

Anticonvulsant Activity of *Diospyros cordifolia* Bark Against Experimentally Induced Convulsions in Swiss Albino Mice

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Abstract: Background: *Diospyros cordifolia* Roxb. (Ebenaceae), commonly known as Indian ebony is used traditionally for several medicinal purposes. The present study was designed to investigate the anticonvulsant and sleep potentiation effect of methanolic extract of *Diospyros cordifolia* stem bark (MEDC) in Swiss albino mice. **Results:** The anticonvulsant effect of the MEDC at the doses of 25, 50 mg kg⁻¹ body weight (b.wt.), intraperitoneally (i.p.), was examined against pentylenetetrazole (PTZ, 80 mg kg⁻¹ b.wt; i.p.) and strychnine (STR, 2.5 mg kg⁻¹ b.wt; i.p.) induced convulsions in mice. MEDC (50 mg kg⁻¹ b.wt; i.p.) significantly delayed (p<0.01) the onset and antagonized PTZ and STR induced seizures. Diazepam (2 mg kg⁻¹ b.wt; i.p.) was used as reference drug for anticonvulsant assessment. Further the study was undertaken to evaluate the pentobarbitone induced sleep potentiation effect of MEDC (25 and 50 mg kg⁻¹ b.wt; i.p.) in mice and the extract significantly increased pentobarbitone (45 mg kg⁻¹ b.wt; i.p.) induced sleeping time in a dose dependent manner. **Conclusion:** Therefore, the present study demonstrated that *D. cordifolia* bark possessed remarkable anticonvulsant efficacy demonstrating depressant action on the central nervous system.

Key words: Anticonvulsant, pentylenetetrazole, strychnine, pentobarbitone, *Diospyros cordifolia*

INTRODUCTION

Seizure is a characteristic feature in epilepsy and is associated with disordered and rhythmic high frequency discharge of impulses by a group of neurons in the brain. Status epilepticus is characterized by repeated episodes of epilepsy without the patient having recovered from the previous attack (Sonavane *et al.*, 2002). Convulsions may also be precipitated as adverse effects or overdose of some drugs.

Diospyros cordifolia Roxb. (Ebenaceae), commonly known as Indian ebony, is a deciduous tree and used traditionally in India for several medicinal purposes. The plant holds very important position in Indian folk medicine where it is mostly used as the treatment of liver disorders, whooping cough, leprosy, dysentery, abdominal pain, wounds, gonorrhoea, fever, inflammation, as emetic and anthelmintic (Nadakarni, 1954; Chopra *et al.*, 1956). Previous workers reported hepatoprotective, wound healing, analgesic and anti-inflammatory activities of its stem bark (Krishnsa *et al.*, 2005; Das *et al.*, 2011). Ursolic acid, α -amyrin, β -amyrin, lupeol, taraxerol,

nentriacontane, hentriacontanol and β -sitosterol were isolated from the leaves of *D. cordifolia* (Suresh and Sastry, 1989).

There are several established antiepileptic drugs used therapeutically but certain adverse effects and weak effectiveness of them has led to the search for more effective agents. The major merits of herbal medicine seem to be their perceived efficacy, low incidence of serious adverse effects and low cost. There is therefore the need for research into medicinal plants with possible anticonvulsant effects.

The objective of the present study was to investigate anticonvulsant activity of methanol extract of *Diospyros cordifolia* (MEDC) against the Pentylenetetrazole (PTZ) and Strychnine (STR) induced seizures in Swiss albino mice and also to find out sleep potentiation effect of the extract to justify the folkloric beliefs.

MATERIALS AND METHODS

Plant material: The plant *Diospyros cordifolia* Roxb. (Ebonaceae) was collected in the month of November 2008

from the forest region of West Bengal, India. The taxonomical identification of the plant was done by Botanical Survey of India, Shibpur, India and the voucher specimen (PMU-5/JU/2008) has been preserved in Pharmacology Research Laboratory, Jadavpur University, Kolkata for future reference.

Preparation of extract: The stem bark of the *Diospyros cordifolia* was dried under shade and then powdered with mechanical grinder. The powdered plant material was extracted with 80% methanol using soxhlet extraction apparatus. The solvent was completely removed under reduced pressure and semisolid mass was obtained (MEDC, yield: 14.5 % w/w). The extracts were stored in a vacuum desiccator for further use. Preliminary phytochemical studies were performed on MEDC as per reported methods (Harborne, 1998).

