

EVALUATION OF ANTIPILEPTIC ACTIVITY OF *DATURA METEL* LEAF EXTRACT IN EXPERIMENTAL ANIMAL

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ABSTRACT

Objective: the study was designed to evaluate the antiepileptic activity of *Datura metel* leaf extract in experimental animal. **Material and Methods:** In the present study ethanolic extract of *Datura metel* was studied for its anticonvulsant effect against Maximum Electroshock (MES) and Pentylentetrazole (PTZ) induced convulsions in Swiss Albino mice. In MES method Seizures were elicited with 50 Hz alternating current of 60 mA intensity for 0.2 sec. The mice were divided randomly into 5 groups containing 5 animals each. After 1 hour of oral and 30 minutes of intra peritoneal drug treatments, convulsions were induced by convulsometer. In PTZ induced convulsions method animals were treated with ethanolic extract *Datura metel* one hour prior to PTZ (80mg/kg) induced convulsion and standard group animals were treated with Diazepam (0.5 mg/kg, i.p.), and Phenytoin(25 mg/kg, i.p.), 30 min before convulsing agent PTZ(80mg/kg). **Results:** It is found that treatment with ethanolic extract of *Datura metel* (200 mg/kg and 400mg/kg) extremely significantly ($p < 0.001$) reduced the duration of flexion and hind limb tonic extensor (HLTE) when compared to control group in MES induced epilepsy. *Datura metel* extract significantly delayed the onset of myoclonic jerks at doses of 200 mg/kg and 400 mg/kg. Reduction in the duration of tonic convulsions by the extract was profound at both the doses used ($p < 0.001$). **Conclusion:** The ethanolic extract of *Datura metel* was showed extremely significant ($p < 0.001$) dose dependent protection in swiss mice against MES and PTZ induced convulsions. , the results indicate that the leaves of *Datura metel* produces anticonvulsant effects through central mechanisms which support the traditional use of the plant to treat epileptic fits.

Key Words: Antiepileptic Activity, *Datura metel*, Flexion, Hind Limb Extension.

INTRODUCTION

Epilepsy is the second most common neurological disorder after stroke, affecting at least 50 million persons worldwide.¹ Epilepsy shows a prevalence rate in 1-2% of the world population.² It affects an estimated 7 million people in India and 50 million worldwide, approximately 40% of them are women. Epilepsy is a chronic and often progressive disorder characterized by the periodic and unpredictable occurrence of epileptic seizures, i.e., involuntary contraction of striated muscle repeatedly. Convulsion arises due to sudden excessive and rapid discharge of cerebral neurons in the grey matter of the brain. It has been observed that presently available antiepileptic drugs are unable to control seizure effectively in as many as 25% of the

patients.³ A variety of different electrical or chemical stimuli can easily give rise to a seizure in any normal brain. There is important role of neurotransmitters especially, γ -amino butyric acid (GABA) and glutamate in epileptogenesis, since they are the major inhibitory and excitatory transmitters in the central nervous system, respectively, and the fact that generation of seizures has been attributed to imbalance between excitatory and inhibitory neurotransmission in epileptic brains. GABA plays an important role in regulation of neuronal excitability and impairment of GABA function produces seizures.⁴ Drug therapy of epilepsy with currently available Antiepileptic Drugs (AEDs) is associated with side effects, dose-related and chronic toxicity that involve virtually every organ system. . Thus it is

