

GASTRIC ANTIULCER ACTIVITY OF A PUNGENT SPICE *FERULA ASSAFOETIDA* L. IN RATS

SALEH ALQASOUMI¹, MOHAMMED AL-DOSARI¹, TAWFEQ AL-HOWIRINY¹, MOHAMMED AL-YAHYA¹, IBRAHIM AL-MOFLEH², SYED RAFATULLAH^{1*}

¹Department of Pharmacognosy and Medicinal, Aromatic & Poisonous Plants Research Center (MAPPRC), College of Pharmacy, P.O. Box 2457, King Saud University, Riyadh 11451, Saudi Arabia

²Gastroenterology Unit (59), College of Medicine and KCUH, King Saud University, P.O. Box 2925, Riyadh-11461, Saudi Arabia

* corresponding author: srafat@ksu.edu.sa

Abstract

Ferula assafoetida 'Assafoetida' is known to possess various therapeutic properties. We evaluated the anti-ulcerogenic property of an aqueous suspension of assafoetida in different ulcer models on Wistar albino rats. The suspension at doses of 250 and 500 mg/kg body weight, orally (i.p. in Shay rat model) had significant effects in the gastric ulceration induced by basal gastric acid secretion, indomethacin and noxious chemicals (80% ethanol, 0.2 M NaOH and 25% NaCl). They showed significant protection in all models used. These findings were supported by histopathological assessment of gastric tissue and by the determination of gastric wall mucus (GWM) contents of the stomach, as these parameters showed protection of various indices and replenishing the depleted (GWM) level by the suspension treatment, respectively. Conclusively, the ulcer protective effect of assafoetida may possibly be due to its anti-secretory action along with antioxidative and cytoprotective through a prostaglandin mediated mechanism.

Rezumat

Ferula assafoetida (asafoetida) este o plantă cu numeroase proprietăți terapeutice. În studiul de față am evaluat acțiunea anti-ulcerogenă a unei suspensii apoase de asafoetida, folosind diferite modele experimentale de ulcer gastric indus la șobolanul alb Wistar. Dozele de 250, respectiv 500 mg suspensie/kg corp, administrate oral, au prezentat efecte semnificative asupra ulcerărilor gastrice induse, pentru toate modelele experimentale. Au fost efectuate teste histopatologice pentru țesutul gastric precum și evaluări ale mucusului peretelui gastric. Acțiunea protectoare a plantei *Ferrula assafoetida* în ulcerul gastric se poate datora acțiunii sale antisecretorii, antioxidante și citoprotectoare, printr-un mecanism mediat de prostaglandine.

Keywords: *Assafoetida*, *Ferula assafoetida*, gastric secretion, antiulcer.

Introduction

The plant kingdom has provided a remarkable source of medicinal plants including spices [28]. Spices have comprised the major part of the indigenous pharmacopoeia. The spices are recognized and scientifically

evaluated for their beneficial effects on human health and reported to be anti-inflammatory, bowel-restorative and liver-detoxifying agents [1].

Ferula assafoetida L. is an oleo-gum resin (commonly known as assafoetida) (*Apiaceae*). In foods, it is used as a source of natural food flavoring and medicine [13]. In Unani, Ayurveda and Arab traditional medicine *assafoetida* is used for various ailments, including flatulence, constipation, flatulent colic as carminative. In the past it was supposed to exercise a stimulant action upon the CNS [20, 22]. Its beneficial effect is also described in the literature as its administration is useful in hysteria and conditions of nervous exhaustion and epilepsy [9].

Previously, some pharmacological effects of assafoetida have been reported, such as antispasmodic and hypotensive in rats. The present study was carried out to investigate the antisecretory, antiulcer and cytoprotective properties of *Ferula assafoetida* suspension on laboratory animals to substantiate its use in Unani, Ayurvedic and Arab traditional medicine for the treatment of various gastro-intestinal disorders.

Materials and Methods

Assafoetida was purchased from local herbal medicine material shop, and identified by an experienced taxonomist and herbal medicine practitioner. A voucher specimen has been deposited at the herbarium of the Department of Pharmacognosy, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia.

A known amount of assafoetida was dissolved in water and the obtained aqueous suspension was used for the further studies.

