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ANTI-SEIZURE ACTIVITY OF METHANOL EXTRACT OF *TECTONA GRANDIS* L. ON MAXIMAL ELECTROSHOCK INDUCED SEIZURE IN ALBINO WISTAR RATS

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ABSTRACT

The aim of the present study was to investigate antiseizure effect of the methanol extract of bark of *Tectona grandis* Linn (METG) on Maximal electroshock (MES) induced seizures. The methanol extract of *Tectona grandis* Linn (METG) was subjected to acute toxicity and then screened for anticonvulsant activity on Maximal Electroshock (MES) induced seizures models in albino wistar rats. Also estimated the effect of methanolic extract of *Tectona grandis* Linn (METG) on biogenic amine concentrations in rat brain after induction of seizures by MES. Study results showed, the mean duration of extensor phase of treated groups reduced significant level than compared to control group. In MES model, METG (200 & 400 mg/kg) showed significantly restored the decreased levels of brain monoamines such as Noradrenaline (NA), Dopamine (DA), Serotonin (5HT) and GABA. Thus, this study suggests that methanolic extract of *Tectona grandis* Linn increased the monoamines on rat brain, which may be decreased the susceptibility to MES induced seizure in rats.

Keywords: Antiseizure Activity, *Tectona grandis*, Maximal electroshock, Monoamines.

INTRODUCTION

Many unknown and lesser known plants are used in folk and tribal medicinal practices in India. The medicinal values of these plants are not much known to the scientific world. *Tectona grandis*, (family Verbenaceae) is one such medicinal plant[1]. It is commonly known as sagwan (Hindi), saka (Sanskrit) and teak tree (English). It is a large deciduous tree with a height up to 35 m bark simple, opposite, broadly elliptical or acute or acuminate, with minute glandular dots; the flowers are white in colour and small with a pleasant smell [2] . Phytoconstituents of *Tectona grandis* Linn present in various parts are Triterpenic hemiterpenic compound, Lignins, Quinones, Steroidal compounds, Phenolic acids and Flavonoids [3-5].

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According to Ayurveda, traditional and ethnopharmacological uses of *Tectona grandis* Linn. are in the treatment of various disorders like anthelmintic, anti-inflammatory, antioxidant and brain disorders, anti-diabetic, bronchitis, constipation and diuretic[1,6,7]. In the light of the above information, the present investigation was undertaken to evaluate the claimed anti-seizure activity of *Tectona grandis* L in albino Wistar rats.

MATERIALS AND METHODS

Plant collection

The Plant material of *Tectona grandis* L. used for investigation was collected from Tirunelveli District, in the Month of August 2014. The plant was authenticated by Dr.V.Chelladurai, Research Officer Botany. C.C.R.A.S., Govt. of India. The voucher specimen of the plant was deposited at the college for further reference.

Preparation of extracts

The bark of *Tectona grandis* L. was dried in shade, separated and made to dry powder. It was then passed through the 40 mesh sieve. A weighed quantity (100gm) of the powder was subjected to continuous hot extraction in Soxhlet Apparatus. The extract was evaporated under reduced pressure using rotary evaporator until all the solvent has been removed to give an extract sample. Percentage yield of methanolic extract of *Tectona grandis* L. was found to be 11.5 % w/w.

Preliminary phytochemical screening

The phytochemical examination of methanol extract of bark of *Tectona grandis* L. was performed by the standard methods [8]**.**

Experimental Animals

Wister albino rats weighing between 200-250gm 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**.**

Acute toxicity study

Acute toxicity study of methanol extract of *Tectona grandis 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 [9].

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 methanol extract of *Tectona grandis L.* (*METG*) (200 and 400 mg/kg body weight) *p.o* respectively for 20 days. On the 20th day, Seizures are induced to all the groups by using an Electroconvulsiometer. 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 Hind leg Tonic Extension (or) extension not greater than 90° [10].

A fluorimetric micro method 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 litre *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 [11].

Nor-Adrenaline, Dopamine and Serotonin Assay

The assay represents a miniaturization of the tri hydroxide method and 395-485nm for NA, 360-470 nm for serotonin and 330-375nm for DA uncorrected instrument values. Nor-Adrenaline, Dopamine and Serotonin was estimated according to the method of Schlumpf *et al.* (1974) [11].

Estimation of brain GABA content

The brain amino butyric acid (GABA content was estimated according to the method of Lowe *et al.*, (1958) [12].

Statistical analysis

The data were expressed as mean ± standard error mean (S.E.M).The significant difference among the groups was assessed using one way and multiple way analysis of variance (ANOVA). The test followed by Dunnet's test *P* values less than 0.05 were considered as significance.

RESULTS

The results of preliminary phytochemical screening of the methanol extract of bark of *Tectona grandis* L. revealed that presence of alkaloids, carbohydrates, glycoside, phenolic compounds, flavonoids, protein and amino acids, saponins, sterols, acidic compounds, mucilage resins, lipids/ fats etc.

