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EVALUATION OF ANTI-SEIZURE ACTIVITY OF THE *THOTTEA SILIQUOSA* EXTRACT ON MAXIMAL ELECTROSHOCK AND PENTYLENETETRAZOLE INDUCED SEIZURES IN ALBINO WISTAR RATS

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ABSTRACT

The present study is an investigation of anti-seizure activity of *Thottea siliquosa* Lamk. (Family- Aristolochiaceae) is a well-known plant which is being used in Indian traditional medicines for treating epilepsy, diarrhoea, dysentery, cholera and ulcers. The ethanol extract of *Thottea siliquosa* Lamk. (EETS) 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 EETS 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 pentylenetetrazole induced seizure model, onset of myoclonic spasm and clonic convulsion was delayed in the EETS treated groups. EETS showed anti-seizure activity against MES and PTZ animal models.

Key words: Anti-seizure activity, *Thottea siliquosa* Lamk., Maximal Electroshock (MES), Pentylenetetrazole (PTZ).

INTRODUCTION

A shrub found in evergreen forests of Western Ghats from Konkan to Kerala. An erect medicinal plant with smooth yellowish grey bark, Alternate, aromatic leaves, purple or greenish flowers in clusters. The roots are used as medicine in the treatment of many diseases like diarrhoea, dysentery, cholera, ulcers. The root of *Apama siliquosa* Lamk is used for snake treatment (Anonymous 1; Yoganarasimhan SN, 1996). 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 antiseizure activity of this plant. Since the antiseizure effect of *Thottea siliquosa* has been experimentally not confirmed. Therefore, the aim of the present investigation was to evaluate the claimed anti-seizure activity of *Thottea siliquosa* Lamk. in albino wistar rats.

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MATERIALS AND METHODS

Plant material

The root of *Thottea siliquosa* was collected from Tirumala hills, Tirupati, Andhra Pradesh. India. The plant was identified and authenticated by Dr.K.Madhava Chetty, Department of botany, S.V.University, Tirupathi. The voucher specimen of the plant was deposited at the college for further reference. The root were dried under shade, powdered and stored in an air tight container.

Preparation of extract

The collected root was dried at room temperature, pulverized by a mechanical grinder, sieved through 40mesh. About 120g 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 normal saline and used for the experiment. The percentage yield of prepared extract was around 12.5%w/w.

Phytochemical analysis

The ethanol extract of *Thottea siliquosa* Lamk. was subjected to qualitative analysis for the various phyto-constituents. Standard methods were used for preliminary qualitative phytochemical analysis of extract (Harbone JP, 1973).

Experimental Animals

Wister albino rats weighing between 150-200gm each maintained in a 12 h light/dark cycle at a constant temperature 25 ºC 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 (CLBM/IAEC/ 037-0070/2012).

Acute toxicity study

Acute toxicity study of ethanol extract of *Thottea siliquosa* Lamk. 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 (OECD, 2002).

ANTI-SEIZURE 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 ethanol extract of *Thottea siliquosa* Lamk. (EETS) (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° (Balakrishnan S *et al*., 1998).

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, ethanol extract of *Thottea siliquosa* Lamk. (EETS) (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 (Kulkarni SK and George B, 1999).

Statistical analysis

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

RESULTS

Phytochemical analysis

The ethanol extract of *Thottea siliquosa* Lamk. revealed the presence of alkaloids, triterpenoids, Reducing sugars, tannins, gums, flavonoids.

Effects of EETS on MES Induced Seizures

The duration of tonic hindleg extension in rats treated with vehicle was 16.59±0.24 seconds. The EETS 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 6.12±0.1.05 and 3.35±0.77 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 whereas EETS 250 mg/kg and 500 mg/kg have shown 63.11% and 79.81% protection respectively (Table 1).

Effect of EETS on PTZ Induced Seizure

In rats treated with vehicle, clonic convulsion appeared for 147.85±2.33 seconds after PTZ and all rats died after seizures. The EETS at doses of 250 mg/kg and

500 mg/kg significantly delayed the onset of clonic convulsions for 419.69±2.46 ($p<0.01$) and 552.28±3.54 ($p<0.01$) seconds respectively in dose dependent manner. Whereas, the standard drug diazepam (4mg/kg, *i.p*) delayed the onset of clonic convulsions for 742.22±2.19 seconds. Diazepam treated animals have shown 84.26% protection against PTZ induced seizures whereas EETS 250 mg/kg and 500 mg/kg have shown 54.04% and 66.59% protection respectively (Table 2).

Table 1. Effect of Ethanolic Extract of *Thottea siliquosa* Lamk. (EETS) On MES induced Seizures in rats

Group	Design of treatment	Flexion (sec)	Extensor (sec)	Clonus (sec)	Stupor (sec)	Recovery (sec)	% protection
I	Vehicle control	8.21±0.22	16.59±0.24	79.52±0.33	41.26±0.42	192.33	0
II	Phenytoin 25mg/kg,i.p.	2.85±0.31**	0**	9.14±0.24**	15.44±0.33**	95.52	100
III	EETS 250mg/kg,p.o	5.62±0.52*	6.12±0.15**	13.28±0.37**	32.16±0.48*	138.26	63.11
IV	EETS 500mg/kg,p.o	3.95±0.33**	3.35±0.77**	11.22±0.46*	17.22±0.29**	106.42	79.81

Table 2. Effect of Ethanolic Extract of *Thottea siliquosa* Lamk. (EETS) On PTZ induced Seizures in rats

Group	Design of Treatment	Onset of convulsions (sec.)	Duration of convulsion (sec)	Protection convulsion%	Protection mortality %
I	Vehicle control	147.85±2.33	72.19±1.29	0	50
II	Diazepam (4mg/kg), i.p	742.22±2.19**	11.36±0.52**	84.26	100
III	EETS 250	419.69±2.46**	33.18±0.64*	54.04	83.33
IV	EETS 500	552.28±3.54**	24.12±0.33**	66.59	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 Dunnett's test. *$p<0.05$;** $p<0.01$; ns-non significant.

