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Evaluation of Antiulcer Activity of Ethanolic Extract of Tubers of *Gloriosa superba Linn* in Albino Rats

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Abstract

Aim: The Plant *Gloriosa superba Linn* has been reported for a variety of ethnic medicinal uses. The present study aimed to evaluate the antiulcer activity of ethanolic extract of tubers of *Gloriosa superba Linn* (EEGS) in albino rats. **Methods:** Antiulcer activity was evaluated using Pylorus ligation induced ulcer animal model in albino rats. The dried and powdered tubers of *Gloriosa superba* were successively extracted with petroleum ether and ethanol. The ethanolic extract was subjected to phytochemical screening to identify various phytoconstituents. The LD_{50} value of EEGS was found to be 1260 mg/kg. The Volume, pH, total acidity and free acidity of gastric fluid, ulcer score, ulcer index and percent inhibition of ulcer were measured. Data were analyzed using One-way Analysis of Variance followed by Tukey's post hoc test, and $P<0.05$ was considered as statistically significant. The test group received two doses (80mg/kg and 120mg/kg) of the extract for seven days before induction of the ulcer. Positive control group received the standard drug Omeprazole(20mg/kg) and negative control group received vehicle (1% CMC). **Results:** Preliminary phytochemical screening of the extract showed the presence of carbohydrates, alkaloids, steroids, flavanoids, tannins and phenolic compounds. The antiulcer activity of EEGS was evidenced by the significant attenuation of gastric volume, pH, free acidity, and total acidity in the gastric juice of pyloric-ligated rats in a dose-dependent manner. **Conclusions**: The results of the present study suggest that EEGS possessed significant dose dependent antiulcer effect in Pylorus ligated ulcer model.

Keywords
Albino rats, Antiulcer, *Gloriosa superba*, Pylorus ligation.

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INTRODUCTION:

Natural products have played a key role in Pharmaceutical Research, as many medicines are either natural products or derivatives thereof. It is estimated that about 40% of all medicines is either natural products or their semisynthetic derivatives. Natural products research continues to explore a variety of lead structures, which may be used as templates for the development of new drugs by pharmaceutical industry. Rapidly increasing attention in herbal medicines has been improved systematic examination of its therapeutic effectiveness and harmless utilization. Various medicinal plants have dramatic effects on the body and few plants have the capacity to cure different kind of diseases [1,2,3].

Peptic ulcers are lesions in stomach due to aggressive action of peptic juices. Peptic ulcer is one of the widespread gastrointestinal disorders. The various peptic ulcers are as follows:

1. Duodenal ulcer
2. Gastric ulcer
3. NSAID induced ulcer
4. Stress ulcer

The aggressive and protective factors in the stomach are acid pepsin secretion, mucosal barrier, blood flow, cellular regeneration, prostaglandins and epidermal growth factors. Sometimes the gastric mucosa is continuously exposed to potentially injurious agents such as pepsin, bile acids, food ingredients, bacterial products and drugs. Factors such as stress, smoking, nutritional deficiency, ingestion of NSAID's all can increase the incidence of gastric ulcers. It is reported that prolonged anxiety, emotional stress, haemorrhagic surgical shock, burns and trauma are known to cause severe gastric irritation [4].

The peptic ulcer is found on that portion of the stomach which is immersed by gastric acid. The first portion of duodenum and the stomach are immersed by acid gastric juice. Bile and pancreatic juice flow in the second portion of duodenum. Hence, this is the reason, the peptic ulcer occurs in the stomach and the initial part of the duodenum as these portions are acted upon by acid peptic juice. It generally occurs due to an imbalance between mucosal defense factors and injurious factors [5].

