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Evaluation of the anticonvulsant activity of the seed acetone extract of *Ferula gummosa* Boiss. against seizures induced by pentylenetetrazole and electroconvulsive shock in mice

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Abstract

Ferula gummosa Boiss. (Apiaceae) which has been used as an antiepileptic remedy in Iranian traditional medicine was evaluated for anticonvulsant activity against experimental seizures. The seed acetone extract of *F. gummosa* protected mice against tonic convulsions induced by maximal electroshock (the median effective dose [ED_{50}] = 198.3 mg/kg) and especially by pentylenetetrazole (ED_{50} = 55 mg/kg). Neurotoxicity (sedation and motor impairment) of the extract was assessed by the rotarod test and the median toxic dose (TD_{50}) value of 375.8 mg/kg was obtained. Preliminary phytochemical analysis showed the presence of terpenoids and alkaloids in the extract. The acceptable acute toxicity of the extract recommends further studies to determine the mechanism(s) and compound(s) involved in the anticonvulsant activity.

Keywords: *Ferula gummosa*; Seizure; Maximal electroshock; Pentylenetetrazole

1. Introduction

Ferula gummosa Boiss. (Apiaceae) is a perennial plant native to central Asia, growing in the northern and western parts of Iran (Zargari, 1989). In Iranian ancient medicine, the gum obtained from the aerial parts of this plant has been used for stomach pain, chorea, epilepsy and as a wound-healing remedy (Aqili Khorasani, 1991; Zargari, 1989). In recent years there are some reports regarding the central effects of this plant. An antinociceptive activity has been shown for the hydroalcoholic extract of aerial parts of *F. gummosa,* which is mediated in part through opioid receptors activation (Fazly Bazaz et al., 1997). Furthermore, a methanol-chloroform (1:1) extract of *F. gummosa* and its fractions have alleviated the morphine withdrawal syndrome induced by naloxone (Ramezani et al., 2001). We recently reported the anticonvulsant potential of an essential oil of the seed of *F. gummosa* (Sayyah et al., 2001). In the present work, we have examined the possible protective effect of the several seed extracts of *F. gummosa* against seizures induced by maximal electroshock (MES) or pentylenetetrazole (PTZ). In order to evaluate the therapeutic value and safety, the neurotoxicity (sedation and motor impairment) and lethality of the extracts were determined as well.

2. Materials and methods

2.1. Plant materials

Seeds of *F. gummosa* were collected from Polour, 90-km northeast of Tehran, in May 2001. *F. gummosa* was authenticated by M. Kamalinejad and a voucher specimen (no. 563) was deposited in the herbarium of Faculty of Pharmacy, Shaheed Beheshti University of Medical Sciences, Tehran, Iran.

2.2. Extracts preparation

Air-dried seeds of the plant were macerated, in portions of 200 g, with acetone or methanol or ethyl

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acetate (2 l) for 3 days. The mixtures were then filtered and the filtrates were concentrated with a rotaevaporator apparatus. The residues then were dried at room temperature. The final weight of the crude extracts were 13.5 g. The extracts were maintained at 4 °C throughout experiments.

2.3. Drugs

Acetone, methanol, ethyl acetate and Tween 80 were purchased from E. Merck (Darmstadt, Germany). PTZ, phenytoin and ethosuximide were purchased from Sigma (Poole, UK). PTZ and ethosuximide were dissolved in saline solution (0.9%). Phenytoin sodium was dissolved in saline that was alkalinized slightly with potassium hydrochloride 0.1 mM. The extracts were dissolved in 5% v/v Tween 80 in distilled water. All drugs and the extracts were administrated intraperitoneally (i.p.) in volume of 0.1 ml/10g of mice body weight.

2.4. Animals

Male NMRI mice (Institute Pasteur of Iran), weighing 18–28 g 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 23 ± 3.0 °C with a 12-h light/dark cycle (light on from 06:00 to 18:00 h). The ethical guidelines for the investigation of experimental seizures in conscious animals were followed in all tests. All efforts were made to minimize animal suffering and to reduce the number of animals used.

