ABSTRACT

Objective: The present report is an investigation of anti seizure activity of Barringtonia asiatica (L.), a well known plant which is being used in Indian Traditional Medicines for epilepsy, nervous disorders, bronchitis and liver ailments. Method: The methanolic (90%) extract of Barringtonia asiatica (L.) (MEBA) was subjected to acute toxicity and then screened for antiepileptic activity on Maximal Electroshock (MES) and Pentylenetetrazole (PTZ) induced seizures models in albino wistar rats. Acute toxicity of extract was non toxic up to the recommended dose 2000mg/kg body weight orally as per OECD guidelines No.423. Animals were pretreated with MEBA at the doses of 250 and 500mg/kg body weight. Results and Discussion: The study reported the significant delay in clonic seizure induced by PTZ and dose dependent decrease in duration of hindleg extensor phase in MES model. In MES model, MEBA showed significant reduction in duration of hindleg extension with 250 mg/kg dose and effect was dramatically reduced with 500mg/kg. Similar dose dependent results were obtained in PTZ model by delayed the onset of clonic convulsions. The complete protective effect against mortality was reported in both the tests. Conclusion: This study predicted possible mechanism of the formulation mediated through chloride channel of the GABA or benzodiazepine receptor complex. However, the exact mechanism of action is not clear, but attributed to its antiepileptic effect. The methanolic extract of Barringtonia asiatica (L.) deserves further investigation for detailed elucidation of active constituents and the mechanisms of action.

KEYWORDS: Antiseizure activity, Traditional Medicine, Barringtonia asiatica, Pentylenetetrazole.
INTRODUCTION

Epilepsy is among the most prevalent of the serious neurological disorders, affecting from 0.5 to 1.0% of the world’s population.[1] In India, studies have reported the prevalence rate of epilepsy varying from 1710 to 9780 cases per million populations.[2] Despite the optimal use of available antiepileptic drugs (AEDs), many patients with epilepsy fail to experience seizure control. Moreover, many patients suffer with the strong side effects of chronic treatment, which may include chronic toxicity, cognitive impairment, sedation and teratogenesis.[3] The increasing knowledge on the basic mechanisms of epilepsy, the most important convulsive disorder, has led to the rational development of compounds that block seizure onset or spread targeting specific neuronal substrates.[4,5]

There is still a great demand for new anticonvulsant drugs, as the existing drugs fail to treat all types of convulsive disorders.[6] Medicinal plants used for the therapy of epilepsy in traditional medicine have been shown to possess promising anticonvulsant activities in animal models and can be invaluable sources of new antiepileptic compounds.

Barringtonia asiatica (L.) Kurz (Family – Barringtoniaceae) is a tree to 25 m tall with glossy alternate, petiolate, entire bark, obovate, 12-40 cm long, 10-20 cm broad. Flowers are large and showy, petals white, calyx green, with pinkish filaments with yellow anthers. Fruit a large fibrous drupe (up to 12 cm long), shiny green, quadrangular (square in cross section), containing a large single seed. This tree usually forms large spreading branches as well as a large, spreading buttress root system. It is common along the sea shore, edges of mangroves, lowland river margins and coastal forests. It is widespread throughout the tropical Pacific and Indian Oceans and widely cultivated in tropical areas. Gallic acid, saponins (including barrinin A1), hydrocyanic acid, monosaccharides, triterpenoids (bartogenic acid, 19-epibartogenic acid, and anhydrobartogenic acid).[7] Traditional used In the Cook Islands, the seed is grated, mixed with coconut cream and rubbed onto burns and wounds. In Fiji, a decoction of the bark is used to treat hernia. A decoction of the bark is used to treat constipation and epilepsy. In Samoa, the fruit or bark is used to treat yaws, seed to treat ringworm and the bark is used in treating tuberculosis. In Solomon Islands and Samoa it is used to stun fish.[8,9] Hence, the present study was designed to verify the claims of the native practitioners.
MATERIALS AND METHODS

Plant collection
The bark of Barringtonia asiatica Linn. was collected from abirami botanicals of Tuticorin, Tamilnadu, India. It was identified and authenticated by Prof. Jayaraman, Taxonomist, Tambaram, Chennai, Tamilnadu, India. The voucher specimen of the plant was deposited at the college for further reference.

Preparation of extracts
The bark of plants were dried in shade, separated and made to dry powder. It was then passed through the 40 mesh sieve. A weighed quantity (220gm) of the powder was subjected to continuous hot extraction in Soxhlet Apparatus. The extract was evaporated under reduced pressure using rotary evaporator until all the solvent has been removed to give an extract sample. The methanolic extract of Barringtonia asiatica Linn. (MEBA) yielded thick violet semi-solid residues. Percentage yield of MEBA was found to be 16.4% w/w.

Preliminary phytochemical screening Phytochemical Screening
The phytochemical examination of the methanolic extract of Barringtonia asiatica was performed by the standard methods.[10] Further investigation was carried out using the ethanol extract suspended in 1% w/v Sodium carboxy methylcellulose (SCMC).

