Pharmacological Evaluation Of *Gloriosa Superba* Linn Flower Extract For Antiulcer Activity

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Abstract

In present study the antisecretory and antiulcer potential of hydroalcoholic and petroleum ether extract of *Gloriosa superba* Linn flower were studied. Both extracts were tested orally at the doses of 100, 200 and 400 mg/kg using pylorus ligation, ethanol and cold stress as toxicants. Both the extracts showed significant ulcer protection at the highest dose of 400mg/kg as compared to standard. To support its antiulcer potential, the plant was investigated for free radical scavenge ring activity by lipid peroxidation. The extract showed comparative significant result. The antioxidant property may be due to presence of flavonoids and polyphenols present in extracts. From the results it was concluded that both hydroalcoholic and petroleum ether extracts of flower of *Gloriosa superba* is having gastric ulcero protective activity.

Keywords: *Gloriosa superba* Colchicaceae.; Antiulcer; Ethanol, pylorus ligation, stress

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Introduction

The Peptic ulcers occur due to the inequity between offensive (acid, pepsin, and *Helicobacter pylori*) and defensive factors (mucin, prostaglandin, bicarbonate, nitric oxide). The stress due to modern existence, disease state and anxiety as well contribute to the creation of ulcer. In general, stress induced ulcers due to mucosal damage and ischemic are far more frequent than hyper secretion of gastric acid in cancer patients.[1] The regulation of mucosal microcirculation of gastric, intimately involved in the maintenance of gastric integrity and endogenous nitric oxide (NO) has been established to have a role in this regulation. Reduced glutathione (GSH) is also important for mucosal integrity since depletion of GSH from the gastric mucosa by electrophilic compounds induces macroscopic mucosal ulceration.[2] The treatment of peptic ulcer is directed against either reduction of aggressive factors or enhancement of mucosal defense of stomach and duodenum with cytoprotective agents. The allopathic treatment of peptic ulcer is still unsatisfactory since they decline the morbidity and mortality but may generate adverse reactions like arrhythmias, impotence, gynaecomastia and haematopoietic changes.[3] Besides, reappearance rates are high. *Gloriosa superba* reported to possess hepatoprotective, anti-inflammatory activity in various investigational models.[4] In traditional Ayurvedic literature *Gloriosa superba* is reported to posses' antiulcer activity. Since gastric and duodenal ulcers are inner wounds, we have considered the antiulcer potential of this herb on different models of gastric ulceration.

2.0 Materials and methods

2.1. Plant material

The flowers of *Gloriosa superba* was collected locally. The specimens were authenticated by renowned botanist and the voucher specimen is deposited for prospect reference. The shed dried flowers powder was subjected to exhaustive uninterrupted hot extraction in Soxhlet apparatus using petroleum ether and hydroalcoholic solution (70% water and 30% alcohol). The chemical constituents of the both extracts were identified by qualitative analysis.[5] The obtained masses were dried and stored in an air tight container in cold environment for further use.

2.2. Experimental animals-

Albino rats and mice of either sex weighing between 150-230 gm were selected for the study. The experimental protocol was approved by Institutional Animal Ethics Committee and animal were maintained under standard condition. They were allowed free access to standard dry pellet diet and water *ad libitum* under strict hygienic conditions.

2.3. *Toxicity study*

Acute toxicity study of both extracts of the *Gloriosa superba* was carried out for determination of LD_{50} by using OECD guidelines.[6] The female albino mice of 20-30 g were used for the study. The animals were continuously observed 12 h to detect changes in behavioral responses. Mortality was observed for 24 hours. The doses of 100, 200 and 400 mg/kg, p.o. were selected based on the results.

2.4. Ethanol-induced acute gastric ulcers[7]

Thirty six rats were deprived of food for 18 h but water was allowed *ad libitum*, and then randomly divided into six treatment groups: Normal control, Toxicant, and low, medium, high doses of petroleum ether and hydroalcoholic extracts. All the rats were treated orally with ethanol 70% 0.5 ml. The toxicant group received only ethanol. One hour later all the animals were sacrificed using ether anaesthesia, the stomachs removed and the number of the ulcers were scored on an arbitrary 0-3 point scale. The ulceration index of each stomach was the sum of its scores.

