Lipophosphoglycan (LPG) is a major cell surface glycoconjugate of Leishmania parasites. This unusual glycoconjugate is present throughout the various phases of growth in Leishmania parasites. The promastigotes plasma membrane contains about 1.25 million copies/cell of LPG, accounting for at least 25% of its cell wall. Structurally, LPG is composed of a neutral cap and a variable composition of a repeating phosphorylated disaccharide units attached via a conserved phosphosaccharide core to a phosphatidylinositol (PI) lipid anchor. All LPG molecules contain multiple unit of a backbone structure of PO -6-Gal(β1-4)Man α 1-. These phosphorylated disaccharide repeat units are attached by α-glycosidic linkage. The disaccharide repeat units of L. donovani LPG has no substitutions in their backbone sequence whereas repeating units of L. mexicana LPG has approximately 30% of its galactose residues substituted at the C 3 hydroxyl with glucose (Glc) residues. The repeating units of the L. major LPG are the most complex, as more than 85% of the galactose residues are further substituted with small saccharide chains containing one to four residues of galactose, glucose, or the pentose arabinose. It has been demonstrated that the number of repeating units per LPG molecule directly depends on the growth stage of promastigotes. The average number of repeat units per molecule reported for different species are: 16 for L. donovani, 20 for L. mexicana and 14 for procyclic, 30 for metacyclic, 36 for amastigotes of L.
The overall structure of LPGs isolated from *L. donovani*, *L. major*, and *L. mexicana* promastigotes are similar, with a linear arrangement of caps, repeating units, glycosylated core and lyso-alkylglycerol lipid moiety. The most striking differences in LPG structure between the *Leishmania* species lies in the phosphorylated oligosaccharide repeats.

LPG plays an important role in the biology of the parasite due to its surface location, its developmental regulation during the life cycle and the reduces virulence of the LPG-deficient organisms. A variety of functions and activities of LPG in the mammalian host have been experimentally demonstrated or suggested. These include, involvement in attachment and entry of promastigotes into mammalian macrophages, protection of parasite within phagolysosomal compartment and as a recognition molecule for the T-lymphocyte dependent immune responses characteristic of leishmaniasis. LPG is an inhibitor of protein kinase C, inhibitor of oxidative burst, inhibitor of viral fusion, signal transduction and scavenger of oxygen free radicals. Lipophosphoglycan has also been proposed to induce a protective immune response in mice and therefore, it is considered to be a candidate vaccine against leishmaniasis.

In recent years, the promastigote cell surface has received considerable attention in view of its importance in interaction with the immune system and for parasite recognition, uptake and survival in macrophages. The cell surface glycoconjugate, lipophosphoglycan (LPG) has been isolated, purified and characterized from *Leishmania* parasites of different species.
However, no attempt has been made to study the LPG from non-pathogenic strains. Hence, LPG from promastigotes of non-pathogenic strain UR6 was isolated, purified, characterized and its potential as immunoprophylactic agent and/or candidate vaccine against experimental visceral leishmaniasis was evaluated. Isolation of LPG was carried out using different organic solvent mixtures. Crude LPG was purified by size-exclusion, and hydrophobic affinity chromatography. The elution profile of LPG from Sephadex G-200 is indicating that LPG fractions eluted just after void volume, suggesting that it is a high molecular weight molecule. Single broad peak was obtained indicating the heterogeneous nature of LPG. The desaltation of LPG molecule which is eluted from Sephadex G-200 was necessary for removal of unwanted salts like, EDTA, NH$_4^+$, Mg$^{++}$ etc. These salts were found to interfere in further purification and characterization studies. LPG purified by conventional methods failed to remove LPG-associated protein contaminants as they were tightly bound to LPG. These associated proteins were removed by the treatment with proteinase K enzyme and protein/peptide free LPG was obtained after purification from octyl Sepharose column. From 25.0 ml (27.0 gm) packed cells, about 150 mg of crude LPG was obtained. This on partial purification yielded 31 mg of partially purified LPG which on octyl Sepharose CL-4B column gave a yield of about 15.0 mg of pure LPG. Characterization of purified LPG was carried out by thin layer chromatography (TLC), SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and NMR
spectroscopy. The mobility of purified LPG on silica gel G coated plate in the solvent mixture 1-propanol/pyridine/H O (1:1:1, V/V) is shown a single diffuse spot. The R value of LPG in this solvent system was found to be about 0.66±0.02, which is close to 0.62 reported earlier for the L. donovani promastigotes LPG of pathogenic strain. The purity of LPG molecule was assessed by SDS-PAGE followed by periodic acid schiffs (PAS) staining. On PAS staining of gel, LPG was the only carbohydrate staining species observed as it migrated as a single diffused band. The molecular weight of UR6 LPG obtained by the migration of standard proteins on SDS-PAGE, was found to be in the range of 20-25 kDa. One dimensional 1 H - NMR and two dimensional COSY NMR studies were carried out for structural characterization of the LPG molecule. The signal at $\delta$ 5.52 ppm was unambiguously assined to H-1 of mannose and connectivities could be traced out as cross peak to the H-2 ($\delta$ 3.71 ppm) and H-3 ($\delta$ 3.53 ppm) respectively. Similarly, the signal at $\delta$ 4.47 ppm can be unambiguously assinged to H-1 proton of galactose and connectivities could be traced out for H-2 ($\delta$ 3.54 ppm) and H-3 ($\delta$ 3.83 ppm) respectively. Further, connectivities could not traced out due to the in phase properties of diagonal peak. Hence, it is suggested that LPG molecule is obtained after final purification was pure. The LPG samples are devoid of any protein contaminants which was supported by the NMR spectroscopy as no signal of any -NH was found during NMR studies except carbohydrates corresponding peaks
were observed.

