

Short Communication

Anticonvulsant activity of *Morinda tinctoria*-Roxb

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Accepted 16 January, 2009

Anticonvulsant activity for *Morinda tinctoria* was evaluated in albino mice of either sex at 3 different dose levels (200, 400 and 600 mg/kg ip) by MES and chemical methods. The extract showed significant ($p < 0.001$) against both MES (maximal electroshock) and chemical methods.

Key words: *Morinda tinctoria*, anticonvulsant, PTZ, MES.

INTRODUCTION

In the traditional system of medicine, leaves and roots of *Morinda tinctoria* are used as astringent, deobstrent, emmenagogue and to relieve pain in the gout (Nadkarni, 1998). The objective of the present study was to assess the anticonvulsant activity of petroleum ether extract (PEE) of *M. tinctoria* (PEEMT) against seizures induced by MES (maximal electroshock) and PTZ (Pentylene-tetrazol).

MATERIALS AND METHODS

Preparation of extract

M. tinctoria Roxb. (Rubiaceae) leaves were collected from Krishnankoil, Tamil Nadu, India in August 2002. The plants were authenticated by the taxonomist, Dr. V. Ganaesan of Anja College of Arts and Science, Sivakasi. A voucher specimen is preserved in the Ultra College of Pharmacy, Mellur, India. The dried leaves were powdered and extracted with petroleum ether and concentrated under vacuum (9.82 g). The petroleum ether extract of *M. tinctoria* (PEEMT) was then tested for anticonvulsant activity.

Animals

Albino Swiss mice weighing 22-25 g of either sex were obtained from the animal house of the Ultra College of Pharmacy, Mellur, India. Animals had free access to food and water; food was withdrawn 8 h before the experiments.

Drugs

Agents (pentylene-tetrazol from Sigma-USA and diazepam, calm-

posse Injection from Ranbaxy-India) were injected intraperitoneally (i.p.) with a dose volume of 1 ml/100g. The lyophilized extract was suspended in Tween 80 (0.2%)

Assessment of activity

Maximum electroshock (MES) induced seizure, (Fisher, 1989): The animals were divided into 5 groups of 6 numbers each and were administered as follows:

- Group I received vehicle
- Group II received diazepam
- Group III received 200 mg/kg of PEEMT suspended in Tween 80
- Group IV received 400 mg/kg of PEEMT suspended in Tween 80
- Group V received 600 mg/kg of PEEMT suspended in Tween 80

All these were administered by ip 30 min before application of electroshock (42 MA, 0.2 sec) using corneal electrode. The duration tonic hind leg extension was noted.

Pentylene-tetrazol (PTZ) induced seizures: The PEE was administered ip in increased doses (200, 400 and 600 mg/kg) 30 min before the subcutaneous injection of PTZ (80 mg/kg) and the mice were observed for the onset of myoclonic spasm and clonic convulsions.

One group received vehicle while the other group received diazepam (4 mg/kg) as reference standard. The animals were observed for the onset of convulsion up to 30 min after PTZ (Swinyard et al., 1952).

Statistical analysis

The data are presented as mean \pm SEM for PTZ and MES. The data of PTZ test & MES were analyzed by one way analysis of variance (ANOVA) followed by Dunnett's test. Differences were considered to be statistically significant when $p < 0.05$.

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Table 1. Effect of PEE of *Morinda tinctoria* (PEEMT) on MES induced seizure in mice.

Treatment	Time duration (sec)			
	Tonic flexion (mean \pm SEM)	Tonic extension (mean \pm SEM)	Clonic stupor (mean \pm SEM)	Mortality
Control	45 \pm 1.80	70 \pm 4.90	200 \pm 4.9	100
Diazepam 4mg/kg	53.6 \pm 6.1*	95 \pm 17.9*	378 \pm 43.73*	00
PEEMT 200 mg/kg	40.3 \pm 5.7*	60.0 \pm 4.86**	210 \pm 51.5*	20.2
PEEMT 400 mg/kg	60.2 \pm 4.7*	200 \pm 54.36**	360.6 \pm 54.5*	21.00
PEEMT 600 mg/kg	65.3 \pm 5.7	220 \pm 55.38**	377.6 \pm 51.3*	22.2

*P < 0.05, **P < 0.001 compared with control, n = 6.

Table 2. Effect of PEE *M. tinctoria* (PEEMT) on pentylenetetrazol induced seizure in mice.

Treatment	Dose mg/kg	Onset of clonic convulsions in sec (Mean \pm SEM)	Incidence of convulsions (%)
Control	Vehicle	146.2 \pm 12.9	100
Diazepam	4	A	0
PEEMT	200	667.6 \pm 95.0*	67.1
PEEMT	400	780.0 \pm 12.4*	16.6
PEEMT	600	A	0

n = 6 in each group, *P < 0.001 when compared to the vehicle treated group. A = Absence of convulsions.

RESULTS

In MES, the duration of hind limb extension for control mice was 200 \pm 15.7 s where 100% mortality was observed. Diazepam (4 mg/kg) decreased the duration of hind limb extension phase where it protected 100% mortality of animals. The PEEMT exhibited significant delay on the onset of convulsion on a dose depended manner (Table 1).

In animals treated with vehicle, clonic convulsions appeared 146.2 \pm 12.9 s after PTZ administration and all animals died after seizures. The petroleum ether extract of *M. tinctoria* (PEEMT) significantly and on a dose depended manner inhibited the onset and incidence of convulsions. The convulsions were completely abolished by PEEMT at a dose of 600 mg/kg and also by diazepam (4 mg/kg) (Table 2).

DISCUSSION

The observation emanated in the present study indicated that PEEMT possesses anticonvulsant activity against seizures induced by MES or PTZ, in a dose dependent manner. It was effective against MES induced seizures, since inhibition of the MES test predicts activity against generalized tonic-clonic and cortical focal seizures. PEEMT was active against MES induced seizures.

PTZ is a most frequently used substance as well as an acute experimental model in the preliminary screening to

test potential anticonvulsant drugs. The mechanism by which PTZ is believed to exert its action is by acting as an antagonist at the GABAA receptor complex. Several biochemical hypotheses have been advanced involving the inhibitory GABAergic system and the system of the excitatory amino acid glutamate and aspartate (Mc Donald et al., 1993). The mechanism by which PTZ is believed to exert its action is by acting as an antagonist at the GABAA receptor complex (Ramanjaneyulu et al., 1984). Drugs protecting against tonic-clonic seizures induced by PTZ are considered to be useful to control myoclonic and absence seizures in humans (Loscher et al., 1988). In this study's experiments, the extract of *M. tinctoria* replicated the effect of this anti epileptic drug by delaying tonic convulsion and mortality. The benzodiazepine site in the GABAA receptor and T-type Ca²⁺ currents could be targets for future studies to know the mechanisms of action of *M. tinctoria* extracts. It suggests that PEEMT is useful in suppressing generalized tonic-clonic seizures. Anticonvulsant activity of PEEMT in inhibiting seizures may be by regulating GABA – mediated synaptic inhibition through action at distinct sites of the synapse. It is hasty to attribute the effect of PEEMT to the GABAergic system. It is suggested to make picrotoxin or bicucullin tests. Summarizing the data obtained in this study, the results suggest a possible anticonvulsant effect of PEEMT in rodents. The precise mechanisms of possible anticonvulsant effect of PEEMT are not clear. Further research is in progress to isolate the compound respon-

sible for this activity.

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