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### Anticonvulsant activity of *Tabernaemontana divaricata* extract in experimental mice

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#### ABSTRACT

Epilepsy is a neuropsychological disorder which causes seizures to occur due to over discharge of neuro transmitter substances result into an imbalance between excitatory and inhibitory neurotransmitters. In the present study, antiepileptic activity of *Tabernaemontana divaricata* leaves extracts viz: aqueous and ethanolic at different doses (50, 100 and 150 mg/kg, i.p.) were studied on electrically and chemically induced seizures. Among the extracts of *Tabernaemontana divaricata*, ethanolic extract shows a significant ( $P < 0.01$ ) anti seizure properties while aqueous extract did not show any significant contribution to reduce the seizure in both PTZ and MES induced seizures. Thus the present study reveals the anticonvulsant activity of *Tabernaemontana divaricata* evaluating the traditional folklore medicinal use of the plant.

**Key words:** *Tabernaemontana divaricata*, Epilepsy, PTZ, MES.

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#### INTRODUCTION

Approximately 7 million people in India and 50 million worldwide are affected by epilepsy which is the second most common neurological disorder in India. The WHO estimated that approximately 80% people with epilepsy live in developing countries and most of them do not get adequate medical treatment [1-3].

Epilepsy is a neuropsychological disorder which causes seizures to occur due to over discharge of neuro transmitter substances result into an imbalance between excitatory and inhibitory neurotransmitters [4-6]. At neuronal level, seizure activity often occurs when glutamatergic excitatory neurotransmitters over rides gamma-aminobutyric acid (GABA) mediated inhibition [7]. A wide range of synthetic anticonvulsant drugs are available in the market for the management, control and treatment of individuals from epilepsy. However, most of the synthetic drugs are not only inaccessible and affordable, but also possess many toxic adverse effects.

Herbs have been used traditionally to treat epileptic seizures. Considering the above fact, search for the development of cheap, effective and safe anticonvulsant agents from plants and other sources were done in the past.

*Tabernaemontana divaricata* (Family: Apocynaceae, synonym *Ervatamia coronaria*) shrub or small tree, usually glabrous, distributed in tropical countries as a garden plant and found in Konkan, North Kanara, Western ghats in Malabar, throughout North India and Travencore upto 3000 ft. [8-10]. In traditional medicine *Tabernaemontana*

*divaricata* is used to treat various diseases like epilepsy, abdominal tumours, eye infections, fractures, fever, headache, inflammation, mania, oedema, leprosy, diarrhea [11]. It is also used as anthelmintic, antihypertensive, aphrodisiac, emmenagogue, purgative, remedy against poisons and tonic to the brain, liver and spleen [12, 13].

Considering the traditionally reported activity associated with the plant *Tabernaemontana divaricata* (TD), it was planned to study the effects of leaves extract (viz: ethanolic and aqueous) on CNS mainly for its anticonvulsant activity induced by Maximal electroshock (MES) and Pentylene-tetrazole (PTZ).

## MATERIALS AND METHODS

### Plant material

The leaves of *Tabernaemontana divaricata* (TD) were collected in January, 2010, from Bhopal, M.P., India. The plant was identified and authenticated by Dr. D. V. Amla, Deputy Director, National Botanical Research Institute, Lucknow, India, and a voucher specimen No. Tit/NBRI/CIF/141/2009 was deposited in Department of Pharmacognosy and Phytochemistry, TIT-Pharmacy, Bhopal.

### Preparation of extract

The leaves were dried in shade and stored at 25°C, powdered, passed through sieve no.40. The dried powdered leaves of TD (500g) were first defatted with Petroleum Ether (60- 80°C) and later extracted with ethanol and distilled water separately by maceration for 5 days. After completion of the extraction, the solvent was removed by distillation and concentrated *in vacuo* (40°C) to yield ethanolic and aqueous extract respectively.

### Preliminary phytochemical screening of TD

The preliminary phytochemical investigation was carried out with ethanolic and aqueous extracts of leaves of *T. divaricata* for qualitative identification of phytochemical constituents. Phytochemical tests were carried out by standard methods [14-15].

