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Effects of *Anethum graveolens* L. seed extracts on experimental gastric irritation models in mice

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Abstract

Background: As a folk remedy, *Anethum graveolens* seed (dill) is used for some gastrointestinal ailments. We aimed to evaluate aqueous and ethanolic extracts of anti-ulcer and acute toxicity effects of the *Anethum graveolens* in mice.

Results: Gastric mucosal lesions were induced by oral administration of HCl (1 N) and absolute ethanol in mice. The acidity and total acid content of gastric juice were measured in pylorus-ligated mice. LD50 values of the aqueous and ethanolic extracts were 3.04 g/kg, i.p., (1.5, 6.16) and 6.98 g/kg, i.p., (5.69, 8.56), respectively. The efficacy of high dose of extracts (p.o.) was similar to sucralfate. The acidity and total acid content were reduced by the orally or intraperitoneally administration of the extracts.

Conclusions: The results suggest that *A. graveolens* seed extracts have significant mucosal protective and antisecretory effects of the gastric mucosa in mice.

Background

Anethum graveolens L. (dill) is a sparse looking plant with feathery leaves and tiny yellow flowers. Some pharmacological effects have been reported, such as antimicrobial [1,2], antihyperlipidaemic and antihypercholesterolaemic [3] activities. As a folk remedy, dill is considered for some gastrointestinal ailments such as flatulence, indigestion, stomachache and colic [4]. Dill fruit has an antispasmodic effect on the smooth muscles of the gastrointestinal tract [5].

On the basis of above observations, the antisecretory and mucosal protective effects of *A. graveolens* seed extracts were evaluated in mice.

Results

The maximum non-fatal doses of aqueous and ethanolic extracts were 0.45 g/kg and 5 g/kg (i.p.), respectively. LD50 values of the aqueous and ethanolic extracts were 3.04 g/kg, i.p. (1.5–6.16) and 6.98 g/kg, i.p. (5.69–8.56), respectively.

The aqueous and ethanolic extracts of *A. graveolens* seed significantly decreased the occurrence of gastric lesions induced by HCl (ED50 values were 0.12 g/kg (0.18–7.88) and 1.12 g/kg (0.81–1.55), respectively) and ethanol (ED50 values were 0.34 g/kg (0.26–0.43) and 1.73 g/kg (0.43–7.05), respectively) (Tables 1,2,3,4). The potency ratio of the aqueous extract versus the ethanolic extract was significant ($P < 0.05$). At a high dose (5 g/kg), the pro-

Table 1: Effects of the oral administration of *Anethum graveolens* seed aqueous extract and sucralfate on the cytoprotective action against HCl-induced (1 N) gastric lesions in mice.

Treatment	Dose (g/kg)	Inhibition (%)
Saline	0.3 ml	---
Extract	0.05	38 ± 2.23***
Extract	0.18	57 ± 1.21***
Extract	0.32	63 ± 1.47***
Extract	0.45	67 ± 1.6***
Sucralfate	0.1	72.2 ± 1.32***

Values are the mean ± S.E.M. of 6 mice, ***P < 0.001, Compared to control, Tukey-Kramer.

Table 2: Effects of the oral administration of *Anethum graveolens* seed ethanolic extract and sucralfate on the cytoprotective action against HCl-induced (1 N) gastric lesions in mice.

Treatment	Dose (g/kg)	Inhibition (%)
Saline	0.3 ml	---
Extract	0.5	38 ± 1.4***
Extract	2	57 ± 1.1***
Extract	3.5	67 ± 1.21***
Extract	5	76 ± 2.09***
Sucralfate	0.1	72 ± 1.17***

Values are the mean ± S.E.M. of 6 mice, ***P < 0.001, Compared to control, Tukey-Kramer.

Table 3: Effects of the oral administration of *Anethum graveolens* seed aqueous extract and sucralfate on the cytoprotective action against absolute ethanol-induced gastric lesions in mice.

Treatment	Dose (g/kg)	Lesion score
Saline	0.3 ml	4 ± 0
Extract	0.05	3.5 ± 0.3
Extract	0.18	3 ± 0.3
Extract	0.32	2 ± 0.4**
Extract	0.45	1.5 ± 0.4***
Sucralfate	0.1	0.5 ± 0.4***

Values are the mean ± S.E.M. of 6 mice, **P < 0.01, ***P < 0.001, Compared to control, Dunn's test.

Table 4: Effects of the oral administration of *Anethum graveolens* seed ethanolic extract and sucralfate on the cytoprotective action against absolute ethanol-induced gastric lesions in mice.

Treatment	Dose (g/kg)	Lesion score
Saline	0.3 ml	4 ± 0
Extract	0.5	3 ± 0.4
Extract	2	2.5 ± 0.3
Extract	3.5	1.5 ± 0.3**
Extract	5	0.5 ± 0.4***
Sucralfate	0.1	0.5 ± 0.4***

Values are the mean ± S.E.M. of 6 mice, **P < 0.01, ***P < 0.001, Compared to control, Dunn's test.

tection of the ethanolic extract against ethanol induced lesion was also equal to sucralfate (Table 4). All the extracts showed anti-ulcer activity in a dose-dependent manner.

