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Research Paper

Effect of *Guettarda speciosa* extracts on antioxidant enzymes levels in rat brain after induction of seizures by MES and PTZ

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ABSTRACT

The inner bark of *Guettarda speciosa* Linn. is used traditional Indian medicine to treat epilepsy. In present study the effect of ethanolic (95%) extract of *Guettarda speciosa* (EEGS) on antioxidant enzymes in rat brain after induction of seizures by MES and PTZ were observed. In which Superoxide dismutase, glutathione peroxidase, glutathione reductase and catalase was significantly ($P<0.01$) decreased in rat brain due to seizure and it was significantly ($P<0.01$) restored by administration of ethanol extract of *Guettarda speciosa* treated rats. Similar dose dependent results were obtained in PTZ model also. Whereas EEGS significantly decreased lipid peroxidation in both models. The anticonvulsant activity of EEGS might be presents of antioxidant properties and it delays the generation of free radical in MES & PTZ induced epilepsy.

Keywords: Antioxidant enzymes; *Guettarda Speciosa*, Superoxide Dismutase; Glutathione Peroxidase; Glutathione Reductase; Catalase; Lipid peroxidation.

INTRODUCTION

Epilepsies constitute a large group of neurological diseases with an incidence of 0.5–1% in the general population. (Yegin, et al., 2002) 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. (Sudha, et al., 2001) Brain is susceptible to free radical damage, considering the large lipid content of myelin sheaths and the high rate of brain oxidative metabolism. (Choi, 1993) Thus, it appears that free radicals may be responsible for the development of convulsions.

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), (Dal-Pizzol, et al., 2000; Glucket, al., 2000) iron-salt induced seizures, (Kabuto, et al., 1998) electroshock induced seizures (Rola, et al., 2002**)** and in the kindling model of complex partial seizures. (Frantseva, et al., 2000) 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. (Barichello, et al., 2004) 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. Hence, the present study to evaluate the status of the antioxidant enzymes in rat brain after induction of seizure by MES and PTZ.

Guettarda speciosa Linn. (Family-*Rubiaceae)* is widely distributed from East Africa to India and throughout to Malaysia into the South Pacific. This A decoction of the leaves is used to treat coughs, colds and sore throats. The native practitioners in and around Tirunelveli District, India, have claimed that the inner bark of this plant are being traditionally used in epilepsy. (Weiner, 1984; Weiner, 1971) Upon literature review it was found that the plant contains loganic acid and secologanin. (Inouye, et al., 1988; Cambie and Ash, 1994) Anti epileptic and antidiarrhoeal activity of Guettarda specoisa was reported. 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 Pentylenetetrazole (PTZ) induced seizures models in albino wistar rats. (Saravanakumar, et al., 2009; Gandhimathi, et al., 2009) Therefore, the present study was performed to verify the effect of *Guettarda speciosa* on antioxidant levels in rat brain after induction of seizure by MES and PTZ model.

MATERIALS AND METHODS

***Plant collection*:** The Plant material of *Guettarda Speciosa* 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: Inner bark 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 (60g) 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 17.5 % w/w.

Animals used: Albino wistar rats (150-230g) of either sex were obtained from the animal house in C.L. Baid Metha College of Pharmacy, Chennai. 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/ 01 / CLBMCP / 2007 - 2008 dt.24-07-2007).

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 inner bark of *Guettarda speciosa* (L.) (EEGS) (200 and 400 mg/kg body weight) by oral 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) was administered by subcutaneous 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, (Aebi, 1983) glutathione peroxidase, (Lawrence and Burk, 1976) superoxide dismutase, (Marklund and Marklund, 1974) glutathione reductase (Dobler and Anderson, 1981) and lipid peroxidation. (Luck, 1965)

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 analysis of variance (ANOVA). The test followed by Dunnet's test *P* values less than 0.05 were considered as significance.

RESULTS

Effect of EEGS on antioxidant enzymes in seizure induced rats by MES and PTZ: The levels 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 EEGS 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 EEGS 200 and 400 mg/kg (Table-1 and 2).

Table-1: Effect of EEGS 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 Nmol MDA/mg protein
I	Vehicle Control (SCMC 1ml/100gm)	13.83 ± 0.60	21.83 ± 0.60	31.16 ± 0.60	25.33 ± 0.76	1.33 ± 0.21
II	MES (SCMC 1ml/100gm)	8±0.36^{a**}	13.32±0.33^{a**}	24.16±0.3^{a**}	15.33±0.49^{a**}	4±0.36^{a**}
III	EEGS 200mg/kg,*p.o*	11.83±0.47^{b**}	17.50±0.42^{b**}	26.66±0.33^{b**}	16.33±0.55^{b**}	3.33±0.33^{b*}
IV	EEGS 400mg/kg *p.o*	12.83±0.30^{b**}	19.33±0.42^{b**}	24.50±0.22^{b**}	23±0.85^{b**}	2.83±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 't' test.
- *$P<0.05$;** $P<0.01$

Table- 2: Effect of EEGS 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 Nmol MDA/mg protein
I	Vehicle Control (SCMC 1ml/100gm)	13.83± 0.60	21.83 ± 0.60	31.16 ± 0.60	25.33 ± 0.76	1.33 ± 0.21
II	PTZ (SCMC 1ml/100gm)	8.83±0.3^{a**}	13.66±0.33^{a**}	23.16±0.79^{a**}	18.33±0.49^{a**}	3.83±0.4^{a**}
III	EEGS 200mg/kg,*p.o*	11.50±0.42^{b*}	19.33±0.42^{b**}	25.83±0.3^{b**}	20.50±0.42^{b**}	3.16±0.3^{b*}
IV	EEGS 400mg/kg,*p.o*	12.16±0.47^{b**}	19.83±0.30^{b*}	28.50±0.84^{b**}	21.83±0.6^{b**}	3.28±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 't' 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. (Sayre, et al., 1999) The free radicals generated cause cascade of neurochemical events leading to neurodegeneration and cell death. (Vesna, et al., 2003) It was reported that the content of reactive oxygen species in the brain might be elevated by the seizure activity. (Choi, 1993)

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 (Rola, et al., 2002; Barichello, et al., 2004**)** and PTZ seizure models. (Rauca, et al., 1999)

Inactivation of oxygen free radicals can be carried out by antioxidative enzymes, like superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase. (Halliwell, 1992; Sies, 1993) Previous study was reported, MES induced seizure shows marked reduction of antioxidant enzymes like glutathione peroxidase, catalase, glutathione reductase, Superoxide dismutase (Nieoczym, et al., 2008) and the intraceribroventricularly administered glutathione (GSH) inhibited pentylenetetrazole (PTZ) induced convulsions in mice. (Abe, et al., 1999) The results of this study showed that EEGS 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. (Paglia and Valentine, 1967; Garey, et al., 1994; Sudha, et al., 2001) 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 H_2O_2 detoxification. H_2O_2 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. (Frindivich, 1978; Halliwell and Gutteridge, 1999; Marnett, 2002) 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 EEGS 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. (Kabuto, et al., 1998)

CONCLUSION

Thus EEGS 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 EEGS treated rats. Hence the antioxidant properties of EEGS 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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