

Anticonvulsant activity of *Erythrina mysorensis* bark extract in an animal model of epilepsy

Sir,

Epilepsy is a common neurological disorder characterized by paroxysmal dysrhythmia, seizure, with or without body convulsions and sensory or psychiatric phenomena. There are many mechanisms by which seizures can develop in either normal or pathologic brains. Three common mechanisms include: (i) diminution of inhibitory mechanism (especially synaptic inhibition due to GABA), (ii) enhancement of the excitatory synaptic mechanism (especially those mediated by NMDA), and (iii) enhancement of endogenous neuronal burst firing (usually by enhancing voltage dependent calcium currents).^[1] *Erythrina mysorensis* is a small tree with few or no prickles.^[2] Literature survey reveals that there are no scientific reports regarding anti-epileptic activity of *Erythrina mysorensis*. Hence this study was undertaken to evaluate anti-epileptic activity of extract of *Erythrina mysorensis* bark using different *in vivo* models such as maximum electroshock (MES), pentylenetetrazole, and locomotor activity.

The stem bark of *Erythrina mysorensis* Gamb. (Fabaceae) was collected from the regions of Shimoga District, Karnataka, India, and authenticated by a plant taxonomist. Coarse powder was extracted successively with petroleum ether 60–80, chloroform, and alcohol in a Soxhlet apparatus, finally with chloroform water by maceration. All the extracts were distilled, dried, and used for this study. The preliminary phytochemical screening^[3] of the extracts revealed the presence of secondary metabolites such as glycosides, alkaloids, flavonoids, tannins, triterpenoids and saponins.

Male Swiss albino mice (18–22 g) were used in this study. All experiments were carried out during the light period (08.00–16.00 h). The Institutional Animal Ethics Committee approved the experimental protocol (SSCPT/IAEC/88/2010-11) dated 27/07/2010.

The animals were divided into 27 groups, each containing six mice. The groups of mice were assigned to receive one of the following: (i) 1 ml of 1% Tween 80 (Ranbaxy Laboratories Ltd.) p.o. (ii) Phenytoin (Sigma Chemical Co.) (90 mg/kg, i.p.) reference standard for locomotor and MES activity. (iii) Diazepam (Ranbaxy Pharma, India) (4 mg/kg, i.p.) reference standard for PTZ activity. (iv) to (xi) Pet. ether, chloroform, ethanolic, and aqueous extracts 200 mg/kg and 400 mg/kg, p.o. for MES activity, (xii) to (ixx) pet. ether, chloroform, ethanolic and aqueous extracts 200 mg/kg and 400 mg/kg, p.o. 60 min before administration of the PTZ (90 mg/kg, i.p.), for PTZ activity, (xx) to (xxvii) pet. ether, chloroform, ethanolic and aqueous extracts 200 mg/kg and 400 mg/kg, p.o. for locomotor activity.

Acute oral toxicity study was carried out as per OECD–423 guidelines. The petroleum ether, chloroform, ethanol, and aqueous extracts of *Erythrina mysorensis* bark were found to be non-toxic up to the dose of 2000 mg/kg and did not cause any death, therefore 200 mg/kg and 400 mg/kg dose levels were selected.

Treatment with petroleum ether, ethanolic, aqueous extracts of 200 mg/kg and 400 mg/kg, chloroform extract in the dose of 200 mg/kg did not produce significant MES, PTZ, and locomotor activity.

The maximal electro shocks (MES) induced epileptic seizures in animals represent grand mal type of epilepsy. All the animals received maximal electro shock (150 mA, 60 Hz for 2 s). The animals were observed individually for 30 min from the time of electric shock for different phases of epileptic seizures.^[4]

Higher dose of chloroform extract (400 mg/kg) significantly ($P < 0.01$) reduced hind limb extensor phase of convulsion as compared to vehicle-treated animals [Table 1]. In PTZ-induced convulsion,^[5] the onset of general clonus was used as the endpoint. The chloroform extract in higher dose significantly ($P < 0.001$) increased the time of onset of convulsion, decreased duration of convulsion, prevented the death of animals, and increased percentage protection of seizure or mortality on comparison with the reference standard diazepam 4 mg/kg [Table 2].

Locomotor activity is an index of alertness and its decrease is indicative of sedative effect. The locomotor activity^[6] was measured using an actophotometer. Each mouse was placed individually in the actophotometer for 10 min and the basal activity score was obtained. Chloroform extract in higher doses of 400 mg/kg produced significant ($P < 0.001$) reduction in locomotor activity as compared to the control animals [Table 2].