2.5. Cold stress-induced acute gastric ulcers[8]

In this method male wister rats of either sex weighing between 150-200 gm were fasted for 24 hours with free access to water. All groups were given 7 days herbal drug therapy. On the day 7^{th} the overnight fasted rats were restrained in a metallic restraint chamber 30 min after the administration of test drug and were kept for 2 hours in a refrigerator at $4\text{-}6^0$C. After the period of immobilization the rats were sacrificed by cervical dislocation and the stomach was removed for ulcer scoring. The numbers of the ulcers were scored on an arbitrary 0-3 point

scale. The ulceration index of each stomach was the sum of its scores.

2.6. Pylorus Ligation induced gastric ulcers[9-13]

Male albino rats were fasted in individual cages for 24 hours. All the extracts of the *Gloriosa superba* (100, 200, 400 mg/kg) or standard drug (Lansoprazole, 8 mg/kg) was administered orally 30 minutes prior to pyloric legation. Under light ether anesthesia, the abdomen was opened and the pylorus was legated. The abdomen was then sutured. At the end of 4 hours after ligation, the animals were sacrificed with excess of anesthetic ether, stomach was dissected out. Gastric juice was collected and its volume, pH, free acidity and total acidity were determined. The glandular portion was then exposed and examined for ulceration. Ulcer index was determined. Determination of total acid and free acid were estimated from gastric juice collected from the 4-hour pyloric ligated rats. Total acid output of the gastric juice as estimated by titration of 0.1 ml of gastric juice with 0.01N sodium hydroxide using phenolphthalein as indicator. Total acid output was expressed as mEq/L per00 gm of body weight.

2.7 Method for estimation of lipid peroxidation[14]

Lipid peroxidation was estimated in terms of thio barbituric acid reactive species (TBARS), using malondialdehyde (MDA) as standard by the method of Buege and Aust. 1.0 ml of the sample extract was added with 2.0 ml of the TCA- TBA- HCl reagent (15% w/v TCA, 0.375% w/v TBA and 0.25 N HCl). The contents were boiled for 15 minutes, cooled and centrifuged at 10000 rpm to remove the precipitate. The absorbance was read at 535 nm and malondialdehyde concentration of the sample was calculated using extinction coefficient of 1.56×10^5 M^{-1} cm^{-1}.

3. Results

Effect of PE and HAL extracts of *GS flowers* on ethanol-induced gastric ulcers:

Pretreatment of rats with either PE or HAL extract of *GS* flowers produced a dose dependent protection from ethanol induced ulceration, as compared to control animals. However, the protection was statistically significant at higher dose 400mg/kg. Lansoprazole (8mg/kg) produced significant gastric ulcer protection as compared to control group. (Table 1).

Effect of PE and HAL extracts of *GS* flowers on cold stress-induced gastric ulcers:

Pretreatment of rats with either PE or HAL extract produced a dose dependent protection from the cold stress-induced ulceration, as compared to control animals. The protection was statistically significant at 200, 400 and 600 mg/kg dose. Lansoprazole (8mg/kg) produced significant protection as compared to control group (Table 2).

4. Discussion

Rapid mechanization of lifestyle has pushed the public into a stressful condition, almost round the clock. This added with increased environmental pollution and lack of hygienic knowledge in common man has provided an avenue for the emergence of varieties of organ related complications. The principle organs of GIT affected for such complications are stomach and proximal part of intestine. Unfortunately, despite tremendous strides in the modern medicine, treatment of some of the gastrointestinal diseases like ulcer is not to the satisfaction of the clinician. Though some synthetic drugs may produce symptomatic relief in such conditions, they are not devoid of adverse incidences, which compel the clinician to stop the therapy. Hence, there is a greater need to explore the alternative therapy particularly herbal sources in this regard. Further, there is increasing inclination towards the herbal therapies across the globe as they are believed to be safe.

Stress causes an ischemic condition in the gastric mucosa by activation of parasympathetic and sympathetic nervous system resulting in vasoconstriction, which in turn causes free radical generation.[15]

The results of current study suggest that, in the stress models, pretreatment with either petroleum ether or hydroalcoholic extract of *Gloriosa superba* flowers significantly suppressed the ulcer index as compared to control. This effect is due to its antioxidant

property. Since antioxidant property of several phyto-constituents like flavonoids, tannins etc which are present in the *Gloriosa superba* flowers extract are well documented.