necessary to investigate for an antiepileptic agent that is highly efficacious as well as safe in terms of drug related toxicity. Natural products and plants for that matter, used in traditional medicine can be an invaluable source for search for novel antiepileptic compounds⁵. *Datura metel* is also known as devil's trumpet is a shrub or woody herb up to 2 m in height that is often grown as an annual in temperate zone⁶. The leaves and seeds are widely used in herbal medicine as anaesthetic, antispasmodic, antitussive, bronchodilator and as hallucinogenic⁷. The roots, dried leaves and flowering tops have been used in India for their narcotic and antispasmodic properties in treatment of numerous ailments and conditions. In ayurveda plant is considered bitter, acrid, astringent, germicidal, anodyne, antiseptic, narcotic and sedative. The warmed leaves are used externally to expel guinea worms in Rajasthan. The young, fresh leaves are used internally in the treatment of amenorrhoea among tribal women in central Orissa. The juice of fresh leaves, or a poultice of them, is used in painful swelling earache. In ayurveda, the seeds are considered heating; tonic, febrifuge, anthelmintic, alexiteric and emetic. They are used to treat leucoderma, skin disease, ulcer, bronchitis, jaundice and piles. It is reported that the plant seeds are used to treat leprosy. The seeds and roots possess antidiarrhoeal, antipyretic properties and are used to treat insanity and fever. The roots powder is reportedly given for 15 days after menstruation to induce sterility among Gond women in Uttar Pradesh⁸. Present study was aimed to investigate the pharmacological effect of *Datura metel* against antiepileptic activity by using Maximum Electro Shock (MES) and Pentylentetrazole (PTZ) methods in Swiss albino mice.

MATERIAL AND METHODS

PLANT COLLECTION

The plant *Datura metel* was collected from botanical garden of university of Rajasthan and smriti van area of Jaipur city.

PREPARATION OF PLANT EXTRACT

Plant materials were treated and analyzed at the Pharmacognosy Lab of Mahatma Gandhi College of Pharmaceutical Sciences, Jaipur. The leaves (100gm) were dried on the laboratory bench for 10 days. The dry sample was milled and ground into powder. 40 g of powdered leaves of *Datura metel* was extracted successively with 200 ml of ethanol at 56-60°C and ethyl acetate at 40-50°C in Soxhlet extractor until the extract was clear. The extracts then evaporated to dryness, after

that weigh the accurate amount of dried extract and calculate the percentage yield of extract. The resulting paste form extracts were stored in a refrigerator at 4°C for future use¹²⁸

EXPERIMENTAL ANIMALS

Swiss Albino mice of strain weighing around (20-25 g) were procured from Animal house, Department of Pharmacology, Mahatma Gandhi College Of Pharmaceutical Sciences, Jaipur (Rajasthan), All animals were housed in polypropylene cages in a temperature controlled animal house room at 24 ±1°C temperature, 60 ± 5 % relative humidity and 12 hour light and 12 hour dark cycle. The animals were fed with pelleted feed with standard rat diet and tap water throughout the experiment. The experiment were designed and conducted in accordance with the ethical norms approved by Institutional Animal Ethical Committee Guidelines (reg. no. 1356/ac/10/CPCSEA/9/July /10). The animal experimentation was carried out in accordance to the guidelines mentioned in the CPCSEA.

DRUGS AND CHEMICALS

The drugs and chemicals used in this study include: Diazepam (INTAS, Gujarat, India), Pentylentetrazole (Abbott), Phenytoin (Brooks)

ACUTE TOXICITY STUDY¹⁰

Methodology: Paragraph 22 of OECD Guideline 425 suggests two types of acute oral toxicity tests i.e. limit test and main test. The limit test is primarily used in situations where the experimenter has information indicating that the test material is likely to be nontoxic, i.e., having toxicity below regulatory limit doses. However, in those situations where there is little or no information about its toxicity, or in which the test material is expected to be toxic, only the main test should be performed. Because the literature survey of this herb indicates about its potential toxicity, therefore, the main test was performed.

Limit test: Doses should not exceed 2000 mg/kg which is considered the upper limit dose. When the first animal is dosed with the upper limit dose and survives, the second animal receives the same dose. When a total of three animals have been dosed with the limit dose and no deaths have occurred, then three animals of the other sex should be tested at the limit dose level. If there is again no lethality, the test can be terminated.

Body weight: Individual weights of animals were recorded before the administration of drug on 1st day of the study and thereafter on the 7th and 14th day of the experiment.

