

IMMUNOMODULATORY ACTIVITY OF ETHANOLIC EXTRACT OF STEM BARK OF *BAUHINIA VARIEGATA* LINN.

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Summary

Immunomodulatory effect of ethanolic extract of stem bark of *Bauhinia variegata* in swiss albino was evaluated mice. Specific cell mediated immune response was studied by performing delayed type of hypersensitivity (DTH) model in mice treated with ethanolic extract of stem bark of *Bauhinia variegata* (EBV). The non specific immune response was studied by performing the model of cecal ligation and puncture (CLP) induced abdominal peritonitis in mice treated with EBV. In DTH model EBV at the dose of 250 and 500 mg/kg p.o. showed significant rise in the mean difference of footpad thickness in immunosuppressed group when compared with cyclosporine control. In the cecal ligation and puncture induced abdominal peritonitis model, EBV at the dose of 500 mg/kg p.o. showed significant increase in survival of animals. EBV shows the specific activation of cellular immune system in the immunosuppressed animal and also non specifically enhances the immune system by activation of the monocyte macrophage system and natural killer cells. Thereby it can be concluded that EBV holds promise as immunomodulatory agent which acts by stimulating both specific and non-specific arms of immunity.

KEYWORDS: *Bauhinia variegata*, Rasayana, immunomodulatory.

Introduction

Modulation of immune responses to alleviate the diseases has been of interest for many years and the concept of Rasayana is based on related principle.^[1] More specifically, rasayana is anti-ageing, increases the life-span, promotes intelligence and memory and increases resistance to disease” (presumably infections and therefore indicating potential immunostimulant effects). Rasayana herbs act as adaptogen, immunomodulator, pro-host probiotic and antimutagenic. Many plants used in traditional medicine have immunomodulating activities. Some of these stimulate both humoral and cell mediated immunity while other activate only the cellular components of the immune system, i.e. phagocytic function without affecting the humoral or cell mediated immunity. Some of these plants also suppress both humoral and cell mediated immunity.^[2] About 34 plants are identified as rasayanas in Indian ayurvedic system of medicine having various pharmacological properties such as immunostimulant, tonic, neurostimulant, antiageing, antibacterial, antirheumatic, anticancer, adaptogenic, antistress. Many plants are reported to possess potential immunomodulatory activity, a lot more are still to be explored and offer scope for further investigation.

Bauhinia variegata Linn (Caesalpinaceae) is an indigenous medicinal plant with properties similar to rasayanas.^[3] *Bauhinia variegata* is a commonly found plant in moist waste ground, and open plantation. It is cultivated throughout India and forests lands in central India. According to Ayurveda, *Bauhinia variegata* is used as tonic for the liver, in treatment of leprosy, menorrhagia, impurities of blood, tuberculous glands, wounds, ulcers, asthma etc.^[4, 5] Bark powder of the plant is a major ingredient of herbal tonic kanchanar guggul an ayurvedic remedy prescribed to increase the white blood cells. The stem bark of *Bauhinia variegata*, phytochemical characterisation shows the presence of tannins, steroids, alkaloids, flavonoids, and saponin.^[6] The ethanolic extract of stem bark of *Bauhinia variegata* Linn contains β sitosterol, lupeol, vitamin C, kaempferol, flavonone, and quercetin. Some studies have reported its antitumour^[7, 8], anti-ulcer^[9], antibacterial and antifungal activity.^[10] The present investigation was aimed to study the immunomodulatory activity of ethanolic extract of stem bark of *Bauhinia variegata* Linn using reported methods in order to justify the traditional claims endowed upon the drug as rasayana.

Materials and methods

Plant material

The stem bark of *Bauhinia variegata* Linn were collected from Pune, India. The plant specimen was authenticated by "Botanical Survey of India" Pune, India, (voucher specimen no.Sasp2)

Preparation of ethanolic extract of stem bark of Bauhinia variegata

The stem bark of *Bauhinia variegata* was dried in the shade and pulverized. The powder was treated with petroleum ether for dewaxing as well as to remove chlorophyll. The powder was cold macerated using ethanol (95%) as solvent. The extract was dried at 60°C (yield 9%w/w) and then suspended in 5% gum acacia for the pharmacological studies.

