Short Report

Anticonvulsant Effect of *Ferula Gummosa* Root Extract against Experimental Seizures

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ABSTRACT

In Iranian traditional medicine, there are some report regarding the anticonvulsant effect of *Ferula gummosa* Boiss. In this study, anticonvulsant activity, neurological deficits and lethality of the root acetone extract of this plant were evaluated in mice. The extract exhibited dose-dependent prevention of tonic seizures induced by pentylenetetrazole (ED_{50} = 154.4 mg/kg). However, the extract produced sedation and motor impairment with TD_{50} value of 546 mg/kg. Preliminary phytochemical analysis showed the presence of terpenoids, alkaloids and a little amount of cardenolids in the extract. It seems that the anticonvulsant and neurotoxic effects of the extract is related in part to the terpenoid compounds. The acceptable protective index of the extract (3.5) recommends further studies on *Ferula gummosa.* *Iran. Biomed. J. 7 (3): 139-143, 2003*

Keywords: Ferula gummosa; Anticonvulsant activity; Root; Acetone extract

INTRODUCTION

Ferula gummosa Boiss. (Apiaceae) is a perennial plant native to central Asia, growing in the northern and western parts of Iran. In Iranian ancient medicinal literatures, there are some reports regarding the anticonvulsant, antispasmodic, expectorant and wound-healing activities of this plant.

Aerial parts of *F. gummosa* have been demonstrated to be antinociceptive [1] that alleviate morphine withdrawal syndrome [2] in part through opioid receptor activation. We recently reported the anticonvulsant activity of the seed acetone extract of *F. gummosa* against experimental seizures [3]. However, the main product of this plant, which is used as a traditional medicine, is a gum obtained from the stem and the root. Therefore, we decided to evaluate the antiseizure effect of *F. gummosa* root. Our preliminary studies on the different non-polar extracts of the root demonstrated that among the methanolic, ethyl acetate and acetone extracts of the root, the acetone extract has low toxicity and is a good candidate for further studies [unpublished data]. Here, we report the anticonvulsant effect of *F. gummosa* root acetone extract against tonic seizures induced by maximal electroshock (MES) and pentylenetetrazole (PTZ). Neurotoxicity and lethality of the extract have been determined as well.

MATERIALS AND METHODS

Plant material. Root of the wild growing *F. gummosa* was 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.

Extract preparation. Air-dried root of the plant (300 g) was macerated with acetone (2 L) for 3 days. The mixture was filtered and then concentrated with a rotaevaporator apparatus and the residue was dried at room temperature. The final weight of the crude extract was 14 g. The extract was maintained at 4˚C throughout the experiments.

Drugs. Acetone 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 physiologic saline solution. Phenytoin sodium was dissolved in saline that was alkalinized slightly with 0.1 mM potassium hydrochloride. The extracts were dissolved in Tween 80 in distilled water 5% v/v. All drugs and the extracts were administrated intraperitoneally (i.p.) in volume of 0.1 ml/10g of mice body weight.

Animals. Male NMRI mice (the Pasteur Institute 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 6:00 A.M to 6:00 P.M). 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.

Convulsions tests:

MES-induced seizures. Electro-convulsive shock, inducing hind limb tonic extension (HLTE) in 99.9% of the animals [4], was previously determined by a current-percent effect curve [5]. The electrical stimulus (50 mA, 50 Hz, 1s duration) was applied through ear-clip electrodes using a stimulator apparatus (MGH-777, Electronic Industry Development Co., Iran). Six groups of 12 mice each pretreated i.p. with the extract (300, 500 and 750 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 [6]. 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.

PTZ-induced seizures. The minimal i.p. dose of PTZ at which 99.9% of the animals showed HLTE [4] was determined by a dose-percent effect curve [5]. This dose (100 mg/kg) was then given to 8 groups of 12 mice each pretreated i.p. with the extract (50, 100, 300, 400 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 [6]. 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 observation, the animals were considered protected.

Rotarod performance and lethality tests. Five groups of 12 mice, each were treated i.p. with the Tween preparation (10 ml/kg, as control) or the extract (300, 400, 500 and 600 mg/kg) and tested on the rotarod at a 30-min interval according to the method described by others [7-9]. 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 that did not remain on the rotarod for at least two consecutive periods of 120 s. After injections, animals were given three opportunities to remain continuously on the rod for 120 s.

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

Lethality was determined for six groups of 15 mice by i.p. injection of the extract (750, 1000, 1200, 1400 and 1500 mg/kg) and 10 ml/kg of the Tween preparation (control). After 24h the number of dead animals was recorded.

Preliminary phytochemical tests:

F. gummosa root extract was screened for alkaloids, cardenolids, cumarins, amino acids and terpenoids [10].

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 [5], using a commercial computer program (PHARM/PCS v n 4.2). The protective index (PI) and the therapeutic index (TI) of the extract were calc d by dividing the TD_{50} and LD_{50} by the ED_{50}, respectively. Data obtained from convu tests were expressed as percer of convulsions and Fisher's exact test was used to yze the data. Data obtained from rotarod test were expressed as mean ± SEM and wer alyzed by one-way analysis of variance (ANOVA) followed by the Tukey-Kramer mult comparisons test. $P<0.05$ was the critical criterion for statistical significance.

RESULTS

F. gummosa root acetone extract (300-500 mg/kg, i.p.) significantl nced seizures in a dose-dependent manner (Table 1). The ED_{50} value o g/kg was obtained for the extract. In PTZ test, the latency of HLTE and percent of mice mortality were obtained at 2-3 min and 100%, respectively. However, these parameters were not affected by the extract.