Changes in the weight of individual animals were calculated and compared with that of the control animals

Wellness parameters: Animals were observed continuously during the first 30 min after dosing and observed periodically (with special attention given during the first 4 hours) for the next 24 hours and then daily thereafter, for 14 days. All observations were systematically recorded with individual records being maintained for each animal. Observations included changes in skin and fur, eyes and mucous membranes and behavioral pattern. Attention was given for observations of tremors, convulsions, salivation, diarrhea, lethargy, sleep, coma and mortality. Changes in wellness parameters were compared with that of control animals

ANTI-EPILEPTIC ACTIVITY

MAXIMAL ELECTROSHOCK SEIZURES (MES) INDUCED CONVULSIONS¹¹

Swiss albino mice weighing 25 to 30 gm were randomized and divided into five groups of five animals each ($n=5$) After 1 hour and 30 minutes of oral and intraperitoneal drug treatments, respectively tonic convulsions of the hind limb extremities of mice were induced by passing alternating electrical current (50 Hz, 60 mA and 0.2 s) through ear electrodes. This was the maximal current (60 mA) that induced tonic hind limb extension in all the trial mice and it was determined previously before commencement of the experiment. The number of animals protected from tonic hind limb extension seizure and the time spent in this position were determined in each dose group.

Group I: vehicle control group treated with distilled water (10 ml/kg *p.o.*, 1 hr) prior to the induction of convulsion.

Group II: standard group S1 treated with diazepam 0.5 mg/kg, *i.p.*, 30min), prior to the induction of convulsion.

Group III: standard group S2 treated with phenytoin (25 mg/kg, *i.p.*, 30 min), prior to the induction of convulsion.

Group IV: test groups T1 treated with the extract (.dose 200 mg/kg, *p.o.*1 hr) , prior to the induction of convulsion.

Group V: test groups T2 treated with the extract (dose 400 mg/kg, *p.o.*1 hr) , prior to the induction of convulsion.

Seizures were induced to all the groups by using an Electro Convulsimeter. Maximal electroshock seizures were elicited by a 50 Hz alternating current of 60 mA intensity for 0.2 sec.14. The duration of phases of epilepsy

(Flexion and hind limb extension) were observed.

PENTYLENETETRAZOLE (PTZ) INDUCED CONVULSIONS¹²

Swiss albino mice weighing 25 to 30 gm were randomized and divided into five groups of five animals each ($n=5$). Pentylenetetrazole (PTZ) (80mg/kg body weight was administered to all the groups to induce clonic convulsions.

Group I: control group treated with distilled water (10ml/kg *p.o.* 1 hr) prior to the PTZ 80mg/kg, *i.p.* induced convulsion.

Group II: Standard groupS1 treated with diazepam 0.5 mg/kg, *i.p.*, 30min), prior to the PTZ 80mg/kg, *i.p.* induced convulsion.

Group III: Standard groupS2 treated with phenytoin (25 mg/kg, *i.p.*, 30 min), prior to the PTZ 80mg/kg, *i.p.* induced convulsion.

Group IV test groups T1 treated with the extract (dose 200 mg/kg, *p.o.* 1 hr) prior to the PTZ 80mg/kg, *i.p.* induced convulsion.

Group V: test groupsT2 treated with the extract (dose 400 mg/kg, *p.o.*, 1 hr) prior to the PTZ 80mg/kg, *i.p.* induced convulsion.

Animals will be observed for 30 min for tonic convulsion episode. Hind limb extension will taken as tonic convulsion. The onset of tonic convulsion and the number of animals convulsing or not convulsing within the observation period will be noted.

STATISTICAL ANALYSIS

In all experiments, a sample size of five or ten ($n=5$ or 10) was utilized. All data are presented as mean \pm SEM. To compare differences between groups, ANOVA was performed with Newman-Keuls" test. GraphPad was used for all statistical analysis.

