CONCLUSION

Since no immunization trial is completely successful against Leishmaniasis therefore a preliminary trial was made in our studies to identify a candidate vaccine. In order to achieve the goal *L. donovani* were cultured *in vitro*, using Brain Heart Infusion Agar medium in which a satisfactory growth was obtained. Different fractions of Leishmania antigen prepared from these cultures were found to be immunologically active against positive kala-azar serum.

Previous studies in our laboratory indicated that Leishmania antigen alone was unable to elicit sufficient protection, therefore, a strong adjuvant, BCG, was used in association with Leishmania antigen to boost up the immunological system of experimental animals. A considerably higher protection after challenge with live *L. donovani* promastigotes was obtained in animals immunized with soluble antigen in association with BCG, compared to animals immunized with particulate and whole antigen in association with BCG. The extent of immunization, or protection, was detected by assaying the antileishmanial antibodies, immunoglobulins levels and DTH responsiveness to leishmania antigens. Animals immunized with soluble antigen in association with BCG always showed higher antibody titres compared to animals immunized with particulate and whole
antigen in association with BCG. Also, the level of IgG was found to be higher compared to IgM in hyperimmune sera samples. However, all the animals immunized either with antigen only or in association with BCG showed negative DTH responsiveness to Leishmania antigens.

Percent protection in immunized animals was detected by intraperitoneally injecting the experimental animals with 1 x 10^6 promastigotes. Again the antibody titres were higher in animals immunized with soluble antigen in association with BCG compared to animals immunized with particulate and whole antigen in association with BCG. Also the level of IgG was always higher compared to IgM in hyperimmune sera. All the animals immunized with Leishmania antigen in association with BCG were found to be positive for DTH responsiveness after challenge. The macrophage migration inhibition was also higher in animals immunized with soluble antigen in association with BCG compared to animals immunized with particulate and whole antigen in association with BCG after challenge. Furthermore, minimum decrease in acid phosphatase activity was observed in animals immunized with soluble antigen in association with BCG compared to animals immunized with particulate and whole antigen in association with BCG after challenge.
In our haematological studies we found that TLC was higher in animals immunized with Leishmania antigen in association with BCG. The animals immunized either with BCG alone or with Leishmania antigen in association with BCG showed higher TLC with slight increase in lymphocytes and monocytes compared to animals immunized with Leishmania antigen only. Furthermore, in organ parasite count studies, a significant reduction in splenic and liver parasite amastigotes was observed in animals immunized with Leishmania antigen in association with BCG, especially the animals immunized with soluble antigen in association with BCG after challenge. Histopathology of liver and spleen from antigen immunized group showed moderate number of L.D. bodies in the hyperplased macrophages compared to animals immunized with Leishmania antigen in association with BCG. The liver and spleen architecture in antigen-BCG immunized groups showed almost no changes or alterations. Some fibrous reactions were observed in spleen, but L.D. bodies were very rarely seen in hyperplased macrophages.

On the basis of our results it was concluded that significant protection against L. donovani infection can be achieved by intraperitoneal immunization of golden hamsters, in association with BCG. Furthermore best results were observed in animals immunized with soluble Leishmania antigen in association with BCG.