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RAPID COMMUNICATION

Aqueous suspension of anise "*Pimpinella anisum*" protects rats against chemically induced gastric ulcers

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Abstract

AIM: To substantiate the claims of Unani and Arabian traditional medicine practitioners on the gastroprotective potential effect of a popular spice anise, "*Pimpinella anisum* L." on experimentally-induced gastric ulceration and secretion in rats.

METHODS: Acute gastric ulceration in rats was produced by various noxious chemicals including 80% ethanol, 0.2 mol/L NaOH, 25% NaCl and indomethacin. Anti-secretory studies were undertaken using pylorus-ligated Shay rat technique. Levels of gastric non-protein sulfhydryls (NP-SH) and wall mucus were estimated and gastric tissue was also examined histologically. Anise aqueous suspension was used in two doses (250 and 500 mg/kg body weight) in all experiments.

RESULTS: Anise significantly inhibited gastric mucosal damage induced by necrotizing agents and indomethacin. The anti-ulcer effect was further confirmed histologically. In pylorus-ligated Shay rats, anise suspension significantly reduced the basal gastric acid secretion, acidity and completely inhibited the rumenal ulceration. On the other hand, the suspension significantly replenished ethanol-induced depleted levels of gastric mucosal NP-SH and gastric wall mucus concentration.

CONCLUSION: Anise aqueous suspension possesses significant cytoprotective and anti-ulcer activities against experimentally-induced gastric lesions. The anti-ulcer effect of anise is possibly prostaglandin-mediated and/or through its anti-secretory and antioxidative properties.

Key words: Spice; Anise; *Pimpinella anisum*; Cytoprotection; Gastric ulcer; Gastric secretion; Sulfhydryls; Gastric wall mucus

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INTRODUCTION

Peptic ulcer is one of the most common gastrointestinal diseases. Nowadays proton pump inhibitors and H_2-recptor antagonists are the most widely used drugs to treat peptic ulcer disease. However, the use of these anti-secretory drugs may be associated with adverse events and ulcer relapse[1]. Thus, there is a need for more effective, less toxic and cost-effective anti-ulcer agents. In recent years, a widespread search has been launched to identify new anti-ulcer drugs from natural sources. Spices comprising the most important products used for flavouring foods and medicinal herbs are considered nowadays as potential bioactive agents that can interfere positively or negatively with different cellular processes. They are extensively used in medicine, pharmaceuticals, perfumery and cosmetics. Additionally they possess antioxidant, antispasmodic, carminative, anti-inflammatory and other properties[2]. A number of spices, namely large cardamom[3], black pepper[4], caraway[5], cardamom[6], clove[7], coriander[8], ginger[9], peppermint[10], saffron[11], turmeric[12] among others have been shown to possess significant gastroprotective activities. The fruits of anise plant, *pimpinella anisum* L. are locally known as aniseed and yansoon. The powder and concoction of anise in hot water are used as carminatives, antiseptics, diuretics, digestives, aphrodisiacs, and as a remedy for insomnia and constipation[13]. Furthermore, anise is used to promote digestion, improve appetite, alleviate cramps and nausea, and relieve flatulence and colic. In Unani and Arabian traditional medicine, anise fruit and its oil have been used for the treatment of various conditions including dyspepsia, nausea, abdominal colic,

seizures and epilepsy[14]. The phytotherapeutic applications of anise are based on its digestive, carminative, diuretic and expectorating action[15]. It has been recently reported that the essential oil of anise is highly effective as both larvicidal and ovicidal agents[16]. The principal constituents of anise are volatile oil, coumarins, fatty acids, flavonoid glycosides, proteins and carbohydrates. Among others, anise oil contains anethole and caryophyllene[17]. Since we have not come across a scientific report on potential gastroprotective claims of anise aqueous suspension, the present study was carried out to assess its effect on chemically induced gastric ulcers in rats.

