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Anticonvulsive activity of *Butea monosperma* flowers in laboratory animals

Veena S. Kasture[a], S.B. Kasture[a,*], C.T. Chopde[b]

[a]*Natural Products Laboratory, NDMVP Samaj's College of Pharmacy, Nāshik 422 002, India*
[b]*Department of Pharmaceutical Sciences, Nagpur University, Nagpur 440 010, India*

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Abstract

The bioassay-guided fractionation of dried flowers of *Butea monosperma* (BM) was carried out to isolate the active principle responsible for its anticonvulsant activity. The petroleum ether extract was fractionated by column chromatography using solvents of varying polarity such as *n*-hexane, *n*-hexane:ethyl acetate, ethyl acetate, and methanol. The anticonvulsive principle of *B. monosperma* was found to be a triterpene (TBM) present in the *n*-hexane:ethyl acetate (1:1) fraction of the petroleum ether extract. TBM exhibited anticonvulsant activity against seizures induced by maximum electroshock (MES) and its PD_{50} was found to be 34.2 ± 18.1 mg/kg. TBM also inhibited seizures induced by pentylenetetrazol (PTZ), electrical kindling, and the combination of lithium sulfate and pilocarpine nitrate (Li-Pilo). However, TBM was not effective against seizures induced by strychnine and picrotoxin. TBM exhibited depressant effect on the central nervous system. After repeated use for 7 days, the PD_{50} (MES) of TBM increased to 51.5 ± 12.1 mg/kg. Similarly, after repeated use of TBM, the duration of sleep induced by pentobarbital was not reduced significantly. Further studies are required to investigate its usefulness in the treatment of epilepsy. © 2002 Elsevier Science Inc. All rights reserved.

Keywords: Anticonvulsant; Maximum electroshock; Pentylenetetrazol; Lithium–pilocarpine; Electrical kindling; *Butea monosperma*

1. Introduction

Antiepileptic drug (AED) therapy remains far from optimal. In many patients, the presently available AEDs such as phenobarbital, phenytoin, benzodiazepines, sodium valproate, carbamazepine, ethosuximide, trimethadione, etc., are unable to control seizures efficiently. Furthermore, the dose-related neurotoxicity and other side effects associated with established AEDs limit their clinical use. The newer AEDs like oxcarbazepine, vigabatrin, lamotrigine, gabapentin, felbamate, etc., represent a real progress in the treatment of nonresponders or refractory patients. However, the problem of adverse effects has also not been circumvented completely (McNamara, 1996). Hence, search should continue to develop newer, more effective, and safer neuroprotective agents for treatment of epilepsy.

In the Ayurvedic system of medicine, flowers of *Butea monosperma* (Lam) Kuntz (also known as Bastard Teak; family: Fabaceae) are used as tonic, astringent, aphrodisiac, and diuretic (Nadkarni, 1998). Roots are useful in the treatment of filariasis, night blindness, helminthiasis, piles, ulcers, and tumors. The alcoholic concentrate of petals has antiestrogenic activity and decoction of flowers is useful in treatment of diarrhoea (Kirtikar and Basu, 1989; Shah et al., 1990). Rane and Grampurohit (1998) have reported the hepato-protective activity of *B. monosperma*. We have previously reported the anticonvulsive activity of the acetone-soluble part of the petroleum ether extract of *B. monosperma* in experimental animals (Kasture et al., 2000). The acetone-soluble part of petroleum ether and ethanolic extract has also exhibited nootropic activity in laboratory animals (Gawale et al., 2001). The objective of the present study was to identify the active ingredient responsible for the anticonvulsant activity of shade-dried flowers of *B. monosperma* by bioassay-guided separation and to study its spectrum of anticonvulsive activity against different chemoconvulsants like pentylenetetrazol (PTZ), picrotoxin, strychnine, and lithium sulfate–pilocarpine.

* Corresponding author. Tel.: +91-253-346-266.
E-mail address: kasture_sb@hotmail.com (S.B. Kasture).

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2. Materials and methods

2.1. Plant material

B. monosperma is commonly grown in India. The flowers of *B. monosperma* (Voucher no. 165413) were collected in April and May and were deposited at the Botanical Survey of India, Pune.

2.2. Extraction

Shade-dried flowers of *B. monosperma* (1.0 kg) were extracted with petroleum ether (pet ether, 60–80 °C; yield: 1.25 g). The extract exhibiting anticonvulsant activity against maximum electroshock (MES) and PTZ-induced seizures was fractionated into acetone-soluble and -insoluble parts. Only the acetone-soluble part (ABM, yield 1.0 g) exhibited anticonvulsant activity against seizures induced by MES and PTZ (Kasture et al., 2000). Using silica gel as a stationary phase in column chromatography, ABM was successively fractionated using *n*-hexane, *n*-hexane:ethyl acetate (1:1), ethyl acetate, and methanol as mobile phases. These fractions were subjected to the anticonvulsant screen using MES- and PTZ-induced seizures. The *n*-hexane:ethyl acetate (1:1) fraction (yield, 0.85 g) possessed anticonvulsant activity. This fraction upon chemical analysis indicated the presence of a triterpene (Harborne, 1965). The fraction was spotted on the thin layer of silica gel and the plate was developed in the solvent system comprising of *n*-hexane:ethyl acetate (7:3). The major component in the *n*-hexane:ethyl acetate (1:1) fraction was isolated by eluting the silica gel column by mixture of *n*-hexane and ethyl acetate (1:1). The yield of triterpene (TBM) was 750 mg. The pH of TBM dissolved in polyethylene glycol 400 (PEG) was found to be 7.1. TBM was administered intraperitoneally. For further experiments, TBM was obtained in more quantity using the same procedure.

