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Research Article

EVALUATION OF ANTIASTHMATIC AND ANTIANAPHYLACTIC ACTIVITY OF *BALANITES AEGYPTIACA* (DELILE), (BALANITACEAE)

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ABSTRACT

Objective: To evaluate the antiasthmatic and antianaphylactic activity of *Balanites aegyptiaca*.
Materials and Methods: The n-butanolic fraction (NBF) of fruit pulp of *Balanites aegyptiaca* was prepared. The antiasthmatic and antianaphylactic activity of NBF of *Balanites aegyptiaca* was evaluated using various experimental models like active and passive anaphylaxis in rats, acetylcholine and histamine induced bronchospasm in guinea pigs, studies on isolated guinea pig ileum and goat tracheal strip preparation.
Results: Treatment with NBF of *Balanites aegyptiaca* showed a dose dependent (at 50, 100 and 200 mg/kg p.o.) beneficial effect on degranulation rate of actively and passively sensitized mesenteric mast cells of albino rats when challenged with antigen (horse serum). NBF of *Balanites aegyptiaca* also significantly reduced the serum IgE level and number of eosinophil cell count in rats compared to untreated control. NBF of *Balanites aegyptiaca* treatment resulted in significant protection against acetylcholine and histamine aerosol induced bronchospasm in guinea pigs. NBF of *Balanites aegyptiaca* produced dose dependent inhibition of ileal contractions induced by histamine and acetylcholine. NBF of *Balanites aegyptiaca* showed significant antihistaminic activity in histamine induced contraction in goat tracheal chain preparation.
Conclusion: Antiasthmatic and antianaphylactic activity NBF of *Balanites aegyptiaca* may be possibly due to the membrane stabilising potential, suppression of antibody production, reduction in eosinophil cells count and inhibition of antigen induced histamine or acetylcholine release.

Key words: *Balanites aegyptiaca,* Antiasthmatic activity, Antianaphylactic activity, Mast cell, Eosinophil, IgE.

INTRODUCTION

Asthma is a common and chronic inflammatory condition of the airways whose cause is not completely understood. As a result of inflammation the airways are hyperresponsive and they narrow easily in response to a wide range of stimuli. This may result in coughing, wheezing, chest tightness, and shortness of breath and these symptoms are often worse at night. Narrowing of the airways is usually reversible, but in some patients with chronic asthma the inflammation may lead to irreversible airflow obstruction. Characteristic pathological features include the presence in the airway of inflammatory cells, plasma exudation, oedema, smooth muscle hypertrophy, mucus plugging, and shedding of the epithelium.[1]

Despite the availability of a wide range of drugs, the relief offered by them is mainly symptomatic and short lived. Moreover the side effects of these drugs are also quite disturbing. Hence a continuous search is on going to identify effective and safe remedies to treat bronchial asthma.[2] Medicinal plants are of great importance in providing healthcare to a large portion of the population in India. Ayurveda, an Indian system of medicine, has described several drugs from indigenous plant sources for use in the treatment of bronchial asthma and allergic disorders and have been successful in controlling the disease as well.

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-3β-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.*

As part of an ongoing programme to validate the use of some reputed herbs in traditional Ayurvedic medicines, present investigation was undertaken to evaluate the anti-asthmatic and anti-anaphylactic activity 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 various *in vivo* and *in vitro* methods for evaluation of anti-asthmatic and anti-anaphylactic activity.

MATERIALS AND 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 (yield 18.87 %w/w). The Phytochemical investigation were 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 2-4^0 C, protected from direct sunlight until use.

Chemicals

Histamine diphosphate (23297-93) was procured from Sigma chemicals, Acetylcholine chloride (57608), 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

Dunkey-Hartley strain guinea pigs (350-500 g) of either sex, male albino mice Swiss strain (22- 25 g) and Albino wistar rats (200-250g) 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.