Effects of *METG* on MES Induced Seizure

The duration of tonic hind leg extension in rats treated with vehicle was 15.27±0.37 seconds. The METG at doses of 200 mg/kg and 400 mg/kg were protect animals

from seizures and significantly ($p<0.01$) reduced the 3.17±0.26 seconds respectively. Whereas, the standard drug phenytoin treated animals exhibits abolished tonic hind leg extension. Phenytoin treated animals have shown 100% protection against MES induced seizures whereas *METG* 200 mg/kg and 400 mg/kg have shown 66.67% and 83.33% protection respectively (Table 1).

Effect of METG on monoamines levels in seizure induced rats by MES

In MES model, Nor-Adrenaline, Dopamine and Serotonin levels were decreased significantly ($p<0.01$) in forebrain of epileptic control animals. METG at the doses duration of tonic hind leg extension for 6.57±0.19 and of 200 & 400mg/kg, standard drugs phenytoin and diazepam treated animals showed a significantly ($p<0.05$ & $p<0.01$) increased in Nor-Adrenaline, Dopamine and Serotonin levels in forebrain of rats. (Table 2).

Gamma amino butyric acid

In MES model, GABA levels significantly ($p<0.01$) decreased in forebrain of epileptic control animals were observed. METG at the doses of 200 & 400mg/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 2).

Table 1. Effect of methanolic extract of *Tectona grandis* L. (METG) on MES induced Seizures in rats

Group	Design of treatment	Flexion	Extensor	Clonus	Stupor	Recovery	% protection
I	MES (SCMC 1ml/100gm)	9.41±0.24	15.27±0.37	71.24±0.37	39.67±0.24	195.33	0
II	Phenytoin 25mg/kg, i.p.	2.52±0.17**	0**	8.24±0.54**	14.33±0.57**	92.17	100
III	METG 200mg/kg,p.o	6.22±0.36*	6.57±0.19**	12.18±0.46**	30.27±0.14*	141.67	66.67
IV	METG 400mg/kg,p.o	3.48±0.21**	3.17±0.26**	10.12±0.41*	16.17±0.34**	105.23	83.33

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.

Table 2. Effect of METG on neurotransmitters levels in rat brain after MES induced epilepsy

Group	Design of Treatment	Noradrenaline	Dopamine	Serotonin	GABA
I	MES (SCMC 1ml/100gm)	422.92±4.67 a **	445.62±4.38 a **	72.19±2.42 a **	226.64±3.29 a **
II	Phenytoin 25mg/kg,*i.p*	552.248±4.64 b **	622.32±4.29 b **	102.54±3.64 b **	296.17±3.54 b **
III	METG 200 mg/kg,*p.o*	573.16±4.99b**	528.36±3.69b**	128.34±3.59b**	254.21±2.19b**
IV	METG 400 mg/kg,*p.o*	748.17±4.36b*	642.21±5.28b*	148.61±4.28b*	275.46±3.62b**

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 CONCLUSION

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" [13, 14]**.** This model is based on observation of the stimulation by repeated electrical pulses induce in different neuronal structures one characteristic standard of seizure activity [15]**.** In our present study, it is found that treatment with METG on rats significantly reduces in tonic hind leg extensor stage in MES induced epilepsy. The MES model is used to identify compounds which prevent seizure spread, corresponding to generalized tonic-clonic seizures in humans**.** 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 [16, 17]. Since, METG significantly inhibited generalized tonic-clonic seizures in MES test; it suggests the presence of anticonvulsant compounds.

The role of biogenic amines in epileptogenesis and in recurrent seizure activity is well-documented. Spontaneous and experimentally induced deficiencies in gamma amino butyric acid (GABA), noradrenaline (NA), dopamine (DA) and/or serotonin (5-hydroxy- tryptamine or 5-HT). It has been implicated in the onset and perpetuation of many seizure disorders many experimental procedures designed to increase monoaminergic activity have proven antiepileptic properties [18-21]

In present study, the established antiepileptic drugs such as phenytoin restored the monoamine levels on brain [16]. Similarly, METG 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 [22]. MES is probably the best validated

method for assessment of anti-epileptic drugs in generalized tonic-clonic seizures [23].

GABA is a major inhibitory neurotransmitter of CNS and increase in its level in brain has variety of CNS dependent effects including anticonvulsant effect [24]. In addition to the GABA binding site, the $GABA_A$ receptor complex appears to have distinct allosteric binding sites for benzodiazepines, barbiturates etc [25, 26]. We therefore studied the effect of *Tectona grandis* L. extract on brain GABA content. *Tectona grandis* L. extract showed significant ($p<0.05$ & $p<0.01$) increased GABA content in brain dose dependently. This suggests that the anticonvulsant activity of *Tectona grandis* L. extract is probably through elevation of brain GABA content. 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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