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Research Article

ANTIEPILEPTIC ACTIVITY OF THE HYDROALCOHOLIC EXTRACT OF *ERYTHRINA FUSCA* LOUR BARK AGAINST THE ANIMAL MODELS OF MES, PTX- AND PTZ - INDUCED EPILEPTIC SEIZURE MODELS

SUBAL DEBNATH[1*], M. KANNADASAN[2], SOUMIK GHOSH[3], NANSRI SAHA GHOSH[4], RAJA CHAKRABORTY[5], SAIKAT SEN[5]

[1]Srikrupa Institute of Pharmaceutical Sciences, Vil. Velkatta, Kondapak (mdl), Dist. Medak, Siddipet, Andhra Pradesh – 502277, [2]College of Pharmacy, SRIPMS, Coimbatore, Tamilnadu. [3]Department of Pharmaceutics, Annamalai University, Annamalai Nagar - 608002, Tamil Nadu, India. [4]SSJ College of Pharmacy, Vattinagula pally, Gandipet, .Rangareddy Dist., Hyderabad, A.P. – 500075, [5]Creative Educational Society's College of Pharmacy, Chinnatekur, Kurnool, Andhra Pradesh – 518218. Email: subal_2007@yahoo.co.in

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ABSTRACT

The objective of the present study was to evaluate the anti-epileptic activity of hydroalcoholic extract of the bark of *Erythrina fusca* Lour**.** (HAEEF) using different *in vivo* experimental models. Experimental models of epileptiform seizures (EMES) have constituted the nearest physiological approach to the development of new antiepileptic drugs for seizure control in epileptic patients. Animals are used for the demonstration of an injury by exogenous agents of epileptic seizure on the brain with its physiological significance. Epileptic seizure challenged animals treated with HAEEF at doses of 250 mg/kg and 500 mg/kg showed reduction of maximal electro shock (MES) -, picrotoxin (PTX) - and pentylenetetrazole (PTZ) - induced epileptic seizure. Seizure duration was found to be lower and onset of seizure found to increase in the extract treated animals compared to control group. Thus, it can be inferred that the hydro-alcoholic extract of *Erythrina fusca* Lour**.** bark possess antiepileptic effect against the animal models of epilepsy. HAEEF significantly (P<0.01) antagonized the PTX - and PTZ - induced epileptic seizures and reduce MES - induced tonic hind limb extension and clonic phase of epileptic seizure. Extracts significantly and dose-dependently delayed the onset of clonic convulsion induced by PTZ and PTX. HAEEF significantly (P<0.01) reduced the duration (in sec) of MES - induced convulsion. Thus, the result suggested that the HAEEF possess anticonvulsive activity.

Key words*: Erythrina fusca* Lour., Hydroalcoholic extract, Epilepsy, Maximal electro shock, Picrotoxin, Pentylenetetrazole

INTRODUCTION

Erythrina fusca Lour**.** (Family: Fabaceae) is a deciduous tree with spiny bark and light orange flowers. Its legume pods reach 20 cm in length and contain dark brown seeds. The seeds are buoyant, allowing them disperse across oceans. The tree is highly adapted to coastal conditions, tolerant of both flooding and salinity. Like many other species in the genus *Erythrina*, *E. fusca* contains toxic alkaloids which have been utilized for medicinal value but are poisonous in larger amounts. The most common alkaloid is erythraline, which is named for the genus. The new buds and leaves are eaten as a vegetable. The easy-to-grow and attractive flowering tree is cultivated as an ornamental shade and hedge plant. It is a common shade tree in cacao plantations. It attracts hummingbirds, which pollinate its flowers**.**

Epilepsy is a common neurological disorder characterized by paroxysmal dysrhythmia, seizure, with or without body convulsion and sensory or psychiatric phenomena[1]. There are many mechanisms by which seizures can develop in either normal or pathologic brains. Three common mechanisms include, 1) Diminition of inhibitory mechanism (especially synaptic inhibition due to GABA 2). Enhancement of the excitatory synaptic mechanism (especially those mediated by NMDA). 3) Enhancement of endogenous neuronal burst firing (usually by enhancing voltage dependent calcium currents). Different forms of human epilepsy may be caused by any one or combination of the above mechanisms[2, 3].