Active anaphylaxis

Thirty rats were sensitised by injecting subcutaneously 0.5 ml of horse serum along with 0.5 ml of triple antigen containing 20,000 million Bordetella pertussis organisms (Serum Institute of India Ltd., Pune).[7] The rats were divided into 6 groups of 6 animals each. Group I served as control. Group II served as sensitized control received water (vehicle). Rats of Group III, IV and V were administered NBF of *Balanites aegyptiaca* at 50, 100 and 200 mg/kg, p.o. respectively, once a day for 14 days. Group VI rats received 10 mg/kg of Prednisolone (reference drug) orally for the same duration. Eosinophil cell count on 1st, 3rd, 5th, 7th, 9th, 11th, 13th and 14th days was noted using method described by Godkar.[6] On day 14th, two hours after treatment with drugs, the rats were anesthetized by anesthetic ether and blood sample was collected from retro-orbital plexus, serum was separated and IgE analysis was done.

Then the rats were sacrificed and the intestinal mesentery was taken for the study on mast cells. Mesenteries of sacrified rats along with intestinal pieces were kept in Ringer-Locke solution (NaCl 9.0, KCl 0.42, CaCl**2** 0.24, NaHCO3 0.15, Glucose 1.0 gm/l of distilled water) at 37^{0}C. The mesenteric pieces were challenged with 5% horse serum for 10 min after which the mast cells were stained with 1% Toluidine blue and examined microscopically for the number of intact and degranulated mast cells.[7, 8]

Passive anaphylaxis

Following active anaphylaxis, the rats were sacrificed and the blood was collected by decapitation and the serum was separated. One ml of serum of the actively sensitized rat was injected intraperitoneally to 30 normal rats.[7] The rats were divided into 6 groups of 6 animals each. Group I served as control. Group II served as sensitized control received water (vehicle). Group III, IV and V were administered NBF of *Balanites aegyptiaca* at 50, 100, 200 mg/kg, p.o. respectively. Group VI received Prednisolone 10 mg/kg (reference drug) orally. Forty eight hours later, passively sensitised rats were challenged by the intraperitoneal injection of horse serum (1 ml). Ten minutes after the antigen challenge, the rats were sacrificed and the intestinal mesentery was collected in Ringer-Locke solution. The mesenteric mast cells were stained with 1 % Toluidine blue and examined microscopically. The numbers of intact and disrupted mast cells were counted in at least 10 randomly selected high power fields for each tissue.[7,8]

Histamine and Acetylcholine induced bronchospasm in guinea pigs

Thirty guinea pigs of either six were exposed to aerosol of 1.0 % Histamine diphosphate using nebuliser with constant pressure 40mm/Hg in histamine chamber and time for preconvulsion dyspnoea (PCD) was recorded from the time of aerosol exposure to the onset of dyspnoea leading to the appearance of asphyctic convulsions i.e. Pre-convulsion time (PCT).[7] As soon as PCD commenced, animals were removed from the chamber and placed in fresh air to recover. All the animals were randomly divided into 5 groups each containing six animals. Two and a half hours later, the animals of groups I received distilled water (vehicle) and served as a control. Group II received Chlorpheniramine maleate 2 mg/kg p.o and served as standard. Group III, IV and V received NBF of *Balanites aegyptiaca* at 50, 100 and 200 mg/kg p.o. respectively. One and half hour after the treatment, animals were exposed to Histamine diphosphate aerosol and time taken for onset of PCD was noted. Animals which withstood exposure to histamine aerosol for 15 min were considered to be completely protected.[9] Similar procedure was repeated by exposure of aerosol of 10% Acetylcholine chloride in another five groups of animals using Atropine sulphate 2 mg/kg as a standard. The percentage protection offered by the treatment was calculated by the following formula,

Percentage protection = {1- T1/T2} × 100

Where, T1 = time in second for PCD before treatment; T2 = time in second for PCD after treatment.

