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Anti-ulcerogenic Evaluation of *Gloriosa Superba* Tuber Extracts *In-vivo* and Anthelmintic Activity *In-vitro*: A Comparison.

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Research Article

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ABSTRACT

Gloriosa Superba (Liliaceae) is a semi-woody herbaceous climber native of tropical Asia, Africa and reported by tribal people of India for its several clinical uses. Traditionally, the tubers and other parts of the plant are widely being used in the treatment of ulcers, piles, inflammation, abdominal pain, infertility, parasitic skin infections and leprosy. The present study was carried out to investigate the antiulcerogenic activity of ethanolic extract of Gloriosa superba L. tuberous rhizomes (GST) and compare with *Zingiber officinale* (rhizomes) and *Glycyrrhiza glabra* (roots) ethanolic extracts on experimental ulcers induced by aspirin (chemically induced ulcers) couple with stress and on pylorus ligation-induced ulcers in Wistar rats. The anthelmintic activity of various extracts of GST was also evaluated *in vitro* against two types of intestinal worms; a) Earthworms and b) Ascardia Galli (a parasitic roundworm). Test extracts were administered orally 60 and 30 min before for aspirin and pylorus ligation-induced ulcer models respectively. GST extract showed superior protection when compared with other test extracts on both the ulcer models tested. The extract significantly increased the pH and decreased both the volume and total acidity of gastric secretion in pylorus ligation-induced ulcer model. The antiulcerogenic effect exhibited by GST extract was comparable with ranitidine (standard drug) in particular at higher dose. Aqueous, alcoholic and chloroform extracts of GST showed significant anthelmintic activity *in vitro* against both types of intestinal worms. Therefore, the results indicate that the herbal preparations containing GST would be beneficial to treat gastric ulcers more effectively for improved clinical outcome.

INTRODUCTION

Gloriosa Superba (Liliaceae) is a semi-woody herbaceous climber native of tropical Asia and Africa. Several parts of the plant were used traditionally by tribal people for the treatment of various diseases [1,2,3]. It is known as 'Malabar glory lily' in English, 'Agnisikha' in Sanskrit and its trade name is 'Glory lily' [4,5,6]. Glory lily is an industrial medicinal crop in South India because of its high colchicine content and potential long list of reported clinical uses. Glory lily is one of the most valued medicinal plants, due to its over-exploitation is on the verge of extinction [7, 8]. Both its tuber and seeds have similar medicinal properties [9].

Traditionally the tubers and other parts of the plant are widely being used in the treatment of ulcers, piles, inflammation, abdominal pain, bruises, parasitic skin infections, infertility, leprosy and as anthelmintic and laxative [2, 3]. In Folklore it is being used to treat pimples, skin eruptions, baldness, killing lice in the hair and also as sedative [10, 11].

Aspirin is well known for its undesirable side effects mainly inducing gastrointestinal ulcers and bleeding in stomach especially at higher doses. The induction of gastric damage by aspirin is due to activated macrophages and characterized by infiltration of neutrophils, growth factor inhibition and elevation of cytokines [12, 13]. Cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6) and interleukin-10 (IL-10) play important roles in

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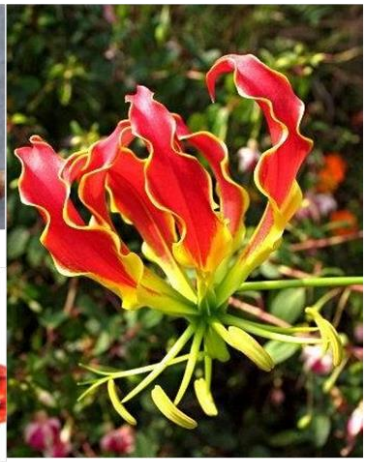
the acute phase inflammation as well as in maintenance and regulation of the severity of gastric ulcer [14]. Extreme mental and psychological stresses to rodents, stimulates gastric secretion related to central nervous system as well as pituitary renal hormonal axis [15-18]. Long-term pylorus ligation induces retention of gastric acid secreted by cholinergic activation and stimulates activity of pepsin, resulting in gastric wall injury [16, 17, 19].