Experimental animal

All experimental procedures were carried out in strict accordance with the guidelines prescribed by the Committee for the Purpose of Control and Supervision on Experimentation on Animals (CPCSEA) and were approved by the Institutional Animal Ethics Committee.

Swiss albino mice weighing between 18 to 25 gm of either sex were used. The above animals of either sex were purchased from National Toxicology Center, Pune. Animals had free access to standard pellet diet and water *ad libitum*. Fresh animals were used for each experiment.

Drugs and chemicals:

Cyclosporine 100 μ g/mouse (i.p.) was used as a standard immunosuppressant. Sheep red blood cells (SRBCs) were procured from nearby slaughter house and were washed three times with normal saline and adjusted to the required concentration for immunization and for challenge as antigen.

Cell mediated immune response- Delayed Type Hypersensitivity

Animals were divided into 8 groups of six animals each. Animals in group I received 1ml of 5% gum acacia p.o. for 21 days. Group II received cyclosporine 100 μ g/mouse, i.p. on 14th day of the study. Animals in group III, IV and V with normal immune status were administered EBV at doses of 125, 250 and 500 mg/kg/day, p.o. respectively for 21 days. Animals in group VI, VII and VIII were administered EBV at doses of 125, 250, 500 mg/kg/day, p.o. respectively for 21 days plus cyclosporine 100 μ g/mouse, i.p. on day

14th as a immunosuppressant. Mice from all the groups were immunized with 0.1 ml of 20% SRBC's in normal saline intraperitoneally on 14th day of the study. On day 21st of the study, animals from all the group were challenged with 0.03 ml of 1% SRBC's in subplantar region of right hind paw. Foot pad reaction was assessed after 24 hr. i.e. on 22nd day, in terms of increase in the thickness of footpad due to oedema caused as a result of hypersensitivity reaction, with the help of a digital vernier calliper. The footpad reaction was expressed as the difference in the thickness (mm) between the right foot pad injected with SRBC and the left footpad injected with normal saline. [11, 12]

Non specific immune response - Cecal ligation and puncture induced abdominal peritonitis

The animals were divided into five groups each containing 15 mice which were further subdivided in 3 groups for the same drug treatment. Animals in group I (sham laparotomy) received 1 ml of 5% gum acacia p.o., at 18 hr \pm 2 hr and 2 hr before laparotomy. Animals in group II, (sham CLP) received distilled water 1ml/kg, p.o. at 18 hr \pm 2 hr and 2 hours before CLP. Animals in group III, IV, and V were administered EBV at the doses of 125, 250 and 500 mg/kg, p.o. at 18 hr \pm 2 hr and 2 hours before CLP. Animals in group II-IV were aneshetised by ketamine 100 mg/kg, i.p. 1 to 2 cm midline incision was made through the abdominal wall (Fig.1.1a) the cecum was identified and ligated with a 3-0 silk tied 1 cm from the tip. Care was taken not to cause bowel obstruction. A single puncture of the cecal wall was performed with a 20-gauge needle (Fig. 1.1b). The cecum was lightly squeezed to express a small amount of stool from the puncture site in order to assure a full thickness perforation. The cecum was returned to the abdominal cavity and the incision was closed with surgiclips (Fig. 1.1c). Group I Sham mice underwent anesthesia and midline lapratomy, the cecum was exteriorized, returned to the abdomen and the wound was closed with surgiclips. Measurement of mortality was carried out for 7 days after CLP. [13]

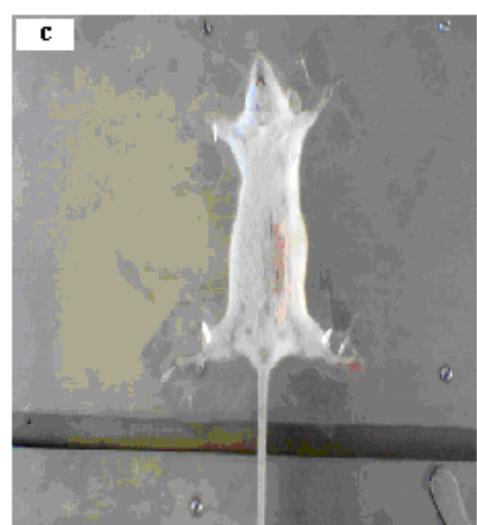
Fig.1.1a



Fig.1.1b



Fig.1.1c



Statistical analysis

The results are expressed as mean \pm S.E.M. Data was analyzed by one-way ANOVA followed by Tukey-Kramar multiple comparison test. Value of *p* less than 5% (i.e. *p*<0.05) was considered statistically significant.

