Evaluation of Anti-epileptic Activity of *Xanthium strumarium L.*

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Summary

The present study is an investigation of anti-epileptic activity of *Xanthium Strumarium L.* (Family-compositae) is a well known plant which is being used in Indian traditional medicines for treating epilepsy, inflammation and tuberculosis. The petroleum ether extract of *Xanthium Strumarium L.* (PEXS) was subjected to acute toxicity and then screened for anticonvulsant activity on Maximal Electroshock (MES) and Pentylenetetrazole (PTZ) induced seizures models in albino wistar rats. Acute toxicity of extract was non toxic up to the recommended dose 2000 mg/kg. p.o. Animals were treated with PEXS at doses of 250 and 500 mg/kg body weight. Study results showed, the mean duration of extensor phase of treated groups reduced significant level than compared to control group. In Pentylenetetrazol induced seizure model, onset of myoclonic spasm and clonic convulsion was delayed in the PEXS treated groups. PEXS showed anti-epileptic activity against MES and PTZ animal models. However, further studies still needed to be carried on exposure of the extract to humans.

Keywords: Anti-epileptic activity, *Xanthium Strumarium L.*, Maximal Electroshock (MES), Pentylenetetrazole (PTZ).

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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]. 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.

Phytochemical analysis

The petroleum ether extract of *Xanthium Strumarium L.* was subjected to qualitative analysis for the various phyto-constituents. Standard methods were used for preliminary qualitative phytochemical analysis of extract [2].

Experimental Animals

Wister albino rats weighing between 150-200gm 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). They were housed in polypropylene cages and maintained at 27±2^{o}C; The animals were given standard diet. Ethical committee clearance was obtained from IAEC (Institutional Animal Ethics Committee) of CPCSEA (Ref. No. /IAEC/XI/07/SPCP/2009-10.

Acute toxicity study

Acute toxicity study of petroleum ether extract of *Xanthium Strumarium L.* was determined by acute toxic class method of OECD guidelines. In acute oral toxicity study mortality was not observed up to 2000mg/kg body weight [3].

Anti-epileptic activity

Effect on Maximal electroshock (MES) induced seizures

Albino wistar rats of either sex weighing 160 to 220 gm were divided into four groups of six animals each. The first group received vehicle control (1% w/v SCMC, 1ml/100 g) whereas Group-II received standard drug (Phenytoin, 25mg/kg) intraperitoneally, Group-III and IV, 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. Maximal electroshock seizures were elicited by a 60 Hz alternating current of 150 mA intensity for 0.2 sec. A drop of electrolyte solution (0.9% NaCl) with lignocaine was applied to the corneal electrodes prior to application to the rats. This increases the contact and reduces the incidence of fatalities. The duration of various phases of epilepsy were observed. The percentage protection was estimated by observing the number of animals showing abolition of Hindleg Tonic Extension (or) extension not greater than 90° [4].

Effect on Pentylenetetrazole (PTZ) induced seizures

Albino wistar rats of either sex weighing 160 to 220 gm were divided into four groups of six animals each. The first group received vehicle control (1% w/v SCMC, 1ml/100 g) whereas Group-II received standard drug (Diazepam, 4mg/kg) intraperitoneally, Group-III and IV, 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, Pentylenetetrazole (PTZ) (90mg/kg body weight, *s.c*) was administered to all the groups to induce clonic convulsions. Animals were observed for a period of 30mins post – PTZ administration. The parameters noted were mean onset time of convulsions, duration of convulsion and recovery/Death (% recovery or % of survival) due to PTZ [5].

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

Phytochemical analysis

The petroleum ether extract of *Xanthium Strumarium L.* revealed the presence of steroids, Alkaloids, Reducing sugars, tannins, gums, flavonoids.

Effects of PEXS on MES Induced Epilepsy

The duration of tonic hindleg extension in rats treated with vehicle was 14.54±0.24 seconds. The PEXS at doses of 250 mg/kg and 500 mg/kg were protect animals from seizures and significantly ($p<0.01$) reduced the duration of tonic hindleg extension for 5.14±0.1.54 and 2.64±0.16 seconds respectively. Whereas, the standard drug phenytoin treated animals exhibits abolished tonic hindleg extension. Phenytoin treated animals have shown 100% protection against MES induced seizures where as PEXS 250 mg/kg and 500 mg/kg have shown 64.65% and 82.01% protection respectively (Table-1).

Effect of PEXS on PTZ Induced epilepsy

In rats treated with vehicle, clonic convulsion appeared for 162.52±0.67 seconds after PTZ and all rats died after seizures. The PEXS at doses of 250 mg/kg and 500 mg/kg significantly delayed the onset of clonic convulsions for 523.64±1.21 ($p<0.01$) and 571.36±1.62 ($p<0.01$)

seconds respectively in dose dependent manner. Whereas, the standard drug diazepam (4mg/kg, *i.p*) delayed the onset of clonic convulsions for 654.24±1.18 seconds. Diazepam treated animals have shown 100% protection against PTZ induced seizures where as PEXS 250 mg/kg and 500 mg/kg have shown 44.90% and 60.74% protection respectively (Table-2).

