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Antioxidant activity of the hydro-alcoholic extract of *Erythrina fusca* Lour. bark against the animal models of epilepsy

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ABSTRACT

The objective of the present study was to evaluate the anti-oxidant activity of the hydro-alcoholic extract of the bark of Erythrina fusca Lour (HAEEF) using different in vivo experimental models. 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 antioxidant activity. Thus, it can be inferred that the hydro-alcoholic extract of Erythrina fusca Lour bark possess antioxidant effect against the animal models of epilepsy. The malondialdehyde levels were significantly increased in serum and brain tissue, the levels of SOD is significantly decreased in brain tissue and epileptic seizures caused a significant ($P<0.01$) depletion in reduced glutathione (GSH) level in the rat brain tissue with intoxication of epileptic seizures by MES, PTX and PTZ models of epilepsy. The effect of HAEEF on oxidative stress in MES, PTX and PTZ models of epileptic seizures was evaluated and there was a simultaneous significant decrease in the SOD and reduced glutathione (GSH) levels. The decrease in MDA levels and increase in SOD and GSH levels in HAEEF-treated epileptic models may be due to its antioxidant property.

Key Words: *Erythrina fusca* Lour., Antioxidant.

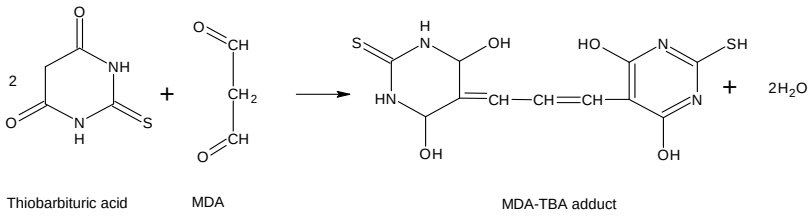
INTRODUCTION

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* L. 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 [1].

Drug therapy of epilepsy with currently available Antiepileptic Drugs (AEDs) is associated with side effects, dose related and chronic toxicity that involves virtually every organ system. Hazards of antiepileptic therapy in pregnancy and teratogenic effects are well known. Different type of epileptic seizures have varied susceptibility to currently available AEDs and on the whole approximately two third of patients with epilepsy can have potential for adverse effects on cognitions and behaviours [2, 3]. The problems with current AED therapy of epilepsy are more prevalent in undeveloped nations due to lack of facilities. The cost of new AED, i.e., lamotrigine (LMG), oxacarbazine (OCBZ), felbamate (FBM), vigabatrin (VGB) and gabapentin (GBP) is 3 – 6 times higher than the conventional AED, i.e., phenytoin (PHT), Phenobarbital (PHB), carbamazepine (CBZ) and valporic acid (VPA). AED therapy does not alter the course or natural history of epilepsy and though AED suppress the seizures but they may not affect the underlying epileptogenic process itself and thus the prognosis is determined in terms of seizure control and the removal of underlying disorder.

Lipid peroxidation is well estabilished mechanism of cellular in both animals and plants, and is used as an indicator of oxidative stress in cells and tissues [4]. The estimation of peroxidation of lipids has been carried out by number of methods of which thiobarbituric acid reactive substances is selected because of its high sensitivity simplicity in operation. The TBARS test is often said to measure malondialdehyde (MDA) formed in peroxidising lipids system by lipid peroxidation and reacts with TBA under high temperature (90-100°c) and acidic condition the reaction yield a pink MDA – TBA adduct **[5].** This coloured solution measured by simple fluorimetry or spectrophotometry (colorimetrically) using wave length 532 nm. So, the results are frequently expressed as micromole MDA equivalents.



Superoxide dismutase (SOD) is one of the most important antioxidant enzymes and present in all oxygen-metabolizing cells. Superoxide dismutase is divided in four classes considering their metal content. One of the classes is Cu^{2+} - Zn^{2+} enzyme which is found in the cytosol of eukaryotes, in chloroplasts, and in the periplasm of some prokaryotes; the second class is Mn3+ protein which is found in bacteria, archaea, mitochondria and chloroplasts whereas Fe^{3+} enzyme is present in both aerobic and anaerobic bacteria, archaea and plants and the fourth type Ni – SOD has been discovered in several Streptomyces species. Superoxide dismutase scavenges the superoxide ($2O_2$) to hydrogen peroxide and molecular oxygen in the following manner.

$$2H_2O + 2O_2 \rightarrow 2H_2O_2 + O_2$$

Epileptic and oxidative stress is the thought to be closely interrelated. In the case of GSH was reduced whereas lipid peroxidation and nitrite content were increased after epileptic seizures. Lipid peroxidation in the brain can be induced by many chemical compounds and brain injury such as epilepsy. The brain is more vulnerable to injury by lipid peroxidation products then other tissues. Moreover, lipid peroxidation is an index of irreversible neuronal damage of cell membrane phospholipids and has been suggested as a possible mechanism of epileptic seizures.