Figure 1. Effect of Ethanolic Extract of *Thottea siliquosa* Lamk. (EETS) On MES induced Seizures in rats

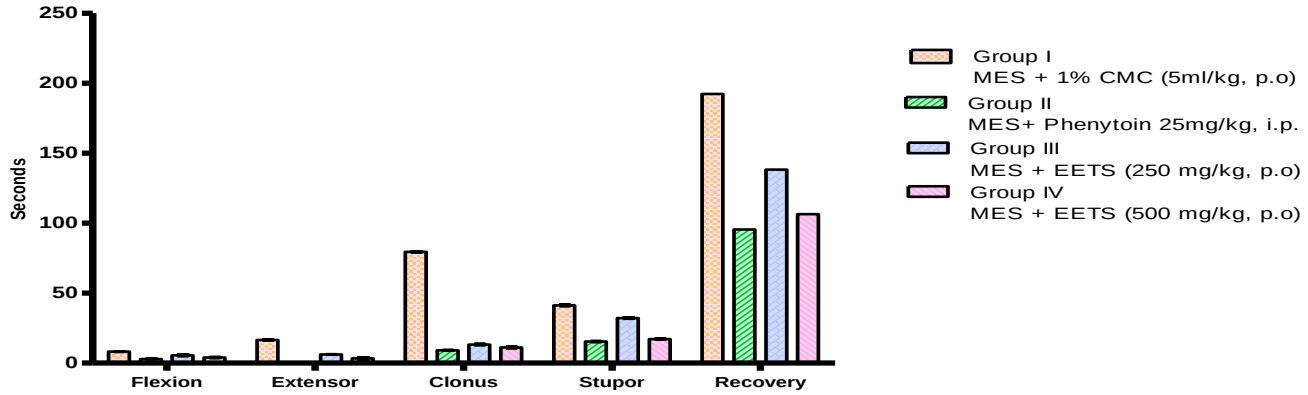
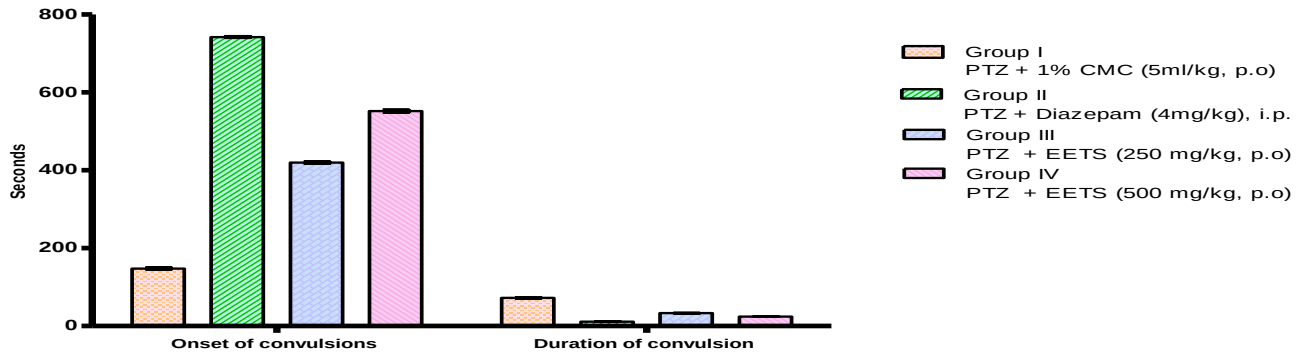


Figure 2. Effect of Ethanolic Extract of *Thottea siliquosa* Lamk. (EETS) On PTZ induced Seizures in rats



DISCUSSION AND CONCLUSION

In India, studies have reported the prevalence rate of epilepsy varying from 1710 from 9780 cases per million population. The modern conventional antiseizure drugs (AEDs) are effective in approximately 50% of patients, many cases still remain resistant to AED treatment (Heinemann UE *et al*., 1994). These drugs are associated with vast array of side effects including chronic toxicity, teratogenecity, adverse effects on cognition and behavior among others (Raza MF *et al*., 2001). 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" (Loscher W and Schmidt D, 1988; Oliveira FA *et al*., 2001)**.** This model based on observation of the stimulation by repeated electrical pulses induce in different neuronal structures one characteristic standard of seizure activity (Quintans-Júnior LJ *et al*., 2002)**.** In our present study, it is found that treatment with EETS 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 (Stables JP and Kupferberg HJ, 1995; Kupferberg HJ, 1989)**.** 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 (Macdonald RL and Kelly KM, 1995; White HS, 1997)**.** Since, EETS significantly inhibited generalized tonic-clonic seizures in MES test; it suggests the presence of anticonvulsant compounds.

We found that treatment with EETS 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 (Ramanjaneyulu R and Ticku MK, 1984).

Since PTZ has been shown to interact with the GABA neurotransmission (De Deyn PP *et al*., 1992) 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 (Coulter DA *et al*., 1989)**,** the antagonism of PTZ- induced seizures suggests the interaction of the EETS with the GABA-ergic neurotransmission.

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

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