Experimental animals: Adult male Swiss albino mice weighing (20-27 g) were maintained in identical laboratory conditions (25-30°C temperature and relative humidity of 55-65% with alternate light and darkness 12 h each) and fed with commercial dry pellet diet (Hindustan Lever, Kolkata, India) and water *ad libitum*. All procedures described were reviewed and approved by Institutional Animal Ethical Committee, Jadavpur University.

Chemicals: Pentylenetetrazole (PTZ), Strychnine (STR) (HiMEDIA Laboratories Pvt. Ltd., Mumbai, India), Diazepam, Pentobarbitone (Ranbaxy, Mumbai, India) were used for the present study.

Assessment of anticonvulsant activity

Pentylenetetrazole (PTZ)-induced seizure: Thirty Swiss albino mice (20-27 g) were randomly divided into 5 groups (n = 6). Group I served as saline control (received normal saline 5 mL kg⁻¹ b.wt; i.p.). Group II received a convulsive dose of PTZ 80 mg kg⁻¹ b.wt; i.p. and served as PTZ-control. Group III, IV and V received MEDC at the doses of 25 and 50 mg kg⁻¹ b.wt; i.p. and diazepam 2 mg kg⁻¹ b.wt; i.p., respectively, 30 min prior to the administration of PTZ (80 mg kg⁻¹ b.wt; i.p.). Group V served as 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 (Kulkarni, 1999).

Strychnine (STR)-induced seizure: Thirty Swiss albino mice (20-27 g) were randomly divided into 5 groups (n = 6). Group I served as saline control (received normal saline 5 mL kg⁻¹ b.wt; i.p.). Group II received STR 2.5 mg kg⁻¹ b.wt; i.p. and served as STR-control. Group

III, IV and V received MEDC at the doses of 25 and 50 mg kg⁻¹ b.wt; i.p. and diazepam 2 mg kg⁻¹ b.wt; i.p. respectively, 30 min prior to the administration of STR (2.5 mg kg⁻¹ b.wt; i.p.). Group V served as reference group. The percentages of protection were observed and recorded (Bum *et al.*, 2001).

Pentobarbitone-induced sleeping time in mice: Eighteen Swiss albino mice (20-27 g) were randomly divided into 3 groups (n = 6). Group I received pentobarbitone (45 mg kg⁻¹ b.wt; i.p.) and served as pentobarbitone control. Group II and III received MEDC (25 and 50 mg kg⁻¹ b.wt; i.p.), 30 min prior to the administration of pentobarbitone (45 mg kg⁻¹ b.wt; i.p.). The time between the loss of the righting reflex and the regain of this reflex measured as the sleeping time (Kulkarni, 1999).

Statistical analysis: All results are expressed as the Mean ± standard Error of Mean (SEM). The results were analyzed for statistical significance by one-way (ANOVA) followed by Dunnett's test using computerized Graph Pad InStat version 3.05, Graph pad software, USA. The p-value < 0.01 were regarded as statistically significant.

RESULTS AND DISCUSSION

In the present study, PTZ (80 mg kg⁻¹ b.wt; i.p.) and STR (2.5 mg kg⁻¹ b.wt; i.p.) produced hind-limb tonic seizures in all mice except saline control. The MEDC (50 mg kg⁻¹ b.wt; i.p.) significantly delayed the onset and antagonized PTZ (p < 0.01) and STR (p < 0.01) induced seizures. The results of treated group are comparable with that of reference drug diazepam (2.0 mg kg⁻¹ b.wt; i.p.) (Table 1, 2).

The total sleeping time induced by pentobarbitone increased significantly from 55.80 ± 3.760 min in the control group to 66.82 ± 2.36 and 97.26 ± 2.48 min in the extract treated group at the doses of 25 and 50 mg kg⁻¹ b.wt, respectively. The sleeping time of extract treated group was approximately doubled at the dose of 50 mg kg⁻¹ b.wt. (Table 3).

Preliminary phytochemical analysis on MEDC performed in this study shows that the flavonoids, tannins, saponins, steroids and terpenoids are the major components of the extract. There are some evidences about anticonvulsant effect of some flavonoid compounds (Du *et al.*, 2002; Griebel *et al.*, 1999). It has been shown that anxiolytic effects of some natural and synthetic flavonoids exerted their action through the central benzodiazepine receptors in rats (Salgueiro *et al.*, 1997). Therefore, it seems that the anticonvulsant effect of MEDC may be related in part to flavonoid constituents present in the extract.

