

ANTIASTHMATIC AND ANTIALLERGIC ACTIVITY OF *BALANITES AEGYPTIACA* (DELILE) BALANITACEAE

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Summary

The present study was designed to evaluate the antiasthmatic and antiallergic activity of n- butanol fraction (NBF) of *Balanites aegyptiaca* (50, 100 and 200 mg/kg) on both *in vivo* and *in vitro* animal models. In milk induced eosinophilia model, treatment with NBF of *Balanites aegyptiaca* at 200 mg/kg, p.o. in mice significantly decreased blood eosinophilia ($P < 0.01$) as compared to sensitized control. In Clonidine induced mast cell degranulation, NBF of *Balanites aegyptiaca* at 200 mg/ml, p.o. showed about 85 % of intact mast cells as compared to toxicant (about 20 %). NBF of *Balanites aegyptiaca* reduced the paw edema significantly in dose dependent manner in passive paw anaphylaxis model. Thus, NBF of *Balanites aegyptiaca* showed antieosinophilic, mast cell stabilizing and antiallergic activity and hence possesses potential role in the treatment of asthma.

Keywords: *Balanites aegyptiaca*, Antiasthmatic activity, Antiallergic activity, Eosinophil, Mast cell degranulation.

Introduction

Over the last 50 yrs. the prevalence of asthma and allied allergic disorders have progressively increased on a worldwide scale, in both developed and developing countries. Whatever the underlying causes for these rising trends, asthma has now become a public health issue [1].

Large number of drugs belonging to α_2 agonist, corticosteroids, mast cell stabilizers, methylxanthins, leukotriene antagonists and others are in use for treating asthma. However none of them seems to be an ideal drug. The search for new drug is still the need of the day. There is high prevalence of usage of alternative traditional system of medicines for the treatment of asthma. Ayurveda offers a unique insight into comprehensive approach to asthma management through proper care of the respiratory tract. More than 400 medicinal plant species have been used ethnopharmacologically and traditionally to treat the symptoms of asthmatic and allergic disorders worldwide. The world health organization (WHO) has recognized herbal medicine as an essential building block for primary health care of vast countries like India and China. Herbal medicines are a treasure house of the information, from which we may derive leads to fill many blank spots in the modern medicine [2].

Balanites aegyptiaca (Delile) is the plant which belongs to the family *Balanitaceae*, (commonly known as Desert date) is a genus of small, thorny shrubs or trees, distributed from Africa to Burma. It is also found in Rajasthan, Gujarat, Bengal, Maharashtra and drier parts of India. The plant contains Diosgenin, Balanitisin 4, 5, 6 and 7, Steroidal saponins, Deltoin, Protodeltoin, Alkaloids, Balanitoside, Cryptogenin, Balanitisin -3 & 6-methyl Diosgenin, Flavonol glycoside, Isorhamnetin-3-O-robinobioside, Isorhamnetin-3-O-rutinoside, (25R and S)-spi-rost-5-en-3 β -ol, Bergapetin, (+)-marmesin [3]. *Balanites aegyptiaca* seems to be a promising plant for treatment of bronchial asthma because of its reported anti-inflammatory activity [4]. Earlier studies from our own laboratory have shown the effectiveness of methanol extract in animal models of bronchial asthma. Traditionally plant used in cough [3]. But no scientific studies have been carried out to reveal and corroborate the anti-asthmatic properties of *Balanites aegyptiaca*.

Present investigation was undertaken to evaluate the anti-asthmatic and antiallergic activity of NBF of *Balanites aegyptiaca* and to try to understand its mechanism of action. In the present study, effect of NBF of *Balanites aegyptiaca* was studied on both *in vivo* and *in vitro* methods for evaluation of anti-asthmatic and antiallergic activity.

Methods

Plant material: The fruits of the plant were collected from local area of shirpur situated in Dhule district of Maharashtra (India), in month of May 2009. The plant was identified and authenticated by Dr. D. A. Patil, Department of Botany, Late Karmveer Dr. P. P. Ghogrey Science College, Dhule, Maharashtra (India).

Preparation of plant extract and fraction: Fruits of the plant were shade dried; the mesocarp (pulp) was scraped. The scraped mesocarp was freeze-dried and then powdered. The powdered material (200 g) was macerated overnight with methanol with the help of mechanical shaker at room temperature. The maceration was repeated twice. The filtered extracts were combined and evaporated under reduced pressure (yield 48.28 %w/w). The extract was subjected to fractionation with n-butanol using separating funnel, filter and solvent was concentrated using vacuum evaporator under controlled temperature below 50° C. The Phytochemical investigation was done according to method described by Khandelwal [5].

