STUDY OF THE ANTICONVULSANT POTENTIAL OF LEAVES OF CLITORIA TERNATEA LINN. IN PENTYLENETETRAZOLE AND MAXIMUM ELECTROSHOCK SEIZURE INDUCED-CONVULSIONS IN EXPERIMENTAL ANIMALS

JOONMONI LAHON1*, SWOPNA PHUKAN2, UPAMA SHARMA3

1Department of Pharmacology, North Eastern Indira Gandhi Regional Institute of Health and Medical Sciences, Shillong, Meghalaya, India. 2Department of Pharmacology, Gauhati Medical College, Guwahati, Assam, India. 3Apcer Pharma, Delhi, India. Email: joonlahon@yahoo.co.in

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ABSTRACT

Objectives: To study the anticonvulsant potential of leaves of Clitoria ternatea Linn. in maximal electroshock seizure (MES) and pentylenetetrazole (PTZ)-induced convulsion in experimental animals.

Methods: The anticonvulsant potential of the ethanolic extracts of C. ternatea Linn. (EECT) was tested in the MES and PTZ models, seizures were induced, respectively, by delivering electroshock of 50 mA for 0.2 s via a pair of transauricular electrodes using an electro-convulsiometer and by injecting 80 mg/kg intraperitoneally PTZ. For MES model, parameters measured were the duration of hindlimb tonic extension (HLTE), total recovery time, and percentage protection. For the PTZ model, parameters measured were the duration of time taken for the onset of clonic convulsions, duration of clonic convulsions, percentage reduction of clonic phase, and the mortality percentage.

Results and Observation: The EECT at both the test doses (200 mg/kg and 400 mg/kg) reduced the duration of HLTE and total recovery time, and increased the percentage protection from MES-induced convulsions, suggesting a dose-dependent anticonvulsant effect of EECT on MES-induced seizures. The extract also produced a dose-dependent anticonvulsant effect on PTZ-induced seizures in albino mice as suggested by prolongation of the latency of clonic convulsion, reduction in the duration of convulsion and seizure score.

Conclusion: The present study concludes that the EECT leaves have an anticonvulsant effect on PTZ and MES-induced convulsion in albino mice.

Keywords: Antiepileptic, Clitoria ternatea Linn., Ethanoic extract, Maximal electroshock, Pentylenetetrazole, Seizure score.

INTRODUCTION

Clitoria ternatea L. commonly known as butterfly pea in English, is a perennial leguminous twiner, belongs to the family Fabaceae (Leguminosae) and sub-family Papilionaceae [1]. It bears white and blue flowers and is distributed mostly within the tropical regions with a few species in temperate areas [2,3]. Its indigenous names are Aparajita (Sanskrit and Bengali) and Aparajit (Hindi) [3]. It is rich in many phytochemicals such as triterpenoids such as β and γ sitosterol, pentacyclic triterpenoid, taraxerol and taraxerone, alkaloids, flavonoids, quercetin, lactones aparajitin and clitorin, saponins, carbohydrates, proteins, tannins, resins, and starch [4-6]. It has great importance in the traditional system of medicine; roots being used for fever, arthritis, chronic bronchitis, and epilepsy; leaves are employed for relieving headache, swollen joints, etc. [7]. For centuries, it has been used as a memory enhancer (Medhya), as well as a nootrop, anxiolytic, antidepressant, anticonvulsant, tranquilizing, and sedative agent [8]. Its roots are administered with honey and ghee as a general tonic to children for improving mental functions, muscle strength, and epilepsy [9]. Moreover, phytochemicals such as triterpenoids, saponins, flavonoids, tannins, alkaloids, isolated from other plants have been reported to have anticonvulsant property in various animal models of epilepsy such as pentylenetetrazole (PTZ), maximal electroshock seizure (MES), and electrical kindling [10-12].

In view of its traditional use in convulsion and epilepsy and also due to the presence of phytochemicals (having anticonvulsant property), this plant, C. ternatea (butterfly-pea), has been investigated for its anticonvulsant potential in the present study.

METHODS

The present study has been carried out in the Department of Pharmacology, Gauhati Medical College and Hospital, Guwahati, Assam to study the anticonvulsant potential of leaves of C. ternatea Linn. (butterfly-pea) in PTZ and maximum electroshock seizure (MES)-induced convulsion in albino mice after obtaining due approval from the Institutional Animal Ethics Committee No. MCI 32/2012/1. The study was performed according to the CPCSEA guidelines.