EXPERIMENTAL SECTION

Collection and authentication of plant

The fresh bark of *Erythrina fusca* L. was collected from the Coonoor, Nilgiris district, Tamil Nadu. The bark was authenticated by a taxonomist from Botanical Survey of India (BSI), Tamil Nadu Agricultural University (TNAU), Coimbatore.

Preparation of the extract

Fresh stem bark of *Erythrina fusca* L. were 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

Pentylenetetrazol (PTZ), picrotoxin (PTX), adrenaline tartrate was obtained from Himedia Ltd., Mumbai, India. Thiobarbituric acid (TBA), 5’5, 1-dithiobis-2 nitrobenzoic acid [DTNB], glutathione was obtained from Sigma chemical company, USA. All other drugs and chemicals used in the study were obtained commercially and were of analytical grade.

Instruments

Electro convulsio meter, Actophotometer, Rotarod, Shimadzu; Jasco V-530 UV/VIS spectrophotometer.

Experimental animals

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

Acute toxicity studies

Wistar albino rats (100-150 g) maintained under standard laboratory conditions was used. A total of five animals were used which received a single oral dose of (2000 mg/kg body weight) of hydroalcoholic extract of *Erythrina fusca* (HAEEF). Animals were observed individually at least once during first 30 minutes after dosing, periodically during the first 24 hrs (with special attention during first 4 hrs) and daily thereafter for a period of 14 days. Once daily cage side observation included changes in skin and fur, eyes and mucus membrane (nasal), and also respiratory rate, circulatory (heart rate and blood pressure), autonomic (salivation, lacrimation, perspiration, piloerection, urinary incontinence and defecation) and central nervous system (drowsiness, gait, tremor and convulsion) changes [6].

Measurement of lipid per oxidation level in serum

After 30 minutes of observation of each model of epileptic seizure, blood was collected from cornea by retro – orbital puncture for the estimation of serum malondialdehyde (MDA). After blood collection the blood samples were left to coagulate at room temperature for 1 hrs and serum was separated by centrifugation at 5000 rpm at 4^{o}C for 10 minutes. The extent of lipid peroxidation was quantified by measuring the thiobarbituric acid reactive substances (TBARS) – malondialdehyde produced during peroxidation of lipids [7].

To 1 ml of serum, 1.5 ml of 20% cold trichloroacetic acid (TCA) was added and was allowed to stand for 15 min at room temperature. The tube was centrifuged and to 2 ml of the supernatant, 1.5 ml of 0.67% of thiobarbituric acid (TBA) was added and the mixture was heated in a boiling water bath for 15 min. After cooling at room temperature, the mixture was centrifuged for 10 min at 1500 rpm. The absorbance of the pink coloured chromophore was measured at 532 nm. The level of lipid peroxidation was expressed as nanomoles of malondialdehyde per gm of wet tissue.

Preparation of brain homogenate

The brain was separated by sacrificing the animals in all models after 30 min observation period. Brain tissues were homogenized with 10 times (w/v) ice – cold 0.1 M phosphate buffer pH (7.4). Aliquots of homogenates from rat brain were used to determine lipid peroxidation, superoxide dismutase and glutathione.

Measurement of lipid peroxidation in brain tissues

One ml of supernatant was added to 1.5 ml of 20 % TCA solution, and allowed to stand for 15 min at room temperature. The tube containing this mixture was centrifuged and supernatant was separated. To this 2 ml of the supernatant was added to 1.5 ml of 0.67% of TBA solution and this mixture was heated in a boiling water bath for 15 minutes. After cooling at room temperature, a pink coloured chromophore was formed. The absorbance of the chromophore was measured at 532 nm. The concentration of MDA was expressed as nanomole of MDA/gm brain tissue [8].

