicum* (Von brand, 1973).

In case of *L. donovani* infection the decline in glycogen may be due to the fact that amastigotes residing in macrophages drain glycogen from the hepatic cells for deriving energy. Disturbance in other aspects of host liver metabolism during *L. donovani* infection may also contribute to this decline. Similar findings were found in other protozoan diseases such as trypanosomiasis and malaria. Earlier, Sadun et al., (1965) and Srivastava et al., (1984) have reported depletion of glycogen contents in liver during malarial infection. Devakul and Maegraith (1958) have observed considerable decrease of liver glycogen in *Macaca*
mulatta infected with *P. knowlesi*. Chatterji and Gupta (1957) have reported similar findings in female rats infected with *P. berghei*.

Glucose contents were also estimated in the infected and treated experimental animals. In our studies, we have found the depletion in glucose content, in the liver of infected hamsters. The depletion of glucose was more effectively controlled by the combined therapy compared to the animals treated with lower concentrations of drug alone. Fall in glucose content of liver was also observed by Saxena et al., (1981) in *Mastomys natalensis* during *P. berghei* infection. Recently, Paul et al., (1991) also reported hypoglycaemia in albino mice infected with *P. berghei* infection. Possible mechanism for hypoglycaemia during leishmaniasis infection includes accelerated tissue metabolism and increased metabolic requirements of the parasite. Impaired hepatic gluconeogenesis with fatty infiltration causes hypoglycaemia (Filkins and Cornell, 1974). Increase in the concentration of lactic acid in the blood of host causes toxicity leading to tissue damage due to the utilization of glucose through glycolytic pathway by the parasites.

Present findings also revealed decreased amount of total ribonucleic (RNA) and deoxyribonucleic (DNA) contents. In other protozoal diseases, such as malaria, the
reduction in nucleic acid values as observed in the present studies has not been shown by Rao et al., (1969). Kreier (1980) and Weber (1988) have suggested that nucleic acids are made up of purine and pyrimidine nucleotides. In mammals, purines are both synthesized denovo and obtained through salvage pathways. While leishmania parasites are incapable of synthesizing purine de novo and as such are totally dependent on salvage pathways. Absorption of nucleotides by the parasites from host under such conditions might be one reason for reduction of nucleic acid contents in liver during leishmaniasis.

Nucleic acid contents in infected liver were further assayed by histochemical techniques in present thesis. The depletion was overcome after treatment with the drug. When the drug (10 mg) was combined with TDM, the depletion was controlled more effectively. The values such obtained were comparable only with higher drug concentration.

There is a significant fall in the rate of synthesis of liver proteins in the infected, experimental hamsters. This would suggest an appreciable alteration in the protein status of the organ of the host. An extensive proteolysis makes readily available a pool of free amino acids which are needed for rapid proliferation of parasites (Saxena et al., 1981, Fern et al., 1985). The decrease in the protein level is another reason for the depletion of RNA and DNA contents.
within the liver of the infected animals. Infact, poor availability of nucleic acid could be responsible for lower levels of protein (Chander and Kapoor, 1990).

It is obvious that the interaction of *L. donovani* and the host gives rise to certain somatic alterations, which may ultimately be reflected in biochemical changes in the body fluids. Therefore, correlation of clinical observations with multiple quantitative determination of serum components may contribute to the diagnosis and progress of this infection and may even permit an early assessment of the effect of various chemotherapeutic agents (Sadun, 1966). The liver damage caused by the parasites during leishmaniasis can be more conveniently demonstrated by means of several routine functional tests. The enzymes generally used as indicators of liver dysfunction are glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT) acid phosphatase and alkaline phosphatase.

