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Short communication

The fruit essential oil of *Pimpinella anisum* exerts anticonvulsant effects in mice

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Abstract

This study investigates anticonvulsant effects of an essential oil of the fruits of *Pimpinella anisum* (Umbelliferae), a folkloric remedy in the Iranian traditional medicine, against seizures induced by pentylenetetrazole (PTZ) or maximal electroshock (MES) in male mice. The essential oil suppressed tonic convulsions induced by PTZ or MES. It also elevated the threshold of PTZ-induced clonic convulsions in mice. The essential oil produced motor impairment. However, this effect was not observed at the doses and time courses needed for anticonvulsant activity.

Keywords: *Pimpinella anisum*; Essential oil; Anticonvulsant effects; Pentylenetetrazole; Maximal electroshock; Mice

1. Introduction

Pimpinella anisum L., Umbelliferae, is an annual herb indigenous to Iran, India, Turkey and many other warm regions in the world (Zargari, 1989). In Iranian folk medicine, the plant and especially its fruit essential oil have been used for treatment of some disease including seizures and epilepsy (Avicenna, 1988). The aqueous extract of the collection of flowers, stems and leaves of *P. anisum* has been reported to delay the onset of picrotoxin-induced seizures in mice (Abdul-Ghani et al., 1987). However there is no study on the neurological properties of the essential oil of the fruits of this plant.

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Chemical studies have demonstrated the presence of eugenol (Monod and Dortan, 1950), anethole (Fujita and Nagasawa, 1960), methylchavicol and anisaldehyde (Wagner et al., 1984) and estragole (Zargari, 1989) as the major compounds of the fruit essential oil of *P. anisum*. Eugenol and estragole has reported to show anesthetic, hypothermic, muscle relaxant and anticonvulsant activities (Dallmeier and Carlini, 1981). Furthermore, anethole possesses muscle relaxant effect (Albuquerque et al., 1995).

The present work was undertaken to evaluate the effects of the fruit essential oil of *P. anisum* on seizures induced by pentylenetetrazole (PTZ) and maximal electroshock (MES). Moreover, it was assessed whether at the anticonvulsant doses the essential oil causes motor impairment.

2. Materials and methods

2.1. Plant material

Fruits of *P. anisum* were obtained from a local market. The plant was authenticated by M. Kamalinejad (Department of Pharmacognosy, Faculty of Pharmacy, Shaheed Beheshti University of Medical Sciences, Tehran, Iran) and a voucher specimen coded P-544 has been deposited at the herbarium of Department of Pharmacognosy, Faculty of Pharmacy, Shaheed Beheshti University of Medical Sciences, Tehran, Iran.

2.2. Preparation of the essential oil

The fruits were processed by steam distillation over a period of 4 h in all glass apparatus, to obtain the essential oil with 2% yield. The essential oil was diluted with sesame oil to obtain the desired doses and was immediately administered intraperitoneally (i.p.) to mice as a single dose expressed as ml of the essential oil per kg body weight.

2.3. Drugs

PTZ, Ethosuximide and Phenytoin sodium were purchased from Sigma (Poole, UK). Sesame oil was obtained from a local market. Different concentrations of the drugs were prepared freshly by dissolving in distilled water. The doses of the essential oil and time intervals were determined in preliminary tests. All i.p. injections were done in volumes of not higher than 10 ml/kg of body weight for mice.

2.4. Animals

Male NMRI mice (21–27 g; Razi Institute, Iran) were used throughout this study. The animals were maintained at constant room temperature (22.0 ± 3.0°C) and submitted to 12-h light/12-h dark cycle with food and water available ad libitum. They were housed in standard polycarbonate cages and acclimated at least 2 days before experiments. The experiments took place between 13:00 and 18:00 h.

2.5. Assessment of motor impairment

The rotarod test was used to determine the effect of the fruit essential oil on motor coordination. This test used a custom built apparatus (rotarod treadmill for mice, Hugo Sachs Elektronik, Germany), that consisted of an elevated rod (diameter 2 cm) that rotated at a constant speed (10 rpm). Mice were trained to walk continuously on the rod for a period of 120 s. The animals were then evaluated for motor coordina-

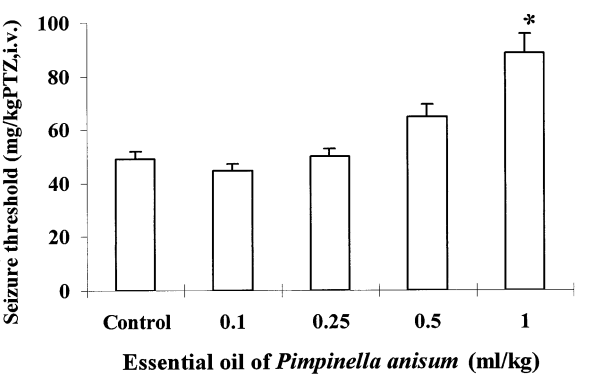

Fig. 1. Effect of *P. anisum* essential oil on clonic seizures threshold induced by pentylenetetrazole in male mice. Histograms represent mean ± S.E.M. for 10–30 animals. $*P < 0.01$.

