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Full Length Research Paper

Gastric antisecretory and antiulcer effects of ajowan "*Carum copticum*" in rats

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An aqueous suspension of ajowan "*Carum copticum*" was evaluated for its possible gastric antisecretory and antiulcer activities in various ulcer models in Wistar albino rats. The suspension at 250 and 500 mg kg/body weight, orally (i.p. in Shay rat model) has significant effect in pyloric ligation induced basal gastric acid secretion, indomethacin and noxious chemicals (80% ethanol, 0.2 M NaOH and 25% NaCl) induced gastric ulceration; showed significant protection in all models used. Histopathological assessment of gastric tissue and the determination of gastric wall mucus (GWM) and non protein sulfhydryl (NP-SH) contents of the stomach, characterized the protection of various indices and replenishing the depleted (GWM) and NP-SH levels by the suspension treatment, respectively. Conclusively, the ulcer protective effect of ajowan may possibly be due to its antisecretory along with antioxidative and cytoprotective effects through prostaglandins mediated mechanism.

Key words: Ajowan, *Carum copticum,* gastric secretion, antiulcer.

INTRODUCTION

The fruit of *Carum copticum* (L.) Sprague ex Turill (Family: Apiaceae) locally known as Ajowan and Nakhwa; in the Middle East, and is primarily grown and used in the Indian subcontinent, but also in Egypt, Iran and many other countries as a spice, flavoring agent and a condiment. Raw ajowan smells almost exactly like thyme, because it also contains thymol (Aman, 1969). Ajowan is being used as a carminative medicine from the ancient times, even Greek physicians Dioscorides, Galen, used ajowan as carminative. The fruits are used as a medicament in Unani, Ayurvedic and Arab traditional medicine for its diuretic, analgesic, antiasthmatic, anthelmintic and antispasmodic properties (Zahin et al., 2010). The ajowan has also been mentioned to have therapeutic effect on flatulence, indigestion, colic and dyspepsia (Dashti-Rahmatabadi et al., 2007). The fruit contains an aromatic volatile essential oil, and a crystalline substance stearoptene (crude thymol), and a phenolic glucoside (Ballba et al., 1973). To ascertain the gastric antiulcer activity of the ajowan in the powdered dosage form (suspension) (a commonly used dosage form in Arab and Unani medicine); the present investigation was carried out in rats.

MATERIALS AND METHODS

Plant material and suspension

Fruits of ajowan were purchased from a local crude herbal drugs store in Riyadh, and identified by an experienced 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 ajowan fruits were finely pulverized (Mesh # 70 µ), dissolved in water and the freshly obtained aqueous suspension was used for treatment in different experiments.

Animal stock

Wistar albino rats of either sex (home bred) aged 7 to 8 weeks and weighing 150 to 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±5%), temperature (22±2°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.

Table 1. Effect of an aqueous suspension of *C. copticum* on the volume of gastric secretion, titratable acidity and the degree of ulceration in 6 h pylorus ligated (Shay) rats.

Groups	Volume of gastric content (ml)	Titratable acid (mEq/l)	Ulcer index
Control (distilled water, 1 ml/rat)	7.33±0.51	123.33±2.98	1.5±0.34
C. copticum suspension (250 mg/Kg)	4.83±0.77*	94.99±4.36***	0.33±0.21*
C. copticum suspension (500 mg/Kg)	2.16±0.90***	71.11±4.84***	00***

Mean ± SEM (n=6). *P < 0.05; ***P < 0.001 compared with control (Student's t-test).

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, care being taken not to cause bleeding or to include blood vessels (Shay et al., 1945). *C. copticum* 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.

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 (0.5 ml/100 g). Rats were treated with ajowan suspension (250 and 500 mg/kg, orally) 30 min before indomathacin. Control rats were treated similarly with an equivalent amount of vehicle (Bhargava et al., 1973). The stomachs of the animals were removed, rinsed with normal saline and studied according to the standard procedure.

Determination of gastric wall mucus (GWM)

Gastric wall mucus was determined according to the procedure of Corne et al. (1974). The glandular part of the stomach (0.5 g) was placed in 10 ml of 1% Alcian blue solution in 0.16 M sodium acetate (pH 5.8) and left to stain for 2 h. The dye complex was extracted with 0.5 M $MgCl_2$ solution, centrifuged and measured spectrophotometrically at 580 nm using a standard curve of Alcian blue.

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 (Robert et al., 1979). Ajowan suspension was given 30 min before the necrotizing agents. The animals were killed under anesthesia, using diethyl ether 1 h after treatment with the necrotic agents. The stomach of each of the animals 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 et al. (1982). 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 by Sedlak and Lindsay (1968). The glandular stomachs of control and treated rats were removed and homogenized in ice-cold 0.02 M ethylenediaminetetraacetic acid. The homogenate was mixed with distilled water and 50% 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 of addition of DTNB, at 412 nm, against a reagent blank with no homogenate.

