

Full Length Research Paper

Anxiolytic effect of *Ferula assafoetida* L. in rodents

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***Ferula assafoetida* 'assafoetida' solution (FAS) has been used in Arab traditional medicine as an intestinal antiseptic, carminative and variety of gastric ailments. It has also been used as a neuroprotective agent for the treatment of epilepsy and hysteria. However, the neuro-pharmacological studies on their gum-resin have not received due attention. The present study was undertaken to study the anxiolytic, analgesic and sedative properties of asrafoetida in rodents, using elevated plus maze, hole-board test, hot plate and motor activity meter. Diazepam was used as a reference anxiolytic agent in this study. The results of this study showed a dose-dependent anxiolytic and analgesic activity of asrafoetida, with a mild sedative effect in high doses. Compared to diazepam, the asrafoetida seems to be a better alternative for the treatment of anxiety disorders. However, further experimental and clinical studies are warranted to accurately assess its safety and efficacy for treatment of chronic anxiety.**

Key words: *Ferula assafoetida*, anxiolytic effect, analgesic effect, mice, rats, neuroprotective agents, sedative effect.

INTRODUCTION

Anxiety is an exaggerated feeling of apprehension, uncertainty, and fear. It is an unpleasant state of tension with an anticipation of imminent danger (Barar, 2005). Anxiety affects one-eighth of the total population worldwide and has become a very important area of research interest in psychopharmacology (Kamal and Jawaid, 2011). Benzodiazepines are widely and frequently used drugs for the treatment of generalized anxiety disorder despite their numerous undesirable side effects (Hassanzadeh et al., 2012). In addition, several types of herbal medicines have been used as anxiolytic agents in different parts of the world (Heinrich and Gibbons, 2001). Drugs derived from traditional herbs may have a possible therapeutic relevance in the treatment of anxiety (Beaubrum and Gray, 2000). The root of the kava plant from the tropical Pacific region, St. John's wort extract from Europe, and the saponin-containing fraction of the leaves of *Albizia lebbbeck* from India are known to possess anxiolytic effects (Rex et al., 2002; Friede and Freudenstein, 2002; Une et al., 2001; Kim et al., 2000).

Ferula assafoetida L. is a plant belonging to the family

Apiaceae/Umbelliferae, and commonly known as asrafoetida (FAS). The oleo-gum-resin obtained from the exudates of this plant has been used as a spice and a folk phytotherapy for centuries (Fenrich, 1971). Asrafoetida has a characteristic sulfurous pungent odor and a bitter taste. It is used as a flavoring agent in a variety of foods. In traditional medicine, it is used as a digestive, aphrodisiac, sedative and diuretic drug (Eigner and Scholz, 1990; Bandyopadhyay et al., 2006). It has traditionally been used for the treatment of different diseases, such as asthma, stomachache, flatulence, intestinal parasites, weak digestion and influenza (Zargari, 1996; Takeoka, 2001; Evans, 2002; Lee et al., 2009).

In Unani medicine, asrafoetida is used for the treatment of several neurological disorders, including hysterias, depression and epilepsy (Alqasoumi et al., 2011). Recent pharmacological and biological studies have also shown several properties, including antioxidant (Dehpour et al., 2009), antifungal (Singh, 2007; Sitara et al., 2008; Angelini et al., 2009), anticancer (Aruna and Sivaramakrishnan, 1992; Saleem et al., 2001),

anti-diabetic (Abu-Zaiton, 2010), antispasmodic and hypoten-sive (Fatehi et al., 2004), and molluscicidal (Kumar and Singh, 2006) from this oleo-gum-resin. Recently, gastric antiulcer activity of the asafoetida aqueous suspension in rats has also been reported (Alqasoumi et al., 2011). Although various pharmacological activities of *F. asafoetida* have been reported, its neurological properties have not received due attention. The present study was, therefore, undertaken to examine its folkloric claims as an anti-anxiety drug.

