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ANTICONVULSANT ACTIVITY OF THE ETHANOLIC EXTRACT OF *TAMARINDUS INDICA* LEAVES IN EXPERIMENTALLY INDUCED (PTZ AND MEST) CONVULSIONS IN MICE

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ABSTRACT

Aim of the study is pharmacological screening of leaves of *Tamarindus indica* for anticonvulsant activity. Pentylenetetrazole-induced seizure and Maximal electroshock-induced seizure study were used to evaluate anticonvulsant activity. Results obtained in this study indicate that the ethanol leaf extract of *Tamarindus indica* possesses bioactive principles that have anticonvulsant activity and that *Tamarindus indica* is a partial neuromuscular agonist.

KEY WORDS: *Tamarindus indica,* Pentylenetetrazole-induced seizure, Maximal electroshock-induced seizure.

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INTRODUCTION

Nature has always stands as a golden mark to demonstrate the outstanding phenomenon of symbiosis. Today a vast store of knowledge concerning therapeutic properties of different plants has accumulated. India has a rich flora that is widely distributed throughout the country (1).

Herbal medicines have been used for the treatment and cure of various diseases and physiological conditions in traditionally practiced methods such as Unani, Ayurveda, and Siddha (2). Many plant species have been used by most ethnic groups for treating different disease conditions ranging from small infections to skin diseases, asthma, dysentery, malaria and other multiple indications (3). Herbal Medicine also termed as Botanical medicine or Herbalism is usage of herbs for therapeutic and medicinal importance. An herb is a variety of chemical substances that act upon the body (4).People tend to rely on traditional and other forms of complementary and alternative medicine for chronic conditions which do not respond well to conventional or modern drug treatments. Among these are neurological disorders such as anxiety, pain and epilepsy. Centuries before the advent of modern medicine, synthetic chemistry and the

pharmaceutical industry, virtually all medicines came from plants. These medicinal plants have been an important source for the discovery of novel bioactive compounds which served and continue to serve as lead molecules for the development of new drugs. Aspirin, atropine, scopolamine, taxol, theophylline, tubocurarine, vincristine and vinblastine are a few examples of such invaluable therapeutic tools for today‟s physicians (5).Medicinal plants used for the therapy of epilepsy in traditional medicine practice possess promising anticonvulsant activities in animal models of anticonvulsant screening and these can be an invaluable source for search for new antiepileptic compounds. Majority of epilepsy patients rely on medicinal plants for therapy. For example, a sample in Nigeria found 52% of epilepsy patients using some form of traditional medicine. Also, the use of traditional medicine and medicinal herbs is currently enjoying a renaissance in popularity in the West as well, and in fact, it is the primary form of medicine in many parts of the world. Epilepsy in particular is a condition where traditional healers are very critical in providing treatment in the rural settings. Considering the great reliance on traditional medicinal plants for treatment of diseases and the potential for drug discovery, it becomes relevant to search for potent, effective and relatively safe plant medicines as well as to scientifically validate success claims about plants already in use by traditional medicine practitioners (7).

Epilepsy is one of the major neurological disorders affecting approximately 0.8% of the population. There has been considerable progress in the pharmacotherapy of epilepsy over the last few decades, including the introduction of new antiepileptic drugs such as felbamate, lamotrigine, etc.. However, current drug therapy of epilepsy is complicated by side-effects, teratogenic effects; long term toxicity and about a third of patients are refractory to pharmacotherapies. Furthermore, there is currently no drug available which prevents the development of epilepsy e.g. after head trauma and all currently available AEDs drugs are synthetic molecules (6) Epilepsy is a disease in which selectively effective drugs are still under clinical trials but in the case of convulsion there is a sudden violent involuntary skeletal muscle contraction. It is a chronic central nervous system disorder in which there is a brief sudden abnormal motor, sensory which results in sudden neuronal discharge. Epilepsy occurs due to many neurons are under high excited stage which delivers massive discharges suppressing the integrity of the brain" seizures occurs due to occasional sudden rapid excessive and local discharges of grey matter" as soon as this is initiated due to abnormal focus there is a attack of the neighboring cells of the brain which leads to generalized convulsions. Seizures are symptoms of a disturbance in brain function. Most cases of seizures have no immediate identifiable cause. Seizures occurs due to electrical stimulation in the brain as soon as this is initiated seizure is initiated by reentry of excitatory impulses .There is total depletion of neurotransmitter, accumulation of CO_2 & adenosine which leads to depletion of oxygen and energy rich phosphate intermediates.

