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EFFECT OF *ENICOSTEMA AXILLARE* EXTRACT ON ANTIOXIDANT ENZYMES LEVELS IN RAT BRAIN AFTER INDUCTION OF SEIZURES

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ABSTRACT

The whole plant of *Enicostema axillare* is used in Indian traditional medicine to treat epilepsy. The purpose of the present study is to investigate the effect of Chloroform extract of *Enicostema axillare* (CEEA) on antioxidant enzymes in rat brain after induction of seizures by MES and PTZ. Our aim of study was relationship between seizure activities and altered the levels of antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GP), glutathione reductase (GR), catalase and lipid peroxidation on rat brain. Superoxide dismutase, glutathione peroxidase, glutathione reductase and catalase was decreased in rat brain due to seizure and it was restored significantly by administration of Chloroform extract of *Enicostema axillare* treated rats. Similar dose dependent results were obtained in PTZ model also. Whereas CEEA significantly decreased lipid peroxidation in both models. The anticonvulsant activity of CEEA might be because of the presence of antioxidant properties and so it delays the generation of free radical in MES & PTZ induced epilepsy.

Keywords: Antioxidant Enzymes, *Enicostema axillare*, Superoxide Dismutase (SOD), Glutathione Peroxidase (GP), Glutathione Reductase (GR), Catalase and Lipid Peroxidation.

INTRODUCTION

Epilepsy constitutes a large group of neurological diseases with an incidence of 0.5–1% in the general population [1]. Many reports suggest a cascade of biological events underlying development and progression of epilepsy. Generalized epilepsy is a chronic disorder characterized by recurrent seizures which can increase the content of reactive oxygen species (ROS) generation in the brain [2]. Brain is susceptible to free radical damage, considering the large lipid content of myelin sheaths and the high rate of brain oxidative metabolism [3]. Thus, it appears that free radicals may be responsible for the development of convulsions.

Hence, the aim of the study is to evaluate the status of some of the antioxidant enzymes in rat brain after induction of seizure by MES and PTZ.

***Enicostema axillare* w.p** (Family: Gentianaceae) is native to tropical Africa, India, Southeast Asia and Malaysia. It is a perennial herb found throughout India and is common in coastal areas**.** The plant is used in folk medicine to treat epilepsy, Diabetes mellitus, Rheumatism, Cancer and Hepatic disorders [4]. Therefore, the present study was performed to verify the effect of *Enicostema axillare* on antioxidant levels in rat brain after induction of seizures by MES and PTZ model.

MATERIALS AND METHODS

Plant collection

The whole plant materials of Enicostema axillare were collected in month of January from Tirupati, Chittoor (Dist), Andhra Pradesh, India. The taxonomical identification of the plant was done by Dr. K. Madhava chetty, Assistant Professor, Department of Botany, Sri Venkateshwara University (SVU), Tirupati. The voucher specimen of the plant was deposited at the college for further reference.

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Preparation of extracts

The whole plants were dried in shade, separated and made to dry powder. It was then passed through 40 mesh sieve. A weighed quantity (60gm) of the powder was subjected to continuous hot extraction in Soxhlet Apparatus. The extract was evaporated under reduced pressure using rotary evaporator until all the solvent has been removed to give an extract sample. Percentage yield of ethanolic extract of *G. Speciosa* was found to be 14.5 % w/w.

Animals used

Albino Wistar rats (150-200g) of either sex were obtained from the animal house in Sree Vidyanikethan College of Pharmacy, Tirupati. The animals were maintained in a well-ventilated room with 12:12 hour light/dark cycle in polypropylene cages. The animals were fed with standard pellet feed (Hindustan Lever Limited., Bangalore) and water was given ad libitum. Ethical committee clearance was obtained from IAEC (Institutional Animal Ethics Committee) of CPCSEA. (Ref No. **IAEC / XIII / 05 / SVCP / 2010)**

Experimental Design

Albino wistar rats were divided into four groups of six animals each. Group I received vehicle control (0.9% normal saline, 1ml/100gm) whereas Group-II and III, received chloroform extract of the whole plant of *Enicostema axillare* (CEEA) (200 and 400 mg/kg body weight) *p.o* respectively for 14 days. On the 14th day, seizures are induced to all the groups by using an Electro convulsiometer. The duration of various phases of epilepsy were observed.

Pentylenetetrazole (90mg/kg b.w, *s.c*) was administered to other groups to induce clonic convulsions after above respective treatment. Animals were observed for a period of 30mins post– PTZ administration.

