Madhan Mohan E. et al. / International Journal of Pharmacy & Therapeutics, 1(1), 2010, 40-43.

ISSN 0976 – 0342

International Journal of Pharmacy & Therapeutics

Journal homepage: www.ijptjournal.com

IJPT

EFFECT OF *INDIGOFERA TINCTORIA* EXTRACTS ON ANTIOXIDANT ENZYMES LEVELS IN RAT BRAIN AFTER INDUCTION OF SEIZURES

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ABSTRACT

The leaves of *Indigofera tinctoria* traditionally used for epilepsy, nervous disorders, bronchitis and liver ailments. Previous studies have demonstrated that the methanolic extract of *Indigofera tinctoria* Linn (MEIT) was subjected to acute toxicity and screened for antiepileptic activity on Maximal Electroshock (MES) and Pentylenetetrazole (PTZ) induced seizures models in albino wistar rats. The purpose of the present study is to investigate the effect of MEIT on antioxidant enzymes in rat brain after induction of seizures by MES and PTZ. Aim of study was relationship between seizure activities and altered the levels of antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GP), catalase and lipid peroxidation on rat brain. Superoxide dismutase, glutathione peroxidase and catalase were decreased in rat brain due to seizure and it was restored significantly by administration of MEIT treated rats. Similar dose dependent results were obtained in PTZ model also. Whereas MEIT significantly decreased lipid peroxidation in both models. The anticonvulsant activity of methanolic extract of *Indigofera tinctoria* might be presents of antioxidant properties and it delays the generation of free radical in MES & PTZ induced epilepsy.

Keywords: *Indigofera tinctoria,* Antioxidant Enzymes, Superoxide Dismutase, Glutathione Peroxidase, Catalase and Lipid Peroxidation.

INTRODUCTION

Indigofera tinctoria Linn. (Family: Fabaceae) was one of the original sources of indigo dye. It has been naturalized to tropical and temperate Asia, as well as parts of Africa, but its native habitat is unknown since it has been in cultivation worldwide for many centuries (Annonymous 1). Decoction of the leaves used in blennorrhagia, roots in urinary complaints and hepatitis. Extract is used in the epilepsy and other nervous disorders, bronchitis and liver ailments (Nadkarni, 1926). The whole plant of *Indigofera tinctoria* Linn. contains glycoside indican, indigotine, indiruben, galactomannan composed of galactose and mannose, 2.5% of alkaloids, rotenoids and flavanoids (Chopra *et al*., 1996) and their pharmacological activities Hepatoprotective (Singh *et al*., 2001), antidyslipidemic (Narender *et al*., 2006), antiproliferative (Kameswaran and Ramanibai, 2008) , and antileukaemia (Hoessel *et al*., 2009) were reported. In previous study, the methanoloic extract of *Indigofera tinctoria* Linn (MEIT) was subjected to acute

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toxicity and then screened for antiepileptic activity on Maximal Electroshock (MES) and Pentylenetetrazole (PTZ) induced seizures models in albino wistar rats was reported (Saravana Kumar *et al*., 2009). Therefore, the present study was performed to verify the effect of *Indigofera tinctoria* on antioxidant levels in rat brain after induction of seizure by MES and PTZ model.

MATERIALS AND METHODS

Plant collection

The leaves of *Indigofera tinctoria* Linn. was collected from abirami botanicals of Tuticorin, Tamilnadu, India. It was identified and authenticated by Prof.Jayaraman, Taxonomist, Tambaram, Chennai, Tamilnadu, India. The voucher specimen (IT-P-08-S5) of the plant was deposited at the college for further reference.

Preparation of extracts

The leaves of plants were dried in shade, separated and made to dry powder. It was then passed through the 40 mesh sieve. A weighed quantity (220gm) 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. The methanolic extract of *Indigofera tinctoria* Linn. (MEIT) yielded thick violet semi-solid residues. Percentage yield of MEIT was found to be 18.9% w/w.

Animals used

Albino wistar rats (150-200g) 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 (Reference No: IAEC/XIII/06/CLBMCP/2008-2009 Dated on 4-09-2008. IAEC/XIII/10/CLBMCP/2008-2009 Dated on 11-12-2008).