Animals used
Albino wistar rats (150-200g) of either sex were obtained from the animal house in Vaagdevi Institute of Pharmaceutical Sciences, Bollikunta, Warangal. The animals were maintained in a well-ventilated room with 12:12 hour light/dark cycle in polypropylene cages. The animals were fed with standard pellet feed (Hindustan Lever Limited., Bangalore) and water was given ad libitum. Ethical committee clearance was obtained from IAEC (Institutional Animal Ethics Committee) of CPCSEA.

Acute Toxicity Study
The acute toxicity of 90% methanolic extract of Barringtonia asiatica was determined as per the OECD guideline no. 423 (Acute Toxic Class Method). It was observed that the test extract was not mortal even at 2000mg/kg dose. Hence, 1/8th (250mg/kg) and 1/4th (500mg/kg) of this dose were selected for further study.[11]
**Antiepileptic Activity**

**Effect on Maximal electroshock (MES) induced seizures**

Albino wistar rats of either sex weighing 150 to 230 gm were divided into four groups of six animals each. The first group received vehicle control (1% w/v SCMC, 1ml/100 g) whereas Group-II received standard drug (Phenytoin, 25mg/kg) intraperitoneally, Group-III and IV, received methanolic extract of the Barringtonia asiatica (L.) (MEBA) (250 and 500 mg/kg body weight) *p.o* respectively for 14 days. On the 14th day, Seizures are induced to all the groups by using an Electro convulsiometer. Maximal electroshock seizures were elicited by a 60 Hz alternating current of 150 mA intensity for 0.2 sec. A drop of electrolyte solution (0.9% NaCl) with lignocaine was applied to the corneal electrodes prior to application to the rats. This increases the contact and reduces the incidence of fatalities. The duration of various phases of epilepsy were observed. The percentage protection was estimated by observing the number of animals showing abolition of Hindleg Tonic Extension (or) extension not greater than 90°.[12]

**Effect on Pentylene tetrazole (PTZ) induced seizures**

Albino wistar rats of either sex weighing 150 to 230 gm were divided into four groups of six animals each. The first group received vehicle control (1% w/v SCMC, 1ml/100 g) whereas Group-II received standard drug (Diazepam, 4mg/kg) intraperitoneally, Group-III and IV, received methanolic extract of Barringtonia asiatica (L.) (MEBA) (500 and 250 mg/kg/body weight) *p.o* respectively for 14 days. On the 14th day, Pentylene tetrazole (PTZ) (90mg/kg body weight, *s.c*) was administered to all the groups to induce clonic convulsions. Animals were observed for a period of 30mins post – PTZ administration. The parameters noted were mean onset time of convulsions, duration of convulsion and recovery/Death (% recovery or % of survival) due to PTZ.[13]

**Statistical analysis**

The data were expressed as mean ± standard error mean (S.E.M).The Significance of differences among the groups was assessed using one way and multiple way analysis of variance (ANOVA). The test followed by Dunnet’s test *P* values less than 0.05 were considered as significance.

**RESULTS**

**Phytochemical screening**

The results of preliminary phytochemical screening of the methanolic extract of Barringtonia
asiatica (L.) revealed that presence of alkaloids, flavonoids, carbohydrates, glycosides, tannins, terpenoids, phenols and absence of saponins and steroids.

**Effects of MEBA on MES Induced Epilepsy**

The duration of tonic hindleg extension in rats treated with vehicle was 13±0.36 seconds. The MEBA at doses of 250 mg/kg and 500 mg/kg were protect animals from seizures and significantly (p<0.001) reduced the duration of tonic hindleg extension. Whereas, the standard drug phenytoin treated animals exhibits abolished tonic hindleg extension. Phenytoin treated animals have shown 100% protection against MES induced seizures where as MEBA 250 mg/kg and 500 mg/kg have shown 71.73 % and 81.47 % protection respectively (Table-1).

**Effect of MEBA on PTZ Induced epilepsy**

In rats treated with vehicle, clonic convulsion appeared for 176.21±2.57 seconds after PTZ and all rats died after seizures. The MEBA at doses of 250 mg/kg and 500 mg/kg significantly delayed the onset of clonic convulsions for 478.67±4.05 (p<0.001) and 568.10±3.36 (p<0.001) seconds respectively in dose dependent manner. Whereas, the standard drug diazepam (4mg/kg, i.p) delayed the onset of clonic convulsions for 698.42±1.54 (p<0.001) seconds. Diazepam treated animals have shown 100% protection against PTZ induced seizures where as MEBA 250 mg/kg and 500 mg/kg have shown 61.94% and 73.45% protection of convulsion and 83.33% and 100% protection of mortality respectively (Table-2).