RESULTS AND DISCUSSION

PERCENTAGE YIELD OF EXTRACT

$$\begin{aligned} \% \text{ yield} &= \text{weight of extract /weight of powdered drug taken} \times 100 \\ &= 5.80 /20 \times 100 \\ &= 29.00\% \end{aligned}$$

ACUTE TOXICITY STUDY

Oral acute toxicity test

Sample: ethanolic extract of *Datura metel* leaf
Test guideline 425 (modified October 2006) limit test

Animal: Swiss Albino Mice

Sex: female, non pregnant

Weight range: 20-25

Route of administration: oral

Dose: 2000mg/kg

Fasted for: 3-4 hours (pre experimental) and 1-2 hours (post experimental)

Total Number of animal used: 03

Table 1: Effect of *Datura metel* leaf extract on the body weight of mice at 2,000 mg/kg dose

Group	Weight of animal			
	Before treatment	After treatment		
		1 day	7 day	14 day
Control	23	23	22.9	23.2
Treated	24	23.8	23.6	24

Table 2: Wellness parameters

Observations (post sample administration)	
1. first 4 hours :	slight sedation
2. within 24 hours :	normal and animals were active
3. after 24 hours :	normal and animals were active
4. after 7 days :	normal and animals were active
5 after 14 days :	normal and animals were active. All five animals survived during 14 days of observation.

Conclusion: Ethanolic extract of *Datura metel* leaf is found to be safe upto 2000mg/kg b.w.

MAXIMAL ELECTROSHOCK SEIZURES (MES) INDUCED CONVULSIONS

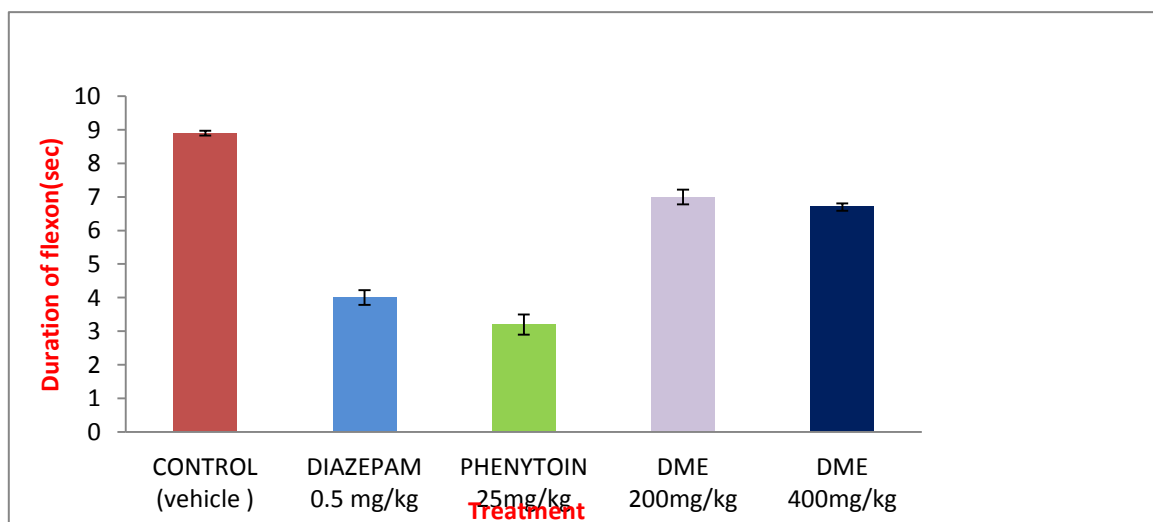
The extract caused significant decrease in the duration of flexon and tonic hind limb extension (THLE) induced by maximal electroshock but was unable to completely prevent its occurrence. *Datura metel* leaf extract 400 mg/kg produced extremely significant reduction of the duration of flexon ($p < 0.001$) and THLE ($p < 0.001$) when compared to control group, however, THLE ($p < 0.05$) i.e significantly reduced when

compared to standard group S1 (DIAZEPAM) .200mg/kg kg produced extremely significant reduction of the duration of flexon ($p < 0.001$) and THLE ($p < 0.0001$) when compared to control group. Diazepam and Phenytoin extremely significantly reduced the duration of flexon ($p < 0.001$) and THLE ($p < 0.001$) when compared to control group while Phenytoin has non significant effect on duration of flexon when compared to the S1 (Diazepam) . The results were shown in Table.3 and Figure.1 and Figure 2.

