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Research Article

STUDIES ON ANALGESIC ACTIVITY OF BALANITES ROXBURGHII IN MICE

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ABSTRACT

To evaluate the analgesic activity of fractions and aqueous residue of methanolic extract of pericarpium of *Balanites roxburghi,* at the doses of 50 and 100 mg/kg. In Ayurvedic, the fruit of the allied species *Balanites aegyptiaca* is being used as analgesic. The tribal people are using the aqueous juice of pericarpium of *B. roxburghii* for relieving the pain. Mice were used as experimental animals and administered test drugs orally. Analgesic activity was studied using the hot plate and acetic acid induced writhing. The butanolic fraction showed promising activity in both the tests. The analgesic effect of the butanolic fraction at a dose of 100mg/kg was more than that observed with the same fraction at a dose of 50mg/kg. No analgesic activity was observed with dichloromethane fraction and aqueous residue. The observations suggest that the pericarpium of *B. roxburghii* possesses potential analgesic activity.

Keywords: *Balanites roxburghi;* analgesic activity; Mice.

INTRODUCTION

Balanites roxburghii is a medicinal herb, found in drier parts, in Bengal India and Myanmar. In Ayurvedia, the fruit of *Balanites aegyptiaca* (allied species of *B. roxburghii*) [1] has a analgesic, alexipharmic, bitter sharp taste and in Unani system of medicine for treatment of boils, leucoderma and other skin diseases [2], in Sudanese folk medicine for treatment of jaundice [3], in tropical Africa as CNS depressant [4].

Preliminary phytochemical investigations have revealed the presence of saponin glycosides, flavonoids, tannins, alkaloids, phenols from different parts of this plant. Chemically, Saponins [5] have been isolated from the fruit pulp. Anti-nociceptive activity of methanol, butanol extracts and of two new saponins isolated from bark of *Balanites aegyptiaca* was evaluated [6]. The literature survey revealed that there is no analgesic activity pericarpium (epicarp and mesocarp) of this plant. Hence the present study was aimed to determine the same.

MATERIALS AND METHODS

Experimental Animals

Studies were carried out using male Wistar albino mice (20-25 g). They were procured from the animal house of Mahaveera Enterprises (Reg. No.146/1999/CPCSEA), Ranga Reddy District, India. The animals were grouped and housed in polyacrylic cages with not more than six animals per cage and maintained under standard laboratory conditions (temperature 25 ± 2°C) with dark and light cycle (12/12 h). They were allowed free access to standard dry pellet diet and water *ad libitum*. All procedures described were reviewed and approved by the Institutional animal ethical committee.

Preparation of pericarpium extract and fractions

The plant growing in Karimnager Dist, Andhra Pradesh, India was authenticated by Prof. Raju S. Vastavaya, Taxonamist, Department of Botany, Kakatiya University, Warangal. Fresh fruits (Voucher number: PLB-050, deposited in: Herbarium, director: Prof. Raju S.V.) from the plant were collected in October 2007. The pericarpium was separated, dried and powdered. The powdered material was macerated with methanol and filtered. The concentrated extract was fractionated with dichloromethane (DCM-BPME) and 1-butanol (BOH-BPME) in succession to yield fractions of DCM-BPME (10%), BOH-BPME (50%) respectively. The remaining residue after fractionation of the methanolic extract was aqueous residue (AQR, 40%). They were stored at -20°C until being used.

Drugs

Pentazocin (Pure pharm Ltd. Mumbai, India) and Diclofenac sodium (Dr Reddy's Lab., Hyderabad) were used as reference standards in this study. The solvents used were of analytical grade. Methanol (BDH, Mumbai, India), 2% w/v Gum acacia in water (M/S Hi-media, Mumbai, India) used as solvent and vehicle respectively.

Drug administration

Suspensions of the fractions and AQR were prepared in 2% w/v gum acacia in water. The animals were divided into eight groups each consisting of six animals. The control group received the vehicle (2% w/v gum acacia in water, 1 ml/kg) orally, whereas the test groups received fractions and AQR at a dose of 50 and 100 mg/kg orally. The standard group received pentazocin 10mg/kg (i.p) or Diclofenac sodium 20mg/kg (orally).

