Neuropharmacological Profile of *Balanites roxburghii*

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Summary

To investigate the neuropharmacological properties of methanolic extract of pericarpium of *Balanites roxburghii* (BPME). Locomotor activity, prodepressant activity, skeletal muscle relaxant activity, pentobarbital-induced sleeping time and spatial learning were carried out using mice and rats. The extract affected locomotor activity, reduced spontaneous motility, produced prodepressant activity, produced relaxation of skeletal muscle, significantly prolonged pentobarbital-induced sleeping time and reduced Spatial learning. A comparison was made between the action of BPME and control. The more significant depressant effect was observed with extract at a dose of 300mg/kg than 100mg/kg.The observations suggest that the fruit of *Balanites roxburghii* possesses potential dose dependent CNS-depressant action.

Introduction

Balanites roxburghii Planch. (Zygophyllacea) is a medicinal herb, common in drier parts of peninsular India, western Rajasthan and from south-East Punjab to West Bengal and Sikkim. It is also found in Myanmar. The fruit is considered useful in whooping cough and in skin diseases [1]. The fruit pulp, mixed with goat milk, is rubbed on the chest to cure pneumonia in children. In Ayurvedic, the fruit has a bitter sharp taste, analgesic actions [2]. It is also reported to possess anticonvulsant [3], antifertility, and hepatoprotective. Phytochemical investigations have revealed the presence of saponin glycosides, flavonoids, tannins, alkaloids, phenols from different parts of this plant [4]. Furostanol saponin, 3-rutinoside and 3-rhamnogalactoside were recorded from the fruits of related species Balanites *aegyptiaca* [5].

The pericarpium of this plant is used at Warangal, Andhra pradhesh in traditional medicine as a nervous tonic. The local people use the pericarpium as a remedy for the treatment of nervous conditions. Relative species *B.aegyptiaca* is used as CNS depressant [6]. Plants containing flavonoids and saponins have been shown to have neuropharmacological actions. Therefore, in view of the above observations, it was aimed to study the neuropharmacological profile of the plant.

Materials and Methods

Experimental Animals

Studies were carried out using male Wistar albino rats (180–220 g) and male 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 (38 x 23 x 10 cm) with not more than six animals per cage and maintained under standard laboratory conditions (temperature 25 ± 2^{o}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 (Regn.No.169/1999/CPCSEA).

Preparation of pericarpium extract

The plant (*B.roxburghii* planch) 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 2006. The pericarpium was separated, dried and powdered. The BPME was prepared by maceration of pericarpium powder (1000g) with methanol (3L) for 7 days with intermittent stirring. After extraction, the solvent was filtered and concentrated under reduced pressure. The extract obtained was stored at -20^{o}C until being used. Preliminary phytochemical investigations of the extracts were conducted as per the procedures described by Kokate [7] where revealed the presence of flavonoids, saponins, carbohydrates, phenolic compounds and alkaloids.

Drugs

Diazepam (Ranbaxy Laboratories, India), Pentobarbital (Sigma, Hyderabad), Piracetam (Intas Laboratories, India), Fluoxetine (Sigma) were used as reference standards in this study. They were administrated in the form of suspensions using gum acacia in water (5% w/v) as the suspending agent. The solvents used were of analytical grade. Methanol (BDH, Mumbai, India), Gum acacia in water (5% w/v) (M/S Hi-media, Mumbai, India) used as solvent and vehicle respectively. Diazepam is a drug known to have a anxiolytic and anti-stress agent while piracetam is a known nootropic agent.

Drug administration

Suspension of the BPME was prepared in 5% w/v gum acacia in water. The animals were divided into four groups each consisting of six animals. The control group received the vehicle, a 5% w/v gum acacia in water (1 ml/kg), whereas the test groups received BPME at a dose of 100 and 300 mg/kg and the standard group received the drugs like piracetam (100 mg/kg) or diazepam (2 mg/kg) or pentobarbital (50mg/kg) or fluoxetine (10mg/kg). In all the animal models, a single dose regimen was employed orally or intraperitoneally. Diazepam, pentobarbital, fluoxetine and piracetam were used to study the locomotor activity or skeletal muscle relaxant activity, sleeping time, antidepressant activity and nootropic activity respectively.

