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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 9 (6): 1009-1013, 2006
ISSN 1028-8880

Antigastric Ulcer Studies on 'Saffron' *Crocus sativus* L. in Rats

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Abstract: Saffron, *Crocus sativus* Linn. (Family Iridaceae) commonly known as Zaa'fran is used as a flavoring agent and an important ingredient of Arabic coffee (Gahwa). It is also used in many gastric ailments. An aqueous suspension of saffron was subjected for evaluating gastric antiulcer activity induced by pylorus ligation (Shay rats), indomethacin and various necrotizing agents including (80% ethanol, 0.2 M NaOH and 25% NaCl) in rats. Gastric wall mucus and non-protein sulfhydryl contents were also estimated in rats. Histopathological assessment of rat stomach was carried out. The saffron aqueous suspension at doses (250 and 500 mg kg^{-1}) exhibited decrease in basal gastric secretion and ulcer index in Shay rats and indomethacin treated groups. Gastric wall mucus elevation was observed. No significant histopathological changes were noted. A large margin of safety was observed in animals after acute and chronic treatment.) Saffron exhibited significant antisecretory and antiulcer activities without causing any deleterious effects on acute and chronic toxicity in rodents.

Key words: Saffron, *Crocus sativus*, Arabian coffee, Gahwa, gastric antiulcer

INTRODUCTION

Stigma of Saffron *Crocus sativus* Linn. (Family Iridaceae) locally known as Zaa'fran is an important ingredient of Arabian coffee (Gahwa) and used as a flavoring agent. It is mostly used as a domestic spice in eastern and western cuisine. Saffron and its extracts are used in alcoholic and non-alcoholic beverages, candy, baked goods and other food products. In cosmetics, saffron extracts are used as fragrance components in perfumes (Leung, 1980). It is included in polyherbal formulations of Unani and Ayurvedic medicine (Anonymous, 1969). It is commonly used in Indian, Arabian and oriental traditional medicines for flatulent colic, to increase appetite, to relieve abdominal pain and to promote menstrual bleeding and as an aphrodisiac (Chopra *et al*., 1956; Said *et al*., 1996). Zhang *et al*. (1994), described its action on CNS as a stimulant and found it to ameliorate the impairment effects of ethanol on learning and memory processes. Saffron has been reported to possess both platelet aggregation-inducer and-inhibiting factors (Liakopoulou-Kyriakides and Skubas, 1990). Earlier studies (Nair *et al*., 1991, 1992), saffron have shown to be cytotoxic in sarcoma-180 and Ehrlich ascites carcinoma solid tumors in mice and increase life span of tumor bearing mice.

Saffron is known to contain various chemical constituents including crocin-1, picrocrocin, startry, vitamins, B1 and B2, fixed oils, carotenoids, colichicine, quercitin, proteins, wax and mucilage (Tarantilis *et al*., 1995).

However, no experimental data are available regarding its gastric antiulcer potential. Therefore, the present studies were carried out in rodents to find rationale for its medicinal use and gastroprotection claims in Arabian and Indian systems of medicine.

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MATERIALS AND METHODS

Plant material: Saffron was purchased from the local market of Riyadh and authenticated by our taxonomist, College of Pharmacy, King Saud University. A voucher specimen of saffron has been deposited at the herbarium of the Department of Pharmacognosy, College of Pharmacy, King Saud University, Riyadh.

Preparation of the aqueous suspension: The saffron was finely ground in an electric blender, sieved and mixed with distilled water. After thorough mixing in a vortex mixer and used for treatment of the animals.

Pharmacological studies: Wistar albino rats of either sex, approximately of the same age, weighing 150-200 g were used. They were divided into groups of six animals each.

The solutions of necrotizing agents and ulcerogenic drugs were freshly prepared before administration. Saffron suspension was administered orally in the dose of 250 and 500 mg kg^{-1} body weight. The stomach was removed, opened along the greater curvature, washed with saline and the inner surface was examined with a 6.9×binocular magnifier. Lesions were also assessed by two observers unaware of experimental protocols.