MATERIALS AND METHODS

Plant material and preparation of aqueous suspension

Seeds of anise "*Pimpinella anisum* L" (family, Apiaceae) were purchased from local herb shops in Riyadh and identified by an expert taxonomist. The sample was preserved (voucher # Sp.Pr.17-16-37) at the herbarium of Department of Pharmacognosy, College of Pharmacy, King Saud University, Riyadh, for future reference. The seeds were ground to very fine powders (75 micron), and used as an aqueous suspension for treatment in different experiments.

Animals

Wistar albino rats of either sex, approximately at the same age, weighing 150-200 g were obtained from Animal Care Center, College of Pharmacy, King Saud University, and maintained under standard conditions of temperature, humidity and light (12 h dark, 12 h light) with free access to Purina chow and water. Before testing, the animals were fasted for 36 h with access to water *ad libitum*. 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.

Dose selection and route of administration

The doses (250 and 500 mg/kg, body weight) selected for the experiments were based on the maximum tolerable dose value (MTD) (30 g/kg, body weight) and the preliminary experiments conducted on the pharmacological activity of anise. The aqueous suspension was administred intragastrically (i.g.) through gastric intubation in all experiments, unless stated otherwise intraperitoneally (i.p.) in anti-secretory studies.

Gastric lesions induced by necrotizing agents

The rats were administered ig 1 mL of different necrotizing agents (80% ethanol, 0.2 mol/L NaOH and 25% NaCl)[18]. Anise suspension was given 30 min before the administration of necrotizing agents. One hour after the administration of ethanol and alkalis, the rats were sacrificed and examined for lesions in the stomach. The scoring of lesions, assays of gastric wall mucus and sulfhydryls as well as histological changes in the stomach were observed as follows. The patchy lesions of stomach induced by ethanol were scored according to the method described by Schiantarelli *et al*[19] using the following scale: 0 = normal mucosa, 1 = hyperemic mucosa or up to 3 small patches, 2 = 4 to 10 small patches, 3 = more than 10 small or up to 3 medium-sized patches, 4 = 4 to 6 medium-seized patches, 5 = more than 6 medium-sized or up to 3 large patches, 6 = 4 to 6 large patches, 7 = 7 to 10 large patches, 8 = more than 10 large patches or extensive necrotic zones. "Small" was defined as up to 2 mm across (max. diameter), "medium-sized" between 2 and 4 mm across and "large" more than 4 mm across.

Histopathological evaluation

Gastric tissue samples were fixed in neutral buffered formalin for 24 h. Sections of gastric tissue were histopathologically examined to study the ulcerogenic and/or anti-ulcerogenic activity of anise. The tissues were fixed in 10% buffered formalin and processed using a VIP tissue processor. The processed tissues were embedded in paraffin blocks and sections of about 5 μm thickness were cut by employing an American optical rotary microtome. These sections were stained with haematoxylin and eosin using routine procedures[20]. The slides were examined microscopically for pathomorphological changes such as congestion, hemorrhage, edema and erosions using an arbitrary scale for severity assessment of these changes.

Gastric lesions induced by indomethacin

Indomethacin was suspended in 1.0% carboxy-methylcellulose (CMC) in water (6 mg/mL) and administered orally to the rats fasted for 36 h at a dose of 30 mg/kg, body weight. Control rats were treated similarly with an equivalent amount of the vehicle[21]. The animals were sacrificed 6 h after the treatment. Stomachs of the animals were excised off the body, rinsed with normal saline and studied accordingly [22].

