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REGULATION OF ANTIOXIDANT ENZYMES LEVELS IN RAT BRAIN – A SIGNIFICANT ROLE OF *RORIPPA SARMENTOSA* (DC.) MACBR. EXTRACTS

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ABSTRACT

The whole plant of *Rorippa sarmentosa* (DC.) Macbr. is used traditional Indian medicine to treat epilepsy. Previous studies have demonstrated that extracts of these plants was subjected to acute toxicity and then screened for antiepileptic activity on Maximal Electroshock (MES) and Pentylene tetrazole (PTZ) induced seizures models in albino wistar rats. The purpose of the present study is to investigate the effect of ethanolic (95%) extract of *Rorippa sarmentosa* (DC.) Macbr. (EERS) 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 ethanol extract of *Rorippa sarmentosa* (DC.) Macbr. treated rats. Similar dose dependent results were obtained in PTZ model also. Whereas EERS significantly decreased lipid peroxidation in both models. The anticonvulsant activity of EERS might be presents of antioxidant properties and it delays the generation of free radical in MES & PTZ induced epilepsy.

Keywords: Antioxidant Enzymes, *Rorippa sarmentosa* (DC.) Macbr. , Superoxide Dismutase (SOD), Glutathione Peroxidase (GP), Glutathione Reductase (GR), Catalase and Lipid Peroxidation.

INTRODUCTION

Epilepsies constitute 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.

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A number of studies suggest that oxidative stress plays an important role in the etiology of epilepsy. In previous studies, this problem was addressed in many experimental models of epilepsy, such as kainic acid (KA) [4, 5], iron-salt induced seizures [6], electroshock induced seizures [7] and in the kindling model of complex partial seizures [8]. In case of chemically induced seizures, the presence of oxygen free radicals may be caused by inducing agents themselves and it might not be solely connected with seizures [9]. 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.

Rorippa sarmentosa (DC.) Macbr. (Family: Brassicaceae) is common in damp waste areas from near sea-level to more than 1000 m elevation. Probably it is native to Melanesia and now widely distributed throughout most of the tropical Pacific. In Fiji, the plant is used to induce miscarriages and to cure convulsions in children [10-12]. Therefore, the present study was performed to verify the effect of *Rorippa sarmentosa (DC.) Macbr.* on antioxidant levels in rat brain after induction of seizure by MES and PTZ model.

MATERIALS AND METHODS

Plant collection

The Plant material of *Rorippa sarmentosa (DC.) Macbr.* used for investigation was collected from Tirunelveli District, in the Month of August 2007. The plant was authenticated by Dr.V.Chelladurai, Research Officer Botany. C.C.R.A.S., Govt. of India. The voucher specimen (CHE-SA-GS-01) of the plant was deposited at the college for further reference.

Preparation of extracts

Whole plant of the whole plants were dried in shade, separated and made to dry powder. It was then passed through the 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 *Rorippa sarmentosa (DC.) Macbr.* was found to be 17.5 % w/w.

Animals used

Albino wistar rats (150-230g) of either sex were obtained from the animal house in Vaagdevi Institute of Pharmaceutical Sciences, Bollikunta, Warangal. 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.

Experimental Design

Albino wistar rats were divided into four groups of six animals each. Group I received vehicle control (1% w/v SCMC, 1ml/100 g) whereas Group-II and III, received 95% ethanolic extract of the whole plant of *Rorippa sarmentosa (DC.) Macbr.* (EERS) (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 Electroconvulsimeter. 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 seizure

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 [13], glutathione peroxidase [14], superoxide dismutase [15], glutathione reductase [16] and lipid peroxidation [17].

Statistical Analysis

The data were expressed as mean \pm 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 EERS 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 EERS 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 EERS 200 and 400 mg/kg. (Table 1 & 2).