Narcotizing agent such as ethanol when administered intragastrically to rats, it produces severe gastric erosions. The genesis of ethanol induced gastric lesion is multifactorial. It involves depletion of gastric walls mucus content as one of involved factors and this damage induced by ethanol may be due to mucosal leukotrienes release[16]. Ethanol induced gastric lesion formation may be due to stasis in gastric blood flow which contribute to the development of hemorrhage and necrotic aspects of tissue injury.[17-39] Ethanol treatment caused significant increase in the ulcer index whereas pretreatment with either petroleum ether or hydroalcoholic extract of *Gloriosa superba* flowers showed significant inhibition ($p<0.01$) in ethanol induced gastric damage. The antiulcerogenic activities of *Gloriosa superba* flowers also involve its antioxidant effect apart from its effects on other defensive factors.

5. Conclusion

Ulcer protective and ulcer healing facilitation effects of petroleum ether and hydroalcoholic extracts of *Gloriosa superba* flowers is due to its effects on both offensive and defensive factors. The antioxidant property of *Gloriosa superba* flowers also has the significant contribution towards its antiulcer activity. Further, work on other mucosal factors like nitric oxide, prostaglandins, cAMP etc. would provide more insight into the activity of petroleum ether and hydroalcoholic extracts of *Gloriosa superba* flowers. Hence it can be opined that petroleum ether and hydroalcoholic extracts of *Gloriosa superba* flowers to be a good antiulcer agent. It is worthwhile to consider this aspect for clinical application in patients of gastric ulcer conditions for further evaluation.

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Table 1. Effect of methanolic extract of the flowers of *Gloriosa superba* against aspirin-and alcohol-induced gastric ulcer in rats

Group and dose	Ulcer positive animals	Ulcer index	%Ulcer protection
Control(2% w/v gum acacia)	6	8.597±0.08	-
Lansoprazole (8mg/kg)	1	1.900±0.04**	77.90%
Low dose PE (100mg/kg)	4	6.717±0.15**	21.90%
Medium dose PE(200mg/kg)	3	5.167±0.06**	39.90 %
High dose PE(400mg/kg)	2	3.517±0.10**	59.09 %
Low dose HAL (100mg/kg)	4	6.717±0.15**	21.90%
Medium dose HAL(200mg/kg)	3	5.167±0.06**	39.90 %
High dose HAL (400mg/kg)	2	3.517±0.10**	59.09 %

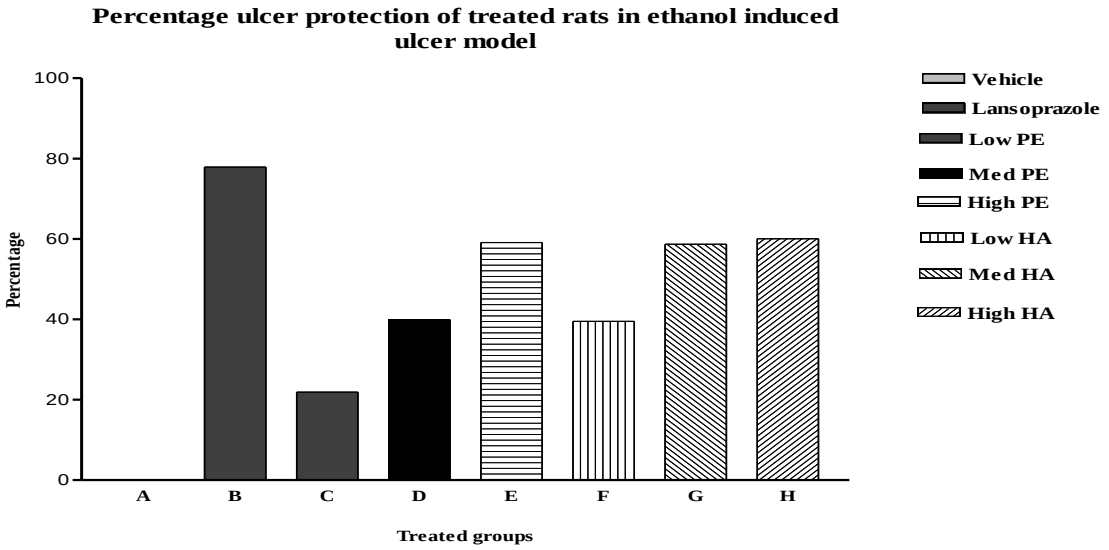
Table 2. Effect of petroleum ether and hydroalcoholic extracts of *Gloriosa superba* flowers against cold stress induced gastric ulcers in rats.