Both *in vivo* and *in vitro* models are available for the evaluation of anti epileptic activities of drugs. In the *in vivo* methods, animals are used for the demonstration of an injury by exogenous agents of epileptic seizure on the brain with its physiological significance. *In vitro* models are employed to elucidate specific aspects of the mechanisms of injury. *In vivo* animal models have been categorized by external agents and chemical agents that initiate the epileptic seizures, for e.g., maximal electro shock (MES) - induced epilepsy, pentylenetetrazol (PTZ) - induced epilepsy, picrotoxin (PTX) - induced epilepsy and also other chemical agents like isoniazid, biccuculine, strychnine, 4-aminopyridine, kainic acid - induced epilepsy models also kindled rat seizures. Mechanical methods like epilepsy induced by focal lesion, and genetic animal models of epilepsy, audiogenic models of epilepsy are available methods to screen the antiepileptic activities of drugs[4,5]. Present aim of this study to evaluate anti epileptic activity of hydroalcoholic extract of *Erythrina fusca* L. bark using different *in vivo* models like by MES -, PTX - and PTZ - induced convulsion in rats.

MATERIALS AND METHODS

Collection and authentication of plant material

The plant material consists of dried powdered stem bark of *Erythrina fusca* Lour belonging to the family Fabaceae. The fresh bark of *Erythrina fusca* Lour was collected from the Connur, Nilgris district, Tamil Nadu. The bark was authenticated by a taxonomist from Botanical Survey of India (BSI), Tamil Nadu Agricultural University, Coimbatore.

Preparation of the extract

Fresh stem bark of *Erythrina fusca* Lour was collected and air dried in shade under the room temperature. The dried stem bark material was powdered mechanically and sieved through No. 20 mesh sieve. The fine powder was kept separately in an airtight container until the time of use. Around 100 g of finely powdered bark material was evenly packed in a *soxhlet* apparatus and the extraction was done with water : ethanol in the ratio of 30 : 70 for 48 hours. The solvent was then evaporated under reduced pressure. The percentage (%) yield of the extract was calculated.

Drugs and chemicals

Standard drugs like diazepam, ketamine, haloperidol, chlorpromazine, phenobarbitone, and phenytoin were purchased from local pharmacy, Coimbatore. All other drugs and chemicals used in the study were obtained commercially and were of analytical grade.

Experimental animals

Wistar rats of either sex weighing between 100-150 g were used for the study. They were housed in well ventilated room. The animals were fed with commercial rat feed pellets and given drinking water

ad libitum. The experiment protocol has been approved by the Institutional Animals Ethical Committee.

Acute toxicity studies

Acute oral toxicity study was carried out as per OECD - 423 guidelines[6]. A group of six *Wistar* rats of either sex selected by randomly and were used for acute toxicity study. The extracts were administered orally at the dose level of 5 mg/kg body weight by gastric intubation to overnight fasted animals and observed for 14 days. If mortality was observed in 2 out of 3 animals, then the dose administered was assigned as toxic dose, but if mortality was observed in one animal, then the same dose was repeated again to verify the toxic dose. If mortality was not observed, the procedure was repeated for further higher doses such as 50, 300 and 2000 mg / kg body weight.

Selection of dose of the extract

The hydroalcoholic extract of *Erythrina fusca* bark (HAEEF) was found to be non toxic up to the dose of 2000 mg/kg and did not cause any death, therefore it is considered as safe. The biological evaluation was carried out at 250 mg/kg and 500 mg/kg dose levels.

Evaluation of antiepileptic activity

Maximal electro shock (MES) - induced epileptic seizure

The maximal electro shocks (MES) induced epileptic seizures in animals represent grand mal type of epilepsy. In MES induced epilepsy, electroshock is applied through the corneal electrodes. Rats were divided into five groups consisting of six animals each. Group I served as epileptic control which received normal saline only (10 ml/kg, *p.o.*). Animals of groups II and III received HAEEF orally at doses of 250 mg/kg and 500 mg/kg respectively. Group IV received the standard drug phenytoin at a dose of 300 mg/kg, *p.o*. One hour after normal saline and drug administration, all the aminals received maximal electro shock (150 mA, 50 Hz for 2 sec). The animals were observed individually for 30 min from the time of electric shock applied for different phases of epileptic seizures[7].

Picrotoxin (PTX) - induced epileptic seizure method

Animals were grouped into four groups consisting of six animals each. Group I served as epileptic control group and received normal saline (10 ml/kg, *p.o.*). The animals of groups II and III received HAEEF orally at doses of 250 mg/kg and 500 mg/kg respectively. Group IV received the standard drug diazepam (10 mg/kg, *p.o.*). All the groups received convulsive dose of 3.5 mg/kg of picrotoxin by intraperitoneally (*i.p.*). The animals were observed for 30 minutes from the time of picrotoxin injection for onset and duration of different phases of epileptic seizures[8].

