Anticonvulsant Activity of Pericarpium Extract of *Balanites Roxburghii Planch* in Mice

K. Thirupathi, D.R. Krishna, B. Ravi Kumar, P. Tirumala Rao[1] and G. Krishna Mohan *

University College of Pharmaceutical Sciences, Kakatiya University, Warangal- India
Rajah RSRKRR College, Bobbili, Vijaya nagaram, Andhra University[1]

Summary

The anticonvulsant effect of methanolic extract from the pericarpium of *Balanites roxburghii* on maximal electroshock (MES) or pentylenetetrazole (PTZ) in male mice examined in this study. This medicinal plant belongs to the zygophyllaceae family and is popularly used in tropical africa as central nervous system depressant. The extract of *Balanites* (orally) was administered in mice at single doses (100 or 300 mg/kg). The extract suppressed hind limb tonic extensions (HLTE) induced by MES and also exhibited protector effect in PTZ-induced seizures, at 300 mg/kg dose. In conclusion, we showed that the methanolic extract of *Balanites* has anticonvulsant effect in the both models, suggesting their possible depressant action in the central nervous system. The activity reported was dose dependent.

Keywords: *Balanites roxburghii*; Anticonvulsant effects; Maximal electroshock; Pentylenetetrazole; Mice

* Corresponding author. drgkrishnamohan@yahoo.co.in Tel.: +009849793783

Introduction

Balanites roxburghii is a medicinal herb, found in Bengal, drier parts of India and Myanmar. In Ayurvedic, the fruit has a bitter sharp taste, analgesic action [1]. and in Sudanese folk medicine for treatment of jaundice[2]. In Egyptian folk medicine, the fruits of *Balanites* (after removal of the apocarps) are commonly used as an oral antidiabetic drug and in tropical Africa as CNS depressant [3]. It is also reported to possess antifertility [4], modest hepatoprotective, anti-inflammatory, antinociceptive and antioxidant activities [5]. Preliminary phytochemical investigations have revealed the presence of saponin glycosides, flavonoids, tannins, alkaloids, phenols from different parts of this plant [6]. Furostanol saponin, 3-rutinoside and 3-rhamnogalactoside were recorded from the fruits of the same plant [7,8].

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. Plants containing flavonoids have been shown to have neuropharmacological actions [9]. Therefore, in view of the above observations, we planned to study the neuropharmacological profile of the plant.

Materials and Methods

Plant material

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 2008.

Preparation of pericarpium extract

The pericarpium was separated, dried and powdered. The Powdered material was macerated with methanol and filtered. So obtained filtrate was concentrated and stored at -20^{o}C until being used. Preliminary phytochemical investigations of the extracts were conducted as per the procedures described by Kokate [10] where revealed the presence of flavonoids, saponins, carbohydrates, phenolic compounds and alkaloids.

Drugs

PTZ, Diazepam and Phenobarbitone were purchased from Sigma Chemical Co (Hyderabad, INDIA). Different concentrations of the drugs were prepared freshly by suspending in gum acacia in water. The solvents used were of analytical grade. Methanol (BDH, Mumbai, India) and Gum acacia in water (M/S Hi-media, Mumbai, India) used as solvent and vehicle respectively.

Animals

Male Swiss albino mice (20–25 g; Mahaveera Enterprises, No.146/1999/CPCSEA, India) were used throughout this study. The animals were grouped and maintained at constant room temperature (25 ± 2^{o}C) and submitted to 12-h light: 12-h dark cycle with food and water available ad libitum. They were housed in standard polyacrylic cages (38 x 23 x 10 cm) and acclimated at least 2 days before experiments. All procedures described were reviewed and approved by the Institutional animal ethical committee (Regn.No.169/1999/CPCSEA).

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Drug administration

Suspension of the methanolic extract of pericarpium was prepared in 2% 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 2% w/v gum acacia in water (1 ml/kg), whereas the test groups received methanolic extract at a dose of 100 and 300 mg/kg and the standard group received the drugs like Diazepam (3mg/kg) or Phenobarbitone (20mg/kg). In all the animal models, a single dose regimen was employed.

Toxicity assessment

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 was noted beginning with 24 h up to a period of 7 days [11].

