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EVALUATION OF ANTI-EPILEPTIC ACTIVITY OF CHLOROFORM EXTRACT OF LEAVES OF *BARRINGTONIA RACEMOSA* IN MICE

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Abstract:
Objective: The present study was aimed to evaluate antiepileptic activity of chloroform extract of leaves of Barringtonia racemosa (CBR) in mice. Methods: The antiepileptic activity of CBR was evaluated in mice at 30, 100, and 300 mg/Kg, p.o. by the convulsions induced by maximum electroshock (MES), Pentylenetetrazole (PTZ) and Isoniazid (INH) methods. Statistical analysis was carried out by one-way analysis of variance (ANOVA) followed by Dunnett's t test. Results: In MES method, CBR (30, 100, and 300 mg/Kg) inhibited convulsions significantly potent than Diazepam. In PTZ method, CBR inhibited convulsions potent than Phenobarbitone sodium (PS). In INH method, CBR delayed the onset of convulsions less potent than Diazepam. Conclusion: In Present investigation, CBR showed significant dose dependent antiepileptic effect potent than Diazepam and PS.
Keywords: *Epilepsy, Barringtonia racemosa, Pentylenetetrazole, Isoniazid.*

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INTRODUCTION:

Epilepsy is a brain disorder characterized by convulsive seizures or loss of consciousness or both. It is a major neurological disorder and upto 5% of the world population have epilepsy in their lifetime. Drug therapy of epilepsy with currently available anti-epileptic drugs (AED) is associated with dose-related side effects and chronic toxicity that involves virtually every organ system [1]. This made man to search for alternative medicine from natural source.

Barringtonia racemosa belongs to the family of Lecythidaceae. In Unani medicine, leaves of this plant were used traditionally to treat epilepsy [2]. Till today there were no reports to justify this claim. Hence the present study was carried out to evaluate antiepileptic activity of various extracts of *Barringtonia racemosa*.

MATERIALS AND METHODS:

Drugs and Chemicals:

Isoniazid (S.D Fine-Chem. Ltd), Diazepam (Ranbaxy), Phenobarbitone sodium (Bayer AG) and Pentylenetetrazole (Sigma Aldrich Chemical Co.). All other chemicals used are of Merck, India (LR grade).

Plant collection:

The leaves of *Barringtonia racemosa* were collected from Tirupati, Andhra Pradesh, India. Leaves were identified and authenticated by Botanist, Dr. K. Madhava Chetty, Department of Botany, Sri Venkateswara University, Tirupati. The plant specimen was deposited at Sri Venkateswara University Herbarium, Tirupati with voucher number 529.

Preparation of the extract:

The fresh leaves of *Barringtonia racemosa* were collected; shade dried and was made in to coarse powder. Then chloroform extract (CBR) was prepared by following maceration method [3].

Preliminary Phytochemical Studies:

Chloroform extract of plant was subjected to qualitative chemical tests for various phytoconstituents like alkaloids, carbohydrates, flavonoids, lipids, proteins, saponins, steroids and tannins [4].

Pharmacological Investigations:

Animals:

Young adult Swiss albino rats of either sex weighing (150-180 g) and Swiss albino mice of either sex, weighing (25–30 g) were procured from M/s Mahavir Enterprises, Hyderabad. They were housed in standard polypropylene cages and kept under controlled room temperature (24±20C; relative humidity 60-70%) in a 12h light – dark cycle. The rats and mice were given a standard laboratory diet and water *ad libitum.* The animals were acclimatizated before the study. The experimental protocol was approved by the Institutional Animals Ethics Committee (IAEC) of Talla Padmavathi College of Pharmacy, Warangal, Andhra Pradesh (CPCSEA no. 1505/PO/a/11/CPCSEA).

Acute toxicity studies:

Acute toxicity study was performed for the extract to ascertain safe dose by acute oral toxic class method of Organization of Economic Co-operation and Development, as per 420 guidelines (OECD). Young adult Swiss albino rats and Swiss albino mice of either sex were used for the study. The chloroform extract of *Barringtonia racemosa* leaves was tested in both the species upto a dose of 2000 mg/kg, body weight [5].

Evaluation of Anti-epileptic activity:

Maximum Electroshock (MES) in mice:

Mice were divided into five groups of six mice each. Treatment schedule is as follows.

Group I: Control (DMSO)
Group II: CBR (30 mg/kg), p.o.
Group III: CBR (100 mg/kg), p.o.
Group IV: CBR (300 mg/kg), p.o.
Group V: Diazepam (3 mg/kg), p.o.

The test was started one hour after oral treatment with the extract or the vehicle or the standard. Tonic hind limb extensions (THLE) were induced by an apparatus with corneal electrodes. The intensity of the stimulus was dependent on the apparatus, eg: 45 mA, 50Hz for 0.2 sec has been used. Percentage of inhibition of convulsions relative to control was calculated [6].

$$\text{Percentage of Inhibition} = \frac{\text{Control} - \text{Treated}}{\text{Control}} \times 100$$

Pentylenetetrazole (PTZ)-induced convulsions:

Mice were randomly allotted to five different groups of six each. Treatment schedule is as follows.