MATERIALS AND METHODS

Plant material and preparation of dosage form

Asafoetida gum-resin was purchased from local herb seller shop, Riyadh, and identified by an expert taxonomist. A voucher specimen has been deposited at the herbarium of the Department of Pharmacognosy, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia. The asafoetida was accurately weighed and dissolved in known volume of distilled water and filtered to remove any insoluble particles. The freshly prepared solution was used in all experiments.

Phytochemical screening

A preliminary phytochemical analysis of *F. assafoetida* was conducted for the detection of alkaloids, cardiac glycosides, flavonoids, tannins, anthraquinones, saponins, volatile oils, cyanogenic glycosides, coumarins, sterols and/or terpenes (Farnsworth, 1966).

Animals and diet

Healthy male adult Swiss albino mice, weighing between 20 - 25 g and Wistar albino rats weighing 140 - 180 g, obtained from the Experimental Animal Care Centre, College of Pharmacy, King Saud University, Riyadh, were used. They were housed in polyethylene cages in groups of six mice per cage and were kept at a constant temperature ($22 \pm 2^\circ\text{C}$), humidity (55%) and 12 h light-dark conditions for 7 days. The animals were provided with a purina chow diet and free access to drinking water. The conduct of experiments and the procedure of sacrifice (using ether) were approved by the Ethics Committee of the Experimental Animal Care Society, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia.

Acute toxicity test

The acute toxicity of the *F. assafoetida* was evaluated in mice using the up and down procedure (OECD, 2001). Six female mice (weight: 20 - 25g) received *F. assafoetida* solution starting from 0.1, 0.3, 1, 1.5 and 2 g/kg orally by gavage. The animals were observed for toxic symptoms continuously for the first 4 h after dosing. Finally, the number of survivors were noted after 24 h and these animals were then maintained for further 14 days with observations made daily (OECD, 2001).

Hot plate method

This method was originally developed by Woolfe and MacDonald

(1944). The response is in the form of jumping, withdrawal of the paws or the licking of the paws (Eddy and Leimback, 1953; O'Neil et al., 1983; Vogel and Vogel, 1997). The animals were placed on hot plate (Hot Plate Analgesia meter, Harvard Apparatus) kept at a temperature of $55 \pm 0.5^\circ\text{C}$. A cut-off period of 30 s was observed to avoid damage to the paw. Reaction time and the type of response were noted using a stopwatch. Control rats were treated with vehicle (1 ml/kg). Solution of asafoetida was administered (250 and 500 mg/kg body weight, p.o.). The latency was recorded before and after 15, 30, 60 and 120 min, following oral administration of 250 and 500 mg/kg of each of the asafoetida solution to different groups of six animals each. The average reaction times were then calculated and the percentage variation calculated using the following ratio:

$$\text{Percentage protection} = \frac{\text{Drug latency} - \text{Baseline latency}}{\text{Baseline latency}} \times 100$$

Behavioral studies

The behavioral studies, elevated plus maze, hole-board test and locomotive activity were performed in adult mice. All the experiments were carried out between 8 - 11 am.

Elevated plus-maze model of anxiety

The plus-maze apparatus, consisting of two open arms (16×5 cm) and two closed arms ($16 \times 5 \times 12$ cm) having an open roof, with the plus-maze elevated (25 cm) from the floor was used to observe anxiolytic behavior in mice (Kulkarni and Reddy, 1996; Vogel and Vogel, 1997). Each mouse was placed at the center of the elevated plus maze with its head facing the open arm. During the 5 min experiment, the behavior of the mouse was recorded as: (i) The number of entries into the open arms (ii) average time spent by the mouse in open arms (average time = total time spent in open arms/number of entries in arms). The solution of *F. assafoetida* was administered orally using a tuberculin syringe fitted with oral cannula. Dose administration schedule was adjusted so that each mouse took its turn on the elevated plus-maze apparatus 1 h after administration of the dose. During the entire experiment, mice were allowed to socialize. Every precaution was taken to ensure that no external stimuli, other than the height of the plus-maze could invoke maze anxiety.

