

**EVALUATION OF ANTIPILEPTIC AND ANTIOXIDANT ACTIVITY
OF METHANOLIC EXTRACT OF TERMINALIA TOMENTOSA
{ROXB.} WIGHT AND ARN IN RATS**

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ABSTRACT

Aim: To evaluate antiepileptic activity and the antioxidant activity of methanolic extract of *Terminalia tomentosa leaves*. **Materials and**

Methods: Anti epileptic activity was studied by maximum electric shock induced convulsions and pentylenetetrazole induced convulsions in rats. **In MES induced convulsions:** Rats were divided into five groups each consisting of six. Group I Served as control with Tweens-80 per kg). Group II, III and IV mice were treated with 150, 300, 600mg/kg of methanolic extract of *Termanalia tomentosa* (TTLME) and after 60min to each rats given Maximum electroshock (MES) with electrical stimulus 150 mA, 0.2 second was delivered was applied through corneal electrodes. Group V rats were treated with standard Phenytoin (20mg/kg, i.p) and MES after 30min of phenytoin

administration. After giving MES, various phases of convulsion i.e. tonic flexion, tonic extensor, clonic convulsion, stupor and recovery/death were observed and noted. **In PTZ-induced convulsions:** Group I served as toxic control treated with pentylenetetrazole (80mg/kg i.p). Group II, III and IV rats treated with TTLME at the dose levels of 150, 300 and 600 mg/kg orally. Group V RATS treated with standard diazepam (4 mg/kg, i.p). All

groups simultaneously treated with PTZ after 60 mins in case of Group II, III and IV whereas after 30 min in case of standard group. After treatment each rats was observed initially for 30 min & later up to 24hrs. The following parameters were recorded during test session of initial 30 min and up to 24 hrs:latency (onset of clonus), duration of tonic convulsion, & status of animal after 30 min & percentage protection. In addition to antiepileptic activity the antioxidant activity of TTLME was also investigated using DPPH assay method. **Antioxidant activity:** *In-vitro* antioxidant activity was done with different doses of methanolic extract of *Terminalia tomentosa* leaves against the standard Ascorbic acid using with spectrophotometer at 510nm. **Results:** At 150, 300 and 600mg/kg the extract exhibited significant anti epileptic activity ($p<0.05$) which may be attributed to the presence of various phytochemicals such as proteins, carbohydrates, flavonoids, glycosides, amino acids, tannins. Its antioxidant activity was studied by DPPH method *invitro*. The results clearly indicating that the formulation is potent in scavenging free radicals *in vitro*. **Conclusion:** In conclusion, the methanolic extract of *Terminalia tomentosa* treated groups shows a significant effect when compared to control group animals which indicating that the plant having the anticonvulsant activity. The effectiveness of the plant's extract in the experimental convulsion used probably suggests that the herb could be used in both Petitmal and Grandmal types of epilepsy. And also the results showed that the methanolic extract of the *Terminalia tomentosa* leaves having the antioxidant activity. The results suggest that the crude methanol extract of *terminalia tomentosa* contains bioactive compounds which possess antiepileptic activity, thus giving credence to the traditional use of this plant in the treatment of epilepsy.

KEYWORDS: Terminalia tomentosa leaf methanolic extract (ttlme), Anti epileptic activity, maximum electro shock(MES), pentylene tetrazole (PTZ) induced convulsions.

INTRODUCTION

The word epilepsy is derived from the Greek word meaning “to seize” or “take hold of”, Indicating that the person having a seizure is “possessed” or at least out of control.^[1] Epilepsy is the second most common serious neurological disorder after stroke, which affects a wide range of people throughout the world.^[2] Epilepsy is a neurological disorder characterized by unprovoked seizures, and affects at least 50 million people worldwide. There is a continuing demand for new anticonvulsant agents as it has not been possible to control every kind of seizure with the currently available antiepileptic drugs. About one third of patients do not respond well to current multiple drugs therapy.^[3] Traditional medicine occupies an important

place in the health care systems of developing countries. The people in developing countries depend on traditional medicine, because it is cheaper and more accessible than orthodox medicine.^[4] Antiepileptic therapy has many drawbacks such as long duration of therapy, adverse effects of drugs, need for therapeutic drug monitoring.^[5] There is clearly a need for more specific and effective drugs.^[6] Medicinal plants have been an important source of new drugs. To search a new anti-epileptic drug from the plant kingdom, which may be therapeutically effective and would be safe, cheap and that would be accepted by developing country like India.