Results

Cell mediated immune response- Delayed Type Hypersensitivity

In DTH, the degree of footpad oedema was measured after 24 hours. The DTH skin response requires antigen specific memory T cells and produces inflammation. The inflammation results from the production of local cytokines and chemotaxis at the site of injection, which results in the recruitment of large number of neutrophils and mononuclear cells. In group II where cyclosporine was administered on day 14th as a standard immunosuppressant has shown significant decrease ($p < 0.001$) in the mean difference, in the foot pad thickness as compared to control group. There was no significant rise in the mean difference, in the foot pad thickness in EBV administered group with normal immune status. In the immunosuppressed group, where the immunity was suppressed by administration of cyclosporine, EBV administration potentiated the DTH response in terms of significant rise ($p < 0.01$) and ($p < 0.001$) in the mean difference in foot pad thickness at doses of 250 and 500 mg/kg, respectively **Table 1**.

Table 1. Effect of ethanolic extract of stem bark of *Bauhinia variegata* on delayed type of hypersensitivity.

Group no.	Group	Footpad thickness (mean difference) (mm)
I	Control	1.015 ± 0.033
II	CsA	0.711 ± 0.027 *** a
III	EBV	0.991 ± 0.035
IV	EBV	1.016 ± 0.047
V	EBV	1.018 ± 0.042
VI	EBV + CsA	0.805 ± 0.041
VII	EBV+ CsA	0.931 ± 0.026 ** b
VIII	EBV + CsA	0.961 ± 0.034 *** b

Values are expressed as mean± S.E.M, n=6 in each group,

** $p < 0.01$ and *** $p < 0.001$

a: when compared with control (Group I).

b: when compared with Cyclosporine control (Group II).

EBV : Ethanolic extract of stem bark of *Bauhinia variegata*

CsA: Cyclosporine

Non specific immune response- Cecal ligation and puncture induced abdominal peritonitis

In cecal ligation and puncture induced abdominal peritonitis model, mortality due to CLP induced abdominal peritonitis was observed and the results were expressed as percentage survival. There was no significant survival in the EBV administered groups at the doses of 125 and 250 mg/kg, p.o. Whereas in EBV administered group at dose of 500 mg/kg, p.o. the percentage survival after 12 hrs was found to be significant ($p < 0.05$) and when observed after 24, 48, 72, and 168 hrs the percentage survival was also found significant ($p < 0.01$) as against in control CLP group. In the sham laparotomy control group, percentage survival was 100% after 168 hrs (7th day) **Table 2**.

Tabel 2. Effect of Ethanolic extract of stem bark of *Bauhinia variegata* in Cecal Ligation and Puncture induced abdominal peritonitis.

Group no.	Drug Treatment	% Survival (After 12 hr)	% Survival (After 24 hr)	% Survival (After 48 hr)	% Survival (After 72 hr)	% Survival (After 168 hr)
I	Sham laprotamy (LAP)	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00
II	Control-CLP	53.33 ± 6.66	46.66 ± 6.66	26.66 ± 6.66	6.66 ± 6.66	0.00 ± 0.00
III	EBV - CLP	53.33 ± 6.66	53.33 ± 6.66	46.66 ± 6.66	26.66 ± 6.66	6.66 ± 6.66
IV	EBV - CLP	66.66 ± 6.66	66.66 ± 6.66	53.33 ± 6.66	33.33 ± 6.66	13.33 ± 6.66
V	EBV - CLP	93.33 ± 6.66 *	93.33 ± 6.66 **	73.33 ± 6.66 **	53.33 ± 6.66 **	46.66 ± 6.66 **

Values are expressed as mean ± S.E.M. of three separate experiments in which n=5 mice were used per experiment.