Acute toxicity test

The methanolic extract was administered orally in doses of 100, 300, 1000 and 2000 mg/kg to groups of mice (n = 6) and percentage mortality and behavioral changes were noted beginning with 24 h up to a period of 7 days [7].

ANALGESIC ACTIVITY

Hot Plate Method

The hot plate test was used to measure analgesic activity by following the method, described by Eddy and Leimbark [8] with minor modifications. In this experiment, the hot plate was maintained at 55 ± 0.5°C. All animals were selected 24 h prior to the experimentation on the basis of their normal reaction time i.e., pain response to the hot plate to the minimum and maximum of 3-8 sec respectively. A latency period of 20 sec was defined as complete analgesia. Latency time was noted at 0, 30, 60, 90, 120, 150 and 180 min. after the administration of vehicle, standard, fractions and AQR. The standard group received pentazocin as a reference. Animals were placed individually on to the hot plate and the time from placing the animal on the hot plate to jumping of the animal from the hot plate was recorded as the reaction time or latency of the pain response.

$$\text{Percentage Variation} = \frac{\text{Drug latency} - \text{Base line latency}}{\text{Base line latency}} \times 100$$

Acetic acid-induced writhing response

Fractions and AQR at doses ranging from 50 to 100 mg/kg, were administered to mice 1 h before i.p. injection of 0.6% (v/v) acetic acid, at a dose of 10 ml/kg. 2% Acacia in water was used as a control treatment while the reference group received Diclofenac sodium (DFS) as a standard. Writhings (a syndrome characterized by a wave of contraction of the abdominal musculature followed by extension of hind limbs) that occurred between 5 and 15 min after acetic acid were counted. A reduction in the writhing number as compared to

the control group was considered as evidence for the analgesia, which was expressed as percent inhibition of writhings [9].

Statistical evaluation

Data were expressed as means ± standard error mean. Statistical comparisons were made by using one-way ANOVA followed by Dunnet's t-test. The obtained parameters following administration of each fraction and AQR at each dose and standard were compared with those of control.

RESULTS

Acute toxicity

In mice, oral administration of the methanolic extract of pericarpium at a dose of 100–2000 mg/kg did not produce any overt changes in behavior or symptoms of toxicity. The animals showed sign of depression characterized by a decrease in spontaneous activity. The extract was found to be safe up to a dose 2000 mg/kg in mice.

Analgesic activity

The BOH-BPME, when given in doses of 50 and 100 mg/kg, elicited a significant analgesic activity in the hot plate as evidenced by increase in latency time in seconds (Table 1) as compared with vehicle control. The increase in latency time was dose dependant. BOH-BPME, 50 mg and 100mg showed significant (P < 0.01) increase in latency time and produced percentage protection were 120.28% and 156.91% respectively, while the standard showed 194.72 % protection. The effect at 100 mg/kg was comparable to the standard drug.

Table 1: Effect of BPME fractions, pentazocine on pain induced by hotplate

Treatment group	Dose mg/kg (p.o)	Latency period (sec)						
		0 h	30 min	60 min	90 min	120 min	150 min	180 min
Control (Group-I)	-	4.02±1.21	3.15±1.14	2.81±1.08	2.50±0.58	2.45±0.58	3.03±0.82	2.75±0.65
Pentazocin (Group-II)	10	3.22±0.97	7.51±1.31 (133.23) **	9.49±1.63 (194.72) **	8.54±1.56 (165.22) **	7.18±1.46 (122.98) **	6.27±1.35 (94.72) **	4.83±1.22 (54.97) *
BOH -BPME (Group-III)	50	2.86±0.85	5.68±1.27 (98.60) *	6.30±1.34 (120.28) **	5.44±1.20 (90.21) **	4.80±1.08 (67.83) *	4.18±0.96 (46.15)	3.48±0.82 (21.68)
BOH -BPME (Group-IV)	100	3.04±0.90	6.55±1.29 (115.46) **	7.81±1.52 (156.91) **	7.08±1.31 (132.89) **	6.82±1.25 (124.34) **	6.12±1.08 (101.32) **	4.70±0.97 (55.26) *
DCM- BPME (Group-V)	50	3.54±1.15	3.42±0.84 (-3.39)	3.31±1.06 (-6.50)	3.23±1.12 (-8.76)	2.86±0.94 (-19.21)	2.32±0.76 (-34.46)	2.37±0.73 (-33.05)
DCM- BPME (Group-VI)	100	3.80±1.24	3.20±1.20 (-15.79)	3.27±1.13 (-13.95)	2.90±0.94 (-31.32)	2.60±0.94 (-37.37)	2.61±1.08 (-26.05)	2.38±0.95 (-31.58)
AQR- BPME (Group-VII)	50	3.90±1.07	4.81±1.11 (7.95)	4.21±1.02 (14.25)	3.88±0.99 (-0.51)	3.07±0.79 (-21.28)	2.99±0.74 (-19.23)	2.50±0.51 (-18.72)
AQR- BPME (Group-VIII)	100	4.20±1.05	4.40±1.61 (4.76)	4.82±1.47 (14.76)	4.26±1.06 (1.43)	3.96±0.81 (-5.71)	3.78±0.89 (-10.00)	3.69±1.02 (-12.14)