Extraction of plant material

The leaves of C. ternatea Linn. used in the study were collected from in and around Guwahati during April-June 2011. The collected leaves were shade dried and powdered in an electric grinder. 300 g of the powdered leaves were extracted with 99.9% ethanol using Soxhlet apparatus at a temperature of 60°C for 24 hrs [13,14]. The solvent was taken in glass Petri dishes and evaporated in a controlled water bath (temperature 40-50°C) which gave semisolid mass [15,16]. The extract was finally stored in air tight containers in a refrigerator at 2-8°C for further use in the experiment. A final yield of 33 g, i.e., 11% w/w with respect to the original air-dried powder was obtained.

Experimental animals

Healthy albino mice of either sex weighing between 25 and 30 g were taken from the Institute’s Central Animal House, Gauhati Medical College and Hospital, Guwahati. The animals were acclimatized to the laboratory conditions for at least 7 days before the experiments. The animals were housed in an animal room in groups, in polypropylene cages as per the standard laboratory conditions at 25°C with 12:12 hrs light and dark cycle, with alternating light-dark cycle of 12 hrs each. The animals were maintained on a standard animal diet with water ad libitum but fasted prior to dosing (food but not water was withheld for 3-4 hrs).

Induction of convulsion

The anticonvulsant effects of the ethanolic extracts of C. ternatea Linn. (EECT) were tested in the MES and PTZ animal models. In MES model, electroshock of 50 mA was delivered for 0.2 s by means of an electro-convulsiometer through a pair of transauricular (ear clip) electrodes

OBJECTIVES

To study the anticonvulsant potential of leaves of Clitoria ternatea Linn. in maximal electroshock seizure (MES) and pentylenetetrazole (PTZ)-induced convulsion in experimental animals.

METHODS

The anticonvulsant potential of the ethanolic extracts of C. ternatea Linn. (EECT) was tested in the MES and PTZ models, seizures were induced, respectively, by delivering electroshock of 50 mA for 0.2 s via a pair of transauricular electrodes using an electro-convulsiometer and by injecting 80 mg/kg intraperitoneally PTZ. For MES model, parameters measured were the duration of hindlimb tonic extension (HLTE), total recovery time, and percentage protection. For the PTZ model, parameters measured were the duration of time taken for the onset of clonic convulsions, duration of clonic convulsions, percentage reduction of clonic phase, and the mortality percentage.

RESULTS AND OBSERVATION

The EECT at both the test doses (200 mg/kg and 400 mg/kg) reduced the duration of HLTE and total recovery time, and increased the percentage protection from MES-induced convulsions, suggesting a dose-dependent anticonvulsant effect of EECT on MES-induced seizures. The extract also produced a dose-dependent anticonvulsant effect on PTZ-induced seizures in albino mice as suggested by prolongation of the latency of clonic convulsion, reduction in the duration of convulsion and seizure score.

CONCLUSION

The present study concludes that the EECT leaves have an anticonvulsant effect on PTZ and MES-induced convulsion in albino mice.

KEYWORDS

Antiepileptic, Clitoria ternatea Linn., Ethanoic extract, Maximal electroshock, Pentylenetetrazole, Seizure score.
to induce seizures [11]. In PTZ model, PTZ in the dose of 80 mg/kg (convulsive dose in 97% of the animals) was injected intraperitoneally (i.p.) to induce seizures [17,18]. Experimental animals were grouped and administered the study drugs and standard drug for both the models as shown in the Tables 1 and 2.

### MES model

Pretesting of the mice was done with a current of 50 mA for 0.2 s via a pair of transauricular (ear clip) electrodes, using an electro-convulsiometer. Only those mice where tonic HLE component of MES was produced and was selected for the main study. A recovery period of 3-4 days was given before doing the main test. The mice were allowed free access to food and water except during the short time when they were removed from their cages for testing [19]. The mice were taken out randomly from the cages and weighed in an electronic weighing machine and marked according to groups. The control, standard, and test groups mice received normal saline, standard drug (phenytoin), and test extracts (suspended in 1% gum acacia) orally, respectively. 1 hr (60 minutes) after administration of the test extracts/drugs/vehicle the animals were subjected to MES as done in the pretest. MES produced various phases of convulsions, i.e. tonic flexion of the forelimbs and hindlimbs, hindlimb tonic extension (HLE), clonus, and stupor followed by recovery [19,20]. Parameters which were measured in this study were (a) duration of HLE, (b) total recovery time, and (c) percentage protection.

The percentage protection was calculated as:

\[
\text{Duration of HLE in control} - \text{Duration of HLE in test / standard} \times 100
\]

The duration of the tonic extension of hindlimb was used as an end point, i.e., prevention or decrease in the duration of hindlimb extension was considered as a protective action against convulsion [21].