The incidence of peptic ulcer is gradually increasing due to stressful life style and fast growth of human civilization. The common estimation of peptic ulcer is between 3 -10%. About fifteen thousand patients are died every year due to peptic ulcer diseases (PUD). The ratio of duodenal ulcer and the gastric ulcer between male and female is 3:1 and 1.5:2.1 respectively. The current drug treatment of Peptic Ulcer aims at controlling gastric acidity, hypermotility and spasm and thus relieving the associated pain, promoting ulcer healing, reinforcement of gastric mucosal production there by prevention of complication and recurrence [6].

The currently used antiulcer drugs like H_2–receptor blockers, Proton pump inhibitors, antimuscarinic agents etc produce adverse reactions such as hypersensitivity, arrhythmia, impotence and haemopoietic changes with a possibility of increased rate of ulcer recurrence within one year after cessation of the treatment. Because of the above mentioned demerits reported with the current antiulcer therapy there is a need for the search of newer therapeutic antiulcer agents from plant sources

The plant *Gloriosa superba* (Liliaceae) is one of the herbaceous medicinal climber which is a striking tuberous plant with brilliant wavy edged yellow and red flowers that appears from November to March every year. It is used traditionally to cure cancer, gout, asthma, leprosy, arthritis, piles, ulcer. It is also used to treat intestinal worms, bruises, infertility, skin problem and impotence. It is also used in folklore medicine as an abortifacient and anti-inflammatory agent. The plant is one of the seven upavishas in the Indian medicine, which cure many ailments but may prove fatal on misuse. The tuberous root stocks of *Gloriosa superba* boiled with *Sesamum* oil reduces arthritis pain in joints [7,8,9]. The high medicinal value of this plant could be due to the presence of many secondary metabolites which act as bioactive compounds against diseases. No scientific data is available in support of traditional uses of this plant for antiulcer effect. Hence the present study was planned to evaluate the antiulcer activity of ethanolic extract of tubers of *Gloriosa superba* (EEGS) in pylorus ligated induced gastric ulcer model in experimental animals, albino rats.

MATERIALS AND METHODS:

Collection of Plant material

The underground tubers of *Gloriosa superba Linn* were considered for the present study. The Plant specimens were collected during the month of June from its natural habitat from Botanical Garden, Palode, Thiruvananthapuram and from the Botanical garden, Kuzhippallam, Thiruvananthapuram, Kerala, India. Care was taken to select healthy plants and normal organs.

The tubers were authenticated by the Botanist Dr P M Radhamany, Professor and Head, Department of Botany, University of Kerala, Karyavattom, Thiruvananthapuram, Kerala. The voucher specimen was deposited in the Botany Department of the institution for further documentation. (Voucher no. KUBH6028).

Preparation of the Extract

The tubers were washed thoroughly with running tap water to remove adhering soil. Then they were cut into smaller pieces and were dried in shades until they were free from moisture. The dried specimen were pulverized mechanically to a coarse powder. The air-dried, powdered tubers of *Gloriosa superba Linn* was freed from fatty substances by Soxhlet extraction with petroleum ether for 24 hours at room temperature. Defatted and dried plant materials were subjected to Continuous hot extraction in Soxhlet apparatus with ethanol. Extracts were filtered off and separated from solid materials using Whatman No. 1 filter paper. The extract so obtained

was then evaporated under reduced pressure in a rotary evaporator at temperature 50^{0}C until all the solvent had been completely removed to get an extract sample. The extract was subjected to preliminary phytochemical screening for detecting various phytochemical constituents [10].

Experimental animals

Adult healthy Wistar albino rats weighing between 150-220g were used for the study. The animals were procured from animal house, Sree Chithra Thirunal Institute of Biotechnology, Thiruvananthapuram, Kerala. The animals were housed at a standard environmental condition (at 25±2^{0}C, humidity 60 ±10% with 12 hours light and dark cycles) with food and water ad libitum. All the experimental procedures and protocols used in this study were reviewed by Institutional Animal Ethics Committee. (IAEC No.01/05/2014/MCT)

Pharmacological Studies

Acute toxicity studies along with gross behavioural studies of the ethanolic extract of GS were done in albino mice and LD50 of the drug was determined graphically [11].