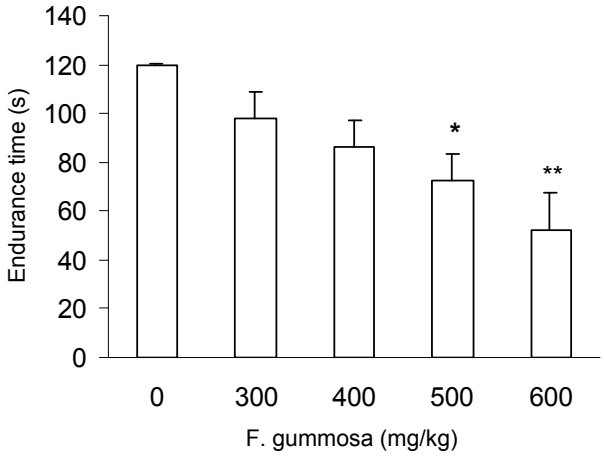

Table 1. Effect of *F. gummosa* root acetone extract, administered i.p. on tonic seizures induced by PTZ (100 mg/kg, i.p.) in mice.

Treatment	Dose (i.p.)	Convulsions (%)
Saline	10 ml/kg	100.0
Tween 80, 5% v/v, (control)	10 ml/kg	100.0
Ethosuximide	150 mg/kg	00.0#
F. gummosa	50 mg/kg	83.3
F. gummosa	100 mg/kg	66.6
F. gummosa	300 mg/kg	41.6*
F. gummosa	400 mg/kg	16.6**
F. gummosa	500 mg/kg	8.3**

Data represent percentage of tonic seizures (n = 12); *p<0.01 and **p<0.001 compared to control, #p<0.001 compared to saline.

F. gummosa extract showed a significant protective effect against MES-induced seizures at doses of 500 and 750 mg/kg (60% and 70% protection, respectively). The higher doses were toxic.

Tween 80 (5%) and saline had no effect on rotarod performance of mice. However, from dose of 500 mg/kg and at 30 min after administration, the extract produced reduction in time spent on rotarod (Fig. 1). This sedation and motor impairment were dose-dependent with TD_{50} value of 546 (394-757) mg/kg. In this regard, PI value of 3.5 was obtained for the extract against seizures induced by PTZ. The extract at the doses employed did not exert any adverse effect on the animals' behavior including irritability, agitation, ataxia or impaired righting reflexes. LD_{50} value of 1252 (1148-1364) mg/kg

Fig. 1. The effect of *F. gummosa* root acetone extract on rotarod performance 30 min after i.p. administration to mice. Histograms represent mean ± SEM (n = 12); *p<0.05 and **p<0.001 compared with control (0) value.

was obtained for the extract and TI value was 8.1 against seizures induced by PTZ.

Preliminary phytochemical screening of *F. gummosa* showed that the acetone extract of the root contains terpenoids, alkaloids and a little amount of cardenolids, while amino acids and cumarin compounds were absent.

DISCUSSION

The present results indicate that *F. gummosa* root acetone extract possesses dose-dependent anti-convulsant effect. This protective effect is more potent against seizures induced by PTZ with ED_{50} value of 154.4 mg/kg than MES-induced seizures, where the dose of 750 mg/kg of the extract partially inhibited the seizures.

The most popular and widely used animal seizure models are the traditional MES and PTZ tests. The MES test is considered to be a predictor of likely therapeutic efficacy against generalized tonic-clonic seizures. By contrast, the PTZ test represents a valid model for human generalized myoclonic seizures and also generalized seizures of the petitmal (absence) type [8]. Thus, our results suggest that *F. gummosa* acetone extract may be effective against human generalized myoclonic seizures and also absence ones. It has been indicated that PTZ-induced seizures can be prevented by drugs that reduce T-type Ca^{2+} currents, such as ethosuximide and also by drugs that enhance gamma amino butyric acid type A ($GABA_A$) receptor-mediated inhibitory neurotransmission such as benzo-diazepines and phenobarbital [11-13].

Preliminary phytochemical analysis performed in this study shows that terpenoids and alkaloids are the major components of the extract.

Some researchers have reported anticonvulsant activity of monoterpenes. SL-1, a synthetic monoterpene homologue of GABA, demonstrated anticonvulsant activity in PTZ-induced seizures [14]. Linalool is another monoterpene compound, which has protective effect against PTZ-, picrotoxin- and NMDA-induced convulsions [15]. Moreover, pinene, eugenol and methyleugenol exhibited anticonvulsant profile in some experimental seizures such as MES and PTZ tests [16, 17]. Modulation of glutamatergic and GABAergic transmission is some mechanisms indicated for anticonvulsant action of the monoterpenes like linalool and eugenol [15, 18, 19]. Therefore, it seems that the antiseizure profile of *F. gummosa* root may be related in part to monoterpens and terpenoid compounds present in the root.

Results of the present study revealed that the extract produces sedation and motor deficits at some anticonvulsant doses. PI value of 3.5 was obtained for the extract against PTZ-induced seizures. Anesthetic, muscle relaxant and inhibitory effect on locomotion have been demonstrated for some terpene compounds such as eugenol, methyleugenol and cineol [17, 20]. It is possible that the motor impairment and sedative effects observed in this study is related in part to terpenoid compounds of the extract.

TI value of the extract for PTZ-induced seizures (8.1) suggests acceptable therapeutic effect for the extract. However, further investigations including chronic toxicity studies and activity-guided fractionation must be performed in order to assess the real toxicological profile and the active compounds of the extract.

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