### PTZ seizure model

The animals were allowed free access to food and water except during the short time when testing was done [22,23]. The mice were taken out randomly from the cages and weighed in an electronic weighing machine and marked according to groups. The standard drug and test extracts were suspended in 1% gum acacia and administered orally to the respective groups (Table 2). 1 hr after administration of the test extracts/drugs/vehicle the animals were given PTZ (80 mg/kg i.p.) after dissolving in distilled water [11]. Each animal was placed into individual plastic cages and was selected for the main study. A recovery period of 3-4 days was given before doing the main test. The mice were allowed free access to food and water except during the short time when they were removed from their cages for testing [19]. The mice were taken out randomly from the cages and weighed in an electronic weighing machine and marked according to groups. The control, standard, and test groups mice received normal saline, standard drug (phenytoin), and test extracts (suspended in 1% gum acacia) orally, respectively. 1 hr (60 minutes) after administration of the test extracts/drugs/vehicle the animals were subjected to MES as done in the pretest. MES produced various phases of convulsions, i.e. tonic flexion of the forelimbs and hindlimbs, hindlimb tonic extension (HLE), clonus, and stupor followed by recovery [19,20]. Parameters which were measured in this study were (a) duration of HLE, (b) total recovery time, and (c) percentage protection.

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### Results

#### Acute toxicity study

NOAEL of EECT leaves was found to be 2000 mg/kg/day. Hence, 1/10(200 mg/kg) and 1/5(400 mg/kg) doses were taken for further study.

### Anticonvulsant study

The results obtained from the study have been summarized in the Tables 4-6, and the values are expressed in specific units for each of the parameters as mentioned in the tables.

#### Table 3: Seizure scoring, the scale described by Velisek et al. (1992)

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Scoring Changes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>0</td>
</tr>
<tr>
<td>2.</td>
<td>0.5</td>
</tr>
<tr>
<td>3.</td>
<td>1</td>
</tr>
<tr>
<td>4.</td>
<td>2</td>
</tr>
<tr>
<td>5.</td>
<td>3</td>
</tr>
<tr>
<td>6.</td>
<td>4</td>
</tr>
<tr>
<td>7.</td>
<td>5</td>
</tr>
</tbody>
</table>

#### Table 5: Grouping for MES model

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Groups</th>
<th>Drugs</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Group IA: Control group</td>
<td>10 ml/kg of 0.1% gum acacia in saline p.o.</td>
</tr>
<tr>
<td>2.</td>
<td>Group IIA: Standard group</td>
<td>Phenytoin 25 mg/kg p.o</td>
</tr>
<tr>
<td>3.</td>
<td>Group IIA</td>
<td>EECT 200 mg/kg p.o</td>
</tr>
<tr>
<td>4.</td>
<td>Group IVA</td>
<td>EECT 400 mg/kg p.o</td>
</tr>
</tbody>
</table>

ECT: Ethanolic extracts of C. ternatea Linn. C. ternatea: Clitoria ternatea, MES: Maximal electroshock seizure

#### Table 6: Grouping for PTZ model

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Groups</th>
<th>Drugs</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Group IB: Control group</td>
<td>10 ml/kg of 0.1% gum acacia in saline p.o.</td>
</tr>
<tr>
<td>2.</td>
<td>Group IIB: Standard group</td>
<td>Phenytoin 25 mg/kg p.o</td>
</tr>
<tr>
<td>3.</td>
<td>Group IIB</td>
<td>EECT 200 mg/kg p.o</td>
</tr>
<tr>
<td>4.</td>
<td>Group IIB</td>
<td>EECT 400 mg/kg p.o</td>
</tr>
</tbody>
</table>

ECT: Ethanolic extracts of C. ternatea Linn. C. ternatea: Clitoria ternatea, PTZ: Pentylenetetrazole

#### Table 4: MES-induced seizures in mice

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Mean±SEM</th>
<th>Duration of HLE (s)</th>
<th>Total recovery time (s)</th>
<th>% Protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>IA</td>
<td>Control</td>
<td>19.17±0.75</td>
<td>183.33±9.80</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>HIA</td>
<td>Phenytoin</td>
<td>0.67±0.33*</td>
<td>22.50±0.62*</td>
<td>86.07</td>
<td></td>
</tr>
<tr>
<td>HIA</td>
<td>EECT</td>
<td>15.8±0.54*</td>
<td>136.67±0.99*</td>
<td>17.42</td>
<td></td>
</tr>
<tr>
<td>IVA</td>
<td>EECT</td>
<td>10.67±0.49*</td>
<td>74.50±1.46*</td>
<td>44.34</td>
<td></td>
</tr>
<tr>
<td>One-way ANOVA</td>
<td>df=3.20</td>
<td>df=3.20</td>
<td>F=170.55</td>
<td>F=199.13</td>
<td>p&lt;0.05</td>
</tr>
</tbody>
</table>

*p<0.05 when compared with the control group (Group IA). EECT: Ethanolic extracts of C. ternatea Linn. C. ternatea: Clitoria ternatea, SEM: Standard error of mean, HLE: Hindlimb tonic extension
**Table 5: PTZ-induced seizures in mice**