### Animals

Adult male Swiss albino mice weighing 20-25 g were provided by the animal house of TIT Pharmacy, Bhopal, from the stock originally purchased from, National Institute of Nutrition, Hyderabad, India. Animals were made available with the standard animal feed and water supply *ad libitum* before the experiments. The animal studies were approved by the Institutional Animal Ethics Committee (Reg. no. 831/bc/04/CPCSEA), New Delhi, India. For each experimental study mice were starved for 18h with access to water only.

### Drug and chemicals

Pentylene-tetrazole (PTZ) (Sigma-Aldrich Pvt.Ltd.), Phenytoin (Sun Pharmaceutical Industries) and diazepam (Ranbaxy Laboratories Ltd.) were used in present study.

### Acute toxicity study

Acute toxicity study was carried out for the extracts of TD considering the protocol of Henriques *et al.*[16] following Organization of economic co-operation and development (OECD) guidelines (OECD guideline, 2001)[17].

### Pentylene-tetrazole-induced seizures test

Mice were divided into eight groups each containing six animals. Group I received saline (1 ml. /kg, i.p.), group II received diazepam (3 mg/kg, i.p.), group III-V received ethanolic extract of TD (TDEE) (50, 100 and 150 mg/kg, i.p.) and group VI-VIII received aqueous extract of TD (TDAE) (50, 100 and 150 mg/kg, i.p.). Thirty minutes later seizures were induced by the pentylene-tetrazole (80 mg/kg, i.p.). The animals were observed during the first 30 min for number of animals with convulsions i.e. latency and duration of myoclonic jerks, number of deaths and percent protection against convulsion and mortality [18].

### Maximal electroshock-induced seizures test

Mice were divided into eight groups each containing six animals and treated with saline (Group I), phenytoin (Group II, 25 mg/kg, i.p.), ethanolic (Group III-V) and aqueous (Group VI-VIII) extracts of TD (50, 100 and 150 mg/kg, i.p.). Thirty minutes later seizures were induced by a current stimulus (18 mA, 50 Hz for 0.2 s) delivered by using corneal electrodes by a shock generator (Inco, India). The percent protection and duration of tonic hind limb

extension (i.e., the hind limbs of animals outstretched at 180° to the plane of the body axis) was observed. Protection was defined as complete absence of tonic hind limb extension [19].

### Statistical analysis

The results are expressed as mean  $\pm$  S.E.M. Data were analyzed using one-way analysis of variance (ANOVA) after Dunnett's tests.  $P < 0.05$  was considered statistically significant in all the cases.

## RESULTS AND DISCUSSION

### Preliminary phytochemical screening of TD:

Phytochemical screening of TD revealed the presence of alkaloids, tannins, resins, proteins, amino acids, flavonoids, saponins, phenols, glycosides, steroids, tri-terpenoids, fixed oils and fats.

### Pentylenetetrazole-induced seizures test:

Standard drug diazepam treated group showed no convulsion after PTZ treatment. As shown in **Table 1**, the seizure latency was prolonged by all the test groups as compared to vehicle treated mice. The onset of seizure was delayed in the animals treated with TD extracts at 50, 100 and 150 mg/kg dose (**Table 1**). The onset was most significantly delayed in TDEE at a dose of 100 and 150 mg/kg, i.p. ( $P < 0.01$ ) when compared with the vehicle (saline) treated group but TDAE at the tested doses did not show any significant results.

**Table 1** Effects of *T. divaricata* extract on pentylenetetrazole-induced seizures.

Group	Treatment	Dose (mg/kg)	Time to seizure onset (s)
Group I	Saline	1 ml	97.7 $\pm$ 30
Group II	Diazepam	3	795.0 $\pm$ 0.00*
Group III-V	TDEE	50	412 $\pm$ 0.81 <sup>ns</sup>
		100	599.6 $\pm$ 0.80*
		150	645.9 $\pm$ 0.34*
Group VI-VIII	TDAE	50	350 $\pm$ 0.17 <sup>ns</sup>
		100	451.3 $\pm$ 0.45 <sup>ns</sup>
		150	552.7 $\pm$ 0.30 <sup>ns</sup>

Values are expressed as the mean  $\pm$  SEM of six observations. \*  $P < 0.01$  vs. saline treatment, ns- not significant ( $P > 0.05$ ) (One-way ANOVA followed by Dunnett's test).

