

Cytoprotective Effect of UIGen, a Polyherbal Formulation against Physical and Chemical Factor Induced Gastric Ulcers in Wister Albino Rats

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Abstract: Problem statement: UIGen, a Polyherbal formulation, was investigated for its possible Ulcero-protective activity in ulcerogen and Cold-restraint stress induced Wister rats (*Rattus rattus*). Oral administration of UIGen, at a dose of 800 mg kg⁻¹ significantly protected the onset of cold-resistance stress induced ulcerations. **Approach:** It significantly inhibited gastric ulceration induced by alcohol and aspirin. Control group: Alcohol 80% induced ulcer was 42.00±2.30 and in UIGen treated rats showed 10.00±1.62. Using alcian blue stain to study the mucus secretion by mucosal cells carried out histological examination of gastric glandular mucosa. **Results:** The volume and acidity of gastric juice in pyloric-ligated rats was reduced in treated rats. Gastric volume (mL 100 g⁻¹) in control rats showed 4.12±0.32 and in UIGen treated rats showed 2.50±0.20. **Conclusion:** UIGen, Cytoprotective effect may be due to the enhancement of defensive mechanism through an improvement of gastric cytoprotection as well as acid inhibition.

Key words: UIGen, gastric ulceration, cold-resistance stress, pyloric-ligated, gastric cytoprotection, ulcer index

INTRODUCTION

Onset of peptic ulcers has been described as an imbalance between the luminal acid peptic attacks versus the mucosal defense. Acid and pepsin components form the aggressive factor and the mucus layer of mucin-bicarbonate secretions; phospholipid layer, tight junctions, cell proliferations, prostaglandins and the urogastrone/Epidermal Healing Factors (URO/EHF) form the defensive factors^[17].

Since its recognition of the peptic ulcer has an important chemical entity, various efforts have been made to assess reliable remedial measures. In peptic ulcer disease, the basis of using antacids as an H₂ receptor antagonists and proton pump inhibitors like omeprazole^[8].

However, not all patients with gastric ulcers have increased acid secretion. It has been reported by various researchers that acid secretion remains within the normal range. The decreased mucosal resistance could be the dominant factor. Recently, the role of mucosal factor in peptic ulceration has received much attention and the term 'cytoprotection' has been incorporated^[11].

It is now well established that strengthening the defensive mechanisms of gastric mucosa rather than attenuating the factors of aggression causing ulceration can prevent peptic ulcer disease^[9].

This led to the introduction of carbonoxolone sodium, sucralfate and Prostaglandin (PGs) in the therapeutic interventions for the treatment of peptic ulcer disease. However, some of them, including carbonoxolone sodium and PGs, have harmful side effects^[2,3].

Because of these reasons, efforts were made to find a suitable nontoxic and curative agent for the treatment of peptic ulcer disease in natural products from plant origin^[14].

A large section of the World's population relies on traditional and Medicinal herbs they are an indispensable part of the traditional medicine^[23] practiced all over the world due to less cost, easy access and Vedic expertization.

In this study, as per the complied data on traditional herbs from ancient Ayurvedic scripts, antiulcerogenic activity was selected. The herbal formulation consists of four medicinal plants, namely

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Glycyrrhiza glabra (Papilionaceae; Root), *Saussurea lappa* (Compositae; Root), *Aegle marmelos* (Rutaceae; Fruit) and *Santalum album* (Santalaceae; Stem).

MATERIALS AND METHODS

Selected healthy animals of Wistar albino rats strain of either sex (180-220 g) were housed at room temperature of $22\pm 2^{\circ}\text{C}$ under a 12:12 light dark cycle and were fed a synthetic diet (Lipton India Ltd.). Water was allowed *ad libitum*.

The plants constituents were procured from authentic sources and were identified and the voucher specimen was deposited in the herbarium of our Dept of Biotechnology. All plant powders were individually weighed in equal proportions and mixed. Drugs were administered as oral aqueous suspension and the animals of the control group received water as vehicle. Experimental gastric ulcers were produced in rats as follows:

Drug induced gastric ulcers in rats: Aspirin (0.2 g kg^{-1} for 3 days) and alcohol (1ml of 80% rats as a single dose) were administered once per day to groups of animals for the number of days specified^[6,10]. Animals of control group received water (10 mL kg^{-1}) and test groups received UIGen (0.8 g kg^{-1}) for 10 days orally. From day 8 the animals received water/UIGen, 2 h prior to the administration of aspirin.

Alcohol was administered on day 10, 2 h after administration of water/UIGen. Overnight fasted animals were sacrificed by cervical dislocation 1hr after the last dose of ulcerogen. The stomach was incised along the greater curvature and examined for ulcers.

Cold-restraint stress induced ulcers: UIGen (0.8 g kg^{-1}), were administered orally for 7 days. On day 7 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 $4-6^{\circ}\text{C}$ ^[1]. After the period of immobilization, the rats were sacrificed by cervical dislocation and the stomachs were removed for ulcer scoring.