Anticonvulsants drug therapy

The selection of the drug is based on the type of the epilepsy and probable Pathophysiology mechanism" because a single drug may have different mechanism it may produce its actions different receptors.[21]Epilepsy is a major neurological disorder characterized by recurrent, spontaneous brain seizures or convulsions and its prevalence in developing countries is generally higher than in developed countries. Epilepsy is the second most common neurological disorder after stroke and it is estimated that approximately 0.8% of the population is affected by some form of epilepsy. Recent studies suggest an increased risk of dying and a greater proportion of deaths that are epilepsy-related in Africa as high as a six-fold increase in mortality in people with epilepsy. This is higher than the two-to-three fold increase reported in developed countries. Though not clear, the reasons for this gap might be due to social deprivation. Recent data suggest that people from socio-economically deprived backgrounds in

developed countries are more likely to develop epilepsy. This neurological disorder is viewed as a shameful disorder and has severe social implications in African communities as it carries a stigma. Sufferers are often shunned and discriminated against with respect to education, employment and marriage.

Drug therapy of epilepsy with currently available Antiepileptic Drugs (AEDs) is associated with side effects, dose-related and chronic toxicity that involve virtually every organ system. Moreover, all the currently available AEDs have potential for adverse effects on cognition and behaviour. The practice of polypharmacy in the therapy of epilepsy that has doubtful background increases the risk of side effects and drug interactions. It can be said that all problems with the current AED therapy of epilepsy are more prevalent in underdeveloped countries due to lack of facilities for proper diagnosis, treatment and monitoring of serum levels of AEDs. Another critical issue associated with currently available AEDs is recent clinical and experimental data that strongly suggest that AED therapy does not alter the course or natural history of epilepsy and though AEDs suppress the seizures, they may not affect the underlying disorder. Only a very few AEDs have been shown to be antiepileptogenic including valproate and phenobarbitone and levetiracetam but these are not well substantiated. There is pressing need for further research especially in the field of pharmacotherapy of epilepsy to find drugs which are not only anticonvulsant but also antiepileptogenics that either prevent epilepsy or alter its natural course. Natural products and plants for that matter, used in traditional medicine can be an invaluable source for search for novel antiepileptic compounds (8).

Based on literature review we aimed pharmacological screening of leaves of *Tamarindus indica* for anticonvulsant activity.

MATERIALS AND METHOD (9-11)

Preparation of *Tamarindus Indica* Leaves *Extract*

The powdered sample (200g) was extracted in 500 ml of ethanol for 72 hours. The extract was filtered using a vacuum pump and concentrated by removing the solvent completely using a water bath.

Method of Anti Epilepsy Activity

The whole plant of the *Tamarindus Indica* selected for the study was collected from the local areas of Hyderabad and authenticated.

Pentylenetetrazole-induced seizure test

The method used was adapted from that described . Female ICR mice were divided into seven groups (n=5). The extract (30, 100 and 300 mg/kg *p.o.*) was administered to three groups while diazepam (0.1, 0.3 and 1.0 mg/kg *i.p*) was given to three other groups and the last group administered 10 ml/kg *p.o* of the vehicle to serve as control. After 1 hour and 30 minutes of treatment with drugs orally and intraperitoneally respectively, each mouse was administered pentylenetetrazole, 85 mg/kg subcutaneously. The animals were placed individually in clear plastic observation chambers (15 cm x 15 cm x 15 cm) placed on a large plain glass elevated above the floor (80 cm) and a mirror placed behind the glass at an angle of 45^0 to the glass on the floor to enable clear and complete view of the animals. A digital video camera was positioned in front of the mirror to videotape test sessions. The Behaviour Tracker Software Version 1.5 (http://www.behaviortracker.com/) was used to analyse the videos for the latency to the first myoclonic jerks, the latency to tonic convulsions and the frequency and duration of tonic convulsions for each mouse.

