Anticonvulsant Effect of Leaf and Bark of Erythrina Variegata Linn and Butea Monosperma (LAM) Taub in different Experimental Convulsion Model in Rats

Prakash T. Sangale{*}, Dhananjay B. Deshmukh{2}, Rajesh Bhambere{3}
{1}Department of Quality Assurance Techniques,
{2}Department of Pharmacology,
VJSM’S Vishal Institute of Pharmaceutical Education and Research,
Junnar, Pune, Maharashtra
*prakashsangale091@gmail.com

ABSTRACT

Epilepsy is a chronic disorder characterized by the occurrence of epileptic seizures, with or without characteristic body movements (convulsion) affecting about 50 million people worldwide. Synthetic drugs for the treatment of epilepsy are associated with severe side effects and addiction liabilities upon long term uses. Thus, researchers around the globe are searching for natural resources. Erythrina variegata and Butea monosperma is a traditional medicinal plant used to treat a seizure. The present studies reveal that the anticonvulsant activity by Erythrina variegata & Butea monosperma of bark & leaf PTZ and MES induced convulsions in wistar rats using Erythrina variegata & Butea monosperma of bark & leaf ethanolic extracts extracted successively. However, the anticonvulsant activity of this plant has not been studied in depth.

In conclusion, we showed that the ethanolic extract of Erythrina variegata and Butea monosperma has anticonvulsant effect in the both models, suggesting their possible depressant action in the central nervous system. EEBM and EEEV gave significant protection (P<0.001) against PTZ & MES induce convulsion.

Keywords: Ebola haemorrhagic fever, EBOV, WHO, NHP, EVD

INTRODUCTION

Epilepsy is a neurological disorder that affects a wide range of people throughout the world. It is a disorder of brain characterizes by unpredictable and periodic occurrence of a transient alteration of behavior due to the disordered, synchronous and rhythmic firing of populations of brain neurons. It has been observed that the presently available antiepileptic drugs are do not provide cure nor prevent relapse and they are often associated with serious side-effects, including teratogenicity, chronic toxicity and adverse effects on cognition and behavior and unable to control seizures effectively in as many as 25% of the patients. The clinical effectiveness, minimal side effect profile and relatively low costs of herbal drugs are the reason for their various applications in traditional medicine. However, only limited efforts have been made to evaluate the potentials of such plants for their use in modern medicine or to scientifically justify their traditional use in the treatment of CNS disorders including epilepsy.

Many drugs that increase the brain contents of GABA have exhibited anticonvulsant activity against seizures induced by maximum electroshock (MES), pentylenetetrazol (PTZ) and lithium-pilocarpine (Li-Pilo). The MES is probably the best-validated method for assessment of anti-epileptic drugs in generalized tonic-clonic seizures. The PTZ induced seizures are similar to the symptoms observed in the absence seizures and drugs useful in treatment of absence...
The objective of the present study was to investigate anticonvulsant activity of ethanolic extract of *Erythrina variegata* (EVEE) and *Butea monosperma* (BMEE) against the seizures induced by MES, PTZ.

**MATeRIAL & METHODS**

**Animals**
Healthy adult albino Wistar Rat strain weighing 180-250g were used for this study. The animals were obtained from animal house, Dr.D.Y.Patil Medical College, Pune, India. On arrival, the animals were placed randomly and allocated to treatment groups in polypropylene cages with paddy husk as bedding. Animals were housed at temperature of 24±2 ºC and relative humidity of 30-70%. A12:12 light: day cycle was followed. All the animals were allowed free access to water and fed with standard commercial pelleted rat chow (M/S Hindustan Lever Ltd. Mumbai). All the experimental procedures and protocols used in this study were reviewed by the Institutional Animal Ethics committee (and were in accordance with the guidelines of the CPCSEA. Approval was obtained from the CPCSEA/IACE for animal studies in this project by proposal no: VIPER/IAEC/UG/2013-14

The plant material:
Plant material bark & leaf of *Erythrina variegata* & *Butea monosperma* respectively were collected from different areas of Alephata, Junnar and it was identified and confirmed by Dr. Mrs. Savita Rahangdale B.J.college Ale. The authenticated samples were used for the Preparation of extract. They were put in shed drying then crushed to produce powdered material.

Preparation of extract
Dried powdered Bark & leaf of *erythrina variegata* and *Butea monosperma* were extracted by using ethanol in soxhlet apparatus. The total extract obtained was dried at 60 °C on steam bath Followed by a vacuum oven (50 °C) to obtain dried extracts. The extractive Value was calculated as % w/w yield was found to be 5.92% & 6.34%.