Values expressed as mean ± S.D. of six mice in each group and units are in seconds. Percentages of protection against thermally induced pain by hotplate are in parentheses. *P < 0.05, **P < 0.01.

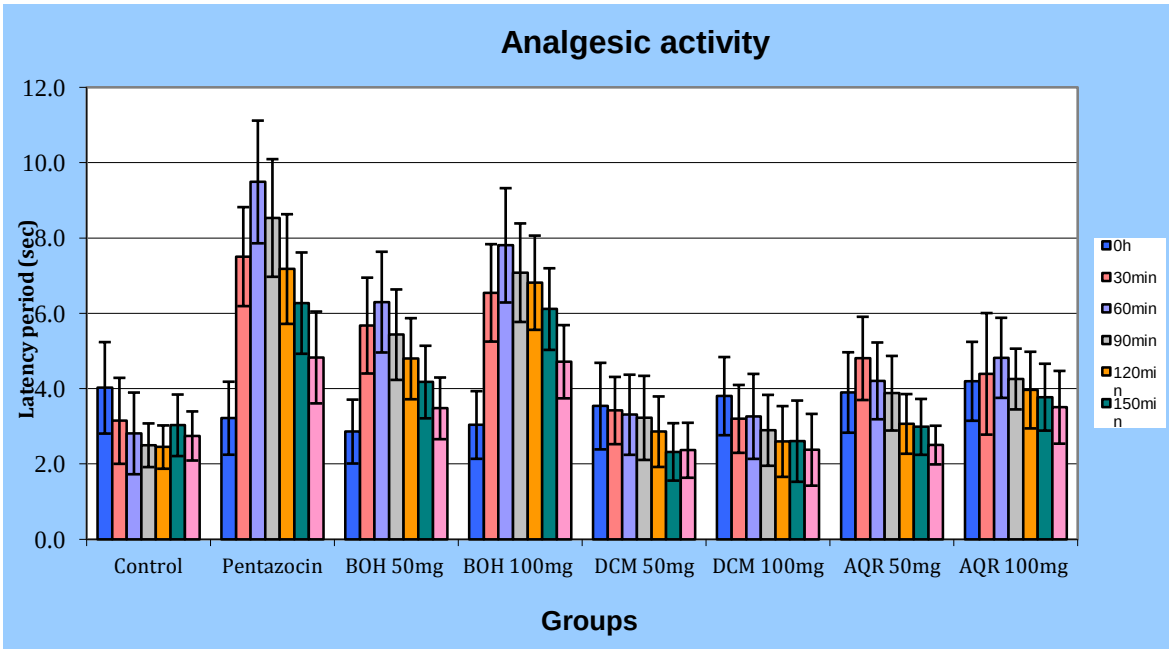

Fig. 1: Effect of BPME fractions, pentazocine on pain induced by hotplate

In acetic acid induced writhing test, the BOH-BPME significantly decreased the number of acetic acid induced writhing in mice when compared with vehicle control (Table.2). The decrease in writhing was dose dependant. The percentage inhibition of writhings with BOH- BPME at doses of 50 and100 mg was 23.33% and 37.50% respectively, while the standard showed 71.37 %. The effect at 100 mg was comparable to the standard drug. There was no significant activity observed with DCM-BPME and AQR in the both tests.

