Original article

Anticonvulsant activity of methanolic extract of *Clerodendron infortunatum* Linn. in Swiss albino mice

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Abstract:

The present study was designed to investigate the anticonvulsant and sleep potentiation effect of methanolic extract of *Clerodendron infortunatum* Linn. (MECI) leaves in Swiss albino mice. The anticonvulsant effect of the MECI (250, 500 mg/kg body weight b.w, intraperitoneal i.p.) was examined against pentylenetetrazole- (PTZ, 80 mg/kg b.w; i.p.) and strychnine- (STR, 2.5 mg/kg b.w; i.p.) induced convulsion. MECI (500 mg/kg b.w; i.p.) significantly delayed ($p < 0.01$) the onset and antagonized PTZ-and STR-induced seizures. Diazepam (2 mg/kg b.w; i.p.) was used as a reference drug for anticonvulsant activity. Further, the study was undertaken to evaluate the sleep potentiation effect of MECI (250 and 500 mg/kg b.w; i.p.) in mice and the extract significantly increased pentobarbitone (45 mg/kg b.w; i.p.)-induced sleeping time in a dose dependent manner.

Keywords: Anticonvulsant; *Clerodendron infortunatum;* Pentobarbitone; Pentylenetetrazole; Strychnine

Introduction

Clerodendron infortunatum Linn. belonging to family Verbenaceae, has been used in Indian folk medicine in the treatment of bronchitis, asthma, fever, burning sensation, disease of blood, inflammation and epilepsy [1]. Traditionally, the plant is used as an antipyretic and antihelmentic. Leaves of the plant are prescribed for tumour, certain skin diseases and scorpion sting [2, 3]. Previous phytochemical investigation of the plant revealed the presence of alkyl sterols [4] and 2,-(3, 4-dehydroxyphenyl) ethanol 1-*O*-α-2-rhamnopyranosyl-(1→3)-β-D-(4-*O*-caffeoyl)-glycopyranoside (acteoside) [5]. Earlier pharmacological investigation was revealed that the assessment of anticonvulsant activity of *C. infortunatum* by leptazol-induced seizures [6].

The pentylenetetrazole (PTZ)-induced seizures are similar to the symptoms observed in the absence seizures and drugs useful in treatment of absence seizures suppress PTZ-induced seizures [7, 8]. The objective of the present study was to investigate anticonvulsant activity of methanolic extract of *C. infortunatum* (MECI) against the pentylenetetrazole-(PTZ) and strychnine-(STR) induced seizures and also to find out sleep potentiation effect of the extract.

Materials and Methods

Plant material

The plant *C. infortunatum* Linn. was collected in the month of November 2008 from the forest region of Midnapore, West Bengal, India. The taxonomical identification of the plant was done by Botanical Survey of India, Shibpur, India and the voucher specimen (PMU-4/JU/2008) has been preserved in Pharmacology Research Laboratory, Jadavpur University, Kolkata for future reference.

Preparation of extract

The leaves of the *C. infortunatum* were dried under shade and then powered by mechanical grinder. The powder plant material was extracted with 80% methanol using soxhlet extraction apparatus. The solvent was completely removed under reduced pressure and semisolid mass was obtained (yield 13.5% w/w). The extracts were stored in a vacuum dessicator for further use. Preliminary phytochemical screening of the plant extract exhibited the presence of flavonoid, tannin and saponin.

Animals used

Male Swiss albino mice weighing (20-27 g) were maintained in identical laboratory conditions (25-30°C and relative humidity of 55-65% with alternate light and darkness 12 h each) and fed with commercial pellet diet (Hindustan Lever, Kolkata, India) and water *ad libitum.* All procedures described were reviewed and approved by the University Animal Ethical Committee (ref no. 367001/C/CPCACA).

Chemicals

Pentylenetetrazole (PTZ), strychnine (STR) from HiMEDIA Laboratories Pvt. Ltd., Mumbai, diazepam and pentobarbitone from Ranbaxy, Mumbai were used for the study.

Assessment of anticonvulsant activity

Pentylenetetrazole (PTZ)-induced seizure [9]

Thirty male Swiss albino mice (20-27 g) were randomly divided into 5 groups (n = 6). Group I served as a saline control (5 ml/kg b.w; i.p.). Group II received a convulsive dose of PTZ 80 mg/kg b.w; i.p. and served as PTZ-control. Group III, IV and V received MECI at the doses of 250 and 500 mg/kg b.w; i.p. and diazepam 2 mg/kg b.w; i.p. respectively, 30 min prior to the administration of PTZ (80 mg/kg b.w; i.p.). Group V served as a reference group. The animals were observed for onset of myoclonic spasm and clonic convulsion up to 30 min after PTZ injection. The percentages of protection were observed and recorded.

Strychnine (STR)-induced seizure [10]

Thirty male albino mice (20-27 g) were randomly divided into 5 groups (n = 6). Group I served as a saline control (5 ml/kg b.w; i.p.). Group II received STR 2.5 mg/kg b.w; i.p. and served as STR-control. Group III, IV and V received MECI at the doses of 250 and 500 mg/kg b.w; i.p. and diazepam 2 mg/kg b.w; i.p.

respectively, 30 min prior to the administration of STR (2.5 mg/kg b.w; i.p.). Group V served as reference group. The percentages of protection were observed and recorded.

