

ASSESSMENT OF IMMUNOMODULATORY ACTIVITY OF METHANOLIC EXTRACT OF *Boerrhaviadiffusa* L.

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ABSTRACT

Boerrhavia diffusa L. (F. Nystagiaceae) has been used in Ayurvedic medicines for the treatment of liver, gall bladder, urinary and many other diseases since ages. The present study was aimed to assess the immunomodulatory effect of the methanolic extract by testing its effect in the haematological parameters of rats and circulating Abtitre and antibody forming cells in the spleen of Balb/c mice. The extract was given at a dose rates *i.e.*, 200 mg/kg and 400 mg/kg to 6 rats each for each parameter. The hematological parameters like total RBC count, total and differential WBC count and haemoglobin concentration did not show significant difference for both doses tested. The effect on plaque forming cells in the spleen was tested by the Jernes plaque assay using a modified slide technique. The extract at both dose rates produced increase in antibody forming cells in the spleen (as shown by the number of plaque forming cells) when compared to that of the control. Similarly the extract at both dose rates also produced increase in circulating Abtitre when compared to that of the control. The results showed for the methanolic extract at the dose rates tested had stimulant activity on the humoral immunity.

Keywords:- *Boerrhaviadiffusa* L., immuno modulatory, plaque forming cells.

INTRODUCTION

Boerrhavia diffusa L. has a long history of use in Ayurvedic medicine in India and has been used for the treatment of many ailments including liver, gall bladder, renal and urinary disorders (Abraham, 1975). Chopra *et al.* (1956) described the use of the plant as asthma, oedema, anaemia, jaundice, anasarca and as an antidote to snake poisoning.

Kirtikar and Basu (1975) recommended the use of the plant in anaemia, inflammations, vatha and kapha. The plant was included one of the extensively investigated medicinal plants in India (Vohora, 1989).

Mugantiwar *et al.* (1997) studied the effect of the alkaloidal fraction of *B. diffusa* on stress-induced changes in plasma and adrenal cortisol levels and immune responsiveness in rats. The drug was found to possess restorative activity against stress induced changes in plasma and adrenal cortisol levels and augmented antibody production.

The present study was undertaken to find out the immunomodulatory effect of the plant.

MATERIALS AND METHODS

The experiment was conducted in Balb/c mice of either sex. which were maintained on identical feeding and managemental practices in the laboratory for one week before the

commencement of study. Work was carried out after getting approval from Institutional Animal Ethics Committee. The whole plants were collected locally, dried under shade, pulverized and extracted with methanol using a Soxhlet extractor and evaporated to dryness using a vacuum flash evaporator. The study was performed using 3 different parameters.

1. Effect on haematological parameters

18 albino rats of either sex, divided into 3 groups of 6 each were used for the study. The first group was given treatment distilled water, the 2nd group was given the extract at the rate of 200 mg/kg and the 3rd group was given the extract at the rate of 400 mg/kg respectively orally for 5 consecutive days.

On the 6th day, blood was collected and the hematological parameters like total RBC count, total WBC count, Differential Count(DC) and Hemoglobin(Hb) count were assessed by standard procedures described by Schalm *et al.* (1975).

2. Effect on plaque forming cells in the spleen

This was performed by the Jerne's plaque assay (Jerne and Nordin, 1963)⁷ using a modified slide technique described by Mehrotra (1983)⁸. 30 Balb/c mice of either sex divided into 3 groups of 10 each were used for the study. All the mice immunized with 0.1 ml each of 20% SRBC in PBS given i/p using aseptic precautions. The first group was kept

as the control and was given no drug. The 2nd group was administered the extract @ 200 mg/kg orally daily till the mice were sacrificed. On the 3rd, 4th, 5th, 6th and 7th day after immunization, 2 mice from each group were sacrificed and spleen collected. Spleen cells were processed and plaque formation assay performed by the modified slide method, as given below.

The spleens were processed into single cell suspension (8×10^6 cells/ml) in HBSS. To 0.5 ml of spleen cell suspension were added, mixed well and poured over a glass slide. The slides were allowed to solidify and incubated with fresh rabbit serum as source of complement for 1 hour at 37°C. The plaque formed were counted over a light source and represented as PFCs/million spleen cells (Lefkowitz and Cosenza, 1979)⁹.

3. Effect on circulating Antibody (Ab) titre/ Hemagglutination titre (HA titre)

This was done by the method described by Nelson and Davy (1992)¹⁰. 15 Balb/c mice were divided into 3 groups of 5 each. The 3rd group was kept as the control. All the mice were immunized with 0.1 ml each of 20% SRBC given i/p. The 2nd group was administered the extract @ 200 mg/kg orally daily till day 25 of immunization. The 3rd group was administered the extract @ 400 mg/kg orally. Similarly blood was collected on day 5, 10, 15, 20 and 25 from all the mice. Sera were separated and heat inactivated as 56°C for

Table 1. Effect of methanolic extract of *B. diffusa* on hematological parameters in rats

Groups	Hb g%	RBC millions/ cu.mm	WBC 10^3 / cu.mm	DC		
				Lymphocyte %	Neutrophils %	Eosinophils %
Control	15.1 ± 1.0	8.28 ± 0.59	5.076 ± 1.328	61.9 ± 6.4	31.3 ± 6.4	1.3 ± 0.9
Extract @ 200 mg/kg	11.22 ± 0.41	8.68 ± 1.11	6.480 ± 2.423	67.8 ± 1.52	26.4 ± 1.13	4.8 ± 0.82
Extract @ 400 mg/kg	12.52 ± 0.17	8.09 ± 1.64	5.45 ± 0.987	63.0 ± 3.32	33.0 ± 2.52	4.0 ± 1.16