2.3. Characterization of TBM

The active compound TBM was purified using column chromatography by utilizing the same eluting solvents, i.e., *n*-hexane:ethyl acetate (1: 1). TBM was a yellow amorphous powder and its melting point was 132–134 °C. The Lieberman–Burchard test (Harborne, 1965) was positive for triterpene. The mass spectrum of TBM showed molecular peak at 392. The UV spectrum yielded two absorption peaks at 255 and 374 nm. The FTIR spectrum showed peaks at 3020.3, 2929.7, 2858.3, 2349.1, 2148.6, 1716.5, 1637.5, 1406.0, 1217.0, 1168.8, 1045.3, 927.7, 763.8, 669.3, 624.9, 553.5, and 418.5 cm^{-1}. In the mass spectrum, molecular ion peaks were obtained at 392 (11.8%), 259 (4%), 167 (4.1%), 157 (5.2%), 148 (4.5%), 139 (5.4%), and 133 (8.3%).

The proton magnetic resonance of TBM generated the following data: H δ 0.856 (s), H δ 1.286 (s), H δ 2.059 (t), H δ 2.293 (q), H δ 2.563 (s), H δ 3.58 (h), H δ 5.5 (dd), H δ 7.167 (dd), H δ 7.748 (dd), H δ 8.015 (dd), H δ 8.118 (s), H δ 8,371 (s).

From elemental analysis, the proportion of carbon, oxygen, and hydrogen was 58.2%, 32.7%, and 9.1%, respectively. Thus, from the molecular weight (392) and elemental analysis of TBM, the empirical formula was found to be $C_{19}H_{36}O_{8}$.

2.4. Animals

Adult male Sprague–Dawley rats weighing 200–220 g and male mice (National Institute of Nutrition; Hyderabad, India) weighing 22–25 g were housed in groups of five under standard laboratory conditions of temperature (23 ± 1 °C), lighting (0800–2000 h), and relative humidity (55 ± 5%), with food (pellets by Lipton, Mumbai, India) and water freely available. They were fasted overnight before the experiments and were transferred to the laboratory at least 1 h before the start of the experiment. The experiments were performed during the light portion (0800–1600 h).

2.5. Drugs

Phenytoin and diazepam injections (Ranbaxy, New Delhi, India), pilocarpine (FDC, Mumbai, India), and lithium sulphate (Glenmark Laboratories, Nāshik, India) were gifts. Picrotoxin, PTZ, and strychnine were purchased from Modern Scientific (Nāshik, India).

2.6. Acute toxicity and effect on gross behaviour

Varying doses of the TBM (50, 100, 200, 400, and 1000 mg/kg ip) were administered to groups of mice, each containing 10 animals. The mortality, if any, was observed after 24 h and the LD_{50} was determined by the graphical method of Miller and Tainter (1944). Since one animal died at the dose of 200 mg/kg, to assess effects on gross behaviour, TBM was administered in a dose of 100 mg/kg and after 60 min, the animals were observed for the change in gross behaviour by an unbiased blind observer (Irwin et al., 1968). The effect on gross behaviour was observed between 1300 and 1500 h. The procedure involved an initial phase of undisturbed observations and a later manipulative phase during which the animals were subjected to the least provoking stimuli. The assessment of animal's behavior always began by observing its undisturbed behavior within a transparent cage (i.e., body position, locomotion, exophthalmos, respiration, tremors, and twitches). The touch response was tested by lightly stroking the sides and body of the animal. The animals were then observed for effect on gait, grip strength, passivity, presence or absence of righting reflex, pain response, lacrimation, and salivation.

2.7. Motor toxicity

2.7.1. Inverted screen test

The inverted screen test was used to assess the motor toxicity of TBM. This test was an adaptation by Ginski and Witkin (1994) of that initially described by Coughenour et al. (1977). In this test, compounds with sedative and/or ataxic properties produce a dose-dependent increase in screen test failures, whereas other classes of drugs (e.g., psychomotor stimulants) do not (Ginski and Witkin, 1994). Mice (eight animals in each group) were treated with either vehicle or varying doses of TBM (25–200 mg/kg) and returned to their home cages. After 30 min, they were placed on a 14 × 14-cm wire mesh screen elevated 30 cm above the ground. After slowly inverting the screen through an angle of 180°, the mice were tested during a 2-min trial for their ability to climb to the top. Mice not climbing to the top (all four paws on upper surface) were counted as failures. The median toxic dose (TD_{50}) of the TBM was calculated from a dose–response curve as the dose in milligrams per kilogram that produced screen failure in 50% of the mice tested.

