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Immunomodulatory activity of *Bauhinia Racemosa* Lam.

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RESUMEN

Objetivo: Evaluar la actividad del extracto metanólico de la corteza del tallo de la *Bauhinia Racemosa* Lam en ratones albinos suizos.

Material y métodos: La inmunidad humoral específica fue evaluada mediante el ensayo de hemaglutinación de antincuerpos (H.A. Titer) y la inmunidad no específica fue evaluada mediante el test de aclaramiento de carbono y el test de adhesión de neutrofilos.

Resultados: se encontró que el extracto del tallo de *Bauhinia racemosa* (MEBR) era efectivo para el incremento del H.A Titer. La respuesta primaria y secundaria no mostró un ascenso significativo en el H.A Titer en el grupo con estado inmune normal al compararlo con el grupo control. Sin embargo, en el grupo de inmunodeprimidos donde la inmunidad estaba suprimida mediante ciclofosfamida se observó un aumento significativo en el H.A Titer ($p<0.01$) a dosis de 200mg/kg cuando se comparaba con la ciclofosfamida. El extracto del tallo de Bauhinia racemosa mostró un aumento significativo ($p<0,05$) en la actividad fagocítica a dosis de 200mg/kg (p.o) en el test de aclaramiento de carbón. En el test de adhesión de neutrófilos el extracto del tallo de Bauhinia racemosa mostró un aumento significativo ($p<0,01$) del porcentaje de adhesión de neutrófilos a dosis de 200mg/kg (p.o)

Conclusión: El presente estudio sostiene al MEBR como un prometedor agente inmunomodulador.

PALABRAS CLAVE: *Bahunia Racemosa*, adhesión inmunomoduladora, neutrófilos, fagocitosis.

ABSTRACT

Aim: To evaluate immunomodulatory activity of methanolic extract of stem bark of *Bauhinia racemosa* Lam swiss albino mice.

Material and Methods: The specific humoral immunity was assessed by performing hemagglutinating antibody titer (H.A.Titer) and the non-specific immunity was assessed by performing carbon clearance test and neutrophil adhesion test.

Results: The methanolic extract of stem bark of *Bauhinia Racemosa* (MEBR) was found effective in increasing the H.A.Titer. Primary and secondary antibody response showed no significant rise in H.A.Titer in normal immune status group when compared with control group, whereas in immunosupressed group, where immunity was suppressed by cyclophosphamide, significant rise in H.A.Titer ($p<0.01$) was observed at dose of 200 mg/kg (p.o.) when compared with cyclophosphamide. MEBR showed significant increase ($p<0.05$) in phagocytic activity at dose of 200 mg/kg (p.o.) in carbon clearance test. In neutrophil adhesion test MEBR showed significant ($p<0.01$) rise in percentage neutrophil adhesion at dose of 200 mg/kg (p.o.).

Conclusion: Present study, therefore, reveals that MEBR) holds promise as immunomodulatory agent.

KEY WORDS: *Bauhinia Racemosa*, immunomdulatory, neutrophil adhesion, phagocytosis.

INTRODUCTION

Herbal medicines derived from plants are being increasingly utilized to treat a wide variety of diseases. There is a growing interest in the pharmacological evaluation of various plants used in Indian traditional system of medicine. The plant *Bauhinia Racemosa* Lam. belongs to the Caesalpiniaceae family. It is popularly known as *Apta* (Marathi) and occurs frequently in India, Srilanka and China. The stem bark of the plant is an astringent and is used in the treatment of headache, fever, skin diseases, tumors, diseases of the blood, dysentery and diarrhea[1]. Pharmacological studies of the plant revealed that the methanolic extract of stem bark of *Bauhinia Racemosa* (MEBR) shows analgesic, antipyretic, anti-inflammatory[2], antimicrobial[3], antioxidant, hepatoprotective[4], and antitumor activity[5]. Chemical evaluation of the stem bark of *Bauhinia Racemosa* afforded 30-norhopane, ethyl cerotate, friedelin, palmitic acid, b-sitosterol, aplysterol, taraxeran-3a-ol, naringenin, kaempferol, quercetin, n-octacosane, b-sitosterol palmitate and docosanoic acid[6].