Hole-board test

The hole-board apparatus consisted of gray panels (40 cm \times 40 cm, 2.2 cm thick) with 16 equidistant holes 3 cm in diameter in the floor (Vinade et al., 2003). The board was positioned 25 cm above a table. Animals were placed singly in the center of the board facing away from the observer and numbers of head-dip and edge sniff's were recorded over 5 min. Mice were treated with *F. assafoetida* solution or vehicle 1 h before they were placed on the board.

Locomotor activity

Since the plus maze experiment was affected by changes in locomotor activity, an additional experiment was carried out with the specific aim of monitoring the activity. The horizontal and vertical locomotor activity of mice was registered by the locomotor activity

Table 1. The effect of *Ferula assafoetida* solution (FSA) on latency parameters using hot plate analgesiometer.

Group	Treatment	Dose (mg/kg, orally)	Hot-plate analgesia		
			Time (min)	Latency (sec)	% Protection
1	FSA	250	0 (Basal value)	11.38 ± 0.79	-
			15	13.16 ± 1.01	17.84
			30	14.01 ± 1.64	25.34
			60	15.65 ± 1.51	40.01
			120	14.91 ± 1.31	33.39
2	FSA	500	0 (Basal value)	12.77 ± 1.30	-
			15	14.88 ± 1.43	16.55
			30	16.33 ± 2.49	27.90
			60	18.75 ± 1.40	46.83
			120	18.10 ± 1.44	41.74

Data are given as mean ± SEM. Six rats were used in each group.

apparatus (Ugo Basile 7431) containing two pairs of 16 photocells. Interruptions of light beams to the photocells during horizontal and vertical movements of the animal were registered. One hour after the administration of asafoetida solution, horizontal and vertical loco-motor activities was registered for a period of 5 min.

Statistical analysis

Values are given as arithmetic means ± standard error of the mean (S.E.M.). Data was statistically analyzed by using the Student's *t*-test.

RESULTS

Phytochemical screening

The preliminary qualitative phytochemical screening of *F. assafoetida* revealed the presence of resins (which contains ferulic acid esters), free ferulic acid, coumarin derivatives, volatile oils and monoterpenes.

Acute toxicity test

The *F. assafoetida* solution up to a maximum dose of 2 g/kg was found to be safe, as the mice did not show any symptoms of toxicity and mortality during a period of 14 days of observation.

Hot plate method

In the hotplate test, *F. assafoetida* mucilage treatment caused increases in analgesia in a dose-dependent manner. The analgesic effects of *F. assafoetida* aqueous solutions (250 and 500 mg/kg) were observed between

15 and 120 min and reached a maximum analgesia (peak central effect) of 40.01 and 46.83%, respectively, at 60 min (Table 1).

Plus-maze test

The behavioral effects of solution of *F. assafoetida* on mice behavior in the elevated plus-maze are summarized in Table 2. Asafoetida solution at a dose of 500 mg/kg significantly increased the percentage of entries in the open arms ($P < 0.05$) and percentage of time spent in the open arms ($P < 0.01$). In a similar fashion, diazepam increased the percentage of entries in the open arms ($P < 0.01$) and percentage of time spent in the open arms ($P < 0.001$). Moreover, asafoetida solution at 250 mg/kg had significant effect on the percentage of time spent in the open arms ($P < 0.05$), but showed no significant effect on percentage of entries in the open arms.

Hole-board test

The effect of asafoetida solution on the head-dipping and edge sniffing behavior in mice is shown in Table 2. Assafoetida solution at a dose of 500 mg/kg significantly increased the number of head dips ($P < 0.001$) and number of edge sniffs ($P < 0.01$). In a similar way, diazepam significantly increased the number of head dips ($P < 0.001$) and number of edge sniffs ($P < 0.01$). The solution at 250 mg/kg had a significant effect on the number of head dips ($P < 0.05$), but it showed no significant effect on the number of edge sniffs.

Locomotor activity

The effect of *F. assafoetida* solution on the horizontal and

Table 2. The effect of *Ferula assafoetida* solution (FSA) on anxiety parameters using elevated plus maze and hole-board test.