2.7.2. Rotarod test

The method of Kupferberg (1989) was used. In this test, mice were first trained to remain for 2 min on the rotarod that is 2.5 cm in diameter and revolving at 20 rpm. Mice maintaining equilibrium on the rotating rod for more than 2 min on three successive trials were randomly divided in different groups, each containing 10 animals. Animals were administered vehicle, diazepam (1.0 mg/kg), or TBM (10–100 mg/kg), and the latency to fall from the rotarod was noted 30 min after the treatments.

2.8. Assessment of anticonvulsant activity

2.8.1. MES test

The TBM was administered in varying doses (10–100 mg/kg ip) to groups of mice, each containing eight, and after 30 min, a current stimulus (45 mA for 0.2 s) was delivered using corneal electrodes. The incidence and duration of tonic hindleg extension were noted (Swinyard et al., 1952). The dose required to protect 50% of animals from tonic seizures (PD_{50}) was calculated as described by Miller and Tainter (1944). Protection was defined as complete absence of tonic hindleg extension. Phenytoin (10–100 mg/kg ip) was used as a reference standard. In further experiments, animals were treated with 2 × PD_{50} dose of TBM.

2.8.2. Electrical kindling seizures in rats

Five rats were used in each group. The animals received two subconvulsive electric shocks per day (21 mA for 0.1 s, 3 h apart, using corneal electrodes) until all animals exhibited (Grades 4–5 seizure score) full-blown clonic convulsions as described by McNamara et al. (1993). All rats were kindled an equal number of times (11 electric shocks) although some demonstrated the Grade 4 convulsions after fewer stimulations. The severity of seizure was scored as: 0 = *normal*; 1 = *facial movements*; 2 = *facial movements with head nodding*; 3 = *symptoms as in Step (2) plus raising of forelimbs with mild forelimb clonus*; 4 = *marked rearing to a vertical position and moving the head from side to side and forelimb clonus*; and 5 = *symptoms as in Step (4) progressing to falling followed by forelimb and hind limb clonic seizures*.

In the test for drug efficacy in established kindled seizures, the rats required 11 stimuli. On the next day, TBM (2 × $PD_{50\ MES}$) or vehicle was administered intraperitoneally and the kindling stimulus was given 30 min later. Seizure scores of test drug-treated rats were compared with vehicle and diazepam (2 mg/kg ip)-treated animals.

To observe whether a compound would retard the development of the kindled seizure process, in another series of experiments, the test compound TBM (2 × PD_{50}) or vehicle was administered once daily, intraperitoneally, 30 min before the first stimulation (of the same intensity as used in earlier experiment) and the second electrical stimulus was given 3 h after the first. TBM was administered once daily for 5 days followed by a washout period (2 days) and on Day 8, electric stimulus was given. The severity of seizures was observed as described above.

2.8.3. Chemically induced seizures

In these studies, doses of chemoconvulsants were chosen to be close to their ED_{95} values as determined during pilot experiments. PTZ (80 mg/kg), picrotoxin (3 mg/kg), or strychnine (1.2 mg/kg) was injected subcutaneously to groups of mice 30 min after dosing with TBM (10–150 mg/kg ip).

2.8.3.1. PTZ-induced seizures. The TBM was administered in varying doses (10–100 mg/kg ip) to albino mice ($n = 6$) 30 min before subcutaneous injection of PTZ. The onset of clonic convulsions and percentage protection in each group were noted (Swinyard et al., 1952). Diazepam (0.5–10 mg/kg) was used as a reference standard. The PD_{50} was determined as described by Miller and Tainter (1944).

2.8.3.2. Picrotoxin- or strychnine-induced convulsions (Swinyard et al., 1952). The TBM was administered in a dose of 35 or 150 mg/kg ip to groups of mice, each containing six, 30 min before administration of picrotoxin (3 mg/kg sc) or strychnine (1.2 mg/kg sc). The presence or absence of clonic convulsions was noted for 30 min following administration of picrotoxin. In case of strychnine, the latency to convulsion, latency to death, and percentage protection were recorded.

2.8.4. Lithium–pilocarpine-induced status epilepticus in rats

The animals, divided into groups of five each, received lithium sulphate in a dose of 3 mEq/kg ip, 24 h before pilocarpine (30 mg/kg ip). The animals were treated with the vehicle or TBM ($2 \times PD_{50\ MES}$), or diazepam (2 mg/kg ip) 30 min before pilocarpine, and the progression of limbic seizures was rated once every 15 min for 75 min as described by Patel et al. (1988). The severity of status epilepticus was scored as follows: 0 = *no response*; 1 = *gustatory movements and/or fictive scratching*; 2 = *tremor*; 3 = *head bobbing*; 4 = *forelimb clonus*; 5 = *rearing, clonus, and falling*. In addition, the latency to attain forelimb clonus (a score of 4) was also recorded for each rat.

