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Protective effect of UL-409, a herbal formulation against physical and chemical factor induced gastric and duodenal ulcers in experimental animals

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Abstract

UL-409, a herbal formulation, was investigated for its possible ulcero-protective activity in Wistar rats of either sex and male guinea pigs. Oral administration of UL-409 at a dose of 600 mg/kg significantly prevented the occurrence of cold-restraint stress induced ulcerations. It significantly inhibited gastric ulceration induced by alcohol and aspirin, as well as cysteamine and histamine induced duodenal ulcers in rats and guinea pigs, respectively. The volume and acidity of gastric juice in pyloric ligated rats was reduced by UL-409. It also significantly, and dose dependently, promoted gastric mucus secretion in normal as well as in stress, drug and alcohol induced ulceration in animals. On the basis of these observations, we conclude that UL-409 possesses antiulcer activity and that the observed activity may be due to the modulation of defensive factors by improvement in gastric cytoprotection.

Keywords: UL-409; Wistar rats; Antiulcer activity; Gastric cytoprotection

1. Introduction

Traditionally, peptic ulcers have been described as an imbalance between the luminal acid peptic attack versus the mucosal defense. Acid and pepsin components form the aggressive factors and the mucus layer of mucin-bicarbonate secretion, phospholipid layer, tight junctions, cell proliferations, prostaglandins, and the urogastrone/epidermal healing factors (URO/EHF) form the defensive factors (Sanyal et al., 1983).

Since its recognition of the peptic ulcer as an important chemical entity, various efforts have been made to find suitable remedial measures. For several decades, the dogma 'no acid — no ulcer' has dominated the peptic ulcer disease (Feldman, 1989; Freston, 1990; Schubert and Shamburek, 1990). This forms the basis of using antacids, H_2 receptor antagonists and proton pump inhibitors like omeprazole.

However, not all patients with gastric or duodenal ulcers have increased acid secretion. It has been reported by various workers that acid secretion remains within the normal range in

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40%–70% of cases of duodenal ulcers and normal or below normal in gastric ulcer patients (Gupta et al., 1980). Thus, decreased mucosal resistance could be the dominant factor. More recently, the role of mucosal factor in peptic ulceration has received much attention and the term 'cytoprotection' has been introduced. It is now well established that peptic ulcer disease can be prevented by strengthening the defensive mechanisms of gastric and duodenal mucosa rather than attenuating the factors of aggression causing ulceration. This led to the introduction of carbenoxolone sodium, sucralfate and prostaglandins (PGs) in the therapeutic armamentarium for the treatment of peptic ulcer disease. However, some of them, including carbenoxolone sodium and PGs, have harmful side effects (Barrowman and Pfeiffer, 1982).

Because of these facts, efforts were made to find a suitable palliative and/or curative agent for the treatment of peptic ulcer disease in natural products of plants and mineral origin. A large section of the world's population relies on traditional remedies to treat a plethora of diseases. Medicinal herbs are an indispensable part of the traditional medicine practised all over the world due to the low costs, easy access and ancesteral experience (Marini-Bettolo, 1980).

In the present study, a crude drug formulation comprising herbs derived from the traditional system of medicine in India, Ayurveda, has been evaluated for its antiulcer action. The herbal formulation consists of six medicinal plants, namely *Glycyrrhiza glabra* L. (Papilionaceae; Root), *Saussurea lappa* C.B. Clarke (Compositae; Root), *Aegle marmelos* Corr. (Rutaceae; Fruit), *Foeniculum vulgare* Mill. (Umbeliferae; Seed), *Rosa damascena* Mill. (Rosaceae; Flower Petals) and *Santalum album* L.(Santalaceae; Stem).

2. Materials and methods

The antiulcerogenic effects of UL-409 were studied on Wistar albino rats strain of either sex (180–220 g) and on male guinea pigs (450–600 g). The animals were housed at a room temperature of 22 ± 2°C under a 12:12 light–dark cycle, and were fed a synthetic diet (Lipton India Ltd pellets). Guinea pigs were fed a synthetic diet supplemented with lucerne. Water was allowed ad libitum.