Studies on isolated Guinea pig ileum

Overnight fasted guinea pigs of either sex weighing 400-600 g were sacrificed using stunned by head-blow. Ileum was quickly dissected out and mounted in an organ bath maintained at 37 ± 1°C and containing 10 ml Tyrode solution under basal tension of 500mg. The composition of solution in mM/L was NaCl, 137; CaCl2, 1.8; KCl, 2.7; glucose, 5.55; NaHCO3, 11.9; MgCl2, 1; NaH2PO4, 0.4. The solution was continuously bubbled with air. The responses to drug were recorded on kymograph paper on Sherrington rotating drum. The issue was allowed to equilibrate for 30 min, during which, the bathing solution was changed at every 10 min. The contractile responses of ileum to various agonists (Acetylcholine and Histamine) were recorded in presence and absence of NBF of *Balanites aegyptiaca*.

Goat tracheal strip preparation

This method is a modification to the tracheal chain model where the knitting/connecting of the tracheal rings is not performed. In this method, goat trachea brought from slaughterhouse was cut into zig-zag fashion thereby exposing large portion of the tissue using the method described by Kulkarni.[10] It was suspended in a organ bath of 20 mL containing Krebs-Henseleit solution (Concentration in mM/L as NaCl, 118; KCl, 4.7; CaCl2, 2.5; MgSO4, 1.2; NaHCO3, 25.0; KH2PO4, 1.2; Glucose, 11.1) maintained at 37 ± 1^{0}C a stream of O_2 was bubbled through the organ tube. One end was tied to aerator tube and other attached to isotonic frontal writing lever to kymograph paper on Sherrington rotating drum. Tissue was allowed to equilibrate for 45 min. during which, the bathing solution was changed at every 15 min. under to load of 400 mg.[2] The contractile responses of tracheal strip to Histamine were recorded in presence and absence of NBF of *Balanites aegyptiaca*.

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 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).

Table 1: Effect of NBF of *Balanites aegyptiaca* on mast cell degranulation in actively sensitised rats

Group	Mast cells %	
	Intact	Disrupt
Control	87±0.4	13±0.4
Sensitized control	18±0.58	82±0.58
B.aegyptiaca 50mg/kg	63±0.68***	37±0.68***
B.aegyptiaca 100 mg/kg	71±0.58***	29±0.58***
B.aegyptiaca 200 mg/kg	81±0.68***	19±0.68***
Standarddrug (Prednisolone)	80±0.55***	19±0.37***
One-way ANOVA		
P	P < 0.0001	P < 0.0001
F	1900	2000
Degree of freedom (df)	29	29
R squared	1.0	1.0

Values given as mean ± SEM, n=6 in each group, *p<0.05, **p<0.01, ***p<0.001 as compared to sensitized control (Bonferroni's multiple comparison post-hoc test).

Table -2: Effect of NBF of *Balanites aegyptiaca* on mast cell degranulation in passively sensitised rats

Group	Mast cells %	
	Intact	Disrupt
Control	94±1.7	5.6±1.7
Sensitized control	17±1.4	81±0.75
B.aegyptiaca 50mg/kg	62±0.63***	38±0.63***
B.aegyptiaca 100 mg/kg	88±0.32***	12±0.32***
B.aegyptiaca 200 mg/kg	93±0.32***	6.6±0.75***
Standard drug (Prednisolone)	90±0.75***	10±0.45***
One-way ANOVA		
P	P < 0.0001	P < 0.0001
F	880	1100
Degree of freedom (df)	29	29
R squared	0.99	1.0

Values given as mean ± SEM, n=6 in each group
*p<0.05, **p<0.01, ***p<0.001 as compared to sensitized control (Bonferroni's multiple comparison post-hoc test).

