Although enormous efforts have been made to eradicate leishmaniasis through vaccination but complete protection has never been achieved. Therefore in the present study we have made a vaccination trial against leishmaniasis in golden hamsters. Three different leishmania antigenic fractions constituting whole, particulate and soluble (alone and in association with BCG) antigens were used for immunization experiments.

In vitro cultivation of Leishmania donovani (NICT: C II strain) was carried out in Brain Heart Infusion Agar medium. Promastigotes obtained from 6 day cultures were washed 6 times in HBSS by centrifugation at a speed of 2000-2500 rpm for 30 min. A clear homogenate was prepared in a homogenizer for obtaining the whole antigen. The whole antigen was centrifuged at 105,000 x g for 1 h, in an ultracentrifuge at 4°C to get particulate and soluble antigen fractions.

Leishmania antigen, before being used for immunization were characterized by estimating protein, carbohydrate and DNA contents. The SDS-PAGE was carried out for molecular weight determination and separation of protein sub-units of each fraction. The antigenicity of these antigenic fractions was checked against antisera obtained from cases of kala-azar. IFA, IHA and ELISA tests were employed to detect the
antigenic activity of whole, particulate and soluble antigenic fractions.

Immunization experiments were carried out in golden hamsters of 80-100 gms. In the first experiment, the animals were intraperitoneally injected with whole, particulate and soluble antigenic fractions. In the second set of experiments, the animals were intraperitoneally injected with different antigenic fractions in association with BCG (Bacille Calmette-Guerin) as an immunomodulator. Control animals received either BCG or saline only. Every hamster was intraperitoneally injected four times with 0.2 mg antigen protein with or without BCG ($2 \times 10^6$ Bacille Calmette-guerin) at four day intervals.

The antibody titres were detected by IHA and ELISA at day 30, 45 and 60 following last immunizing dose. Animals immunized with antigen plus BCG showed titre values greater than those which received antigen only. In IHA and ELISA tests, the highest antibody titres were found in animals immunized with soluble antigen in association with BCG following particulate and whole antigen in association with BCG. The immunoglobulin (IgG and IgM) levels were also detected by single radial immunodiffusion technique in hyperimmune sera on day 45 following last immunizing dose. The animals immunized with antigen in association with BCG
showed higher immunoglobulin levels compared to animals immunized with antigen only. Moreover, highest immunoglobulin levels were found in animals immunized with soluble antigen in association with BCG following particulate and whole antigen in association with BCG. The appearance of cell mediated immunity in these animals was demonstrated by delayed type hypersensitivity (DTH) response. The DTH was measured by foot pad swelling response to phenol suspension of promastigotes. This test was negative in all hamsters immunized with leishmania antigens (with or without BCG), when tested at day 30, 45 and 60 following the last immunizing dose. Hamsters which received parasite antigen plus BCG showed leukocytosis, while hamsters which received parasite antigen only showed almost normal leukocyte counts compared to control hamsters. The percentage of polymorphonuclear cells was higher in the antigen plus BCG group than in the antigen group only.

Experimental and control hamsters were challenged by intraperitoneal injection of viable *L. donovani* promastigotes on day 45 following last immunizing dose. Each hamster received a challenge dose containing 1 ml of $1 \times 10^7$ promastigotes from log phase. The antibody titres were assessed by employing IHA and ELISA on day 30, 45 and 60 post challenge. Hamsters which received antigen in association with BCG showed higher antibody titres than those given
antigen only. The highest antibody titres were recorded in animals immunized with soluble antigen in association with BCG following particulate and whole antigen in association with BCG. Also, highest immunoglobulin levels were found in animals immunized with soluble antigen in association with BCG following particulate and whole antigen in association with BCG. The DTH was positive only in animals which received parasite antigen in association with BCG. The highest percentage of foot pad swelling was recorded in hamsters immunized with soluble antigen in association with BCG. Parasaemia and foot pad lesions were developed only in control animals. In vitro cell mediated immune response by macrophage migration inhibition test was also assessed. Highest percentage of migration inhibition values were obtained from hamsters which received soluble antigen in association with BCG following particulate and whole antigen in association with BCG. The hamsters of antigen-BCG group showed higher TLC with slight increase in lymphocyte and monocyte counts. The control animals and those which received parasite antigen only showed leukopenia accompanied with lymphocytosis and slight increase in monocytes.

All animals immunized with leishmania antigen in association with BCG showed significant reduction in hepatic and splenic parasite counts on day 15, 30, 45 and 60 post challenge compared to animals immunized with leishmania
antigen only. Animals immunized with soluble antigen in association with BCG showed highest parasite reduction followed by particulate and whole antigen in association with BCG. Our histopathological studies showed almost no change in animals immunized with antigen in association with BCG. However, moderate number of LD bodies were observed in hyperplased macrophages of animals immunized with antigen only.

Since complete protection of immunized animals was not obtained in this study therefore a small dose of drug (Stibogluconate) was used in order to get one hundred percent protection. All hamsters were intramuscularly injected with 2.5 mg/kg body weight of stibogluconate. The protection in drug treated animals was seen through organ smear impressions, where no parasite was seen in these slides. Our studies clearly showed that sufficient protection can be elicited in golden hamsters against L. donovani infection by immunizing the animals with soluble antigen in association with BCG. And, complete protection can be achieved by infecting a very small dose of drug to the vaccinated animals.