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ANTIOXIDANT PROPERTIES OF *OCHNA OBTUSATA* EXTRACT DELAYS THE GENERATION OF FREE RADICAL IN PTZ INDUCED EPILEPSY

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ABSTRACT

The leaves of *Ochna obtusata DC.* is traditionally used for treats epilepsy. In present study the effect of ethanol extract of *Ochna obtusata DC.* (EEOO) on antioxidant enzymes in rat brain after induction of epilepsy by PTZ were observed. In which Superoxide dismutase, glutathione peroxidase, glutathione reductase and catalase was significantly ($P<0.01$) decreased in rat brain due to epilepsy and it was significantly ($P<0.01$) restored by administration of ethanol extract of *Ochna obtusata DC.* treated rats. Similar dose dependent results were obtained in PTZ model. Whereas EEOO significantly decreased lipid peroxidation in PTZ model. The anticonvulsant activity of EEOO might be presents of antioxidant properties and it delays the generation of free radical in PTZ induced epilepsy.

Keywords: Antioxidant enzymes, *Ochna obtusata DC*, Superoxide Dismutase, Glutathione Peroxidase, Glutathione Reductase; Catalase, Lipid peroxidation.

INTRODUCTION

Ochna obtusata DC (Family- Ochnaceae). Habit: Small trees up to 8 m tall. Trunk & Bark: Bark greyish, smooth; blaze pinkish. Branches and branchlets: Branchlets terete, lenticellate, glabrous. Leaves: Leaves simple, alternate, distichous; stipules caducous and leaving scar; petioles ca. 0.4 cm long, planoconvex, glabrous; lamina 16 x 5 cm, elliptic or elliptic-oblong to obovate, apex acute to rounded, base acute to rounded, margin serrate, shining above, chartaceous, glabrous beneath; midrib raised above; secondary_nerves ca. 12 pairs, ascending towards apex; tertiary_nerves slender, reticulo-percurrent. Inflorescence / Flower: Inflorescence axillary or lateral racemes; flowers yellow; pedicels up to 2.5 cm long. Fruit and Seed: Drupe, 3-5 distinct drupes seated on the enlarged disk; seeds 1 drupe. Distribution: South Asia; in the Western_Ghats- South, Central and Maharashtra Sahyadris. The leaves and roots of Ochna obtusata is used for fits, fever, ulcer, asthma and bronchitis [1]. From the source of literature documentation and relevant traditional approaches on plant drugs, the present investigation was carried out to investigate the anti-convulsant activity of the ethanol extract of Ochna obtusata leaves on antioxidant enzymes in rat brain after induction of epilepsy by PTZ is being reported here.

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MATERIALS AND METHODS

Plant material

The leaves of Ochna obtusata was collected from Tirumala hills, Tirupati, Andhra Pradesh. India. It was identified and authenticated by Prof. Madhava Chetty, K., Taxonomist, S.V. University, Tirupati, Andhra Pradesh,

India. A voucher specimen has been kept in our laboratory for future reference.

Preparation of plant extract

The collected leaves were dried at room temperature, pulverized by a mechanical grinder, sieved through 40mesh. About 100g of powdered materials were extracted with Ethanol (90%) using soxhlet apparatus. The extraction was carried out until the extractive becomes colourless. The extracts is then concentrated and dried under reduced pressure. The solvent free semisolid mass thus obtained is dissolved in tween 80 and used for the experiment. The percentage yield of prepared extract was around 14.5%w/w.

Animals Used

Albino rats (180–200 g) of either sex were maintained in a 12 h light/dark cycle at a constant temperature 25 ◦C with free access to feed (Sai durga feeds and foods, Bangalore) and water. All animals were fasted prior to all assays and were allocated to different experimental groups each of 6 rats. Moreover the animals were kept in specially constructed cages to prevent coprophagia during the experiment. All experiments were carried out according to the guidelines for care and use of experimental animals and approved by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). 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 ethanol extract of *Ochna obtusata DC.* (EEOO) (250 and 500 mg/kg body weight) *p.o* respectively for 14 days. On the 14th day, 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 [2], glutathione peroxidase [3], superoxide dismutase [4], glutathione reductase [5] and lipid peroxidation [6].

Statistical analysis

The data were expressed as Mean ± S.E.M. and statistically analyzed using one way ANOVA followed by Tukey-Kramer's Multiple comparison test, $p<0.05$ was considered significant.