Table -3: Effect of NBF of *Balanites aegyptiaca* on agonists induced contractions of guinea pig ileum

Conc.of *B.aegyptiaca* fraction (mg/ml)	% inhibition of Acetylcholine contractions	% inhibition of Histamine contractions
10	4.17	100
20	25	-
50	37.5	-
100	75	-
150	91.67	-
200	100	-

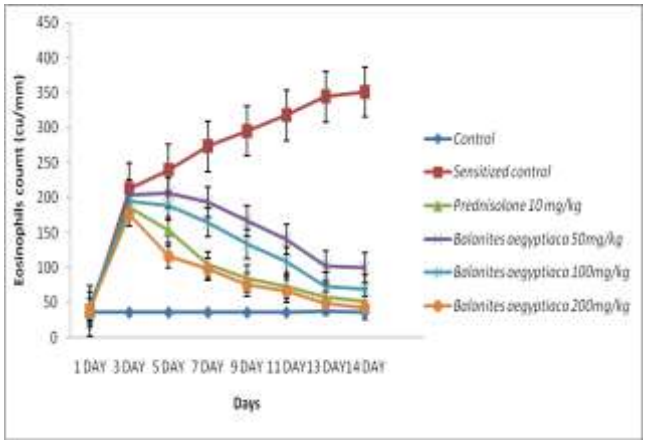

Figure -1: Effect of NBF of *Balanites aegyptiaca* on eosinophil cell count in active anaphylaxis. Value represents, Mean ± S.E.M. (n=5) ANOVA: Dunnett's Multiple Comparative test *p< 0.05, **p<0.01, ***p<0.001 as Compared with control.

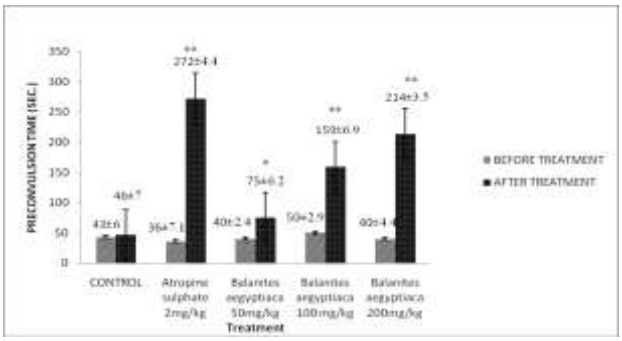

Figure -2: Effect of NBF of *Balanites aegyptiaca* on Histamine induced bronchospasm.Value represents, Mean ± S.E.M. (n=5) ANOVA: Dunnett's Multiple Comparative test *p< 0.05, **p<0.01, ***p<0.001 as Compared with control

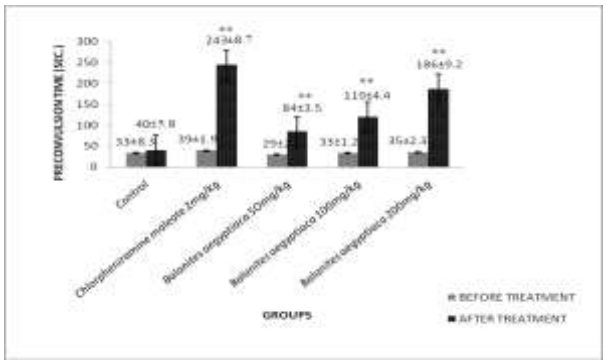

Figure -3: Effect of NBF of *Balanites aegyptiaca* on Acetylcholine induced bronchospasm. Value represents, Mean ± S.E.M. (n=5) ANOVA: Dunnett's Multiple Comparative test *p< 0.05, **p<0.01, ***p<0.001 as Compared with control.

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.

