SUMMARY

The leishmaniases are a group of diseases caused by protozoan parasites of the genus *Leishmania* and it affects millions of people worldwide. Human leishmaniasis is distributed worldwide specifically in the tropical and subtropical regions, with a prevalence of 12 million cases and an approximate incidence of 0.5 million cases of VL and 1.5 million cases of cutaneous leishmaniasis (CL) (http://www.who.int/tdr/disease/leish/diseaseinfo.htm). The current treatment of leishmaniasis relies on few drugs with serious limitations so, the development of an effective anti-leishmanial vaccine remains the best hope. The present study was carried out to evaluate the immunoprophylactic potential of different cocktails of three antigens of *Leishmania donovani* i.e. 31kDa, 36kda and 51kDa in combination with adjuvants ALD, saponin and liposome. The immunogenicity and protective efficacy of sixteen different formulations was tested in the present study.

**Electro-elution and identification of 31, 36 and 51 kDa antigens**

The 31, 36 and 51 kDa antigens were identified in the gel with the help of molecular weight markers. The desired bands of interest were taken in the electrophoresis buffer (0.025M Tris, 0.192M glycine, 1% SDS) in the electro-eluter and a constant voltage of 50 V for 5-6 min was applied through the gel. After elution, the proteins were dialyzed, lyophilized and resuspended in PBS (pH-7.2). The antigens were quantified by Lowry’s method. The eluted proteins were also checked on SDS-PAGE gel. The antigens were identified by 2D-gel electrophoresis.

**Preparation of different vaccine formulations**

Sixteen types of vaccine formulations were prepared.

The cocktails of 31+36kDa, 36+51kDa, 31+51kda and 31+36+51kDa antigens were formulated with ALD (Autoclaved *L. donovani*), saponin and cationic liposomes as adjuvants. In addition, the cocktails of 31+36kDa, 36+51kDa, 31+51kda and 31+36+51kda antigens (without any adjuvant) were also used as a vaccine candidate for immunization.
**Immunization of animals and challenge infection**

Mice were immunized with cocktail of different vaccine formulations (31+36kDa, 36+51kda, 31+51kDa and 31+36+51kDa alone and in combination with three adjuvants i.e. ALD, saponin and liposome). Two booster doses with the respective vaccine combination were given to all immunized groups at an interval of 2 weeks each. Two weeks after the last booster dose, mice of control and immunized groups were challenged with $1 \times 10^7$ promastigotes. Various parasitological and immunological studies were carried out in six mice of each group at 15 days post immunization and 30, 60 and 90 days of post challenge.

**DTH responses**

The delayed type hypersensitivity responses (DTH) were assessed by measuring the percentage increase in footpad thickness of leishmanin injected footpad in comparison to the control (PBS) footpad. In all the immunized groups, a profound DTH response was induced. Maximum DTH responses were elicited by animals immunized with cocktail of 31+51+liposome followed by 36+51+liposome, 31+36+liposome and 31+36+51+liposome.

**Parasite Load**

To check the protective efficacy of different vaccine formulations groups, the parasite load was assessed in terms of Leishman Donovan Units (LDU) on Giemsa stained impression smears of liver. The parasite load in all the immunized groups of animals was significantly reduced as compared to infected controls. However, maximum protection was conferred by groups of animals immunized with cocktail of 31+51+liposome.

**Cytokine responses**

The cytokine responses generated by different vaccine formulations were assessed by quantifying the levels of cytokines (IFN-γ, IL-12, IL-4 and IL-10) in serum samples. In mice model of leishmaniasis Th1 type of immune response is considered to be protective and Th2 type is considered non-protective. Cytokines
IFN-γ and IL-12 indicate the generation of Th1 type of immune responses whereas IL-4 and IL-10 point towards the generation of Th2 type of immune responses.

Higher levels of Th1 cytokines and lower levels of Th2 cytokines were observed in immunized groups as compared to infected controls. Maximum levels of Th1 cytokines (IFN-γ, IL-12) and minimum levels of Th2 cytokines (IL-4, IL-10) were observed in groups of animals immunized with a cocktail of 31+51+liposome followed by those immunized with a cocktail of 36+51+liposome, 31+36+liposome and 31+36+51+liposome.

**Humoral immune responses**

Antibody responses in infected and immunized mice were analyzed by IgG1 and IgG2a antibodies in their respective serum samples by ELISA. The IgG1 antibodies were determined for the assessment of Th2 type, whereas IgG2a was determined for the assessment of Th1 type of immune responses.

The Th2 specific antibody, IgG1 was found to be maximum in the infected controls whereas minimum levels were observed in groups of animals immunized with a cocktail of 31+51+liposome. Conversely, minimum levels of IgG2a, a Th1 specific antibody were observed in the infected controls and maximum levels were observed in groups of animals immunized with a cocktail of 31+51+liposome.

From these results following conclusions can be withdrawn:

1. The immunized groups of animals showed heightened DTH responses suggesting the generation of cell mediated immune responses.

2. A decline in parasite load was observed in all the immunized groups suggesting the protective role of all the vaccine formulations.

3. A remarkable increase in the levels of IFN-γ and IL-12 pointed towards the generation of protective Th1 type of immune responses in all the immunized groups. Decreased levels of IL-4 and IL-10 were observed in all the immunized groups suggesting the suppression of non-protective Th2 type of immune responses.
4. Amongst the antigen cocktails, most promising results were obtained with a cocktail of 31+51kDa and liposome proved to be the best adjuvant. Besides this, results of cocktail of two antigens were better as compared to cocktail of three antigens.

5. Further studies should be carried out in higher animal models.