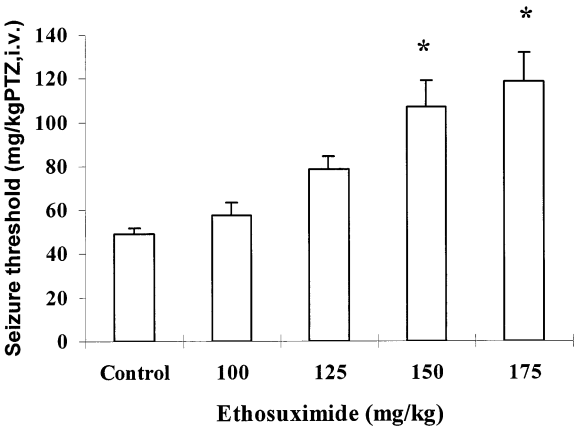

Fig. 2. Effect of ethosuximide on clonic seizures threshold induced by pentylenetetrazole in male mice. Histograms represent mean ± S.E.M. for 10–30 animals. $*P < 0.001$.

tion at 30 and 60 min after i.p. administration of the doses of 0.5, 0.75 and 1 ml/kg of the essential oil. The time each animal could walk continuously on the rod was recorded. A control group was also used which mice only received sesame oil (10 ml/kg, i.p.).

2.6. Convulsion tests

2.6.1. Threshold of PTZ-induced seizures

PTZ (1.0%) was infused into the tail vein of freely moving mice at a constant rate (1.0 ml/min) via a 30-gauge dental needle which was connected by polyethylene tubing to a Hamilton microsyringe. The onset of general clonus was used as the endpoint. The general clonus was characterized by forelimb clonus followed by full clonus of the body. The volume of PTZ solution required to attain the endpoint was recorded.

Considering the results obtained in the rotarod test, doses of 0.1, 0.25, 0.5 and 1 ml/kg of the essential oil were administered i.p. 30 min before PTZ infusion.

As positive control, doses of 100, 125, 150 and 175 mg/kg of ethosuximide were injected i.p. to mice 30 min before infusion of PTZ. Two other control groups were also used which mice received sesame oil or distilled water.

2.6.2. PTZ-induced seizures

PTZ at the dose of 85 mg/kg (minimal dose needed to induce convulsions) was injected i.p. to induce clonic-tonic convulsions in mice. Doses of 0.25, 0.5, 0.75 and 1 ml/kg of the essential oil were administered i.p. 30 min before PTZ injection. After injection of PTZ the mice were observed for 30 min to detect the occurrence of hind limb tonic extensions (HLTE) and mortality. If no HLTE occurred during the time limit, the animals were considered protected. Sesame oil (10 ml/kg, i.p.) or ethosuximide (150 mg/kg, i.p.) were injected into two groups of animals 30 min before PTZ injection as control and positive control groups, respectively.

2.6.3. MES-induced seizures

Electroconvulsive shock (150 volts, 25 ohms, 50 pulses per second, 0.2 s duration) was delivered through ear-clip electrodes to induce HLTE in mice. The essential oil was administered i.p. at the doses of 0.1, 0.25, 0.5 and 1 ml/kg and 30 min later electroconvulsive shock was delivered. After electrical stimulation occurrence of HLTE and incidence of mortality were noted. The animals that did not exhibit HLTE were considered protected. Sesame oil (10 ml/kg, i.p.) or phenytoin (20 mg/kg, i.p.) were injected into two groups of animals 30 min before electrical stimulation as control and positive control groups, respectively.

2.7. Toxicity assessment

Doses of 0.5, 0.75, 1, 1.5, 2 and 2.5 ml/kg of the essential oil were administered i.p. to mice and incidence of mortality was noted up to 24 h after injection. The control group received sesame oil.

2.8. Data analysis

The one-way analysis of variance (ANOVA) followed by Tukey–Kramer multiple-comparisons test was used to analyse data obtained from clonic seizures threshold determination and rotarod test. Fisher's exact test was used to analyse data obtained from MES and PTZ-induced seizures. A P-value of less than 0.05 was the critical criterion for statistical significance.