Active anaphylaxis

Horse serum challenge resulted in degranulation of mesenteric mast cells (about 83%). Pretreatment of sensitized animals with NBF of *Balanites aegyptiaca* (50, 100 and 200 mg/kg p.o.) for fourteen days resulted in significant reduction in number of disrupted mast cells (P<0.001) when challenged with horse serum. Animals treated with 200 mg/kg were shows better protection as compared with that of Prednisolone (Table 1). Sensitized control group showed gradual increase in eosinophils cells count near about (352 cu/mm) on 14th day, while rats treated with NBF of *Balanites aegyptiaca* at 50, 100 and 200 mg/kg p.o. showed gradual decrease in eosinophils cell count on 14th day. Animals treated with 200 mg/kg were shown better decreased in eosinophil count on 14th day as compared with standard drug Prednisolone (Figure 1). Treatment with NBF of *Balanites aegyptiaca* at 50 mg/kg (8.8±0.35 IU/ml, P < 0.001), 100 mg/kg (6.2±0.11 IU/ml, P < 0.001), and 200 mg/kg (3.6±0.045 IU/ml, P < 0.001) showed gradual decreased in serum IgE level as compared to sensitized control group (11±0.075 IU/ml). Serum IgE level in control group was 3.4±0.035 IU/ml (P < 0.001 as compared to sensitized control).

Passive anaphylaxis

When the serum of the actively sensitized rats from sensitized control group were administered i.p. to fresh rats and challenged with horse serum 48 h later, the percentage degranulation of the peritoneal mast cells was about 82%. The percentage degranulation was significantly reduced in the rats which received the serum from NBF of Balanites aegyptiaca (50, 100 and 200 mg/kg) treated group. Treatment with standard drug Prednisolone showed better protection on the mast cells in passively sensitised rats (Table 2).

Effect of *Balanites aegyptiaca* on Histamine and Acetylcholine induced bronchospasm

Balanites aegyptiaca significantly prolonged the latent period of convulsion as compared to control following exposure to Acetylcholine chloride and Histamine diphosphate aerosols (Figure 2 and 3).

Table -4: Effect of NBF of *Balanites aegyptiaca* on Histamine induced contractions of goat tracheal preparation

Conc. of *B. aegyptiaca* Fraction (mg/ml)	% inhibition of Histamine induced contraction of goat tracheal preparation
10 mg/mL	27.78 %
20 mg/mL	38.89 %
50 mg/mL	66.67 %
100 mg/mL	100 %

Effect of *Balanites aegyptiaca* on agonists induced contractions of guinea pig ileum

The n-butanol fraction of *Balanites aegyptiaca* (10-200 mg/ml) dose dependently inhibited ileum contractions induced by Acetylcholine 5 µg/ml, Histamine 2 µg/ml (Table 3).

Goat tracheal strip preparation

Histamine (20 µg /ml) was able to produce notable contraction on isolated goat tracheal chain (18 mm taken as 100 %). *Balanites aegyptiaca* (10-100 mg/ml) dose dependently inhibited tracheal strip contraction induced by Histamine 20 µg/ml (Table 4).

DISCUSSION

The present study was undertaken to evaluate the antiasthmatic and antianaphylactic activity of NBF of *Balanites aegyptiaca* fruit pulp. *Balanites aegyptiaca* seems to be a promising plant for treatment of bronchial asthma because plant contains steroidal saponins & flavonoids which inhibit the release of several mediators of the phlogistic agents such as prostaglandins, histamine, serotonin and bradykinin by inhibiting the biosynthetic pathways of inflammatory mediators. Evidence strongly suggests that steroidal saponins present in the extracts obtained from *Balanites aegyptiaca* may interfere with lipoxygenase and/or cyclo-oxygenase pathway.[4] Earlier studies from our own laboratory have shown the effectiveness of methanol extract in animal models of bronchial asthma.

