Vol 1 | Issue 1 | Jul –Dec 2010 | 50-53.

Research Article

Effect of *Xanthium strumarium L.* Extracts on Biogenic Amines Concentrations in Rat Brain after Induction of Seizure

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ABSTRACT

The whole plant of *Xanthium Strumarium L.* is used traditional Indian medicine to treat epilepsy. The purpose of the present study is to investigate the effect of petroleum ether extract of *Xanthium Strumarium L.* (PEXS) on biogenic amines concentrations in rat brain after induction of seizures by MES and PTZ. Our aim of study was relationship between seizure activities and altered the monoamines such as noradrenaline (NA), dopamine (DA), serotonin (5-HT) and Gamma amino butyric acid (GABA) in forebrain of rats in MES and PTZ seizure models. In MES model, PEXS (250 & 500 mg/kg) showed significantly restored the decreased levels of brain monoamines such as NA, DA, 5-HT and GABA. Similarly in PTZ model, PEXS showed significantly increased the monoamines in forebrain of rats. Thus, this study suggests that petroleum ether extract of *Xanthium Strumarium L.* increased the monoamines on rat brain, which may be decreased the susceptibility to MES and PTZ induced seizure in rats.

Keywords: Antiepileptic activity, Traditional Medicine, *Xanthium Strumarium L.,* biogenic amines, NA, DA, 5-HT and GABA

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 anthelmentic, 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]. Our aim of this study was relationship between seizure activities and altered the monoamines such as noradrenaline (NA), dopamine (DA), serotonin (5-HT) and Gamma amino butyric acid (GABA) in forebrain of rats in MES and PTZ seizure models.

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

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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 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.

A fluorimetric micromethod for the simultaneous determination of serotonin, noradrenaline and dopamine

On the 14th day after observed the convulsion all groups rats were sacrificed, whole brain was dissected out and separated the forebrain. Weighed quantity of tissue and was homogenized in 0.1 mL hydrochloric acid - butanol, (0.85 ml of 37% hydrochloric acid in one liter *n*- butanol for spectroscopy) for 1 min in a cool environment. The sample was then centrifuged for 10 min at 2,000 rpm. 0.08 mL of supernatant phase was removed and added to an Eppendorf reagent tube containing 0.2 mL of heptane (for spectroscopy) and 0.025 mL 0.1 M hydrochloric acid. After 10 min of vigorous shaking, the tube was centrifuged under same conditions to separate two phases. Upper organic phase was discarded and the aqueous phase (0.02 mL) was used for estimation of Serotonin, Nor Adrenaline and Dopamine assay [2].

Estimation of brain GABA content
The brain amino butyric acid (GABA content was estimated according to the method of Lowe et al., (1958) **[3].**

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 PEXS on monoamines levels in seizure induced rats by MES and PTZ:
Noradrenaline

In MES and PTZ models, Noradrenaline levels significantly ($p<0.01$) decreased in forebrain of epileptic control animals. PEXS at the doses of 250&500mg/kg, standard drugs phenytoin and diazepam treated animals showed a significantly ($p<0.05$ & $p<0.01$) increased in Noradrenaline levels in forebrain of rats (Table 1 and 2).

Dopamine

In MES and PTZ models, Dopamine levels significantly ($p<0.01$) decreased in forebrain of epileptic control animals. PEXS at the doses of 250&500mg/kg, standard drugs phenytoin and diazepam treated animals showed a significantly ($p<0.05$ & $p<0.01$) increased in Dopamine levels in forebrain of rats **(**Table 1 and 2).

Serotonin

In MES and PTZ models, Serotonin levels significantly ($p<0.01$) decreased in forebrain of epileptic control animals were observed. PEXS at the doses of 250&500mg/kg, standard drugs phenytoin and diazepam treated animals showed a significantly ($p<0.05$ & $p<0.01$) increased in Serotonin levels in forebrain of rats (Table 1 and 2).

Gamma amino butyric acid

In MES and PTZ models, GABA levels significantly ($p<0.01$) decreased in forebrain of epileptic control animals were observed. PEXS at the doses of 250&500mg/kg, standard drugs phenytoin and diazepam treated animals showed a significantly ($p<0.05$ & $p<0.01$) increased in GABA levels in forebrain of rats **(**Table 1 and 2).

Table: 1. Effect of PEXS on neurotransmitters levels in rat brain after MES induced epilepsy

Group	Design of Treatment	Noradrenaline	Dopamine	Serotonin	GABA
I	Vehicle Control(SCMC ml/100gm)	756±2.06	624.32±3.26	184±4.52	274±1.64
II	MES (SCMC 1ml/100gm)	424.33±2.54 ^{a**}	452.34±5.02 ^{a**}	72±1.25 ^{a**}	242.32±2.36 ^{a**}
III	Phenytoin 25mg/kg,*i.p*	584±4.14 ^{b**}	686.34±3.64 ^{b**}	94.28±2.92 ^{b**}	294.32±2.54 ^{b**}
IV	PEXS 500 mg/kg,*p.o*	574.24±4.58^{b**}	642±1.66^{b**}	91.33±0.24^{b**}	262.4±1.26^{b**}
V	PEXS 250 mg/kg,*p.o*	762.66±4.24^{b*}	554.26±2.33^{b*}	81±0.94^{b*}	264.59±1.33^{b**}

Values are expressed as mean ± SEM of six observations. Comparison between: **a**- Group I Vs Group II, **b**- Group III Vs Group IV and Group V. Statistical significant test for comparison was done by ANOVA, followed by Dunnet's 't' test *p<0.05;** p<0.01; Units = pg/mg of wet tissue.