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Onset of clonus (s)</th>
<th>Duration of convulsion (s)</th>
<th>% reduction of clonus</th>
<th>% Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>IB</td>
<td>Control</td>
<td>129.50±6.82</td>
<td>77.17±4.09</td>
<td>-</td>
<td>100</td>
</tr>
<tr>
<td>IIB</td>
<td>Phenytoin 25 mg/kg p.o.</td>
<td>419.67±8.88*</td>
<td>88.3±0.31</td>
<td>88.56</td>
<td>16.67</td>
</tr>
<tr>
<td>IIB</td>
<td>EECT 200 mg/kg p.o.</td>
<td>173.33±2.86*</td>
<td>60.00±1.59**</td>
<td>22.25</td>
<td>83.33</td>
</tr>
<tr>
<td>IIB</td>
<td>EECT 400 mg/kg p.o.</td>
<td>261.67±6.72*</td>
<td>37.9±2.26**</td>
<td>50.98</td>
<td>50</td>
</tr>
</tbody>
</table>

One-way ANOVA

df=3.20
F=36.77,77
F=14.2±82
p<0.05
p<0.05

**Table 6: PTZ-induced seizure score**

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Seizure score (mean±SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IB</td>
<td>Control</td>
<td>5.00±0.00</td>
</tr>
<tr>
<td>IIB</td>
<td>Phenytoin 25 mg/kg p.o.</td>
<td>2.50±0.22**</td>
</tr>
<tr>
<td>IIB</td>
<td>EECT 200 mg/kg p.o.</td>
<td>4.83±0.17</td>
</tr>
<tr>
<td>IIB</td>
<td>EECT 400 mg/kg p.o.</td>
<td>3.67±0.21***</td>
</tr>
</tbody>
</table>

One-way ANOVA

df=3.20
F=44.2±4
p<0.05

**p<0.05 when compared with the control group (Group IB).** EECT: Ethanolic extracts of *C. ternatea* Linn. *C. ternatea*: Clitoria ternatea, PTZ: Pentylentetrazole

**DISTRIBUTION**

The MES test, in which tonic hindlimb extensions are induced by bilateral corneal or transauricular electrical stimulation, is thought to be predictive of efficacy of anticonvulsant drugs against generalized tonic-clonic seizures, while the PTZ test, in which generalized myoclonic and clonic seizures are induced by systemic (usually s.c. or i.p.) administration of convulsant doses of PTZ, is thought to represent a valid model for generalized absence and/or myoclonic seizures in humans [26].

The results of acute toxicity of EECT in the present study revealed that the extract was not lethal up to 2000 mg/kg orally. Patil and Patil [21] reported that the petroleum ether, chloroform, and methanol extract of *C. ternatea* roots were not toxic in mice up to 2000 mg/kg orally [27]. Sini et al. found that the median lethal dose of the methanolic extract of *C. ternatea* was >5000 mg/kg bodyweight [28]. Boominathan et al. found out significant neuropharmacological activity in the ethanol extract of the root of *C. ternatea* at doses of 100 and 150 mg/kg in rats and mice [29]. PTZ is an antagonist of gamma-aminobutyric acid (GABA) at GABA-A receptor which has been widely implicated in epilepsy. Furthermore, drugs which protect animals against the seizure induced by PTZ, like drugs that reduce the F-type of Ca²⁺ currents or drugs that inhibit GABA-mediated neurotransmission, act by elevating the seizure threshold and are effective in myoclonic and absence seizures. The antiepileptic drugs that block the MES-induced tonic extension act by blocking seizure spread. Moreover, MES-induced tonic extension seizure can be prevented either by drugs that inhibit voltage-gated Na⁺ channels such as phenytoin or by drugs that inhibit glutamatergic excitation mediated by N-methyl-D-aspartate receptors such as felbamate. In addition, drugs that are effective in protecting animals against the tonic-clonic extensor spasm induced by MES are effective in the management of and/or protecting against grand mal epilepsy [11].

**CONCLUSION**

EECT has shown significant anticonvulsant potential in PTZ and MES-induced convulsion in Swiss albino mice. However, the mechanism of anticonvulsant action and the components of the extract responsible for this effect were not investigated in this study. Further investigations are needed for identification of the active compounds and their exact molecular mechanism of action, responsible for the anticonvulsant activity of this plant extract. The results of the present study provide scientific evidence to the ethnomedicinal use of these plants in treating convulsion and epilepsy.

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**REFERENCES**

1. Zingare ML, Zingare PL, Dubey AK, Ansari MA. *Clitoria ternatea* (Aparajita): A review of the antioxidant, anti-diabetic and...