Many plants used in traditional medicine have immunomodulating activities, some of which stimulate both humoral and cell mediated immunity while others 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[7]. During the functioning of the immune system, such as in phagocytosis, reactive oxygen species and nitrogen species are generated. If they are left unchecked they can affect the components of the immune system by inducing oxidative damage. This is more so in the elderly or during inflammation where there is excess generation of these reactive species than can be taken care of by defenses in the form of antioxidants. There are some indications of possible benefits of antioxidant supplementation. Natural compounds from medicinal plants having antioxidant have potential as therapeutic agents in this regard. Indian medicinal plants with the activities of antioxidant and immunomodulator have been identified; the few gargling examples are *Allium sativum, Azadirachta indica, Emblica officinalis, Withania somnifera.* Hence immunomodulation can go hand in hand with antioxidant[8]. Recent reports indicate that several types of flavonols stimulate human peripheral blood leukocyte proliferation. They significantly increase the activity of helper T-cell, cytokines, Interleukin 2, g-interferon, and macrophages and are thereby useful in the treatment of several diseases caused by immune dysfunction[9]. MEBR shows the presence of flavonoids, triterpenoids, steroids, tannins, and saponins[4], the class of compounds which are known to have immunostimulant properties. In the present study methanolic extract of stem bark of *Bauhinia Racemosa* (MEBR) was studied for its potential immunomodulatorty activity, driven by the presence of its antioxidant property and presence of class of compounds which are known to posses the immunostimulant properties.

MATERIAL AND METHODS

Plant material

The stem bark of *Bauhinia Racemosa* Lam. was collected from Solapur, Maharashtra, India. The plant specimen was authenticated by "Botanical Survey of India" Pune, India, (voucher specimen no.Sasp1)

Preparation of methanolic extract of stem barks of *Bauhinia Racemosa* Lam

The stem bark of *Bauhinia Racemosa* Lam. was dried in the shade and pulverized. The dried powder material of the stem bark of *Bauhinia Racemosa* was extracted with methanol (95%) by cold maceration (yield 9.25 %w/w).

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

Cyclophosphamide 100 mg/kg was used as a standard immunosuppressant.

Carbon ink suspension: Pelican AG,Germany,ink was diluted eight times with saline and used for carbon clearance test in a dose of 10µl/gm body weight of mice.

Sheep red blood cells (SRBCs) were washed three times with normal saline and adjusted to the required concentration for immunization and for challenge as antigen.

Humoral antibody response[10-12]

Animals were divided into 8 groups of six animals each. Animals in group I received orally 1ml of 5% gum acacia, for 21 days. Group II received cyclophosphamide 100mg/kg, p.o, on 9th and 16th day. Animals in group III, IV and V with normal immune status were administered MEBR at doses of 50, 100, 200 mg/kg/day, p.o, respectively for 21 days. Animals in group VI, VII and VIII received MEBR at doses of 50, 100, 200 mg/kg, p.o, respectively for 21 days

in addition to cyclophosphamide 100 mg/kg, p.o, on day 9th and 16th. On 7th and 14th day of the study, mice from all the groups were immunized and challenged respectively, with 0.1 ml of 20% sheep red blood cell's (SRBC) in normal saline intraperitoneally. Blood was withdrawn on 14th and 21st day from retro-orbital plexus under mild ether anesthesia from all animals and centrifuged to obtain serum. The antibody titer was determined using microtiter plates. Each well of a microtiter plate was filled initially with 25 µl of normal saline, 25 µl of serum was mixed with 25 µl of normal saline in the first well of microtiter plate. Twofold dilutions of serum were made till the last well of the second row of microtiter plate, so that the antibody concentration of any of the dilutions is half of the previous dilution. 25 µl of 1% SRBC were added to each well and the microtiter plates were incubated at 37°C for one hour and then observed for hemagglutination. The highest dilution giving hemagglutination was taken as the antibody titer. The antibody titers were expressed in the graded manner, the minimum dilution (1/2) being ranked as 1, and mean ranks of different groups were compared for statistical significance. Antibody titer obtained on 14th day after immunization and on 21st day after challenge with SRBCs was considered as primary and secondary humoral immune response respectively.