Assay of antioxidant enzymes

Assay of superoxide dismutase (SOD)

To 150 ml of homogenate, 1.8 ml of carbonate buffer, 0.7 ml of distilled water and 400 ml of epinephrine were added and the absorbance was measured at 480 nm using a spectrophotometer. Auto – oxidation of epinephrine to adrenochrome was performed in a control tube without the homogenate. Activity was expressed as units/gm brain tissue [9].

Assay of reduced glutathione (GSH)

To 1 ml of homogenate was mixed with equal quantity of 10% TCA solution and centrifuged at 5000 rpm for 10 min. To 0.01 ml of this supernatant, 2 ml of phosphate buffer (pH-8.4), 0.5 ml of Ellman's reagent [5'5-dithiobis (2- nitrobenzoic acid)] and 0.4 ml of double distilled water were added. The absorbance was measured at 412 nm against blank which contained only 2 ml of phosphate buffer solution and 0.5 ml of Ellman's reagent (DTNB). A standard graph was constructed using 50 g of reduced glutathione. The activity was expressed as units/g brain tissue [10].

RESULTS

Effect of HAEEF on serum and brain tissue lipid peroxidation levels

The malondialdehyde levels were significantly increased in both serum and brain tissue with intoxication of epileptic seizures by MES, PTX and PTZ models of epilepsy. The increase in MDA content of the serum and brain tissue was significantly (P<0.01) prevented by HAEEF treated animals at both the selected doses (250 mg/kg and 500 mg/kg) and these levels were similar to the standard drugs (Table 1- 3).

Assay of Antioxidant Enzymes

Effect of HAEEF on superoxide dismutase activity [11]

The levels of SOD is significantly decreased to 34.18 ± 0.71, 14.28 ± 0.64 and 19.00 ± 0.381 units/g brain tissue in the MES, PTX and PTZ models respectively when compared with the control group. Oral administration of the HAEEF at doses of 250 mg/kg and 500 mg/kg did not significantly increase the brain tissue content of SOD as compared to the normal control. But both the doses of HAEEF inhibited the depletion of SOD levels as compared to epileptic control rats (Table 1-3).

Table 1: Effect of HAEEF on serum and brain tissue MDA level and antioxidant enzymes in MES-induced epileptic animals

Treatment	MDA nmol/g tissue		Antioxidant enzymes Units/g tissue	
	Serum	Brain tissue	SOD	GSH
Control (Saline 10 ml/kg-*p.o.*)	8.45 ± 0.653	15.72 ± 0.719	46.68 ± 1.662	110.29 ± 1.350
Epileptic control (MES 150 mA, 50HZ, 2 sec)	27.34 ± 1.243[a]	58.65 ± 0.716[a]	5.46 ± 0.214[a]	34.18 ± 0.710[a]
HAEEF(250 mg/kg-*p.o.*) + MES (150 mA, 50HZ, 2 sec)	20.18 ± 1.027[a]	38.23 ± 1.262[a]	6.89 ± 0.432[a]	53.11 ± 0.490[a]
HAEEF (500 mg/kg-*p.o.*) + MES (150 mA, 50HZ, 2	10.32 ± 1.327[a]	16.05 ± 0.910[a]	16.21 ± 0.098[a]	80.593 ± 0.481[a]
PHT(300 mg/kg-*p.o.*) + (MES 150 mA, 50HZ, 2 sec)	12.87 ± 0.701[a]	14.90 ± 0.641[a]	25.43 ± 0.348[a]	92.34 ± 0.284[a]

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

Effect of HAEEF on the reduced glutathione levels [12]

Epileptic seizures caused a significant (P<0.01) depletion in reduced glutathione (GSH) level in the rat brain to 36.23 ± 0.809, 32.65 ± 0.423 and 21.25 ± 0.96 units/g brain tissue in the epileptic models of MES, PTX and PTZ respectively, when compared to control group (110.29 ± 1.35). HAEEF at doses of 250 mg/kg and 500 mg/kg significantly increased the level of reduced glutathione in different models of epileptic rat brain as compared to the standard drug (Table 1-3).