The oral and intraperitoneal administrations of both extracts induced a significant decrease in total gastric acid together with an increase in pH values (Tables 5,6,7,8).

Table 5: Effects of the oral administration of *Anethum graveolens* seed aqueous extract and cimetidine on the pH and total gastric acid from pylorus-ligated mice

Treatment	Dose (g/kg)	pH	μEq H^+
Saline	0.3 ml	3.37 ± 0.13	20.83 ± 2.04
Extract	0.05	3.59 ± 0.04***	12.50 ± 0***
Extract	0.18	3.65 ± 0.08***	12.50 ± 0***
Extract	0.32	3.83 ± 0.09***	10.41 ± 1.21***
Extract	0.45	4.45 ± 0.10***	5.41 ± 1.02***
Cimetidine	0.032	4.54 ± 0.11***	5.00 ± 0***

Values are the mean ± S.E.M. of 6 mice, ***P < 0.001, Compared to control, Tukey-Kramer.

Table 6: Effects of the oral administration of *Anethum graveolens* seed ethanolic extract and cimetidine on the pH and total gastric acid from pylorus-ligated mice

Treatment	Dose (g/kg)	pH	μEq H^+
Saline	0.3 ml	3.46 ± 0.13	20.83 ± 2.04
Extract	0.5	4.01 ± 0.08***	7.5 ± 0***
Extract	2	4.14 ± 0.06***	5.83 ± 1.29***
Extract	3.5	4.30 ± 0.11***	5.00 ± 0***
Extract	5	4.60 ± 0.08***	4.58 ± 1.21***
Cimetidine	0.032	4.54 ± 0.11***	5.00 ± 0***

Values are the mean ± S.E.M. of 6 mice, ***P < 0.001, Compared to control, Tukey-Kramer.

Table 7: Effects of the intraperitoneal administration of *Anethum graveolens* seed aqueous extract and cimetidine on the pH and total gastric acid from pylorus-ligated mice

Treatment	Dose (g/kg)	pH	μEq H^+
Saline	0.3 ml	3.73 ± 0.09	20.00 ± 0
Extract	0.05	3.96 ± 0.08***	7.08 ± 1.88***
Extract	0.18	4.43 ± 0.09***	5 ± 0***
Extract	0.32	5.04 ± 0.17***	2.5 ± 0***
Extract	0.45	5.58 ± 0.11***	2.5 ± 0***
Cimetidine	0.032	6.23 ± 0.06***	2.5 ± 0***

Values are the mean ± S.E.M. of 6 mice, ***P < 0.001, Compared to control, Tukey-Kramer.

Table 8: Effects of the intraperitoneal administration of *Anethum graveolens* seed ethanolic extract and cimetidine on the pH and total gastric acid from pylorus-ligated mice

Treatment	Dose (g/kg)	pH	μEq H^+
Saline	0.3 ml	3.76 ± 0.08	20.0 ± 0
Extract	0.5	4.45 ± 0.07***	5.0 ± 0***
Extract	2	5.05 ± 0.06***	5.0 ± 0***
Extract	3.5	5.51 ± 0.17***	2.5 ± 0***
Extract	5	6.07 ± 0.05***	2.5 ± 0***
Cimetidine	0.032	6.23 ± 0.07***	2.5 ± 0***

Values are the mean ± S.E.M. of 6 mice, ***P < 0.001, Compared to control, Tukey-Kramer.

These effects were dose-dependent. Both extracts with higher doses showed antisecretory activity as effective as cimetidine. The ED50 values of the aqueous and ethanolic extracts oral treatment were 0.17 g/kg (0.43–2.04) and 0.07 g/kg (0.03–0.15), respectively. The ED50 values of the aqueous and ethanolic extracts intraperitoneal treatment were 0.02 g/kg (0.01–0.03) and 0.03 g/kg (0.01–0.08), respectively. The potency ratio of the aqueous extract versus the ethanolic extract was not significant in these tests.

Discussion

These results indicate that the extracts of *A. graveolens* seed have effective antisecretory and anti-ulcer activity against HCl- and ethanol-induced stomach lesions.

In respect to LD50 values, the aqueous extract was more toxic than the ethanolic extract. Compare with a toxicity classification [6], the aqueous and ethanolic extracts are relatively and little toxic, respectively. As high doses of ethanolic extract was used in this study, in clinical trial the toxicity of dill should be considered.

The oral administration of the extracts diminished HCl-induced gastric lesions in mice. This may be related to an antacid effect or cytoprtotective properties in gastric mucus. The cytoprotective action against ethanol showed that the effects of extracts are not a simple acid neutralizing activity but the dill extracts have a cytoprotective effect against the gastric mucosa in ethanol-induced gastric lesion in mice.