Table 1
Effect of *P. anisum* essential oil on pentylenetetrazole-induced seizures in male mice

Treatment	Dose	% Of convulsions	% Of mortality
Control (distilled water)	10 ml/kg	100	100
Control (sesame oil)	10 ml/kg	100	100
Control (ethosuximide)	150 mg/kg	0**	0**
P. anisum	0.25 ml/kg	80	80
P. anisum	0.5 ml/kg	50*	40**
P. anisum	0.75 ml/kg	40**	20**
P. anisum	1.0 ml/kg	20**	10**

Data represent percentage of convulsions and mortality ($n = 10$).
*$P < 0.01$; **$P < 0.001$.

The dose of the essential oil needed to produce an anticonvulsant (ED_{50}) or lethal (LD_{50}) effect in 50% of animals and its associated 95% confidence limits was calculated by the method of Litchfield and Wilcoxon (Litchfield and Wilcoxon, 1949) using a commercial computer program (PHARM/PCS version 4.2).

3. Results

3.1. Effect on motor function

The essential oil did not induce statistically significant disturbance in motor coordination up to a dose of 1 ml/kg at 30 min post-administration period. However, the dose of 1 ml/kg of the essential oil 60 min after injection and also the doses higher than 1 ml/kg 30 min after injection produced severe sedation (endurance time on rotarod = 0).

3.2. Toxicity assessment

LD_{50} value of 0.93 (1.11–0.79) ml/kg was obtained for the essential oil.

3.3. Convulsion tests

3.3.1. Effect on the threshold of PTZ-induced seizures

The essential oil increased, in a dose dependent manner, the dose of i.v. infused PTZ needed to induce clonic seizures in mice (Fig. 1). Ethosuximide also did so (Fig. 2).

3.3.2. Activity against PTZ-induced seizure

The essential oil at the doses of 0.5, 0.75 and 1 ml/kg significantly suppressed PTZ-induced HLTE and mortality (Table 1).

ED_{50} value of 0.52 (0.35–0.76) ml/kg was obtained for the essential oil.

3.3.3. Effect on MES-induced seizures

The essential oil significantly blocked HLTE and mortality with an i.p. ED_{50} of 0.20 (0.12–0.33) ml/kg (Table 2).

4. Discussion and conclusion

The present study revealed that *P. anisum* increases the threshold of clonic seizures induced by i.v. infusion of PTZ and it can also block tonic convulsions induced by i.p. injection of PTZ. Moreover, *P. anisum* possesses anticonvulsant activity against tonic seizures induced by MES. It has often been stated that antiepileptic drugs that prevent or delay clonic convulsions induced by i.v. infusion of PTZ, act by elevating the seizure threshold, whereas drugs that block MES-induced tonic extensions, act by blocking seizure spread (Loscher and Schmidt, 1988; Rogawski and Porter, 1990). Such observations suggest that the essential oil may act both by increasing seizure threshold and also inhibiting seizure spread.

Our results showed that the essential oil induces disturbance in motor coordination. However, this effect was not significant at the doses and time intervals in which the anticonvulsant activity was observed.

Table 2
Effect of *P. anisum* essential oil on tonic seizures induced by maximal electroshock in male mice

Treatment	Dose	% Of convulsions	% Of mortality
Control (distilled water)	10 ml/kg	100	20
Control (sesame oil)	10 ml/kg	100	20
Control (phenytoin)	20 mg/kg	0**	0
P. anisum	0.1 ml/kg	80	10
P. anisum	0.25 ml/kg	40*	10
P. anisum	0.5 ml/kg	10**	10
P. anisum	1 ml/kg	10**	10

Data represent percentage of tonic seizures and mortality ($n = 10$).
*$P < 0.01$; **$P < 0.001$.

The anticonvulsant activity of *P. anisum* observed in the present study may be related to estragole and eugenol presented in the essential oil of the plant (Zargari, 1989; Monod and Dortan, 1950) which their anticonvulsant activity has been shown by some researches (Dallmeier and Carlini, 1981). Furthermore, eugenol, anethole, and estragole presented in the essential oil of *P. anisum* (Monod and Dortan, 1950; Fujita and Nagasawa, 1960; Zargari, 1989) possess muscle relaxant property (Albuquerque et al., 1995). The motor impairment observed in our study may be related to these compounds.

We conclude that the fruit essential oil of *P. anisum* possesses anticonvulsant activity against PTZ and MES induced convulsions. The mechanisms and the active compound(s) involved in these pharmacological effects are unknown and need to be elucidated in further studies.

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