Pylorus ligated (Shay) rats: The pylorus was ligated by means of the technique of Shay *et al.* (1945). The animals were fasted for 48 h prior to pylorus ligation. The freshly prepared suspension of saffron was administered at a dose of 250 and 500 mg kg^{-1} intraperitoneally just after pylorus ligation. The animals were killed 6 h after the pylorus ligation using anaesthetic ether. The gastric contents were measured and the stomachs were opened along the greater curvature and the inner surface was examined for the degree of ulceration.

Gastric mucosal damage induced by indomethacin: Indomethacin was suspended in 0.5% carboxymethylcellulose (CMC) in water and administered orally in the dose of 40 mg kg^{-1} body weight to the fasted rats according to the method of Bhargava *et al.* (1973). The suspension was administered orally 30 min before the ulcerogenic drug. The animals were sacrificed 6 h after indomethacin administration. The incidence and extent of ulceration was recorded.

Cytoprotection studies: Gastric lesions induced by Necrotizing agents: The experiments were conducted on male Wistar albino rats starved for 36 h with access to drinking water *ad libitum*. The animals were given 1 mL of 0.2 M NaOH and 25% NaCl (w/v) 30 min after the oral administration of 250 and 500 mg kg^{-1} saffron suspension. One hour after the necrotizing agents administration, the animals were sacrificed and the stomachs were examined for the total area or the lesions (Robert *et al.*, 1983).

Biochemical studies

Estimation of gastric wall mucus in rats: Gastric wall mucus was determined according to the modified procedure of Corne *et al.* (1974). The glandular segments of the stomach, which included corpus and antrum were separated from the rumen of the stomach and weighed. Each segment was transferred immediately to 10 mL of 0.1% w/v Alcian blue solution (in 0.16 M sucrose solution buffered with 0.05 mL of 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 M sucrose, first for 15 and then for 45 min. Dye complexed with the gastric wall mucus was extracted with 10 mL of 0.5 M $MgCl_2$ which was shaken intermittently for 1 min at 30 min intervals for 2 h. Four milliliter of blue extract were then shaken vigorously with an equal volume of diethyl ether. The resulting emulsion was centrifuged at 3600 rpm for 10 min and the absorbance of aqueous layer was recorded at 580 nm. The quality of Alcian blue extracted per gram of wet glandular tissue was then calculated.

Estimation of non-protein sulfhydryl (NP-SH) groups: The animals were fasted for 36 h before oral administration of saffron suspension and 30 min later the rats were treated with 1 mL of 80% ethanol. After 1 h the animals were sacrificed and their stomachs removed. Gastric mucosal NP-SH was measured according to the method of Sedlak and Lindsay (1968). The glandular part of the stomach was homogenized in the ice-cold 0.02 M EDTA. Aliquots (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. The tubes were shaken intermittently for 10 to 15 min and centrifuged at 3000 ×g. Two milliliter of supernatant were mixed with 4 mL Tris buffer, pH 8.9; 0.1 mL of 5,5'-dithio-bis-2 (nitrobenzoic acid) at 412 nm against a reagent blank with no homogenates.

Histopathological studies: The gastric tissue was fixed in 10% ethanol buffered formalin and processed through 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.

Acute toxicity: Acute toxicity studies were undertaken in mice (10 male and 10 female mice were used in each group). The observation on acute toxicity involved autonomic responses, motor activity, CNS excitation, sedation, calmness and mortality (Chan *et al*., 1986).

Chronic toxicity: This study was done on mice for a period of 90 days (10 male and 10 female mice were used in each group). The parameters include, sign of toxicity, impact on body and vital organ weight and observation of visceral organs for any obvious change (Chan *et al*., 1986).

Statistical analyses: The data was statistically analyzed using and Student's t-test.

