ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 9, Issue 2, 2016

Online - 2455-3891
Print - 0974-2441

Research Article

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

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Received: 06 January 2016, Revised and Accepted: 27 January 2016

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., Ethanolic 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 nootropic, 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

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-day 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 (HLTE), clonus, and stupor followed by recovery [19,20]. Parameters which were measured in this study were (a) duration of HLTE, (b) total recovery time, and (c) percentage protection.

The percentage protection was calculated as:

$$\frac{\text{Duration of HLTE in control} - \text{Duration of HLTE in test / standard}}{\text{Duration of HLTE in control}} \times 100$$

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 for observation lasting 1 hr. The onset of a general clonus (characterized by forelimb clonus followed by full clonus of the body) was used as the endpoint. The time taken for the onset of clonic convulsions (latency period), the duration of clonic convulsions, the percentage reduction of clonic phase, and the percentage mortality were recorded [24].

Table 1: Grouping for MES model

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

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

Table 2: Grouping for PTZ model

S. No.	Groups	Drugs
1.	Group IB: Control group	10 ml/kg of 0.1% gum acacia in saline p.o.
2.	Group IIB: Standard group	Phenytoin 25 mg/kg p.o
3.	Group IIIB	EECT 200 mg/kg p.o
4.	Group IVB	EECT 400 mg/kg p.o

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

The percentage reduction of clonic convulsion was calculated as:

$$\frac{\text{Duration of clonus in control} - \text{Duration of clonus in test / standard}}{\text{Duration of clonus in control}} \times 100$$

PTZ seizure scoring was done as per the scale described by Velisek *et al*. (1992) as described in Table 3 [25].

Statistical analysis

All the data were entered into the statistical software, SPSS 16.0. Data were expressed as mean ± standard error of mean. Results were analyzed by one-way analysis of variance, followed by Dunnett multiple comparison test. $p<0.05$ was considered as statistically significant.

RESULTS

Acute toxicity study

NOAEL of EECT leaves was found to be 2000 mg/kg/day. Hence, $1/10^{th}$ (200 mg/kg) and $1/5^{th}$ (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)

S. No.	Scoring	Changes
1.	0	No change in behavior
2.	0.5	Atypical behavior (e.g., intensive grooming, sniffing, and moving arrests)
3.	1	Isolated myoclonic jerks and ear and facial twitching
4.	2	Atypical minimal seizures and convulsive waves throughout the body
5.	3	Fully developed minimal seizures, clonus of the head muscles and forelimbs, and the presence of the righting reflex
6.	4	Major seizures (i.e., generalized, without the tonic phase
7.	5	Generalized tonic-clonic seizures beginning with running followed by lost righting ability and a short tonic phase (i.e., flexion or extension of forelimbs and hindlimbs) progresses to the clonus

Table 4: MES-induced seizures in mice

Group	Treatment	Mean±SEM		% Protection
		Duration of HLTE (s)	Total recovery time (s)	
IA	Control	19.17±0.75	183.33±9.80	-
IIA	Phenytoin 25 mg/kg p.o	02.67±0.33#	22.50±0.62#	86.07
IIIA	EECT 200 mg/kg p.o.	15.83±0.54#	136.67±0.99#	17.42
IVA	EECT 400 mg/kg p.o.	10.67±0.49#	74.50±1.46#	44.34
One-way ANOVA		df=3.20 F=170.55 p<0.05	df=3.20 F=199.13 p<0.05	

#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, HLTE: Hindlimb tonic extension

Table 5: PTZ-induced seizures in mice

Group	Treatment	Onset of clonus (s)	Duration of convulsion (s)	% reduction of clonus	% Mortality
IB	Control	129.50±6.82	77.17±4.09	-	100
IIB	Phenytoin 25 mg/kg p.o	419.67±8.88*	8.83±0.31*	88.56	16.67
IIIB	EECT 200 mg/kg p.o.	173.33±2.86*	60.00±1.59*	22.25	83.33
IVB	EECT 400 mg/kg p.o.	261.67±6.72*	37.83±2.26*	50.98	50
One-way ANOVA		df=3.20 F=367.77 p<0.05	df=3.20 F=142.82 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: Pentylenetetrazole

Table 6: PTZ-induced seizure score

Group	Treatment	Seizure score (mean±SEM)
IB	Control	5.00±0.00
IIB	Phenytoin 25 mg/kg p.o	2.50±0.22**
IIIB	EECT 200 mg/kg p.o.	4.83±0.17
IVB	EECT 400 mg/kg p.o.	3.67±0.21**
One-way ANOVA		df=3.20 F=44.24 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*, SEM: Standard error of mean

MES-induced seizure

As suggested in Table 4, the extract of EECT showed dose-dependent reduction in the HLTE and total recovery time. The mean duration of the HLTE were 19.17±0.75, 2.67±0.33, 15.83±0.54, and 10.67±0.49 s in Group IA, Groups IIA, IIIA, and IVA, respectively. The mean duration of total recovery time was 183.33±9.80, 22.5±0.62, 136.67±0.99, and 74.50±1.46 s, respectively, in Group IA, IIA, IIIA, and IVA. Analysis of variance followed by Dunnett's *t*-test showed that the reduction in the HLTE and total recovery time were statistically significant (p<0.05) in the standard and the test groups when compared with the control group. The percentage protection in the phenytoin-treated group was 86.07%, whereas the groups treated with EECT 200 mg/kg and EECT 400 mg/kg showed percentage protection of 17.42% and 44.34%, respectively.

PTZ-induced seizures study

The mean duration of the onset of clonic convulsion (latency) was 129.50±6.82, 419.67±8.88, 173.33±2.86, and 261.67±6.72 s in Group IB, IIB, IIIB, and IVB, respectively (Table 5). The mean duration of convulsion in Group IB, IIB, IIIB, and IVB were 77.17±4.09, 8.83±0.31, 60±1.59, and 37.83±2.26 s, respectively. Analysis of variance followed by Dunnett's *t*-test showed that prolongation of the duration of the onset of convulsion was statistically significant (p<0.05) in Groups IIB, IIIB, and IVB when compared with the control group (Group IB). Similarly, reduction in the duration of convulsion was found to be statistically significant (p<0.05) in the standard and the test groups when compared with the control group (Group IB). The percentage reduction of convulsion in the phenytoin-treated group was 88.56%, whereas the percentage reduction of convulsion in the groups treated with EECT at doses of 200 mg/kg and EECT 400 mg/kg were 22.25% and 50.98%, respectively. Prolongation of the latency of clonus, reduction of the duration of convulsion, and percentage reduction of convulsion by EECT were dose dependent. The control group showed 100% mortality with PTZ. The mortality of the mice in the phenytoin-treated group was 16.67%, whereas the groups treated with EECT at the doses 200 mg/kg and 400 mg/kg showed mortality of 83.33% and 50%, respectively.

PTZ-induced seizure score

Phenytoin-induced a significant decline in the seizure score (2.50±0.22) as compared to the control group (5.00±0.00) (Table 6). While the 200 mg/kg dose of EECT showed a decline in seizure score (4.83±0.17) but is not statistically significant (p>0.05) when compared with the control group. EECT at 400 mg/kg dose showed a statistically significant reduction in the seizure scores (3.67±0.21) when compared to the control group.

DISCUSSION

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 (2011) 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 T-type of Ca^{++} 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.

ACKNOWLEDGMENT

The authors take the opportunity to acknowledge DBT Nodal Cell for Medical Colleges and Biomedical Research Institutes of NE India, Napam, Tezpur, Assam for providing fund for the research.

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