Preparation of the drug: The fraction was weighted (200 mg) and dissolved in 1 ml of distilled water to prepare required concentration (50, 100, 200 mg/kg) and administered by gavage. Fraction was stored at 2-4⁰ C, protected from direct sunlight until use.

Chemicals: Yellow eosin and Toluidine blue were purchased from Loba chemicals, India and Horse serum from Hi-media Laboratories limited, India. Clonidine was purchased from Unichem Laboratories, India.

Animals: Male albino mice Swiss strain (22- 25 gm) and Albino wistar rats (200-250 gm) obtained from Veterinary College, Mhow (M.P.) India. All animals were housed at ambient temperature (22 ± 1°C), relative humidity (55 ± 5%) and 12/12 h light/dark cycle. Animals had free access to standard pallet diet and water *ad libitum*. The protocol of the experiment was approved by the Institutional Animal Ethical Committee of R.C.P.I.P.E.R, Shirpur, India (Resolution no: RCPIPER/IAEC/2009-10/16), as per the guidance of the Committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India.

Milk induced eosinophilia: A blood eosinophilia is hallmark of both allergic and non allergic asthma. Mice were divided into six groups, five animals each. Blood samples were collected from retro-orbital plexus under light ether anaesthesia, the eosinophil count was done in each group before drug administration and 24 h after the milk injection (boiled and cooled, 4 ml/kg, s.c.). [6]. Difference in the eosinophil count before and 24 h after milk administration was noted using modified method described by Godkar [7].

Mast cell degranulation by Clonidine: Mesenteric specimens from freshly sacrificed albino rats were dissected out and cut into small bits of 1 cm each, washed with normal saline and incubated in Tyrode solution (1mL), then tissue was incubated in distilled water for 10 min, served as control and likewise the tissues were incubated in suspension of NBF of *Balanites aegyptiaca* in distilled water at

various dose level like 50, 100 and 200 mg/ml for 10 min. A mast cell degranulator, Clonidine 1 mL of 50 µg / mL was added to the above solution and the incubation continued for another 10 min. Clonidine (50 µg / mL) alone served as positive control. After the incubation was over, the mesenteric bits were removed, washed with Tyrode solution and spread over glass slides. Mast cells were stained by the 1% toluidine blue 1% Toluidine blue and examined microscopically for the number of intact and degranulated mast cells [8, 9].

Passive paw anaphylaxis: The rats were sensitized by injecting subcutaneously 0.5 ml of horse serum along with 0.5 mL of triple antigen containing 200,000 million *Bordetella pertussis* organisms (Serum institute, Pune, India), fourteen day after the first immunization, blood was collected from retro-orbital plexus under light ether anesthesia and serum was separated [10].

Rats were randomly divided into six groups each containing six animals. Group I served as control group. Group II served as sensitized control and received distilled water. Group III, IV and V received NBF of *Balanites aegyptiaca* at 50, 100 and 200 mg/kg respectively and Group VI received reference drug Prednisolone 10 mg/kg. Two hours after the drug administration, rats were passively sensitized with 0.1 ml of undiluted serum of the actively sensitized rats was injected sub planter (in left hind paw). 24 hours after sensitization rats were challenged in the left hind paw with 0.1 ml of horse serum. The hind paw volume is measured by volume displacement method using plethysmometer (Ugo Basile, 21025, Comerio-Varese-Italy) before the injection of the horse serum (time 0) and 1, 2, 3, 4, 5, 6, 12 and 24 hours after the challenge [11, 12].

Statistical analysis

The results of various studies were expressed as mean ± SEM and analysed statistically using one-way ANOVA followed by followed by Bonferroni's multiple comparison post-hoc test or Dunnett's multiple comparison post-hoc test or Tukey-Kramer multiple comparison post-hoc test to find out the level of significance. P < 0.05 was considered statistically significant. The analysis was performed using Graphpad Prism software package (version 4.0).

Results

The result of preliminary phytochemical investigation of NBF of *Balanites aegyptiaca* showed the presence of steroids, saponins and phenolic compound. The yield of NBF of *Balanites aegyptiaca* was found to be 18.87 %w/w.

Effect of NBF of *Balanites aegyptiaca* on milk induced eosinophilia

Mice treated with milk (boiled and cooled, 4 ml/kg, s.c.) showed a significant increase in total eosinophil count in sensitized control group (287 ± 8.9 cells/cumm). Mice treated with NBF of *Balanites aegyptiaca* at 50 mg/kg ($P < 0.01$), 100 mg/kg ($P < 0.01$) and 200 mg/kg ($P < 0.01$) showed significant dose dependent decrease in eosinophil cell count while standard drug Dexamethasone 1mg/kg ($P < 0.01$) showed significant decreased in eosinophil cell count (Figure 1).

