Anticonvulsant effects of 3, 4-Dimethoxy toluene, the major constituent of *Phoenix dactylifera L* Spathe in mice

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Abstract:

The anticonvulsant effects of 3,4-Dimethoxy toluene (DMT), the major constituent of the date palm (*Phoenix dactylifera L.*) spathe, were investigated using pentylenetetrazole (PTZ), picrotoxin (Pic), nicotine (Nic) and maximal electroshock (MES)-induced seizure models. In PTZ (85 mg/kg)-induced seizures, the intraperitoneally injection of DMT at a dose of 100 mg/kg significantly delayed the onset time of seizures and produced 50% protective effect against mortality. In MES model, DMT showed complete inhibition of tonic hind-limb extension (THLE) and exhibited complete protection against mortality. After mice were injected with picrotoxin (12 mg/kg), DMT (100 mg/kg) significantly delayed the onset time of convulsions and death. DMT exhibited complete protection against nicotine (0.8 mg/kg)-induced convulsions**.** These results indicate that DMT may have a promising anticonvulsant activity.

Key words: 3, 4-Dimethoxy toluene**,** anticonvulsant**,** *Phoenix dactylifera L.***,**

Introduction

Epilepsy is a condition in which a person has a tendency to have recurring seizures. The problem of antiepileptic drugs (AEDs) arise from their inability to control seizure efficiently and adverse effects which have not been circumvented completely (Gates, 2000). Hence, search for antiepileptic compounds with more selective activity and lower toxicity should continue to develop newer agents for treatment of epilepsy.

Traditional medicine, especially medicinal plants, has been practiced for long time in most parts of the world. In Saudi Arabia, there are many medicinal plants with claim of neurological properties. But these claims of treatment successes are often made without any scientific basis. *P*henox *dactylifera* spathe which is commonly grown in Saudi Arabia was believed to have certain neurological properties. Chemical constituents of the *P*henox *dactylifera* spathe were identified by both (Al-Yahya 1986; Mikki *et al.,* 1988). However, the pharmacological activities have not been scientifically evaluated.

The present work was aimed to evaluate the neurological effects of DMT, the main constituent of *P*henox *dactylefera* spathe, on seizures induced by MES, PTZ, Pic and Nic.

Materials and Methods

Animals. Male albino mice, weighing 25-30 g obtained from the animal house (King Faisal University, Saudi Arabia) were used. The animals were housed in standard cages with free access to food (standard laboratory rodent's chow) and water. The animal house temperature was maintained at 22 ± 2.0°C with a 12-h light/dark cycle (light on at 6:00 a.m. to 6:00 p.m.). DMT was given 30 min before the convulsing agents was injected. The ethical guidelines for the investigation of experimental seizures in conscious animals were used. All efforts were made to minimize animal suffering and to reduce the number of animals used.

Chemicals:
3,4-Dimethoxy toluene, pentylenetetrazole, picrotoxin, nicotine (Aldrich-Sigma, USA)

Convulsions tests:

Maximal electroshock -induced seizures

MES-inducing tonic hind-limb extension (THLE) in 99.9% of the animals (Swinyard, 1969), was previously determined. The electrical stimulus (50 mA, 75 Hz, 2s duration) was applied through ear-clip electrodes using a current generator (Ugo Basile, ECT Unit, 7801). The DMT (100 mg/kg, i.p.) was administered and 10 min later electroconvulsive shock was delivered. THLE was accepted as maximal electroshock seizure. Mice which did not show THLE were considered to be protected from MES. Consequently, abolition of THLE after drug treatment was regarded as the endpoint of protection (Swinyard *et al*., 1989).

Pentylenetetrazole-induced convulsions:

DMT (100 mg/kg) was injected intraperitoneally (i.p) 30 minutes before administration of PTZ (85 mg/kg, i.p.). The time taken before onset of myoclonic spasm and clonic convulsions and percentage mortality was recorded (Swinyard *et al*., 1952). The animals were observed for onset time of convulsions and lethality up to 30 min after PTZ. Each group contained six animals.

Picrotoxin-induced convulsions :

Chemical seizures were achieved using Pic (Lloyd and Worms, 1981). Each animal in both the treatment (DMT 100 mg/kg, i.p.) and control groups were injected with Pic (12 mg/kg, i.p.). The behavioral changes of the mice were observed for 2 h after Pic injection. The onset time of generalized clonic seizures, the survival time, and the survival percentage were calculated as a percent of the control. The number of animals protected in each group was also recorded and the percentage protection was considered as the anticonvulsant parameter. (Abdul-Guani *et al*., 1987).