2.9. Tolerance studies

To study whether tolerance develops to anticonvulsant activity, the TBM was administered daily, intraperitoneally, in double the PD_{50} (MES) for 7 days and subjected to the MES test in mice. Groups of mice (10 animals per group) were treated daily (intraperitoneally) for 7 days with either vehicle or $2 \times PD_{50\ MES}$ of TBM. On the eighth day (i.e., 24 h after the last dose), the same dose of TBM was administered and MES test was carried out 30 min later. Comparison of resultant PD_{50} was made with the PD_{50} of TBM (acute administration) to evaluate development of tolerance.

2.10. Spontaneous motor activity

Spontaneous motor activity was measured using an actophotometer (Centronics, Mumbai, India), equipped with six infrared light sources and photocells. Briefly, mice were randomly divided into groups ($n = 6$) and groups were placed in the actophotometer for a duration of 30 min for acclimatization. After the acclimatization period, a baseline locomotor activity was recorded for 5 min. These animals were then treated with TBM ($2 \times PD_{50\ MES}$) or diazepam (0.5 mg/kg) or vehicle and the activity counts were recorded again for each group at 30, 60, and 90 min of these treatments. The locomotor activity was compared with the baseline count.

2.11. Open field test

The exploratory behavior was tested using an open field apparatus made of plywood ($24 \times 24 \times 15$ in.), the floor being divided in 16 squares of equal size as described earlier (Sakina and Dandiya, 1990). The animal was placed gently in one corner of the apparatus and was observed for transfer latency—the time taken to move out of the square—the number of squares traversed, and the number of rearing in a test interval of 5 min. The observations were made 30 min after the intraperitoneal injection of vehicle or TBM ($2 \times PD_{50\ MES}$). Diazepam (0.5 mg/kg ip) was used as a reference drug. Each group consisted of six animals.

2.12. Effect on pentobarbital sleeping time on single and repeated use

Male mice ($n = 10$) were treated intraperitoneally either once or repeatedly for 7 days with the vehicle or TBM ($2 \times PD_{50\ MES}$). Pentobarbital (40 mg/kg ip) was administered 30 min after vehicle or TBM, and duration of sleep was measured. The duration of sleeping time was measured

Table 1
Effect of TBM on CNS activity screen in mice

Behavior	Dose of 100 mg/kg ip
(A) Awareness	
Alertness	@
Passivity	p
Stereotypy	–
(B) Mood	
Grooming	@
Vocalization	–
Restlessness	–
Irritability	–
(C) Motor activity	
Spontaneous motor activity	↓
Reactivity	↓
Touch response	@
Pain response	@
Central excitation	
Startle response	–
Straub response	–
Tremor	–
Convulsions	–
(D) Posture and tone	
Body position	↓
Limb position	↓
Gait	s
Limb tone	↓
Grip strength	↓
Body tone	↓
Abdominal tone	↓
(E) Reflexes	
Corneal	–
Pinna	–
Righting	@
(F) Secretory signs	
Salivation	–
Urination	–
Lacrimation	–
(G) General signs	
Respiration	–
Body temperature	–
Piloerection	–
Defecation	–

(@) Normal (no change); (–) none; (↓) decrease; (s) staggering; (p) present. $n = 10$ mice. Observations were made at 60 min after intraperitoneal injection of TBM. Behavioral effects were not observed in the animals treated with PEG 400 alone.

as the period that mice lost the righting reflex after pentobarbital (Turner, 1972).

2.13. Statistical analysis

The data are presented as mean ± S.E.M. One-way ANOVA was followed by Dunnett's test. For nonparametric data, Kruskal–Wallis ANOVA was followed by Dunn's test for multiple comparisons. Fisher's exact test was used to determine the presence or absence of activity.

3. Results

3.1. Acute toxicity and effect on gross behavior

The median lethal dose of TBM was found to be 500 ± 32 mg/kg. After 60 min of administration, TBM (100 mg/kg ip) reduced spontaneous activity and reactivity, limb tone, body tone, abdominal tone, and grip strength. Body positions and limb positions were also affected. Gait was staggering and the animal was unresponsive when placed in an unaccustomed position indicating passivity. The observations are given in Table 1.

3.2. Motor toxicity

3.2.1. Inverted screen test

Intraperitoneal administration of TBM dose-dependently (Table 2) increased the percentage of mice failing to climb the screen within 2 min. The median toxic dose of TBM was found to be 83.1 ± 11.16 mg/kg, whereas that of diazepam was 3.13 ± 1.1 mg/kg.

3.2.2. Rotarod test

The animals treated with TBM in doses of 10 and 20 mg/kg remained on the rotating rod. However, at higher doses, the drug produced significant neurological deficit and at 100 mg/kg dose, 50% animals could not remain on the rotating rod (Table 3).

Table 2
Effect of varying doses of TBM on falling of mice from inverted screen in mice

Treatment	Dose [mg/kg ip]	Number of mice climbing the screen	Percentage of mice falling from the screen	TD_{50}
TBM	10	8/8	0	83.1 ± 11.1
	25	8/8	0	
	50	7/8	12.5	
	75	5/8	37.5	
	100	3/8#	62.5	
Diazepam	0.5	8/8	0	3.13 ± 1.1
	1.0	6/8	25	
	2.0	5/8	37.5	
	4.0	3/8#	62.5	
	5.0	1/8#	87.5	
	10	0/8	100	

$n = 8$ in each group.
# $P < .05$ (Fisher's exact test).