It has been observed that liver GPT and GOT were decreased significantly. The results are in agreement with the earlier observations made by Lal and Hussain (1978) in other protozoal diseases. They have also observed decreased levels of GPT and GOT activity in the liver of *P. berghei* infection indicating a dysfunction of liver during infection. We have also observed marked alterations in the SGPT and SGOT levels in the serum of infected hamster. SGPT and SGOT
levels are increased during leishmaniasis. Similar findings were also reported by Sadun et al., (1965), Lal and Hussain (1978) and Khanna et al., (1986) in malarial infections. During leishmanial infections, an increase in the number of macrophages, lymphocytes and kupffer cells were also observed in the liver. It was confirmed by estimating the acid and alkaline phosphatase levels in the liver tissues. Biochemical estimations have shown a significant elevation of alkaline phosphatase activity. The increased levels of acid phosphatase activity in the liver was observed in our studies and was found increased significantly. Saxena et al., (1985) also reported similar findings in various animal models including mice, rats, and Mastomys natalensis. During P. berghei infection Banyal et al., (1980) also reported acid and alkaline phosphatase activity in monkeys infected with P. knowlesi. They noted altered levels of these enzymes due to the membrane disruption of the cytoplasmic organelles which ultimately leads to liver damage. When the infected animals were treated with drug the elevated levels of enzymes were more effectively controlled with the combined therapy of drug and TDM. These results are comparable to the higher drug treatment alone. It has been found that the combined therapy repaired the oxidative damage effectively and normalized the level of enzymes.
In our study, we found that in experimentally induced leishmaniasis, there is a significant reduction of parasites in the liver and spleen by using the combined therapy of TDM and sodium stibogluconate as compared to the infected controls. The effect of TDM was dependent upon the amount of TDM given to each mouse. One of the advantages of TDM is certainly the long duration of its action. The immunostimulation by TDM is supposed to increase gradually over the weeks. Mice were better protected against Babesia microti seven weeks after an intravenous infection of 200 TDM in aqueous suspension than after three or five weeks (Clark, 1979). These workers have also reported that mice which were inoculated with 10 ug or 50 ug TDM intravenously were not protected, Olds et al., (1980) also showed that number of Shistosomulae recovered from mice treated with 200 ug TDM was reduced, but an intravenous dose of the 100 ug per mouse was not protective. TDM in a 1% squalane in water emulsion was also found to protect mice against Toxoplasma gondii four weeks after intra peritonial infection (Masini et al., 1986). Lederer (1986) also obtained protection in mice following administration of TDM against P. berghei, Toxoplasma and Mesocestoides corti. They showed infection rate ranging from 40 to 100 per cent and the reduction in the infected rate from 30 to 100 percent. Recently, very good protection was observed in mice against P. berghei infection (Lederer, 1988). TDM has been shown to enhance non specific immunity.
against various infections. It can also induce local immunity against an airborne, tuberculosis infection. Pimm et al., (1979) also demonstrated that intraperitoneal inoculation of TDM causes suppression of an ascitic rat tumor. Similar antitumor activity of TDM in mice has also been reported by Sakurai et al., (1988). Yarkoni et al., (1977) showed that after intra-peritoneal administration of TDM into mice, phagocytosis of L. monocystogenes by peritoneal macrophages increased. On the basis of these facts, Yarkoni's et al., (1977) concluded that TDM activates macrophages. Similarly, Kierszenbaun (1984) observed macrophages a few days after intraperitoneal administration of TDM in mice. These macrophages produced large quantities of \( H_2O_2 \) following triggering by phorbol myristate acetate (Lepoivre et al., 1982). On the basis of various reports there are sufficient evidences to believe that the non-specific protection in mice following TDM administration, is mainly achieved due to macrophage activation a few days after its intra-peritoneal inoculation of TDM in mouse. In our laboratory, Pathak (1987) had carried out immunization studies in golden hamsters by inoculating purified leishmania antigen with TDM.

The use of drug and an immuno modulator combination has opened a new era in the field of chemotherapy and immuno therapy. The work of Haidaris and Bonventre (1983) as claimed is the first documented report that the two therapeutic modalities concurrently employed can be of
advantage in the treatment of experimental *L. donovani* infection. In an *in-vitro* experiment, macrophages from C$_5$7 BL/6 mice were infected with *L. donovani* amastigotes. Partial or complete activation of macrophages was found as pitting of tumor cells significantly enhanced the efficacy of sodium stibogluconate (Pentostam). It should be appreciated that the problem of therapeutic control of leishmaniasis is not only confined to the discovery of a drug with antileishmanial effect, but also that of the delivery or targeting of other drugs to the actual site in the macrophages where parasites are lodged.