Estimation of non-protein sulfhydryls

Gastric mucosal non-protein sulfhydryls (NP-SH) were measured according to the method of Sedlak and Lindsay[23]. The glandular part of the stomach was homogenized in ice-cold 0.02 mmol/L ethylenediam inetetraacetic acid (EDTA). Aliquots of 5 mL of the homogenates were mixed in 15 mL test tubes with 4 mL of distilled water and 1 mL of 50% trichloroacetic acid (TCA). The tubes were shaken intermittently for 10 min and centrifuged at 3000 *g*. Two milliliters of supernatant was mixed with 4 mL of 0.4 mol/L Tris buffer at pH 8.9, 0.1 mL of 5, 5'-dithio-bis- (2-nitrobenzoic acid) (DTNB) was added and the sample was shaken. The absorbance was measured within 5 min after addition of DTNB at 412 nm against a reagent blank.

Pylorus-ligated rats (anti-secretory studies)

The rats were fasted for 36 h with access to water *ad libitum* before the pylorus was ligated under ether anesthesia and care was taken to avoid bleeding and occlusion of blood vessels [24]. Anise suspension was administered immediately after pylorus ligation (Shay) by ip route. The rats were sacrificed at 6 h after the pylorus ligation. The stomachs were removed, with the contents collected, volumes measured, centrifuged and analyzed for titratable acidity

Table 1 Effect of aqueous anise suspension on gastric lesions induced by various necrotizing agents (mean ± SD)

Group serial	Treatment	Dose (mg/kg, i.g.)	Ulcer index		
			80% EtOH	0.2 mol/L NaOH	25% NaCl
1	Control (distilled water)	-	7.16 ± 0.40	8.00 ± 0.0	7.66 ± 0.33
2	*Pimpinella anisum*	250	6.00 ± 0.51[b]	4.66 ± 0.80[d]	6.00 ± 0.44[d]
3	*Pimpinella anisum*	500	4.00 ± 0.44[d]	3.66 ± 0.66[d]	4.00 ± 0.51[d]

Six rats were used in each group. [b]$P < 0.01$, [d]$P < 0.001$ *vs* control (distilled water) group, Student' *t*-test.

Table 2 Effect of aqueous anise suspension on ethanol-induced histopathological changes in rat stomach

Group serial	Treatment and dose (mg/kg, body weight/day)	Histopathological changes							
		Congestion	Haemorrhage	Edema	Necrosis	Inflammatory changes	Dysplastic changes	Erosions	Ulceration
1	Control (distilled water) (1 mL/rat)	-	-	-	-	-	-	-	-
2	Ethanol, 80% (1 mL/rat)	++	+++	++	++	+	+	+++	+
3	Pimpinella anisum (250) + ethanol, 80% (1 mL/rat)	+	+	+	-	-	-	+	-
4	Pimpinella anisum (500) + ethanol, 80% (1 mL/rat)	+	+	+	-	-	-	+	-

-: normal, +: moderate, ++: severe, +++: intensely severe.

against 0.01 mol/L NaOH (pH 7) and the titratable acidity was calculated.

Determination of gastric wall mucus

Gastric wall mucus was determined according to the modified procedure of Corne *et al*[25]. 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 for 15 min and then for 45 min. Dye complexed with the gastric wall mucus 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 was then vigorously shaken with an equal volume of diethyl ether. The resulting emulsion was centrifuged at 4000 r/min for 10 min and the absorbance of aqueous layer was recorded at 580 nm. The quantity of Alcian blue extracted from per gram of wet glandular tissue was then calculated.

Statistical analysis

The readings shown are means ± SD. The mean determination of treatment groups was compared statistically with that of control group using *t*.

RESULTS

Effect of anise suspension on gastric lesions induced by necrotizing agents

The treatments of rats with 80% ethanol, 0.2mol/L NaOH and 25% NaCl produced extensive gastric lesions mainly confined to glandular part of the stomach in all the control (only necrotizing agents treated) animals. The ulcer index in ethanol, sodium hydroxide and sodium chloride treatment groups was 7.16 ± 0.40, 8.00 ± 0.0 and 7.66 ± 0.33, respectively. Pretreatment of rats with anise suspension at the dose of 250 mg/kg significantly prevented gastric mucosal lesions induced by all necrotizing agents used. The ulcer index was 6.00 ± 0.51 ($P < 0.01$), 4.66 ± 0.80 ($P < 0.001$), 6.00 ± 0.44 ($P < 0.001$), respectively. In stomach of rats treated with 500 mg/kg of anise suspension, the ulcer index was 4.00 ± 0.44 ($P < 0.001$), 3.66 ± 0.66 ($P < 0.001$) and 4.00 ± 0.51 ($P < 0.001$) in ethanol, sodium hydroxide and sodium chloride groups respectively (Table 1).

