

ANTICONVULSANT ACTIVITY OF *TOONA CILIATA* M. ROEM IN ALBINO MICE

Pharmacology

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ABSTRACT

Background: Epilepsy is the second most common neurological disorder after stroke contributing to significant morbidity and mortality worldwide. Out of the 70 million persons with epilepsy worldwide, India contributes to nearly one-sixth of the global burden. Currently available antiepileptic drugs (AEDs) mostly have side effects and about 20% to 40% of sufferers are categorized as treatment resistant.

Objective: To evaluate the anticonvulsant activity of ethanolic extract of leaves of *Toona ciliata* M. Roem (EETC) in albino mice.

Method: Healthy albino mice of either sex were allocated into 5 groups of 6 mice in each group. The anticonvulsant activity of EETC (100 mg/kg, 200 mg/kg and 400 mg/kg) was assessed by using maximum electroshock seizure (MES) and pentylenetetrazol (PTZ) induced seizure models.

Results: EETC at doses 200 mg/kg and 400 mg/kg significantly ($p < 0.001$) reduced the duration of tonic hind limb extension (THLE) and offered seizure protection (35.3 % and 62.2 % respectively) in MES induced seizure. In PTZ induced seizure EETC at 400 mg/kg significantly ($p < 0.001$) delayed the latency and reduced the duration of seizures as well as offered seizure protection (68 %). Standard drugs-inj. phenytoin and inj. diazepam in MES and PTZ induced seizure respectively showed 100% protection.

Conclusion: The ethanolic extract of leaves of *Toona ciliata* M. Roem (EETC) showed anticonvulsant activity in seizures produced by maximal electroshock and pentylenetetrazol.

KEYWORDS

Epilepsy, *Toona ciliata* M. Roem, maximum electroshock seizure (MES), pentylenetetrazol (PTZ).

INTRODUCTION

Epilepsy refers to a neurological disorder due to rapid firing of brain neurons characterized by periodic and unpredictable occurrence of convulsions.^{1,2} The mortality rate increases by two to three times among people with epilepsy and the risk of sudden death is 24 times greater compared to the general population.³ Out of the 70 million persons with epilepsy worldwide, India contributes to nearly one-sixth of the global burden.⁴ DALYs estimates the burden of epilepsy to about 1% of the total burden of disease in the world, which excludes that due to social stigma and isolation, which further add to the disease burden.⁵ Currently available antiepileptic drugs (AEDs) mostly have side effects such as somnolence, drowsiness, lethargy, ataxia and behavioral changes which reduce the quality of life. Moreover about 20% to 40% of sufferers are categorized as treatment resistant as they do not respond to current medications.⁶ Herbal drugs have gained importance and popularity in recent years as a consequence of their efficacy, safety and cost effectiveness. Indian traditional medicine like Ayurveda, Unani, Siddha, have long been using plant medicines as the remedy of different ailments.

Due to the unique geographical location and different climatic condition, the Himalayan region is enriched with variety of plant species.⁷ *Toona ciliata* M. Roem of the 'Meliaceae' family, known as 'Indian Mahogany' in English and 'Tun' in Hindi, is one such species with many medicinal properties.

The plant *Toona ciliata* M. Roem possesses many important biological properties that account for its traditional uses in medicinal treatments and dye preparations. The bark is useful in chronic dysentery, leprosy, fever, headache and blood complaints, etc. Due to its powerful astringent property the bark also finds its utility in various forms of ulceration and rheumatism. The flowers are used as dye and also as

emmenagogue in menstrual disorders. Leaves are used in vomiting, diarrhoea, skin diseases, small pox and as insect repellent. The leaf extract has beneficial effect on decreasing blood glucose levels and also decrease hyperlipidaemia.

Various previous studies suggested that leaves of *Toona ciliata* M. Roem exhibit anti-diabetic, anti-ulcer, anti-oxidant, anti-inflammatory, anti-microbial and anti-fungal activities.^{8,9} Oxidative stress and consequent lipid peroxidation has been found to be increased in brain after electroshock as well as chemical induced seizure.¹⁰ Therefore neuroprotective effect of antioxidants could slow or even prevent epileptogenesis and consequently decrease seizure severity and frequency.¹¹ So, the present study was undertaken to evaluate the anticonvulsant activity of ethanolic extract of leaves of *Toona ciliata* M. Roem in MES (Maximal electroshock seizure) and PTZ (Pentylenetetrazol) induced seizure in albino mice.