The quantity of drug required for elimination of parasites from immunostimulated cells was considerably lower than that required to achieve comparable amastigote killing in thiglycollate cultured macrophages (Haidarais and Bonventre, 1983). In an *in vivo* study C$_5$7 BL/6 mice infected with *L. donovani* were treated alone and in combination with *C. parvum* and sodium stibogluconate at 60 or 120 mg/kg body weight. The combined therapy was significantly more effective than the immunotherapy or chemotherapy alone (Haidarais and Bonventre, 1983, Berger and Fairlamb, 1992).

It is well known that cellular, rather than the humoral immunity, is responsible for acquired resistance against visceral leishmaniasis (Garham and Humphrey, 1969; Zuckerman, 1975). In the active state of visceral leishmaniasis, the
cell mediated immunity is generally depressed; usually the skin test (delayed hypersensitivity) becomes positive only after 6-8 weeks of treatment. It is usually such cases which later become immune to a subsequent infection. Generally speaking, antibodies in leishmaniasis are not of much critical value in the immune protection of host (Garnham and Humphrey, 1969; Neal et al., 1969; Stauber 1970).

In this thesis the humoral immune response against leishmaniasis in golden hamsters was estimated by using IHA and ELISA tests. ELISA has been found more useful and more sensitive than IHA and immuno electrophoresis, etc. which were used for the diagnosis of kala-azar. (Gupta et al., 1993). ELISA technique was first used by Hommel (1976) and subsequently by Voller et al., (1976) for the detection of antileishmanial antibodies showing promising results. ELISA has also been used by Arora et al., (1985) in large scale seroepidemiological surveys for kala-azar and found it to be more practical than IFA. Jalees et al., (1982, 1983) used IHA and ELISA for the detection of leishmanial antibodies. Obaid et al., (1989) used ELISA for the detection of humoral immune responses during immunization studies against visceral leishmaniasis. In this study, elevated levels of antibody titres were found in infected animals and the level was found to be normal in the drug treated; and more effectively by using the combined therapy of drug and TDM.
In vitro, the CMI responses were detected by the development of delayed type hypersensitivity response (DTH) following intradermal antigen inoculation. The test has been widely used in studies on the epidemiology of kala-azar in Kenya and of mucocutaneous leishmaniasis in South African countries (Pessoa and Barretto, 1948). The leishmanian test was found slightly more specific (Manson Bahr, 1961). Dacost et al. (1988) reported DTH to have a similar relationship to leishmaniasis, since this test measures the delayed hypersensitivity of cell mediated immunity. In this study, foot pad swelling in response to animal challenge by phenol suspension of promastigotes was taken as a measure of DTH responsiveness. The skin test was found to be less positive in only drug treated animal groups and negative in the group which were treated with a combination of drug and TDM. The experiment on maximum macrophage migration inhibition (MMI) was also done. It was found to be positive in infected animals. The inhibition was less in the drug treated group and negative in the group in which a combination of lower drug and TDM was used.

The results from DTH and MMI tests were reproducible and in agreement with one another. The main immunological feature of visceral leishmaniasis is the total suppression of cellular hypersensitivity to leishmanias antigens. This is associated with high parasite multiplication accompanied by
high levels of specific antibodies and polyclonal IgM and IgG (Report of WHO Expert Committee, 1990). Earlier workers have suggested from the *in vitro* experiments that the activity may result from interactions with several cell types (Parant, 1980). Halder et al., (1983) reported the absence of lymphocyte blastogenesis to leishmaniasis antigen in an *in vitro* test. Such impairment in cell mediated immunity is attributed to the development of suppressor T cells. However, these cells do not interfere with the activity of helper T-cells which along with B-cells help in the production of high antibody levels (Preston, 1987).

Generally speaking, antibodies in leishmaniasis are not usually of much critical value in the immune protection of host (Garham and Humphrey 1969; Stauber 1970). While Zukerman and Lainson (1977) suggested that antibodies in leishmaniasis have a limited role in providing immune resistance. Our data showed that infected animals which received drug in combination with TDM were found to have raised antibody titres than only drug treated animal groups.

