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Immunomodulatory Potential of Ethanolic Extract of Stem Bark of *Balanites roxburghii* Planch

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Abstract: This work have done to investigate the immunomodulatory property of ethanolic extract of stem bark of *Balanites roxburghii* Planch (Family; Simarubaceae). The extract was given at the doses of 50, 100 and 200 mg/kg p.o. and Septilin (Himalaya, India) was used as a standard drug at a dose of 500 mg/kg, p.o. for 14 days to wister albino rats. The assessment of immunomodulatory activity were carried out by carbon clearance test for phagocytic index, haemagglutination antibody titre for humoral immune response and delayed type of hypersensitivity for cell-mediated immune response. The results of present study clearly indicates that the rate of elimination of carbon particles is more in ethanolic extract of stem bark of *Balanites roxburghii* treated group in dose dependent manner when compared with control group and standard drug. The augmentation of the humoral immune response to Sheep Red Blood Cells (SRBCs) by ethanolic extract is evidenced by significant ($P<0.05$) enhancement of antibody titres in the blood in dose dependent manner when compare with control group. In the cell-mediated immunity; the extract showed increase in DTH reaction in rats. This study clearly indicates the immunomodulatory activity of *Balanites roxburghii* in dose dependent manner when compared with standard drug and control group. Thus, the immunostimulatory effect produced by ethanolic extract of *Balanites roxburghii* may be due to cell mediated immune response by T lymphocytes and humoral antibody mediated response by B lympocytes. It can therefore be concluded that the ethanolic extract of *Balanites roxburghii* is potent immunostimulant and can be used as a complimentary therapeutic agent.

Key words: *Balanites roxburghii* · Immunomodulatory activity · Phagocytic index · DTH

INTRODUCTION

Herbal drugs are known to possess immunomodulatory properties and generally act by stimulating both specific and non-specific immunity [1]. Many plants used in traditional medicine are reported to have immunomodulating activities. Some of these 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 [2].

Balanites roxburghii Planch. (Simarubaceae) locally known as Hingota, is one of the most common but neglected wild plant species of the dry land areas of India. Traditionally it is used as emetic, anthelmintic, anti-fungal, purgative, cathartic, colic, in whooping cough, skin diseases and dog bite. According to Ayurveda, bark is anthelmintic, spasmolytic, used in cough and skin diseases. Leaf is anthelmintic whereas root is emetic. Fruits are used in treatment of whooping cough and in skin diseases. The paste of bark is prepared and applied externally on the affected part of the body [3]. The whole plant is used in treatment of snake-bite. Seeds are used as expectorant (given in the treatment of cough) and colic [4]. Kernel is used in skin diseases and burns [5]. Roots and fruits contain 0.2-2.2 % and 0.3-3.8 % diosgenin (used in contraceptives), respectively. The steroids (sapogenin) are employed in the synthesis of drug including sex hormones and oral contraceptives. In case of pain and swelling, the bark of plants is used by traditional healers. The plant *Balanites roxburghii* having antifertility efficacy [6] and anti-

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inflammatory activity [7]. *Balanites roxburghii* which contains steroidal saponins has spermicidal, cardiovascular, molluscidal properties [8]. *Balanites roxburghii* pericarp extract show contraceptive efficacy in male mice [9]. Stem bark of *Balanites roxburghii* showed antimicrobial activity and anti-asthmatic activity [10, 11].

Exhaustive literature survey indicated that systematic pharmacological work has not been done so far on this plant. Hence, this plant was selected to find its immunomodulatory activity.

MATRIALS AND METHODS

Animal: Wister albino rats (120-150 gm) of either sex were used. The animals housed under standard laboratory conditions maintained at 25±1°C and under 12 / 12 h light/ dark cycle and fed with standard pellet diet (Gold Mohur brand, Lipton India Ltd.) and water ad libitum. All experimental procedures were followed in strict accordance with the guideline prescribed by the Committee for the Purpose of Control and Supervision on Experimental on Animals (CPCSEA) and the protocol was approved by the Institutional Animal Ethical Committee (Registration no. 1030/a/07/CPCSEA). Albino rats were divided into five groups viz. Group A, received 0.5 ml of 2 % w/v sodium carboxy methyl cellulose suspension p.o. for 14 days as a control group, Group B, received 500 mg/kg, p.o. of Septilin 14 days, Group C, received 50 mg/kg, p.o. of ethanolic extract of *Balanites roxburghii* (EEBR-I) for 14 days, Group D, received 100 mg/kg, p.o. of ethanolic extract of *Balanites roxburghii* (EEBR-II) for 14 days and group E, received 200 mg/kg, p.o. of ethanolic extract of *Balanites roxburghii* (EEBR-III) for 14 days.

Determination of Phagocytic Index: All groups were administered with 0.2 ml/animal of carbon suspension (Pelikan Tuschea Ink, Germany) intravenously, through tail vein on seventh day. Blood samples were collected from retro-orbital plexuses immediately before and 5, 10, 15 and 20 min after the injection of carbon suspension. An aliquot of each and 25 µl of blood sample lysed with 2 ml of 0.1 % acetic acid and absorbance was observed at 675 nm. The graph were plotted between absorbance against time for each animal and its respective test groups. The phagocytic index was calculated by the slope of time concentration curve [12].