Pentobarbitone-induced sleeping time in mice [9]

Eighteen male Swiss albino mice (20-28 g) were randomly divided into 3 groups (n = 6). Group I received pentobarbitone (45 mg/kg b.w; i.p.) and served as pentobarbitone control. Group II and III received MECI (250 and 500 mg/kg b.w; i.p.) 30 min prior to the administration of pentobarbitone (45 mg/kg b.w; i.p.). The time between the loss of the righting reflex and the regain of this reflex measured as the sleeping time.

Statistical analysis

All results are expressed as the mean ± SEM. The results were analyzed for statistical significance ($p < 0.05$, $p < 0.01$) by one-way (ANOVA) followed by Dunnett's test using computerized Graph Pad InStat version 3.05, Graph pad software, U.S.A.

Results and Discussion

PTZ (80 mg/kg b.w; i.p.) and STR (2.5 mg/kg b.w; i.p.) produced hind-limb tonic seizures in all mice except a saline control group. The MECI (500 mg/kg b.w; i.p.) significantly delayed the onset and antagonized PTZ- ($p < 0.01$) and STR- ($p < 0.01$) induced seizures. The results of MECI-treated group were comparable with those of reference drug, diazepam (2 mg/kg b.w; i.p.) (Tables 1 and 2).

The total sleeping time induced by pentobarbitone increased significantly from 55.80 ± 3.76 min in the control group to 62.20 ± 1.24 and 93.80 ± 1.16 min in the extract treated group at the doses of 250 and 500 mg/kg b.w. respectively. The sleeping time of extract-treated group was approximately doubled at the dose of 500 mg/kg b.w. (Table 3).

Table 1 Effect of methanolic extract of *C. infortunatum* (MECI) leaves on pentylenetetrazole (PTZ)-induced seizures (n = 6)

Group	Treatment	Dose (mg/kg)	Onset of convulsion in minute (Mean ± SEM)	Duration of convulsion in minute (Mean ± SEM)	Mortality (%)	Protection or survival (%)
I	Saline control	5 ml	0	0	0	100
II	pentylenetetrazole	80	1.66 ± 0.05	1.36 ± 0.07	100	0
III	MECI	250	3.56 ± 0.07	2.80 ± 0.37	70	30
IV	MECI	500	5.34 ± 0.06*	9.20 ± 0.97*	0	100
V	Diazepam	2	5.52 ± 0.04*	10.80 ± 1.07*	0	100

*P < 0.01 when compared with control group

Table 2 Effect of methanolic extract of *C. infortunatum* (MECI) leaves on strychnine (STR)-induced seizures (n = 6)

Group	Treatment	Dose (mg/kg)	Onset of convulsion in minute (Mean ± SEM)	Duration of convulsion in minute (Mean ± SEM)	Mortality (%)	Protection or survival (%)
II	Saline control	5 ml	0	0	0	100
II	Strychnine	2.5	3.60 ± 0.40	1.60 ± 0.29	100	0
III	MECI	250	4.80 ± 0.66	2.80 ± 0.37	60	40
IV	MECI	500	7.20 ± 0.37*	6.80 ± 0.58*	0	100
V	Diazepam	2	7.60 ± 0.51*	7.40 ± 0.51*	0	100

*P < 0.01 when compared with control group

Table 3 Effect of methanolic extract of *C. infortunatum* (MECI) leaves on pentobarbital-induced sleeping time in mice (n = 6)

Group	Treatment	Dose (mg/kg)	Onset of sleep in minute (Mean ± SEM)	Duration of sleep in minute (Mean ± SEM)
I	Pentobarbitone	45	2.50 ± 0.22	55.80 ± 3.76
II	MECI	250	1.60 ± 0.04	62.20 ± 1.24
III	MECI	500	1.60 ± 0.04	93.80 ± 1.16*

*P < 0.01 when compared with control group

Preliminary phytochemical analysis performed showed that the tannin, saponin and flavonoid are the major components of the extract. There are some evidences about anticonvulsant effect of some flavonoid compounds [11, 12]. It is shown that anxiolytic effects of some natural and synthetic flavonoids exerted their action through the central benzodiazepine receptors in rats [13]. Therefore, it seems that the anticonvulsant effect of *C. infortunatum* may be related in part to flavonoid compound present in the extract.

The observations emanated in the present study indicate that MECI produced a depressant effect on the central nervous system as motor coordination was impaired to a significant extent and duration of pentobarbitone-induced sleep was prolonged [14]. MECI at both doses (250 and 500 mg/kg b.w, i.p) inhibited PTZ-induced and STZ-induced convulsions. These observations indicate that the anticonvulsant effects of MECI are possibly mediated by chloride channels of GABA/benzodiazepine receptor complex and by chloride channel of glycine receptor [15]. GABA plays a critical role in the etiopathology of epilepsy [16]. GABAergic mechanisms have been implicated in protection from a variety of chemo and electroshock induced seizures. MECI at both doses has been found to be effective against PTZ-induced seizure convulsion. This finding suggests that GABAergic system may involve in the action of MECI, since PTZ acts by interfering with GABA transmission [17].

Conclusion

From the above study, it was concluded that the methanol extract of *C. infortunatum* Linn. exhibited anticonvulsant activity.

Acknowledgement

The financial assistance of Department of Pharmaceutical Technology, Jadavpur University, Kolkata, India is gratefully acknowledged.

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