Pylorus-ligation induced ulcer model [12]

The method of Shay Rat ulcer was adopted for the study (Shay et al,1945).

Animals: Albino rats randomly divided into five groups of six animals each. They were fasted in individual cages with measures taken to avoid coprophagy for 24 h prior to the experiment with free access to water

Experimental Design

Group I: Normal Control-Received 0.1mg/ml CMC, p.o

Group II: Disease Control (Undergone Pylorus ligation), received 0.1mg/ml CMC, p.o

Group III: Test group - Received 80mg/kg EEGS, p.o

Group IV: Test group - Received 120mg/kg EEGS, p.o

Group V: Positive Control-Received Omeprazole (20mg/kg), p.o

Experimental Procedure

On the day of experiment, animals of Group III, IV and V were treated with low, high doses of EEGS and the standard drug Omeprazole respectively. The normal control (Group I) and disease control (Group II) groups were received vehicle only. After 1 hour of drug treatment all the animals except in the normal control group were anaesthetized (ketamine 75mg/kg i.p), abdomen were opened by a small midline incision below the xiphoid process of the sternum. Pyloric portion of the stomach was slightly lifted out and ligated avoiding traction to the pylorus or damage to the blood supply. Then the stomach was replaced back carefully and the abdominal wall was closed by sutures. Skin was cleared from any blood spots and bleeding. Collodion was applied over the wounds. The animals were sacrificed after 4 hours of pylorus ligation. The abdomen was opened, cardiac end of the stomach was dissected out and the contents were drained into a glass tube and centrifuged at 1000 rpm for 10 minutes and volume was noted. The pH of gastric juice was recorded by pH meter. The gastric contents were subjected to analysis for free and total acidity. The stomachs were then washed and the number of ulcers per stomach were examined microscopically with the help of a hand lens (10x) and the scoring of ulcers were done as per standard procedure. The Ulcer index and percentage of Ulcer inhibition were determined as follows: UI = UN+US+UP×10-1

Where, UI = ulcer index,

UN = average number of ulcers per animal,

US = mean of ulcer score,

UP = percentage of animals with ulcers. ulcer probability for each group.

$$\text{Ulcer Inhibition (\%)} = \left\{ \frac{UI_{control} - UI_{treated}}{UI_{control}} \right\} \times 100$$

Biochemical estimations

Determination of gastric volume

After sacrificing the rat, the stomach portion was removed. The gastric contents were transferred into the centrifuge tube, centrifuged and filtered. The supernatant liquid was then transferred to a measuring cylinder and the volume was measured.

Determination of pH of gastric content

One ml of the gastric juice was collected and the pH was directly measured by using Digital pH meter.

Determination of ulcer index

Ulcer scoring was done by the scoring system specified in Table [1].

The Ulcer index and percentage of Ulcer inhibition were determined as follows:

UI = UN+US+UP×10-1

Where, UI = ulcer index,

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UP = percentage of animals with ulcers. ulcer probability for each group.

$$\text{Ulcer Inhibition (\%)} = \left\{ \frac{UI_{control} - UI_{treated}}{UI_{control}} \right\} \times 100$$

Determination of free acidity and total acidity:
The total volume of gastric content was measured. The gastric contents were centrifuged and filtered. One ml of the gastric juice was pipetted out and the solution was titrated against 0.1N sodium hydroxide using 2 to 3 drops of Topfer's reagent (Dimethylaminoazobenzene 0.5% in absolute ethanol) as indicator until all traces of red colour disappears and the colour of the solution turned to yellowish orange colour. The volume of alkali added was noted. This indicated the volume of NaOH required for neutralizing the free hydrochloric acid present in the gastric juice. Then 2 to 3 drops of phenolphthalein solution was added and titration was continued until a definite red colour appears. Again the total volume of sodium hydroxide added was noted. The sum of the volumes of alkali added corresponds to the total acid present in the gastric juice.