Pentylenetetrazole (PTZ) - induced epileptic seizure models

Animals were divided into four groups consisting of six animals each. Group I served as epileptic control receiving normal saline only (10 ml/kg, *p.o.*) and the animals of groups II and III received HAEEF orally at doses of 250 mg/kg and 500 mg/kg respectively. Group V received standard drug phenobarbitone (30 mg/kg, *p.o.*). Groups I–IV received convulsive dose of 80 mg/kg, *i.p* of pentylenetetrazole intraperitoneally. The animals were observed for onset and duration of different phases of epileptic seizures[9].

RESULTS AND DISCUSSION

The anticonvulsant activity of hydroalcoholic extract of the bark of *Erythrina fusca* Lour. (250 and 500 mg/kg) in *Wistar* rats were assessed using maximum electroshock seizures, pentylenetetrazole - and picrotoxin - induced epileptic seizure. The epileptic convulsive agent picrotoxin blocks, some but not all, responses to inhibitory nerve stimulation or GABA. There are two kinds of GABA receptors, one of which is blocked by bicuculline but not by picrotoxin, and the other which is blocked by both. Picrotoxin can act locally to disrupt the balance of inhibition and excitation and create epileptogenic focus. The epileptic effect of picrotoxin is considered simple partial and generalized tonic clonic seizures. Pentylenetetrazol is a central nervous system stimulant, and also widely used systematically administered epileptic agent. Repeated injections of PTZ and at high dose of PTZ reliably produces tonic clonic epileptic seizures in rats or mice. The epileptic effect of this drug is considered to be analogus to petit mal epilepsy in man. Recently pentylenetetrazol has been reported to act through GABA-benzodiazepine receptor mechanisms in the brain by antagonize the GABA receptors[10,11].

The HAEEF was significantly antagonized PTX - and PTZ - induced epileptic clonic seizures. However, it was found that extract produced more inhibition of clonic seizures than its the extensor and tonic phase seizures induced by MES. Both PTX and PTZ are GABA-ergic blockers, by selective blocker of the chloride ionophore complex to the $GABA_A$ receptor. Therefore the mechanism of antiepileptic effect of HAEEF may due to enhancing GABA receptor and blocking multineuronal pathways in the spinal cord.

Effect of HAEEF on maximal electro shock-induced epileptic seizure

The HAEEF did not completely abolish the tonic hind limb extension. However, HAEEF at a dose of 500 mg/kg, reduced the duration of tonic hind limb extension by almost 47.4% and clonic phase of epileptic seizure by 70.4%. The epileptic activity induced by MES was significantly ($P<0.01$) prevented in the animals treated with HAEEF extract (Table 1 and Fig. 1).

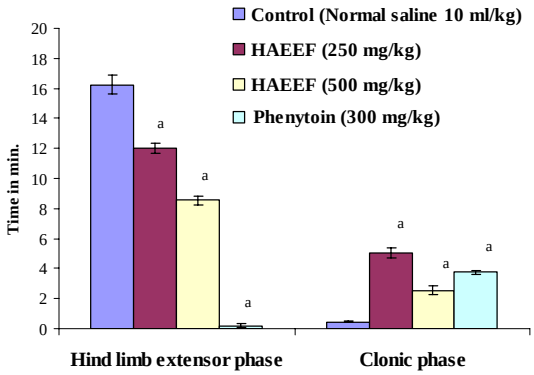

Fig. 1: Effect of HAEEF on MES - induced epilepsy

Effect of HAEEF on picrotoxin - induced epileptic seizure

The effect of HAEEF on PTX induced epilepsy is summarized in the Table 2 and Fig. 2. The HAEEF treated animals, at doses of 250 mg/kg and 500 mg/kg significantly ($P<0.01$) delayed the onset of effect (20.43 and 25.00 min respectively). The extract was also significantly ($P<0.01$) decrease in the clonic phase of epileptic seizures, when compared to the control group.

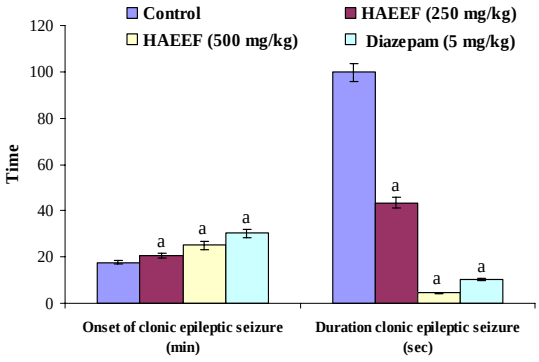

Fig. 2: Effect of HAEEF on PTX - induced epilepsy

Effect of HAEEF on pentylenetetrazole - induced epileptic seizure

The anti epileptic effect against PTZ was tabulated in the Table 3 and Fig. 3. In epileptic control animals, the onset of myoclonic epileptic

seizures were observed 122.49 sec after the administration of PTZ and the duration of seizures was found to be 137.21sec. HAEEF at doses of 250 mg/kg and 500 mg/kg delayed the onset of spasm (188.00 and 268.21 sec respectively) and the duration of epileptic seizures was reduced to 103.09 and 33.00 sec repectively The dose of 500 mg/kg of HAEEF significantly (P<0.01) inhibited spasm as well as epileptic seizures when compared to 250 mg/kg dose level.