Effect of anise suspension on histopathological changes

Histological examination of gastric mucosa showed various histopathological changes including congestion, haemorrhage, edema, necrosis, inflammatory and dysplastic changes, erosions and ulcers in ethanol-treated rats. The histological indices such as necrosis, inflammatory and dysplastic changes and ulceration were completely inhibited in rats pretreated with both doses of anise suspension (Table 2).

Effect of anise suspension on gastric lesions induced by indomethacin

To study the anti-ulcerogenic effects of anise suspension on indomethacin-induced gastric lesions in rats, two does of anise were used (250 and 500 mg/kg). Data on ulcer index in rats pretreated with both doses are reported in Table 3. The increased ulcer index in the gastric mucosa of

Table 3 Effect of aqueous anise suspension on indomethacin-induced gastric mucosal lesions (mean ± SD)

Group serial	Treatment	Animals (*n*)	Dose (mg/kg, i.g.)	Ulcer index
1	Control (indo only)	6	-	32.16 ± 5.22
2	*Pimpinella anisum* + indo	6	250	9.66 ± 1.96[d]
3	*Pimpinella anisum* + indo	6	500	6.00 ± 2.68[d]

[d]$P < 0.001$ *vs* control (indo only) group. Indo: indomethacin.

Table 4 Effect of aqueous anise suspension on the levels of non-protein sulfhydryles (NP-SH) in glandular stomach of rats treated with 80% ethanol (mean ± SD)

Group serial	Treatment and dose (mg/kg, body weight)	NP-SH concentration (μmol/100 mg wet tissue)
1	Control (distilled water, 1 mL/rat)	11.70 ± 0.86
2	Control (80% ethanol, 1 mL/rat)	6.31 ± 0.23[b]
3	*Pimpinella anisum* (250) + 80% ethanol (1 mL/rat)	6.71 ± 0.33
4	*Pimpinella anisum* (500) + 80% ethanol (1 mL/rat)	7.68 ± 0.37[a]

Six rats were used in each group. [a]$P < 0.05$ *vs* control (80% ethanol) group, [b]$P < 0.01$ *vs* control (distilled water) group.

Table 5 Effect of anise suspension on gastric secretion, acidity and gastric lesion index in pylorus-ligated Shay rats (mean ± SD)

Group serial	Treatment	Dose (mg/kg, i.g)	Volume of gastric content (mL)	Titratable acid (mEq/L)	Ulcer index
1	Control (distilled water)	-	7.83 ± 0.38	127.21 ± 2.64	0.66 ± 0.21
2	*Pimpinella anisum*	250	3.00 ± 0.51[b]	109.33 ± 4.64[d]	0.00[d]
3	*Pimpinella anisum*	500	0.33 ± 0.33[d]	108.33 ± 5.00[d]	0.00[d]

Six rats were used in each group. [b]$P < 0.01$, [d]$P < 0.001$ *vs* control (distilled water) group.