Determination of Humoral Immune Response: The animals were immunised with 0.1 ml of 1×10^8 SRBC, intraperitoneally on day 0. Blood samples were collected from individual animals from the retro-orbital plexuses on day 7. Antibody levels were determined by the haemagglutination technique [13]. Two-fold dilutions sera in saline (0.025 ml) were mixed with 0.025 ml of 0.1% v/v SRBC suspension in 96 well microtitre plates. The plates were incubated at 37±1°C for 1 h and then inspected for haemagglutination. The highest dilution giving rise to macroscopic haemagglutination was taken as antibody titre. Antibody titres were expressed in a graded manner, the minimum dilution (1/2) being ranked as 1 and the mean ranks of different groups were compared for statistical significance.

Determination of Cell Mediated Immune Response: The animals were immunized by injecting 0.1 ml of SRBC suspension containing 1×10^8 cells, intraperitoneally, on day 0 and challenged on day 7 with 0.05 ml of 2×10^8 SRBC in the right hind foot pad. The contra lateral paw received an equal volume of saline. The footpad thickness was measured at 0, 24 and 48 h after challenge using Mitutoyo Dail Caliper (Mitutoyo Manufacturing Company, Japan). The difference in the thickness of the right hind paw and left hind paw was used as a measure of DTH reaction [14].

Statistical Analysis: Values expressed are mean ± SEM (Standard error of mean); using Student's t-test. $P < 0.05$ were considered as significant.

RESULTS AND DISCUSSION

In the present study, phagocytic index were determined by carbon clearance method. When the carbon suspension is injected intravenously, the rate of clearance of carbon from blood by macrophage is governed by an exponential equation. This seems to be the general way in which inert particulate matter is cleared from the blood. The results of present investigation clearly indicates that the rate of elimination of carbon particles is more in extract of stem bark of *Balanites roxburghii* treated group in dose dependent manner, when compared with control group and standard drug (Table 1). The haemagglutination antibody titre was used to assess humoral immune response. At the selected dose both primary and secondary antibody titre was observed in rats treated with ethanolic extract of *Balanites roxburghii.* The augmentation of the humoral immune response to SRBCs by ethanolic extract is evidenced by increase in the antibody titres in the blood of rats (Table 1). The results of this experiment demonstrated that *Balanites roxburghii* extract administration significantly

Table 1: Effects of ethanolic extract of *Balanites roxburghii* on phagocytic index, haemagglutination antibody titre and delayed type hypersensitivity

		Haemagglutination Antibody Titre		Delayed Type Hypersensitivity (footpad thickness)	
Treatment	Phagocytic Index	Primary	Secondary	24 h	48 h
Control	-	7.02±0.21	10.38±0.13	22.39±0.10	15.08±0.02
Septilin	0.667	18.35±0.11^{c}	25.01±0.35^{c}	20.44±0.44^{c}	4.38±0.30^{c}
EEBR-I	0.570	11.10±0.24^{b}	16.33±0.15^{a}	26.01±0.02^{b}	11.08±0.24^{c}
EEBR-II	0.613	14.75±0.12^{c}	20.10±0.11^{c}	24.09±0.13^{c}	8.18±0.11^{c}
EEBR-III	0.691	17.55±0.21^{c}	24.86±0.22^{c}	21.21±0.24^{c}	5.48±0.14^{c}

Values are expressed as the mean ± SEM; (n = 6). Statistics significant vs Control, $^{a}P<0.05$, $^{b}P<0.01$ and $^{c}P<0.001$

rise the primary and secondary antibody levels in dose dependent manner. Antibody molecules, a product of B lymphocytes and plasma cells, are central to humoral immune responses; IgG and IgM are the major immunoglobulins which are involved in the complement activation, opsonization, neutralization of toxins, etc.

In the present investigation, SRBC-induced delayed-type hypersensitivity was used to assess the effect of the fraction on cell-mediated immunity. Cell-mediated immunity (CMI) involves effector mechanisms carried out by T lymphocytes and their products (lymphokines). CMI responses are critical to defence against infectious organisms, infection of foreign grafts, tumor immunity and delayed-type hypersensitivity reactions. Therefore, increase in DTH reaction in rats response to T cell dependent antigen revealed the stimulatory effect of ethanolic extract of *Balanites roxburghii* on T cells (Table 1). This study clearly indicates the immunoprotective activity of *Balanites roxburghii* in dose dependent manner when compared with standard drug and control group. The immunostimulatory effect produced by ethanolic extract of *Balanites roxburghii* may be due to cell mediated and humoral antibody mediated activation of T and B cells. It can therefore be concluded that the ethanolic extract of *Balanites roxburghii* is a potent immunostimulant and can be used as a complimentary therapeutic agent.

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