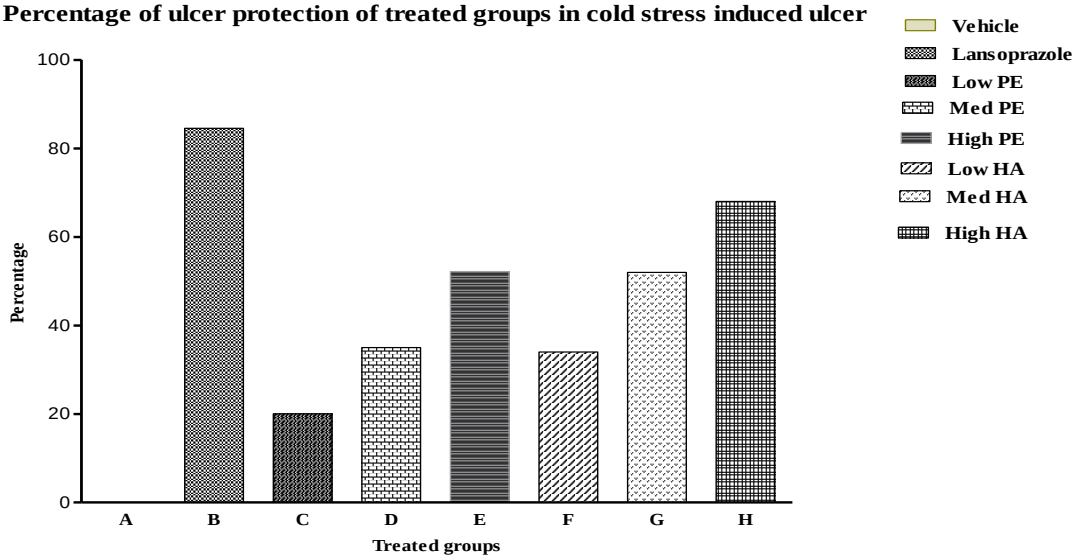
Group and dose	Ulcer positive animals	Ulcer index	%Ulcer protection
Control(2% w/v gum acacia)	6	10.73±0.14	-
Lansoprazole (8mg/kg)	1	1.657±0.03**	84.00%
Low dose PE (100mg/kg)	5	8.616 ± 0.07**	20.00%
Medium dose PE(200mg/kg)	4	6.98 ± 0.16**	35.00%
High dose PE(400mg/kg)	3	5.133 ± 0.06**	52.17%
Low dose HAL (100mg/kg)	4	7.08 ± 0.15**	34.00%
Medium dose HAL(200mg/kg)	3	5.15 ± 0.07**	52.00%
High dose HAL (400mg/kg)	2	3.43 ± 0.07**	68.00%

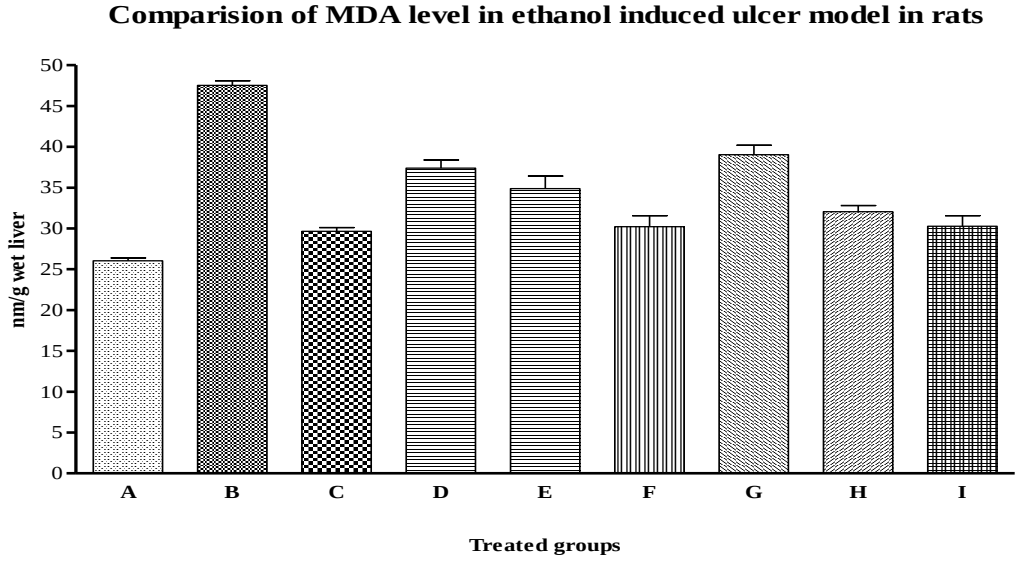
Lipid Peroxidation Estimation in treated groups in ethanol induced ulcer model in rats