Acidity was calculated by using formula

$$\text{Acidity (mEq/L)} = \frac{\text{Volume of NaOH X Normality of NaOH}}{\text{Volume of gastric juice used}}$$

Statistical analysis
The results were represented as Mean ±Standard error mean [Mean ± S.E.M.]. The data were analyzed using a one-way ANOVA followed by Tukey's multiple comparison post-hoc test. Significance is represented as in case of *p < 0.05, **p < 0.01, ***p < 0.001. The Statistical analysis is done with the help of Graph Pad Prism (Version 9).

RESULTS AND DISCUSSION:
The percentage yield of the EEGS was 9.4% w/w. The extract was pungent dark brown thick paste, partially soluble in water, sparingly soluble in dilute acetic acid and completely soluble in dilute alkali.

Preliminary Phytochemical Screening
The results of phytochemical screening of EEGS are shown in Table [2]. The extract showed the presence of carbohydrates, alkaloids, steroids, flavonoids, tannins and phenolic compounds. The LD50 value of EEGS was found to be 1260 mg/kg. Two different doses of the extract namely low ($1/15^{th}$) and high ($1/10^{th}$) such as 80 mg/kg and 120 mg/kg were selected with respect to the LD50 dose for the present study.
The gross behavioural studies showed mild sedation of animals. There were no autonomic and behavioural changes but CNS depression noted at higher doses.

Effect of EEGS on Pylorus ligation induced Gastric ulceration
The effect of EEGS was studied in Pylorus ligated gastric ulcer model in rats. Table [3] shows the effect of EEGS on gastric volume, gastric pH, free and total acidity, ulcer index and percentage ulcer inhibition of pylorus ligated rats.
In the present study in Pylorus ligated rats (Disease control group), the gastric volume was 3.2 ±0.12 and it was significantly (P>0.001) decreased by the standard drug Omeprazole, the low dose and high doses of EEGS to 1.73 ±0.08, 2.16 ±0.012 and 1.86 ±0.08 respectively. The pH of gastric juice in Normal Control group is 3.0 ±0.05 and it was significantly increased to 4.88 ± 0.04 in Omeprazole treated group and to 3.8 ±0.07 and 4.1 ±0.04 in low dose and high dose of EEGS respectively. The total acidity of the standard drug treated group (13.16 ±0.04), low and high doses of EEGS (17.33 ±0.33 and 14.66 ±0.21) were found to be decreased significantly (P<0.001) compared to disease control group (21.5 ±0.5). Ulcer Index in the disease control animals was 36.62 ±0.18 and it was significantly (P<0.001) decreased by the drug treated groups (17.16 ±0.74 and 12.0 ±0.73) and standard drug treated group (10.8 ±1.20).
In this study the percentage inhibition of ulceration was found to be 52.77% and 72.22% in Low dose EEGS and High dose EEGS groups, respectively. The standard drug Omeprazole also decreased the ulcer formation by 72.9% which is comparable with that of High dose EEGS group. Pylorus ligation induced ulcer was used to study the effect of EEGS on gastric acid secretion and mucus secretion. The ligation of the pyloric end of the stomach causes accumulation of gastric acid in the stomach.
This increase in the gastric acid secretion causes ulcers in the stomach. The fasting of rats for 24h followed by ligation of pyloric end of the stomach and then the ulcer index is determined 4h after pylorus ligation. The lesions produced by this method are located in the lumen region of the stomach. The ethanolic extract of tubers of *G superba* and omeprazole significantly decreased the total acidity and free acidity and significantly enhanced the pH of the gastric juice. This suggests that it is having an antisecretory effect. Pylorus ligation induced ulcer control rats showed perforated ulcer, deep ulceration of granular epithelium and almost reducing the sub-mucosa. The ethanolic extract at 80 mg/kg dose has shown mucosal erosion, partial healing of ulcer with few inflammatory cells and the

extract at dose 120 mg/kg has shown the healed ulcer, normal mucosa and no inflammatory cells.