**PHYTOCHEMICAL EVALUATION**

The ethanolic extract obtained above was subjected to qualitative analytical test for the detection of various chemical constituents viz. steroids, terpenoids, flavanoids, carbohydrates, proteins, tannins, glycosides saponins.

**PHARMACOLOGICAL EVALUATION**

1. **PTZ to study the anticonvulsant activity**

   Animals in Group I served as control were, treated with vehicle (4 % acacia) orally. Group II served as standard received diazepam (5 mg/kg i.p.). Group III and Group IV received EVE at the dose levels of 250 mg/kg and 250 mg/kg i.p. respectively. Group V and Group VI received BMEE at the dose levels of 250 mg/kg and 500 mg/kg i.p., respectively. One hour after administration of vehicle, standard drug, EVE and BMEE to the respective groups, the animals were treated with PTZ (Pentylene tetrazole, 80 mg/kg) subcutaneously. Each animal was placed in to individual polypropylene cage and were observed initially for 30 min and later up to 24 hrs. The following parameters were recorded during test session of initial 30 min and up to 24 hrs: Latency (onset of clonus) onset of tonic convulsion and status of animal after 30 min, status of animal after 24 hrs and percentage protection.

2. **MES Induced convulsion in rat**

   MES seizures were induced by electroconvulsiometer. Maximal seizures were elicited by 60 Hz alternating current of 150 mA intensity for 0.2 sec using corneal electrodes. A drop of electrolyte solution 0.9% sodium chloride with lignocaine was applied to the corneal electrodes, which ensures better contact and the mortality rate to zero. This current intensity elicited complete tonic extension of the hind limbs in control rats. For recording various parameters, rats were placed in a clear
rectangular polypropylene cage with an open top, permitting full view of the animal motor responses to seizure. In the pilot study of various phases of convulsion like tonic flexion, extension, stupor, and mortality due to convulsions were observed.

RESULTS

2. PTZ induced convulsion model

Table No. 1- Effect of EVEE & BMEE on PTZ induced convulsion model

<table>
<thead>
<tr>
<th>Gr.</th>
<th>Treatment</th>
<th>Latency onset of clonic convulsion sec/min</th>
<th>Onset of tonic convulsion sec/min</th>
<th>Status of animal after 30 min</th>
<th>Status of animal after 24 hrs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>No of alive animal</td>
<td>% protection</td>
</tr>
<tr>
<td>I</td>
<td>Control 4% acacia</td>
<td>51.16±0.60</td>
<td>544.33±1.22</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>II</td>
<td>Diazepam(25mg/kg)</td>
<td>No colonus</td>
<td>No tonic</td>
<td>All</td>
<td>100</td>
</tr>
<tr>
<td>III</td>
<td>EVEE(250mg/kg)</td>
<td>210.83±0.94</td>
<td>374.01±0.76</td>
<td>4</td>
<td>67</td>
</tr>
<tr>
<td>IV</td>
<td>EVEE(500mg/kg)</td>
<td>236.50±0.68</td>
<td>356.21±2.67</td>
<td>5</td>
<td>84</td>
</tr>
<tr>
<td>V</td>
<td>BMEE(250mg/kg)</td>
<td>144.20±1.12</td>
<td>175.83±0.47</td>
<td>3</td>
<td>50</td>
</tr>
<tr>
<td>VI</td>
<td>BMEE(500mg/kg)</td>
<td>191.41±0.92</td>
<td>416.52±1.22</td>
<td>4</td>
<td>67</td>
</tr>
</tbody>
</table>

Values expressed are mean SEM from rats. *p < 0.001*** as compared to control group.

Graph No. 1: Effect of EVEE & BMEE on PTZ induced convulsion model indicating onset of clonic & tonic convulsion