Terminalia is a genus of large trees of the flowering plant family Combretaceae, comprising around 100 species distributed in tropical regions of the world. *Terminalia tomentosa* (syn. *Terminalia alata* Heyne ex Roth, *Terminalia elliptica* (Roxb.) Wight & Arn) is a species of *Terminalia* native to southern and South east Asia in India, Nepal, Bangladesh, Myanmar, Thailand, Laos, Cambodia and Vietnam. It is a prominent part of both dry and moist deciduous forests in southern India up to 1000 m. *Terminalia tomentosa* {Roxb.} Wight & Arn has been traditionally the stem bark of the plant is used as cardiac stimulant and in treatment of atonic diarrhea & callous ulcer.^[7] This study was therefore designed to evaluate the antiepileptic activities of TTLME in order to scientifically justify its use in traditional medicine to treat epilepsy.

METHODS

Plant Material Collection

Leaves of *Terminalia tomentosa* were collected during August 2014 from the botanical garden of madhapur, Hyderabad, Ap and taxonomically identified by Dr. pratibha devi at the osmania university and A voucher specimen (0265) has been preserved in the herbarium for future reference.

Preparation of extract

The plant material was cleaned, air dried for 7 days and then crushed into coarse powder with a pestle and mortar and passed through sieve. 150 g of the powdered plant was extracted in Soxhlet apparatus with methanol for 48 hours with occasional shaking. The solvent was distilled off and the extract was concentrated on a water bath. The concentrated extract was then weighed and the percentage yield was calculated as 3.99g.

Phytochemical screening

Phytochemical screening of Terminalia Tomentosa was carried out on the methanol extract using standard methods of analysis.^[8]

Animals

Adult male Wistar rats (150-200gm) were used to evaluate anti-epileptic activity by maximal electroshock (MES) and pentylenetetrazole (PTZ) induced convulsions respectively. The animals were maintained under standard laboratory conditions in polypropylene cages under 12 hr light/dark cycle, controlled temperature(24±2°C), fed with commercial pellet diet and water *ad-libitum* in an animal house approved by the Committee for the Purpose of Control and Supervision on Experiments on Animals.

Acute Toxicity Studies in Rats

The median lethal dose (LD50) was determined using the method described by Lorke (1983). LD50 determination was conducted using the method of Lorke (1983). In the initial phase, 3 groups of three animals each were treated with the methanolic extract of the plant at doses of 150,300 and 1000 mg/kg body weight *i.p.* and observed for 24 hours. In the second phase, 4 groups of one animal each were injected with the methanolic extract at doses of, 150,300 600mg/kg *p.o.* The LD50 value was determined by calculating the geometric mean of the lowest dose that caused death and the highest dose for which the animal survived (0/1and 1/1). The same procedure was repeated using the oral route of drug administration.

Evaluation of Anti Epileptic Activity

Maximum electroshock-induced convulsion in rats

The modified method Swinyard and Kufferberg (1985): and Browning.(1992) was employed (Shalini et al 2010) Adult male wister rats (150-200mg) were randomly divided into 5 groups of 6 rats per groups. Adult wister rats (150-200mg) were randomly divided into 5 groups of 6 rats per groups. The first group received Tween-80 per kg body weight *ip.* The third, fourth and fifth group received the third fourth fifth group received (150,300,600 mg) methanolic extract of terminalia TOMENTOSA per kg body weight *Po.* While diazepam 4mg per kg *ip* body weight.

An electroshock of 150 mA, 0.2 second was delivered through ear-electrode to induce hind limb tonic extensor phase (HLTE) in rats using electroconvulsimeter (INCO, Ambala, India). The current was delivered 30 mins after intra-peritoneal administration of respective

treatments. Different parameters observed were the time spent by rats in flexion, extensor, clonus and stupor phases of tonic-clonic seizures. The occurrence and duration of HLTE and incidence of mortality were noted.^[9] The number of animals protected from hind limb tonic extension seizure (HLTE) and the time spent in this position were determined for each dose group.

Pentylentetrazole-induced convulsion in Rats

The method of Swinyard *et al.* (1989) was employed. Adult male wister rats (150-200mg) were randomly divided into 5 groups of 6 rats per groups. The first group received Tween-80 per kg body weight ip. The second group received diazepam 4mg per kg ip body weight while the third fourth, fifth group received (150,300,600 mg) methanolic extract of terminalia tomentosa per kg body weight Po.30 min later all rats received 80mg/kg ip 30 mins after administration of standard drug and test extract doses, clonic seizures and tonic-clonic convulsions were induced in rats by intra-peritoneal injection of Pentylene tetrazole (80 mg/kg). The latency to the onset of myoclonic spasm, onset of convulsions and mortality in treated rats was recorded.^[9]

