EVALUATION OF IMMUNOMODULATORY ACTIVITY AND PROTECTIVE EFFICACY OF HERBAL DRUGS WITHANIA SOMNIFERA, ASPARAGUS RACEMOSUS AND TINOSPORA CORDIFOLIA IN COMBINATION WITH CISPLATIN AGAINST MURINE VISCERAL LEISHMANIASIS

SUMMARY

For the assessment of antileishmanial effect of cisplatin alone and in combination with herbal drugs, 6 mice from each group were sacrificed on 0, 15 and 30 p.i.d./p.t.d. The liver of mice from each group was removed and weighed. Impression smears were made and the parasite load was calculated in terms of Leishman Donovan Units. The group of L. donovani infected BALB/c mice treated with cisplatin showed significant (p<0.0001) reduction in parasite load as compared to infected controls. However, a further reduction in parasite load was observed in infected mice treated with cisplatin in combination with herbal drugs on different p.t.d.

The levels of IgG1 and IgG2a reflect Th1/Th2 responses. Their levels were detected in sera samples of all the groups on 0, 15 and 30 p.i.d/p.t.d. by doing indirect ELISA. The levels of IgG1 were found to be highest in infected controls. However, treatment of infected mice with cisplatin resulted in decreased levels of IgG1. Moreover, the levels of IgG1 were found to be decreased further in the groups of infected mice treated with cisplatin in combination with herbal drugs. Treatment of infected mice with cisplatin increased the levels of IgG2a, however, infected mice showed decreased levels of IgG2a. A further increase in levels of IgG2a was observed in infected mice treated with cisplatin in combination with herbal drugs on different p.t.d.

DTH was determined as an index of cell mediated immune response. In L. donovani infected mice disease severity is associated with hampered DTH responses. The DTH responses were assessed by measuring the percentage increase in foot pad thickness of leishmanin injected footpad in comparison to the control footpad (PBS). The profound DTH responses were induced by treatment of infected mice with cisplatin, suggesting the generation of cell mediated immune responses. Moreover, in the groups of infected mice treated with cisplatin in combination with herbal drugs, DTH responses increased further on different p.t.d. Also, increase in DTH responses was also observed in infected
mice treated with herbal drugs alone, thus, suggesting the immunomodulatory role played by the herbal drugs.

The cellular immune responses (protective Th1 or non protective Th2) generated during the course of infection and treatment were assessed by quantifying the cytokines viz. IFN-γ, IL-2, IL-4 and IL-10, produced by the splenocytes of mice from different groups. Cytokines IFN-γ and IL-2 indicate towards the generation of Th1 type of immune response whereas increased levels of IL-4 and IL-10 are predictors of Th2 type of immune response. The levels of IFN-γ were increased during the treatment of infected mice with cisplatin as compared to infected controls. However, treatment of infected mice with cisplatin along with herbal drugs showed further increase in the levels of IFN-γ. This increase was also found in infected mice treated with herbal drugs alone as compared to infected control. Alike, IFN-γ, IL-2 levels were also increased in infected mice treated with cisplatin as compared to infected control. Moreover, the levels of IL-2 increased further in infected mice treated with cisplatin in combination with herbal drugs. In contrast, the levels of IL-4 and IL-10 were found to be higher in infected control. However, the levels of IL-4 were found to be decreased in infected mice treated with cisplatin and a further decrease in the levels of IL-4 was observed in infected mice treated with cisplatin in combination with herbal drugs. The levels of IL-10 were also found to be decreased in infected mice treated with cisplatin and least levels of IL-10 were observed in infected mice treated with cisplatin along with herbal drugs.

All the groups of BALB/c mice showed similar percentage of CD3+ cells. In the group of infected mice, percentage of CD4+ cells was found to be decreased with increase in percentage of CD8+ cells. However, cisplatin alone and cisplatin in combination with herbal drugs treated infected mice showed increased percentage of CD4+ cells and decrease in percentage of CD8+ cells. Similarly, the ratio of CD4+/CD8-/CD8+CD4+ cells was also found to be decreased in infected mice. However, infected mice treated with cisplatin alone and in combination with herbal drugs showed increase in cell ratio. Infected mice showed increase in percentage of total B cells (CD19). However, the percentage of CD19 cells was found to be decreased in infected mice treated with cisplatin alone and in combination with herbal drugs. The percentage of NK 1.1 cells was found to be decreased in the group of infected mice, however, the percentage of NK1.1 cells...
was observed to be increased in infected mice treated with cisplatin alone and cisplatin in combination with herbal drugs.

The group of infected mice treated with cisplatin showed signs of hepatotoxicity as an increase in levels of SGOT, SGPT and LDH was found. However, treatment of infected mice with cisplatin along with herbal drugs brought about a reduction in the levels of SGOT, SGPT and LDH and were within the normal range. The levels of serum alkaline phosphatase, acid phosphatase and bilirubin were within the normal range in all the groups of BALB/c mice.

Treatment of infected mice with cisplatin resulted in nephrotoxicity and caused an increase in the levels of blood urea, blood urea nitrogen, serum creatinine and uric acid. However, to ameliorate the toxicity of the drug cisplatin, herbal drugs were administered along with cisplatin. It was found that after the treatment of infected mice with cisplatin along with herbal drugs, normal levels of blood urea, blood urea nitrogen, serum creatinine and uric acid were observed. Also, treatment of infected mice with cisplatin caused electrolyte disturbances like hypomagnesia, hypocalcemia, hyponatremia and hypochloremia. However, after the treatment of infected mice with cisplatin along with herbal drugs, normal levels of electrolytes were observed.

Treatment of infected mice with cisplatin caused lymphocytic infiltration, damage in brush border epithelium of distal convoluted tubules and proximal convoluted tubules with less tubular distinction and lumen of these tubules was also found to be filled with cellular debris. However, treatment of infected mice with cisplatin along with herbal drugs prevented kidneys from cisplatin induced damage as no pathological changes were observed and kidneys appeared normal in architecture. Cisplatin treatment in infected mice showed histopathological changes such as microvascular fatty change, vacuolization and reduction in sinusoidal space, however, liver sections of infected mice treated with cisplatin along with herbal drugs showed no morphological alterations and liver sections were normal. The group of infected mice treated with cisplatin showed amyloid deposits and presence of megakaryocytes with expanded marginal zone. However, infected mice treated with cisplatin in combination with herbal drugs showed normal spleen structure with no pathological changes.