Table 3
Effect of TBM on the rotarod performance in mice

Treatment	Dose [mg/kg ip]	Duration of stay on rotating rod [s]	Rotarod deficit in percent of animals
Vehicle	–	120.0	0
TBM	10	120.0	0
	20	120.0	0
	40	116.0 ± 8.9	20
	100	96.0 ± 16.8 *	50
Diazepam	1.0	108.0 ± 8.4 *	30

$n = 10$ in each group.
* $P < .05$ (one-way ANOVA followed by Dunnett's test).

3.3. Assessment of anticonvulsant activity

3.3.1. MES test

Vehicle-treated mice subjected to the MES test developed a typical seizure pattern in all the animals. The tonic flexion of the limbs occurred immediately after the shock, which then progressed into tonic extension of hind limbs followed by stupor and recovery. TBM inhibited MES-induced seizures dose-dependently. The PD_{50} of TBM in the MES test was 34.2 ± 18.1 mg/kg, whereas the PD_{50} of phenytoin was 45.2 ± 4.2 mg/kg. The observations are given in Table 4.

3.3.2. Electrical kindling seizures in rats

In fully kindled rats, the administration of TBM ($2 \times PD_{50\ MES}$) significantly reduced the severity of electrically kindled seizures. The compound was effective in reducing severity of established seizures from 3.8 ± 0.2 (in vehicle-treated group) to 1.9 ± 0.2 ($P < .05$). Diazepam inhibited the seizures completely. TBM on repeated use reduced the

Table 4
The effect of TBM on MES-induced seizures in mice

Treatment [dose: mg/kg]	Duration of tonic hindleg extension in seconds [mean ± S.E.M.]	Percentage of animals protected	PD_{50}
Vehicle	14.3 ± 1.9	0	
TBM			
10	10.2 ± 1.9	12.5	34.2 ± 18.1
25	8.3 ± 2.4*	37.5#	
50	5.9 ± 1.8**	62.5#	
75	4.2 ± 0.5**	75.0#	
100	3.2 ± 0.2**	75.0#	
Pheny			
10	14.1 ± 1.8	0	45.2 ± 4.2
25	11.2 ± 0.9	25.0	
50	7.5 ± 1.4*	50#	
75	2.9 ± 1.1**	75.0#	
100	nil	100#	

$n = 8$ in each group. Pheny, phenytoin.
* $P < .05$ (one-way ANOVA followed by Dunnett's test).
** $P < .01$ (one-way ANOVA followed by Dunnett's test).
# $P < .05$ (Fisher's exact test).

severity of kindling and the compound retained its anticonvulsant activity even after a washout period of 48 h. The observations are given in Table 5.

3.3.3. Chemically induced seizures

3.3.3.1. PTZ-induced seizures. Administration of PTZ to vehicle-treated mice produced clonic convulsions in all animals and the onset of such convulsions was 104.5 ± 9.5 s. Prior administration of TBM dose-dependently either delayed or completely abolished clonic seizures. The PD_{50} value of TBM was 50.11 ± 22.9 mg/kg. The observations are given in Table 6. As a measure of the separation in potencies between anticonvulsant effects (as measured using MES test) and motor impairment (as determined using the inverted screen test), protective indices were calculated as ratio of TD_{50} to PD_{50}. The protective index of TBM (against MES-induced seizure) was 2.42 while that against PTZ-induced seizure was 1.66, whereas the protective index of diazepam (against PTZ-induced seizures) was 9.7.

3.3.3.2. Picrotoxin- or strychnine-induced convulsions. After receiving picrotoxin (3 mg/kg ip) or strychnine (1.2 mg/kg ip), all mice exhibited clonic convulsions. In the vehicle-treated group, picrotoxin induced convulsions after 315.0 ± 7.9 s, whereas the strychnine-induced convulsions were observed after 264.3 ± 12.4 s. The TBM, in both doses (35 and 150 mg/kg ip), failed to inhibit seizures and only diazepam inhibited the convulsions induced by picrotoxin as well as strychnine. The observations are given in Table 7.

3.3.3.3. Lithium–pilocarpine-induced status epilepticus. Administration of pilocarpine 30 min after the injection of lithium sulphate produced a time-dependent increase in the severity of seizures as evidenced from the increase in the behavioral score. TBM ($2 \times PD_{50\ MES}$) effectively delayed the onset of seizures and also reduced the severity of

Table 5
Effect of TBM seizure intensity during the development of kindling in rats