Histopathological studies

The gastric tissue was fixed in 10% ethanol buffer formalin and processed though graded ethanol, xylene and impregnated with paraffin wax; sections were made by microtome. After staining with haemotoxylin and eosin stain (Culling, 1974), the sections were examined under a research microscope by a person who was not aware of experimental protocols. The different histopathological indices screened were: congestion, hemorrhage, edema, necrosis, inflammatory and dysplastic changes erosions and ulcerations.

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 was <0.05.

RESULTS

Aqueous suspension of *C. copticum* significantly inhibited gastric secretory volume, acidity and ulceration caused by pylorus ligation of rats (Table 1). It also resulted in a significant reduction of gastric ulceration induced by indomethacin (Table 2). Furthermore, the treatment with ajowan, at a higher dose, was found to significantly increase the gastric wall mucus content depleted by ethanol, whereas there was no significant protection at the lower dose (Table 3). It showed a protective effect on gastric mucosal damage induced by various necrotizing agents. However, there was no significant protection at the lower dose (250 mg/kg, body weight) against an 80% ethanol challenge (Table 4). Suspension pretreatment also replenished the NP-SH contents depleted by ethanol. However, the protection was significant only at a higher dose (500 mg/kg, body weight) (Table 5). On isolated smooth muscle of guinea pig ileum preparation, the suspension of ajowan produced an inhibitory effect on acetylcholine induced contraction of the muscle.

Histological assessment of gastric tissue after ethanol treatment induced intensely severe congestion, haemorrhage and erosions, severe edema, necrosis and

Table 2. Effect of an aqueous suspension of *C. copticum* on the gastric mucosal damage induced by indomethacin in rats.

Groups	Ulcer index
Control (indomethacin only, 30 mg/Kg t)	33.66 ± 2.88
C. copticum (250 mg/Kg) + indomethacin only (30 mg/Kg	23.50 ± 1.38**
C. copticum (500 mg/Kg) + indomethacin only (30 mg/Kg	16.16 ± 1.74***

Mean ± SEM (n=6). **P < 0.01; ***P < 0.001 compared with control (Student's *t*-test).

Table 3. Effect of an aqueous suspension of *C. copticum* on ethanol-induced gastric wall mucus changes in rats.

Groups	Gastric wall mucus (µg Alcian blue of wet glandular tissue)
1. Control (distilled water, 1 ml/rat)	462.70 ± 9.44
2. 80% Ethanol only (1 ml/rat)	286.58 ± 7.77***
3. *C. copticum suspension* (250 mg/Kg) + 80% ethanol	307.60 ± 10.852
4. *C. copticum suspension* (500 mg/Kg) + 80% ethanol	320.63 ± 11.45*

Mean ± SEM (n=6). ***P < 0.001 compared with control, *P < 0.05 compared with 80% ethanol treated group (Student's *t*-test). Group 2 was statistically compared to group 1 and groups 3 and 4 were statistically compared to group 2.

Table 4. Effect of an aqueous suspension of *C. copticum* on the gastric lesions induced by various necrotizing agents.

Groups	Ulcer index		
	80% EtOH	0.2 M NaOH	25% NaCl
Control (distilled water, 1 ml/rat)	7.50±0.34	7.83±0.16	7.00±0.36
C. copticum suspension (250 mg/Kg)	7.33±0.42	6.16±0.40**	5.00±0.63*
C. copticum suspension (500 mg/Kg)	4.83±0.79*	4.33±0.42**	3.33±0.94**

Mean ± SEM (n=6). *P < 0.05; **P<0.01 compared with control group (Student's t-test).

Table 5. Effect of an aqueous suspension of non-protein sulfhydryl (NP-SH) concentration in gastric tissue.

Groups	NP-SH concentration (µmol/100 mg wet tissue)
1. Control (distilled water, 1 ml/rat)	10.98 ± 0.83
2. 80% ethanol (1 ml/rat)	6.23 ± 0.16**
3. *C. copticum* suspension (250 mg/kg) + 80% ethanol	6.37 ± 0.05
4. *C. copticum* suspension (500mg/kg) + 80% ethanol	6.67 ± 0.05*

Mean ± SEM (n=6). *P < 0.05; **P<0.01 compared with control group (Student's *t*-test).Group 2 was statistically compared with group 1 and group 3 and 4 were statistically compared with group 2.

a moderate effect on inflammatory changes, dysplastic changes, and erosions. The pretreatment with ajowan suspension totally inhibited edema, necrosis, dysplastic changes and ulceration at both lower (250 mg/kg., body weight) and higher (500 mg/kg., body weight) doses (Table 6). There was moderate protection of congestion, haemorrhage and erosions at these doses. The inflammatory changes induced by ethanol were not protected at any of the doses of the *C. copticum*.