Animals

Wistar albino rats of either sex (home bred) aged 7–8 weeks and weighing 150–200 g, were obtained from the Experimental Animal Care Centre, King Saud University, Riyadh, Saudi Arabia. The animals were fed on Purina chow diet and water *ad libitum* and were maintained under standard conditions of humidity ($55\pm 5\%$), temperature ($22\pm 2^\circ\text{C}$) and light (12-h light/12-h dark cycle). The rats were randomly assigned to different control and treatment groups. 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.

Antisecretory Studies

Pylorus ligated (Shay) rats

The animals were fasted for 36 h with access to water *ad libitum* before the pylorus was ligated under ether anesthesia, taking care not to

cause bleeding or to occlude blood vessels [30]. Assafoetida suspension (250 and 500 mg/kg body weight) was administered immediately after pylorus ligation by intraperitoneal injection. The animals were sacrificed 6 h after the pylorus ligation, stomachs were removed, and contents were collected, measured, centrifuged, and subjected to analysis for titratable acidity against 0.01 N NaOH to pH 7. Each stomach was examined for lesions as described above.

Indomethacin-induced gastric ulcer

Indomethacin was suspended in 1% carboxymethylcellulose in water (6 mg/mL) and administered to the fasted rats in a dose of 30 mg/kg b.w. (0.5 mL/100 g b.w.). Rats were treated with assafoetida suspension (250 and 500 mg/kg b.w. orally) 30 min before indomethacin. Control rats were treated similarly with an equivalent amount of vehicle [6]. The stomachs of the animals were removed, rinsed with normal saline and studied.

Determination of Gastric Wall Mucus (GWM)

GWM was determined according to the modified procedure of Crone [10]. The glandular segment of the stomach was separated from the rumen of the stomach, weighed, and transferred immediately to 10 mL of 0.1% w/v Alcian blue solution (in 0.16 mmol/L sucrose solution buffered with 0.05 mL sodium acetate at pH 5). Tissue was stained for 2 h in Alcian blue and excess dye was removed by two successive rinses with 10 mL of 0.25 mmol/L sucrose, first after 15 min and then after 45 min. Dye complexed with the GWM was extracted with 10 mL of 0.5 mmol/L magnesium chloride, which was intermittently shaken for 1 min at 30 min intervals for 2 h. Four milliliters of blue extract were then vigorously shaken with an equal volume of diethyl ether. The resulting emulsion was centrifuged at 4,000 rpm for 10 min and the absorbance of the aqueous layer was recorded at 580 nm. The quantity of Alcian blue extracted per gram of wet glandular tissue was then calculated.

Gastric lesions induced by necrotizing agents

The animals in the test groups were given 1 mL of necrotic agents, either 80% ethanol, 0.2 M NaOH or 25% NaCl, which are known to produce gastric lesions [27]. Hypertonic saline and NaOH (0.2 M) were used only in cytoprotection studies. Assafoetida suspension was given 30 min before the necrotizing agents. The animals were killed under anesthesia, using diethyl ether 1 h after the treatment with the necrotic agents. The stomach of each animal was excised and opened along the greater curvature. After washing with normal saline the gastric lesions were quantified using a binocular magnifier. The ulcers were scored according to the method of Valcavi [31]. Control animals were treated with vehicle only.

Estimation of nonprotein sulfhydryl groups (NP-SH)

Gastric mucosal NP-SH was measured according to the method reported earlier [29]. The glandular stomachs of control and treated rats were removed and homogenized in ice-cold 0.02 M ethylenediaminetetraacetic acid (EDTA). The homogenate was mixed with distilled water and 50% trichloro acetic acid (TCA), and centrifuged; the supernatants were mixed with Tris buffer, 5,5'-dithio-bis(2-nitrobenzoic acid) (DTNB) was added and the sample was shaken. The absorbance was measured, within 5 min after the addition of DTNB, at 412 nm, against a reagent blank with no homogenate.

Statistical analysis

The differences between control and treated groups were compared using the ANOVA and Student's *t*-test as appropriate and were considered significant if $p < 0.05$.

Histopathological studies

The gastric tissue was fixed in 10% ethanol buffer for malin and processed through graded ethanol, xylene and impregnated with paraffin wax; sections were made by microtome. After staining with haemotoxylin and eosin stain [11], the sections were examined under a research microscope by a person who was not aware of the experimental protocols. The different histopathological indices screened were: congestion, hemorrhage, edema, necrosis, inflammatory and dysplastic changes erosions and ulcerations.