2.5. MES-induced seizures

Electro-convulsive shock, inducing Hind Limb Tonic Extension (HLTE) in 99.9% of the animals (Swinyard, 1969), was previously determined by a current-percent effect curve (Litchfield and Wilcoxon, 1949). The electrical stimulus (50 mA, 50 Hz, 1 s duration) was applied through ear-clip electrodes using a stimulator apparatus (MGH-777, Iran). 13 groups of 12 mice each pretreated i.p. with the acetone extract (100, 150, 200, 250, 300 and 350 mg/kg), methanolic extract (250 and 500 mg/kg), ethyl acetate extract (250 and 500 mg/kg) phenytoin (25 mg/kg, as positive control), saline (10 ml/kg, as control) and Tween preparation (10 ml/kg, as control) received the transauricular electroshock.

The time of peak effect of phenytoin (30 min after administration) was previously established (Pourgholami et al., 1999). The time for the extract to reach its maximum effect was determined as 30 min after i.p. injection. The criterion for the anticonvulsant effect was abolition of HLTE within 10 s after delivery of the electroshock.

2.6. PTZ-induced seizures

The minimal i.p. dose of PTZ at which 99.9% of the animals showed HLTE (Swinyard, 1969) was determined by a dose-percent effect curve (Litchfield and Wilcoxon, 1949). This dose (100 mg/kg) was then given to 14 groups of 12 mice each pretreated i.p. with the acetone extract (12.5, 25, 50, 100, 150, 200 and 250 mg/kg), methanolic extract (250 and 500 mg/kg), ethyl acetate extract (250 and 500 mg/kg), ethosuximide (150 mg/kg, as positive control), saline (10 ml/kg, as control) or Tween preparation (10 ml/kg, as control). The time of peak effect of ethosuximide (30 min after administration) was previously established (Pourgholami et al., 1999). The time for the extract to reach its maximum effect was determined as 30 min after i.p. injection. If no HLTE occurred during a 30-min period of observation, the animals were considered protected.

2.7. Neurotoxicity and lethality tests

Six groups of 12–20 mice each were treated i.p. with the Tween preparation (10 ml/kg, as control) or the acetone extract (100, 200, 300, 500 and 600 mg/kg) and tested on the rotarod at 30-min interval according to the method of Dunham and Miya, 1957. The apparatus (MGH- 778, Iran) consisted of a horizontal rod with 3.5-cm diameter moving on its axis at 15 rpm and subdivided into five compartments by Plexiglas disks. Predilection was done on the experimental day by eliminating the animals which did not remain on the rotarod for at least two consecutive periods of 120 s. After injections, animal were given three opportunities to remain on the rod continuously for 120 s.

For determination of the neurotoxic dose for 50% of the mice (TD_{50}), the percentage of the animals that fell off the bar within 1 min was considered.

Lethality was determined by i.p. injection of 750, 1000, 1500, 2000 and 2500 mg/kg of the acetone extract and 10 ml/kg of the Tween preparation (control) to six groups of 15 mice. The number of deaths was recorded after 24h.

2.8. Preliminary phytochemical tests

The *F. gummosa* seed acetone extract was screened for alkaloids, coumarins, amino acids and terpenoids (Wagner and Bladt, 1996).

2.9. Data analysis

The dose of the extract required to produce an anticonvulsant effect (ED_{50}) or motor impairment (TD_{50}) or death (LD_{50}) in 50% of animals and its associated 95% confidence limit was calculated by the method of Litchfield and Wilcoxon, 1949, using a

commercial computer program (PHARM/PCS version 4.2). The protective index (PI) and the therapeutic index (TI) of the extract were calculated by dividing the TD_{50} and LD_{50} by the ED_{50}, respectively. Data obtained from convulsion tests were expressed as percentage of convulsions and Fisher's exact test was used to analyze the data. Data obtained from rotarod test were expressed as mean ± SEM and were analyzed by one-way analysis of variance (ANOVA) followed by the Tukey–Kramer multiple comparisons test. P value of less than 0.05 was the critical criterion for statistical significance.

3. Results

3.1. Anticonvulsant activity

3.1.1. MES-induced seizures

The methanolic and ethyl acetate extracts at the dose of 250 mg/kg did not exert any anticonvulsant effect against MES-induced seizures and at the dose of 500 mg/kg were very sedative.

However, the acetone extract produced dose-dependent anticonvulsant effect with an ED_{50} of 198.34 (171.93–228.8) mg/kg (Table 1).