The extract contains various types of compounds such as flavonoids, polyphenolic compounds, saponins and tannins. Some phytoconstituents extracted from medicinal plants possess antiulcerogenic activity and act by various mechanisms. Phenolic compounds and flavonoids possess antiulcer effect due to their antisecretory, cytoprotective, antioxidant, anti-inflammatory and anti-H. pylori actions. Phenolic compounds and flavonoids also promote prostaglandin synthesis, stress defense, antioxidant enzymes synthesis and wound healing properties [13-17]. Moreover, flavonoids increase capillary resistance and improve microcirculation. Tannins directly protect the outermost layer of mucosa and change the mucosal structure that can resist to chemical and mechanical injury [18].

The gastro protective effect exhibited by the ethanolic extract may be attributed to the presence of flavonoids, polyphenolic compounds, saponins and tannins. These compounds most likely inhibit gastric mucosal injury.

Table [1]. Ulcer scores

Sl no	Stomach colour	Ulcer score
1	Normal colour	0
2	Red colour	0.5
3	Red spots	1
4	Hemorrhagic streaks	1.5
5	3>5 ulcers	2
6	<5 ulcers	3

Table [2]. Phytochemical constituents of *Gloriosa superba Linn*

Sl no	Phytochemicals	Present (+)/Absent (-)
1	Carbohydrates	+
2	Proteins and Amino acids	-
3	Glycosides	-
4	Alkaloids	+
5	Steroids	+
6	Flavanoids	+
7	Saponins	-
8	Tannins and Phenolic compounds	+
9	Fixed Oils and Fats	-
10	Gums and Mucilage	-

Table [3]. Effect of extract on Gastric volume, pH, Acidity, Ulcer index and Ulcer inhibition

Group	Dose (mg/kg)	Gastric volume (ml)	Gastric pH	Acidity(mEq/100gm)		Ulcer Index	Ulcer Inhibition (%)
				Free acidity	Total Acidity		
Group I (Normal Control)	CMC (0.1mg/ml)	1.06 ±0.17	3.0± 0.05	2.05± 0.3115	6.083± 0.04	2.0± 0.2	-
Group II (Disease Control)	CMC (0.1mg/ml)	3.2± 0.12a*	3.03± 0.05	17.33± 0.3849a*	21.5± 0.53a*	36.67± 0.18a*	-
Group III (Low dose of EEGS)	EEGS (80mg/kg)	2.16 ±0.12b*	3.8± 0.07b*	12.03± 0.0192b*	17.33± 0.33b*	17.16 ±0.75 b*	52.77
Group IV (High dose of EEGS)	EEGS (120mg/kg)	1.86 ±0.08b*	4.1± 0.04b*	10.93± 0.0384b*	14.66 ±0.21b*	12.0 ±0.73 b*	72.22
Group V (Standard)	Omeprazole (20mg/kg)	1.73± 0.08b*	4.88 ±0.04b*	10.1± 0.0408b*	13.16± 0.04b*	10.8± 1.2 b*	72.22

All Values are expressed as Mean ± SEM; N =6. The data were analyzed using a one-way ANOVA followed by Tukey's multiple comparison test. a* indicates P<0.001 compared to the normal control group and b* indicates P<0.001 compared to that of disease control group.

Figure [1]