MATERIALS AND METHODS

The study was conducted in the Department of Pharmacology, Regional Institute of Medical Sciences, Imphal from March 2019 to September 2019.

Materials Preparation of the plant materials:

Leaves of *Toona ciliata* M. Roem, mixer grinder, soxhlet apparatus, petroleum ether, ethanol, evaporating dish

For Seizure model: Albino mice, electro-convulsimeter, (Techno electronics, Lucknow; Instrument no. C-2, Sl. No. 710510), ear clip electrodes, pentylenetetrazol, stop watch, markers, syringes, distilled water

Drugs and others: Gum acacia, inj. Phenytoin, inj. Diazepam,

weighing machine and electronic balance (Shimadzu, Japan; No. D455007340)

Authentication and collection of plant material:

The plant had been identified and authenticated by Botany Department, D.M College, Imphal, having the Acc. No. DM 201/2010. The leaves of *Toona ciliata* M. Roem were collected from foot hills of Langol Range, Imphal, Manipur.

Extraction of plant material:

The ethanolic extract of *Toona ciliata* M. Roem leaves (EETC) was obtained by the extraction procedure using soxhlet apparatus as described by Knevel AM et al.¹⁶ The extract thus obtained was investigated for anticonvulsant property in albino mice.

Toxicity testing:

Acute toxicity testing was carried out as per OECD guidelines 423¹⁷. Solubility test¹⁷ was done by dissolving plant extract in solvent (normal saline). But it is not completely dissolved, so 1% gum acacia was used as vehicle.

Method

Healthy albino mice of either sex weighing approximately 25-30g were used. These animals were obtained from the Animal house, RIMS, Imphal and transported to the Departmental animal room 7 days before the test. They were housed in groups of six per cage under the natural light-dark cycle at the room temperature with free access to standard food and water.

Ethics committee approval

Ethical clearance was obtained from Institutional Animal Ethics Committee, RIMS, Imphal.

Methods for assessment of anticonvulsant potential

A. Maximal electroshock seizure (MES) model

Selection of animals:

Pretesting of the mice was done with a current of 50 mA (milliamper) for 0.2 sec via a pair of ear clip electrodes (transauricular) using an electro-convulsimeter. The occurrence of a hind limb tonic extension (HLTE) was taken as a positive response for MES. HLTE was defined by hind limb extension more than 90° from the body and sustained for more than 3 sec following 10 sec after stimulation.¹⁴ Thirty mice which show hind limb tonic extension (HLTE) were selected for the study.

Experimental design:

Selected animals were allocated into 5 groups of 6 mice in each and treated as follows:

- Group I: Control
- Group II: Standard
- Group III: EETC - 100 mg/kg
- Group IV: EETC - 200 mg/kg
- Group V: EETC - 400 mg/kg

Control group was given 1% gum acacia solution in distilled water alone and test drugs were suspended in 1% gum acacia solution and given at corresponding doses in 10 ml/kg volume p.o. using feeding tubes. Standard group was given inj. phenytoin 25 mg/kg i.p. All the drugs were administered in the different groups for 3 consecutive days. The animals were evaluated on the next day after 1h of drug administration. Except for the short duration of experimentation, the animals were allowed free access to food and water. All the experiments were carried out in a quiet room under controlled light condition between 11:00 a.m. and 3:00 p.m. Each animal was tested only once.

Procedure:

Mice were subjected to maximal electroshock seizure (MES) by an electrical stimulus of 50 mA of alternating current from an electro-convulsimeter for 0.2 sec via ear clip electrodes. The resulting seizure passed through various phases: tonic flexion, tonic extension, clonic convulsions, stupor and recovery or death.¹⁸ The reduction or complete of abolition of hind limb tonic extension (HLTE) phase was considered as protection action against convulsion.^{18,19,20} The number of animals exhibiting HLTE, duration of HLTE and mortality was recorded for the different groups. The percentage protection²¹ will be calculated as follows:

$$\% \text{ Protection} = \frac{\text{Duration of HLTE in control} - \text{Duration of HLTE in test / standard}}{\text{Duration of HLTE in control}} \times 100$$

B. Pentylenetetrazol (PTZ) induced seizure

Experimental design:

Thirty healthy albino mice of either sex was allocated into 5 groups of 6 mice in each and treated as follows:

- Group I: Control
- Group II: Standard
- Group III: EETC - 100 mg/kg
- Group IV: EETC - 200 mg/kg
- Group V: EETC - 400 mg/kg

Control group was given 1% gum acacia solution in distilled water alone and test drugs were suspended in 1% gum acacia solution and given at corresponding doses in 10 ml/kg volume p.o. using feeding tubes. Standard group was given inj. diazepam 4 mg/kg i.p. All the drugs were administered in the different groups for 3 consecutive days. The animals were evaluated on the next day after 1h of drug administration. Except for the short duration of experimentation, the animals were allowed free access to food and water. All the experiments were carried out in a quiet room under controlled light condition between 11:00 a.m. and 3:00 p.m. Each animal was tested only once.

Procedure:

The animals in the different groups were given the treatments as per the experimental design for 3 consecutive days. The next day, after one hour of drug administration mice were given 1% solution of PTZ in distilled water (D/W) at the dose of 60 mg/kg i.p. and observed for 30 minutes. There was a sequence of excitement, myoclonic jerks, clonic seizures, one or more maximal tonic seizures and death. The occurrence of clonic seizure with loss of righting reflex will be taken as a positive seizure response. Abolition or suppression of the clonic seizure was considered as protection against PTZ seizures.²² The presence or absence of the clonic seizure, time of its onset (seizure latency) and duration, and mortality in the different treated groups was recorded. The percentage reduction of clonic phase²¹ was calculated as:

$$\% \text{ Reduction of clonus} = \frac{\text{Duration of HLTE in control} - \text{Duration of HLTE in test / standard}}{\text{Duration of HLTE in control}} \times 100$$

Analysis of results

The data obtained in the studies was expressed as mean ± standard error of mean (SEM) and was analyzed by One-way analysis of variance (ANOVA) followed by Bonferroni test. P < 0.05 was considered significant.

Waste Disposal

The waste materials eg. cotton swabs, gloves, needles, disposable syringes were put into suitable containers and disinfected at source by chemical treatment (1% hypochlorite solution). The sharp wastes were mutilated or shredded after chemical disinfection. Then, these materials were sent for further treatment to the institutional waste management plant. After the experiment the animal carcasses were buried deep in the ground covered with lime and disinfectants by laboratory attendants.

Results and Discussion

Table 1. Effect on THLE in MES induced seizure model

Group	Duration of THLE (sec)	Seizure protection
Control	17.38 ± 0.44	0 %
Standard(Phenytoin)	0.00 ± 0.00	100%
EETC 100	14.94 ± 0.32 ^{a***d**}	35.3 %
EETC 200	5.64 ± 2.53 ^{a**b**}	35.3 %
EETC 400	2.19 ± 1.38 ^{a**b**}	62.2 %

Results were expressed in Mean + SEM. Symbols a, b, c and d were used to compare with control group, EETC 100, EETC 200 and EETC 400 group respectively. P value < 0.05 was considered significant. ** p<0.001

Table 2. Effect on onset and duration of seizure in PTZ induced seizure model

Group	Onset of seizure(min)	Duration of seizure(sec)	Reduction in clonus%	Mortality
Control	2.30 ± 0.06	39.82 ± 1.00	0 %	66.6 %

Standard	0.00 ± 0.00	0.00 ± 0.00	100 %	0 %
EETC 100	9.57 ± 0.50	32.67 ± 2.77 ^{a***}	17.9 %	33.3 %
EETC 200	14.67 ± 0.26	29.90 ± 0.69 ^{a***b**}	24.9 %	16.6 %
EETC 400	27.48 ± 0.38 ^{a*}	12.73 ± 0.45 ^{a***b**}	68 %	0%

Results were expressed in Mean ± SEM. Symbols a, b, c and d were used to compare with control group. EETC 100, EETC 200, EETC 400 group respectively. P value < 0.05 was considered significant. ^{*}p<0.001, ^{**}p<0.01 and ^{***}p<0.05.

