

IMMUNOMODULATORY ACTIVITY OF *BALANITE ROXBURGHII*

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ABSTRACT:

Besides having traditional use as an immunomodulatory action, it is not well scientifically establish. Hence present study was undertaken to screen the immunomodulatory activity of ethanolic extract of *Balanite roxburghii* leaves. Traditionally, leaves of *Balanite roxburghii* are claimed to possess immunomodulatory activity and hence the reason behind evaluating the immunomodulatory activity of ethanolic extract of *Balanite roxburghii* (EBR) leaves. The animals were distributed into three groups consisting of six animals each. The first group served as control, the second and third group received low dose and high dose of ethanolic

extract of *Balanite roxburghii* (EBR) at 200 mg/kg, p.o. and 400 mg/kg, p.o. respectively. The immunomodulatory activity of EBR was evaluated in Carbon clearance test, Effect on serum immunoglobulin, Cyclophosphamide induced neutropenia. In all these paradigms 400mg/kg dose of EBR was more effective than its 200 mg/kg of dose. These results suggest that *Balanite roxburghii* can be use as immunomodulator.

Keywords: Immunomodulator, *Balanite roxburghii*, Carbon clearance test, serum immunoglobulin, neutropenia.

1. INTRODUCTION:

Natural products of plant and animal origin offer vast resources of newer medicinal agents with potential in clinical use [1]. Some of these are believed to promote positive health and maintain organic resistance against infection by re-establishing the body's equilibrium and conditioning the body tissue [2]. The historic use of herbal medicines to treat and prevent infectious disease has been supplanted with the emergence of specific synthetic drugs and antimicrobial agents. However, the use of plant remedies, known to possess natural antioxidant, immunomodulatory and other activities, has increased in the last decade in human and animal medicine, as it is perceived as a natural approach to treat disease. Intensive farming system rely heavily on the use of pharmaceuticals, but there is increasing public concern regarding their use, mostly for the emergence of drug resistance [3], the associated risk of developing antibiotic resistance in human pathogens [4] and contamination in the food chain [5]. *Balanite roxburghii* is native of Africa [6]. Most part of the plant is considered to posses various medicinal properties. Traditionally, leaves of *Balanite roxburghii* are claimed to possess immunomodulatory activity [7] and hence the reason behind evaluating the immunomodulatory activity of ethanolic extract of *Balanite roxburghii* leaves.

2. OBJECTIVE:

Besides having traditional use as an immunomodulatory action, it is not well scientifically establish. Hence present study was undertaken to screen the immunomodulatory activity of ethanolic extract of *Balanite roxburghii* lea

3. MATERIALS AND METHODS:**3.1 Experimental animals:**

Albino wistar rats weighing between 180–220 g and Swiss albino mice weighing between 25–35 g were used. Institutional Animal Ethics Committee approved the experimental protocol. Animals were maintained under standard conditions in an animal house approved by the Committee for Purpose of Control and Supervision on Experiments on Animals (CPCSEA). The animals were given pellet food and water *ad libitum*.

3.2 Materials:

3.2.1 Chemicals : Ethanol, anhydrous sodium sulphate, 2% carboxy methyl cellulose, Indian ink, 0.1% sodium carbonate, zinc sulphate, standard barium sulphate (BaSO₄) solution, cyclophosphamide, etc.

3.2.2 Equipments: Soxhlet apparatus, UV-Visible Spectrophotometer, micro-centrifuge, pH meter, TLC.

3.3 Treatment:

The animals were distributed into three groups consisting of six animals each. The first group served as control, the second and third group received low dose and high dose of ethanolic extract of *Balanite roxburghii* (EBR) at 200 mg/kg, p.o. and 400 mg/kg, p.o. respectively.

3.4 Preparation of extract:

The leaves were dried in shade at room temperature and coarsely powdered. The ethanolic extract was prepared using ethanol by soxhlet method at temperature of 40–60° c. The extract was dried over anhydrous sodium sulphate. The ethanolic extract yielded semisolid, viscous, dark coloured mass. The extract was administered in the form of suspension by using 2% carboxy methyl cellulose as a suspending agent.

3.5 Phytochemical screening of extract of leaves of *Balanite roxburghi* :

Quantitative chemical tests were conducted for the extract of leaves of *Balanite roxburghi* to identify the various phytoconstituents like alkaloid, carbohydrates, tannins etc [8].