Acute toxicity test
The animals were divided into four groups each consisting of six animals. The BPME was administered orally in doses of 100, 300, 1000 and 2000 mg/kg to groups of mice and percentage mortality was noted beginning with 24 h up to a period of 7 days [8].

Statistical evaluation
Data were expressed as means ± standard deviation. Statistical comparisons were made by using one-way ANOVA followed by Newman-Keuls multiple comparison test.

Neuropharmacological activity

Locomotor activity
The locomotor activity (horizontal activity) was measured using an actophotometer. The movement of the animal (mouse) cuts off a beam of light falling on the photocell and a count was recorded and displayed digitally. The animals were divided into four groups and each group of mice was placed individually in the actophotometer for 10 min and basal activity score was obtained. The vehicle (1 ml/kg), BPME in a dose of 100 and 300 mg/kg and diazepam 2 mg/kg (standard) were administered orally and after 1 h, 2 h, 3 h, 4 h, 5h, 6h the mice were placed again in the actophotometer for recording the activity score as described earlier [9].

Forced swimming test in mice
The study was carried out according to the method of Porsolt et al (1977). The mice were placed in glass cylinders (40 cm height and 18 cm in diameter) containing water up to a height of 15 cm and maintained at 25°C. After 15 min in the water the mice were removed and allowed to dry in heated enclosure (32°C) before being returned to their home cages room for 30 min. They were placed in a cylinder 24 hrs later and the total duration of immobility was recorded for a period of 6 min. A mouse was regarded as immobile when it remained floating on water, making only small movements to keep its head above water. Test drugs (100mg and 300mg) or standard (Fluoxetine) were administered one hour prior to testing orally [10, 11].

Tail suspension test in mice
Mice were transported from the housing room to the testing area in their own cages and allowed to adapt to the new environment for 1 h before testing. Animals were divided in to four groups and treated with the test compounds or the vehicle by intraperitoneal injection 30 min prior to testing. For the test the mice were suspended on the edge of a shelf 58 cm above a table top by adhesive tape placed approximately 1 cm from the tip of the tail. The duration of immobility was recorded for a period of 6 min. Mice were considered immobile when they hang passively and completely motionless for at least 1 min [12].

Skeletal muscle relaxant activity
Animals (mice) remaining on Rota-Rod for 2-3 min at the speed of 16 rpm were selected for testing. The animals were divided in to four groups. Groups of animal received vehicle (1 ml/kg), BPME (100 and 300 mg/kg.), or diazepam (2 mg/kg.) orally , sixty min after the administration the same test was repeated at intervals of 30 min for 3h [13].

Effect on pentobarbital-induced sleeping time

Mice were divided into four groups. The test group received doses of the BPME, while the control group received an equal volume of the vehicle intraperitoneally. After 10 min, all the animals received 50 mg/kg (i.p.) of pentobarbital. The time that elapsed between loss and recovery of the righting reflex was taken as the sleeping time and was recorded, both for control and for drug-pretreated animals [14].

Spatial learning in the rectangular maze

The apparatus is a rectangular maze, divided in to chamber A, Maze and Chamber B. In chamber A, the rat is placed, which has a sliding door that is opened to allow the rat to enter the maze. The Maze, the animal has to explore. Chamber B, at other end of the maze in which the food pellets (reward) are kept. All the three divisions of the maze are covered by hinged separate top-lids so as to maintain a uniform environment inside the maze. Electrical system provides indication when the rat is placed in Chamber A, when it leaves and enters the maze, and when enters chamber B, thus enabling the reaction time to be noted without observing the animal. Animals were trained on a daily basis in the maze to collect the food pellets for three days. They were grouped and administered with test or standard drug (piracetam-100 mg/kg) orally, one hour prior to testing. The total time taken by the animal in traversing the maze was recorded in seconds [15].

Results

Acute toxicity

In mice, oral administration of the BPME 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, relaxation of skeletal muscle and decreased struggling when handled. The extract was found to be safe up to a dose 2000 mg/kg in mice.

Asessment of locomotor activity

The BPME at a dose of 100 and 300mg/kg produced significant reduction ($p<0.001$) in locomotor activity as compared to the control animals receiving only the vehicle. There is significant reduction with low and high doses up to 4 hours and up to 5h with Diazepam (Table 1).