Table 2: Effect of BPME fractions on acetic acid-induced writhing in mice

Treatment group	Dose mg/kg (p.o)	Number of writhings	% Inhibition
Control (Group-I)	-	41.33±2.16	-
DFS (Group-II)	20	11.83±1.94**	71.37
BOH- BPME (Group-III)	50	31.67±2.73**	23.33
BOH- BPME (Group-IV)	100	25.83±2.14**	37.50
DCM- BPME (Group-V)	50	41.83±3.06	-01.20
DCM- BPME (Group-VI)	100	38.83±2.73	06.04
AQR- BPME (Group-VII)	50	40.17±3.25	02.80
AQR- BPME (Group-VIII)	100	37.67±2.94	08.85

Values expressed as mean ± S.D. of six animals in each group **P < 0.01, compared to control

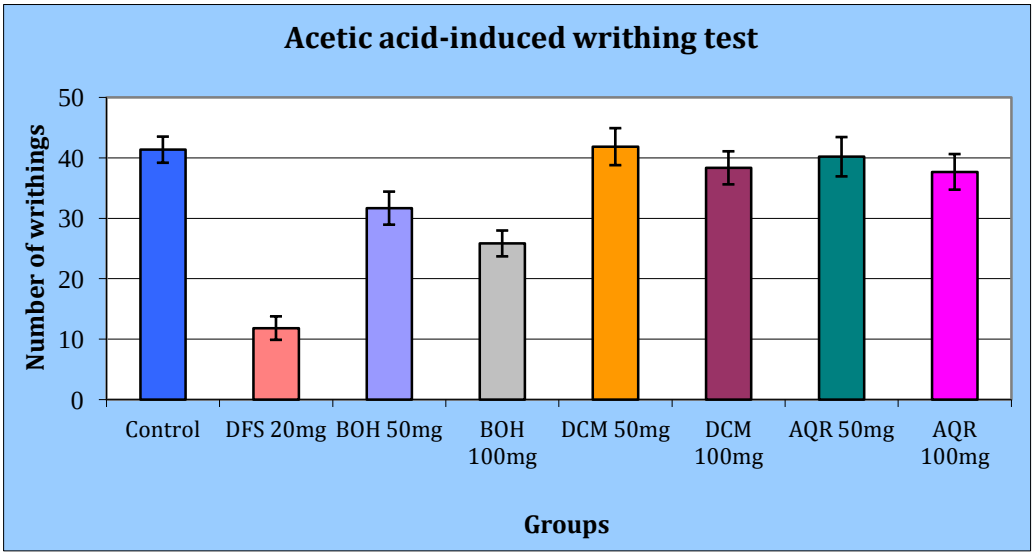

Fig 2: Effect of BPME fractions on acetic acid-induced writhing in mice.

DISCUSSION

Using two models of nociception activity it was found that BOH-BPME was effective in producing significant and dose-dependent analgesic activity. The antinociceptive tests used here involved both chemical visceral nociceptive stimuli (acetic acid) and thermal stimulus (hot plate). It was essential to use more than one test to confirm the analgesic activity, as it has been shown that some "false-positive" activity can be observed with agents that are not normally considered as analgesics [10]. The two tests used confirmed the analgesic activity of the BOH-BPME.

The increase in the reaction time of the mice on the hot plate and tail flick, following administration of the fractions suggests that the BOH-BPME possess central analgesic activity. The suppression of the BOH-BPME on the acetic acid - induced writhing suggests, however, that the extracts may act via local peritoneal receptors [11, 12]. As opioid receptors are also present in the periphery [13, 14], the possibility of the extracts acting on peripheral sites to cause anti-nociceptive effects has been ruled out. Therefore, it can be inferred that the extract may have produced antinociception via central and peripheral mechanisms.

This may provide some pharmacological rationale for the traditional use of this plant in pain. Several flavonoids isolated from medicinal plants have been reported to possess analgesic activity [15, 16]. As the fruit of this plant contain flavonoidal compounds; the analgesic activity of pericarpium of *B .roxburghii* may be due to them.

Isolation of the active components from this fraction is being carried out in the attempt to invent new compounds. Further research would be of interest to explain the exact mechanism of this analgesic effect.

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