Table 3: Effect of *Datura metel* leaf extract on the duration of MES induced flexon and hind limb extension

Treatment (mg/kg)	Duration of flexon (sec)	Duration of tonic hind limb extension (sec)
CONTROL (vehicle)	8.9±0.07	12.2±0.09
DIAZEPAM 0.5 mg/kg	4±0.22 ^{a3}	6±0.26 ^{a3}
PHENYTOIN 25mg/kg	3.2±0.30 ^{a3,bNS}	4±0.30 ^{a3,b2}
DME 200mg/kg	7±0.22 ^{a3,b3,c3}	9±0.15 ^{a3,b3,c3}
DME 400mg/kg	6.7±0.11a ^{3,b3,c3}	7±0.22 ^{a3,b1,c2}

Values are mean ± SEM of n=5 animals a group.,SEM = standard error mean
P value: a = when compared to control group ,b = when compared to standard group S1 (DIAZEPAM),c = when compared to standard group S2 (PHENYTOIN)
NS = Not Significant , 1 = $p < 0.05$, 2 = $p < 0.01$, 3 = $p < 0.001$

**Fig. 1: Effect of DME (200-400 mg/kg) and standard drug Diazepam (0.5mg/kg), Phenytoin(25mg/kg) on the duration of flexon**

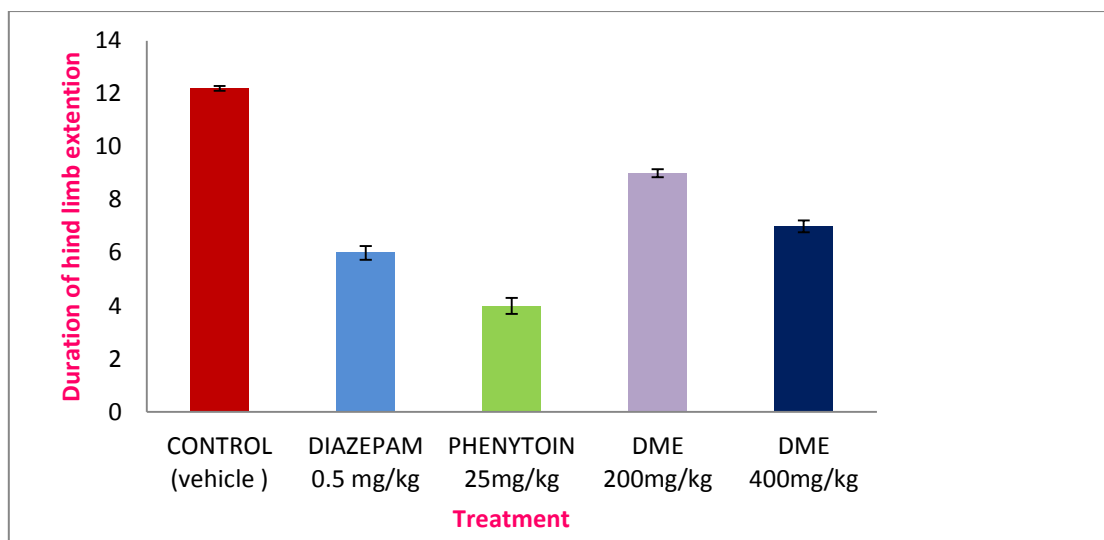


Fig. 2: Effect of DME (200-400 mg/kg) and standard drug Diazepam (0.5mg/kg), Phenytoin(25mg/kg) on the duration of hind limb extension

CHEMICALLY-INDUCED SEIZURES

The extract showed significant anticonvulsant activity against PTZ-induced seizures. It significantly and dose-dependently delayed the onset of myoclonic jerks (fig. 3) and decreased the duration of tonic convulsions (fig 4). DME significantly delayed the onset of myoclonic jerks at doses of 200 mg/kg and 400 mg/kg). Reduction in the duration of tonic convulsions

by the extract was profound at both the doses used (p<0.001). Diazepam, an anticonvulsant, produced effects similar to that of the extract against PTZ-induced seizures and the effects were dose-dependent. The drug significantly delayed the onset of myoclonic jerks (P<0.001, table 4). Also, Phenytoin caused significant reduction of the latency and duration of tonic convulsions.