Table 2: Effect of HAEEF on serum and brain tissue MDA level and antioxidant enzymes in PTX–induced epileptic animals

Treatment	MDA level nmol/g tissue		Antioxidant enzymes Units/g tissue	
	Serum	Brain tissue	SOD	GSH
Control (Saline 10 ml/kg)	6.36 ± 0.731	12.65 ± 0.637	43.18 ± 1.29	103.29 ± 1.731
Epileptic control PTX (3.5 mg/kg)	14.21 ± 1.320	90.65 ± 1.000	14.28 ± 0.640	32.65 ± 0.423
HAEEF (205 mg/kg-*o.p*) + PTX (3.5 mg/kg)	10.21 ± 0.512[a]	75.36 ± 1.310[a]	22.731 ± 0.471[a]	43.34 ± 0.421[a]
HAEEF (500 mg/kg*o.p.*) + PTX (3.5 mg/kg-*i.p*)	7.21 ± 0.814[a]	34.47 ± 1.932[a]	33.72 ± 0.551[a]	71.42 ± 0.721[a]
DZP (5 mg/kg-*o.p*) + PTX (3.5 mg/kg-*i.p*)	6.12 ± 0.418[a]	32.73 ± 1.827[a]	41.52 ± 0.660[a]	86.21 ± 0.337[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 3: Effect of HAEEF on serum and brain tissue MDA level, antioxidant enzymes in PTZ– induced epileptic animals

Treatment	MDA nmol/g tissue		Antioxidant enzymes Units/g tissue	
	Serum	Brain tissue	SOD	GSH
Control (saline 10 ml/kg)	8.32 ± 0.772	12.15 ± 0.821	38.04 ±1.964	100.57 ± 1.055
Epileptic control PTZ(80 mg/kg-*i.p.*)	51.03 ± 1.321	98.47 ± 1.354	19.00 ± 0.381	21.25 ± 0.960
HAEEF (250 mg/kg-*o.p*) + PTZ (80 mg/kg-*i.p.*)	33.12 ± 2.291[a]	71.22 ± 1.662[a]	21.48 ± 0.491[a]	38.21 ± 0.996[a]
HAEEF (500 mg/kg-*o.p.*) + PTZ (80 mg/kg-*i.p.*)	17.49 ± 0.759[a]	56.97 ± 1.107[a]	32.30 ± 0.559[a]	80.24 ± 1.435[a]
PHB(20 mg/kg-*o.p.*) + PTZ (80 mg/kg)	17.85 ± 1.009[a]	53.25 ± 1.806[a]	46.21 ± 0.391[a]	100.21 ± 0.929[a]

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

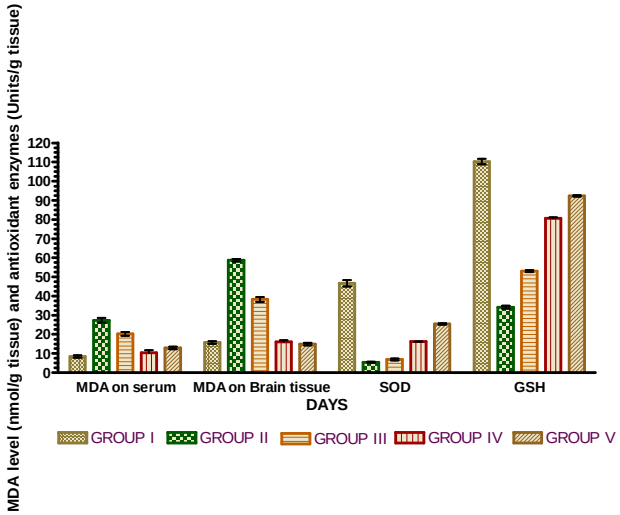


Fig. 1: Effect of HAEEF on serum and brain tissue MDA level and antioxidant enzymes in MES-induced epileptic animals.

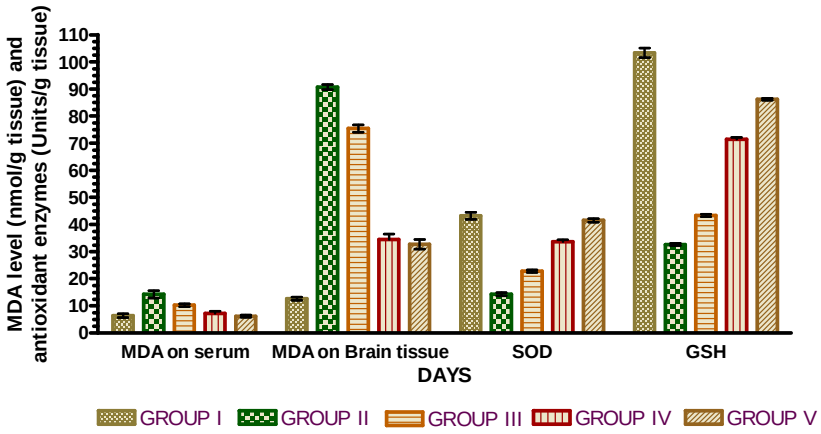


Fig. 2: Effect of HAEEF on serum and brain tissue MDA level and antioxidant enzymes in PTX– induced epileptic animals.