Eosinophils, mast cells, and their preformed de novo synthetized mediators, play a pivotal role in the pathogenesis of allergic disorders. These molecules are potent vasoactive and bronchoconstrictor agent and they modulate local immune response and inflammatory cell infiltration.[11] Antigen Challenge in sensitized animals, results in degranulation of mast cells, which is an important features of anaphylaxis.[12] Mast cell stabilizing activity of NBF of *Balanites aegyptiaca* may be due to mast cell stabilizing potential against antigen antibody reaction and/or due to the suppression of IgE antibody production, which is responsible for degranulation of mast cells.[13] NBF of *Balanites aegyptiaca* also showed marked protection against eosinophil cell count, which is hallmark of allergic asthma as compared to sensitized control group. Significantly reduction in eosinophil cell count means that NBF of *Balanites aegyptiaca* may inhibit clustering of eosinophil cell around nerves or inhibit eosinophil cell recruitment and inhibition of interleukins such as IL4, IL-5 and IL-13 which play important role in eosinophil cell recruitment. The anti-anaphylactic action of NBF of *Balanites aegyptiaca* was due to the suppression of antibody production which was studied indirectly by passive anaphylaxis. The serum of the actively sensitized rats which contains the appropriate elevated IgE antibodies containing serum was injected intraperitoneally into the fresh recipient rats. The present IgE antibodies become fixed to the peritoneal mast cells and, when exposed to antigen (horse serum ex vivo) will show anaphylactic degranulation.[7] This was further confirmed by the reduction in the number of degranulated mast cells in the rats which received serum from NBF of *Balanites aegyptiaca* treated animals. This anti-anaphylactic effect may be due to stabilisation of the mast cell membrane, inhibition of antigen-induced histamine release or non-availability of antibodies on the mast cell surface in NBF of *Balanites aegyptiaca* treated animals. In the early stage of asthma, release of inflammatory mediators like histamine, tryptase, acetylcholine, leukotrienes, and prostaglandins are triggered by exposure to allergens, irritants, cold air or exercise. Some of these mediators directly cause acute bronchoconstriction. In the present study, we have used Histamine and Acetylcholine as spasmogens in the form of aerosols to cause immediate bronchoconstriction in guinea pigs. Chlorpheniramine maleate (2 mg/kg) and atropine sulphate (2 mg/kg) were used as reference standard against histamine and acetylcholine induced bronchospasm respectively. NBF of *Balanites aegyptiaca* significantly prolonged latent period of convulsions due following exposure to either histamine or acetylcholine aerosol. The bronchodilatory effect of n-butanol fraction was found comparable to the protection offered by both the reference standard drug Chlorpheniramine maleate and atropine sulphate.

Spasmolytic effect of *Balanites aegyptiaca* was also evaluated by observing the effect of its n-butanol fraction on Histamine and Ach induced ileal contractions. NBF of *Balanites aegyptiaca* produced dose dependent inhibition of ileal contractions induced by Histamine and Ach. These indicate that *Balanites aegyptiaca* has a nonspecific spasmolytic activity on smooth muscle.

Histamine contracts the trachea-bronchial muscle of guinea pig, goat, horse, dog and man.[14] Goat tracheal chain is much more sensitive than guinea pig and easier to handle.[2] In the present study the isolated goat tracheal chain preparation; there is right side shift of Dose Response Curve (DRC) of histamine in the presence of NBF of *Balanites aegyptiaca* indicating antiasthmatic activity. Antihistaminic activity of *Balanites aegyptiaca* may possess due to inhibition of H1 receptor.

Balanites aegyptiaca has nonselective direct in-vivo antihistaminic and anticholinergic activity with moderately activity in active and passive anaphylactic conditions. *Balanites aegyptiaca* has antihistaminic activity may be due to H_1 receptor antagonism while anticholinergic activity may be due to M_3 receptor antagonism. *Balanites aegyptiaca* shows antianaphylactic activity may be due to moderately mast cell stabilization, lowering of elevated IgE antibody and may inhibit clustering of eosinophil cell around the nerves or inhibition of eosinophil cell recruitment. *Balanites aegyptiaca* has smooth muscle relaxation properties. Further studies are needed to isolate the steroidal saponins from fruit pulp of *Balanites aegyptiaca* its antiasthmatic activity can be tested further.

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