Table 4: Effect of *Datura metel* leaf extract and standard drug on PTZ induced convulsion

Treatment (mg/kg)	latency to PTZ induced convulsion	Duration of convulsion (sec)
CONTROL (vehicle)+PTZ	110±0.14	35±0.11
DIAZEPAM +PTZ	1000±0.12 ^{a3}	10±0.18 ^{a3}
PHENYTOIN +PTZ	1500±0.09 ^{a3,b3}	8±0.19 ^{a3,b3}
EXTRACT 200mg/kg+PTZ	400±0.08 ^{a3,b3,c3}	20.6±0.04 ^{a3,b3,c3}
EXTRACT 400mg/kg +PTZ	706±0.06 ^{a3,b3,c3}	17.6±0.13 ^{a3,b3,c3}

Values are mean ± SEM of n=5 animals a group. SEM = standard error mean
 P value: a = when compared to control group, b = when compared to standard group S1 (DIAZEPAM +PTZ) , c = when compared to standard group S2 (PHENYTOIN+PTZ)
 NS = Not Significant 1 = p<0.05 , 2 = p<0.01 , 3 = p<0.001

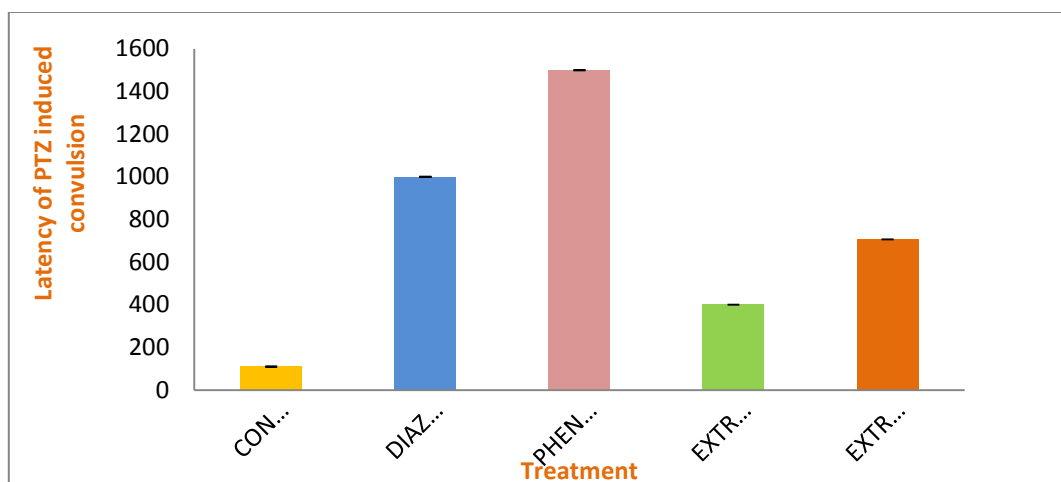


Fig. 3: Effect of DME (200-400 mg/kg) and standard drug Diazepam (0.5mg/kg), Phenytoin(25mg/kg) on latency to PTZ (80mg/kg) induced convulsion