Group	Treatment	Dose (mg/kg, orally)	Elevated plus-maze		Hole-board	
			No. of open arm entries	Time spent in open arms	No. of head dips	No. of edge sniff's
1	Control (distilled water)	-	3.17 ± 0.80	9.67 ± 2.11	20.83 ± 3.37	11.50 ± 2.67
2	Diazepam	0.5	6.5 ± 0.43**	27.17 ± 3.18***	59.33 ± 3.13***	26.33 ± 2.11**
3	FSA	250	3.33 ± 0.50	16.50 ± 2.22*	31.00 ± 2.94*	15.67 ± 0.88
4	FSA	500	5.17 ± 0.31*	23.83 ± 4.01**	54.67 ± 3.22***	23.67 ± 2.82**

Data are mean ± SEM. Six mice were used in each group. *P<0.05; **P<0.01; ***P<0.001, Student's *t*-test as compared with control (vehicle) group.

Table 3. The effect of *Ferula assafoetida* solution (FSA) on locomotor activity.

Group	Treatment	Dose (mg/kg, orally)	Locomotor activity	
			Horizontal	Vertical
1	Control (distilled water)	-	462.5 ± 36.62	23.0 ± 2.02
2	Diazepam	0.5	335.33 ± 16.14**	15.33 ± 0.99**
2	FSA	250	455.67 ± 39.80	21.16 ± 1.01
3	FSA	500	364.83 ± 25.01*	17.50 ± 1.17*

Data are Mean ± SEM. Six mice were used in each group. *P<0.05; **P<0.01; ***P<0.001, Student's *t*-test as compared with control (vehicle) group.

vertical locomotor activity of mice is shown in Table 3. Locomotor activity, both horizontal and vertical were significantly reduced ($P < 0.05$) in animals pretreated with 500 mg/kg of asafoetida solution compared with that in the vehicle group. Also diazepam significantly reduced ($P < 0.01$) both horizontal and vertical locomotor activity. The solution of asafoetida at 250 mg/kg had no significant effect on the locomotor activity parameters. However, *F. assafoetida* mucilage inhibited locomotor activity to a lesser extent than diazepam and thus had a better profile for anxiolytic agents.

DISCUSSION

The present study was undertaken to examine the anti-anxiety and analgesic properties of asafoetida using elevated plus-maze, hole board test as models of anxiety and hot-plate test and locomotor activity for analgesic and sedative activity (Kulkarni, 2002; Dawson and Tricklebank, 1995; File and Pellow, 1985). Diazepam was used as a reference standard anxiolytic drug in the present study (Soderpalm et al., 1989). In the current investigation, it was found that FSA increased the analgesia and the peak effect was achieved after 60 min of drug administration (Table 1). The results obtained herein support the claimed folkloric use of asafoetida (Alqasoumi et al., 2011). On the other hand, the *F. assafoetida* showed a significant anti-anxiety activity with

mild sedation action in higher doses, whereas low dose was free from any sedative activity. The synthetic anxiolytic drugs such as benzodiazepines are also known to possess sedative activity (Treit, 1985)

In addition, it was found that FSA increased the percentage of entries in the open arms and percentage of time spent in the open arms using elevated plus maze. Asafoetida solution also increased the number of head dips and number of edge sniffs using hole-board test. These results showed anxiolytic effects of asafoetida in the models used (Tables 2 and 3). Administration of diazepam in rats produced significant increase in the number of entries into the open arms and in time spent in open arms of elevated plus-maze. There was a significant increase in the number of head dips and number of edge sniffs on hole-board. Diazepam also produced statistically significant decrease in motor activity, as indicated by locomotor activity counts. These data are consistent with numerous previous studies, which have shown that benzodiazepines, including diazepam produce anxiolytic effects accompanied by a sedative effect (Vogel et al., 1971).

In conclusion, these findings clearly suggest that the low doses of asafoetida may be a therapeutic alternative to the presently used anxiolytic drugs. The results of the present study, therefore, substantiate the use of FSA for the treatment of anxiety disorders. However, further clinical and experimental studies are warranted to determine its safety, efficacy and possible mechanism(s) of action.

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