Available online at www.ijpcr.com
International Journal of Pharmaceutical and Clinical Research 2009; 1(2): 62-64

Research Article ISSN 0975 1556

Study of the Immunomodulatory Activity of Brenol Tablet-A Polyherbal Formulation

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ABSTRACT
To study the immunomodulatory effect of brenol tablet-A polyherbal formulation and extracts were administered orally at doses of 200 and 400 mg/kg/day to healthy rats divided into three groups consisting of six animals each. The assessment of immunomodulatory activity was carried out by testing cellular (foot pad swelling) immune responses to the antigenic challenge by sheep RBCs and by neutrophil adhesion test. On oral administration polyherbal formulation showed a significant increase in neutrophil adhesion and delayed type hypersensitivity (DTH) response. Thus polyherbal formulation shows significantly potentiated the cellular immunity by facilitating the footpad thickness response to sheep RBCs in sensitized rats. The responses were statistically significant when they were compared with the control. The study stated that polyherbal formulation shows a significant stimulation of the cell mediated immunity and no effects on the humoral immunity. The study demonstrates that polyherbal formulation shows preferential stimulation of the components of cell-mediated immunity.

Keywords: Polyherbal formulation, Hypersensitivity, immunomodulatory activity.

INTRODUCTION

Immunomodulation is a procedure that can alter the immune system of an organism by interfering with its function. If it results in an enhancement of immune reaction named as Immunomodulatory. [1] Natural products of plant and animal origin offer vast resource of newer medicinal agents with potential in clinical use. [2]

Plants are the essential and integral part in Complementary and Alternative medicine and due to this they develop the ability for the formation of secondary metabolites like proteins, flavonoids, alkaloids, steroids and phenolic substances which are in turn used to restore health and heal many diseases. [3]

Thus the present investigation was aimed at evaluating the immunomodulatory activity of polyherbal formulation and *Bacopa monnieri* extract (Linn.) on standard animal models.

Bacopa monnieri (Linn) (Scrophulariaceae) commonly growing in marshy places throughout India, It contains following chemical constituents: Brahmine, Herpestine, Hersaponin, Bacoside A, Bacoside B, Becogenin A1 and A2, Monnierin and Bacosaponin D. [4]

MATERIALS AND METHODS

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Animals

Healthy albino Wister rats of either sex having (150-180 g) body weight were used for this study. The rats were housed in polypropylene cages and maintained under standard conditions (12 h light and dark cycles, at 25±3°C and 35-60 % humidity). Standard palletized feed and tap water were provided ad libitum. A fresh sheep red blood cell (SRBC) in Alsever's solution was obtained from authentic sources. The animals were divided into three groups consisting of six animals each. A group of six untreated rats were taken as control (Group I).The formulation group were administered with 200 mg/kg/day (Group II) and the extract was fed orally for 7 days at a dose of 400 mg/kg/day (Group III) for assessment of immunomodulatory effect. The study was approved by Institutional Animal Ethical Committee of R. C. Patel College of Pharmacy, Shirpur, India, registered under CPCSEA, India (Registration No.651/02/C/CPCSEA).

Antigen

As an antigen, Sheep Red Blood Cells (SRBC) was used. Fresh sheep blood was collected and aseptically added to sterile alsever's solution in 1:1 proportion. Then the SRBC were washed three times with pyrogen free sterile phosphate buffer saline (PBS) and the count was adjusted approximately to 1×10^6 cells/ml for immunization and challenge. [5]

Neutrophil adhesion test [6]

On the 7th day drug treatment, blood samples were collected (before challenge) by puncturing the retro orbital plexus into heparanized vials and analyzed for total leucocyte count

(TLC) and differential leucocyte count (DLC) by fixing blood smears and staining with Field stain I and II-Leishman's stain. After initial counts, blood samples were incubated with 80 mg/ml of nylon fibers for 15 min at 37°C. The incubated blood samples were again analyzed for TLC and DLC. The product of TLC and % neutrophil gives neutrophil index (NI) of blood sample. Percent neutrophil adhesion was calculated as shown below.

$$\text{Neutrophil adhesion (\%)} = \frac{NI_u - NI_t \times 100}{NI_u}$$

Where NI_u= Neutrophil index of untreated blood samples
NI_t= Neutrophil index of treated blood sample.

Delayed type Hypersensitivity (DTH) response [5]

On the day '0' of experiment, rats in all groups were immunized by intraperitoneal administration of 1×10^6 cells/ml/100gm of SRBCs. Control group received normal saline from day 0 to 7 of the experiment. The extract groups were administered with 400mg/kg orally from day 0 to day 7. Formulation group were administered with 200 mg/kg orally from day 0 to day 7. On day '7' animal in all groups were challenged with 1×10^6 SRBCs in left hind Paw and equal volume of saline in the contra lateral paw. The difference in the volume of left paws at 0 h, 24 h and 48 h was used as a measure of DTH reaction. The paw thickness was measured using plethysmometer.