Table: 1. Effect of EERS on antioxidant enzymes in rat brain after induced seizure by MES

Group	Design of Treatment	Superoxide dismutase Units/mg protein	Catalase Units/mg protein	Glutathione Reductase Units/mg protein	Glutathione Peroxidase Units/mg protein	Lipid peroxidation N mol MDA/mg protein
I	Vehicle Control (SCMC 1ml/100gm)	10.63 ± 0.27	20.28 ± 0.60	31.16 ± 0.60	25.33 ± 0.76	1.33 ± 0.21
II	MES (SCMC 1ml/100gm)	7.8±0.29 ^{a**}	12.28±0.33 ^{a**}	24.16±0.3 ^{a**}	15.33±0.49 ^{a**}	4±0.36 ^{a**}
III	EERS 200 mg/kg, <i>p.o</i>	10.7±0.23 ^{b**}	16.89±0.42 ^{b**}	25.29±0.28 ^{b**}	15.33±0.65 ^{b**}	3.17±0.66 ^{b*}
IV	EERS 400 mg/kg, <i>p.o</i>	11.3±0.30 ^{b**}	20.37±0.42 ^{b**}	23.19±0.73 ^{b**}	22±0.18 ^{b**}	2.98±0.3 ^{b*}

Values are expressed as mean ± SEM of six observations

Comparison between: **a-** Group I Vs Group II, **b-** Group II Vs Group III and Group IV.

Statistical significant test for comparison was done by ANOVA, followed by Dunnet's test

**p*<0.05; ** *p*<0.01

Table: 2. Effect of EERS on antioxidant enzymes in rat brain after induced seizure by PTZ

Group	Design of Treatment	Superoxide dismutase Units/mg protein	Catalase Units/mg protein	Glutathione Reductase Units/mg protein	Glutathione Peroxidase Units/mg protein	Lipid peroxidation N mol MDA/mg protein
I	Vehicle Control (SCMC 1ml/100gm)	12.76 ± 0.60	20.83 ± 0.60	31.16 ± 0.60	25.33 ± 0.76	1.33 ± 0.21
II	PTZ (SCMC 1ml/100gm)	8.67±0.3 ^{a**}	12.37±0.33 ^{a**}	24.16±0.27 ^{a**}	18.33±0.49 ^{a**}	3.82±0.4 ^{a**}
III	EERS 200 mg/kg, <i>p.o</i>	10.28±0.29 ^{b*}	18.90.42 ^{b**}	25.54±0.34 ^{b**}	22.38±0.42 ^{b**}	3.37±0.3 ^{b*}
IV	EERS 400 mg/kg, <i>p.o</i>	11.28±0.29 ^{b**}	19.28±0.30 ^{b*}	28.10±0.29 ^{b**}	20.26±0.6 ^{b**}	3.39±0.25 ^{b*}

Values are expressed as mean ± SEM of six observations

Comparison between: **a-** Group I Vs Group II, **b-** Group II Vs Group III and Group IV.

Statistical significant test for comparison was done by ANOVA, followed by Dunnet's test

**p*<0.05; ** *p*<0.01

DISCUSSION

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 [18]. The free radicals generated cause cascade of neurochemical events leading to neurodegeneration and cell death [19]. 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 [7, 9] and PTZ seizure models [20].

Inactivation of oxygen free radicals can be carried out by antioxidative enzymes, like superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase [21, 22]. Previous study was reported, MES induced seizure shows marked reduction of antioxidant enzymes like glutathione peroxidase, catalase, glutathione reductase, Superoxide dismutase [23] and the intracerebroventricularly administered glutathione (GSH) inhibited pentylenetetrazole (PTZ) induced convulsions in mice [24]. The results of this study showed that EERS 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.

Whereas lipid peroxidation level increases in brain during epileptic seizures [2, 25, 26]. We documented that changes in glutathione peroxidase activity in brain homogenates were inversely correlated with intensity of lipid peroxidation. It may be supposed that decrease in glutathione peroxidase activity causes failure of H₂O₂ detoxification. H₂O₂ accumulated in brain tissue iron 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 [27, 28, 29]. Increases in lipid peroxidation in brain observed in the present study were 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 present study results showed that EERS 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 [6].

CONCLUSION

In conclusion, present study results are in accordance with the previous reports of antioxidant enzymes level in rat brain. EERS 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 decreased in EERS treated rats. Hence the antioxidant properties of EERS 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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