Stimulus day and stimulus number	Mean ± S.E.M. seizure score after treatment with		
	Vehicle	TBM, 68 mg/kg	Diazepam, 0.5 mg/kg
Day 1			
1	0.5 ± 0.1	0.0 ± 0.0	0.0 ± 0.0
2	1.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0
Day 2			
1	1.5 ± 0.1	0.0 ± 0.0	0.0 ± 0.0
2	2.0 ± 0.1	1.0 ± 0.1	0.0 ± 0.0
Day 3			
1	2.1 ± 0.1	0.7 ± 0.1	0.0 ± 0.0
2	2.5 ± 0.1	1.3 ± 0.2	0.5 ± 0.1
Day 4			
1	3.0 ± 0.2	1.0 ± 0.1	0.0 ± 0.0
2	3.5 ± 0.2	1.5 ± 0.4	0.7 ± 0.2
Day 5			
1	3.7 ± 0.2	1.0 ± 0.0	0.0 ± 0.0
2	4.0 ± 0.2	1.9 ± 0.2	1.0 ± 0.0
Day 6	Washout		
Day 7	Washout		
Day 8			
1	4.2 ± 0.2	1.8 ± 0.2	1.5 ± 0.2

$n = 5$ rats in each group. The drugs were administered 30 min before the first stimulus every day. Drugs were not administered on the eighth day. The severity of seizure was recorded as follows: 0 = *normal*; 1 = *facial movements*; 2 = *head nodding*; 3 = *raising of forelimbs with mild forelimb clonus*; 4 = *marked rearing to a vertical position with prominent oral movements, moving the head from side to side, and forelimb clonus*; and 5 = *symptoms as in Step (4), progressing to falling followed by forelimb and hind limb clonic seizures*. Both TBM and diazepam reduced severity of seizures significantly ($P < .05$, Kruskal–Wallis ANOVA followed by Dunn's test).
1 and 2 indicate the electrical stimulus numbers 1 and 2, respectively.

Table 6
The effect of TBM on the PTZ-induced seizures in mice

Treatment	Dose [mg/kg ip]	Onset of convulsions in seconds [mean ± S.E.M.]	Percentage protection	PD_{50}
Vehicle	–	104.5 ± 9.5	0	
TBM	10	108.7 ± 8.4	0	50.1 ± 22.9
	25	139.5 ± 12.2*	16.6	
	50	149.8 ± 5.8**	33.3	
	75	184.2 ± 6.3**	66.6	
	100	225	83.3	
Diazepam	0.2	165.4 ± 9.4**	33.3	0.32 ± 0.05
	0.4	178.5 ± 12.5**	50	
	0.8	197.4 ± 10.5**	66.6	
	1.6	235.6 ± 14.2**	83.3	
	2	nil	100	

$n = 6$ in each group.
* $P < .05$ vs. vehicle (one-way ANOVA followed by Dunnett's test).
** $P < .01$ vs. vehicle (one-way ANOVA followed by Dunnett's test).

Table 7
Effect of TBM on the seizures induced by picrotoxin or strychnine in mice

Treatment [mg/kg]	Incidence of seizures	Latency to convulsions in seconds ± S.E.M.	Latency to death in seconds ± S.E.M.	Percentage protected
Picrotoxin (3 mg/kg ip)-induced seizures				
Vehicle	6/6	315.0 ± 7.9	–	0
TBM				
35	6/6	359.2 ± 16.2	–	0
150	6/6	365.3 ± 12.6	–	0
Diazepam				
2	0/6[#]	nil	–	100
Strychnine (1.2 mg/kg)-induced seizures				
Vehicle	6/6	264.3 ± 12.4	452.5 ± 21.4	0
TBM				
35	6/6	287.3 ± 14.8	424.6 ± 23.2	0
150	6/6	321.5 ± 12.8	402.5 ± 23.5	0
Diazepam				
2	0/6[#]	nil	–	100

$n = 6$ mice in each group. TBM was without any significant effect on strychnine and picrotoxin-induced seizures (one-way ANOVA).
[#] $P < .05$ (Fisher's exact test).

seizures (Table 8). The Stage 4, i.e., forelimb, clonus was observed only in the vehicle-treated group after 67.5 ± 4.5 min and not in any group that received the compound. Diazepam (2 mg/kg) inhibited the seizures completely.

3.4. Tolerance study

On acute administration of TBM, the PD_{50} was found to be 34.2 ± 18.1 mg/kg whereas the PD_{50} on repeated administration for 7 days was 51.5 ± 12.1 mg/kg. The overlapping of PD_{50} values indicated that the increase in the PD_{50} on repeated use was not significant.

3.5. Spontaneous motor activity

The spontaneous motor activity count for a test period of 5 min in the vehicle-treated group varied from 834 ± 45.5 (at 0 min) to 780.4 ± 32.8 (at 90 min) ($P > .05$). Upon administration of TBM, the motor activity was reduced in a time-dependent manner and the motor activity count decreased from 802.6 ± 24.3 at 0 min to 286.2 ± 39.8 at 90 min ($P < .01$).

3.6. Open field test

The number of ambulations at 0 min in the vehicle-treated group was 20.66 ± 3.5 and after 30 min it was 18.3 ± 5.7. Thirty minutes after administration of TBM, the number of ambulations was reduced from 27.0 ± 5.9 to 3.2 ± 0.4. Diazepam reduced ambulations from 25.4 ± 6.3 at 0 min to 8.4 ± 3.6 at 30 min ($P < .05$). Similarly, TBM completely inhibited rearing and diazepam reduced the rearing from 7.4 ± 1.4 to 3.2 ± 1.3 ($P < .05$, Table 9).