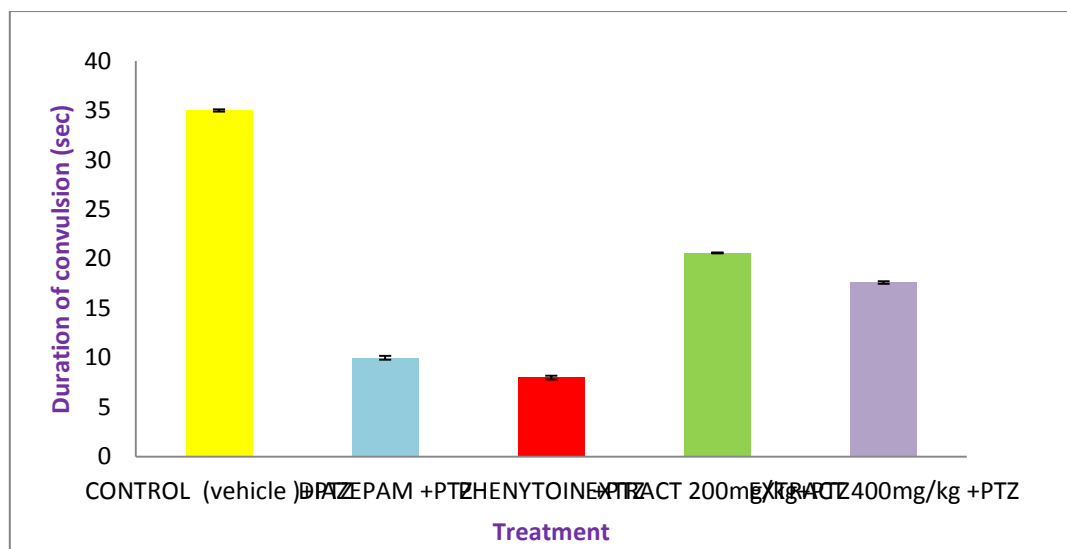


Fig. 4: Effect of DME (200-400 mg/kg) and standard drug Diazepam (0.5mg/kg), Phenytoin (25mg/kg) on the duration of PTZ induced convulsion

DISCUSSION

We had used the most popular two different animal models experiments such as MES and PTZ methods which characteristically described three types of seizures activity. The MES test is the most frequently-used as an animal model for identification of anticonvulsant activity of drugs for the generalized ("grand mal") tonic-clonic seizures and partial seizures. PTZ-induced seizures test is considered as an experimental model for the "generalized absence seizures" and also a valid model for human generalized myoclonic seizures and generalized seizures of the petitmal type. This present study demonstrates that DME has anticonvulsant activities but does not cause neuromuscular impairment. The extract inhibited seizures induced by pentylentetrazole, and maximal electroshocks. Inhibition of seizures induced by pentylentetrazole and maximal electroshock in laboratory animals is the most common predictive screening tests used for characterizing potential anticonvulsant drugs^{13,14}. The maximal electroshock-induced seizure test is considered to be a predictor of likely therapeutic efficacy against generalised tonic-clonic seizures. By contrast, the pentylentetrazole-induced seizure test represents a valid model for human generalised myoclonic and absence seizures¹⁵. *Datura metel* may therefore, contain compounds that have activity against generalised tonic-clonic seizures as well as generalised myoclonic and absence seizures. The fact that the extract inhibited convulsions induced by these agents may suggest that enhancement of GABA neurotransmission may be responsible for its anticonvulsant

activity. The benzodiazepine-like anticonvulsants such as diazepam, which enhance GABA neurotransmission act as anxiolytics at low doses and have anticonvulsant and myorelaxant or neurotoxic effects at higher doses^{16,17}. It was therefore, expected that the extract may have anxiolytic effects. However, unlike centrally acting anticonvulsants such as diazepam, which have skeletal muscle relaxant effects, the extract did not cause defective motor coordination or balance but caused *in vitro* skeletal muscle contractions. The extract is not a pure compound and contains a variety of secondary metabolites including alkaloids, saponins, cardiac glycosides, reducing sugars and flavonoids and any of these could have skeletal muscle contractile effects. From the above observation it could be predicted that the ability of the extract to exhibit activity against these two types of seizures suggests that it may act through different mechanisms to elicit its anticonvulsant effects, such as inhibition of voltage-gated sodium channels or by enhancing the GABAergic pathway. Further studies to establish the active chemical constituent(s) of the extract and the exact mechanism of action is currently going on in our laboratory.

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