In vitro Antioxidant activity DPPH free radical-scavenging activity

The methanolic solution of DPPH (0.1mM, 1 ml) was incubated with 3 ml of different concentrations of the root extract ranging from 10-100 µg/ml. Incubation was carried out at room temperature (25°C) for 30 min. For each concentration, the assay was run in triplicate. At the end of the incubation period, the optical density of each sample was determined at 517 nm (Gopinathan *et al.*, 2004). Ascorbic acid solution was used as a standard. EC₅₀ values (concentration required to scavenge 50% of the free radicals) for both ascorbic acid and the root extract were determined. The radical scavenging activity of the tested sample was expressed as an inhibition percentage (IP).^[10]

$$\text{DPPH Scavenged (\%)} = (\text{ADPPH} - \text{A}_{\text{test}} / \text{ADPPH}) \times 100$$

Where, ADPPH is the absorbance of the 0.1 mM of DPPH solution and A_{test} is the absorbance in the presence of the extract or ascorbic acid. IC₅₀ value was determined from the graph obtained using standard ascorbic acid by using the “y = mx + c” formula from the slope of the graph.^[11]

Statistical analysis

The statistical data was expressed as mean \pm SD (Standard Deviation). Statistical analysis was carried out by using one-way analysis of variance (ANOVA) followed by Dunnett's Multiple Comparison test. using GraphPad InStat version 6 for Windows, GraphPad Software, San Diego California USA.

RESULTS

Acute toxicity studies

Acute oral toxicity study of TTLME was performed in rats as per OECD-423 guidelines. The acute toxicity study reveals that the maximum tolerable dose of TCLME is more than 2000 mg/kg as all the test doses were found to be safe and no mortality or toxicity was observed. Three test doses i.e. (150,300 and 600 mg/kg) of TTLME were chosen for the evaluation of anti-convulsant activity based on the acute toxicity testing.No, mortality or any other autonomic or behavioral responses such as tremors, convulsion, salivation, diarrhea, lethargy, sleep and/or coma were observed.

Evaluation of anti-epileptic activity

1) Maximal electro shock induced convulsions

In Maximal electro shock –induced convulsions model, the methanolic extract of Terminalia Tomentosa at two doses of 300 and 600 mg/kg was produced significant ($P < 0.01$) decrease in the duration of tonic hind limb extensor phase in a dose dependent manner and was comparable to that of diazepam. At the dose of 150 mg/kg of TTLME did not exhibit significant effect of hind limb extensor. Mortality was not observed in any groups treated with methanolic extract of leaf of TTLME. The duration of tonic hind limb extensor phase was analyzed using Analysis of variance (ANOVA) test. The results were represents in table-5 and Fig-8.

2) Pentylene tetrazole induced convulsions

In pentelenetetrazole-induced seizure model, the methanolic extract of Terminalia tomentosa at two doses of 300 and 600 mg/kg was produced significant ($P < 0.01$) reduction in the duration of convulsion and was comparable to that of diazepam. 150 mg/kg extract group non- significant of onset of tonic seizure compared with toxic control group. These were represented in Table-6 and

Table 5: Effect of TTLME against maximal electroshock induced convulsions in rats.

S.No	Group (n=6)	Various phases of convulsions(time in sec)				No.of animals alive	% inhibition
		Tonic flexion	Tonic extensor	Clonic convulsions	stupor		
1	Control	13±1.9	25±2.8	66±8.8	93±7.2	5	83
2	Extract (150mg/kg)	11±1.5 ^{ns}	21±1.9 ^{ns}	53±4.1 ^{ns}	83±6.2 ^{ns}	3	50
3	Extract (300mg/kg)	9±1.4 ^{***}	20±2.6 ^{**}	52±3.5 ^{**}	79±5.8 ^{**}	4	67
4	Extract (600mg/kg)	7.3±1.2 ^{***}	15±1.7 ^{***}	34±6.9 ^{***}	46±5.2 ^{***}	6	100
5	Standard (Phenytoin 20mg/kg)	5.5±1 ^{***}	7.8±1.5 ^{***}	22±4.6 ^{***}	29±3.6 ^{***}	6	100

All treatment groups were compared with control group animals and data was expressed as mean ± S.D (n=6/group).

P values were * p<0.05, ** p<0.01, *** p<0.001, compared with control & treated groups.

Table 8: Effect of TTLME against pentylenetetrazole induced convulsions in rats.

