Leishmania donovani is the obligate intracellular parasite of macrophages that causes visceral leishmaniasis in humans. The worldwide incidence and prevalence of VL cases per year are 0.5 and 2.5 million respectively. Ninety percent of the world’s VL cases occur in rural and suburban areas of India, Bangladesh, Nepal, Sudan and Brazil.

The control of leishmaniasis remains a source of grave concern worldwide. Over the years, control measures have revolved heavily around chemotherapy. However, the treatment of VL is hampered by the intracellular location of the parasite, toxicity of drugs and emergence of drug-resistant strains. The development of effective vaccines represents one of the most promising approaches for providing cost-effective interventions against this disease. However, to date there is no prophylactic vaccine available against any form of human leishmaniasis. Protection in leishmaniasis is dependent on the activation of a Th1 type of immune response with production of a high level of IFN-γ and low levels of IL-4.

The gp63, a membrane-anchored matrix metalloprotease has been used as an experimental subunit vaccine against leishmaniasis. It induces a protective immune response when administered with an appropriate immunoadjuvant.

The heat shock or stress response is one of the most highly conserved adaptive responses in nature. Heat shock proteins play an important role in the control of protective immunity by participating in the assembly of antibody molecules, stabilizing MHC class I and II molecules and through their ability to synthesize cytokines. In addition, because they potently stimulate innate and antigen-specific pathways, they are promising as vaccine adjuvants for a broad spectrum of pathogens. The adjuvant effect of Hsp70 has been demonstrated after immunization with Plasmodium peptides and L. infantum. It has also been used as an adjuvant along with DNA immunization with P4 antigen of L. amazonensis.

In the present study, the immunoprophylactic potential of cocktail of gp63 and Hsp70 against L. donovani in BALB/c mice was investigated. Inbred BALB/c mice were immunized with gp63 and Hsp70 individually and in combination and then challenged with $10^7$ promastigotes of L. donovani. Animals were sacrificed on 30, 60
Abstract

and 90 days post infection/challenge. The protective efficacy of the proteins was assessed by studying the hepatic and splenic parasite load and the generation of cellular and humoral immune responses.

The immunized animals revealed significantly lesser parasite burden in comparison to the infected controls. The maximum reduction in splenic and hepatic parasite burden was observed in mice immunized with the combination of gp63 and Hsp70.

To evaluate the humoral responses generated by gp63 and Hsp70, antileishmanial antibodies (IgG, IgM, IgG1 and IgG2a) were assessed in the serum samples of the immunized animals and the infected controls. Immunization of animals raised the levels of IgG antibody in comparison to the infected controls. The IgG antibody production was observed to be the maximum in animals immunized with a combination of gp63 and Hsp70. In contrast to the IgG levels, IgG1 and IgM levels declined in the immunized animals in comparison to the infected controls. Since both the antibodies point towards the generation of a non-protective Th2 type of immune responses, least antibody production in the mice immunized with the cocktail vaccine proves the protective efficacy of the two proteins in combination. The level of IgG2a increased in the immunized animals in comparison to the infected controls. Maximum production of this antibody in animals immunized with the combination of gp63 and Hsp70 points towards the generation of protective Th1 type of immune response.

The mice immunized with a combination of gp63 and Hsp70 also showed increased cell-mediated immune responses as revealed by heightened delayed type hypersensitivity (DTH) response to leishmanin. These animals also generated maximum levels of IFN-γ and IL-2 and minimum levels of IL-4 and IL-10 indicating again the generation of a protective Th1 response and suppression of the non-protective Th2 type of immune response.

The current study showed that the combination of gp63 and Hsp70 is highly immunogenic and protective against VL in comparison to immunization with gp63 and Hsp70 individually.