Table 6 Effect of aqueous anise suspension on ethanol-induced gastric wall mucus concentration changes (mean ± SD)

Group serial	Treatment	Dosage (mg/kg,i.g.)	Gastric wall mucus (μg Alcian blue of wet glandular tissue)
1	Control (distilled water)	-	474.98 ± 13.86
2	80% ethanol only	-	307.92 ± 10.69[d]
3	*Pimpinella anisum* 80% ethanol	250	372.75 ± 20.87[a]
4	*Pimpinella anisum* 80% ethanol	500	391.60 ± 20.19[b]

[a]$P < 0.05$, [b]$P < 0.01$ *vs* 80% ethanol group only; [d]$P < 0.001$ *vs* control (distilled water) group.

indomethacin-treated rats (control) decreased significantly in animals treated with lower and higher anise doses (32.16 ± 5.22 *vs* 9.66 ± 1.96 and 6.00 ± 2.68, $P < 0.001$), respectively.

Effect of anise suspension on ethanol-induced mucosal NP-SH *depletion*

The level of NP-SH in the gastric mucosa of control rats was 11.70 ± 0.86 mmol/g of tissue, significantly decreased to 6.31 ± 0.23 mmol/g following the administration of ethanol. Pretreatment of rats with anise suspension at a higher dose (500 mg/kg) significantly replenished the ethanol-induced depletion of NP-SH ($P < 0.05$, Table 4).

Effect of anise suspension on gastric secretion in pylorus-ligated rats

In control rats, pylorus ligation for 6 h resulted in an accumulation of 7.83 ± 0.38 mL of gastric secretions, titratable acidity 127.21 ± 2.64 in mEq/L and an ulcer index 0.66 ± 0.33 (Table 5). The volume of gastric secretion in the rats treated with 250 and 500 mg/kg of anise suspension significantly reduced to 3.00 ± 0.51 and 0.33 ± 0.33 mL, respectively ($P < 0.001$). A significant decrease in titratable acid was also observed in the rats treated with 250 mg/kg (109.33 ± 4.64 mEq/L) and 500 mg/kg (108.33 ± 5.00 mEq/L) ($P < 0.001$). A complete inhibition of rumenal ulcers was noted in both groups of rats treated with anise suspension as compared to control group (Table 5).

Effect of anise suspension on ethanol-induced changes in gastric wall mucus

The treatment of rats with ethanol significantly decreased the Alcian blue binding capacity of gastric wall mucus (307.92 ± 10.69 μg Alcian blue/g of tissue) as compared to control rats (474.98 ± 13.86 μg/g). Pretreatment of rats with anise suspension at 250 mg/kg (372.75 ± 20.87 μg/g) and 500 mg/kg (391.60 ± 20.19 μg/g) significantly enhanced Alcian blue binding capacity of gastric mucosa ($P < 0.05$, $P < 0.01$), respectively (Table 6).

DISCUSSION

Anise spice is added to foods in several forms as whole spice, as ground spice or as isolates from its extracts and volatile oils[26]. We adopted the suspension dosage form in our experiments. In necrotizing agents-induced gastric ulcers, the lesions were characterized by multiple haemorrhage red bands of different sizes along the longitudinal axis of the glandular stomach. This model is extensively used to screen drugs for cytoprotection[27]. This study provided a substantial evidence for anti-ulcer and

anti-secretory effects of an aqueous suspension of anise. Anise suspension significantly inhibited the ulcerative lesions in all animals treated with necrotizing agents, which was further confirmed by histological findings in which necrosis, inflammatory, dysplastic changes and ulcers were abolished in rats pretreated with anise suspension. The ability of gastric mucosa to resist injury by endogenous secretions (acid, pepsin and bile) and ingested irritants (e.g., alcohol), can be attributed to a number of factors that have been referred to collectively as mucosal defense[28]. Gastric mucosal lesions induced by necrotizing agents such as ethanol and strong alkalis are due to depression of the gastric defensive mechanisms[29]. Although ethanol-induced ulcers are not inhibited by anti-secretory agents such as cimetidine, they are inhibited by agents that enhance mucosal defensive factors such as prostoglandins[30]. The current results suggest that the anti-ulcerogenic effect of anise suspension may be related to its cytoprotective activity.