These results are more or less in agreement with other workers, who have used BCG, glucan, aluminium hydroxide and HES as adjuvant to enhance the immune resistance in mice and hamsters against *L. donovani* infection. (Smrkovski and Larson, 1977, Jarecki Black et al., 1984, 1986, 1988, Obaid et al., 1989; Cook and Holbrook, 1983 and White and McMohan
Pratt; 1990). The histopathological changes in total infections of *L. donovani* are characterized by hyperplasia of the reticuloendothelial tissues particularly in kupffer cells of the liver and macrophages of the spleen and bone marrow. This condition results in the enlargement of liver and spleen tissues accompanied by some circulatory changes (Melency 1925, WHO Tech Rep. Scr. 1984, 1990, Guitirrez et al., 1984, Oleveira et al., 1985). Under laboratory conditions in *L. donovani* infection, some animals respond in a manner similar to that in fatal human infection with marked macrophage proliferation and in the formation of granulomas in the liver and spleen (Melency 1925; Reiner and Mahmood 1985, Gutierrez et al., 1984). The liver of the infected controls showed hyperplasia in Kupffer cells with the accumulation of macrophages forming granuloma foci. The granuloma formation increased with the progress of infection, leading to an atrophy of the heptic cells and distortion of the normal liver architecture.

A large number of L.D. bodies were seen in the hyperplastic macrophages. The results were found to be in agreement with those of Melency (1925) and Gutierrez (1984). In the lower drug treated animal groups, there is a marked proliferation of kupffer cells and alteration in the pattern of hepatic lobules. Large number of parasitized reticulo endothelial cells were seen. The combination of TDM and lower concentrations of drug caused significant effects. There was
only very little inflammation of the macrophages but the cells did not contain LD bodies. With a high drug dose, the liver appeared to be more or less normal.

The purpose of the present work was to extend the previous studies and to see the synergistic effect of an established drug given at reduced doses along with TDM and to assess and evaluate the anti parasitic activity/efficacy of DM alone and in combination with antileishmanial drug. Although there are several studies confirming the antiparasitic, antibacterial, antiviral and antitumor activities of TDM, but to our knowledge there are no available reports indicating such use of TDM in experimental leishmaniasis.

As for chemotherapy, the antileishmanial drugs commonly in use appear to be quite toxic, if given in adequate doses. Cases of drug resistance are not uncommon either. Currently, a lot of research is going on to investigate the possibility of minimizing the side effects of therapy by introducing newer/safer drug, or alternatively, drug combinations with some suitable immunomodulators.

Based on available information, two possible explanations can be proposed; First it may be quite likely that the microbial armamentarium of activated macrophages acts in parasites within the cells while higher
concentrations of drug are required to kill organisms in non-activated macrophages. Because microbial products such as hydrogen peroxide and similar products are produced in very small amounts, while the toxic substances are produced in considerable quantities by activated cells. Thus sub lethal drug doses in combination with microbicidal effector mechanisms of the immuno stimulated macrophages acting independently may provide more effective therapy than either of the above treatment given alone. The alternative explanation could be that the activated macrophages concentrate antileishmanial drug more effectively so that a great concentration of drug is delivered to the parasitophorous vacuoles than in the unstimulated cell. There is ample evidence in the literature to show a greater pinocytic activity by the activated macrophages. Effect of combined immunostimulation and drug therapy has also been studied in experimental bacterial (Proteus mirabilis, E. Coli, Klebsilla pneumonia, Strepto coccus fecalis) infections (Lahnborg et al., 1982). It was concluded that glucose ( 1-3 linkage) in combination with ampicillin, has a significant effect on the survival rate of rats with induced peritonites. The efficacy of this treatment was probably due to an enhanced activity of the R.E. system - an important segment of the total host resistance.

The findings from our study, suggested that TDM when used as an immunomodulator was able to stimulate the immune
status of the animal. Such a treatment can overcome the infection even when the animals were treated with lesser doses of drug in combination with TDM. Quite evidently, the drug alone cannot possibly have a similar effect on leishmaniasis.