The constituent plants of the formulation were procured from authentic sources and were identified by Dr. S. Farooq, botanist of The Himalaya Drug Co. A voucher specimen was deposited in the herbarium of our R&D Centre, Bangalore. All the constituents were in equal proportion in the whole formulation. All plant powders were individually weighed and mixed. Drugs were administered as oral aqueous suspension and the animals of the control group received water as vehicle. Experimental gastric and duodenal ulcers were produced in rats and guinea pigs as follows.

2.1. Drug induced gastric ulcers in rats

Aspirin (0.2 g/kg × 3 days) and alcohol (1 ml of 80%/rat as single dose) were administered once per day to groups of animals for the number of days specified (Goel et al., 1986; Derelanko and Long, 1981). Animals of control group received water (10 ml/kg) and test groups received UL-409 (0.6 g/kg) for 10 days orally. From day 8 the animals received water/UL-409 2 h prior to the administration of aspirin. Alcohol was administered on day 10, 2 h after administration of water/UL-409. Overnight fasted animals were sacrificed by cervical dislocation 1 h after the last dose of ulcerogen. Ranitidine (50 mg/kg) and omeprazole (5 mg/kg) were used as reference drugs in aspirin and alcohol induced ulcers. The stomach was incised along the greater curvature and examined for ulcers.

2.2. Cold-restraint stress induced ulcers

UL-409 (0.6 g/kg), ranitidine (50 mg/kg) and omeprazole (5 mg/kg) were administered orally for 7 days. On day 7 the overnight fasted rats were restrained on a wooden plank 30 min after administration of test drugs and kept for 2 h in a refrigerator at 4°–6°C (Aguwa and Mittal, 1987). After the period of immobilization, the rats were sacrificed by cervical dislocation and the stomachs were removed for ulcer scoring.

2.3. Duodenal ulcers in rats

The rats were treated with UL-409 (0.6 g/kg) for a period of 7 days. On day 8, the overnight fasted animals were given a single s.c. injection of cysteamine hydrochloride (30 mg/kg) and the animals were killed by cervical dislocation after 18 h (Borella et al., 1979). Duodenum was examined for the presence or absence of ulcers.

2.4. Duodenal ulcers in guinea pigs

After 7 days of assigned treatment with UL-409, the animals were fasted for 18 h and were injected i.m. with 0.25 mg/kg of histamine acid phosphate in the thigh. Eight such injections were given at 30 min intervals. Thirty minutes after the last injection the animals were killed by a blow to the head and each duodenum was examined for the presence or absence of ulcers (Eagleton and Watt, 1967).

2.5. Pylorus ligated rats

UL-409 (0.6 g/kg) was administered for a period of 7 days. On day 7, after the last dose of UL-409,the rats were kept for 24 h fasting and care was taken to avoid coprophagy. Under light ether anaesthesia, the abdomen was opened and pylorus was ligated without causing any damage to its blood supply. The stomach was replaced carefully and the abdominal wall was closed with interrupted sutures. The animals were deprived of water during the post-operative period (Shay et al., 1945). Four hours after ligation, stomachs were dissected out and contents were collected into tubes. Volume, pH, free acid and total acid content (Parmar et al., 1984) of gastric juice were determined. The stomach was opened along the greater curvature and examined for ulcers.

2.6. Estimation of gastric mucus barrier in rats

The rats were treated with UL-409 (0.6 g/kg) once per day orally for 7 days. On day 8 the overnight fasted rats were sacrificed and stomachs were removed. The glandular portion of the stomach was excised and opened along the lesser curvature. The everted stomachs were soaked for 2 h in 0.1% alcian blue dissolved in 0.16 M sucrose buffered with 0.05 M sodium acetate, adjusted to pH 5.8 with hydrochloric acid. Uncomplexed dye was removed by two successive washes at 15 and 45 min in 0.25 M sucrose. Dye complexed with mucus was diluted by immersion in 10 ml aliquots of 0.5 M magnesium chloride for 2 h. The resulting blue solutions were shaken briefly with equal volumes of diethyl ether and the optical density of aqueous phase was measured at 605 nm (Corney et al., 1974). Histological examination of gastric glandular mucosa was carried out by using alcian blue stain to study the mucus secretion by mucosal cells.

The method of Souza-Formigoni et al. (1991) was used to evaluate the ulcer index. Statistical analyses were carried out by using Student's *t*-test for gastric ulcers and Yate's modification of the chi-square test for duodenal ulcers.