*p<0.05 and ** p<0.01

EBV-CLP treated groups were compared with control-CLP.

EBV : Ethanolic extract of stem bark of *Bauhinia variegata*

CLP: cecal ligation and puncture.

Discussion

Different types of immune response fall in two categories specific or adaptive immune response and non adaptive or non specific immune response. In specific immune response there is high degree of specificity as well as the remarkable property of "memory". Typically, there is an adaptive immune response against an antigen within five or six days after the initial exposure to a particular antigen. Exposure to the same antigen some time in the future results in a memory response. The immune response to the second challenge occurs more quickly than the first, is stronger, and is often more effective in neutralizing and clearing the pathogen. Memory response generate a life-long immunity following an infection. The two key features of the adaptive immune response are thus specificity and memory. The major agents of adaptive immunity are lymphocytes, antibodies and the other molecules they produce. Because adaptive immune responses require some time to marshal, innate immunity provides the first line of defense during the critical period just after the host exposure to a pathogen. In general, most of the micro-organism encountered by a healthy individual are readily cleared within a few days by defense mechanism of innate immune system before they activate the adaptive immune system. Non specific immune response provides the first line defense against infection.

Most components of innate immunity are present before the onset of infection and constitute a set of disease resistance mechanism that are not specific to particular pathogen but that include cellular and molecular components that recognize frequently encountered pathogens. Phagocytic cells, such as macrophages and neutrophils, barriers such as skin and a variety of antimicrobial compounds synthesized by the host, all play important roles in innate immunity.

When activated T_{H1} cells encounter certain antigens like SRBC, they secrete cytokines that induce a localised inflammatory reaction called Delayed Type Hypersensitivity. DTH comprises of two phases, an initial sensitisation phase after the primary contact with SRBC antigen. During this period T_{H1} cells are activated and clonally expanded by APC with class II MHC molecule (eg.langerhans cells and macrophages are APC involved in DTH response). A subsequent exposure to the SRBC antigen induces the effector phase of the DTH response, where T_{H1} cells secrete a variety of cytokines that recruits and activates macrophages and other non specific inflammatory mediators. The delay in the onset of the response reflects the time required for the cytokines to induce the recruitment and activation of macrophages.^[14,15]

In the model of Delayed type hypersensitivity, in the groups of mice with normal immune status administered with EBV at the dose of 250 and 500 mg/kg was potentiated, when challenged with SRBC, as compared to control group but it was not found statistically significant. Cyclosporine is widely used as an immunosuppressant reference drug and also used to prevent rejection of allografts and to treat some autoimmune diseases.^[16] In the Cyclosporine induced immunosuppressed groups of mice administered with EBV in the doses of 250 and 500 mg/kg, has significantly shown a potentiating effect of delayed type hypersensitivity in terms of increase in paw oedema, when challenged with SRBC, as compared to cyclosporine treated immunosuppressed control group. Heightened Delayed type hypersensitivity reaction suggests activation of cellular immune system.

The cecal ligation and puncture induced abdominal peritonitis, which is associated with the presence of the pathogens such as *Enterococcus cloacae*, *Escherichia coli*, *Proteus mirabilis*, and *Alcaligenes faecalis*^[17], closely resembles the pathophysiology of human sepsis.^[18] After the CLP, increased adhesion of monocytes was also observed in the animals rendered sepsis.^[17] Most studies indicate that NK cells contribute positively to bacterial clearance mechanisms. Depletion of NK cells has been shown to impair bacterial clearance following CLP or challenge with other bacterial pathogens.^[19] IFN- γ and IL-12 are normally elevated in response to systemic bacterial challenge.^[20] EBV 500 mg/kg, p.o. administered group with CLP showed a significant increase in percentage survival, 46.66 % ($p < 0.01$) when compared with CLP control, suggesting that the drug can overcome the mortality due CLP. EBV probably causes the enhancement of the monocyte macrophage system and NK cell activity after CLP. The present investigation therefore reveals that stem bark of *Bauhinia variegata* Linn certainly possess immunomodulatory properties.

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