Table: 1. Effect of methanolic extract of Barringtonia asiatica (L.) (MEBA) on Maximum electroconvulsive shock (MES)- induced seizures in rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Design of Treatment</th>
<th>Flexion (seconds)</th>
<th>Extensor (seconds)</th>
<th>Clonus (seconds)</th>
<th>Stupor (seconds)</th>
<th>Recovery (seconds)</th>
<th>% Protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Vehicle control (SCMC,1ml/100g)</td>
<td>8.42±0.21</td>
<td>15±0.22</td>
<td>19.67±0.27</td>
<td>44 ±0.13</td>
<td>182.69</td>
<td>0</td>
</tr>
<tr>
<td>II</td>
<td>Phenytoin 25mg/kg, i.p</td>
<td>4.45±0.68**</td>
<td>0</td>
<td>9.57±0.36**</td>
<td>15.54±0.79**</td>
<td>90.54</td>
<td>100</td>
</tr>
<tr>
<td>III</td>
<td>MEBA 250mg/kg, p.o</td>
<td>6.14±0.48ns</td>
<td>4.24 ±0.5***</td>
<td>14.92 ±0.4*</td>
<td>32.24±1.36*</td>
<td>132.57</td>
<td>71.73</td>
</tr>
<tr>
<td>IV</td>
<td>MEBA 500 mg/kg, p.o</td>
<td>5.52±0.15**</td>
<td>2.78±0.13***</td>
<td>13.21 ±1.2*</td>
<td>16 ±0.41***</td>
<td>113.64</td>
<td>81.47</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM of six observations

Comparison between Group I Vs Group II, Group II Vs Group III &Group IV

Statistical significant test for comparison was done by ANOVA, followed by Dunnet’s test

*p<0.05; **p<0.01; ***p<0.001; ns-non significant.
Table: 2. Effect of methanolic extract of Barringtonia asiatica (L.) (MEBA) on Pentylentetrazole (PTZ) induced seizures in rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Design of Treatment</th>
<th>Onset of clonic convulsions (seconds)</th>
<th>Duration of convulsion (Seconds)</th>
<th>Protection convulsion %</th>
<th>Protection mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Vehicle control (SCMC, 1ml/100g)</td>
<td>176.21±2.57</td>
<td>76.24±1.34</td>
<td>0</td>
<td>50</td>
</tr>
<tr>
<td>II</td>
<td>Diazepam (4mg/kg, i.p)</td>
<td>698.42±1.54***</td>
<td>12.87±0.52***</td>
<td>83.11</td>
<td>100</td>
</tr>
<tr>
<td>III</td>
<td>MEBA (250 mg/kg, p.o)</td>
<td>478.67±4.05***</td>
<td>29.02±1.13***</td>
<td>61.94</td>
<td>83.33</td>
</tr>
<tr>
<td>IV</td>
<td>MEBA (500mg/kg, p.o)</td>
<td>568.10±3.36***</td>
<td>20.24±1.17***</td>
<td>73.45</td>
<td>100</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM of six observations

Comparison between Group I Vs Group II, Group II Vs Group III & Group IV
Statistical significant test for comparison was done by ANOVA, followed by Dunnet’s test
*p<0.05; **p<0.01; ***p<0.001; ns - non significant.

DISCUSSIONS AND CONCLUSIONS

The most popular and widely used animal seizure models are the traditional MES and PTZ tests. The MES test is the most frequently used as an animal model for identification of anticonvulsant activity of drugs for the generalized tonic-clonic seizures "grand mal".[14,15]

This model based on observation of the stimulation by repeated electrical pulses induce in different neuronal structures one characteristic standard of epileptic activity.[16] PTZ-induced seizures test is considered as an experimental model for the "generalized absence seizures"[15] and also a valid model for human generalized myoclonic seizures and generalized seizures of the petitmal type.[14]

In our present study, it is found that treatment with MEBA on rats significantly reduces in tonic hindleg extensor stage in MES induced epilepsy. The MES test – to identify compounds which prevent seizure spread, corresponding to generalized tonic-clonic seizures in humans.[17,18] Currently used anticonvulsant drugs (e.g. phenytoin, carbamazepines) effective in therapy of generalized tonic-clonic and partial seizures have been found to show strong anticonvulsant action in MES test.[19,20] Since, MEBA significantly inhibited generalized tonic-clonic seizures in MES test; it suggests the presence of anticonvulsant compounds.

Similarly, we found that treatment with MEBA on PTZ induced rats significantly reduce the duration of convulsion and delayed the onset of clonic convulsion. PTZ may cause seizures by inhibiting chloride ion channel associated with GABA\textsubscript{A} receptors.[14,21,22] Since PTZ has been shown to interact with the GABA neurotransmission[14,23] and PTZ induced seizures can
be prevented by drugs that enhance gamma amino butyric acid type A (GABA<sub>A</sub>) receptor-mediated inhibitory neurotransmission such as benzodiazepines and phenobarbital<sup>[24-26]</sup>, the antagonism of PTZ-induced seizures suggests the interaction of the methanolic extract of Barringtonia asiatica (L.) with the GABA-ergic neurotransmission. The effect of the MEBA in the PTZ test could therefore suggest antiepileptic efficacy against the above mentioned seizures type in man.

Preliminary phytochemical analysis performed in this study shows that alkaloids and flavonoids are the major components of the MEBA. Hence, these properties could be mediated by several compounds present in the extract and could explain the use of this plant in traditional medicine in the treatment of epilepsy. The study concluded with significant antiepileptic activity of methanolic extract of Barringtonia asiatica (L.) against various models of epilepsy but was unable to reveal the exact mechanism of its action.

**REFERENCES**


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