Maximal electroshock-induced seizure test

The method used has been previously described. Male ICR mice were grouped into seven (n=10). Three groups were treated with the extract (30, 100 and 300 mg/kg p.o), three other groups treated with carbamazepine (3, 10 and 30 mg/kg p.o) and the last grouped administered distilled water (10 ml/kg p.o), to serve as control. After 1 hour and 30 minutes of oral and intraperitoneal drug treatments, respectively tonic convulsions of the hind limb extremities of mice were induced by passing alternating electrical current

(50 Hz, 60 mA and 0.2 s) through ear electrodes. This was the maximal current (60 mA) that induced tonic hind limb extension in all the trial mice and it was determined previously before commencement of the experiment. The number of animals protected from tonic hind limb extension seizure and the time spent in this position were determined in each dose group.[85]

Statistical Analysis

To compare differences between groups, one-way ANOVA was performed with Newman-Keuls‟ test as post hoc.

RESULTS AND DISCUSSION

Preliminary Phytochemical Screening

Phytochemical screening of ethanol extract of *Tamarindus indica* leaves was done, the extract showed the presence of Alkaloids, glycosides, saponins, flavonoids, Fixed oils and fats, Proteins, Phenol compounds and tannins.The results of preliminary phytochemical studies of the plant extract are presented in the below Table-1

Table-1 Data Showing Preliminary Phytochemical Screening of the Extract of *Tamarindus indica*

Carbohydrates	+
Glycosides	+
Fixed oils and fats	+
Potein & amino acids	+
Saponins	+
Tannins	-
Phenolic compounds	+
Flavonoids	+
Alkaloids	+

(+) Present (-) Absent

Effect of extract on PTZ-induced seizures

The extract showed significant anticonvulsant activity against PTZ-induced seizures. It significantly and dose-dependently delayed the onset of myoclonic jerks ($F_{3,16}$ =6.29, P=0.0051) and decreased the duration of tonic convulsions ($F_{3,16}$ =9.20, P=.0009). TIE significantly delayed the onset of myoclonic jerks at doses of 100 mg/kg and 300 mg/kg ($p<0.01$ and 0.05, respectively) but 30 mg/kg did not cause a significant delay in the onset of myoclonic jerks ($p>0.05$). Reduction in the duration of tonic convulsions by the extract was profound at all the doses used ($p<0.01$ at 30-300 mg/kg). Again, it delayed the onset of PTZ-induced tonic convulsions and reduced the frequency of convulsions though not statistically significant ($F_{3,16}$=0.95, P=0.4385 and $F_{3,16}$=2.39, P=0.1065, respectively).

Diazepam, an anticonvulsant, produced effects similar to that of the extract against PTZ-induced seizures and the effects were dose-dependent. The drug significantly delayed the onset of myoclonic jerks ($F_{3,16}$=18.18, P<0.0001) as well as the onset of tonic convulsions ($F_{3,16}$ =5.10, P=0.0115). Also, diazepam caused significant reduction of the frequency ($F_{3,16}$ =6.03, P=0.006) and duration of tonic convulsions ($F_{3,16}$ =21.34, P<0.0001) (Table-2-4 and fig-1, 2)

Table-2 Effect of extract on PTZ-induced seizures-Myoclonic jerks

Group	Group	Drug Treatment	Myoclonic jerks
1	Normal	Saline	203.46 ±8.64
2	Test-1	30 mg/kg	458.76±10.47
3	Test-2	100 mg/kg	976.45±20.54
4	Test-3	300 mg/kg	724.65±12.04*
5	D-1	0.1 mg/kg	605.34±10.23**
6	D-2	0.3 mg/kg	1254.37±23.21**
7	D-3	1.0 mg/kg	1905.34±2.14**

