

Effect of *Xanthium strumarium L.* Extracts on Antioxidant Enzymes Levels in Rat Brain after Induction of Epilepsy

***K.K. Senthil Kumar and ¹B. Raj Kapoor**

*Department of Pharmaceutical science, St.Peter's College of Pharmacy, Kazipet, Warangal,
Andhra Pradesh, India-506 001.

¹St.John's Pharmacy College, Vijayanagar, Bangalore, Karnataka, India-560 040.

Summary

The whole plant of *Xanthium Strumarium L.* is used traditional Indian medicine to treat epilepsy. In present study the effect of petroleum ether extract of *Xanthium Strumarium L.* (PEXS) on antioxidant enzymes in rat brain after induction of epilepsy 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 epilepsy and it was significantly ($P<0.01$) restored by administration of petroleum ether extract of *Xanthium Strumarium L.* treated rats. Similar dose dependent results were obtained in PTZ model also. Whereas PEXS significantly decreased lipid peroxidation in both models. The anticonvulsant activity of PEXS might be presents of antioxidant properties and it delays the generation of free radical in MES & PTZ induced epilepsy.

Keywords: Antioxidant enzymes, *Xanthium Strumarium L.*, Superoxide Dismutase, Glutathione Peroxidase, Glutathione Reductase; Catalase, Lipid peroxidation.

Corresponding Author

K.K. Senthil Kumar M.Pharm., (Ph.D)

Department of Pharmaceutical science,
St.Peter's College of Pharmacy, Kazipet, Warangal,
Andhra Pradesh, India-506 001

E-mail: kksenthilqa@yahoo.co.in

Introduction

Xanthium Strumarium L. compositae, is a common weed found in India. The whole plant, specially root and fruit, is used as medicine. According to ayurveda, *Xanthium Strumarium L.* is anthelmintic, antipyretic, antiepileptic, diuretic, cooling laxative, fattening, alexiteric, and tonic, digestive and improves appetite, voice, complexion, and memory. It cures leucoderma, poisonous bites of insects, salivation and fever. Seed yields semi-drying edible oil (30-35%).

Which resembles sun flower oil and used in bladder infection, herpes can be used as manure where shell can be used as activated carbon^[1]. On the basis of the traditional use of the plant for treating convulsion, but no previous pharmacological (or) clinical study was carried out to test the antiepileptic activity of this plant. Since the antiepileptic effect of *xanthium strumarium* has been experimentally not confirmed. Therefore, the aim of the present investigation was to evaluate the claimed antiepileptic activity of *Xanthium Strumarium L.* in albino wistar rats.

Materials and Methods

Plant material: The whole plant of *Xanthium strumarium L.* was collected from Thirupathi, Talakona, Tirumala, Andhra Pradesh, India. The whole plant were dried under shade, powdered and stored in an air tight container.

Preparation of extract: The collected whole plant was dried at room temperature, pulverized by a mechanical grinder, sieved through 40mesh. About 120g of powdered materials were extracted with petroleum ether (60°-80°C) 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 normal saline and used for the experiment. The percentage yield of prepared extract was around 9.3%w/w.

Experimental Animals: Wister albino rats weighing between 160-220gm each were used for this experiment. They were procured from St. Peter's College of Pharmacy, Kazipet, Warangal, Andhra Pradesh,, India. The animals were kept under standard condition in an animal house approved by committee for the purpose of control and supervision of experiments on animals (CPCSEA: Ref. No. /IAEC/X/07/SPCP/2009-10.

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 petroleum ether extract of *Xanthium Strumarium L.* (PEXS) (250 and 500 mg/kg body weight) *p.o* respectively for 20 days. On the 20th day, Seizures are induced to all the groups by using an Electro convulsimeter. 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^[2], glutathione peroxidise^[3], superoxide dismutase^[4], glutathione reductase^[5] and lipid peroxidation^[6].

Statistical analysis: The data were expressed as Mean \pm 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 PEXS 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 ($p < 0.05$) in both models. Administration of PEXS 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 PEXS 250 and 500 mg/kg. (Table 1 & 2).

Table: 1. Effect of PEXS 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)	14.52 ± 1.24	24.34 ± 0.18	34.58 ± 2.24	25.36 ± 0.34	3.66 ± 0.54
II	MES (SCMC 1ml/100gm)	6.25±0.54 ^{a**}	16.34±0.33 ^{a**}	18.34±0.42 ^{a**}	17.02±0.40 ^{a**}	5±0.14 ^{a**}
III	PEXS 250 mg/kg, <i>p.o</i>	11.32±0.47 ^{b**}	19.36±0.26 ^{b**}	29.33±0.33 ^{b**}	19.12±0.18 ^{b**}	4.13±0.26 ^{b*}
IV	PEXS 500 mg/kg, <i>p.o</i>	12.44±0.26 ^{b**}	22.66±0.32 ^{b**}	25.06±0.12 ^{b**}	24±0.06 ^{b**}	3.46±0.82 ^{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 PEXS 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)	13.14 ± 0.72	23.17 ± 0.21	34.22 ± 0.52	27.14 ± 0.72	2.42 ± 0.54
II	PTZ (SCMC 1ml/100gm)	7.54±0.48 ^{a**}	15.64±0.52 ^{a**}	25.12±0.92 ^{a**}	20.64±0.46 ^{a**}	3.14±0.94 ^{a**}
III	PEXS 250 mg/kg, <i>p.o</i>	11.12±0.34 ^{b*}	19.33±0.44 ^{b**}	27.36±0.66 ^{b**}	24.52±0.48 ^{b**}	3.12±0.26 ^{b*}
IV	PEXS 500 mg/kg, <i>p.o</i>	12.54±0.33 ^{b**}	21.16±0.66 ^{b*}	30.64±0.65 ^{b**}	26.54±0.55 ^{b**}	2.64±0.12 ^{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

The study showed that, high level of oxidative damage was detected both in case of electrically generated seizures, viz. electroshock induced seizures^[7,8] and PTZ seizure models^[6]. Inactivation of oxygen free radicals can be carried out by antioxidative enzymes, like superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase^[9,10]. Previous study was reported, MES induced seizure shows marked reduction of antioxidant enzymes like glutathione peroxidase, catalase, glutathione reductase, Superoxide dismutase^[11] and the intracerebroventricularly administered glutathione (GSH) inhibited pentylenetetrazole (PTZ) induced convulsions in mice^[2]. The results of this study showed that PEXS 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^[12,13,14]. 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^[15,16,17]. 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^[18].

In conclusion, PEXS 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 PEXS treated rats. Hence the antioxidant properties of PEXS extract delays the generation of free radical in MES & PTZ induced epilepsy.

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