Results and Discussion

An aqueous suspension of assafoetida administered immediately after pylorus ligation, significantly reduced the volume of basal gastric secretion, acidity and completely inhibited the ulceration (Table I). Table II shows that the aqueous suspension of assafoetida by gavage inhibited gastric ulceration induced by indomethacin in fasted rats in the higher dose (500 mg/kg b.w.) group. Although there was a decrease in the ulcer index in the low dose group (250 mg/kg b.w.), it was not found to be statistically significant. The gastric wall mucus depletion was induced by 80% ethanol in fasted rats. The gastric wall mucus was significantly increased in the animals pretreated with assafoetida in both doses (Table III, $p < 0.05$). Lesion induced by various necrotizing agents were significantly reduced by pretreatment of assafoetida suspension (Table IV, $p < 0.001$).

Table I

Effect of *Ferula assafoetida* on the volume of gastric secretion, titratable acidity and the degree of ulceration in 6-h pylorus ligated (Shay model) rats.

Treatment	Dose (mg/kg b.w., i.p.)	Mean $\pm$ S.E.		
		Volume of content (mL)	Titratable acid (mEq/L)	Ulcer index
Control	-	8.83 ± 0.98	132.21 ± 3.40	1.00 ± 0.36
<i>Ferula assafoetida</i>	250	6.08 $\pm 0.15^*$	131.66 ± 2.95	00***
<i>Ferula assafoetida</i>	500	4.00 $\pm 0.22^{***}$	106.66 $\pm 3.44^{***}$	00***

Six animals were used in each group. The treated groups were compared with the control group.

* $p < 0.05$; *** $p < 0.001$. Student's *t*-test.

Table II

Effect of *Ferula assafoetida* on the gastric mucosal damage induced by indomethacin in rats.

Treatment	No. of animals	Dose (mg/kg b.w., p.o.)	Ulcer Index (Mean $\pm$ S.E.)
Control	6	-	34.66 $\pm$ 4.58
<i>Ferula assafoetida</i>	6	250	24.66 $\pm$ 4.05
<i>Ferula assafoetida</i>	6	500	20.66 $\pm$ 4.05*

Six animals were used in each group. The treated groups were compared with the control group.

* $p < 0.05$. Student's *t*-test.

Table III

Effect of *Ferula assafoetida* on 80% ethanol-induced gastric wall mucus changes in rats.

Group (n = 6)	Dosage (mg/kg b.w. p.o.)	Gastric wall mucus μ g Alician blue of wet glandular tissue
80% Ethanol only.	-	464.31 $\pm$ 12.92
Control	-	327.07 $\pm$ 9.07 ^{a***}
<i>Ferula assafoetida</i> + 80% Ethanol.	250	351.30 $\pm$ 15.76 ^b
	500	367.81 $\pm$ 11.14 ^{b*}

^aAs compared to the control group.

^bAs compared to the 80% ethanol-treated group.

* $p < 0.05$. Student's *t*-test.

Table IV

Effect of *Ferula assafoetida* on the gastric lesions induced by various necrotizing agents in rats.

Treatment (n = 6)	Dose (mg/kg b.w. p.o.)	Ulcer index (Mean $\pm$ S.E.)		
		80% EtOH	0.2M NaOH	25% NaOH
Control	-	7.33 ± 0.33	7.50 ± 0.34	7.33 ± 0.21
<i>Ferula assafoetida</i>	250	2.33 $\pm 0.21^{***}$	5.16 $\pm 0.60^{***}$	2.83 $\pm 0.54^{***}$
<i>Ferula assafoetida</i>	500	1.50 $\pm 0.34^{***}$	2.50 $\pm 0.71^{***}$	2.50 $\pm 0.42^{***}$

***p < 0.001, Student's *t*-test.

The treated groups were compared with the control group.

Administration of ethanol 80% to the fasted rats showed a significant depletion in NP-SH level. The prior treatment of animals with assafoetida aqueous suspension failed to replenish the NP-SH contents in both doses groups (Table V). Histopathological assessment (Table VI) further confirmed that pretreatment with ferula suspension prevented ethanol-induced hemorrhage, necrosis and ulceration of the gastric mucosa.