Tubers

Seeds



Flowers

Gastric ulcer is a serious injury resulting by ingestion of spicy food or alcohol, due to extreme stress or gastric surgery and Helicobacter (H.) pylori [17]. The enzyme H+/K+-ATPase pumps protons in exchange for potassium ions across the apical membrane to secrete gastric acid by parietal cells [19]. Histamine antagonists including cimetidine and ranitidine as well as proton pump inhibitors such as omeprazole and lansoprazole are well known effective drugs for the treatment of gastric ulcers by decreasing the gastric secretions [17].

The objective of this study was to investigate the *Gloriosa superba* L tuber (GST) ethanolic extract for its anti-ulcerogenic activity and compare with *Zingiber officinale* (rhizomes) and *Glycyrrhiza glabra* (roots) ethanolic extracts in aspirin induced ulcers coupled with stress and pylorus ligation-induced ulcer models after per oral administration to male Wistar rats. *Zingiber officinale* [20, 4] and *Glycyrrhiza glabra* [7, 21, 22] were selected in this study for comparison of their anti-ulcerogenic activity based on the published literature. Anthelmintic activity of aqueous, ethanolic and chloroform extracts of GST was also investigated *in vitro* against two types of intestinal worms. Ranitidine and piperazine citrate was selected as standard drugs for the evaluation of antiulcerogenic activity *in vivo* and anthelmintic activity *in vitro* respectively.

MATERIALS AND METHODS

Plant material

The tubers of *Gloriosa superba* (Liliaceae) were obtained and authenticated by Dr. K. Madhava Chetty, Department of Botany, Sri Vankateshwara University, Tirupati, India. Rhizomes of *Z. officinale* and roots of *G. glabra* were collected from different regions of India and authenticated by Prof. Raju of Kakatiya University, Warangal, India. All the voucher specimens have been deposited at the department of botany herbarium, Vaagdevi College of Pharmacy, Warangal, India. The collected tubers, rhizomes and roots were washed, cut in to small pieces, air dried in the shade, reduced to fine powder, packed in tightly closed containers and stored at room temperature for subsequent extraction and pharmacological evaluation.

Drugs and chemicals

Ranitidine, Aspirin and Piperazine citrate were purchased from Dr. Reddy's Laboratories Pvt. Ltd., India. Gum acacia, sodium carboxymethylcellulose, ethanol, chloroform, Whatmann filter papers were purchased from E. Merck India Pvt. Ltd. MilliQ water from MilliQsystem (Millipore, France) was used throughout the study.

Preparation of test extracts

The extracts of *G. superba* (tubers), *Z. officinale* (rhizomes) and *G. glabra* (roots) were prepared using shade dried and coarsely powdered materials by cold maceration extraction process using ethanol as solvent. Each plant material (100 g) was soaked in 500 ml of ethanol in stoppered glass container for 72 hours with intermittent shaking at room temperature and then filtered through Whatmann filter paper no. 41. The last traces of the solvent was removed and concentrated to dryness under vacuum by using a rotary evaporator. The dried extracts were stored in a refrigerator until use. The extracts were suspended in aqueous 1.0 % w/v sodium carboxymethylcellulose before

administration to the test animal. Aqueous and chloroform extracts of *G. superba* (tubers) were prepared by following the above procedure.

Animals

Male Wistar rats weighing 160 to 200 g were purchased from Mahaveer Enterprises, Hyderabad, India. Animals were housed in a room maintained at 22 ± 2 °C with an alternating light-dark cycle. Food and water were available adlibitum. All the animals were acclimatized to laboratory conditions for 7 days when they are out from quarantine. All the procedures described were reviewed and approved by the Institutional Animals Ethics Committee, Vaagdevi College of Pharmacy, Kakatiya University, Warangal, Andhra Pradesh, India and conducted as per the guidelines provided by CPCSEA (Committee for the Purpose of Control and Supervision on Experiments on Animals).