3.7. Effect on pentobarbital sleeping time on single and repeated use

In mice treated with vehicle from the first to the seventh day, pentobarbital induced sleep that persisted for 116.0 ± 16.5 and 121.5 ± 11.8 min, respectively. The TBM on single administration increased the duration of pentobarbital-induced sleep to 363.0 ± 44.5 min ($P < .01$), whereas upon repeated administration, the duration of pentobarbital-induced sleep was to 298.5 ± 21.6 min. Though the duration of sleep decreased after repeated administration of TBM, the difference was not significant.

Table 8
Effect of TBM on lithium–pilocarpine-induced seizures in rats

Treatment [mg/kg]	Behavioral score [mean ± S.E.M.] at various time intervals				
	0	15	30	60	75
Vehicle	0.0 ± 0.0	1.75 ± 0.25	2.75 ± 0.25	3.0 ± 0.57	4.25 ± 0.5
TBM 70	0.0 ± 0.0	0.0 ± 0.0	0.75 ± 0.25*	1.25 ± 0.25*	1.7 ± 0.2*
Diazepam 0.5	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

$n = 5$ rats in each group.
* $P < .05$ (Kruskal–Wallis ANOVA, followed by Dunn's test).

Table 9
Effect of TBM on ambulation and rearing in the open field test

Treatment [dose: mg/kg ip]	Number of ambulation		Number of rearing	
	0 min	30 min	0 min	30 min
Vehicle	20.66 ± 3.5	18.3 ± 5.7	7.33 ± 2.3	6.2 ± 1.0
TBM 2 × 34	27.0 ± 5.9	0.0	8.0 ± 2.6	0.0
Diazepam 0.5	25.4 ± 6.3	8.4 ± 3.6*	7.4 ± 1.4	3.2 ± 1.3 *

$n = 6$ mice in each group.
* $P < .05$ vs. vehicle-treated group (one-way ANOVA followed by Dunnett's test).

4. Discussion

The previous study indicated the anticonvulsant activity of the acetone-soluble part of the petroleum ether extract of *B. monosperma* (Kasture et al., 2000). In the present study, the behavioral signs produced by the triterpene, TBM, isolated from the petroleum ether extract of *B. monosperma* flowers indicated overall depression of central nervous system (CNS) activity. The triterpene also exhibited anticonvulsant activity against seizures induced by MES, electrical kindling, PTZ, and lithium–pilocarpine combination. This observation is congruent with the report of Meckes et al. (1996) stating that the triterpene isolated from the hexane extract of *Psidium guajava* has anticonvulsant activity. TBM was ineffective against seizures induced by the glycine receptor antagonist strychnine, as well as $GABA_A$ antagonist picrotoxin. TBM also inhibited the development of kindling process and also inhibited the established kindling. The acute anticonvulsant efficacy of a drug does not necessarily predict its antiepileptic activity, which is measured by the ability of the drug to attenuate the development of progressive epileptogenic process (Starr, 1996). Kindling seizure in which a subthreshold stimulus (e.g., electrical stimulation of the basolateral amygdala) induces widespread seizure activity through repeated exposure has been used to model the progressive pathogenesis of refractory epilepsy in human (Murray et al., 1993). The test compound TBM protected fully established kindling seizures in rats. In addition, the TBM also blocked the development of kindled seizures induced by electrical stimulation. Moreover, the effect persisted after the washout period, suggesting that the presence of the drug did not simply mask the genesis of kindled seizures.

The TBM was effective against lithium–pilocarpine-induced status epilepticus, suggesting the potential application of the test compound in the treatment of status epilepticus. Numerous biochemical effects have been attributed to lithium (Mork, 1990); however, none of these has been proved to be the basis of its therapeutic action.

Administration of subconvulsive doses of pilocarpine after lithium increases brain levels of acetylcholine and decreases brain level of inositol. Kofman and Belmaker (1993) have shown that inositol prevents the status epilepticus induced by lithium and pilocarpine and benzodiazepines are also effective against the seizures induced by this combination.

Lack of protection against picrotoxin-induced seizures indicates that TBM has no action on the postsynaptic $GABA_A$ receptors, whereas failure to inhibit strychnine-induced seizures indicates lack of effect on the glycine receptors in the spinal cord. The exact mode of action of PTZ is not known (Rang et al., 1999). Therefore, further study is necessary to elucidate the mechanism of the anticonvulsant action of TBM.

The median toxic dose of TBM was determined using the inverted screen test. In this test, the compounds with sedative and/or ataxic properties produce dose-dependent failure to climb the inverted screen. The ataxia induced by TBM is commonly observed with many antiepileptic agents (McNamara et al., 1993). The protective index of TBM [the ratio of median toxic dose (in the inverted screen test) to the median protective dose (in the MES test)] was 2.42, indicating that TBM has a wide margin of safety. TBM diminished spontaneous motor activity in the actophotometer and also decreased both rearing and ambulating in the open field test. It has been reported that a decrease in both rearing and ambulating reveals a general depressant effect on the CNS while a decrease in rearing without any change in locomotion is an anxiogenic effect (Nutt and Glue, 1991).

