

## Pharmacological evidence for the anticonvulsant activity of *Tylophora indica* in experimental animal models

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**Received:** 02 March 2017

**Accepted:** 11 March 2017

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### ABSTRACT

**Background:** Epilepsy is a common neurological disorder. The antiepileptic drugs currently used are unable to manage seizures effectively and are associated with numerous adverse drug effects. Hence, there is a necessity of a newer anticonvulsant drug with high therapeutic index profile. The objective of this pre-clinical research was to investigate the role of *Tylophora indica* on Maximal electric shock [MES] and Pentylene tetrazole [PTZ] provoked convulsions in Wistar albino rats.

**Methods:** 36 Wistar albino rats were used for this study, after obtaining ethical clearance. The ethanolic extract of the leaves of *Tylophora indica* [TIEE] (100 mg/kg, p.o) was used to screen the anticonvulsant effect on MES and PTZ provoked convulsions in Wistar albino rats. In MES seizures, inhibition of the tonic hind limb extension and in PTZ seizures, extent of convulsions was noted.

**Results:** TIEE (100 mg/kg, p.o) significantly ( $p < 0.001$ ) blocked the hind limb extension due to MES. The same dose also significantly ( $p < 0.001$ ) lessened the extent of convulsions induced by PTZ.

**Conclusions:** The data suggests that the ethanolic extract of *Tylophora indica* leaves produce its anticonvulsant effect via different mechanisms since it prevented the hind limb extension induced by MES and decreased the duration of convulsions produced by PTZ.

**Keywords:** Anticonvulsant, Convulsion, Maximal electric shock, Pentylene tetrazole, *Tylophora indica*, Wistar albino rats

### INTRODUCTION

Epilepsy is a central nervous system disorder. This frequently seen chronic neurological disease is characterized by unpredictable and intermittent occurrence of a momentary alteration of behavior due to the distorted firing of brain neurons.<sup>1</sup> The antiepileptic drugs presently available are not capable to control seizures efficiently and encompass various grave adverse effects. Keeping the above facts in mind, the significance of medicinal plants comes to the picture. India has an affluent source of an array of medicinal herbs. Phytoconstituents can play a key role in the treatment of epilepsy as they are well-known for high therapeutic index and comparatively low cost.<sup>2</sup> Anticonvulsant properties of many plants is not scientifically proven.<sup>3,4</sup>

Enormous research works are to be carried out to establish the therapeutic properties of the unexplored plants and additional detailed investigations should be done to pin down the active ingredient of the plant accountable for the proven health benefit.

*Tylophora indica* is a climber seen in India. This plant of Asclepiadaceae family is traditionally used for the treatment of jaundice, inflammation and bronchial asthma. Different parts of this climber have many therapeutic uses. Many pre-clinical studies have proved the antiasthmatic, anti-inflammatory, antitumor, anti-anxiety, antirheumatic, analgesic, hepatoprotective and antioxidant activities of this plant.<sup>5-8</sup> However there is no scientific data about the anticonvulsant activity of *Tylophora indica* till date. The aim of the present study

was to evaluate the anticonvulsant property of ethanolic extract of *Tylophora indica* leaves in experimental animal models.

## METHODS

### Drugs and chemicals

Phenytoin sodium (Abbott Group, Acme formulation Pvt Ltd, Himachal Pradesh), Sodium valproate (Sun Pharma, Sikkim) were purchased from our hospital pharmacy in Mangalore. Pentylene tetrazole (Sigma Aldrich, China) was procured from Rajesh Chemicals, Mumbai.

### Instruments

- Soxhlet apparatus to prepare the ethanolic extract.
- Electro-convulsimeter for induction of convulsions in animals.

### *Tylophora indica* ethanolic extract (TIEE)

*Tylophora indica* plants were cultivated. Fresh leaves of *Tylophora indica* were collected and authenticated by a botanist. They were shade dried, and then made into coarse powder. 500 grams of the powder was extracted with ethanol (90%) in a Soxhlet apparatus. The extract was concentrated on a water bath at a temperature below 60°C. The ethanolic extract was suspended in distilled water.<sup>7</sup> It was administered at a dose of 100mg/kg body weight orally to the animals. The dose was selected based on the acute toxicity study.

### Animals

Thirty six adult Wistar albino rats of both males and females weighing 150-200 g were used in this study after obtaining Institutional Animal Ethical Committee Clearance, Yenepoya University. The rats were maintained under standard conditions in the Animal House (CPCSEA approved, Reg No: 347) under Department of Pharmacology, Yenepoya University, Mangalore. The rats were provided food and water *ad libitum*. The rats were maintained on a 12:12 hour light-dark cycle.