Gastroduodenal ulceration is a major limitation to the use of non-steroidal anti-inflammatory drugs (NSAIDs)[31]. NSAIDs can cause damage to the gastroduodenal mucosa via several mechanisms, including their topical irritant effect on the epithelium, impairment of the mucosal barrier function, suppression of gastric prostaglandin synthesis, reduction of gastric mucosal blood flow and interference with the repair of superficial injury. The presence of acid in the lumen of stomach also contributes to the pathogenesis of NSAIDs-induced ulcers and bleeding by impairing the restitution process, interfering with haemostasis and inactivating several growth factors that are important in mucosal defence and repair[32,33]. In the present study, indomethacin-induced gastric lesions were extensively prevented by anise suspension.

Sulfhydryl compounds have been significantly implicated in the maintenance of gastric integrity, particularly when reactive oxygen species are involved in the pathophysiology of tissue damage[34]. Since anise suspension significantly enhances gastric tissue NP-SH concentration, it is conceivable that it is endowed with antioxidant properties accounting for its gastroprotective action. Hence, it may be presumed that the replenishing potential of sulfhydryl levels might play an important role in the gastroprotective activity of anise suspension-treated rats. Furthermore, the ability of anise suspension to protect against ulcers in NSAID-induced gastric damage may be due to the enhanced synthesis of mucus, bicarbonates and prostaglandins, as well as reduced acid output. Consequently these activities can promote the inhibition of basal gastric acid secretion as observed in our pylorus-ligated shay rat model[35,36]. On the other hand, it is also important to note that NSAIDs can increase gastric acid secretion, through prostaglandin inhibitory effects on parietal cells[37,38]. In the present study, anise aqueous suspension treatment significantly reduced basal gastric acid volume, titratable acidity and completely inhibited ulcer formation in rats. However, to date it is still controversial about relationship between the acid output and the genesis of acute gastric mucosal lesions (AGML). Our results support this correlation as anise suspension significantly reduced basal gastric secretion and prevented the occurrence of AGML in pylorus-ligated rats and thus, supporting the hypothesis of "no acid no ulcer" [39]. It has been postulated that histamine may be involved in the formation of pylorus-ligated ulcers and play a mediating role in the gastric secretion stimulated by gastrin, vagal stimulation, and cholinergic agents[40]. The correlation between gastric mucus and acid secretions in our experiments, clearly demonstrated that the gastric protective activity observed may be associated with correction or normalization of the altered balance between erosive action of acid and gastric mucosal defence. Gastric wall mucus is thought to play an important role as a defensive factor against gastric mucosal damage[41]. The determined gastric wall mucus is used as an indicator for gastric wall mucus secretion[42]. In the present investigation, anise suspension caused a significant enhancement of ethanol-induced gastric wall mucus depletion in rats, which further confirms the ability of anise to prevent and/or ameliorate the effects of damaging agents. These findings indicate that anise suspension preserves gastric mucus secretion and strengthens gastric mucosa defense factors in experimental rats[43,44].

The chemical constituents of anise responsible for its anti-ulcer activity are not known. However, chemical studies demonstrated that anise contains estrarole[45], anethol[46], eugenol[47], anisaldehyde, methylchanicol[48], coumarins[49] and terpenes[50] among others as the major compounds. Anise and its compounds have been identified as free radicals or active oxygen scavengers[51]. In addition, the ability of anise suspension to protect gastric mucosa against lesions induced by chemical irritants is likely by maintaining the structural integrity of gastric epithelium and balance of aggressive factors and inherent protective mechanisms[52]. Furthermore, the mucus gel and its bicarbonate gradient seem to be an important first-line defense against harmful stimuli [53].

In conclusion, anise suspension exhibits an anti-ulcer potential activity through at least one or more possible mechanisms including inhibition of basal gastric secretion, stimulation of mucus secretion, endogenous gastric mucosal prostaglandin synthesis and possible antioxidative activity.

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