Table 2. Effect of methanolic extract of *B. diffusa* on plaque forming cells in spleen

Groups	Number of PFCs/10 ⁶ spleen cells (days after immunization)				
	3	4	5	6	7
Control	40	112	200	280	96
Extract @ 200 mg/kg	56	152	192	416	104
Extract @ 400 mg/kg	32	136	120	304	194

30 min. 2 fold dilutions of sera samples were made using PBS (pH 7.2) in microtitre plates and mixed 1:1 with trypsinised suspension of SRBC in PBS. The plates were incubated at 37°C for 3h. The degree of agglutination was evaluated macroscopically. The HA titre was calculated as the reciprocal of highest dilution of serum which showed visible agglutination.

RESULTS

1. Effect on hematological parameters: There were no significant difference between the 3 groups in Hb concentration count, total and differential WBC counts and RBC count (Table 1).

2. Effect on plaque forming cells (PFCs) in the spleen: Administration of the extract at both dose rates produced increase in antibody forming cells in the spleen (as shown by the number of PFCs), when compared with that of the control (Table 2). The number of PFCs started increasing from day 3 and reached a peak level on day 6. The number gradually decreased in the higher dose group (group

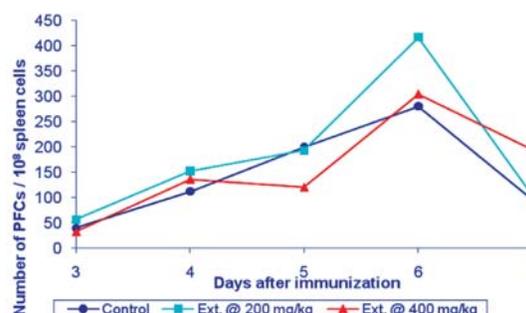


Fig. 1 Effect of methanolic extract of *B. diffusa* on plaque forming cells

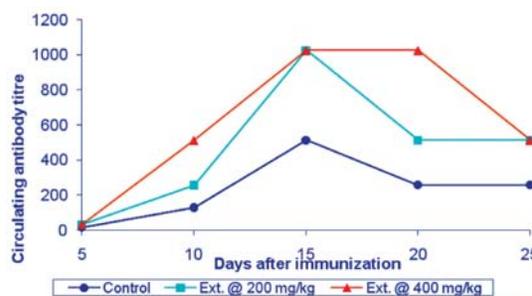


Fig. 2 Effect of methanolic extract of *B. diffusa* on circulating antibody titre

III), while it suddenly returned to near normal values at day 7 for the group II 3. Effect on circulating Ab titre: The experiment at both dose rates produced increase in circulating Ab titre when compared to that of the control group (Table 3). The peak titre was observed at day 15 for the 3 groups but it was maintained upto day 20 in the group III.

DISCUSSION

The results showed that the experiment at both dose rates stimulated the humoral immune response, as shown by an increase in Ab forming cells in spleen and Ab titre against SRBC in Balb/c mice. The normal hematological values for rats are (1) RBC

Table 3. Effect of methanolic extract of *B. diffusa* on circulating antibody titre

Groups	Antibody titre (days after immunization)				
	5	10	15	20	25
Control	16	128	512	256	256
Extract @ 200 mg/kg	32	256	1024	512	512
Extract @ 400 mg/kg	32	512	1024	1024	512

count – 7-10 million/cu.mm (2) WBC count – 6-17 thousands/cu.mm. (3) Hb – 11-18 g/dl, (4) Neutrophil 9-34%, (5) Lymphocyte – 65-85% and (6) Eosinophil -0-6% (Hrapkewicz et al, 1998)¹¹. Since the mean values for all the group fall within these ranges, there is no significant change in the hematological parameters.

Praveenkumar *et al.* (1999b) have found that ‘rasayana’, a multidrug herbal preparation could enhance humoral immune responses as seen from increased number of Ab forming cells & circulating Ab titres and hence had immune stimulant properties.

Geetha and Sangeetha (2000) conducted a controlled experimental study to assess the effect of *B. diffusa* extract @ 2.4 g/kg bodyweight orally to ward off post surgical infection and mortality in albino rats. The results showed that the drug caused (1) better prevention of post surgical infection, (2) better expectancy of life after surgery, (3) normal maintenance of level of water intake and urine output after surgery, (4) maintenance of total and differential WBC count after surgery and infection and (5) prevention of accumulation of peritoneal fluid and onset of gangrene.

Mugantiwar *et al.* (1997) have earlier reported that alkaloidal fraction of *B. diffusa* significantly reversed the depleted adrenal cortisol level and the elevated plasma cortisol level in stressed rats, thus appearing to have a corticosteroid sparing effect in experimental stress. The results of the present study were also complementary to these findings supporting the immunostimulant activity of alcoholic extract of *B. diffusa*.

CONCLUSION

The methanolic extract at the dose rates of 200 mg/kg and 400 mg/kg had immunostimulant properties as determined by an increase in the number of plaque forming cells in the spleen and circulating Ab titre.

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