INTRODUCTION

Leishmaniasis, is stated to be a major disease of the tropical countries in the world. Among six tropical diseases, it has a major impact on public health itself, and so the current efforts are directed-though insufficient, to control this disease. In recent years, India has been experiencing a resurgence of visceral leishmaniasis (kala-azar) which has also been reported for the first time in Afghanistan and Bolivia (Anon, 1987).

The name leishmaniasis comprises of several diseases caused by different species of the intracellular kinotoplastid protozoan parasite of the genus *Leishmania*. Of these about ten infect humans, and the disease is grouped under three headings viz. Cutaneous Leishmaniasis (CL); Mucocutaneous Leishmaniasis (MCL) and Visceral Leishmaniasis (VL) or kala-azar.

The infective agent of leishmaniasis are transmitted by over 50 species of female sandflies of the genus *Phlebotomus*. The breeding and resting sites of sandflies are diverse and widespread, and include rodent burrows, tree trunks and organic debris.

The genus *Leishmania* established by Ross (1903), belongs to Phylum Sarcomastigophora. In the same year, Sir Willium Leishman isolated the parasite from the spleen smears of a soldier in England, who died from a fever contracted at Dum-Dum (Calcutta), known as Dum-Dum fever now called kala-azar. In that year, Charles Donovan (1903) demonstrated the presence of a similar parasite in the spleen smear of a patient suffering from kala-azar in India. The parasite was cultivated successfully by Rogers (1904). The leishmania-form in the blood and flagellate form in the insect gut were demonstrated by Patton (1907).

The life-cycle of this parasite consists of an extracellular flagellated nucleated spindle-shaped bodied Promastigote form or leptomonad form (size 15-25 μm length and 1.5-3.5 μm breadth), found in the gut lumen of sandfly vector *Phlebotomas argentipes* and an intra-cellular aflagellar but nucleated round or oval bodied Amastigote form or L.D. bodies (size 2-4 μm length), in the reticuloendothelial cells of mammalian macrophage like spleen, liver, bone-marrow, lymph nodes, heart and kidney.

The world prevalence of human leishmanisasis is not properly known but is thought to exceed 12 million cases. An incidence of 400,000 new infections per year has previously been reported (Anon 1989). However, in the light of information provided therein, supported by epidemiological studies in the past

decade and the practice of leishmanization in the Middle East, this could now be regarded as an underestimate. The WHO Expert Committee on Leishmaniasis recommended that visceral leishmaniasis should be made a notifiable disease (W.H.O 1984). It is expected the information on occurrence of the disease will now be truely depicted.

About a third of the world's population is at a risk of infection and disease. With the exception of Oceania, the leishmaniasis occur in most parts of the world i.e, the southern regions of North America, most of Central and South America, the Mediterranean basin, East and North Africa, the Caspian littoral, the Arabian Peninsula, the Persian Gulf region, the Indian subcontinent, China and the Southern Soviet Union. The most severe form of leishmaniasis is found in Africa (Ethiopia, Kenya and the Sudan), Latin America and India.

In general, the leishmaniasis specially the Kala-azar appear to be increasing in frequency particularly in India. Kala-azar caused by *Leishmania donovani* has been known to occur endemically in well defined areas in the eastern half of the Indian subcontinent, particularly Assam, Tripura, Bangala Desh, West Bengal, Bihar, certain areas of Nepal and Sikkim lying at the foothills of the Himalayas, Uttarpradesh and Tamil Nadu. Kala-azar foci occur at altitude of 200-700 meter where there is alluvial soil and much rain. A focus has also been discovered in Pakistan at an altitude of 2400 meter. The distribution of the disease closely corresponds to that of *Phlebotomas argentipes* as has been shown by Mackie (1914) and Sinton (1925).

Most forms of leishmaniasis are zoonotic in nature—human being getting infected only secondarily. When no suitable animal reservoir host is available, a human host is suspected, as in the case of anthroponotic of urban cutaneous leishmaniasis (ACL) due to *L.tropica,* and kala-azar, due to *L.donovani* Animal reservoir hosts for leishmaniasis include wild cats, dogs, jackals, foxes, sloths, and rodents.

Animal reservoirs of leishmaniasis have been incriminated in most parts of the world where fresh cases of cutaneaous and/or visceral forms of the disease have been detected. In India, Kala-azar is an anthroponosis; passed from man to man almost exclusively by the sandfly *Phlelotomus argentipes* (Lysenko, 1971). Canines and rodents are the reservoir host in other parts of the world. Srivastava and Chakraborty (1984) confirmed that no animal show any evidence of infection during the epidemic phase. But the reappearence of Kala-azar after lapse of 10-15 years, suggests the possibility of an animal reservoir for *L. donovani* during interepidemic phase or in low endemicity areas. An animal reservoir may well harbour the infection in the interepidemic phase and given suitable conditions for the spread and multiplication of vector

population, the disease could then reach epidemic proportions. The search of animal reservoirs is thus a very important aspect for Leishmania research.

Immune-responses in leishmaniasis have been more defined in recent years. Leishmanial infection in man induces both humoral and cellular immune response, but the balance of their expression varies with the type of disease. In Kala-azar as in leprosy, there is a complete sippression of cell-mediated immunity (CMI) to the leishmania antigen with a normal or exaggerated humoral response. There is a sharp rise of immunoglobulins in the blood, particularly IgG without giving any effective immunity but resposible for the serological reactions. The elevated level of IgG deminishes rapidly when treatment begins. Delayed CMI response to parasite antigens becomes demonstrable only after spontaneous recovery or treatment which suggests the role of cellmediated mechanisms in infection process. But it is not clear how the immune responses operate to influence and modulate the pathogenesis of the disease and development of clinical expression in consequence. The serological tests can provide useful information particularly in the detection of circulating antibodies during different stages of kala-azar infection. A variety of serological methods and antigens have been developed for the detection of specific circulating antibodies but most of these tests can be carried out only in the laboratories equipped with expensive sophisticated apparatus like floures-cent microscope (for IFA), gamma counter (for RIA) and spectrophotometer (for ELISA). The antigens used in these tests needed to be studied keeping in mind the different forms of the infective agent in reservoir hosts and in vectors i.e. *in vivo* and *in vitro,* and the nature of antibodies that could be developed due to transmission of agent by vectors or due to experimental inoculation of the agent grown *in vitro.* Moreover, tests for detecting cell mediated immunity in kala-azar possibly associated with amastigotes has not been applied. Hence, there is a need to develop rapid, sensitive and reliable diagnostic tests which would detect antibodies to promastigotes and amastigotes for use in small laboratories in remote areas engaged in the diagnosis of zoonotic parasitic diseases, and for epidemiological surveillance.

The biochemical characterization of the antigens of *Leishmania donovani* so far made were directed towards unveiling the host-parasite relationship in general and for studying the mechanisms of pathogenesis so as to develop an immunogen to protect against the infection. Studies on biochemical character-isation of antigens participating in different immunological tests are therefore of great importance for the meaningful interpretation of immunological reactions.

The present study was therefore undertaken with the following objectives :-

I. To prepare antigen component(s) of *Leishmania donovani*.

II. To identify biochemically the antigens obtained from promastigote cells.

III. To assess the immunological behaviour of the antigen isolated.

IV. To develop any simple, sensitive, quick test to detect the antibodies of *L. donovani*, in early stage of infection.

V. To apply immunological tests for the detction of *L. donovani* antibodies in animal species.