Experimental procedures

Restraint plus aspirin induced gastric ulcers

Male Wistar rats weighing 160 – 200 g were divided into eight groups, each group comprising of six rats. Animals were deprived of food for 24 h with access to water ad-libitum. After 24 h, test compounds were administered by p.o. route and 60 min after test compound administration aspirin (50 mg/kg, 10.0 mL/kg) was administered in 1.0 % carboxymethylcellulose sodium (Sodium CMC) by p.o. route. The doses of *G. superba* (7.5, 3.75 mg/kg); *Z. officinale* (700, 350 mg/kg); *G. glabra* (250,100 mg/kg), ranitidine (30 mg/kg) were selected from the published results. Gloriosa was tested only at low doses considering possible toxicity of colchicine. All the animals were returned to their cages, and after 30 minutes, they were subjected to manual restraint. Rats were subjected to restraint by placing them individually in a piece of galvanized steel window screen that is molded tightly around and held with adhesive tapes so that the animals cannot move. After 6 hours of restraint, all the animals were sacrificed under ether anesthesia followed by decapitation and the stomachs were cut opened through the greater curvature and tissues were rinsed with saline and examined by 5-fold binocular magnifier to assess the ulcer formation. The number of ulcers was counted and scoring was done as per the reported methods [23, 24,25].

0.0 - Normal stomach, 0.5 - Pink or Red coloration of stomach, 1.0 - Superficial ulcer, 1.5 - Spot ulcer, 2.0 - Hemorrhage spot, 2.5 - Scattered Hemorrhage streak, 3.0 - Bleeding ulcer, 4.0 - Perforated ulcer.

Ulcer index was calculated using the formula

$$U_I = U_N + U_S + U_P \times 10^{-1}$$

Where U_I - Ulcer index, U_N - Avg. no. of ulcer's/ animal, U_S - Avg. of severity score,

U_P - Percentage of animals with ulcers.

And the inhibition percentage was calculated by the formula

$$\% \text{ of inhibition} = [(U_I \text{ control} - U_I \text{ treated}) / (U_I \text{ control})] \times 100$$ [26, 27, 28]

Gastric secretion in Pylorus ligated rats

Male Wistar rats weighing 160 - 200 g were divided into nine groups, each group comprising six rats. Group-I received vehicle (sodium CMC, 10 mL/kg), Group-II and Group-III received *G. superba* at 7.5, 3.75 mg/kg dose; Group-IV and Group-V received *Z. officinale* at 700, 350 mg/kg dose; Group-VI and Group-VII received *G. glabra* at 250,100 mg/kg dose respectively and Group-VIII received ranitidine at 30 mg/kg dose. Test extracts and ranitidine was administered orally for three consecutive days 3 hours prior to the aspirin administration (50 mg/kg) suspended in 1.0 % sodium CMC.

Animals were fasted for 24 h after the last dose, 24 h later all the rats were subjected to ligation of the pylorus by opening their abdomen from midline incision. The pylorus connected to duodenum was ligated, and the peritoneum and skin were sutured [29-31]. The animals were sacrificed 4 hours after pylorus ligation, and their stomachs were removed after clamping the esophagus. The gastric contents were collected into centrifuge tube and centrifuged @ 5000 rpm for 5.0 min. The pH, volume of gastric secretion from each animal was precisely measured and volume was reported as mL/100 g body weight. While the total acidity of the gastric secretions was determined by titrating the undiluted gastric juice with 0.01N NaOH using phenolphthalein as an indicator and expressed as µEq/l.

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Anthelmintic activity

Test worms collection and authentication

Adult earthworms (*Pheretima posthuma),* roundworms (*Ascardia galli) were* used to evaluate anthelmintic activity in *vitro.* Indian adult earthworms were collected from moist soil of the vermiculture plants and washed with normal saline to remove all the fecal and soil matter, while roundworms were collected from freshly slaughtered fowls infested intestines. The intestines were washed with normal saline solution to remove all the fecal matter, dissected; worms were collected and kept in normal saline solution. The average length of earthworm was 6-8 cm and round worm was 5-7 cm. The collected worms were authenticated by experts in the Department of Zoology, Kakatiya University, Warangal and also verified by veterinary practitioners, Warangal, India.

Test sample preparation for *in vitro* assay

Test samples for *in vitro* study were prepared by suspending each extract in ~1.0 % of dimethylformamide (DMF) and the volume was adjusted with normal saline to obtain stock solution concentrations of 2.5, 5.0 and 7.5 mg/mL.

Experimental procedure

The anthelmintic assay was carried out as per the published methods [25, 32, 33] with minor modifications. Piperazine citrate was added at 10 mg/mL concentration and included in the assay as positive control. Approximately equal sized six earthworms to 11 petri dishes and 6 roundworms to second set of 11 petri dishes at room temperature containing the extracts at different concentrations and standard drug at one concentration along with normal control. Each petri dish was observed for time taken to induce paralysis (no movement even after tapping) or death of an individual worm. While the time for death was recorded after ascertaining that worms neither moved when tapping the petri dish nor when dipped in warm water (~50 °C) followed by fading away of their body colors. The results from these experiments are presented in table 3 and table 4.

