Anticonvulsant Activity of *Mitragyna Parvifolia* Leaves Extract

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Summary

The anticonvulsant effect of ethanolic extract from the leaves of *Mitragyna parvifolia* was investigated by studying the effects of seizures induced by pentylenetetrazole (PTZ) and maximal electroshock convulsive methods in mice. The extract was administered orally in mice at three doses (100, 250 and 500 mg/kg). The extract suppressed tonic hind limb extensions (THLE) induced by MES at the doses of 250 and 500 mg/kg ($p<0.05$) and also exhibited protector effect in PTZ-induced seizures only at 500 mg/kg ($p<0.05$). The activity reported was dose dependent in both the models.

Keywords: *Mitragyna parvifolia*, Anticonvulsant effects, Maximal electroshock, Pentylenetetrazole, Mice

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Introduction

Epilepsy is a neurological disorder that affects a wide range of people throughout the world. It is a disorder of brain characterize by unpredictable and periodic occurrence of a transient alteration of behavior due to the disordered, synchronous and rhythmic firing of populations of brain neurons [1]. The current therapeutic treatment of epilepsy with modern antiepileptic drugs [AEDs] is associated with side-effects, dose related and chronic toxicity. Approximately 30% of the patients continue to have seizures with current AED therapy [2]. Natural products from folk remedies have contributed significantly in the discovery of modern drugs and can be an alternative source for the discovery of AEDs with novel structures and better safety and efficacy profiles [3].

Mitragyna parvifolia (Roxb.) Korth belongs to family Rubiaceae is commonly known as Kaim [4]. The plant grows throughout India, in deciduous and evergreen forests. The chemical constituents of the plant are pyroligneous acid, methyl acetate, ketones and aldehydes. It is reported to possess analgesic, anti-inflammatory and antibacterial activities [5] and is widely used by tribal people and other ayurvedic practitioners. The bark and roots are used to treat fever, colic, muscular pain, burning sensation, poisoning, gynecological disorders, cough, edema and as aphrodisiac. The fruit juice augments the quantities of breast milk in lactating mothers and also work as lactodepurant. Wounds and ulcers are dressed with its leaves to alleviate pain, swelling and for better healing [4-8]. Though the plant has great potential for anticonvulsant activity, nobody has not been yet documented this activity neither on this plant nor on any of its parts. So, in this study we have attempted to investigate the anticonvulsant activity of the plant.

Materials and Methods

Plant Material

The leaves of *M. parvifolia* (MP) Roxb. (Rubiaceae) were collected from local areas during the November month of 2008. The plant got identified and authenticated by Department of Botany, Kurukshetra University, Kurukshetra, Haryana, (India) and a voucher specimen of the sample (Sr. No. KUK/IPS/2008/MP-105) has been deposited in the Herbarium collection of Department. The leaves were cleaned and dried in the shade, then powdered to 40 mesh and stored in an airtight container.

Preparation of Extract

The extract was prepared by cold maceration process. The dried leaves powder (750 g) divided in three parts and was treated each three times with fresh ethanol (1000 ml) separately for 48 h. The ethanolic extracts thus obtained were combined, filtered and distilled on a water bath. The last traces of the solvent were evaporated under reduced pressure using rotatory evaporator (Heidolph Laborota 4011 digital). The ethanolic extract (yield = 2.06 % w/w) was used for pharmacological studies by suspending a weighed amount of the extract in normal saline (95 ml): tween 80 (5 ml) ratio.

Test Animals

Swiss albino mice weighing 25-30 gm were obtained from Haryana Agriculture University, Hisar, Haryana, (India). The animals were housed in Animal house, Institute of Pharmaceutical Sciences, Kurukshetra University, Kurukshetra (Haryana) in polycarbonate cages, in a room maintained under controlled room temperature 22 ± 20 C, relative humidity 60 -70% and provided with food and water *ad libitum*. All the experimental procedures and protocols used in the study were reviewed by the Institutional Animal Ethics Committee (Register Number: 562/02/a/CPCSEA) and were in accordance with the guidelines of the CPCSEA, Ministry of Forests and Environment, Government of India. The animals were deprived of food for 24 h before experimentation but allowed free access to water throughout. The animals were divided into five groups of ten each.

Group I received normal saline : tween 80 (p.o),
Group II received Phenytoin (20 mg/kg i.p.)/ Diazepam (1.0 mg/kg, i.p.),
Group III received MP extract (100 mg/kg p.o.),
Group IV received MP extract (250 mg/kg p.o.) and
Group V received MP extract (500 mg/kg p.o.).

Drugs

All the standard drugs (PTZ, Diazepam and Phenytoin) were obtained from various chemical units – Sigma chemical Co. and S. D. Fine Chem. Ltd. (India).

Acute toxicity test

Acute toxicity tests were performed according to OECD – 423 guidelines (acute toxic class method) [9]. Swiss mice (n = 6) of either sex selected by random sampling technique were employed in this study. The animals were fasted for 4 h with free access to water only. The ethanolic extract of *M. parvifolia* suspended in normal saline:tween 80 (95:5) was administered orally at a dose of 5 mg/kg initially and mortality was observed for 3 days. If mortality was observed in 5/6 or 6/6 animals, then the dose administered was considered as toxic dose. However, if the mortality was observed in less than four mice, out of six animals then the same dose was repeated again to confirm the toxic effect. If mortality was not observed, the procedure was then repeated with higher doses such as 100, 300 and 1500 mg/kg.