DISCUSSION

The present study clearly indicates the capacity of ajowan aqueous suspension to significantly inhibit the formation of basal gastric volume, acidity and ulcers in Shay rats and by various ulcerogenic agents including indomethacin, hyperosmotic and strongly alkaline solutions. Ulcers occur when an imbalance allows luminal injurious factors to predominate over mucosal resistance.

Table 6. Effect of an aqueous suspension of *C. copticum* on ethanol-induced histopathological lesions in gastric tissue of rats.

Groups	Histopathological lesions induced							
	Congestion	Haemorrhage	Edema	Necrosis	Inflammatory changes	Dysplastic changes	Erosions	Ulcerations
Control (distilled water, 1 ml/rat)	–	–	–	–	–	–	–	–
80% Ethanol (1 ml/rat).	+++	+++	++	++	+	+	+++	+
C. copticum suspension (250 mg/Kg) + 80% ethanol	+	++	–	–	+	–	+	–
C. copticum suspension (500 mg/Kg) + 80% ethanol	+	++	–	–	+	–	+	–

– = Normal; + = Moderate effect; ++ = Severe effect; +++ = Intensely severe effect.

The chief injurious factors are acid and peptic activity. Bile reflux, drugs and stasis may alter the luminal milieu and act together with acid-peptic activity to induce injury, weaken mucosal resistance, or both (Malagelada et al., 1986). Moreover, gastric acid hypersecretion plays an important role in producing experimental ulcers by pylorus ligation and stress in rats (Kitagawa et al., 1979), which are often termed as “aggressive” factor (Goa and Monk, 1987). The pylorus ligation studies showed that ajowan suspension significantly decreased the volume and acidity of basal gastric secretions and inhibited the ulcer formation. It is generally believed that inhibition of acid secretion is the most important factor for the healing of gastric ulers (Okabe et al., 1977). Indomethacin, a non-selective cyclooxygenase inhibitor is known to induce gastric damage through multiple mechanisms which include suppression of prostaglandin generation, overproduction of leukotrienes, acting as a topical irritant and by reducing the local blood flow (Alqasoumi et al., 2008). Rats pretreated with ajowan suspension produced significant protection in this model. It is possible that an increase in gastric mucus and/or a possible leukotriene antagonism may contribute to the gastroprotective effect of ajowan (Paiva et al., 1998). On the other hand, the suspension found to prevent ethanol induced gastric wall mucus depletion. Furthermore, ajowan also significantly inhibited gastric mucosal damage induced by cytodestructive agents. The preservation of adherent mucus on the glandular mucosa is one of the contributory factors in the prevention of chemical irritants induced gastric mucosal damage (Wong et al., 1986; Al-Mofleh et al., 2008). It is therefore, concluded that the protective effect of ajowan may be partly due to the preservation of mucus level in the gastric mucosa.

The chemical constituents of ajowan responsible for its antiulcer activity are not known. However, ajowan is reported to contain essential oil, which is a principal source of thymol and other phenolic substances (Chopra et al., 1956; Shahidi et al., 1992). Earlier, phenolic compounds have been shown to possess strong antioxidant activity and function as free radical terminators (Madsen et al., 1997). Okuda et al. (1994) have demonstrated that phenolics and several other types of plant polyphenolics have shown inhibition of leukotriene biosynthesis. The observations showed a significant reduction in non-protein sulfhydryls (NP-SH) content of gastric mucosa after 80% ethanol administration. Pretreatment with ajowan suspension prevented NP-SH depletion. Non-protein sulfhydryls are thought to be involved in protecting gastric mucosa against various chemicals (Szabo et al., 1981; Rogers et al., 1988). Decreased levels of endogenous sulfhydryls have been associated with tissue damage by various chemical agents (Miller and Lid, 1985; Rafatullah et al., 1995). These findings suggest that NP-SH may be involved in the ulcer protective effect of ajowan suspension; as ajowan has been shown to possesses strong antioxidant activity (Schaich et al., 1994; Mehta et al., 1994; Simic and Jovanovic, 1994) and may exert its antiulcer activity through its antioxidant potential, moreover, an anti-cholenergic activity of ajowan may also contribute to some extent in protecting ulceration (Rafatullah et al., 1993; Al-Howiriny et al., 2010).

Ajowan pretreatment protected all the ethanol-induced histological lesions except the inflammatory changes. These results confirm the folkloric use of ajowan in flatulence, indigestion, colic, dyspepsia, diarrhoea. In conclusion, the present data also substantiate the use of *C. copticum* in Unani, Ayurvedic and Arab traditional medicine as an anti-ulcer agent through its antisecretory, antioxidant properties of the phytoconstituents present in ajowan suspension. However, the role of prostaglandin in protecting the ulcer cannot be ruled out.

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