Carbon Clearance Test[13]

Mice were divided into 4 groups of six animals each. The control group I received orally 1ml of 5% gum acacia, while animals of treatment group II, III, and IV were administered MEBR at doses of 50, 100, 200 mg/kg/day, p.o. respectively daily for 5 days. Carbon ink suspension 10µl/gm body weight of mice was injected via tail vein to each mouse 48hrs after the 5 day treatment. Blood samples (25 µl) were then withdrawn from retro orbital plexus under mild ether anesthesia at 0 and 15 minutes after injection of colloidal carbon ink and lysed in 0.1% sodium carbonate solution (3ml). The optical density was measured spectrophotometrically at 660 nm. The phagocytic index was calculated using the following formula

$$K = \frac{(\text{In } OD_1 - \text{In } OD_2)}{(t_2 - t_1)}$$

Where OD_1 and OD_2 are the optical densities at time t_1 and t_2 respectively

Neutrophil Adhesion Test[14]

Mice were divided into 4 groups of six animals each. The control group I recived orally 1ml of 5% gum acacia, while animals of treatment groups II, III and IV were administered MEBR at doses of 50, 100, 200 mg/kg/day, p.o.respectively daily for 7 days. On the 7th day of the treatment, blood samples from all the groups were collected by puncturing retro-orbital plexus under mild ether anaesthesia. Blood was collected in vials pre-treated with disodium EDTA and analysed for total leukocyte count (TLC) and differential leukocyte count (DLC) by fixing blood smears and staining with Field stain I and Leishman's stain. After initial counts, blood samples were incubated with nylon fiber (80 mg/ml of blood sample) for 15 min at 37^0C. The incubated blood samples were again analyzed for TLC and DLC. The product of TLC and percentage neutrophil gives neutrophil index (NI) of blood sample. Percent neutrophil adhesion was calculated as follows.

$$\text{Neutrophil adhesion} = \frac{\text{NIu-NIt}}{\text{NIu}} \times 100$$

Where,

NIu:Neutrophil Index before incubation with nylon fibers.

NIt :Neutrophil Index after incubation with nylon fibers.

Statistical analysis

The results are expressed as mean ± 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

Humoral Antibody Response

Primary antibody response on day 14th in MEBR treated group with normal immune status showed no significant rise in H.A.Titer when compared with control group. A significant decrease in H.A.Titer ($p<0.01$) was observed in cyclophosphamide treated group when compared with control group. In immunosuppresed group where the immunity was suppressed by administration of cyclophosphamide on day 9th , MEBR administration produced a significant rise in H.A.Titer ($p<0.01$) at dose of 200 mg/kg when compared with cyclophosphamide treated group. Secondary antibody response on day 21st day in MEBR treated groups with normal immune status group showed no significant rise in H.A.Titer when compared with control group. A significant decrease in H.A.Titer ($p<0.001$) was seen in cyclophosphamide group II when compared with control group I. In immunosuppresed group where the immunity was suppressed by administration of cyclophosphamide on day 16th, MEBR showed Significant rise in H.A.Titer ($p<0.01$) at dose 200 mg/kg respectively when compared with cyclophosphamide group (Table 1).

Carbon Clearance Test

The phagocytic activity of reticuloendothelial system is generally measured by the rate of removal of carbon

Table 1. Effect of methanolic extract of stem bark of *Bauhinia racemosa* on primary and secondary antibody response on H.A.titer.

Group nº	Group	H.A.Titer	
		Primary	Secondary
I	Control	4.66 ± 0.33	7.5 ± 0.22
II	Cyp	3.00 ± 0.25** a	5.5 ± 0.22*** a
III	MEBR	5.33 ± 0.21	7.66 ± 0.42
IV	MEBR	5.66 ± 0.33	8.16 ± 0.16
V	MEBR	5.83 ± 0.30	8.50 ± 0.22
VI	MEBR + Cyp	3.16 ± 0.40	6.0 ± 0.25
VII	MEBR + Cyp	4.33 ± 0.21	6.83 ± 0.30
VIII	MEBR + Cyp	4.66 ± 0.21** b	7.66 ± 0.55 ** b

MEBR: Methanolic extract of stem bark of *Bauhinia Racemosa*; Cyp: Cyclophosphamide; Values are expressed as (Mean± S.E.M), n=6, **p<0.01 and ***p<0.001; a: when compared with control (Group I); b: when compared with Cyclophosphamide Control (Group II); (Statistically analyzed by one way ANOVA followed by Tukey-Kramar multiple Comparison test.)