Table 1. Effect of methanolic extract of *Xanthium Strumarium L.* (PEXS) On MES induced seizers in rats.

Group	Design of treatment	Flexion	Extensor	Clonus	Stupor	Recovery	% protection
I	Vehicle control	7.9±0.12	14.54±0.24	18.78±0.32	38.2±0.58	189.2	0
II	Phenytoin 25mg/kg,i.p.	2.9±0.24	0**	8.91±0.54**	16.29±0.35**	93.5	100
III	PEXS 250mg/kg,p.o	5.58±0.34*	5.14±0.1.54**	14.81±0.36	31.58±0.95	141.79	64.65
IV	PEXS 500mg/kg,p.o	4.09±0.26**	2.64±0.16**	12.82±0.85*	16.23±0.69**	113.51	82.01

Values are expressed as mean ± SEM of six observations. Comparison between Group I *Vs* Group II, Group II *Vs* Group III &Group IV. Statistical significant test for comparison was done by ANOVA, followed by Dunnet's 't' test. *p<0.05;** p<0.01; ns-non significant.

Table 2. Effect of methanolic extract of *Xanthium Strumarium L.* (PEXS) On PTZ induced seizers in rats.

Group	Design of Treatment	Onset of convulsions(sec.)	Duration of convulsion(sec.)	Protection convulsion %	Protection mortality %
I	Vehicle control	162.52±0.67	69.42±0.78	0	50
II	Diazepam(4mg/kg)	654.24±1.18**	12.02±0.13**	100	100
III	PEXS 250	523.64±1.21**	38.25±0.52*	44.90	83.33
IV	PEXS 500	571.36±1.62**	27.25±0.26**	60.74	100

Values are expressed as mean ± SEM of six observations. Comparison between Group I *Vs* Group II, Group II *Vs* Group III &Group IV. Statistical significant test for comparison was done by ANOVA, followed by Dunnet's 't' test. *p<0.05;** p<0.01; ns-non significant.

Discussions and Conclusions

In India, studies have reported the prevalence rate of epilepsy varying from 1710 from 9780 cases per million population. The modern conventional antiepileptic drugs (AEDs) are effective in approximately 50% of patients, many cases still remain resistant to AED treatment [6]. These drugs are associated with vast array of side effects including chronic toxicity, teratogenecity, adverse effects on cognition and behavior among others [7]. Thus, due to aforementioned reasons and others, it is pertinent to look for affordable and conventional alternative medicine with view to providing a better protection and activities- particularly medicinal plants.

The MES test is the most frequently used as an animal model for identification of anticonvulsant activity of drugs for the generalized tonic-clonic seizures "grand mal" [8,9]**.** This model based on observation of the stimulation by repeated electrical pulses induce in different neuronal structures one characteristic standard of epileptic activity [10]**.** In our present study, it is found that treatment with PEXS on rats significantly reduces in tonic hindleg extensor stage in MES induced epilepsy. The MES model – to identify compounds which prevent seizure spread, corresponding to generalized tonic-clonic seizures in humans [11,12]**.** Currently used anticonvulsant drugs (e.g. phenytoin, carbamazepines) effective in therapy of generalized tonic-clonic and partial seizures have been found to show strong anticonvulsant action in MES test [13,14]**.** Since, PEXS significantly inhibited generalized tonic-clonic seizures in MES test; it suggests the presence of anticonvulsant compounds.

We found that treatment with PEXS on PTZ induced rats significantly reduce the duration of convulsion and delayed the onset of clonic convulsion. Although animal models based on pentylenetetrazole (e.g. pentylenetetrazole threshold, and acute convulsions) have still been widely used for drug screening, the mechanism by which pentylenetetrazole elicits its action has not been completely understood. One generally accepted mechanism by which pentylenetetrazole exerts its action is by acting as an antagonist at the picrotoxin sensitive site of the $GABA_A$ receptor complex [15].

Since PTZ has been shown to interact with the GABA neurotransmission [16,8] and PTZ induced seizures can be prevented by drugs that enhance gamma amino butyric acid type A ($GABA_A$) receptor-mediated inhibitory neurotransmission such as benzodiazepines and phenobarbital [17,13]**,** the antagonism of PTZ- induced seizures suggests the interaction of the PEXS with the GABA-ergic neurotransmission.

The study concluded PEXS possesses an anticonvulsant effect which results from potentiate the activity of GABA. However, more precise mechanisms of PEXS anticonvulsant activity and the relationship between the seizure and $GABA_A$ receptor subunits and the other neurotransmitter systems which may explain how PEXS produce anticonvulsant effect must be investigated further.

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