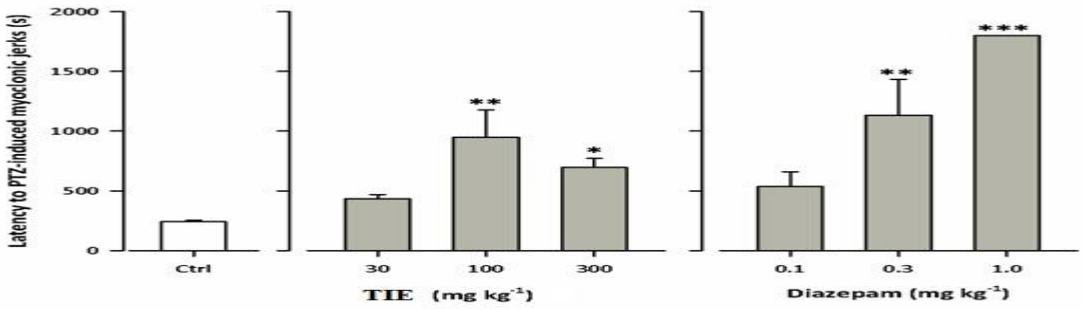

Fig-1 Effect of TIE (30-300 mg/kg, *p.o.*) and diazepam (0.1-1.0mg/kg, *i.p.*) on the latency to PTZ-induced myoclonic jerks. Each column represents the mean ± S.E.M. n=5, ***P<0.001; **P<0.01 and *P<0.05, one-way ANOVA followed by Newman-Keuls test

Table-3 Effect of extract on PTZ-induced seizures-frequency of Convolusions

Group	Group	Drug Treatment	PTZ induced convulsions
1	Normal	Saline	3.74 ±0.32
2	Test-1	30 mg/kg	1.54±0.28
3	Test-2	100 mg/kg	1.23±0.18
4	Test-3	300 mg/kg	0.98±0.14
5	D-1	0.1 mg/kg	1.21±0.18**
6	D-2	0.3 mg/kg	0.38±0.1**
7	D-3	1.0 mg/kg	0.06±0.003**

Table-4 Effect of extract on PTZ-induced seizures-Duration of Convolutions

Group	Group	Drug Treatment	Duration of convulsions
1	Normal	Saline	32.22 ±1.2
2	Test-1	30 mg/kg	16.54±1.1*
3	Test-2	100 mg/kg	9.53±0.67*
4	Test-3	300 mg/kg	9.22±0.54**
5	D-1	0.1 mg/kg	7.22±0.57***
6	D-2	0.3 mg/kg	4.52±0.1***
7	D-3	1.0 mg/kg	0.02±0.001***