3.1.2. PTZ-induced seizures

The methanolic and ethyl acetate extracts at the dose of 250 mg/kg did not exert any anticonvulsant effect against PTZ-induced seizures and at the dose of 500 mg/kg were very sedative.

However, the acetone extract prevented PTZ-induced seizures in a dose-dependent manner (Table 2). The ED_{50} value of 55 (34.65–87.43) mg/kg was obtained for the extract.

3.2. Neurotoxicity and lethality

From the dose of 300 mg/kg and at 30 min after administration, the acetone extract produced a reduction in time spent on rotarod (Fig. 1). This sedation and motor impairment was dose-dependent, with TD_{50} value of 375.8 (246.1–478.8) mg/kg. In this regard PI values of 1.8 and 6.6 were obtained for the acetone extract against seizures induced by MES and PTZ, respectively. However, at the doses employed the extract did not exert any other noticeable effect on the animals' behavior.

LD_{50} value of 1720.7 (1469.5–2015.0) mg/kg was obtained for the acetone extract and TI values were 8.6 and 31.2 against seizures induced by MES and PTZ, respectively.

Table 1
Effect of *F. gummosa* seed acetone extract on tonic seizures induced by maximal electroshock in mice

Treatment	Dose	Convulsions (%)
Saline	10 (ml/kg)	100
Tween 80 (5%, v/v)	10 (ml/kg)	100
Phenytoin	25 (mg/kg)	0**
F. gummosa	100 (mg/kg)	91.6
F. gummosa	150 (mg/kg)	80
F. gummosa	200 (mg/kg)	55*
F. gummosa	250 (mg/kg)	55*
F. gummosa	300 (mg/kg)	10**
F. gummosa	350 (mg/kg)	0**

Data represent percentage of tonic seizures ($n = 12$–20).
* $P < 0.05$ and ** $P < 0.001$ compared to control value.

Table 2
Effect of *F. gummosa* seed acetone extract on seizures induced by pentylenetetrazole in mice

Convulsions (%)	Dose	Treatment
100	10 (ml/kg)	Saline
100	10 (ml/kg)	Tween 80 (5%, v/v)
0**	150 (mg/kg)	Ethosuximide
83.3	12.5 (mg/kg)	*F. gummosa*
66.7	25 (mg/kg)	*F. gummosa*
66.7	50 (mg/kg)	*F. gummosa*
41.6*	100 (mg/kg)	*F. gummosa*
25**	150 (mg/kg)	*F. gummosa*
25**	200 (mg/kg)	*F. gummosa*
0**	250 (mg/kg)	*F. gummosa*

Data represent percentage of tonic seizures ($n = 12$).
* $P < 0.05$ and ** $P < 0.001$ compared to control value.

3.3. Preliminary phytochemical screening

Preliminary phytochemical screening of the *F. gummosa* showed that the acetone extract of seeds contains terpenoids and alkaloids, while amino acids and coumarin compounds were absent.

4. Discussion and conclusions

Current available anticonvulsant drugs are able to efficiently control epileptic seizures in about 50% of the

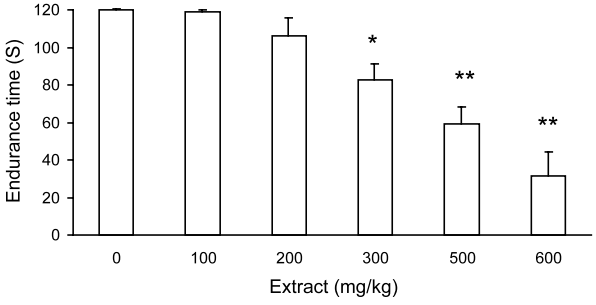

Fig. 1. Effect of *F. gummosa* seed acetone extract on motor function 30 min after i.p. administration to mice. Histograms represent mean ± S.E.M. for 12–20 mice. * $P < 0.01$ and ** $P < 0.001$.

patients; another 25% may show improvement whereas the remainder does not benefit significantly (MacNamara, 1994). Furthermore, undesirable side effects from the drugs used clinically often render treatment difficult; so that a demand for new types of anticonvulsants exists. One of the approaches to search for new antiepileptic drugs is the investigation of naturally-occurring compounds, which may belong to new structural classes.