Group I: Control (DMSO)
Group II: CBR (30 mg/kg), p.o.
Group III: CBR (100 mg/kg), p.o.
Group IV: CBR (300 mg/kg), p.o.
Group V: Phenobarbitone sodium (40 mg/kg), i.p.

Mice belonging to Group I was administered with pentylenetetrazole (PTZ) (75 mg/kg, i.p.) one hour after vehicle. Mice belonging to Group V received PTZ, 15 min after phenobarbitone sodium (40 mg/kg, i.p.). Mice belonging to Group II, III and IV mice

received different doses of CBR, p.o. one hour before PTZ. Onset time as well as duration of convulsions was recorded [7].

Isoniazid (INH)-induced convulsions:
Five Groups of six Swiss albino mice (25–30 g) were used. Treatment schedule is as follows.
Group I: Control (DMSO)
Group II: CBR (30 mg/kg), p.o.
Group III: CBR (100 mg/kg), p.o.
Group IV: CBR (300 mg/kg), p.o.
Group V: Diazepam (4 mg/kg), i.p.
One hour after the administration of vehicle or chloroform extract of *Barringtonia racemosa* leaves (CBR), isoniazid at a dose of 300mg/kg, s.c. was administered to mice belonging to Group I, II, III, IV and 15 min after administration of diazepam to mice belonging to Group V. The mice were placed in isolated perplex chamber and the latency of convulsions was recorded [8].

Statistical analysis:
The data was analyzed using one-way analysis of variance (ANOVA), followed by Dunnett's test and $p<0.05$ was considered as statistically significant. The data was expressed as mean ± Standard deviation (SD).

RESULTS:

Preliminary Phytochemical Studies:
Preliminary phytochemical studies indicated the presence of various phytoconstituents like steroids, flavanoids and lipids (Table 1).

Pharmacological Investigations:
Acute toxicity studies:
In acute toxicity study, the chloroform extract was found to be safe upto 1000 mg/kg, p.o. So, the doses of 30, 100 and 300 mg/kg, p.o. were selected to evaluate antiepileptic activity.

Maximal electroshock-induced convulsions in mice:
The average time of onset, duration of THLE and percentages of inhibition of convulsions were presented in Table 2.
CBR treated mice showed the onset time as 2.96±0.08, 3.25±0.08 and 4.15±0.06 sec ($p<0.01$) respectively at the doses of 30, 100 and 300 mg/kg, p.o. The standard group mice (diazepam 3 mg/kg, p.o.) showed 2.46±0.15 sec ($p<0.01$).
Albino mice pretreated with CBR at the doses of 30, 100 and 300 mg/kg, p.o. showed the duration of 50.06±0.38, 42.09±0.20 and 37.89±0.19 sec ($p<0.01$) respectively. The standard group animals (diazepam 3 mg/kg, p.o.) showed 49.97±0.32 sec ($p<0.01$).
The percentage inhibition achieved in mice pretreated with CBR were 57.90% (30 mg/kg), 64.60% (100 mg/kg) and 68.14% (300 mg/kg) ($p<0.01$) respectively when compared to control group animals.

Pentylenetetrazole (PTZ)-induced convulsions in mice:
The average time of onset, duration of convulsions and percentages of inhibition of convulsions were presented in Table 3.
Animals which received CBR exhibited the onset time as 4.59±0.38, 6.56±0.03 and 7.31±0.09 min ($p<0.01$) respectively at the doses of 30, 100 and 300 mg/kg, p.o. The standard group animals (Phenobarbitone sodium, 40 mg/kg, i.p.) showed 6.46±0.08 min ($p<0.01$).
CBR treated mice exhibited the duration as 4.57±0.02, 3.59±0.23 and 2.59±0.23 min ($p<0.01$) respectively at the doses of 30, 100 and 300 mg/kg, p.o. The standard group mice (Phenobarbitone sodium 40 mg/kg, i.p.) showed 8.43±0.12 min ($p<0.01$).
The percentage inhibition achieved in animals which received CBR at the doses of 30, 100 and 300 mg/kg, p.o. were 73.45%, 79.15% and 84.96% ($p<0.01$) respectively when compared to control group animals.

Isoniazid (INH)-induced convulsions in mice:
The average latency of convulsions were presented in Table 4. Albino mice pretreated with CBR showed the latency of convulsions of 39.49±0.10, 47.06±0.26 and 52.45±0.18 min ($p<0.01$) respectively at the doses of 30, 100 and 300 mg/kg, p.o. The standard group animals (diazepam 4 mg/kg, i.p.) showed 54.44±0.17 min ($p<0.01$).