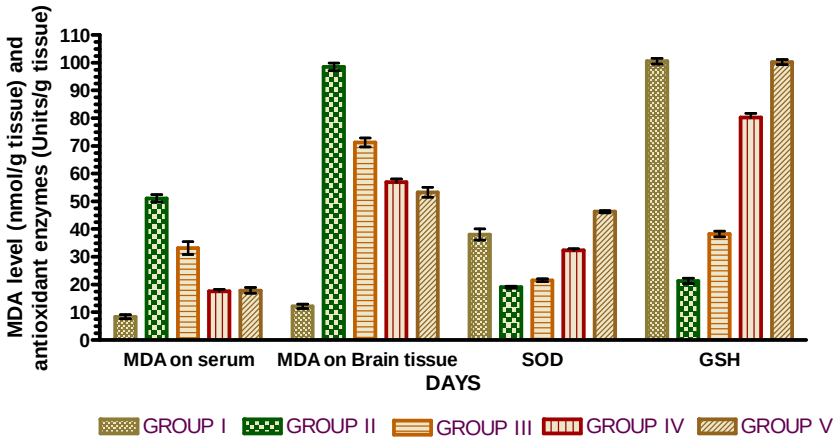


Fig. 3: Effect of HAEEF on serum and brain tissue MDA level, antioxidant enzymes in PTZ– induced epileptic animals

DISCUSSION

Free radicals in addition to contributing to neuronal injury in cerebral ischemia and hemorrhage, may be involved in neuronal degeneration in schizophrenia, normal aging, and Parkinson's and Alzheimer's diseases. Concerning epileptic seizures, excitatory amino acid receptor activation by glutamate or N-methyl-D-aspartic acid (NMDA) has been known to accompany generation of reactive oxygen species (ROS), e.g., superoxide anion radical ($O_2^{\cdot-}$) hydrogen peroxide (H_2O_2) and hydroxyl radical ($^{\cdot}OH$), and reactive nitrogen species (RNS), e.g., nitric oxide (NO) and peroxynitrite anion ($ONOO^-$). In fact, free $^{\cdot}OH$ is detectable after pentylenetrazole-induced seizure and kindling ROS and RNS are related in their metabolic pathway in that $ONOO^-$, formed from NO and $O_2^{\cdot-}$, is a potent oxidant that may exert injurious effects in the brain [13].

Nitric oxides (NO) have been pointed out as potential neurotransmitters (or) retrograde messengers linked to synaptic plasticity and regulation of brain excitability, including the triggering of seizures activity. NO is formed from L-arginine by the enzyme NO synthase (NOS) and the involvement of NO in epileptic disorders has been shown in experiments with

systemic injection of NOS inhibitors however, NOS inhibitor treatment has been reported either to augment (or) to inhibit experimentally, induced seizures.

Free radicals have been suggested to the most likely candidate responsible for producing the neuronal changes mediating the behavioral deficits in neurodegeneration disorders. Number of studies has demonstrated that antioxidants are effective in the rodent models of epilepsy. Currently, there has been an increasing interest in the biochemical effects of medicinal plants with antioxidant properties as they could be potential candidates for the prevention of oxidative damage associated with epileptic seizures.

Since some antioxidants have been shown to be effective in reducing the oxidative stress in the animal models of epilepsy, we evaluated the effect of HAEEF on oxidative stress in MES, PTX and PTZ models of epileptic seizures. There was a simultaneous significant decrease in the SOD and reduced glutathione (GSH) levels. The decrease in MDA levels and increase in SOD and GSH levels in HAEEF-treated epileptic models may be due to its antioxidant property [14].

CONCLUSION

In this study, elevation in the level of end products of lipid per oxidation (MDA) in epileptic seizures induced by MES, PTX and PTZ animals was observed. The increase in MDA level in brain suggests enhanced lipid per oxidation leading to tissue damage and failure of antioxidant defense mechanisms. Treatment with HAEEF significantly prevented these changes and has significantly increased the superoxide dismutase and glutathione content of rat brain. The antioxidant enzymes levels of the epileptic groups were decreased whereas that of HAEEF treated are almost similar to that of control and standard drug treated group.

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