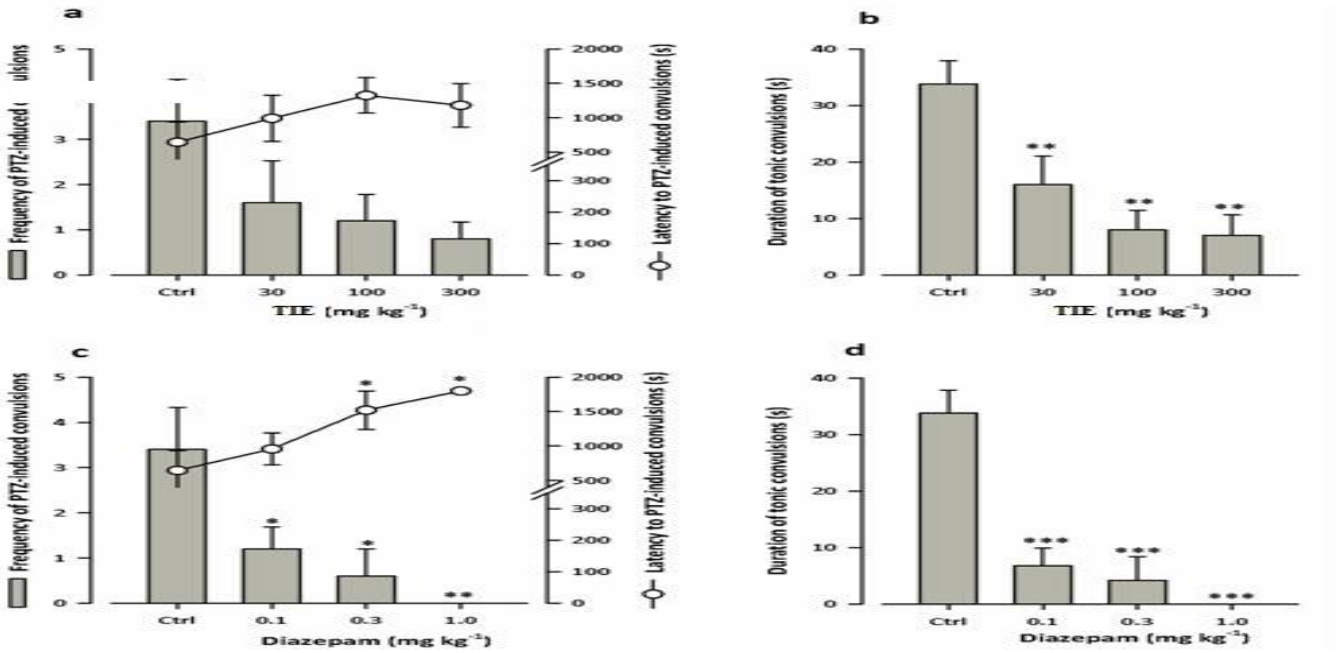

Fig-2 Effect of TIE (30-300 mg/kg, *p.o.*) and diazepam (0.1-1.0 mg/kg, *i.p.*) on PTZ- induced seizures. (a) Effect of TIE on frequency and latency to tonic convulsions. (b) Effect of TIE on duration of tonic convulsions. (c) Effect of diazepam on the frequency and latency to tonic convulsions. (d) Effect of diazepam on duration of tonic convulsions. Each point and column represents the mean ± S.E.M. n=5, ***P<0.001; **P<0.01 and *P<0.05, one-way ANOVA followed by Newman-Keuls test.

Effect of extract in maximal electroshock seizures

The extract caused significant decrease in the duration of tonic hind limb extension (THLE) induced by maximal electroshock ($F_{3,35}$ =5.08, P=0.0050) but was unable to completely prevent its occurrence. TIE at 100-300 mg/kg produced significant reduction of the duration of THLE ($p<0.05$) however, 30 mg/kg did not cause significant effect ($p>0.05$). Carbamazepine (3-30 mg/kg *p.o.*) significantly reduced the duration of MES-induced THLE ($F_{3,36}$=7.35, P=0.0006) and completely prevented the occurrence of this behaviour at 30 mg /kg. CBZ at 3 mg/kg did not produce significant effect compared to the control group ($p>0.05$) (Table-5 and fig-3).

Table-5 Effect of extract on PTZ-induced seizures-Duration of Convolusions

Group	Group	Drug Treatment	THLE
1	Normal	Saline	14.34 ±0.8
2	Test-1	30 mg/kg	13.22±0.7
3	Test-2	100 mg/kg	3.51±0.52*
4	Test-3	300 mg/kg	7.12±0.78*
5	D-1	3 mg/kg	10.01±0.47
6	D-2	10 mg/kg	5.22±0.49*
7	D-3	30 mg/kg	0.04±0.001***

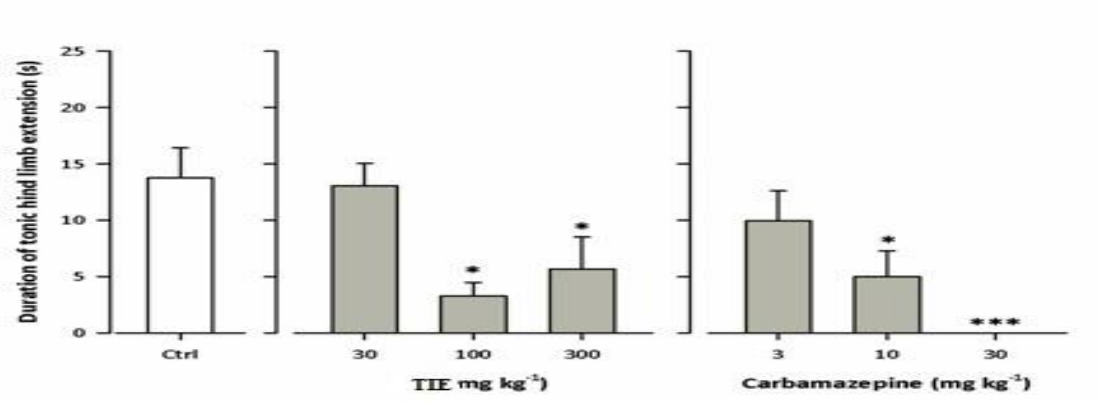

Fig-3 Effect of TIE (30-300 mg/kg) and carbamazepine (3-30 mg/kg) on the duration of MES-induced tonic hind limb extension.Each column represents Mean±S.E.M. n=10 ****P*<0.05; ***P*<0.01 and **P*<0.001 ANOVA followed by Newman-Keuls test.