Decrease in the pentobarbital-induced sleeping time is suggestive of development of metabolic tolerance. In the present study, subchronic doses of TBM showed a decrease in the duration of pentobarbital-induced sleeping time, which could not reach significance. The increase (though not significant as shown by overlapping values) in the PD_{50} of TBM on repeated administration indicates a possibility of functional tolerance to anticonvulsant activity on prolonged use. Thus, it is concluded that the compound, identified as a triterpene, has a broad spectrum of protective activity against different experimental models of seizures and further study is necessary to understand its mode of action.

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References

Coughenour LL, McLeas JR, Parker RB. A new device for the rapid measurement of the motor impairment function in mice. Pharmacol, Biochem Behav 1977;6:351–3.

Gawale NS, Pal SC, Kasture VS, Kasture SB. Effect of *Butea monosperma* on memory and behaviour mediated via monoamine neurotransmitters in laboratory animals. J Nat Rem 2001;1:33–41.

Ginski M, Witkin JM. Sensitive and rapid behavioral differentiation of *N*-methyl-D-aspartate receptor antagonists. Psychopharmacology 1994; 114:573–82.

Harborne JB. Phytochemical methods: a guide to modern techniques of plant analysis. 2nd ed. London: Chapman and Hall, 1965. pp. 120–8.

Irwin S, Taber RI, Fox J, Roth FE. Comparison of perphenzanie and fluphenazine enanthates in rats. Psychopharmacologia 1968;12:441–7.

Kasture VS, Chopde CT, Deshmukh VK. Anticonvulsant activity of *Albizzia lebbeck*, *Hibiscus rosa sinesis* and *Butea monosperma* in experimental animals. J Ethnopharmacol 2000;71:65–75.

Kirtikar KR, Basu BD. *Butea monosperma*. In: Blatter E, Caius JF, Mhaskar KS, editors. Indian medicinal plants. 2nd ed. Allahabad: Lalit Mohan Bose, 1989. pp. 785–8.

Kofman O, Belmaker RH. Biochemical, behavioral and clinical studies of the role of inositol in lithium treatment and depression. Biol Psychiatry 1993;34:839–52.

Kupferberg HJ. Antiepileptic drug development program: a co-operative effort of government and industry. Epilepsia 1989;30:S51–6.

McNamara JO. Drugs effective in the therapy of the epilepsies. In: Hardman LE, Limbird LE, Molinoff PB, Ruddon RW, editors. Goodman and Gillman's the pharmacological basis of therapeutics. New York: Pergamon, 1996. pp. 461–6.

McNamara JO, Bonhaus DW, Shin C. In: Schwartz-Kroin PA, editor. Epilepsy: models, mechanisms and concepts. Cambridge: Cambridge Univ. Press, 1993. pp. 27–47.

Meckes M, Calzado F, Tortoriello J, Gonzalez JL, Martinez M. Terpinoid isolated from *Psidium guajava* hexane extract with depressant activity on central nervous system. Phyto Res 1996;10:600–3.

Miller LC, Tainter ML. Estimation of ED_{50} and its error by means of logarithmic probit paper. Proc Soc Exp Biol Med 1944;57:261–4.

Mork A. Actions of lithium on second messenger activity in the brain. Lithium 1990;1:131–47.

Murray TF, Zhang G, Franklin PH. Manipulation of endogenous adenosine affects seizure susceptibility. Drug Dev Res 1993;28:410–5.

Nadkarni KM. *Sesbania grandiflora*. Indian materia medica 3rd ed. Mumbai: Popular Prakashan, 1998. pp. 52–4.

Nutt DJ, Glue P. In: File SE, editor. Psychopharmacology of anxiolytics and depressants. New York: Pergamon, 1991. pp. 1–28.

Patel S, Millan MH, Meldrum BS. Decrease in excitatory transmission within the lateral habenula and mediodorsal thalamus protects against limbic seizures in rats. Exp Neurol 1988;101:63–74.

Rane A, Grampurohit ND. Hepatoprotective activity if *Pterocarpus marsupium* and *Butea monosperma*. Indian J Pharm Sci 1998;60:182–4.

Rang HP, Dale MM, Ritter JM. Pharmacology. 4th ed. London: Livingstone, 1999. pp. 604–13.

Sakina MR, Dandiya PC. A psycho-neuropharmacological profile of *Centela asiatica* extract. Fitoterapia 1990;61:291–306.

Shah KG, Baxi AJ, Shukla VJ, Dave KK, De S, Ravishankar B. Phytochemical studies and antiestrogenic activity of Butea frondosa (*B. monosperma*) flowers. Indian J Pharm Sci 1990;52:272–5.

Starr MS. The role of dopamine in epilepsy. Synapse 1996;22:159–94.

Swinyard EA, Brown WC, Goodman LS. Comparative assay of antiepileptic drugs in mice and rats. J Pharmacol Exp Ther 1952;106:319–30.

Turner RA. Screening procedures in pharmacology. New York: Academic Press, 1972.