The protective potential of *L. donovani* LPG, against *L. donovani* infection in peritoneal macrophages has been studied. The studies demonstrate that UR6 LPG inhibits binding of promastigotes of Dd8 strain to peritoneal macrophages in a concentration dependent manner. For 10 ng/ml LPG about 50% inhibition of attachment to peritoneal macrophages was observed. This inhibitory activity was enhanced from 60% to 78%, when the LPG concentration was increased from 50 ng/ml to 100 ng/ml, respectively. A maximum inhibition of about 85-90% was observed for 1000 ng/ml of LPG. No further enhancement in inhibition with increase in LPG concentration upto 2000 ng/ml was observed. Similar results were also observed when LPG was incorporated into small unilamellar vesicle (SUV) containing cholesterol. Furthermore, UR6 LPG provided a significant protection (about 85-90%) in peritoneal macrophages against promastigotes infection. This study is also supported by previous study (Kelleher et al., 1995) that LPG was unable to completely block attachment of promastigotes to macrophages, suggesting the involvement of other ligands supporting the earlier observation that the domain of LPG which is involved in binding of promastigotes to the macrophages, is conserved in the promastigotes LPG of both the Dd8 and UR6 strains of *L. donovani*.

In order to look into the possibility whether LPG mediated protection in macrophages system against *L. donovani* was either due to inhibition of attachment of promastigotes to macrophages
or killing of amastigotes inside the macrophages, studies with pre and post treatment of macrophages with UR6 LPG were carried out. It was observed that the peritoneal macrophages which were treated with LPG before being challenged with promastigotes provided significant protection against *L. donovani* infection. However, those macrophages which were first challenged with promastigotes and then treated with LPG provided similar pattern of infection as observed that for control. These observations, suggest that pretreatment of peritoneal macrophages is necessary for achieving protection which depends on the inhibition of attachment of promastigotes to peritoneal macrophages.

In vivo efficacy of LPG molecules against visceral leishmaniasis was evaluated in susceptible golden hamsters using LPG alone or incorporated in liposomes. Pretreatment of hamsters with LPG anywhere between 10-28 days prior to infection did provide significant protection against *L. donovani* infection. The group of animals receiving LPG alone (10 µg/animal) showed a relatively low protection of about 22-25%. The protective efficacy of LPG was enhanced significantly on its incorporation in liposomes. LPG incorporated in cholesterol containing multilamellar liposomes provided 40 percent protection whereas LPG incorporated in small unilamellar vesicles (SUV) provided about 65-70 percent protection. This protection was more than double as compared to LPG alone. Furthermore, for incorporation of LPG in negatively charged liposomes as similar percent
protection as that for neutral liposomes was observed. Studies on concentration dependence of liposomised LPG in protecting hamster against *L. donovani* infection showed that a single dose of 20 μg/animal of liposomised LPG incorporated in SUV provided a maximum protection of about 68 percent. The effectiveness of liposome incorporated LPG compared to LPG alone in protection against *L. donovani* in hamsters can be due to induction of an effective T-cell response. This possibility is indicated from the fact that increased percent protection was observed on increasing the pretreatment schedule with liposomised LPG from 2 days to 21 days.