Table: 2. Effect of PEXS on neurotransmitters levels in rat brain after PTZ induced epilepsy

Group	Design of Treatment	Noradrenaline	Dopamine	Serotonin	GABA
I	Vehicle Control(SCMC ml/100gm)	756±2.06	624.32±3.26	184±4.52	274±1.64
II	MES (SCMC 1ml/100gm)	524.26±2.24 ^{a**}	574.33±4.94 ^{a**}	93.02±3.54 ^{a**}	209.47±1.68^{a**}
III	Diazepam (4mg/kg), *p.o*	619±2.64 ^{b**}	897.59±3.48 ^{b**}	137.33±2.42 ^{b**}	287.69±1.44^{b**}
IV	PEXS 500 mg/kg,*p.o*	754.04±4.06^{b*}	884.14±2.66^{b**}	132.05±1.48^{b**}	234±1.62^{b**}
V	PEXS 250 mg/kg,*p.o*	767.16±4.22^{bns}	771.50±4.35^{b**}	106.33±1.64 ^{b*}	276.34±1.22^{b**}

Values are expressed as mean ± SEM of six observations. Comparison between: **a** - Group I Vs Group II, **b**- Group III Vs Group IV and Group V. Statistical significant test for comparison was done by ANOVA, followed by Dunnet's 't' test *p<0.05;** p<0.01; Units = pg/mg of wet tissue.

DISCUSSIONS AND CONCLUSIONS

In present study, the established antiepileptic drugs such as phenytoin and diazepam restored the monoamine levels on brain [4]. Similarly PEXS significantly (p<0.05 & p<0.01) increased monoamines levels in forebrain of rats. Many drugs that increase the brain contents of GABA have exhibited anticonvulsant activity against seizures induced by MES and PTZ [5]. MES is probably the best validated method for assessment of anti-epileptic drugs in generalized tonic-clonic seizures [6]**.**

GABA is a major inhibitory neurotransmitter of CNS and increase in its level in brain has variety of CNS dependent effects including anticonvulsant effect (Macdonald RL and McLean MJ, 1982). In addition to the GABA binding site, the $GABA_A$ receptor complex appears to have distinct allosteric binding sites for benzodiazepines, barbiturates, methanol etc [7]. We therefore studied the effect of *Xanthium Strumarium L.* extract on brain GABA content. *Xanthium Strumarium L.* extract showed significant (p<0.05 & p<0.01) increased GABA content in brain dose dependently. This suggests that the anticonvulsant activity of *Xanthium Strumarium L.* extract is probably through elevation of brain GABA content.

In Norepinephrine-lesioned rats showed a greater susceptibility to seizures induced by the chemoconvulsant PTZ and electroconvulsive shock [8]. The antiepileptic role of endogenous Norepinephrine was inferred from studies that showed harmful effects of a damage of Norepinephrine system on seizures induced by electrical stimulation or systemic administration of chemoconvulsants [9,10]. In present study, PEXS significantly (p<0.05 & p<0.01) increased the NA in forebrain of rats and proves the antiepileptic activity of *Xanthium Strumarium L.* extract.

Chen et al. [11] demonstrated that pre-treatment with the monoamine-depleting agent reserpine decreased the epileptic threshold to PTZ and caffeine in mice. Reserpine lacks specificity, since this drug also depletes serotonin (5-HT) and DA, in addition to NE. Therefore, increased seizure susceptibility could be due to a multiple deficit of monoamines [12]. Subsequent the present studies confirmed and extended these results. It became clear that PEXS significantly increased the serotonin (5-HT) and DA and NA. It produces significantly decreased the susceptibility to various epileptic stimuli.

In conclusion biogenic amines participate in the control of Maximal electroshock and pentylenetetrazole induced seizure in rat models. Our findings support the hypothesis that decreased the monoamines levels in rat brain after induction of seizure. In *Xanthium Strumarium L.* extract treated rats, monoamines such as NA, DA, 5-HT and GABA levels significantly restored on forebrain. Thus PEXS increases the seizure threshold and decreased the susceptibility to MES and PTZ induced seizure in rats. Hence we suggest that petroleum ether extract of whole plant of *Xanthium Strumarium L.* possess antiepileptic properties that may be due to restore the biogenic amines in rat brain. These results support the ethnomedical uses of the plant in the treatment of epilepsy. However more experimentation, detailed phytochemical and experimental analysis are required for a definitive conclusion.

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