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Antiepileptic Potential and Composition of the Fruit Essential Oil of *Ferula Gummosa boiss*

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ABSTRACT

The fruit essential oil of *Ferula gummosa boiss*., Umbelliferae, which has been used as an antiepileptic remedy in Iranian traditional medicine, was evaluated for anticonvulsant activity against experimental seizures. The essential oil had no effect against seizures induced by maximal electroshock. However, it protected mice against pentylenetetrazole-induced tonic seizures. The protective dose produced neurotoxicity. Moreover, this dose was too close to the LD_{50} of the essential oil. Gas chromatography and gas chromatography-mass spectroscopy analyses of the essential oil revealed the presence of -pinene (50.1%), ~-pinene (18.3%), 3-carene (6.7%), ~-thujene (3.3%) and sabinene (3.1%) as the main components. The anticonvulsant and toxic effects of the essential oil may be related to the compounds pinene and ~-thujene respectively that present in the essential oil. *Iran. Biomed. J. 5 (2 & 3): 69-72, 2001*

Keywords: Constituents, Essential oil, *Ferula gummosa*, Seizure protection

INTRODUCTION

Ferula gummosa boiss., Umbelliferae, is a wild plant indigenous to Iran. It grows in the northern and western parts of the country [1]. In Iranian folk medicine, this plant has been used for stomach pain, epilepsy and as a wound healing remedy [1]. However, there is no report about the neurological effects of the fruit essential of *F. gummosa*. The present study was undertaken to determine the chemical composition and anticonvulsant activity of the fruit essential oil of *F. gummosa* against seizures induced by pentylenetetrazole (PTZ) or maximal electroshock (MES) in mice. In order to evaluate the therapeutic value and safety, the neurotoxicity (sedation and motor impairment) and lethality of the essential oil were determined as well.

MATERIALS AND METHODS

Plant material. *F. gummosa* was collected from Ploor, 30-km northeast of Tehran, in July 1997. Voucher specimen (No. 563) was deposited in the herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Shaheed Beheshti University of Medical Sciences, Tehran.

Preparation of the essential oil. The fruits were obtained from dried plant (at room temperature) and subjected to hydrodistillation for 3 hours using a Clevenger apparatus. The fruits yielded 6-7% (v/w) essential oil. The essential oil was diluted freshly with sesame oil and shaken vigorously to obtain desired doses.

Drugs. PTZ, phenytoin sodium and ethosuximide were purchased from Sigma (Poole, UK). They were prepared in saline solution. The essential oil was diluted with sesame oil. All intraperitoneal (i.p.) injections were administered in volumes not higher than 10 ml/kg of body weight of animals.

Animals. Male NMRI mice (20-28 g, Pasteur Institute of Iran) were used throughout this study. The animals were maintained at constant room temperature (22.0 ± 3.0°C) and submitted to a 12-h light/dark cycle with food and water available ad libitum.

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MES-induced seizures. Electro-convulsive shock inducing Hind Limb Tonic Extension (HLTE) in 99.9% of the animals [2] was previously determined by a current-percent effect curve [3]. The electrical stimulus (50 mA, 50 Hz, 1 s duration) was applied through ear-clip electrodes to 5 groups of 10 mice each previously treated i.p. with doses of 1, 1.5 and 2.5 ml/kg of the essential oil or phenytoin (25 mg/kg, as positive control) or sesame oil (10 ml/kg, as control). The time of peak effect of the essential oil or phenytoin (30 min after administration) was previously established. The criterion for the anticonvulsant effect was abolition of HLTE within 10 s after delivery of the electroshock.

PTZ-induced seizures. The minimal i.p. dose of PTZ that 99.9% of the animals showed HLTE [2] was determined by a dose-percent effect curve [3]. This dose (110 mg/kg) was then given to 5 groups of 10 mice each previously treated i.p. with the essential oil (1, 1.5 and 2.5 ml/kg) or ethosuximide (150 mg/kg, as positive control) or sesame oil (10 ml/kg, as control). The time of peak effect of the essential oil or ethosuximide (30 min after administration) was previously established. If no HLTE occurred during a 30-min period of observation, the animals were considered protected.

Neurotoxicity and lethality tests. Groups of 10 previously selected mice were treated i.p. with the sesame oil (10 ml/kg, as control) or the essential oil (1, 1.5, 2.5 and 5 ml/kg) and tested on the rotarod at 30-min interval according to the method of Dunham and Miya [4]. 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 90 s.

Lethality was determined by i.p. injecting groups of 10 mice each with the doses of 1, 2.5, 4 and 5 ml/kg of the essential oil. The number of deaths was recorded after 24 h and the LD_{50} (the dose needed to produce lethal effect in 50% of the animals) of the essential oil and its associated 95% confidence limit was calculated by the method of Litchfield and Wilcoxon [3].

Gas chromatography (GC) and gas chromatography/mass spectroscopy (GC/MS). GC analysis was performed using a Packard 439 chromatograph equipped with CP Sil 5CB column (25 m × 0.25 mm i.d., film thickness 0.39 μm), temperature programmed 5°C/min from 60°C to 220°C, carrier gas N_2 (0.8 ml/min), split ratio 1:100, injector and detector temperature were 270°C. For mass data a Varian 3700 chromatograph was used with a CP Sil 5CB column (25 m × 0.25 mm i.d., film thickness 0.39 μm) combined with Varian MAT 44S (70 ev), temperature programmed as above, helium as the carrier gas. The identification of the components was based on comparison of their mass spectra with standard spectra and confirmed by comparison of their retention indices relative to C_9-C_{16} n-alkanes with literature values [5, 6].