### Maximal electroshock-induced seizures test

A standard drug phenytoin had exhibited significant anticonvulsant effect ( $P < 0.01$ ) in onset of time to seizure and offered 100% animal protection. High doses of TDEE (150 mg/kg, i.p.) shows a significant value ( $P < 0.01$ ) of time to seizure onset but not in lower dose (50, 100 mg/kg, i.p.) whereas TDAE at the tested doses did not show any significant results (**Table 2**).

**Table 2** Effects of *T. divaricata* extract on MES induced seizures

Group	Treatment	Dose (mg/kg)	Time to seizure onset (s)
Group I	Saline	1 ml	11.83 $\pm$ 1.47
Group II	Phenytoin	25	0.00 $\pm$ 0.00*
Group III-V	TDEE	50	7.10 $\pm$ 0.14 <sup>ns</sup>
		100	5.00 $\pm$ 0.50 <sup>ns</sup>
		150	1.00 $\pm$ 0.00*
Group VI-VIII	TDAE	50	10.88 $\pm$ 0.74 <sup>ns</sup>
		100	9.01 $\pm$ 0.73 <sup>ns</sup>
		150	8.00 $\pm$ 0.10 <sup>ns</sup>

Values are expressed as the mean  $\pm$  SEM of six observations. \*  $P < 0.01$  vs. saline treatment, ns- not significant ( $P > 0.05$ ) (One-way ANOVA followed by Dunnett's test).

Herbs may have antiepileptic effects in several ways. Some herbs may increase brain levels and/or the binding of nerve transmitter gamma aminobutyric acid (GABA), which quiets nerve activity [20].

Grandmal type of epilepsy is induced in animals with MES. Antiepileptic drugs that block MES-induced tonic extension phase act by blocking seizure spread. Antiepileptic drugs either inhibit voltage-dependent Na<sup>+</sup> channels thus prevents MES-induced tonic extension phase or block the glutaminergic excitation mediated by the N-methyl-D-Aspartate (NMDA) receptor such as feblamate [21,22].

TD (ethanolic and aqueous extracts) showed anticonvulsant activity against MES-induced convulsion. Among the extracts of TD, TDEE at a dose of 150 mg/kg, i.p. significantly showed anticonvulsant activity against MES-induced convulsion. TDEE abolished tonic extension phase either by inhibiting voltage-dependent Na<sup>+</sup> channels or act as a NMDA antagonist [21, 22].

PTZ is widely used acute experimental model in the preliminary screening to test potential anticonvulsant drugs. It produces petitmal type epilepsy. PTZ exert its action by acting as an antagonist at the GABA<sub>A</sub> receptor complex. Several biochemical hypotheses have been involved in the inhibitory GABAergic system and the system of the excitatory amino acid glutamate and aspartate [23, 24].

TD increase the time taken for onset of convulsion and tonic convulsion induced by PTZ thus shows the anticonvulsant effect. Among the extracts of TD, TDEE shows a significant anti seizure properties. The significant anticonvulsant effect of TDEE (Dose 100, 150 mg/kg, i.p.) against PTZIC might be due to its action as a GABA<sub>A</sub> agonist [6, 25].

Though the exact mechanism of TD as an anticonvulsant agent is not clear but some chemical constituents of the extract may be attributed with its anticonvulsant effect to one or several active principles among those detected in the screening. Triterpenic steroids and triterpenoidal saponins are reported to possess anticonvulsant activity in some experimental seizure models such as MES and PTZ [26, 27]. Some alkaloids, monoterpenes, flavonoids also have protective effects against PTZ, picrotoxin and NMDLA-induced convulsions [28-31]. As TD consist of all the above constituents which has been evident in phytochemical screening hence the anticonvulsant effects may be attributed to presence of one or more of the above chemical constituent.

### CONCLUSION

Our findings confirmed the anticonvulsant activity of *Tabernaemontana divaricata*, specifically TDEE. From the study it may be concluded that the test drug can be replaced as an alternative agent in preventing and treating the convulsions. However, further studies are needed to evaluate the safety profile of the plant as safe and therapeutic anticonvulsant agent.

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