Relative Lymphoid Organ Weight [7-8]

After measuring DTH responses on 7th day the body weight of rats in all experimental groups were determined. Animals were sacrificed under ether anesthesia. The thymus spleen were excised free from adhering tissues and weighed individually. The relative organ weights were calculated according to the following:

$$\text{Relative lymphoid organ weight} = \frac{\text{Organ weight (g)} \times 100}{\text{Body weight (g)}}$$

Table 1: Effect of extract of Bacopa monnieri and formulation (tablet) on Neutrophil adhesion in rats

Group	Neutrophil index		Neutrophil Adhesion (%)
	UB	FTB	
I (Control)	30.83±0.6009	24.5±0.4282	20.51±0.3757
II (Formulation)	35.83±0.6009	26±0.3651	27.41±0.5080**
III (Extract)	40.34±0.5574	30.16±0.3073	24.54±0.7117**

The values are mean ± S.D. of 6 rats in each group. One way ANOVA followed by Dunnett's test, **p<0.01 Vs group I, UB= untreated blood; FTB= Fiber Treated Blood.

Table 2: Effect of polyherbal formulation and *Bacpoa monnieri* extract on DTH response to antigenic challenge by sheep RBCs in rats.

Groups	DTH response (% increase in paw volume at 24hrs)	DTH response(% increase in paw volume at 48 hrs)
I (Control)	73±1.511	65±1.49
II (Formulation)	64±1.410**	56±1.25**
III (Extract)	53±1.89**	44±1.56**

The values are mean ± S.D. of 6 rats in each group. One way ANOVA followed by Dunnett's test, **p<0.01 Vs group I.

RESULTS

Formulation evoked as a significant increase in neutrophil adhesion (P<0.01, significance value) at a dose of 200 mg/kg.day in rats. The results of neutrophil adhesion test are shown in the table 1. DTH response to SRBC which corresponds to cell mediated immunity showed a dose dependent increase. The differences in DTH response were statistically significant which are shown in Table 2. Thus it can be said that formulation induced a remarkable enhancement in DTH Response to SRBC in animals. The results of relative lymphoid organ weights are shown in Table 3 and 4.

Table 3: Effect of extract and formulation on Relative organ weight (spleen) in rats.

Groups	Relative organ weight
Control	0.38±0.0066
Formulation	0.27±0.0070**
Extract	0.29±0.0057**

The values are mean ± S.D. of 6 rats in each group. One way ANOVA followed by Dunnett's test, **p<0.01 Vs group I.

Table 4: Effect of extract and formulation on Relative organ weight (Thymus) in rats

Groups	Relative organ weight
Control	0.18±0.0057
Formulation	0.12±0.0042**
Extract	0.10±0.0052**

The values are mean ± S.D. of 6 rats in each group. One way ANOVA followed by Dunnett's test, **p<0.01 Vs group I.

DISCUSSION

Immunomodulatory agents obtained from plant and animal origin generally enhances the immune responsiveness of an organism against a pathogen by activating the system. In the present investigation formulation when administered orally, significant increased in the adhesion of neutrophils to nylon fibers which interrelates to the process of margination of cells in blood vessels. It was found to be highly significant when compared with control.[6] as given in the table 1.The DTH response directly correlated the cell mediated immunity and was found significant. Thus in this process the T-lymphocytes gets sensitized when they are challenged by any antigen which there by gets converted in to lymphoblasts and secrets lymphokines, and attracts the scavenger cells to the site of reaction. It is a T-cell mediated immune (CMI) response which requires priming of a specific type of T cell followed by sensitization and efficient recruitment with release of mediators. DTH response is regulated by specific suppressor type of T cell (TS). The increase in the response indicated that formulation has a stimulating effect on the lymphocytes [8]. Thus it can be concluded that formulation was found to be highly stimulating agent for both cell mediated immune responses as given in Table 2. In Relative lymphoid organ the alterations in the weights of lymphoid organs like spleen and thymus also indicate the trafficking and proliferation of the lymphocytes. However, T-cells were mostly in an active form and highly mitotic. Hence the next immunological parameter studied was the spleen index. Due to the predominance of T and B lymphocytes, the spleen is considered as one of the major lymphoid organs. Formulation and extract group decreased the spleen weight as compared to control group which are as described in (Table 3, 4).

The decrease in the weight of these organs indicates suppression of lymphocyte turnover and consequent thinning of their population in these organs. In the Formulation and extract treated group, there was significant decrease in thymus to body weight ratio. This decrease in the weight of thymus may be due to both decrease in epithelial cells and the number of thymocytes. This may reflects decrease in the proliferation of thymic epithelial cells and chemotaxis mediated enhancement in the departure of precursor T-cells

from the thymus. Thus formulation and extract group can be considered to act through suppression of T and B lymphocytes. [7-8]

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