Nicotine-induced convulsions:

Epilepsy was induced using Nic (Damaj *et al*., 1999; Miner and Collins, 1989; Miner *et al.*, 1985). Each animal in both the treatment (DMT 100 mg/kg, i.p.) and control groups was injected with Nic (0.8 mg kg^{-1}, i.p.). The behavioral changes of the mice were observed for 10 min after Nic injection. The latency to the onset time of generalized clonic seizures, the survival time, and the survival percentage were calculated as a percent of the control.

Statistics :

Student's *t*-test was used for the analysis of the results using SPSS (version 10) software and the results were expressed as the means ± S.E.M. A *P* value of less than 0.05 was considered significant.

Results:

MES-induced seizures

In the maximal electroshock seizures, DMT (100 mg/kg) significantly abolished the extension of lower limbs and produced 100% protection against mortality of tonic seizures (Table 1).

Table (1)

Effect of DMT on tonic seizures induced by maximal electroshock-induced seizures in male mice

Treatment	% convulsions	% protection	% mortality
Control (distilled water, 10 ml/kg)	100 %	0 %	50 %
DMT (100 mg/kg i.p.)	0 %	100 %**	0 %**

represented as percentage of tonic seizures and mortality (n = 6). **P < 0.01 when compared to the vehicle treated group.

Pentylenetetrazole-induced convulsions :

In control animals, clonic convulsions appeared 60 ± 6.2 sec after PTZ injection and all animals died after seizures. Intraperitoneal injection of DMT (100 mg/kg) significantly delayed both the onset time of convulsions and onset time of death induced by PTZ. In addition, DMT produced 50% protection against mortality (Table 2).

Table (2)

Protective effect of DMT against PTZ induced-seizures in male mice

Treatment	Onset time of convulsion (Sec.) mean ± SEM	% delay	Onset time of death (min.) mean ± SEM	% delay	% Mortality
PTZ (control)	60 ± 6.2	-	2.5 ± 0.2	-	100 %
DMT + PTZ	230 ± 42.2**	283 %	45 ± 2.9**	1700 %	50 %

Data represented as percentage of tonic seizures and mortality mean ±SEM (n = 6). **P < 0.01 when compared to the vehicle treated group.

Picrotoxin-induced convulsions

Intraperitoneal injection of DMT (100 mg/kg) significantly delayed both the onset time of convulsions and onset time of death induced by Pic but failed to produce protection against mortality (Table 3).

Table (3)

Protective effect of DMT against Pic-induced convulsions in mice

Treatment	Onset time of convulsion (min.) mean ± SEM	% delay	Onset time of death (min.) mean ± SEM	% delay	% Mortality
Pic	3.8 ± 0.3	-	7.2 ± 0.9	-	100 %
DMT + Pic	8.7 ± 1.4**	129 %	28.5 ± 5.3**	296 %	100 %

Data represented as percentage of tonic seizures and mortality mean ± SEM (n = 6). **P < 0.01 when compared to the vehicle treated group.

Nicotine-induced convulsions

Intraperitoneal injection of DMT (100 mg/kg) totally abolished the convulsion induced by nicotine. In addition, it produced 100% protection against mortality (Table 4).

Table (4)
Protective effect of DMT on Nic-induced convulsions

Treatment	Onset time of convulsion (min.) mean ± SEM	% delay	% Mortality
Nic	1.0 ± 0.0	-	83.3 %
DMT + Nic	No convulsion	100 %	0 %

Data represented as percentage of tonic seizures and mortality or mean ± SEM (n = 6). **P < 0.01 when compared to the vehicle treated group.

Discussion

Currently there is an increasing demand for new types of anticonvulsants because undesirable side effects from the drugs used clinically are increasing and often render treatment difficult (MacNamara, 1994). One of the approaches to search for new antiepileptic drugs is the investigation of naturally occurring compounds, which may belong to new structural classes.