3.6 Determination of Acute oral toxicity:

Three female mice were randomly selected and marked for individual identification. Animals were fasted 24 h prior to dosing of ethanolic extract of leaves of *Balanite roxburghi* were administered in a single dose orally. Toxicity study was carried out using a starting dose of 5000 mg/kg body weight. Animals were observed individually after dosing at least once during the first 30 min. periodically during the first 24 h, with special attention given during first 4 h, and subsequently daily for 14 days. The observation comprised the behavior and according to the Guidelines of the Organization for Economic Cooperation and Development (OECD 1998).

3.7 Evaluation of Immunomodulatory activity:

3.7.1 Carbon clearance test [9,10] :

The three groups of Swiss albino mice were administered drug or vehicle for 5 days orally. After 48 h of the last dose of the drug, mice were injected with 0.1 ml of Indian ink via the tail vein. Blood samples were withdrawn at 0 min and 15 min. A 50 µl blood sample was mixed with 4 ml of 0.1% sodium carbonate solution and the absorbance of this solution was determined at 660 nm. The phagocytic index K was calculated using the following equation:

$$K = (\text{Loge } OD_1 - \text{Loge } OD_2) / 15$$

Where OD_1 and OD_2 are the optical densities at 0 and 15 min respectively.

3.7.2 Effect on serum immunoglobulins [11, 12] :

Albino rats were treated with the drug or vehicle orally for 21 days. Six hours after the last dose, blood samples were collected and the serum was separated by centrifugation, the collected serum was used for estimation of immunoglobulin levels. Briefly, for each serum sample to be analyzed, a control tube containing 6 ml of distilled water and a test tube containing 6 ml of zinc sulphate solution were prepared. To each, 0.1 ml of serum was added from a pipette. They were inverted to enable complete mixing of the reagents and left to stand for 1 h at room temperature in plugged tubes. The pH of the solution was monitored throughout the experimental period using pH meter. The first tube served as blank and the second tube was taken as sample. The turbidity developed was measured. The turbidity obtained

(sample-blank) was compared with that obtained with standard barium sulphate ($BaSO_4$) solution. The turbidity obtained with this solution was expressed as zinc sulphate turbidity (ZST) units.

3.7.3 Cyclophosphamide induced neutropenia [13, 14] :

Swiss albino mice received the drug or vehicle orally for 10 days. On 10th day, a neutropenic dose of cyclophosphamide (200 mg/kg, s.c.) was administered and this day was labeled as day zero. Blood samples were collected through retro-orbital vein. The total leucocyte count (TLC) and % reduction in neutrophil count were performed prior to and on day 3 after injection of cyclophosphamide. The TLC and % reduction in neutrophil count in treated groups were compared with the values of the control group.

4. STATISTICAL ANALYSIS :

The Statistical analysis was performed by using One Way ANOVA followed by Dunnett's comparison test. The values are expressed as mean \pm SEM and the $P < 0.05$ was taken as significant.

5. RESULTS :

5.1 Phytochemical screening :

The ethanolic extract of *Balanite roxburghi* showed the presence of alkaloids, glycosides, saponin, flavanoids, steroids, and phenolic compounds.

5.2 Acute oral Toxicity :

It was observed that ethanolic extract of the *Balanite roxburghi* leaves was not lethal even at the dose of 2000mg/kg body weight. Hence 200mg/kg and 400 mg/kg were fixed as dosage.

5.3 Carbon clearance assay :

Administration of EBR at doses 200mg/kg and 400 mg/kg produced increase in clearance of carbon particles from blood as indicated by a significant increase in phagocytic index ($P < 0.01$) as shown in table 1.

5.4 Serum immunoglobulin levels :

The administration of EBR at doses 200 mg/kg ($P < 0.05$) and 400 mg/kg ($P < 0.01$) significantly increased the serum immunoglobulin levels when compared to control as shown in table 1.

5.5 Cyclophosphamide induced neutropenia :

Administration of Cyclophosphamide (200 mg/kg, s.c.) produced a decrease in neutrophil count in all the groups. However, the reduction in neutrophil count was less in EBR 400 mg/kg treated groups

compared to control. The EBR 400 mg/kg administration produced a 46.06% reduction in TLC and 13.19% reduction in neutrophil count and EBR 200 mg/kg administration produced a 52.25%

reduction in TLC and 30.96% reduction in neutrophil count and compared to 46.03% reduction in neutrophil count and 56.91% reduction in TLC in control, as shown in table 2.

Table1. Effect of EBR on Phagocytic index and immunoglobulin level.