Pylorus ligated rats: UIGen (0.8 g kg^{-1}) was administered for a period of 7 days. On the day 7, after the last dose of UIGen, the rats were kept for 24 h fasting and care was taken to avoid coprophagy. Under light ether anesthesia, 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^[20]. Four hours after ligation, Stomachs were dissected out and contents were collected into tubes. Volume, pH, free acid and total acid content^[15] of gastric juice were determined. The stomach was opened along the greater curvature and examined for ulcers.

Estimation of gastric mucus barrier in rats: The rats were with UIGen (0.8 g kg^{-1}) once per day orally for 7 days. On day 8 the over-night 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 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 or 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 ^[5]. Using alcian blue stain to study the mucus secretion by mucosal cells carried out histological examination of gastric glandular mucosa.

The method of^[19] was used to evaluate the ulcer index. Statistical analyses were carried out by using Student's t-test for gastric ulcers.

RESULTS

This project study was undertaken to study the anti-ulcerogenic effect of, a poly herbal preparation containing herbs that are mentioned in Ayurvedic scripts as a remedy for, 'Amlapitta', which closely resembles peptic ulcers^[21].

UIGen was found to prove an antiulcerogenic property in experimental gastric ulcers in rats. UIGen significantly decreased the aspirin-induced ulcers index from 25.00 ± 1.20 in the control group to 15.00 ± 2.02 in the treated group (Table 1). There was a significant reduction in alcohol induced ulcer index from 42 ± 2.30 in the control group to 10.00 ± 1.62 in the treated group (Table 1).

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

	Control	Treated UIGen
Aspirin 200 mg Kg^{-1}	25.00 ± 1.20	$15.00\pm 2.02^{\#}$
Alcohol 80%	42.00 ± 2.30	$10.00\pm 1.62^{\circ}$
Cold Restraint stress	31.50 ± 2.00	$20.60\pm 3.02^*$

Values are mean \pm SE (n = 6). #: $p < 0.01$; °: $p < 0.025$; *: $p < 0.001$. Compared with respective control group

Table 2: Effect of UGen on gastric volume, free acid, total acid and ulcer index and ulcer index in pylorus ligated rats (n=10)

Group	Gastric volume (mL 100 mg ⁻¹)	Free acid (M Eq 100 g ⁻¹ 4 h ⁻¹)	Total acid (M Eq 100 g ⁻¹ 4 h ⁻¹)	Ulcer index
Control	4.12±0.32*	385±32.10*	492.30±27.50*	30.02±0.5*
UGen treated	2.50±0.20*	215±21.60*	321.25±30.07*	16.50±3.2*

*: p<0.005; *: p<0.025 as compared to control

UGen significantly reduced the volume of gastric juice, total acid, free acid and ulcer index as compared to the control group (Table 2).

DISCUSSION

A significant improvement in total gastric barrier mucus was observed after UGen treatment. The gastric mucin content in the control group was 38.5±2.1 as compared to 58.1±5.2 in the UGen treated group. Alcian blue characteristically stains the sialomucin^[4]. Intense staining of gastric mucosa by alcian blue at the apical region and in the deeper mucosal layer as compared to control indicates that UGen treatment promotes mucus secretion by the mucosal cells (microphotograph: Stomachs).

Peptic ulcer is now widely believed to be due to the imbalance between offensive acid-pepsin and defensive mucus factors. UGen was found to increase the mucus and decrease the acid volume, free and total acid contents in the rats. These effects of UGen in these ulcer models that influence the initiation and cytoprotection of ulceration may be considered as highly desirable properties of an anti-ulcerogenic drug. Duodenal ulcers produced by the injection of histamine¹¹ into guinea pigs, are the result of contact of duodenum with the acid gastric juice so their incidence may change with the rate of emptying of stomach. On the other hand production of gastric ulcer would require the revitalizations of gastric mucosa by a high dose of histamine prior to the action of gastric juice. Even before 1962 some authors insisted on different origins of experimental ulcers of the stomach and duodenum^[7].

In another study the evaluation of Aspirin+Pylorus Ligation (APL) and Alcohol (AL) induced acute gastric ulcer models and ulcer-healing activity using acetic acid-induced (AC) chronic ulcer model in rats. Both the antisecretory and cytoprotection hypothesis were evaluated. The antioxidant activity of *Jasminum grandiflorum* L. (JGLE) has been assayed by using *in vitro* methods like 2,2-diphenyl-1-picrylhydrazylhydrate (DPPH) assay^[22].

Phytochemical investigation of *Byrsonima crassa* leaves consists of 5 phenolic compounds may probably revealed antiulcerogenic effects; The MeOH extract reduced the ulceration by 93 and 99% only at the doses of 500 and 1000 mg kg⁻¹. The lower gastroprotective

action (69%) was observed when animals were treated with CHCl₃ extract at the dose of 1000 mg kg⁻¹^[16].