Table 2. Effect of methanolic extract of stem bark of *Bauhinia racemosa* on phagocytic activity by carbon clearance test.

Group nº	Group	Phagocytic index
I	Control	0.011 ± 0.00096
II	MEBR	0.011 ± 0.00073
III	MEBR	0.012 ± 0.00061
IV	MEBR	0.018 ± 0.00088 *

MEBR: Methanolic extract of stem bark of *Bauhinia Racemosa*; Values are expressed as (Mean± S.E.M), n=6; * = p<0.05 ; MEBR treated groups were compared with control. (Statistically analyzed by one way ANOVA followed by Tukey-Kramar multiple Comparison test.)

Table 3. Effect of methanolic extract of stem bark of *Bauhinia racemosa* on neutrophil activation by neutrophil adhesion test.

Group nº	Group	% Neutrophil Adhesion
I	Control	30.77 ± 1.26
II	MEBR	30.89 ± 1.43
III	MEBR	32.07 ± 1.33
IV	MEBR	39.25 ± 2.15 **

MEBR: Methanolic extract of stem bark of *Bauhinia Racemosa*; Values are expressed as (Mean± S.E.M), n=6; ** = p<0.01; MEBR treated groups were compared with control.(Statistically analyzed by one way ANOVA followed by Tukey-Kramar multiple Comparison test.)

particles from blood stream, there was significant increase in phagocytic activity (p<0.05) of MEBR at dose of 200 mg/kg when compared with control (Table 2), indicating that MEBR posses macrophage stimulatory activity.

Neutrophil Adhesion Test

MEBR increased the adhesion of neutrophil to nylon fibres which co-relates to the process of margination of neutrophils in blood vessels. The neutrophil adhesion was significantly increased (p<0.01) with MEBR at dose of 200 mg/kg when compared with control (Table 3).

DISCUSSION

Immunomodulatory agents of plants and animal origin enhance the immune responsiveness of an organism against a pathogen by activating the immune system[15].

Antioxidant supplementation essentially reverses several immune deficiences, resulting in increased levels of interleukin-2, elevated numbers of total lymohocytes and T-cell subsets, enhanced mitogen responsivness, increased killer cell activity, augmented antibody response to antigen stimulation, decreased lipid peroxidation, and decreased prostaglandin synthesis[16].

When mice are sensitized with SRBC, an antigen gets diffused in the extra vascular space and enters the lymph node via the lymphatics. B cells with receptors for antigen bind and internalize it into an endosomal compartment and process and presents it on MHC class II molecules to T_{H2} cells. These B cells are triggered to proliferate, giving rise to clones of large numbers of daughter cells. Some of the cells of these expanding clones serve as memory cells, other differentiates and become plasma cells that make and secrete large quantities of specific antibody. During a primary response, IgM is secreted initially, often followed by a switch to an increasing proportion of IgG[17, 18]. The magnitude of secondary antibody response to the same antigen is amplified in terms of antibody production. In present study assesment of humoral immunity was carried out using H.A.Titer .The anti-SRBC antibody titer in MEBR treated group was raised but was not found statistically significant when compared to control group in both primary and secondary antibody titer response.

In the immunosuppressed groups cyclophosphamide was used as immunosuppressant as, it selectively suppresses humoral immunity by exerting depressive effect on antibody production, if given after antigenic stimulation[19]. This may be due to interference with helper T cell activity[20]. MEBR 200 mg/kg significantly protected cyclophosphamide induced suppression of humoral immunity, probably by counteracting suppression of both primary and secondary humoral response.

The increase in carbon clearance index reflects the enhancement of phagocytic function of mononuclear macrophage and non–specific immunity. MEBR significantly increased the phagocytic activity when compared to control.

MEBR significantly evoked increase in adhesion of neutrophils to nylon fibre which co-relate to the process of margination of cells in blood vessels. The neutrophil adhesion was significantly increased by MEBR when compared to control.

The present investigation therefore reveals that stem bark of *Bauhinia Racemosa* Lam certainly possess immunomodulatory properties.

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