Table 1: Effect of MEDC on pentylenetetrazole (PTZ)-induced seizures

Group	Treatment	Dose	Onset of convulsion (min)	Duration of convulsion (min)	Mortality (%)	Protection or survival (%)
I	Saline control	5 (mL kg ⁻¹)	0	0	0	100
II	PTZ	80 (mg kg ⁻¹)	1.66±0.05	1.36±0.07	100	0.0
III	MEDC	25 (mg kg ⁻¹)	3.38±0.04	3.26±0.18	80	20
IV	MEDC	50 (mg kg ⁻¹)	5.12±0.05*	10.30±0.68*	0	100
V	Diazepam	2 (mg kg ⁻¹)	5.52±0.04*	10.80±1.07*	0	100

Data are Mean±SEM (n = 6), *p<0.01 when compared with saline control group

Table 2: Effect MEDC on strychnine (STR)-induced seizures

Group	Treatment	Dose	Onset of convulsion (min)	Duration of convulsion (min)	Mortality (%)	Protection or survival (%)
I	Saline control	5 (mL kg ⁻¹)	0	0	0	100
II	Strychnine	2.5 (mg kg ⁻¹)	3.60±0.40	1.60±0.29	100	0.0
III	MEDC	25 (mg kg ⁻¹)	5.15±0.48	2.60±0.26	70	30
IV	MEDC	50 (mg kg ⁻¹)	7.40±0.22*	7.10±0.45*	0	100
V	Diazepam	2 (mg kg ⁻¹)	7.60±0.51*	7.40±0.51*	0	100

Data are Mean±SEM (n = 6), *p<0.01 when compared with saline control group

Table 3: Effect of MEDC on pentobarbital-induced sleeping time in mice.

Group	Treatment	Dose (mg kg ⁻¹)	Onset of sleep (min)	Duration of sleep (min)
I	Pentobarbitone	45	2.50±0.22	55.80±3.76
II	MEDC+Pentobarbitone	25+45	1.70±0.03	66.82±2.36
III	MEDC+Pentobarbitone	50+45	1.70±0.05	97.26±2.48

Data are Mean±SEM (n = 6), *p<0.01 when compared with saline control group

In the present study, anticonvulsant activity was studied to assess the effect of MEDC on counteracting convulsions in mice induced by putative central nervous system stimulants viz. PTZ and STR. PTZ is a powerful Central Nervous System (CNS) stimulant and convulsant acting by direct depolarization of central neurones by interfering with GABAergic inhibition (Manocha *et al.*, 2001). PTZ produces jerky type of clonic convulsions in mice analogous to petit mal type of convulsions in humans (Kulkarni, 1981). Furthermore, the 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 (McNamara, 1996; Tripathi, 1999). STR is also a potent CNS stimulant and convulsant from plant source. It acts by blocking the post synaptic inhibition produced by the inhibitory neurotransmitter glycine (Tripathi, 1999). MEDC at both doses (25 and 50 mg kg⁻¹ b.wt., i.p.) inhibited PTZ-induced and STR-induced convulsions. These observations indicate that the anticonvulsant effects of MEDC are possibly mediated by chloride channels of GABA/benzodiazepine receptor complex and by chloride channel of glycine receptor (Meldrum, 1996). GABA plays a critical role in the etiopathology of epilepsy (Meldrum, 1975). GABAergic mechanisms have been implicated in protection from a variety of chemo and electroshock induced seizures. MEDC at both doses has been found to be effective against PTZ-induced seizure convulsion. This finding suggests that GABAergic system may be involved in the action of MEDC. Prolongation of pentobarbitone induced sleeping time by

MEDC in the present study indicated that MEDC 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 (Fujimori, 1995).

CONCLUSION

From the present study, it can be concluded that *Diospyros cordifolia* bark demonstrated promising anticonvulsant activity against experimentally induced convulsions in Swiss albino mice with depressant action on the central nervous system. It may have beneficial effects in epilepsy that holds the hope of new generation of anticonvulsant drugs. However, comprehensive chemical and pharmacological research is required to find out the exact mechanism of this extract for its anticonvulsant effect and to identify the active constituents responsible for this effect.

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