In Iranian traditional medicine, the gum obtained from the aerial parts of *F. gummosa* was used as an antiepileptic remedy (Aqili Khorasani, 1991). We previously reported the anticonvulsant potential of an essential oil of the seed of *F. gummosa* against experimental seizures (Sayyah et al., 2001). However, the essential oil produced neurotoxicity and its LD_{50} was too close to the anticonvulsant dose (Sayyah et al., 2001). In the present study we have evaluated the effect of the seed methanolic, ethyl acetate and acetone extracts of *F. gummosa* on seizures induced by MES and PTZ in mice. The results indicate that only the acetone extract has an anticonvulsant effect, which is dose-dependent. The ED_{50} values obtained for the acetone extract indicate that it possesses more potent protective effect against seizures induced by PTZ ($ED_{50} = 55$ mg/kg) than MES-induced seizures ($ED_{50} = 198.3$ mg/kg).

It has often been stated that antiepileptic drugs that block MES-induced tonic extension act by blocking seizure spread (Rogawski and Porter, 1995). Moreover, MES-induced tonic extension can be prevented either by drugs that inhibit voltage-dependent Na^+ channels, such as phenytoin, valproate, felbamate and lamotrigine (Rogawski and Porter, 1995; MacDonald and Kelly, 1995; White et al., 1995); or by drugs that block glutamatergic excitation mediated by the *N*-methyl-D-aspartate (NMDA) receptor, such as felbamate (Subramaniam et al., 1995; MacCabe et al., 1993).

On the other hand, drugs that reduce T-type Ca^{2+} currents, such as ethosuximide can prevent seizures induced by PTZ (Coulter et al., 1989). This type of seizures can also be prevented by drugs that enhance gamma amino butyric acid type A ($GABA_A$) receptor-mediated inhibitory neurotransmission, such as benzodiazepines and phenobarbital and perhaps valproate and felbamate (Rogawski and Porter, 1995; MacDonald and Kelly, 1995; White et al., 1995).

Gas chromatography/mass spectroscopy analyses of the seed essential oil of *F. gummosa* has been shown that near to 80% of the essential oil composed of the monoterpens β- and α-pinene (Sayyah et al., 2001). Preliminary phytochemical analysis performed in this study, shows the presence of terpenoids and alkaloids in the seed acetone extract of *F. gummosa.*

Some researchers have reported anticonvulsant activity of monoterpenes. SL-1, a synthetic monoterpene homologue of GABA, demonstrated anticonvulsant activity in PTZ-induced seizures (Librowski et al., 2000). Linalool is another monoterpene compound which has protective effect against PTZ-, picrotoxin- and NMDA-induced convulsions (Silva Brum et al., 2001). Moreover, pinene, eugenol and methyleugenol exhibited anticonvulsant profile in some experimental seizures such as MES and PTZ tests (Consroe et al., 1981; Dallmeier and Carlini, 1981). Modulation of glutamatergic and GABAergic transmission are some mechanisms indicated for anticonvulsant action of the monoterpenes like linalool and eugenol (Silva Brum et al., 2001; Szbadics and Erdelyi, 2000; Wie et al., 1997).

Therefore, it seems that the antiseizure profile of *F. gummosa* seed may be related in part to monoterpens and terpenoid compounds present in the seed.

Results of the present study revealed that the acetone extract of *F. gummosa* produces sedation and motor deficits at some anticonvulsant doses. pI values of 1.8 and 6.6 were obtained for the extract against MES and PTZ-induced seizures. Some terpene compounds such as eugenol and methyleugenol and also cineol exhibited anesthetic, muscle relaxant and inhibitory effect on locomotion (Dallmeier and Carlini, 1981; Santos and Rao, 2000).

TI values of the extract for MES and PTZ-induced seizures (8.6 and 31.2, respectively) suggest acceptable therapeutic effect for the extract. However, chronic toxicity studies must be performed in order to assess the real toxicological profile of the extract.

The seed acetone extract of *F. gummosa* possesses protective effect against experimental seizures induced by PTZ and MES. The PIs obtained for the extract show that the extract is more favorable in PTZ-induced seizures. However, the exact mechanism(s) and the active compound(s) involved in these effects need to be clarified in future studies.

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