Estimation of antioxidant enzymes in rat brain after induction of seizures

On the day of experiment, 100 mg of the brain tissue was weighed and homogenate was prepared in 10 ml tris hydrochloric acid buffer (0.5 M; pH 7.4) at 4°C. The homogenate was centrifuged and the supernatant was used for the assay of antioxidant enzymes namely catalase [5], glutathione peroxidase [6], superoxide dismutase [7], glutathione reductase [8] and lipid peroxidation [9].

Statistical Analysis

The data were expressed as mean ± standard error mean (S.E.M). The Significance of differences among the group was assessed using one way and multiple way analyses of variance (ANOVA). The test followed by Dunnet's test p values less than 0.05 were considered as significance.

RESULTS

Effect of CEEA on antioxidant enzymes in seizure induced rats by MES and PTZ:

The levels of antioxidant enzymes such as superoxide dismutase, glutathione peroxidase, glutathione reductase and catalase were significantly reduced ($p<0.01$) due to induction of seizure by MES and PTZ in Group II, whereas lipid peroxidation enzymes significantly increased ($p<0.01$) in both models. Administration of CEEA at the doses of 200 and 400mg/kg significantly increased ($p<0.05$ and $p<0.01$) the levels of the enzymes on the rat brain. Lipid peroxidation was significantly decreased ($p<0.05$) by the administration of CEEA 200 and 400 mg/kg. **(Table 1 & 2)**

DISCUSSION AND CONCLUSION

Oxygen is necessary for many important aerobic cellular reactions but it may undergo electron transfer reactions which generate highly reactive oxygen free radicals such as superoxide anion radical, hydrogen peroxide or the hydroxyl radical. The brain is extremely susceptible to oxidative damage induced by these reactive species [10]. The free radicals generated cause cascade of neurochemical events leading to neurodegeneration and cell death [11]. It was reported that the content of reactive oxygen species in the brain might be elevated by the seizure activity [3].

The study showed that electroshock induced seizure produce changes in levels of oxidative stress and supported previous works which indicated that oxidative stress processes are implicated as contributory factors in epilepsy. High level of oxidative damage was detected both in case of electrically generated seizures, viz. electroshock induced seizures [12, 13] and PTZ induced seizure models [14].

Inactivation of oxygen free radicals can be carried out by antioxidative enzymes, like superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase [15, 16]. In the previous studies also, it was reported that MES induced seizure shows marked reduction of antioxidant enzymes like glutathione peroxidase, catalase, glutathione reductase, Superoxide dismutase [17] and the intracerebroventricularly administered glutathione (GSH) inhibited pentylenetetrazole (PTZ) induced convulsions in mice [18]. The results of this study showed that CEEA at the doses of 200 & 400mg/kg significantly increased the levels of antioxidant enzymes such as superoxide dismutase, glutathione peroxidase, glutathione reductase and catalase on rat brain.

Simultaneously lipid peroxidation level increases in brain during epileptic seizures [2, 19, 20]. It was also documented that changes in glutathione peroxidase activity in brain homogenates were inversely correlated with intensity of lipid peroxidation. It may be supposed

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that decrease in glutathione peroxidase activity causes failure of H_2O_2 detoxification. H_2O_2 accumulated in brain tissue ions present in the brain may undergo Fenton's reaction in which hydroxy radicals are produced. These reactive oxygen species participate in lipid peroxidation processes. Increase in lipid peroxidation in brain observed in the present study was dependent on decrease in glutathione peroxidase activity. They suggested that oxidative stress and lipid peroxidation rise might occur during seizure and participate in the pathophysiology of epilepsy. In the present study, results showed that CEEA significantly decreased lipid peroxidation on rat brain. Participation of oxygen free radicals and oxidative stress in Seizure etiology may indirectly be confirmed by anticonvulsant activity of antioxidant enzymes [21].

In conclusion, the study results are in accordance with the previous reports of antioxidant enzymes level in rat brain. CEEA at the doses of 200 & 400mg/kg significantly increased the levels of antioxidant enzymes such as superoxide dismutase, glutathione peroxidase, glutathione reductase and catalase on rat brain. Inversely lipid peroxidation was decreased in CEEA treated rats. Hence the antioxidant properties of CEEA extract delays the generation of free radical in MES & PTZ induced epilepsy. Participation of oxidative stress in seizure induction and pathophysiology of epilepsy awaits further clarification.

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