It is possible that the inhibitory effects of extracts are due, at least partly, to the presence of terpenes in dill [7,8]. Terpenes were associated to antiulcerogenic activity in other plants [9,10]. Some triterpenes are known as antiulcer drugs and their action has been suggested to be due to the activation of cellular protection, reduction of mucosal prostaglandins metabolism-cytoprotective action and reduction of gastric vascular permeability [19]

Flavonoids have antiulcer and gastroprotective activities [11–13]. The aqueous extracts of *Phoradendron crassifolium* and *Franserio artemisiodes* that contain polyphenolic agents exerted cytoprotective activity in rats [14]. Two flavonoids have been isolated from *A. graveolens* seed, quercetin 3-O-beta-D-glucuronide and isoharmentin 3-O-beta-D-glucuronide, have antioxidant activity and could counteract with free radicals. This effect may help to prevent ulcer peptic [15,16].

The extracts provoked a marked decrease in total gastric acid together with an increase in pH values. As the fruit of dill has an antispasmodic effect on the smooth muscles of the gastrointestinal tract [5], it is possible that both extracts act via cholinergic system (an anticholinergic activity) and block release of HCl. At this stage, other mechanisms such as H2 receptor antagonist effect or the inhibition of gastric H+,K+-ATPase can not be excluded.

In respect to the ED50 values, the aqueous extract was more potent than the ethanolic extract in HCl- or ethanol-induced stomach lesions. However, both extracts showed similar potency in reduction total gastric acid together with an increase in pH values. This may be related to the extracts different mechanisms of action. The ED50 values of extracts were much lower than their LD50 values.

In conclusion, *A. graveolens* seed markedly inhibits acid secretion and the occurrence of lesions in stomach but exact mechanisms are not clear yet and need further investigations.

Materials and Methods

Animals

Male albino BALB/c mice 25–35 g were obtained from the animal house of School of Pharmacy, Mashhad University of Medical Sciences. Animals were housed in colony room 12/12 hr light/dark cycle at 24 ± 1°C. After 24 h fasting, the mice were used for the experiments but were allowed drinking water during the 24 h fasting period. All animal experiments were carried out in accordance with Mashhad University of Medical Sciences, Ethical Committee acts.

Plant material

The seed was collected at Bojnord (a town in Khorassan province, the northeastern of Iran). All samples collected were dried in shade and then powdered. Ferdowsi University properly identified the plant and voucher samples were preserved for reference in the herbarium of Department of Pharmacognosy, School of Pharmacy, Mashhad (293-0107-18).

The preparation of extracts

The seed powder was extracted using maceration with ethanol (80 v/v) or water for 3 days and, subsequently, the mixture was filtered and concentrated under reduced pressure (by a rotaevaporator) at 40°C. The yield (w/w) of the aqueous and ethanolic extracts was 6.46% and 8.5%, respectively.

HCl or ethanol-induced mucosal membrane lesions

Gastric mucosal lesions were induced by the modified method of Mizui and Doteuchi [17]. The mice were divided into groups of 6 animals. After 24 h fasting, the extracts and drugs were administered orally to the mice. 30 min thereafter, each mouse received 0.2 ml of 1 N HCl or absolute ethanol by oral administration. 60 min after administration of the necrotizing agent, each animal was killed by ether, and the stomach was excised, inflated by injecting 2 ml of normal saline and then fixed for 30 min in 5% formalin solution. After opening along the greater curvature, HCl induced gastric damage was observed in the gastric mucosa as elongated black-red lines parallel to the long axis of the stomach of the mice. The lesion index was determined as the sum of erosion length per mouse [18]. Ethanol induced lesion was assessed and scored for severity according to, (0) absence of lesion, (1) superficial 1–5 hemorrhagic points, (2) superficial 6–10 hemorrhagic points, (3) submucosal hemorrhagic lesions with small

erosions (4) severe hemorrhagic lesion and some invasive lesions.

Antisecretory study

One hour after extract or test drug treatment, mice were anesthetized (xylazine 10 mg/kg plus ketamine 100 mg/kg, i.p.) and the pylorus was ligated. The animals were killed 3 h later and their stomach content was drained into a tube which was centrifuged 2000 rpm for 10 min. The pH was recorded with a digital pH meter. Total acid content of gastric secretion was determined by titration against 0.05 N NaOH [19].

ED50 values

ED50 values and the corresponding confidence limits were determined by the Litchfield and Wilcoxon method (PHARM/PCS Version 4).

Acute toxicity

Different doses of extracts were injected intraperitoneally into groups of six mice. The number of death was counted at 24 h after treatment. LD50 values and the corresponding confidence limits were determined by the Litchfield and Wilcoxon method (PHARM/PCS Version 4).

Statistical analysis

The data were expressed as mean values ± S.E.M. and tested with analysis of variance followed by the multiple comparison test of Tukey-Kramer. Ethanol induced lesion was assessed by Dunn's test.

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