Sl. No.	Treatment	Malondialdehyde (MDA) (nm/g gastric tissue)
1	Normal control	26.05 ± 0.3496
2	Control	47.54 ± 0.5670
3.	Lansoprazole	29.64 ± 0.4808**
4.	Low dose (PE) (100mg/kg)	37.38±0.9971**
5.	Medium dose (PE) (200mg/kg)	34.89 ± 1.543**
6.	High dose (PE) (400mg/kg)	30.23±1.339**
7.	Low dose (HAL) (100mg/kg)	39.04±1.169**
8.	Medium Dose (HAL)(200mg/kg)	32.04±0.7735**
9.	High dose(HAL) (400mg/kg)	30.27±1.297**

** P < 0.01 when compared with control

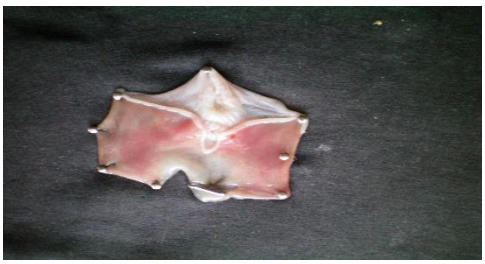




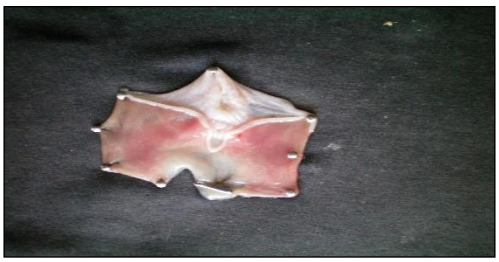


Comparision of MDA levels in ethanol induced ulcer model in rats.

Ethanol induced ulcer model



Cold stress induced ulcer model



Normal Control

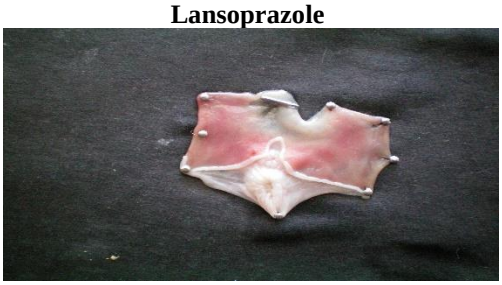
Normal Control

Control

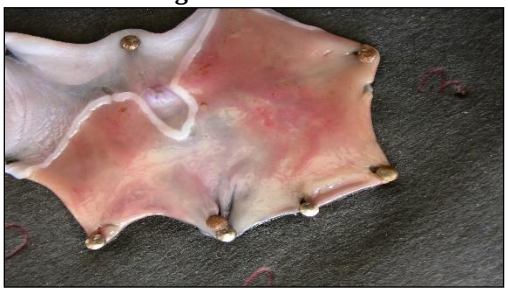
Control

Lansoprazole

Lansoprazole

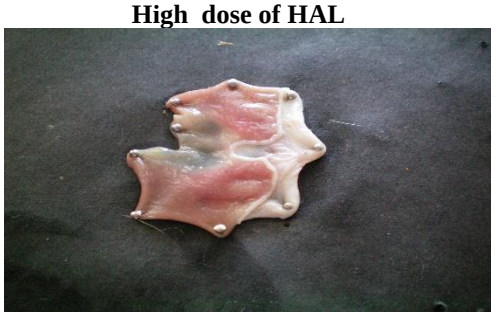


High dose of HAL



High dose of PE

High dose of HAL



High dose of PE