The ability of an agent to prevent or delay the onset of tonic and tonic-clonic convulsion induced by PTZ in animals is an indication of anticonvulsant activity. In this study, the extract, TIE, caused significant dose-dependent anticonvulsant effect against PTZ-induced seizures by delaying the onset of myoclonic jerks and tonic convulsions in mice. It also caused profound decrease in the duration of the tonic convulsions. Anticonvulsant activity in PTZ-induced seizures identifies compounds that can raise seizure threshold in brain. AEDs effective in the therapy of generalised seizures of petit mal type (absence of myoclonic) i.e. phenobarbitone, valproate, ethosuximide and benzodiazepines suppress PTZ-induced seizures in a dose-dependent manner. Diazepam which was used in this study as a reference anticonvulsant agent showed significant activity by delaying the onset of myoclonic jerks and tonic convulsions and decreasing the frequency and duration of tonic convulsions (12, 13).

According to De Sarro *et al.*, (1999), PTZ may be exerting its convulsant effect by inhibiting the activity of GABA at $GABA_A$ receptors. GABA is the major inhibitory neurotransmitter which is implicated in epilepsy. The enhancement and inhibition of the neurotransmission of GABA attenuates and enhances convulsion, respectively. Standard antiepileptic drugs such as diazepam and phenobarbitone are thought to produce their effects by enhancing GABA-mediated inhibition in the brain and in this study with diazepam showed anticonvulsant activity against PTZ seizures. Seizures induced by PTZ are also blocked by drugs such as ethosuximide, by reducing T-type Ca^{2+} currents. Activation of N-methyl-D-aspartate (NMDA) receptor system is also involved in the initiation and propagation of PTZ-induced seizures. In this regard, drugs such as Felbamate that block glutamatergic excitation mediated by NMDA receptor have demonstrated anticonvulsant activity against PTZ-induced seizures. Since the extract delayed the occurrence and decreased the duration of convulsions induced by PTZ, it is possible that the anticonvulsant effects might be due to enhancement of GABA-mediated inhibition and/or inhibition of Ca^{2+} currents or blockade of glutamatergic neurotransmission mediated by NMDA receptor; which is not tested in this study. Effect of extract in maximal electroshock seizures (14, 15)

The extract, TIE, was not able to abolish tonic hind limb extension at all the doses used in this study but significantly reduced the duration of the tonic hind limb extension.Tonic hind limb extension is the universal feature of maximal electroshock in mice, rats, rabbits, cats, monkeys and humans[89]. Abolishing tonic hind limb extension in MEST predicts the ability of testing material to prevent the spread of seizure discharge from the epileptic focus and its effectiveness in MEST correlates well in suppressing generalized tonic-clonic seizures.Also, abolishing hind limb extension indicates the ability of testing material to inhibit or prevent seizure discharge within brainstem seizure substrate. All the

currently available drugs that are clinically effective in the treatment of generalised tonic seizures (phenytoin, carbamazepine, phenobarbitone, valproate, lamotrigine, oxycarbamazepine, etc) are effective in MEST. TIE in this study was not able to abolish tonic hind limb extension but significantly reduced its duration. Carbamazepine in this experiment caused significant reduction of the tonic hind limb extension phase and completely abolished this behaviour at 30 mg/kg. This validates the activity of the extract in this model. Reduction in the duration of tonic hind limb extension but inability to completely abolish it by TIE indicated weak anticonvulsant activity in MEST but suggested strongly the presence of anticonvulsant compounds in the extract

CONCLUSION

Results obtained in this study indicate that the ethanol leaf extract of *Tamarindus indica* possesses bioactive principles that have anticonvulsant activity and that *Tamarindus indica* is a partial neuromuscular agonist. Enhancement of GABAergic neurotransmission and/or calcium ion channel mechanisms may be involved in the anticonvulsant activity of the extract. In conclusion, the extract showed anticovolusant activity in PTZ and MES models.

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