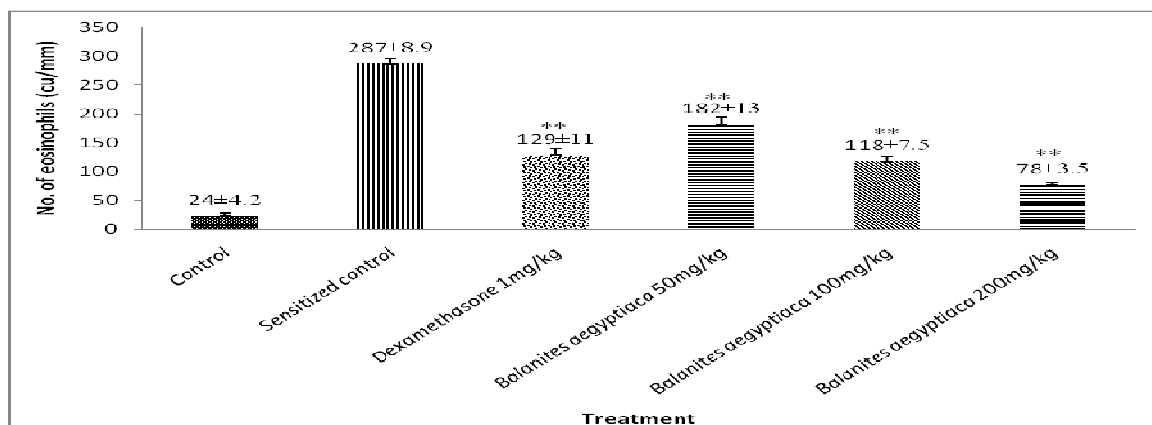


Figure 1: Effect of NBF of *Balanites aegyptiaca* on milk induced eosinophilia

Values are expressed as mean \pm SEM, n=5.

The statistical significance was determined using One-way ANOVA followed by Dunnett's multiple comparison post-hoc test * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ as compared with sensitized control.

Effect of NBF of *Balanites aegyptiaca* on Clonidine induced mast cell degranulation

Clonidine (50 μ g/ml) challenge resulted in significant degranulation of mast cell. Pretreatment of sensitized animal with NBF of *Balanites aegyptiaca* at 50 mg/kg ($P < 0.001$), 100 mg/kg ($P < 0.001$) and 200 mg/kg ($P < 0.001$) showed significant dose dependent decreased in percentage of degranulation of mast cells (Table 1).

Effect of NBF of *Balanites aegyptiaca* on passive paw anaphylaxis

In Passive paw anaphylaxis model, treatment with NBF of *Balanites aegyptiaca* and reference drug Prednisolone suppressed paw edema in a time and dose-dependent manner (Figure 2).

	Mast cells %	
	Intact	Disrupt
Control	94±1.5***	6.5±1.5***
Toxicant (Clonidine treated) 50µg/ml	19±1.5	82±1.5
<i>B.aegyptiaca</i> 125 mg/ml	82±1***	18±1***
<i>B.aegyptiaca</i> 250 mg/ml	88±0***	12±0***
<i>B.aegyptiaca</i> 500 mg/ml	94±2***	06±2***
B.E 50 mg/ml + Clonidine	66±2***	34±2***
B.E 100 mg/ml + Clonidine	77±1.5***	24±1.5***
B.E 200 mg/ml + Clonidine	84±2***	16±2***
One-way ANOVA		
P	P < 0.0001	P < 0.0001
F	250	250
Degree of freedom (df)	15	15
R squared	1.0	1.0

Table 1: Effect of NBF of *Balanites aegyptiaca* on Clonidine induced mast cell degranulation

Values are expressed as mean ± SEM, n=5.

The statistical significance was determined using One-way ANOVA followed by Bonferroni's multiple comparison post-hoc test *p<0.05, **p<0.01, ***p<0.001 as compared with Clonidine treated group.

Discussion

Animal studies showed that bovine γ and β -lacto-globulin (LG) a major cow's milk allergen, which was important precipitants of asthma attacks, activate airway eosinophils, leading to a chain of events similar to that seen after antigen challenge [13]. A blood eosinophilia is hallmark of both allergic and non-allergic asthma. Activated eosinophil cells cause desquamation and damage to respiratory epithelial cells. The eosinophil count increases in body fluids and tissues, emphasis is placed on the number of eosinophils in blood. Eosinophilia is associated with respiratory disorder, often allergic in nature together with pulmonary infiltrates that are detectable on chest films [6]. Observations of the present study indicate that NBF of *Balanites aegyptiaca* significantly reduced milk-induced eosinophilia as compared with sensitized control group.