RESULTS

Aqueous suspension of saffron produced a significant decrease in the volume of gastric secretion and ulcer index in the low dose level group (250 mg kg^{-1}), while there was no decrease found in titratable acidity (Table 1). Aqueous suspension of saffron pretreatment showed significant reduction of ulceration of stomach in rats induced by indomethacin (Table 2). Pretreatment of animals with saffron suspension significantly prevented the depletion of gastric wall mucus induced by 80% ethanol (Table 3). Saffron's suspension, when orally pretreated to animals, failed to inhibit gastric ulcers induced by various necrotizing agents. Although, the lesions were found to be insignificantly decreased in NaOH and NaCl groups (Table 4). The gastric non-protein sulfhydryl (NP-SH) contents were significantly decreased

Table 1: Effect of *Crocus sativus* on the volume of gastric secretion, titratable acidity and the degree of ulceration in 6 h pylorus ligated (Shay) rats

Treatments	Dose (mg kg^{-1}, i.p.)	Volume of gastric content (mL)	Titratable acid (mEq L^{-1})	Ulcer index
Control	–	6.92±0.70	136.77±3.31	1.00±0.52
Crocus sativus	250	4.17±0.28*	130.55±6.41	00*
Crocus sativus	500	4.83±0.83	125.55±4.44	1.00±0.45

Six animals were used in each group.*p<0.001. Student's *t*-test

Table 2: Effect of *Crocus sativus* on the gastric mucosal damage induced by indomethacin in rats

Treatment	No. of animals	Dose (mg kg^{-1}, p.o.)	Ulcer index
Control	6	–	35.33±5.31
Crocus sativus	6	250	13.67±3.25**
Crocus sativus	6	500	16.33±3.77*

*p<0.05; **p<0.01. Student's *t*-test

Table 3: Effect of *Crocus sativus* in 80% ethanol-induced gastric wall mucus changes in rats

Group (n = 6)	Dosage (mg kg^{-1}, p.o.)	Gastric wall mucus µg Alcian of blue wet glandular tissue
Control	–	409.16±24.96
80% Ethanol only	–	311.69±17.403[a]***
Crocus sativus	250	343.15±12.15[b]
+80% ethanol	500	361.07±9.392[b]*

[a]As compared to the control group. [b]As compared to the 80% ethanol-treated group. *p<0.05; **p<0.01.Student's t-test

Table 4: Effect of *Crocus sativus* on the gastric lesions induced by various necrotizing agents in rats

Treatment (n = 6)	Dose (mg kg^{-1}, p.o.)	Ulcer index	
		0.2 M NaOH	25% NaCl
Control	–	7.16±0.65	7.00±0.51
Crocus sativus	250	7.33±0.33	6.00±0.77
Crocus sativus	500	7.50±0.34	5.33±0.66*

*p<0.05. Student's t-test

Table 5: Effect of *Crocus sativus* on glutathione (NP–SH) concentration in gastric tissue of rats

Treatment and dose (mg kg^{-1}, body weight)	NP–SH concentration (µmol/100 mg wet tissue)
Control (distilled water, 1 mL/rat)	11.80±0.95
Control (80% ethanol, 1 mL/rat)	6.05±0.27*[a]
Crocus sativus (250)+80% ethanol (1 mL/rat)	5.58±0.14[b]
Crocus sativus (500)+80% ethanol (1 mL/rat)	5.95±0.17[b]

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<0.001. (Student's t-test)

Table 6: Effect of *Crocus sativus* on ethanol-induced histopathological lesions in gastric mucosa of rats

Group No.	Treatment and dose (mg kg^{-1}, body weight)	Histopathological lesions induced							
		Congestion	Haemorrhage	Edema	Necrosis	Inflammatory changes	Dysplastic changes	Erosions	Ulceration
1	Control (distilled water) (1 mL/rat)	–	–	–	–	–	–	–	–
2	Ethanol, 80% (1 mL/rat)	++	++	+	+	+	+	++	++
3	*Crocus sativus* (250) +ethanol, 80% (1 mL/rat)	++	+	+	–	–	–	+	+
4	*Crocus sativus* (500) +ethanol, 80% (1 mL/rat)	–	–	–	–	–	–	–	–

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

following administration 80% ethanol. Treatment with saffron failed to prevent this depletion in both the groups (Table 5). The results on histopathological investigations (500 mg kg^{-1}) revealed that the pretreatment with saffron suspension absolutely prevented the ethanol-induced congestion, haemorrhage, edema, necrosis, inflammatory and dysplastic changes erosions and ulceration in the gastric mucosa of rats. However, the rats received small dose (250 mg kg^{-1}) showed gastric mucosal erosions and vascular congestion at the lower dose (Table 6). The observation of animals in acute toxicity test, the aqueous suspension of saffron in mice did not produced any apparent toxic symptoms. However, animals were shown sedation and calmness which continued for a period of 8 h.