Statistical Analysis

Statistical analysis was carried out using GraphPad Prism, version 5.02. Data was analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test. All data are presented as mean ± S. D. A "P" value of <0.05 was considered statistically significant.

OBSERVATIONS AND RESULTS

Oral administration of aspirin to Wistar rats produced characteristic mucosal lesions. This damage may be due to its direct action of the gastric epithelium. Aspirin is known to cause gastric injury and to delay ulcer healing. Histologically, an ulcer consists of two major structures; a distinct ulcer margin formed by the adjacent non-necrotic mucosa, the epithelial component, granulation tissue at the ulcer base and the connective tissue component. The latter consists of fibroblasts, macrophages and proliferating endothelial cells forming micro vessels. Ulcer healing is a complex process, which involves cell migration, proliferation, re-epithelialization, angiogenesis and matrix deposition, all ultimately leading to scar formation. All these processes are controlled by growth factors, transcription factors and cytokines [34, 35,36].

Table 1: Comparison of anti-ulcer activity of ethanolic extracts after restraint plus aspirin induced ulcers in rats

Treatment	Dose (mg/kg)	Ulcer index (mean ± SD)	% inhibition
Control	-	10.89 ± 0.07	-
Ranitidine	30	0.31 ± 0.12***	97
Gloriosa	7.5	1.01 ±0.77***	93
	3.75	4.02 ± 1.53**	68
Glycyrrhiza	250	4.78 ± 1.57*	76
	100	5.65 ± 1.53*	48
Zingiber	700	2.89 ± 1.29**	83
	350	5.35 ± 1.78*	50

*P<0.05- significant; **P<0.01- highly significant; ***P<0.001- very highly significant relative to control; (n = 6 in each group)

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In the present study, the antiulcerogenic effects of *Gloriosa Superba* (tuber), Zingiber Officinale (rhizome) and Glycyrhiza Glabra (root) extracts were evaluated and compared in stress plus aspirin induced ulcers and pylorus ligation-induced ulcer models in male Wistar rats. Ranitidine was used as a positive control drug for comparison and the results are presented in table 1. Stress plus aspirin induced linear type of hemorrhagic ulcers with a mean diameter of 10.89 mm (Table 1). Such hemorrhagic lesions were attenuated by pretreatment with ranitidine. The ulcer index was inhibited to 97% (ranitidine), 93% (Gloriosa, 7.5 mg/kg) followed by 83% (Zingiber, 700 mg/kg) and 76% (Glycyrhiza, 250 mg/kg). Such an excellent antiulcerogenic effect of *Gloriosa* could be due to the facilitation of gastric secretion during severe stress. Its cytoprotective activity would be contributed to the observed strong anti-ulcer activity.

Four hours pylorus ligation significantly increased volume and acidity of the gastric juice in control group of animals received vehicle. Ranitidine effectively controlled the volume, acidity and maintained the pH compared to control group. *Gloriosa* exhibited strong anti-ulcerogenic effect comparable to ranitidine at high dose (7.5 mg/kg). However, Glycyrhiza and Zingiber failed to exert similar effect even at high doses (table 2).

Table 2: Comparison of anti-ulcer activity of ethanolic extracts after aspirin plus pylorus ligation-induced ulcers on gastric contents, acidity and pH in rats

Treatment	Dose (mg/kg)	Gastric pH (mean ± SD)	Volume of Gastric juice (mL) (mean ± SD)	Total acidity (µEq/l) (mean ± SD)
Control	-	2.13 ± 0.05	2.58 ± 0.08	42.21 ± 1.20
Ranitidine	30	4.43 ± 0.07***	1.23 ± 0.08**	18.34±0.09***
Gloriosa	7.5	4.18 ± 0.05***	1.36± 0.08**	20.06±0.30***
	3.75	3.34 ± 0.05*	1.55 ± 0.07*	36.46 ± 0.08*
Glycyrrhiza	250	2.38 ± 0.10*	2.65 ± 0.01*	32.25 ± 0.15
	100	2.45 ± 0.08	2.85 ± 0.04	46.25 ±0.85
Zingiber	700	3.62 ± 1.15**	1.85 ± 0.10*	29.80±0.07*
	350	3.83 ± 1.14*	2.41 ± 0.12	42.03 ± 0.24