Treatment	Phagocytic index	Serum immunoglobulin
Control	0.0079 ± 0.000294	17.74± 0.2394
EBR (200 mg/kg)	0.06435± 0.001064**	23.2± 0.3866*
EBR (400 mg/kg)	0.0347 ± 0.00118**	27.19± 0.4193**

Values are expressed as mean ± S.E.M., n=6, EBR 200 mg/kg, EBR 400 mg/kg, *p<0.05, **p<0.01, as compared with control using one way ANNOVA followed by Dunnet test.

Table 2. Effect of EBR on cyclophosphamide induced neutropenia.

Treatment	TLC		Reduction in cell no	% reduction	% neutrophil		Neutrophil reduction	% reduction
	Before	After			Before	After		
	Control	5636± 34.19			2428± 110.90	3208±11		
EBR (200 mg/kg)	5487± 56.04	2620± 70.03	2867±84.23	52.25*	12.66±0.13	8.74± 0.071	3.92±0.16	30.96*
EBR (400 mg/kg)	5369± 37.22	2896± 29.26	2473±21.70	46.06**	10.89±0.32	9.37± 0.065	1.522±0.31	13.97**

Values are expressed as mean ± S.E.M., n=6, EBR 200 mg/kg, EBR 400 mg/kg, *p<0.05, **p<0.01, as compared with control using one way ANNOVA followed by Dunnet test

6. DISCUSSIONS :

The carbon clearance test was carried out to evaluate the effect of drugs on the reticulo- endothelial system (RES). This is a diffuse system comprising of phagocytic cells, comprising of fixed tissue

macrophages and mobile macrophages. The phagocytic cells in this system comprise the mononuclear phagocyte system (MPS), and the macrophage is the major differentiated cell in the MPS. Cells of the RES and MPS are known to be important in the clearance

of particles from the bloodstream. When colloidal ink containing carbon particles are injected directly into the systemic circulation, the rate of clearance of carbon from the blood by macrophage is governed by an exponential equation [9, 10]. *Balanite roxburghi* at both doses showed significant increase in the phagocytic index. Hence, these agents may stimulate the reticulo endothelial system.

The estimation of serum immunoglobulin level is a direct measure to detect the humoral immunity. Serum immunoglobulin refers to a group of serum molecules produced by B-lymphocytes, they are soluble and secreted form of B-cell receptors and are produced to a maximum level to counter the invasion by an antigen, hence, they are also called as antibodies. Blood contains three types of globulins-alpha, beta and gamma, based on their electro phoretic migration rate. In the present study, estimation of serum immunoglobulins was carried out using zinc sulphate turbidity test (ZST). This test determines the amount of immunoglobulins present in the serum. A small amount of serum was added to a zinc sulphate solution and allowed to incubate at room temperature for 1 h. Zinc sulphate causes precipitation of the immunoglobulins, which makes the solution cloudy instead of clear. This test is fairly specific for immunoglobulins, but does not do a very good job of quantitating them and it is difficult to distinguish a borderline problem. However, this test is relatively quick and inexpensive test [11]. Its drawbacks include the dependence of results on a number of factors, such as time, temperature, and particularly pH of the reaction mixture. A serious drawback is the dependence of the final turbidity on pH of the zinc sulphate solution. Prolonged storage or even a short exposure to atmospheric carbon dioxide considerably changes pH and affects the result of the reaction. The

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possible ways of overcoming this problem are buffering or the use of pH indicators [12]. The turbidity was expressed as ZST units, which in turn indicate the amount of immunoglobulin present in the sample. *Balanite roxburghi* at a low dose showed a significant increase in the serum immunoglobulin level.

Cyclophosphamide induces myelo suppression in the experimental animals. It belongs to nitrogen mustard subclass of alkylating agents and acts as an immunosuppressive agent by causing alkylation of DNA, in turn by interfering in DNA synthesis and function. It is also used extensively as immunosuppressant [13]. *Balanite roxburghi* at a 400mg/kg of dose caused a 46.06% reduction in the cyclophosphamide induced neutropenia suggesting that it may have an effect on the haemopoetic system. The prevention of neutropenia induced by cyclophosphamide may be through activation of macrophages, which secrete a large number of substances including colony stimulating factor and interleukin 1 [13, 14].

7. CONCLUSION :

From the given data we conclude that ethanolic extract of *Balanite roxburghi* have immunomodulator activity this might be due to presence of alkaloids, glycosides, saponin, flavanoid, steroids, and phenolic compounds in its ethanolic extract.

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