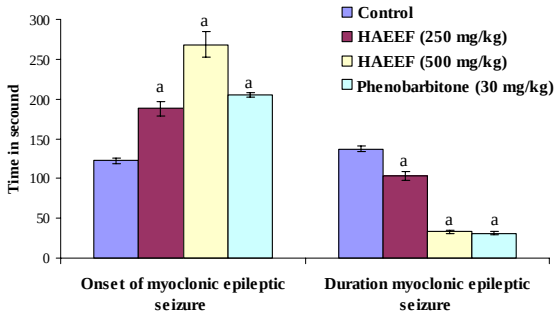

Fig. 3: Effect of HAEEF on PTZ - induced epilepsy

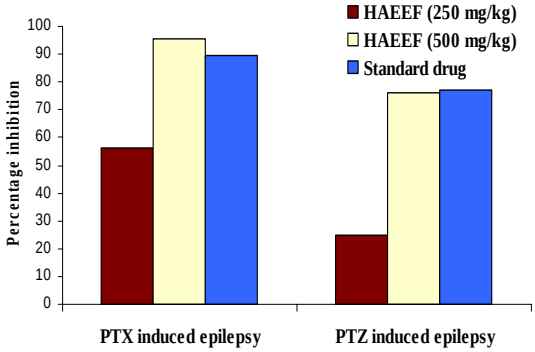

Fig. 4: Percentage inhibition of PTX- and PTZ - induced epilepsy by HAEEF

Table 1: Effect of HAEEF on MES - induced epilepsy

Group	Treatment	Duration of seizure (min)	
		Hind limb extensor phase	Clonic phase
I	Control (Normal saline, *p.o.*)	16.25 ± 0.45	8.45 ± 0.21
II	HAEEF (250 mg/kg, *p.o.*)	12.00 ± 0.31[a]	5.06 ± 0.44[a]
III	HAEEF (500 mg/kg, *p.o.*)	8.55 ± 0.27[a]	2.56 ± 0.31[a]
IV	Phenytoin (300 mg/kg, *p.o.*)	0.21 ± 0.16[a]	3.75 ± 0.10[a]

Values are mean ± SEM; n= 6 in each group, [a]P<0.01 when compared to control (ANOVA followed by Dunnett's test).

Table 2: Effect of HAEEF on PTX - induced epilepsy

Group	Treatment	Onset of clonic epileptic seizure (min)	Duration clonic epileptic seizure (sec)	Percent inhibition
I	Control (Normal saline.)	17.65 ± 0.32	99.77 ± 4.09	-
II	HAEEF (250 mg/kg, *p.o.*)	20.43 ± 0.12[a]	43.43 ± 3.12[a]	*56.46*
III	HAEEF (500 mg/kg, *p.o.*)	25.00 ± 2.34[a]	4.562 ± 0.88[a]	*95.42*
IV	*Diazepam (5 mg/kg, p.o.)*	*30.23 ± 1.79[a]*	*10.23 ± 0.76[a]*	*89.75*

Values are Mean ± SEM; n = 6 in each group, [a]P<0.01 when compared to control. (ANOVA followed by Dunnett's test)

Table 3: Effect of HAEEF on PTZ - induced epilepsy

Group	Treatment	Onset of myoclonic epileptic seizure (sec)	Duration myoclonic epileptic seizure (sec)	Percent inhibition
I	Control (Normal saline.)	122.49 ± 3.61	137.21 ± 3.01	-
II	HAEEF (250 mg/kg, *p.o.*)	188.00± 3.10[a]	103.09 ± 5.03[a]	*24.87*
III	HAEEF (500 mg/kg, *p.o.*)	268.21± 15.65[a]	33.00± 3.24[a]	*75.95*
IV	*Phenobarbitone (30 mg/kg, p.o.)*	*205.43 ± 4.70[a]*	*31.21± 2.99[a]*	*77.25*

Values are Mean ± SEM; n = 6 in each group, [a]P<0.01 when compared to control. (ANOVA followed by Dunnett's test)

CONCLUSION

In conclusion the results of the present studies demonstrated antiepileptic potential of hydroalcoholic extract of the *Erythrina fusca* L. bark and the extract may be to enhancing GABA receptor and block multineuronal pathways in the spinal cord to treat the disease. Further studies are needed to be ascertain its clinical effectiveness and mechanism of action.

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