Fig. 1. Effect on THLE in MES induced seizure model

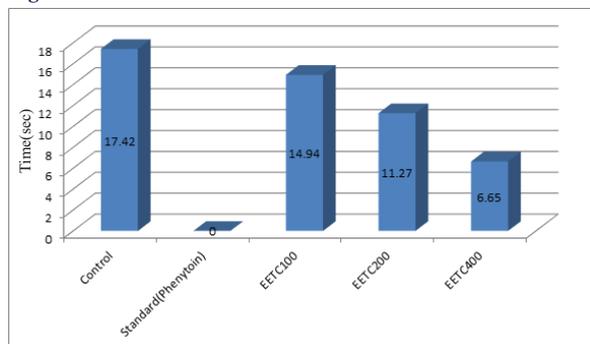


Fig. 2. Effect on onset of seizure in PTZ induced seizure model

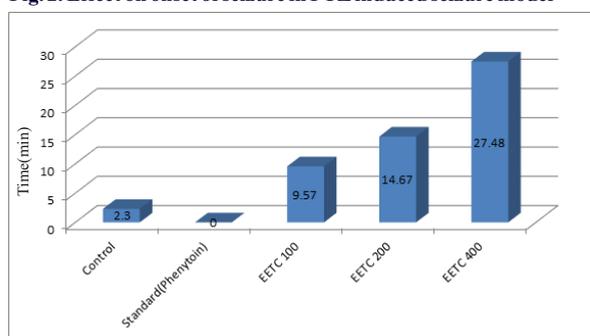
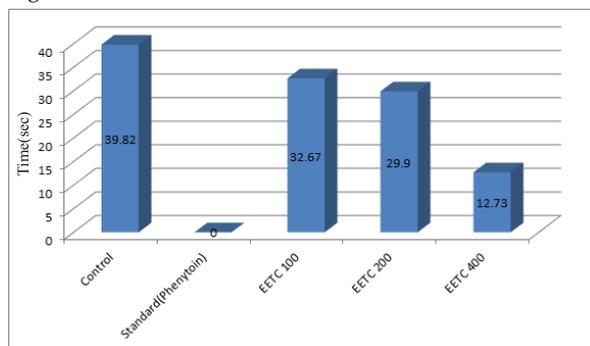


Fig. 3. Effect on duration of seizure in PTZ induced seizure model



The duration of tonic hind limb extension (THLE) in MES model in the present study was observed to be 17.38 ± 0.44 seconds in control group which was comparable to the studies conducted by Debnath et al²¹ (14.83 ± 0.40 sec) and Lahon et al²² (19.17 ± 0.75 sec). The latency of convulsion in PTZ model in control group in the present study was observed to be 2.30 ± 0.06 min which was similar to the study conducted by Lahon et al²² (129.50 ± 6.82 sec). However the duration of convulsion in the present study (39.82 ± 1.00 sec) was less than that observed by Lahon et al²² (77.17 ± 4.09 sec) which was probably due to the lesser dose of PTZ. Standard drug phenytoin and diazepam exhibited 100% protection from maximal electroshock seizure (MES) model and pentylenetetrazol (PTZ) induced seizure respectively which was similar to the study conducted by Debnath et al²¹. Phenytoin inhibits voltage-dependent Na⁺ channels & prevents MES-induced tonic extension.

Diazepam antagonizes PTZ induced seizure by enhancing GABA mediated opening of chloride channel. EETC at the highest dose (400 mg/kg) significantly reduced the duration of THLE (2.19 ± 1.38 sec) in MES model when compared to control group. In PTZ model EETC at the highest dose significantly delayed the latency of convulsion (27.48

± 0.38 min) and reduced the duration of convulsion (12.73 ± 0.45 min) when compared to control group. Moreover EETC (400 mg/kg) exhibited 62.2 % and 68 % seizure protection in MES and PTZ model respectively with no mortality in PTZ model which was comparable to standard drug diazepam. Hence, EETC possesses anticonvulsant activity which could be due to interaction with voltage-dependent Na⁺ channels or GABA mediated opening of chloride channel or neuroprotective effect by its antioxidant property.

CONCLUSION

Maximal electroshock seizure (MES) and pentylenetetrazol (PTZ) induced seizure models remain 'Gold standards' in screening of anticonvulsant activity. The present study showed that ethanolic extract of leaves of *Toona ciliata* M. Roem (EETC) significantly reduced the duration of tonic hind limb extension in MES model; delayed the latency and reduced the duration of seizures in PTZ model. Thus it establishes the anticonvulsant activity of leaves of *Toona ciliata* M. Roem. However the phytochemical responsible and the exact mechanism are yet to be investigated.

Conflict of interest: None

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