### Anticonvulsant activity

#### Maximal electroshock (MES) seizure

Electrical stimulation was given using ear electrodes. The electrodes were dipped in saline before electrical

stimulation. All animals were stimulated with 150mA for 0.2 seconds, with constant voltage stimulators of 250 V.<sup>9</sup>

The animals were divided into three groups. Each group consisted of 3 males and 3 females (n=6).

- Group I: Distilled Water (1ml P.O for 10 days)+MES on 10<sup>th</sup> day
- Group II: TIEE (100 mg/kg P.O for 10 days) + MES on 10<sup>th</sup> day
- Group III: Phenytoin (25mg/kg I.P for 10 days) + MES on 10<sup>th</sup> day

On 10<sup>th</sup> day, 1 hour after the test compounds were administered, convulsions were induced by electrical shock. The parameter observed was duration of tonic hind limb extension.<sup>9</sup>

#### Pentylene tetrazole (PTZ) induced convulsion

The animals were divided into three groups. Each group consisted of 3 males and 3 females (n=6).

- Group I: Distilled Water (1ml P.O for 10 days) + PTZ on 10th day
- Group II: TIEE (100 mg/kg P.O for 10 days) + PTZ on 10th day
- Group III: Sodium valproate (75mg/kg I.P for 10 days) + PTZ on 10th day

On 10<sup>th</sup> day, 1 hour after the test compounds were administered, convulsions were induced by injecting PTZ 70mg/kg I.P. The parameter noted was duration of convulsion.<sup>9</sup>

#### Statistical significance

The results of the study is expressed as mean±SD, n = 6. One Way ANOVA followed by Tukey Kramer multiple comparison tests was used to compare the data.

## RESULTS

### Antiepileptic activity: MES induced seizures

In the case of MES induced seizures there was no hind limb extension in TIEE and Phenytoin groups on comparing with the control group (p <0.001). There were 3 deaths in the control group. No mortality was observed in TIEE and Phenytoin groups (Table 1).

**Table 1: Effect of TIEE on MES seizures.**

Drugs	Dose (mg/kg)	Duration of Hind Limb extension in seconds (mean ± SD) n=6	Mortality
Distilled Water	10ml / kg	13.53± 0.54	3
TIEE	100	0 ± 0.000 <sup>a</sup>	0
Phenytoin	25	0 ± 0.000 <sup>a</sup>	0

n=6. <sup>a</sup>p<0.001, Considered highly significant on comparing TIEE and Phenytoin administered rats with the normal animals

**Table 2: Effect of TIEE on PTZ seizures**

Drugs	Dose (mg/kg)	Duration of convulsions in seconds (mean $\pm$ SD) n=6	Mortality
Distilled Water	10ml / kg	12.161 $\pm$ 0.55	3
TIEE	100	1.01 $\pm$ 0.31 <sup>a</sup>	0
Sodium Valproate	75	1.26 $\pm$ 0.14 <sup>a</sup>	0

<sup>a</sup>p <0.0001, Considered highly significant on comparing TIEE and Sodium valproate administered rats with the normal animals

### Antiepileptic activity: PTZ induced seizures

In the case of PTZ induced seizures there was a significant decrease in the duration of convulsions in TIEE and Sodium Valproate groups on comparing with the control group. There were 3 deaths in the control group. No mortality was observed in TIEE and Sodium valproate groups (Table 2).

## DISCUSSION

The results showed that the TIEE has significant anticonvulsant activity in MES and PTZ induced seizure models. Table-1 indicates that ethanolic extract of *Tylophora indica* leaves completely abolished the hind limb extension in MES models. Table 2 indicates that ethanolic extract of *Tylophora indica* leaves completely prevented the convulsions in PTZ induced seizure models.