Convulsive Tests

MES-induced seizures [10]

Electroconvulsive shock (45 mA, 50Hz for 0.2 sec) was delivered through corneal electrodes to induce tonic hind limb extensions (THLE) in mice. The extract was administered orally at the doses of 100, 250 and 500mg/kg into test groups. Normal saline: tween 80 (95:5) and Phenytoin (20 mg/kg) were administered into two groups of animals as control and positive control groups, respectively. Electroconvulsive shock was delivered 30 min after the administration of drugs. Seizures were manifested as tonic hind limb extension (THLE). The ability to prevent this feature or prolong the latency and/or onset of THLE was considered as an indication of anticonvulsant activity. Percentage of inhibition of seizures relative to controls was calculated.

PTZ-induced seizures [11]
PTZ at the dose of 80 mg/kg (minimal dose needed to induce convulsions) was injected i.p. to induce clonic-tonic convulsions in mice. Doses of 100, 250 and 500 mg/kg of the extract were administered orally into test groups. Normal saline: tween 80 (95:5) and Diazepam (1 mg/kg) were administered orally into two groups of animals as control and positive control groups, respectively. PTZ was injected i.p. 30 min after the administration of drugs. Mice were observed over a period of 30 minutes, absence of an episode of clonic convulsion of at least 5 seconds duration indicated the extract or a compound's ability to abolish the effect of PTZ on seizure threshold. Percentage of inhibition of seizures relative to controls was calculated.

Statistical analysis
All data were represented as mean ± S.E.M. and as percentage. Results were statistically evaluated using student's *t*- test. $P<0.05$ was considered significant.

Results

Acute toxicity test
M. parvifolia leaves extract did not produce any mortality even at the dose of 1500 mg/kg, p.o. All the doses (5, 50 and 300 mg/kg, p.o.) of *M. parvifolia* were thus found to be non-toxic. On the basis of above results, three doses (100, 250, 500 mg/kg, p.o.) of *M. parvifolia* were selected for further pharmacological studies.

MES-induced seizures
Swiss albino mice pretreated with the MP extract have been significantly protected from convulsions induced by electroshock thirty minutes post-dosing. The percentage inhibition achieved at the doses 100, 250 and 500mg/kg were 60%, 80% and 90% respectively. Extract at all the doses, dose dependently prolonged the onset of convulsions in the treated group compared to vehicle treated control group (Table 1).

Table. 1. Effect of leaves extract of *Mitragyna parvifolia* on tonic seizures induced by maximal electroshock in mice

Treatment group	Dose mg/kg (p.o.)	Mean Onset time (Min)	Percentage protection
Control (Group I)	--	4.36±0.31	0.0
Phenytoin (Group II)	20 (i.p.)	0.0	100.0
MP Extract (Group III)	100	3.74±0.54	60.0
MP Extract (Group IV)	250	6.84±.01*	80.0
MP Extract (Group V)	500	7.22±0.02*	90.0

All values are expressed as mean of ten mice in each group. Statistically significant
* $p<0.05$ compared to control.

PTZ-induced seizures

Animals treated with MP extract at a dose of 500 mg/kg showed alteration in the onset of convulsion and duration of seizures significantly as related to controls in the model of convulsion induced by pentylenetetrazole in mice but did not alter significantly at 100 and 250 mg/kg. Percentage of inhibition of seizures for 500 mg/kg relative to controls was 60.0% (Table 2).

Table. 2. Effect of leaves extract of *Mitragyna parvifolia* on pentylenetetrazole induced seizures in mice

Treatment group	Dose mg/kg (p.o.)	Onset time (Sec)	Percentage protection
Control (Group I)	--	8.61±1.21	0.0
Diazepam (Group II)	1 (i.p.)	0.0	100.0
Extract (Group III)	100	9.67±1.76	20.0
Extract (Group IV)	250	11.0±2.08	40.0
Extract (Group V)	500	14.0±0.84*	60.0

All values are expressed as mean of ten mice in each group. Statistically significant * $p<0.05$ compared to control.

Discussion

The most popular and widely used animal seizure models are the traditional maximum electroshock-induced seizure and pentylenetetrazole tests. Prevention of seizures induced by pentelenetetrazole in laboratory animals is the most commonly used preliminary screening test for characterizing potential anticonvulsant drugs. The maximum electroshock-induced seizure test is considered to be a predictor of likely therapeutic efficacy against generalized tonic-clonic seizures. By contrast, the pentylenetetrazole test represents a valid model for human generalized myoclonic and also absence seizures [12].

Data from this study show that *Mitragyna parvifolia* significantly increases the onset time and percentage protection by electroconvulsive shock. The study also revealed that the onset of tonic convulsion produced by PTZ was significantly delayed and increased percentage protection.

The majority of currently available antiepileptic drugs fall in to two pharmacological classes, those that modulate neuronal voltage gated sodium channels (e.g. carbamezepine, phenytoin and topiramate) and those that modulate inhibitory GABAergic neurotransmission (e.g. benzodiazepines, vigabatrin etc.). While some AEDs act through voltage operated calcium channels [13].

The ability of the extract to exhibit activity against MES and PTZ induced convulsions showed that the extract may act through different mechanisms such as voltage gated sodium, calcium and potassium or GABAergic pathway [14, 15].

So, the plant needs further investigation of chemical constituents responsible for the above activity.

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