S.No	Group (n=6)	Latency (onset of clonic convulsions) (sec/30min)	Onset of Tonic convulsions (sec/30min)	Status of animals	
				No.of animals alive	% inhibition
1	Toxic control	49±6.3	608±40.36	0	0
2	Extract (150mg/kg)	185±10.3 ^{***}	625±13 ^{ns}	3	50
3	Extract (250mg/kg)	218±14 ^{***}	530±27 ^{***}	5	83
4	Extract (600mg/kg)	228±24 ^{***}	494±24 ^{***}	6	100
5	Standard (Diazepam 4mg/kg)	No clonus	No tonic	6	100

All treatment groups were compared with control group animals and data was expressed as mean ± S.D (n=6/group).

P values were * p<0.05, ** p<0.01, *** p<0.001, compared with control & treated groups,

Concentration(µg/ml)	%Scavenging activity	
	Ascorbic acid	TTLME
10	92.12	87.4
25	94.09	88.58
50	96.06	89.76
75	96.45	93.30
100	97.63	94.06
IC50 (µg/ml)	37.59 µg/ml	39.40 µg/ml

DISCUSSION

Epilepsy is the second most common neurological disorder which affects an estimated 7 million people in India and 50 million people worldwide (approximately 1-2% of the world population).^[12] Furthermore, undesirable side effects from the drugs used clinically often render treatment difficult; so that a demand for new type of anticonvulsants exists. Due to these problems research focus has shifted towards natural products for new and better sources of drugs. In this process, medicinal plants serve as an alternative source for the development of new anti-convulsant drugs.

Various plants are being studied based on the traditional knowledge of their pharmacological properties and confirmed to be useful in treating and managing various diseases.^[13] Several plants used for the treatment of epilepsy in different systems of traditional medicine have shown activity when tested in modern bioassays for the detection of anticonvulsant activity and many such plants are yet to be scientifically investigated.^[14] In the present study we have evaluated the effect of methanolic extract of *Terminalia tomentosa* against seizures induced by maximal electroshock (MES) and pentylenetetrazole (PTZ) in rats. The results indicate that the methanolic extract produced dose-dependent anticonvulsant effect against MES and PTZ-induced seizure.

In the MES model it has been observed that the duration of hind limb extension of methanolic extracts of *Terminalia Tomentosa* produced a dose dependent reduction in the duration of hind limb extensor phase. The maximum reduction was seen with a dose of 300 mg/kg and 600mg/kg of methanolic extract of *Terminalia Tomentosa*.

The ability of the plant extract to inhibit the hind limb tonic extensor phase in MES model suggests the antiepileptic activity for the management and control of generalized tonic-clonic and partial seizures.

PTZ test represents a valid model for human generalized myoclonic seizures and also generalized seizures of the petitmal (absence) type.^[15]

Drugs protecting against tonic clonic seizures induced by PTZ are considered useful in controlling myoclonic and absence seizures in humans.^[16] Thus, demonstration of activity in this model suggests that the plant possesses anti-convulsant activity which validates the traditional use of this plant for the treatment of epilepsy. Since TTLME antagonized PTZ

induced seizures, this suggests enhancement of GABAergic transmission with general depression of the central nervous system.

Further, in PTZ model TTLME at 600 mg/kg was found to be more beneficial as compared to 300 mg/kg. In PTZ model, TTLME was more effective at dose of 600 mg/kg indicating CNS depressant action as consequence of its GABAergic transmission (since PTZ is selective GABAA receptor antagonist).

DPPH assay is based on the measurement of the scavenging ability of antioxidant towards the stable DPPH radical. DPPH is relatively stable nitrogen centered free radical that can accept an electron or hydrogen radical to become a stable diamagnetic molecule.^[17] DPPH radicals react with suitable reducing agent as a result of which electron become paired off forming the corresponding hydrazine. A higher DPPH radical-scavenging activity is associated with a lower IC₅₀ value. Phytochemical screening of plant leaves methanolic extract showed the presence of carbohydrates, glycosides, alkaloids, flavonoids and tannins. However, a number of investigators have shown that tannins and other polyphenolic compounds (eg., coumarins), flavonoids, triterpenoids and a host of other secondary plant metabolites possess analgesic, anti-inflammatory, anticonvulsant, hypoglycaemic and anti hypertensive properties in various experimental animal models.^[18; 19]

Moreover, previous studies have shown that flavonoids may cause facilitation of GABAergic system as they are structurally similar to benzodiazepines like molecules present in CNS.^[20]

CONCLUSION

In conclusion, the methanolic extract of *Terminalia tomentosa* treated groups shows a significant effect when compared to control group animals which indicating that the plant having the anticonvulsant activity. The findings of the present study lends pharmacological credence to the suggested folkloric, ethno medical uses of *Terminalia tomentosa* as a natural supplementary remedy for the treatment of convulsions and also the results showed that the methanolic extract of the *Terminalia Tomentosa* leaves having the antioxidant activity.

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