Available online at www.ijpcr.com
International Journal of Pharmaceutical and Clinical Research 2009; 1(1): 40-42

Research Article

Preliminary Phytochemical and Anti-asthmatic Studies on Stem Bark of *Balanites roxburghii* Planch.

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ABSTRACT
The present study deals with the phytochemical screening and effect of ethanolic extract of stem bark of *Balanites roxburghii* Planch. on experimental models of bronchial asthma. Phytochemical screening of the ethanolic extract showed the presence of alkaloids, saponins, flavonoids, tannins and phenolic compounds as chemical constituents. Significant increase in preconvulsion time was observed due to pretreatment with *Balanites roxburghii* when the guinea pigs were exposed to acetylcholine (Ach) and histamine. This bronchodilating effect of *Balanites roxburghii* was comparable to Ketotifen fumarate. Thus, present study revealed that the ethanolic extract of stem bark of *Balanites roxburghii* has significant antihistaminic (H_1 receptor antagonist) activity. The *Balanites roxburghii* Planch. by virtue of the said action will prove to be very effective in the antihistaminic therapy of asthma.

Keywords: Anti-asthmatic, bronchodilators, histamine, acetylcholine, *Balanites roxburghii* Planch.

INTRODUCTION

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, expectorant, 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. [1] The whole plant is used in treatment of snake-bite. Seeds are used as expectorant (given in the treatment of cough) and colic. [2] Kernel is used in skin diseases and burns. [3] 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 [4] and anti-inflammatory activity. [5]

Asthma is a chronic condition involving the respiratory system in which the airways occasionally constrict, become inflammed, and are lined with excessive amount of mucous, often in response to one or more triggers. These episodes may be triggered by exposure to environmental stimulants such as an allergen, environmental tobacco smoke, cold or warm air, perfume, pet dander, moist air, exercise or exertion or emotional stress. [6]

During the exhaustive literature survey it was found that systematic pharmacological work has not been done so far on this plant. Hence, this plant was selected to evaluate its anti-asthmatic activity.

MATERIALS AND METHODS

Plant material

The stem bark of *B. roxburghii* was collected from the roadside location of the village- Doniapura, Gormi, Bhind (M.P.) and was authenticated from CDRI, Lucknow. Plant material was preserved in herbarium department of institution (voucher specimen no. J-52). The stem bark of the plant was separated, dried and coarsely powdered.

Preparation of plant extract

400gm of dried powdered stem bark of *B. roxburghii* was defatted with petroleum ether (60-80˚C) in Soxhlet apparatus. Defatted material obtained was air-dried and subsequently extracted with ethanol. After complete extraction, the solvent was removed by distillation under reduced pressure and extract was concentrated to dryness in vacuum. The percentage of ethanol soluble extractives was calculated with reference to air-dried plant material and the yield was found to be 11.18 ± 0.70 % w/w.

Phytochemical screening

Phytochemical screening of the EEBR showed the presence of alkaloids, saponins, flavonoids, tannins and phenolic compounds as chemical constituents by chemical tests in Table 2. The Thin Layer chromatographic study using various solvent systems was carried out. The R_f values and number of spots with respective solvent systems were obsreved. The solvent system Cyclohexane : Ethylacetate and n-Butanol : Glacial acetic acid : Water showed maximum 5 and 4 spots respectively in the EEBR in Table 1.

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Table 1: Thin layer chromatographic profile of EEBR

Drug	Solvent systems	Ratio of Solvents	R_f values of the spots	No. of spots
EEBR	Benzene : Ethanol	9 :1	0.42, 0.66, 0.71	03
	Chloroform : Acetone	7 : 3	0.23, 0.55, 0.64	03
	Cyclohexane : Ethylacetate	8 : 2	0.21,0.29, 0.37, 0.71, 0.75	05
	n-Butanol : Glacial acetic acid : Water	12 : 3 : 5	0.24, 0.28, 0.39, 0.51	04
	Benzene : Ethyl acetate : Acetic acid	7.5 : 2.4 : 0.1	0.52, 0.68	02
	Methanol : Benzene	5 : 5	0.34, 0.55, 0.67	03
	Benzene : Acetic acid	9 :1	0.26, 0.38, 0.57, 0.63	04

Experimental animals

Guinea pigs of either sex (350-450 g) were selected for present study. Six animals were taken in each group and maintained under standard laboratory conditions. They were allowed free access to standard dry pellet diet and water *ad libitum* during the experiment. 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).

Screening of anti-asthmatic activity

***In vitro* studies on isolated guinea pig ileum Preparation** [7-8]

Overnight fasted guinea pigs were sacrificed using cervical dislocation method. Ileum was quickly dissected out and mounted in an organ bath maintained at 30±0.5°C and containing 20 ml Tyrode's solution under basal tension of 500 mg. The solution was continuously bubbled with air. The responses to drug were recorded on student physiograph using isotonic transducer, which exerted a basal tension equivalent to 500 mg load on tissues. The tissues were allowed to equilibrate for 30 minutes, during which, the bathing solution was changed at every 10 minutes. The contractile responses of ileum to Histamine were recorded in presence and absence of EEBR in Table 3.