Complete protection against THLE and death in MES usually predicts the ability of a drug to prevent the spread of seizure discharge from the epileptic focus in the brain. It indicates its potential to protect against tonic-clonic (grand mal) and partial seizures. Thus, DMT protective activity against MES, suggests its potential as an anti-epileptic drug against grand mal and partial seizures (Krall *et al*., 1978; Porter *et al*., 1984).

DMT significantly increased the latency period and reduced the duration of seizures induced by PTZ. PTZ test is assumed to identify anticonvulsant drugs effective against generalized tonic-clonic, partial seizures and generalized clonic seizures (Loscher and Schmidt, 1988). Two mechanisms have been proposed for the mode of PTZ-induced convulsions. It is proposed that PTZ induces convulsion by either inhibiting gamma amino butyric acid (GABA) pathway in CNS (Corda *et al*., 1990), or by increasing the central noradrenergic activity (De Potter *et al*., 1980). The effect of DMT in this model can therefore suggest its involvement in

GABA-ergic or noradrenergic pathways and its efficacy against generalized tonic-clonic and partial seizures in man.

DMT showed a significant anticonvulsant effect against Pic-induced seizures mainly due to a prolongation of the latency of the onset of seizure, and delay in the onset time of death. It has been demonstrated that Pic selectively antagonizes the effect GABA (Ramanjaneyulu and Ticku, 1984). Anticonvulsant drugs such as benzodiazepines and valproic acid increase the GABA level in the brain (Meldrum and Chapman, 1986; Macdonald, 1988). Therefore, in epileptic patients, a deficiency of brain GABA had been suggested (Delgado-Escueta *et al.*, 1986). So, DMT anticonvulsant effect might be due an increase in GABA level.

It has been reported that nicotine-induced seizures are centrally mediated (Dixit *et al*., 1971; Caulfield and Higgins, 1983). So, DMT possesses the ability to cross the blood-brain barrier after peripheral injection to achieve pharmacological action. Two mechanisms have been proposed for the mode of Nic-induced convulsion; either by stimulating *N*-methyl-D-aspartate (NMDA) receptors and or increasing nitric oxide formation through stimulating nitric oxide synthase (Damaj, *et al*., 1999). Thus DMT might exert it action by blocking NMDA or reducing nitric oxide formation through inhibiting nitric oxide synthase.

It can be concluded that the DMT possesses anticonvulsant activity against MES, PTZ, Pic and Nic induced convulsions. The mechanisms involved in these pharmacological effects are unknown and need to be elucidated in further studies.

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تأثير المادة الفعالة في أغاريض نخيل التمر (3, 4 Dimethoxy toluene) كمانع لحدوث التشنج في الفئران

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الملخص :

لقد تم من خلال هذا البحث دراسة تاثير المادة الفعالة في أغاريض النخل (3,4 Dimethoxy toluene) علي التشنج في الفئران بأستخدام اربع طرق مختلفة للبحث معتمدة في مثل هذة النوعية من البحوث: (١) بحقن مادة الـ (pentylenetetrazole) (٢) والمادة السامة (picrotoxin) (٣) ومادة النيكوتين (Nicotine) (٤) وكذلك باستخدام الصعق الكهربائي العالي الأكثر اعتمادا في مثل هذة التجارب. في التجربة الاولى وبعد حقن مادة الـ (pentylenetetrazole) ثم حقن المادة المراد دراستها (3,4 Dimethoxy toluene 100 mg/kg) عن طريق الصفاق وتبين ان لهذه المادة تاثيرا معنويا تمثل في تأخير وقت حدوث التشنجات في الفئران وكذلك حماية ٥٠٪ من الفئران من الموت. أما في تجربة الصعق الكهربائي العالي فأظهرت (3,4 Dimethoxy toluene 100 mg/kg) قدرة فائقة علي التثبيط الكامل للتشنجات للأرجل الخلفية من جراء الصعق الكهربائي العالي مع عدم حدوث أي وفيات في الفئران المصعوقة. أما في تجربة السم (picrotoxin, 12 mg/kg) فأظهرت المادة (3,4 Dimethoxy toluene) قدرة على تأخيرحدوث التشنجات والوفيات بشكل يعتد به إحصائيا وكذلك حماية كاملة من حدوث التشنجات بعد حقن مادة النيوكوتين (Nicotine, 0.8 mg/kg). يتضح من خلال هذة النتائج ان مادة (3,4 Dimethoxy toluene) مادة واعدة لعلاج التشنجات في الانسان.