RESULTS

Effect of EEOO on antioxidant enzymes in seizure induced rats by 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 PTZ in Group II, whereas lipid peroxidation enzymes significantly increased ($p<0.05$) in PTZ model. Administration of EEOO at the doses of 250 and 500mg/kg significantly increased ($p<0.05$) the levels of the enzymes on the rat brain. Lipid peroxidation was significantly decreased ($p<0.05$) by the administration of EEOO 250 and 500 mg/kg. (Table 1).

Table 1. Effect of EEOO 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)	12.33 ± 0.14	24.14 ± 0.11	35.27 ± 0.19	28.12 ± 0.32	2.84 ± 0.24
II	PTZ (SCMC 1ml/100gm)	7.68±0.24^{a}****	14.28±0.12^{a}****	26.57±0.37^{a}****	19.27±0.26^{a}****	3.82±0.14^{a}****
III	EEOO 250 mg/kg,*p.o*	12.04±0.14^{b}***	18.35±0.22^{b}****	29.33±0.28^{b}****	25.33±0.18^{b}****	3.64±0.21^{b}***
IV	EEOO 500 mg/kg,*p.o*	13.52±0.13^{b**}	22.37±0.48^{b*}	32.66±0.15^{b**}	27.51±0.15^{b**}	2.54±0.22^{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 AND CONCLUSION

The present study showed that, high level of oxidative damage was detected in case of chemically seizures, viz. PTZ seizure model [6]. Inactivation of oxygen free radicals can be carried out by antioxidative enzymes, like superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase [7,8]. Previous study was reported, the intracerebroventricularly administered glutathione (GSH) inhibited pentylenetetrazole (PTZ) induced convulsions in mice [2]. The results of this study showed that EEOO at the doses of 250 & 500mg/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 [9-11]. 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 [12-14]**.** 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. Participation of oxygen free radicals and oxidative stress in seizure etiology may indirectly be confirmed by anticonvulsant activity of antioxidant enzymes [15]**.**

In conclusion, EEOO at the doses of 250 & 500mg/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 EEOO treated rats. Hence the antioxidant properties of EEOO extract delays the generation of free radical in PTZ induced epilepsy.

REFERENCES

1. Madhava Chetty K. *Ochna obtusata DC*. Chittoor medicinal plants, Himalaya Book Publications, Tirupati, 2005.
2. Abe K, Nakanishi K, Satio H. The anticonvulsive effect of glutathione in mice. *Biol Pharm Bull*., 1999; 22:1177-1179.
3. Lawrence RA, Burk RF. Glutathione peroxidase activity in selenium deficient rat liver. *Biochem Biophys Res Comm*., 1976; 71: 952-58.
4. Marklund S, Marklund G. Involvement of superoxide anion radical in auto-oxidation of pyrogallol and a convenient assay of superoxide dismutase. *Eur J Biochem*., 1974; 47: 469- 74.
5. Dobler RE, Anderson BM. Simultaneous inactivation of the catalytic activities of yeast glutathione reductase by N-alkyl melimides. *Biochem Biophys Acta*., 1981; 70: 659.
6. Luck H. In: Methods of enzymatic analysis. 2nd ed. New York, Academic Press, 1965; 885-90.
7. Halliwell B. Reactive oxygen species and the central nervous system. *J Neurochem*., 1992; 59: 1609-1623.
8. Sies H. Stratagies of antioxidant defense. *Eur J Biochem*., 1993; 215: 213-219.
9. Sudha K, Rao AV, Rao A. Oxidative stress and antioxidants in epilepsy. *Clin Chim Acta.*, 2001; 303(1– 2):19–24.
10. Garey CE, Schwartzman AL, Rise ML, Seyfried TN. Ceruloplasmin gene defect associated with epilepsy in EL mice. *Nat Genet*., 1994; 6: 426–31.
11. Paglia DE, Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte GP. *J Lab Clin Med*., 1967; 70: 158–69.
12. Frindivich I. The biology of oxygen radicals. *Science*, 1978; 201: 875-880.
13. Halliwell B, Gutteridge JMC. Free radicals in biology and medicine. Oxford University Press, Newyork. 1999.
14. Marnett LJ. Oxy radicals, lipid peroxidation and DNA damage. *Toxicology*, 2002; 181-182: 219-222.
15. Kabuto H, Ykoi I, Ogava N. Melotonin inhibits iron-induced epileptic discharges in rat by suppressing peroxidation. *Epilepsia*, 1998; 39: 237-243.