Experimental design

Albino wistar rats were divided into four groups of six animals each. Group I received 1% w/v SCMC, 1ml/100 g whereas Group-II received Phenytoin, 25mg/kg *i.p*, Group-III and IV, received methanolic extract of *Indigofera tinctoria* (L.) (200 and 400 mg/kg b.w) *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 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) and lipid peroxidation (Sayre *et al*., 1999).

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.

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

Group	Design of Treatment	**Superoxide dismutase** Units/mg protein	**Catalase** (µ mol H_2O_2 decomposed / mg protein/ min)	**Glutathione Peroxidase** Units/mg protein	**Lipid peroxidation** (nmol MDA/ mg protein)
I	Vehicle Control(SCMC 1ml/100gm)	7.833±0.7491	12.50± 0.4282	14.17± 0.5426	3.833± 0.3073
II	MES (SCMC 1ml/100gm)	13.33±0.4944^{**a}	22.67± 1.1450^{**a}	24.33± 0.6146^{**a}	1.500± 0.3416^{**a}
III	MEIT 200 mg/kg, *p.o*	10.17±0.6540^{*b}	16.33± 0.4216^{**b}	16.83± 0.7491^{*b}	3.167± 0.3073^{ns}
IV	MEIT 400 mg/kg, *p.o*	11.00±0.5164^{**b}	18.67± 0.8819^{**b}	20.50± 0.6191^{**b}	2.667± 0.2108^{*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 MEIT 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 Peroxidase** Units/mg protein	**Lipid peroxidation** N mol MDA/mg protein
I	Vehicle Control (SCMC 1ml/100gm)	8.422± 0.6009	14.33± 0.4944	14.33± 0.4216	4.33± 0.2108
II	PTZ (SCMC 1ml/100gm)	15.83± 0.5426**[a]	22.50± 0.8851**[a]	26.33± 0.7601**[a]	1.667± 0.3330**[a]
III	MEIT 200 mg/kg,*p.o*	11.33± 0.3333**[b]	16.83± 0.4014*[b]	20.33± 0.667**[b]	3.333± 0.4216[ns]
IV	MEIT 400 mg/kg,*p.o*	12.67± 0.4944**[b]	18.83± 0.3073**[b]	24.17± 0.4773**[b]	3.000± 0.3651**[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 AND CONCLUSION

Epilepsies constitute a large group of neurological diseases with an incidence of 0.5–1% in the general population (Yegin *et al*., 2002). The imbalance between the concentrations of free radicals and the antioxidant defenses may be related to the pathogenesis of various diseases such as atherosclerosis, stroke, diabetes mellitus, cancer and inflammatory diseases (Kasapoglu and Ozben, 2001; Sudha, 2001). Many reports suggest that 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, 2001). 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 (Sayre *et al*., 1999). 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). The free radicals generated cause cascade of neurochemical events leading to neurodegeneration and cell death (Rauca *et al*., 1999). 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. MES induced seizures (Rola *et al*., 2002) 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, 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 MEIT at the doses of 200 & 400mg/kg significantly increased the levels of antioxidant enzymes such as superoxide dismutase, glutathione peroxidase and catalase on rat brain.

Whereas lipid peroxidation level increases in brain during epileptic seizures (Sudha *et al*., 2001; Frindivich, 1978). We documented that changes in glutathione peroxidase activity in brain homogenates were inversely correlated with intensity of lipid peroxidation (Marnett, 2002; Halliwell and Gutteridge, 1999). 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 MEIT 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, 1998).

In conclusion, the present study demonstrates that MEIT significantly prevented MES and PTZ-induced seizures and attenuated the oxidative stress induced by MES and PTZ. Therefore, use of it could be a potential approach in arresting or inhibiting the seizure genesis caused by excitotoxic agents.

ACKNOWLEDGEMENT

Authors are sincerely thankful to Dr. S. Venkatraman, M.Sc., M.D., Ph.D., Director and Mr. P. Muralidharan, M.Pharm. (Ph.D), Department of Pharmacology, C.L. Baid Metha Foundation for Pharmaceutical Education and Research, for their contribution and facilities provided regarding our Research work.

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