*P<0.05-significant; **P<0.01- highly significant; ***P<0.001- very highly significant relative to control (n = 6 in each group)

In spite of multiple mechanisms, gastric secretion is one of the main factors for ulceration, because most types of the ulcers induced by diverse agents were attenuated by the secretion inhibitors. Inhibition of gastric secretion by irreversibly inhibiting the proton-pump H+/K+-ATPase is well known. In addition to gastric secretion, however, several factors such as cell death and recovery, inflammation, stasis and blood flow are involved in the process of ulcer formation [37].

In the present study, it has been demonstrated that *Gloriosa Superba* tuber extracts exerted strong anti-ulcerogenic effects by controlling gastric acid secretion, acidity and by maintaining the pH of the gastric contents. However, further studies needs to be conducted in appropriate disease models, higher animals and focus should be on isolating and characterizing active constituent(s) responsible for its astounding antiulcerogenic activity in rat model.

Table 3: Anthelmintic activity of *Gloriosa superba* (L.) tuber extracts against Earthworms

Test extract	Concentration (mg/ml)	Paralysis time (min) (mean ± SD)	Death time (min) (mean ± SD)
Normal Saline	-	-	-
Piperazine citrate	10	21.7±0.2**	35.5±0.7**
Aqueous extract	2.5	19.5±0.1	31.2±0.5
	5	10.1±0.4*	26.8±0.1*
	7.5	8.5±0.1**	14.5±0.3**
Ethanolic extract	2.5	20.4±0.1	38.4±0.2
	5	18.7±0.2	34.2±0.2
	7.5	13.3±0.1*	18.3±0.8*
Chloroform extract	2.5	14.2±0.2*	16.7±0.5*
	5	9.2±1.2**	13.1±0.8**
	7.5	5.8±0.3***	8.2±0.1***

*P<0.05-significant; **P<0.01- highly significant; ***P<0.001- very highly significant relative to control.

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Table 4: Anthelmintic activity of *Gloriosa superba* (L.) tuber extracts against *Ascardia galli*

Test extract	Concentration (mg/ml)	Paralysis time (min) (mean ± SD)	Death time (min) (mean ± SD)
Normal Saline	-	-	-
Piperazine citrate	10	13.1±0.2**	23.4±0.7**
Aqueous extract	2.5	11.2±1.1	21.4±0.2
	5	10.13±0.1*	18.9±0.2*
	7.5	8.2±0.4**	15.1±0.1**
Ethanolic extract	2.5	15.8±1.1	22.1±0.3
	5	14.9±0.6	20.7±0.9
	7.5	11.6±0.8*	13.10±0.3*
Chloroform extract	2.5	10.7±0.2*	15.4±0.2*
	5	8.1±0.9**	12.1±0.2**
	7.5	4.1±0.2***	6.8±0.1***

*P<0.05-significant; **P<0.01- highly significant; ***P<0.001- very highly significant relative to control.

Gloriosa Superba tuber aqueous, ethanolic and chloroform extracts were evaluated for the anthelmintic activity *in vitro* against earthworms and roundworms. Results from these studies are presented in table 3 and table 4. Anthelmintic activity exhibited with all the extracts fits this hierarchy from highest to lowest chloroform extract>aqueous extract>ethanolic extract. Chloroform extract exhibited time to death against both the intestinal worms in a dose related way, whereas conventionally used drug piperazine citrate has moderate anthelmintic activity. Our results confirm the reported claims in the literature on its potent anthelmintic activity. However, *in vivo* studies in disease models need to be conducted to establish its efficacy in treating chronic intestinal infections.

CONCLUSION

The results of this comprehensive study reveals that the *Gloriosa superba* tuber extracts have significant antiulcerogenic activity compared to other extracts and standard drug ranitidine. The effects on gastric contents and pH suggest, the extract exerted antispasmodic and acid neutralizing effects. However, experiments in disease models need to be conducted, to delineate its mechanism of action and benefit in the management of gastrointestinal disorders.

Similarly, superior anthelmintic activity found *in vitro* needs to be assessed *in vivo* in appropriate disease models to establish its efficacy.

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