Precise information on receptors and signal transduction mechanisms as well as on intramural neural and paracrine regulatory pathways has led to the development of new drugs capable of inhibiting acid secretion. These include antagonists that interact with stimulatory receptors (histamine H₂-receptor antagonists, muscarinic receptor antagonists and gastrin receptor antagonists), agonists that interact with inhibitory receptors (somatostatin and prostaglandin E analogues) and irreversible inhibitors of the luminal enzyme, H+K(+)-ATPase^[18].

One study demonstrated that Corticotrophin-Releasing Factor (CRF) injected in a picomole amount into the cerebrospinal fluid acts within the brain to inhibit gastric emptying of a liquid meal through vagal-dependent pathways^[21].

The reduction in gastric fluid volume, total acidity and an increase in the pH of the gastric fluid in APL rats proved the anti secretory activity of JGLE. Additionally, JGLE completely healed the ulcer within 20 days of treatment in AC model as evidenced by histopathological studies^[22].

In our study alcian blue stain is used to evaluate the extent of mucus secretion by mucosal cells carried out in histological and histochemical examination of gastric glandular mucosa revealed that as when compared to stress induced stomach (Control): lot of mucosal surface layer distortions and ulceration are visible, (Fig. 1). In UGen treated stomach rats, extensive mucus secretion were evident this could be due to drug response (Fig. 2).

Bioassay-guided fractionation led to the isolation of two bioactive indole alkaloids called canthin-6-one (1) and 4-methoxycanthin-6-one (2). The alkaloid fraction and both alkaloids demonstrated potent antiulcerogenic effects when evaluated in gastric lesion-induced animals, *Simaba ferruginea* St. Hill (Simaroubaceae)^[24].

Phytochemical investigation on hydroethanolic extract of the leaves (HEL) and bark (HEB) from the plant *Alchornea castaneaefolia* and enriched flavonoidic fraction led to the isolation of flavonoids glycosides as the main compounds, thus suggesting that these substances may be involved in the observed antiulcer activity^[12].

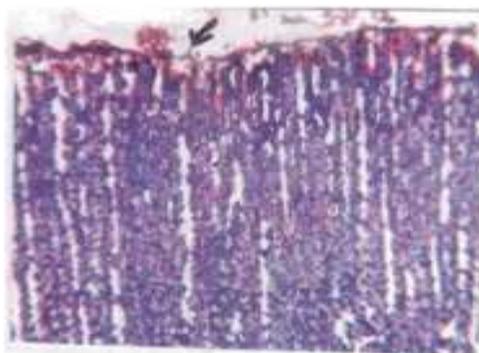


Fig. 1: Control: Stomach-stress induced ulcer (←)

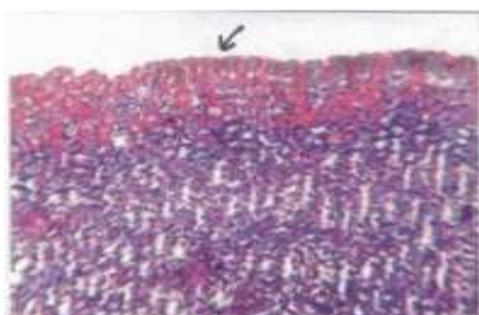


Fig. 2: Treated Ulgen: Ulcer protection (←); (Stomach): Drug response

Solanum nigrum (SNE) fruits extract significantly inhibits H (+) K(+)ATPase activity and decreases the gastrin secretion in EtOH-induced ulcer model.

The severity of the reaction of ulcerogen and the reduction of ulcer size by SNE was evident by histological findings. SNE, thus, offers antiulcer activity by blocking acid secretion through inhibition of H (+).

K (+) ATPase and decrease of gastrin secretion. These results further suggest that SNE was found to possess antiulcerogenic as well as ulcer healing properties, which might also be due to its antisecretory activity^[13].

The extracts of *Byrsonima crassa* leaves, phenolic compounds may probably acting as an antiulcerogenic effect^[6].

After *Helicobacter pylori* eradication was introduced and largely eliminated the need for maintenance therapy for peptic ulcer disease, Gastroesophageal Reflux Disease (GERD) became the main indication for prolonged gastric acid inhibition. The drug effect on GERD depends on the degree of acid inhibition, thus the efficacious proton pump inhibitors are preferred. The proton pump inhibitors

have few immediate side effects, the main concern being the profound hypoacidity and hypergastrinaemia they induce. Gastric hypoacidity also increases gut bacterial infections and the barrier function of acid against viral and prion infections requires further assessment^[25].

CONCLUSION

The experimental data concluded that, UIGen activity may be due to the enhancement of defensive mechanism through an improvement of gastric cytoprotection as well as acid inhibition.

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