Table 1: Effect of chloroform extract of *Erythrina mysorensis* bark on MES-induced convulsions in rats

Treatment	Various phases of convulsions (s)				
	Flexion	Extension	Clonus	Stupor	Recovery
Vehicle (1% Tween 80, 1 ml/kg, p.o.)	2.33 ± 0.33	5.33 ± 0.66	10.83 ± 2.54	28.83 ± 1.38	356.89
Phenytoin (90 mg/kg, i.p.)	2.16 ± 0.30	Absence of extension	5.00 ± 0.68*	7.33 ± 1.14***	86.90
Chl.ext (200 mg/kg, p.o.)	6.16 ± 1.22**	4.16 ± 0.47	7.00 ± 0.68	16.67 ± 1.83***	198.02
Chl. ext (400 mg/kg, p.o.)	3.83 ± 0.70	2.16 ± 0.47**	6.5 ± 0.42	8.00 ± 1.82***	103.73

Values are expressed in mean ± SEM, n = 6. *P < 0.05, **P < 0.01, ***P < 0.001 compared with the vehicle-treated group. One-way ANOVA followed by Tukey's test.

Table 2: Effect of chloroform extract of *Erythrina mysorensis* bark on PTZ-induced seizure in rats and locomotor activity

Treatment	PTZ-induced seizure in rats					Locomotor activity in 10 min		
	No conv/no. used	Onset of convulsion (s)	Duration of convulsion (s)	No. of death	Protection (%)	Before	After	Reduction in activity (%)
Vehi. (1%, Tween 80, 1 ml/kg, p.o.)	6/6	307.7 ± 20.80	43.17 ± 2.44	6	0	144.8 ± 6.95	118.8 ± 12.30	17.96
Diazepam (4 mg/kg, i.p.)	0/6	0***	0***	0	100	215.0 ± 51.71	73.33 ± 41.98	65.89
Chl. ext (200 mg/kg, p.o.)	6/6	380.3 ± 23.26	28.50 ± 3.46**	2	66	295.5 ± 41.11	136.3 ± 16.59	53.87
Chl. ext (400 mg/kg, p.o.)	6/6	600.5 ± 21.38***	9.33 ± 1.05***	1	83	309.5 ± 33.79	80.67 ± 12.32	73.93

Values are expressed in mean ± SEM, where n = 6. *P < 0.05, **P < 0.01, ***P < 0.001 compared with the vehicle-treated group. One-way ANOVA followed by Tukey's test.

The observations emanated in this study indicated that chloroform extract in the dose of 400 mg/kg of *Erythrina mysorensis* bark possesses anticonvulsant activity against PTZ, MES-induced seizures and significant decrease in locomotor activity. The MES is a standard procedure that evaluates the testing materials ability to protect against hind limb extension (HLE) in MES. Toman *et al.* reported that the seizure pattern in MES for all laboratory animals and men are similar except for the time scale. Protection against HLE in the MES predicts anticonvulsant activity of anti-epileptic drugs that prevent the spread of the epileptic seizure from an epileptic focus during seizure activity. Protection against HLE also indicates the ability of the testing material to inhibit or prevent seizure discharge within the brain stem substrate. The effect of most of anti-epileptic agents is to enhance the response to GABA (gamma amino butyric acid), by facilitating the opening of GABA-activated chloride channels. GABA_A receptors have been formed to be involved in epilepsy and their direct activation would have an anti-epileptic effect. Since the chloroform extract showed anti-epileptic activity in the MES, it may act through any of the above-mentioned mechanisms.^[7]

It is well documented that PTZ-induced convulsions are produced due to diminution of the GABA level in brain.^[8] Chloroform extract of *Erythrina mysorensis* bark also exhibited significant and delayed onset of clonic-tonic actions and protection from PTZ-induced mortality by interfering with the

GABAergic mechanism.^[9] Therefore, chloroform extract might possibly be producing anti-epileptic action by increasing the level of GABA, an inhibitory neurotransmitter in the central nervous system. This is in accord with the pharmacological effects of benzodiazepine, and highlights the relevance of the putative anti-epileptic effects of chloroform extract.

Locomotor activity is considered as an index of alertness, and a decrease indicates a sedative effect. In this study, chloroform extract of *Erythrina mysorensis* bark was found to decrease the locomotion, supporting the earlier evidence.^[6] In conclusion, chloroform extract *Erythrina mysorensis* bark exhibited significant activity in MES- and PTZ-induced seizure models, it can be an effective compound against both grand mal and petit mal epilepsies.^[10] Further research is warranted to determine the specific mode of its anticonvulsant activity.

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