DISCUSSION

Results obtained in this study show the antigastric activity of saffron aqueous suspension as evaluated in the most commonly utilized experimental models, including pylorus-ligated Shay rats, indomethacin and various necrotizing agents induced gastric lesions in rats. The saffron suspension has the capacity to significantly inhibit the basal gastric secretion and ulcerogenicity induced by pylorus ligation. Alteration in gastric secretion (Kitagawa *et al*., 1979; Murakami *et al*., 1985), abnormal motility (Garrick *et al*., 1986) has been considered as pathogenic mechanisms responsible for gastric mucosal lesions. It is reasonable to assume that a concomitant increase in gastric mucus and/or increase in prostaglandin generation contribute to protect gastric mucosal wall against chemical aggressors (Sartori *et al*., 1999) as the saffron suspension significantly replenish the gastric wall mucus depletion. The mucus layer is considered important both as a barrier to prevent damage as well as to facilitate repair (Wallace and Whittle, 1986). In the present study aqueous suspension of saffron exhibited a dose-dependent protection against indomethacin-induced gastric mucosal damage. Several non-steroidal anti-inflammatory drugs including indomethacin are known to induce gastric damage by suppression of prostaglandin (Valcavi *et al*., 1982; Konturek *et al*., 1984). Earlier, several compounds with the potential to generate prostaglandins have been reported to protect gastric mucosa against various ulcerogenic agents (Grant *et al*., 1988; Schepp *et al*., 1988). Therefore, a possible involvement of prostaglandin stimulation in the protection of gastric lesions may not be ruled out.

Although, the exact nature of phytoconstituents of saffron, responsible for antiulcer activity is not known, however, saffron is reported to contain phenolic compounds (Chrungoo and Farooq, 1984), crocin (a natural carotenoid) and flavonoids, these components were reported to have strong antioxidant activities on various biological systems including gastroprotective effects in rats (Takenaka *et al*., 1993; Quinn and Tang, 1996; Madsen *et al*., 1997). The suspension has shown protection only on hypertonic saline induced mucosal damage which suggests in part cytoprotective effect. The possible mechanism of this action may appear through adaptive cytoprotection mediated by endogenous prostaglandins (Takenaka *et al*., 1993). The findings on histopathological investigations are in corroboration with antigastric ulcer activity of the suspension observed under the studies on pharmacological and biochemical evaluation. On acute toxicity, the male mice initially showed aggressive behaviour, then calmness and sedation. The female mice were found to be normal. The prolong (12 weeks) treatment with saffron suspension (data not shown), was found to significantly increase the body weight throughout the duration of treatment. The rate of mortality was not affected.

In conclusion, it appears that *Crocus sativus* (saffron) suspension possesses antiulcerogenic principles which protect against gastric mucosal damage induced by indomethacin and necrotizing agent, through inhibition of gastric acid (attenuation of aggressive factors) and stimulation of mucus secretion (potentiation of defensive factors). Probably the antiulcer effect is due, partly at least, to the presence of flavonoids in the saffron, although, the involvement of other compounds in saffron cannot be ruled out. Hence, the prolong use of saffron in a small quantity in Arabian Coffee (Gahwa) as a flavoring agent and its use in oriental traditional medicine is substantiated by the results obtained in the present study as saffron also did not cause any apparent deleterious effects on the animals.

ACKNOWLEDGMENT

The authors are thankful to King Abdulaziz City for Science and Technology (KACST), Riyadh, Saudi Arabia for funding the Project (AR-16-37) on Spices.

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