Table No. 2- Effect of EVEE & BMEE on MES induced convulsion in rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Flexion</th>
<th>Extension</th>
<th>Colonus</th>
<th>Stupor</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control 4% acacia</td>
<td>9.62±0.17</td>
<td>15.21±0.17</td>
<td>17.05±0.20</td>
<td>8.62±0.19</td>
<td>210.33±2.65</td>
</tr>
<tr>
<td>II</td>
<td>Diazepam (25mg/kg)</td>
<td>4.50±0.09***</td>
<td>00</td>
<td>9.42±0.09***</td>
<td>2.42±0.10***</td>
<td>120.50±1.26</td>
</tr>
<tr>
<td>III</td>
<td>EVEE(250mg/kg)</td>
<td>3.50±0.08***</td>
<td>1.53±0.06***</td>
<td>5.25±0.03***</td>
<td>24.14±1.24***</td>
<td>176.12±1.97</td>
</tr>
<tr>
<td>IV</td>
<td>EVEE(500mg/kg)</td>
<td>2.15±0.07***</td>
<td>1.15±0.04***</td>
<td>4.71±0.06***</td>
<td>18.70±0.42***</td>
<td>136.20±0.63</td>
</tr>
<tr>
<td>V</td>
<td>BMEE(250mg/kg)</td>
<td>3.91±0.09***</td>
<td>1.98±0.11***</td>
<td>5.68±0.08***</td>
<td>24.33±0.84***</td>
<td>181.24±1.97</td>
</tr>
<tr>
<td>VI</td>
<td>BMEE(500mg/kg)</td>
<td>2.31±0.06***</td>
<td>1.27±0.07***</td>
<td>5.41±0.16***</td>
<td>21.50±0.61***</td>
<td>143.11±1.67</td>
</tr>
</tbody>
</table>
Values represent mean of six observations.
Comparisons between: a-group I vs. group II, b-group II vs. group III, IV, V & VI.
Statistical significant test for comparison done by ANOVA.

Graph No 2: Effect of EVEE & BMEE on MES induced convulsion model indicating Stupor & Recovery

DISCUSSION

In Pentylene tetrazole induced seizure test parameters like latency, onset of tonic convulsion, clonic convulsions and percent protection were observed in the test groups (p<0.001), showing strong antiepileptic effect.

The death rate was 100% in Group I.

25 mg/kg of Diazepam, prevents tonic and clonic convulsion and offered 100% protection. 250mg/kg and 500mg/kg of EEEV and EEBM exhibited a significant anticonvulsant effect by increasing onset of clonic convulsion and by decreasing onset of tonic convulsion.

After 30 min of interval 67% and 84% of animals survived with a dose of 250mg/kg and 500mg/kg of EEEV. While 50% survived with the dose of 250 mg/kg and 67% survived with 500mg/kg of BMEE.

After 24 hrs, the % protection of animals was, 67% and 84% for 250mg/kg and 500mg/kg of EVEE respectively. 34% and 50% protection after 24 hrs in 250mg/kg and 500mg/kg in BMEE survived (p value is p<0.001 as compare to control). Here, EEEV shows potent anticonvulsant activity compare to EEBM. These results further indicate that strong protective effect of test drug against a known epileptic agent in Maximum Electroshock induced seizure. test, shown anticonvulsant activity by increasing the onset of clonic convulsion time and by decreasing the time of extensor of test groups reduced to significant level as compare to control group(p<0.0001 and 0.001 as compare to control). This result indicates the strong protective effect of 500mg/kg EVEE and 500mg/kg of BMEE against known epileptic agents.

There are some evidences of anticonvulsant effect of his fatty acids and some flavonoids . Therefore it seems that anti seizure effect of Erythrina variegata Linn bark and leaf may be to part of linoleic acid and flavonoid compounds present in extracts. Thus the result of both doses of EEEV, demonstrate a very striking and potent antiepileptic activity, it may be useful in both types of epileptic conditions like Grand mal and petit mal epilepsy. It demonstrated specified nature of pharmacological effect of erythrina variegate Linn bark and leaf.

SUMMARY AND CONCLUSION

The Anticonvulsant activity ethanol extract of Erythrina variegata and Butea monosperma bark & leaf was evaluated on rats by using two anticonvulsant models such as MES induced convulsion in rat &PTZ- induced convulsion in mice. The Pharmacological studies involving phytochemical estimation suggests presence of carbohydrates,
glycosides and tannins in Erythrina variegata and Butea monosperma bark & leaf specimen.

Erythrina variegata and Butea monosperma is a traditional medicinal plant used to treat a seizure. However, the anticonvulsant activity of this plant has not been studied in depth. We therefore sought to evaluate the anticonvulsant activity of ethanolic extract of Erythrina variegata and Butea monosperma bark & leaf on albino Wistar rats. In order to verify traditional use of this plant, Pentylene tetrazole (PTZ) and the maximal electroshock seizure (MES) models were used for assessing the anticonvulsant effects of the ethanolic extract of bark & leaf. In Pentylene tetrazole (PTZ) and maximal electroshock seizure (MES) models test parameters like latency, onset of tonic convulsions, clonic convulsions and percent protection were observed in the different test groups. In conclusion, we showed that the ethanolic extract of bark & leaf of Erythrina variegata and Butea monosperma has anticonvulsant effect in the both models, suggesting their possible depressant action in the central nervous system.

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