Data analysis

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

Convulsive tests **[12]**

MES-induced seizures

Electroconvulsive shock (30 mA, 50Hz for 0.2 sec) was delivered through corneal electrodes to induce hind limb tonic extensions (HLTE) in mice. The extract was administered orally at the doses of 100 and 300mg/kg into test groups. Gum acacia in water and Diazepam were administered orally into two groups of animals as control and positive control groups, respectively. Electroconvulsive shock was delivered 60 min after the administration of drugs. Occurrence of HLTE and duration of seizures were noted closely for 2 min. The animals that did not exhibit HLTE were considered protected. Percentage of inhibition of seizures relative to controls was calculated.

PTZ-induced seizures

PTZ at the dose of 80 mg/kg (minimal dose needed to induce convulsions) was injected i.p. to induce clonic-tonic convulsions in mice. Doses of 100 and 300mg/kg of the extract were administered orally into test groups. Gum acacia in water and Phenobarbitone (20 mg/kg) were administered orally into two groups of animals as control and positive control groups, respectively. PTZ was injected i.p. 60 min after the administration of drugs. Occurrence of HLTE and duration of seizures were noted. If no HLTE occurred during the time limit, the animals were considered protected. Percentage of inhibition of seizures relative to controls was calculated.

Results

Toxicity assessment

In mice, oral administration of the methanolic extract of pericarpium at a dose of 100 to 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 2g/kg in mice.

MES-induced seizures

Male albino mice pretreated with the methanolic extract have been significantly protected from convulsions induced by electroshock one hour post-dosing. The percentage inhibition achieved at the doses 100 and 300mg/kg were 46% ($p<0.001$) and 69% ($p<0.001$) respectively. Extract at both the doses, dose dependently prolonged the onset of convulsions in the treated group compared to vehicle treated control group (Table 1).

Table. 1. Effect of pericarpium extract of *Balanites roxburghii* on tonic seizures induced by maximal electroshock in male mice

Treatment group	Dose mg/kg (p.o)	Onset time (Sec)	Duration of HLTE (Sec)	Percentage inhibition of convulsions
Control (Group-I)	--	1.71±0.34	96.93±4.29	-----
Diazepam (Group-II)	3	0	0	100
Extract (Group-III)	100	6.37±10.63	52.66±4.80†	45.67
Extract (Group-IV)	300	11.09±0.86	30.46±4.04†	68.57

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

PTZ-induced seizures

Animals treated with methanolic extract at a dose of 300mg/kg showed alteration in the occurrence of HLTE and duration of seizures significantly as related to controls in the model of convulsion induced by pentylenetetrazole in mice but did not alter significantly at 100mg/kg. Percentage of inhibition of seizures for 300 mg/kg relative to controls was 44.34% (Table 2).

Table. 2. Effect of pericarpium extract of *Balanites roxburghii* on pentylenetetrazole-induced seizures in male mice

Treatment group	Dose mg/kg (p.o)	Onset time (Sec)	Duration of HLTE (Sec)	Percentage inhibition of convulsions
Control (Group-I)	--	52.12±4.06	38.88±5.10	-----
Phenobarbitone (Group-II)	20	0	0	100
Extract (Group-III)	100	60.10±4.53	34.55±4.26	11.42
Extract (Group-IV)	300	66.74±5.21	21.64±4.19†	44.34

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

Discussion

Data from this study show that *Balanites roxburghii* significantly increases the onset time and decreases the duration of seizures by electroconvulsive shock. The study also revealed that the onset of tonic convulsion produced by PTZ was significantly delayed and also duration of seizures was prolonged.

MES and PTZ may be exerting their convulsant effects by inhibiting the activity of gamma aminobutyric acid (GABA) at $GABA_A$ receptors [13]. Gamma aminobutyric acid is the major inhibitory neurotransmitter which is implicated in epilepsy. The enhancement and inhibition of the neurotransmission of GABA will attenuate and enhance convulsion, respectively [14].

Diazepam and Phenobarbitone, standard antiepileptic drugs, have been shown to exert their antiepileptic effects by enhancing GABA-mediated inhibition in the brain [15]. It is possible that both standard drugs antagonize MES and PTZ convulsions in this study by enhancing GABA neurotransmission.

Since the methanolic extract of *B. roxburghii* delayed the occurrence of MES and PTZ convulsions, it is probable that it may be interfering with gabaergic mechanism(s) to exert their anticonvulsant effect.

Phytochemical tests carried out in the present study show that the pericarpium contains saponins, tannins and flavonoids. The plants containing saponins or flavonoids exhibit anticonvulsant activity [16,17]. As the saponins and flavonoids present in *B. roxburghii* might contribute to the anticonvulsant activity of the plant.

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