Data analysis. Data obtained from convulsion tests were expressed as percent of convulsions and mortality, 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. $P<0.05$ was the critical criterion for statistical significance.

RESULTS

The essential oil of *F. gummosa* had no protective effect against seizures induced by MES. However, at the dose of 2.5 ml/kg, the essential oil significantly suppressed PTZ-induced convulsions and mortality (Table 1). This dose had neurotoxicity producing sedation and motor impairment, which reached to the peak at the dose of 5 ml/kg (Fig. 1). Moreover, LD_{50} value of 2.62 (1.99-3.43) ml/kg was obtained for the essential oil. As shown in Table 2, 17 components were identified, constituting 94.6% of the essential oil in which the principal components were β-pinene (50.1%), α-pinene (18.3%), 3-carene (6.7%), α-thujene (3.3%) and sabinene (3.1%).

DISCUSSION

This study revealed that the fruit essential oil of *F. gummosa* blocked PTZ (but not MES) -induced seizures in mice. Prevention of seizures induced by PTZ in laboratory animals is the most commonly used initial screening test for characterizing potential anticonvulsant drugs. It has been often

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Table 1. Effect of *F. gummosa* fruit essential oil on seizures induced by pentylenetetrazole or maximal electroshock in mice.

Treatment	Dose (Per kg)	Pentylenetetrazole, Convulsions (%), Mortality (%)	Maximal electroshock Convulsions (%), Mortality(%)
Sesame oil	10 ml	90, 90	100, 100
F. gummosa	1 ml	83.3, 83.3	100, 100
F. gummosa	1.5 ml	66.6, 58.3	100, 90
F. gummosa	2.5 ml	[a]25, [a]25	90, 90
Ethosuximide	150 mg	[b]0, [b]0	-
Phenytoin	25 mg	-	[c]0, [c]0

n = 10-12 animals, [a]$p< 0.05$ and [b]$p< 0.01$ compared to control of pentylene-tetrazole group, [c]$p< 0.01$ compared to the control of maximal electroshock group

stated that seizures induced by PTZ, can be blocked by drugs that reduce T-type Ca^{2+} currents, such as ethosuximide [7], and drugs that enhance gamma aminobutyric acid type A ($GABA_A$) receptor mediated inhibitory neuro-transmission, such as benzodiazepines and phenobarbital [8]. So, it is possible that the essential oil exerts its anticonvulsant action by modulation of Ca^{2+} currents or $GABA_A$ receptor.

GC/MS analysis showed that nearly 70% of the essential oil composed of ß- and α-pinene. It has been shown that some analogs of pinene prevent the audiogenic seizures in susceptible rats [9]. The

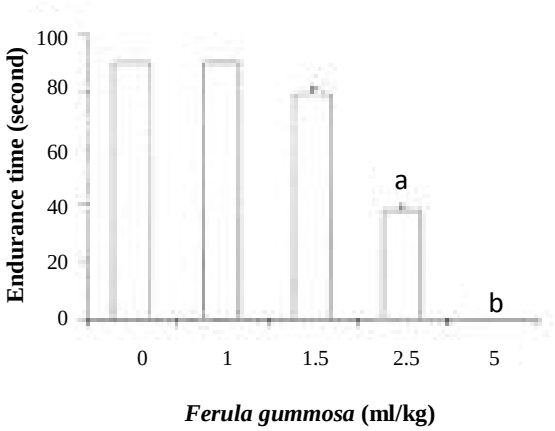

Fig. 1. Effect of the fruit essential oil of *F. gummosa* on motor function, 30 min after i.p. administration to mice. Histograms represent mean ± SEM for 12 animals. [a]$p< 0.05$ and [b]$p< 0.01$ compared to the control group.

Table 2. Constituents of the fruit essential oil of *F. gummosa*obtained by GC/MS analysis.

No	Component (GC/MS)	[a]RI	%
1	α-Thujene	926	3.3
2	α- Pinene	936	18.3
3	Camphene	949	0.2
4	Sabinene	970	3.1
5	β-Pinene	977	50.1
6	α-Phellandrene	998	0.3
7	δ-3-Carene	1007	6.7
8	Allo-ocimene	1024	2.9
9	β-Phellandrene	1028	2.1
10	Myrtenal	1173	0.5
11	α-Cubebene	1333	0.4
12	α-Elemene	1431	0.9
13	Germacrene-D	1478	0.9
14	β-Gurjunene	1480	1.8
15	α-Muurolene	1497	0.8
16	δ- Cadinene	1522	1.2
17	β-Sesquiphellandrene	1668	1.1

[a]Retention indices on CP Sil 5CB column.

anticonvulsant activity of the essential oil, observed in the present study, may be related to the presence of pinene compounds in the plant. However, the essential oil produced neurotoxicity and its LD_{50} value (2.62 ml/kg) was too close to the anticonvulsant dose (2.5 ml/kg). This toxicity may be related to the presence of α-thujene in the essential oil, which possesses toxic and lethal effects [10, 11]. Further studies are needed before any precise conclusions can be drawn.

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