***In vivo* studies on Acetylcholine and Histamine induced bronchospasm in guinea pigs**

Guinea pigs of either sex (350-450 g) were selected and randomly divided into four groups each containing six animals. The animals were kept on fasting overnight before treatment. The ethanolic extract and standard drug were administered orally in 0.5 % CMC. The single dose treatment was given one and half an hour before the study. Later the animals were exposed to an aerosol of 0.25 % histamine and time for preconvulsion state was observed for each animal as described by Sheth *et al.* (1972). [9] After 15 days of washout period, the same animals were treated with the above treatment and time for preconvulsion state was observed for 0.5% acetylcholine bromide aerosol spray. [10-11] The observations are presented in Table 4.

RESULTS

The preliminary phytochemical study revealed the presence of alkaloids, saponins, flavonoids, tannins and phenolic compounds in the ethanolic extract of *B. roxburghii*. The ethanolic extract of stem bark of *B. roxburghii* Planch. was found to be practically nontoxic and its LD_{50} value was found to be 4 g/kg body wt. *In vitro* study of EEBR has been performed on isolated guinea pig ileum. Results showed the increase in the contractile responses of the tissues significantly at the level of $P<0.05$ when treated with EEBR at the different doses of 250, 500 and 1000 µg/ml. Pretreatment with EEBR at the dose of 250, 500, 1000 mg/kg p.o. significantly and dose dependently delayed the onset of convulsion in guinea pigs due to acute bronchospasm induced by acetylcholine (0.5 %) and histamine (0.25 %) aerosol. The bronchodilating effect of EEBR was comparable to Ketotifen (1 mg/kg).

DISCUSSION

Histamine is one of the important mediators of allergy, inflammation and bronchoconstriction, which were released after degranulation of mast cell by an antigen exposure. Targeting histamine, either prevention of its release from mast cell or use of histaminergic receptor antagonist becomes part of antihistaminic therapy in allergic diseases. [12]

Table 2: Qualitative analysis of EEBR

S. No.	Chemical constituents	Results
1.	Alkaloids	+ve
2.	Carbohydrates	+ve
3.	Glycosides	-ve
4.	Saponins	+ve
5.	Phytosterols	-ve
6.	Proteins	-ve
7.	Flavonoids	+ve
8.	Fat & oils	-ve
9.	Tannins & phenolic compound	+ve
10.	Gum and Mucilage	+ve

+ve indicates the presence of chemical constituents and –ve indicates the absence of chemical constituents

In vitro study of EEBR has been performed on isolated guinea pig ileum. Results showed the increase in the contractile responses of the tissues significantly at the level of $P<0.05$ when treated with EEBR at the different doses of 250, 500 and 1000 µg/ml. *In vivo* study of EEBR has been also shown the significant increase in preconvulsion time due to pretreatment with EEBR at the dose of 250, 500 and 1000 mg/kg of bodyweight of guinea pigs, when the guinea pigs were exposed to acetylcholine (ACh) and histamine. The results of EEBR suggested that it is effective in reducing the symptoms of bronchial asthma and also improve the lung function parameters of asthmatic subjects.

Results of the experimental studies of *B. roxburghii* suggested that anti-asthmatic activity could be due to its bronchodilator, mast cell stabilizing and antimicrobial property. The possible mechanism of action may be blockade of H_1 and Ach receptors leading to inhibitory of smooth muscle to respond histamine and Acetylcholine induced spasm leading to inhibition of bronchoconstriction. It has been reported that these patients are resistant to main antibiotics prescribed. It is possible that these patients are suffering from bronchial infection but have been diagnosed,

Table 3: *In vitro* study of EEBR on histamine induced contraction of isolated guinea pig ileum preparation

S. No.	Dose of Histamine in ml (10 µg/ml)	250µg/ml of EEBR		500µg/ml of EEBR		1000µg/ml of EEBR	
		Control group	Test Group	Control Group	Test Group	Control Group	Test Group
1.	0.3	21.22±1.23	14.47±1.04	31.25±2.54	24.54±2.74	36.55±3.61	25.78±1.24
2.	0.6	39.22±4.57	26.67±3.66	59.64±4.57	35.27±3.66	65.33±3.57	37.28±3.46
3.	0.9	52.19±3.54	34.14±4.41	81.94±4.87	51.22±4.55	84.99±3.47	61.31±1.82
4.	1.2	65.21±4.17	48.15±2.41	89.36±5.01	59.05±3.74	90.24±4.11	71.41±4.64
5.	1.5	73.14±5.24	51.44±2.57	97.29±6.11	62.55±3.47	99.05±4.24	65.59±4.79

n=6, values are expressed in Mean ± SD, P <0.05, using student 't' Test

Table 4: *In vivo* study of EEBR on acetylcholine and histamine induced bronchospasm in guinea pigs

S. No.	Group	% increase in preconvulsion	
		Acetylcholine	Histamine
1.	Group I (Ketotifen; 1 mg/kg)	27.64±2.14	34.38±2.11
2.	Group II (EEBR; 250 mg/kg, p. o.)	32.25± 2.54	17.36±1.85
3.	Group III (EEBR; 500 mg/kg, p. o.)	47.58±3.98	34.33±2.11
4.	Group IV (EEBR; 1000 mg/kg, p. o.)	54.91±4.42	39.67±3.88

as asthmatic patients because of their symptoms like breathless. [6, 13] Thus, it can be concluded that EEBR possess significant antihistaminic (H_1- receptor antagonist) activity.

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