Forced swimming

When tested in the forced swimming test, the BPME at a dose of 100mg and 300 mg/kg given by oral route, significantly increased the duration of immobility time in comparison to the vehicle treated group with variation 47% and 78%. It indicated that the extract showing prodepressant activity. Fluoxetine significantly decreased the duration of immobility and exhibited antidepressant activity (Table 2).

Table 1. Effect of BPME and diazepam on locomotor activity in mice, using actophotometer apparatus

Treatment group	Dose mg/kg (p.o)	Locomotor activity scores in 10 min (Mean±S.D)						
		Pretreatment	Post treatment					
		0 h	1 h	2 h	3 h	4 h	5 h	6 h
Control (Group-I)	-	1740±79	1481±70	1449±65	1598±90	1576±98	1601±90	1726±61
Diazepam (Group-II)	2	1755±86	505±79***	572±79***	598±89***	854±96***	1338±92	1718±75
BPME (Group-III)	100	1701±68	820±60***	834±66***	955±102***	1257±61*	1312±96	1567±93
BPME (Group-IV)	300	1719±83	810±93***	643±87***	675±82***	1167±69**	1234±65*	1465±101

All values are expressed as mean of six mice in each group from three observations. Statistically significant *p<0.05, **p<0.01, ***p<0.001 compared to control respective time.

Table-2. Effect of BPME on the forced swimming test

Treatment group	Dose mg/kg (p.o)	Duration of immobility (sec)	Variation (%)
Control (Group-I)	-	130.33±12.01	-
Fluoxetine (Group-II)	10	93.00±9.25	-28.64
BPME (Group-III)	100	191.33±14.21***	46.80
BPME (Group-IV)	300	232.17±10.44***	78.14

All values are expressed as mean of six mice in each group. Statistically significant ***p<0.001 compared to control.

Tail suspension

The BPME at a dose of 100mg and 300 mg/kg significantly increased the duration of immobility time with variation 36% and 63%. It confirmed the forced swimming test that the extract showing prodepressant activity (Table 3).

Table-3. Effect of BPME on the tail suspension test

Treatment group	Dose mg/kg (i.p)	Duration of immobility (sec)	Variation (%)
Control (Group-I)	-	122.67±14.53	-
Fluoxetine (Group-II)	10	80.50±10.50	-34.38
BPME (Group-III)	100	166.83±14.48***	36.00
BPME (Group-IV)	300	199.83±15.61***	62.90

All values are expressed as mean of six mice in each group. Statistically significant ***p<0.001 compared to control.

Skeletal muscle relaxant activity

The BPME 100mg/kg and diazepam significantly produced skeletal muscle relaxation up to 90 min and 300mg/kg produced up to 120 min (Table 4).

Table 4. Effect of BPME and diazepam on muscle-relaxant activity in mice, studied using rota rod apparatus

Treatment group	Dose mg/kg (p.o)	Remaining time in seconds on a revolving rod (Mean±S.D)					
		0 h	60 min	90 min	120 min	150 min	180 min
Control (Group-I)	-	101.40±5.64	99.20±8.67	96.60±7.02	100.40 ±9.13	98.80±8.93	101.60±8.32
Diazepam (Group-II)	2	106.20±6.69	72.80±7.12***	66.40±5.68***	77.80±9.76***	86.80±8.58*	98.00±6.39
BPME (Group-III)	100	97.40±5.78	81.80±8.47**	75.00±3.41***	83.60±6.47**	89.20±7.11	96.20±5.71
BPME (Group-IV)	300	96.20±6.18	84.40±4.84**	62.20±6.76***	78.40±5.15***	83.60±4.50*	92.40±5.61

All values are expressed as mean of six mice in each group. Statistically significant *p<0.05, **p<0.01, ***p<0.001 compared to control respective time.

Pentobarbital-induced sleeping time

The BPME significantly decreased the time required for onset of loss of the righiting reflex, and increased pentobarbital induced sleeping time. It was statistically significant ($p<0.001$) at two doses (Table 5).

Table-5. Potentiation of pentobarbital sleeping time of BPME

Treatment group	Dose mg/kg (i.p)	Duration of sleep (min)	% increase in sleeping time
Pentobarbital (Group-I)	50	67.00± 8.40	-
BPME (Group-II)	100	177.40±23.20***	165
BPME (Group-III)	300	238.60±18.79***	255

All values are expressed as mean of six mice in each group. Statistically significant ***p<0.001 compared to control.