Table No. 1: Preliminary Phytochemical Studies

Phytoconstituents	CBR
Alkaloids	- ve
Steroids	+ ve
Carbohydrates	- ve
Tannins	- ve
Flavonoids	+ ve
Saponins	- ve
Lipids	+ ve
Proteins	- ve

Table No. 2: Effect of CBR on maximal electroshock-induced convulsions in mice

Group (n=6)	Treatment	Onset of THLE (sec)	Duration of THLE (sec)	Percentage inhibition of convulsions
I	DMSO	1.36±0.18	118.91±0.51	-
II	CBR (30 mg/kg)	2.96±0.08**	50.06±0.38**	57.90**
III	CBR (100 mg/kg)	3.25±0.08**	42.09±0.20**	64.60**
IV	CBR (300 mg/kg)	4.15±0.06**	37.89±0.19**	68.14**
V	Diazepam (3 mg/kg)	2.46±0.15**	49.97±0.32**	57.98**

CBR: Chloroform extract of *Barringtonia racemosa*; Values were mean±SD (n=6). Statistical significance was determined by ANOVA, followed by Dunnett's t test (n=6); ***p*<0.01 when compared to Group I (control).

Table No. 3: Effect of CBR on Pentylenetetrazole (PTZ)-induced convulsions in mice

Group (n=6)	Treatment	Onset of convulsions (min)	Duration of convulsions (min)	Percentage inhibition of convulsions
I	DMSO	3.42±0.12	17.22±0.13	-
II	CBR (30 mg/kg)	4.59±0.38**	4.57±0.02**	73.45**
III	CBR (100 mg/kg)	6.56±0.03**	3.59±0.23**	79.15**
IV	CBR (300 mg/kg)	7.31±0.09**	2.59±0.23**	84.96**
V	Phenobarbitone sodium (40 mg/kg, i.p.)	6.46±0.08**	8.43±0.12**	51.04**

*CBR: Chloroform extract of Barringtonia racemosa; Values were mean± D (n=6). Statistical significance was determined by ANOVA, followed by Dunnett's t test (n=6); **p<0.01 when compared to Group I (control).*

Table No. 4: Effect of CBR on Isoniazid (INH)-induced convulsions in mice

Group (n=6)	Treatment	Latency of convulsions (min)
I	DMSO	24.51±0.10
II	CBR (30 mg/kg)	39.49±0.10**
III	CBR (100 mg/kg)	47.06±0.26**
IV	CBR (300 mg/kg)	52.45±0.18**
V	Diazepam (4 mg/kg, i.p.)	54.44±0.17**

*CBR: Chloroform extract of Barringtonia racemosa; Values were mean±SD (n=6). Statistical significance was determined by ANOVA, followed by Dunnett's t test (n=6); **p<0.01 when compared to Group I (control).*

DISCUSSION:

It is a major neurological disorder and upto 5% of the world population have epilepsy in their lifetime [1]. GABA is known to be an important inhibitory neurotransmitter in the brain. One major factor in epileptogenesis seems to be a decreased function of GABAA synapses [6].

Barringtonia racemosa belongs to the family of Lecythidaceae. In Unani medicine, leaves of this plant were used traditionally to treat epilepsy [2]. Preliminary phytochemical studies showed the presence of steroids, flavanoids and lipids.

Based on acute toxicity study, antiepileptic activity was evaluated at three dose levels (30, 100 and 300 mg/kg, p.o.).

Antiepileptic activity of *Barringtonia racemosa* was evaluated in three models namely maximum electroshock (MES), pentylenetetrazole (PTZ) and isoniazid (INH)-induced convulsions in mice which are simple and extensively studied models with minimal animal preparation for evaluating antiepileptic activity.

In MES model, the time of onset of THLE in control group animals was very less when compared to the extract and standard treated animals and duration of THLE was greater when compared to the extract and standard treated animals. CBR treated animals exhibited significant antiepileptic activity and more percentage inhibition of convulsions at both 100 and 300 mg/kg when compared to diazepam 3.0 mg/kg (57.98%, $p<0.01$).

In PTZ model, the time of onset of convulsions in control group animals was very less when compared to the animals which received extract and standard and duration of convulsions was greater when compared to the animals which received extract and standard. Animals which received CBR exhibited significant antiepileptic activity and more percentage of inhibition of convulsions when compared to Phenobarbitone sodium (40 mg/kg, i.p.) treated animals (51.04%, $p<0.01$).

Albino mice pretreated with CBR at the doses of 30, 100 and 300 mg/kg were provided significant protection from convulsions induced by electroshock and PTZ.

In INH model, the latency of convulsions in control group animals was very less when compared to the extract and standard group animals. All the three doses of CBR showed the latency time more than that of control group animals and less than that of standard group animals i.e., diazepam. Although CBR exhibited significant delay in the latency of convulsions but failed to protect the animals from mortality.

CONCLUSION:

Presence of steroids and flavonoids may be responsible for the antiepileptic activity of CBR. Further significant antiepileptic activity of *Barringtonia racemosa* was found in PTZ model. It has been found that *Barringtonia racemosa* can effectively antagonize the action of PTZ which is a GABA-antagonist. The GABA-agonist action of *Barringtonia racemosa* might be responsible for enhanced GABAergic neurotransmission. Present study supports the use of *Barringtonia racemosa* in Unani medicine for the treatment of epilepsy.

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