3. Results and discussion

The present study was undertaken primarily to study the anti-ulcerogenic effect of UL-409, a traditional herbal preparation containing herbs that are mentioned in Ayurvedic texts as a remedy for, among other diseases, 'Amlapitta', which closely resembles peptic ulcer (Varga et al., 1969). UL-409

Table 1
Effect of UL-409 on aspirin, alcohol and cold-restraint stress induced gastric ulcers in rats

Ulcerogen and dose	Ulcer index			
	Control	UL-409	Ranitidine	Omeprazole
Aspirin 200 mg/kg	27.00 ± 1.41	16.40[b] ± 2.80	9.80[d] ± 1.25	18.80[a] ± 3.45
Alcohol 80%	36.00 ± 2.32	9.00[e] ± 1.66	17.40[d] ± 1.46	13.20[d] ± 2.94
Cold-restraint stress	38.66 ± 3.00	22.67[c] ± 4.09	21.00[d] ± 1.22	21.75[d] ± 2.63

Values are mean ± S.E. ($n = 6$).
[a]$P < 0.05$, [b]$P < 0.025$, [c]$P < 0.01$, [d]$P < 0.005$, [e]$P < 0.001$ compared with respective control group.

Table 2
Effect of UL-409 on gastric volume, free acid, total acid and ulcer index in pylorus ligated rats ($n = 10$)

Group	Gastric volume (ml/100 g)	Free acid (μEq/100 g per 4 h)	Total acid (μEq/100 g per 4 h)	Ulcer index
Control	4.43 ± 0.31[a]	363.74 ± 43.18[a]	489.46 ± 37.40[b]	27.29 ± 12.2[a]
UL-409	3.16 ± 0.28[a]	240.26 ± 26.62[a]	315.36 ± 31.90[b]	15.70 ± 2.74[a]

Values are mean ± S.E. ($n = 10$).
[a]$P < 0.005$, [b]$P < 0.025$ as compared with control.

was found to possess an antiulcerogenic property in different experimental gastric and duodenal ulcers in rats and guinea pigs. UL-409 significantly decreased the aspirin-induced ulcer index from 27 ± 1.41 in the control group to 16.4 ± 2.80 in the treated group. Ulcer index in aspirin + ranitidine treated group was 9.8 ± 1.25 and in aspirin + omeprazole treated group was 18.8 ± 3.45 (Table 1). There was a significant reduction in alcohol induced ulcer index from 36 ± 2.32 in the control group to 9 ± 1.66 in the treated group. In the alcohol + ranitidine treated group the ulcer index was 17.4 ± 1.46 and in the alcohol + omeprazole group it was 13.2 ± 2.94 (Table 1). The incidence and severity of cold restraint stress induced ulcerations were significantly reduced by UL-409 and were comparable to ranitidine and omeprazole (Table 1). UL-409 significantly reduced the volume of gastric juice, total acid, free acid and ulcer index as compared to the control (Table 2). Induction of duodenal ulcers in rats with cysteamine showed the presence of ulcers in 9 out of 10 animals in the control group, which was significantly reduced to only 2 animals in the UL-409 treated group ($P < 0.005$). Histamine induced duodenal ulcer in guinea pigs showed the presence of ulcers in 7 out 8 animals in the control group, which was significantly reduced to only 2 animals in the UL-409 treated group ($P < 0.025$).

A significant improvement in total gastric barrier mucus was observed after UL-409 treatment. The gastric mucin content in the control group was 40.9 ± 2.20 as compared to 60.18 ± 6.78 in the UL-409 treated group. Alcian blue characteristically stains the sialomucin (Brown, 1978). Intense staining of gastric mucosa by alcian blue at the apical region and in the deeper mucosal layer as compared to control indicates that UL-409 treatment promotes mucus secretion by the mucosal cells.

Peptic ulcer is now widely believed to be due to the imbalance between offensive acid–pepsin and defensive mucus factors. UL-409 was found to increase the mucus and decrease the acid volume, free and total acid content in the rats. These effects of UL-409 in parameters that influence the initiation and perpetuation of ulceration may be considered as highly desirable properties of an anti-ulcerogenic drug. UL-409 thus has the potential of an anti-ulcerogenic agent, and the observed activity may be due to the modulation of defensive factors through an improvement of gastric cytoprotection as well as acid inhibition.

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