Table V

Effect of *Ferula assafoetida* on nonprotein sulfhydryl groups (NP-SH) concentration in gastric tissue of rats.

S. No.	Treatment and dose (mg/kg body weight)	NP-SH concentration (μ mol/100 mg wet tissue) Mean $\pm$ S.E.
1	Control (distilled water, 1 mL/rat)	8.59 $\pm$ 0.33
2	Control (80% ethanol, 1 mL/rat)	6.38 $\pm$ 0.27 ^{a*}
3	<i>Ferula assafoetida</i> assafoetida (250) + 80% ethanol (1 mL/rat) ¹	6.47 $\pm$ 0.36
4	<i>Ferula assafoetida</i> (500) + 80% ethanol (1 mL/rat)	7.94 $\pm$ 0.80

Six rats were used in each group.

^a = as compared to control (distilled water) group.

^b = as compared to control (80% ethanol) treated group.

*p<0.01; **p < 0.001. Student's *t*-test.

Table VI

Effect of *Ferula assafoetida* on ethanol-induced histopathological lesions in gastric mucosa of rats.

Group #	Treatment and dose (mg/kg. body weight)	Induced Histopathological Lesions							
		Congestion	Haemorrhage	Edema	Necrosis	Inflammatory changes	Dysplastic changes	Erosions	Ulceration
1	Control (distilled water) (1 mL/rat).	-	-	-	-	-	-	-	-
2	Ethanol, 80% (1 mL/rat)	++	+	+	++	+	+	++	++
3	<i>Ferula assafoetida</i> (250mg/kg b.w.) + ethanol, 80% (1 mL/rat)	++	+	+	-	-	-	+	-
4	<i>Ferula assafoetida</i> (500mg/kg b.w.) + ethanol, 80% (1 mL/rat)	+	-	+	-	-	-	+	-

- = Normal; + = Moderate effect; ++ = Severe effect

Conclusions

Our observations show that *Ferula assafoetida* produced a significant inhibition of basal gastric secretion, acidity and ulceration in pylorus ligated Shay rats model. It also significantly prevented the gastric mucosal injury induced by indomethacin and various necrotizing agents. On the other hand, assafoetida suspension also significantly increased the gastric wall mucus level in rats. The chemical constituents of assafoetida, responsible for its gastroprotective activity are not known. However, assafoetida contains resinous material which consists of ferulic acid and other flavonoidal glycosides and coumarins [18, 20, 3]. Ferulic acid is known to exert antioxidant activity by reducing vascular disorders in humans by strengthening the membranes [17, 15, 23, 32].

Previously, Risch [26] also reported a potent antioxidant activity of ferulic acid. It is well established that antioxidants play an important role in preventing gastric mucosal damage by strong cell defense mechanisms [24, 25] are likely to stimulate the endogenous synthesis of prostaglandins [2], or by a protective role as a membrane stabilizing agent [16] and act by scavenging oxygen free radicals [14]. Our findings substantiate the folkloric use of assafoetida exert an anticholinergic activity on smooth muscle of

guinea pig ileum. An earlier study [4] reported similar findings on *Ferula sinaica*, a very close species of *F. assafoetida*. Anticholinergic drugs have been shown to inhibit acid secretion and slow gastric motility [12] and perhaps this could be the one of the mechanism(s) by which the suspension of assafoetida offers its ulcer protective effect [5]. Many of the orally effective cytoprotective agents act by increasing generation of prostaglandins due to a mild irritant action, a phenomenon which has been described as 'adaptive cytoprotection' [8,19]. The results on histological investigation on the gastric tissue of rats revealed that the pretreatment with ferula suspension significantly inhibited the ethanol-induced various pathological indices including ulcers, results that confirm the obtained pharmacological results in the present study. Assafoetida suspension appears to possess significant cytoprotective activity as prior treatment of the suspension significantly inhibited the formation of stomach lesions induced by noxious chemicals and also prevent the indomethacin-induced gastric ulceration in rats. These results support the antiulcerogenic property of assafoetida could be related to a cytoprotective and it could also be through partly prostaglandin-dependent mechanism(s) [21, 7].

In conclusion, it appears that assafoetida possesses antioxidant and strong gastric antiulcer activity in rats and substantiate its use in Unani and Arab traditional medicine.

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