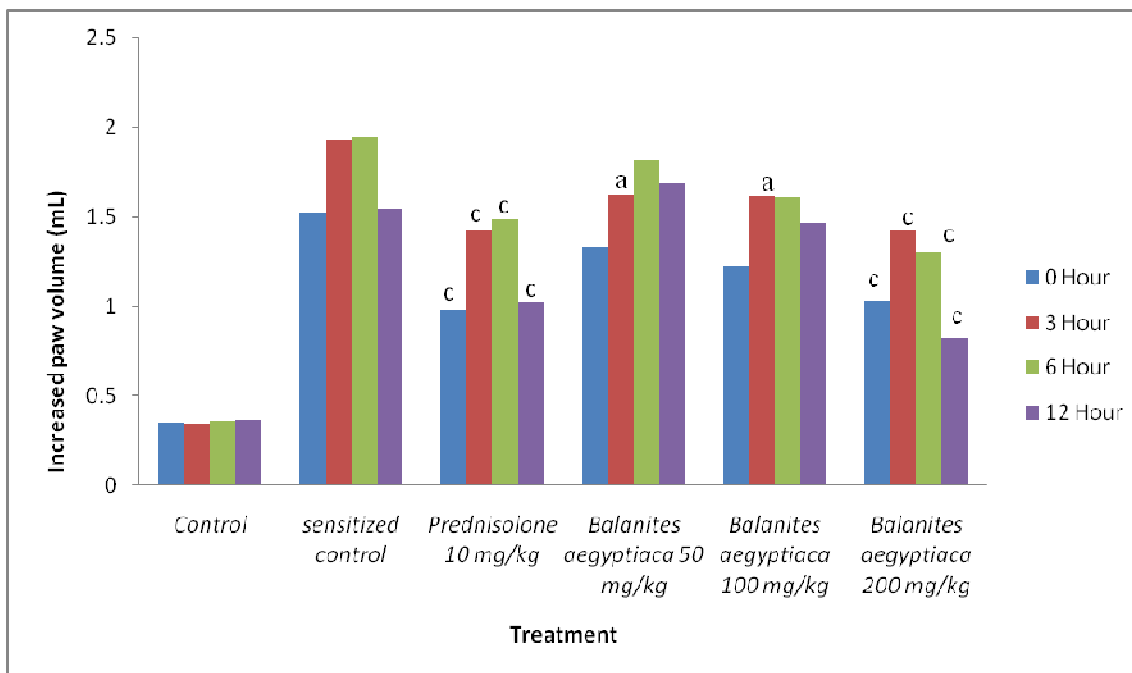


Figure 2: Effect of NBF of *Balanites aegyptiaca* on passive paw anaphylaxis

Values are expressed as mean \pm SEM, n=5 in each group. a=*, b=**, c=***.

The statistical significance was determined using One-way ANOVA followed by Tukey-Kramer multiple comparison post-hoc test *p<0.05, **p<0.01, ***p<0.001 as compared with sensitized control.

While Dexamethasone showed significant protection. NBF of *Balanites aegyptiaca* showed significant effect may be due to inhibition of PGD2 pathway which leads to inhibition of late phase and may possess antieosinophilic activity.

Surface acting agents like Tween 80, compound 48/80, drugs like Tubocurarine, Morphine, Atropine, Polymyxin-B, Clonidine etc. releases histamine from mast cell degranulation [14]. They produce 'anaphylactoid' reactions itching and burning sensation, asthma [15]. It is known that Clonidine releases histamine from mast cells. In present finding NBF of *Balanites aegyptiaca* significantly stabilized the mast cell degranulation in dose dependent manner as compared with toxicant.

Passive paw anaphylaxis model involves passive immunization with serum containing IgE antibody and then second antigen exposure will cause immediate hypersensitivity- I reaction in form of paw edema. The regulation of histamine and inflammatory cytokines has been considered an important strategy in the management of immediate hypersensitivity reaction. Several effect of standard drug Prednisolone may be explained by their capacity to block the release of chemotactic mediators as the metabolites of arachidonic acid and/or cytokines with inflammatory properties [11, 17, 18]. NBF of *Balanites aegyptiaca* showed dose dependent gradual decreased in paw edema induced by type I hypersensitivity reaction was comparable with Prednisolone. The results of the present study showed that NBF of *Balanites aegyptiaca* is effective against Type I allergic disorders. This means that *Balanites aegyptiaca* found to be may be effective in acute allergic conditions like anaphylaxis and asthma.

All the above findings lend credence to the beneficial use of NBF of *Balanites aegyptiaca* in the treatment of asthma and related conditions. Further studies are needed to isolate the steroidal saponins from fruit pulp of *Balanites aegyptiaca* its antiasthmatic activity can be tested further.

Acknowledgment

The authors are thankful to R. C. Patel Institute of Pharmaceutical Education and Research, Shirpur for providing facility for research work.

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