Epilepsy, a neurological disorder is seen globally in approximately 1% of the population, affecting almost all ages and both sex. Some of the contributing factors of this neurological disorder include stroke and oxidative stress.<sup>10,11</sup> This pathological condition is differentiated by localized bursts of electrical over-activity in the cerebral hemispheres.<sup>12,13</sup> Long-lasting plastic changes in the brain affecting the functions of neurotransmitter, the properties of receptors and channels, regulation of gene expression, synaptic re-organization and astrocyte activity lead to epilepsy.<sup>14</sup>

There are concrete evidences that an imbalance between the inhibitory and excitatory transmitters is involved in the etiology of epilepsy. GABA (g-aminobutyric acid), the major inhibitory neurotransmitter in the brain, is vital for the overall balance between neuronal excitation and inhibition that is vital to normal brain function. Changes in GABA-A receptor subunits are strongly implicated in idiopathic generalized epilepsies. Drugs which reduce brain GABA content are often convulsants and drugs which elevates brain GABA content, have anticonvulsive properties.<sup>14-16</sup>

Glutamate being an excitatory transmitter has a major role in the beginning and spread of seizure activity. Unrelenting activation of glutamate receptor signaling pathways by wide release of glutamate in susceptible areas leads to neuronal damage through apoptosis. The NMDA and AMPA receptors of glutamate have a chief

role in this disorder. Various animal studies showed that agents blocking these two glutamate receptors have powerful anticonvulsant property.<sup>17,18</sup>

Role of dopamine in epilepsy came into the picture based on the convulsigenic effect of antipsychotics (i.e., dopaminergic D2-like antagonists). On the contrary, seizure inhibition has been seen in patients administered antiparkinsonian drugs such as pergolide and bromocriptine, both D<sub>2</sub>-agonists.<sup>19,20</sup>

The role of serotonin [5HT] in epilepsy had come into the picture due to the fact that, in patients with epilepsy, a common co-morbidity diagnosed is depression. Elevation in serotonin concentration has proved to have an inhibitory response to epileptic discharge and stabilizes the depressed mood disorder. Studies showed that mono amino oxidase inhibitors are effective in kindling model of epilepsy. Previous reports indicate that fluoxetine, a selective 5-hydroxytryptamine (5-HT) uptake inhibitor, has anticonvulsant effects. Another study has shown that sertraline decreased the intensity of autogenic seizures. Mutant mice lacking 5HT<sub>1A</sub> or 5HT<sub>2C</sub> receptors have proved to have lower seizure threshold, implicating the role of respective 5HT receptors in epilepsy.<sup>13,21</sup>

Amongst the pre-clinical screening tests used for assessment of anticonvulsant activity, the MES and PTZ tests using rodents are the best validated methods. These two models mimic human generalized tonic-clonic seizures and absence seizures respectively. More over the above two seizure models are associated with oxidative damage. MES induced seizures are prevented by the drugs that blocks voltage gated sodium channels like Phenytoin or by the NMDA receptor antagonist like Felbamate. Whereas the drugs like Sodium valproate that block T-type Ca<sup>2+</sup> channels in thalamus or the drugs has GABA mimetic activity like Diazepam prevents PTZ induced seizures.<sup>9</sup>

Abolition of MES induced seizures by the TIEE hint an activity against generalized tonic clonic seizures. The activity against PTZ induced convulsions envisages an activity against petitmal seizures.

Based on the above facts and results the anticonvulsant action of TIEE can be due to:

- Blockade of sodium channels since they abolish MES induced seizures.

- Blockade of T type calcium channels in thalamus since it blocks PTZ induced seizures.
- GABA mimetic activity.
- Glutamate antagonistic property.
- Increase in monoaminergic activity.
- Antioxidant activity.

Phytochemical study revealed that Tylophorinidine, an alkaloid is the active constituent present in the ethanolic extract of *Tylophora indica*.<sup>10</sup> This alkaloid is an isoquinoline derivative. Isoquinoline compounds can inhibit monoamine oxidase enzyme (MAO) in brain. Being an isoquinoline derivative, Tylophorinidine can increase the levels of monoamines like dopamine and serotonin, and thereby preventing the MES and PTZ seizures.<sup>22</sup> This alkaloid also has antioxidant activity.<sup>22</sup> By virtue of this free radical scavenging activity; this compound might have prevented MES and PTZ seizures.

## CONCLUSION

The ethanolic extract of leaves of *Tylophora indica* will be beneficial in the management of grandmal and petitmal epilepsies. Tylophorinidine, an isoquinoline derivative may be responsible for its anticonvulsant property. Further studies are ongoing to elucidate the exact mechanism by which this plant acts as an anticonvulsant agent.

*Funding: No funding sources*

*Conflict of interest: None declared*

*Ethical approval: The study was approved by the Institutional Ethics Committee*

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**Cite this article as:** Hafis TK, Manikkoth SK, Sequeira M, Nayak RP. Pharmacological evidence for the anticonvulsant activity of *Tylophora indica* in experimental animal models. Int J Basic Clin Pharmacol 2017;6:750-3.