Spatial learning

The BPME did not produce any significant change in time for spatial learning at a dose of 100mg or 300 mg/kg. However the piracetam treated group showed a statistically significant increase in the spatial learning (Table 6).

Table-6. Effect of BPME on the spatial learning of rats in the rectangular maze

Treatment group	Dose mg/kg (p.o)	Total time taken by the animal in traversing the maze (sec)	
		Pretreatment	After treatment
Control (Group-I)	-	48.67±6.83	45.5±6.75
Piracetam (Group-II)	100	42.67±7.26	15.33±4.80 ***
BPME (Group-III)	100	46.17±8.35	43.0±9.82
BPME (Group-IV)	300	41.50±6.72	45.17±7.05

All values are expressed as mean of six rats in each group. Statistically significant p<0.05 compared to control.

Discussion

In the light of the literature on *Balanites roxburghii*, an attempt was made for the first time to study the neuropharmacological activity. In preliminary experiments, the toxicity of the extracts of *B. roxburghii* was tested, and judging from the high doses that were tolerated without significant mortality or overt signs of toxicity, it was observed that the plant extract was of relatively low toxicity.

Preliminary chemical investigations revealed the presence of flavonoids, saponins (steroids and triterpenoids) and its glycosides in the extracts of *B. roxburghii*. There is large number of chemical compounds present in the plant endowed neuro pharmacological activity and supported the traditional uses. The neuropharmacological activity of BPME was studied in various behaviour animal models.

Locomotor activity is considered as an index of alertness or a measure of the level of excitability of the CNS [16] and any decrease of this activity may be closely related to sedation resulting from depression of the central nervous system [17].

The decreased locomotor activity of BPME was significant and dose dependent and it could be due to inhibitory effects of the extract on the CNS. Several flavonoids and saponins isolated from medicinal plants have been reported to posses central nervous system depressant activity [18, 19]. The fruit of *B. roxburghii* was reported to posses flavonoids and saponins. These compounds may contribute to the central nervous system (CNS) depressant activity of BPME observed.

Diazepam is a standard CNS depressant drug, has been shown to exert it depressant effect by enhancing GABA-mediated inhibition in the brain [20]. It is possible that depression may be due to enhancing GABA neurotransmission. Since the BPME, showed depressant action, it is probable that it may be interfering with gabaergic mechanism(s) to exert it depressant effect.

Taking into consideration the locomotor activity of BPME observed in the above cited test, it was decided, in addition, to investigate the role of BPME in depressant animal models. For this, it was realized the forced swimming and tail suspension tests, which have been useful experimental models for screening antidepressant activity. Drugs with established antidepressant activity, as imipramine or fluoxetine, reduce the time during which the animals remain immobile [21]. The results showed that BPME, at both doses, was able to increase the total time spent in immobility of mice exposed to those tests, indicating depressant activity, in opposite to the psychostimulants effects.
A deficit in motor coordination would very likely affect performance in the forced swimming and tail suspension tests. In this way, it was aimed to investigate the effects of BPME in the rota rod test, a classical animal model used to evaluate peripheral neuromuscular blockage. The results showed that BPME, similar from diazepam, had significant effect on the motor coordination of the animals on rota rod test.

Thus, the observed decrease in the remaining time probably is related to peripheral neuromuscular blockage, but may involve neurons that control central depressant activity (22).

Pentobarbital sleeping time test was also used to confirm the possible depressive-like effects observed with BPME in the previous tests of this study. Decrease in sleep latency and increase in sleeping time are classically related to central nervous system (CNS) depressant drugs [23]. The results showed that BPME, at both doses, decreased the sleep latency time and increased the duration of sleeping, which possibly confirm the depressant activity of CNS detected before.

Additionally, the decrease in locomotor activity works to the advantage of the plant do not showing nootropic activity. CNS depressants most act on the brain as benzodiazepine and they affect the neurotransmitter gamma-amino butyric acid (GABA). Neurotransmitters are brain chemicals that facilitate communication between brain cells. GABA works by decreasing brain activity and affect the cognition. Although different classes of CNS depressants work in unique ways, ultimately it is their ability to increase GABA activity that produces a drowsy or calming effect. The BPME neither increase nor decrease the cognitive of the animal, but piracetam increased the cognition.

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