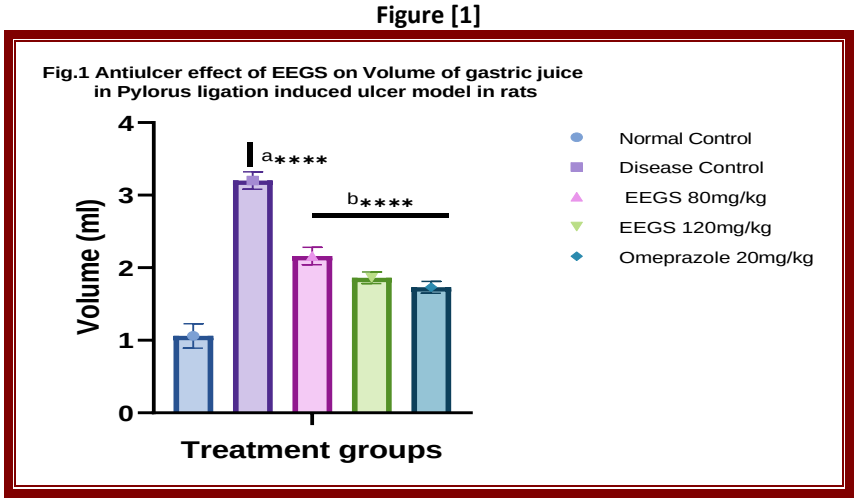

Figure [2]

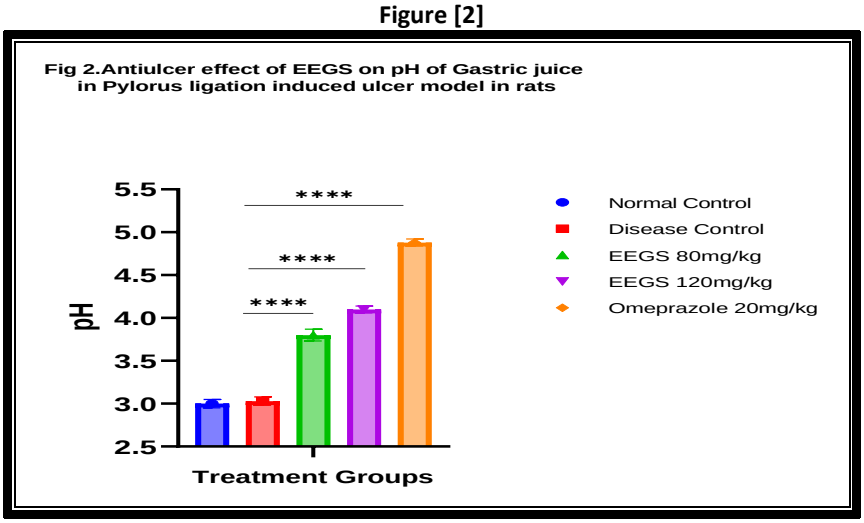

Figure [3]

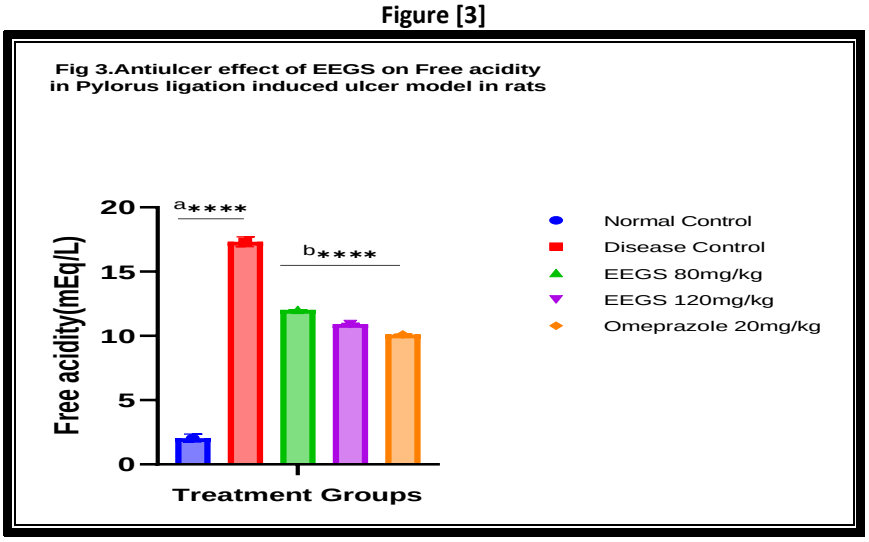

Figure [4]

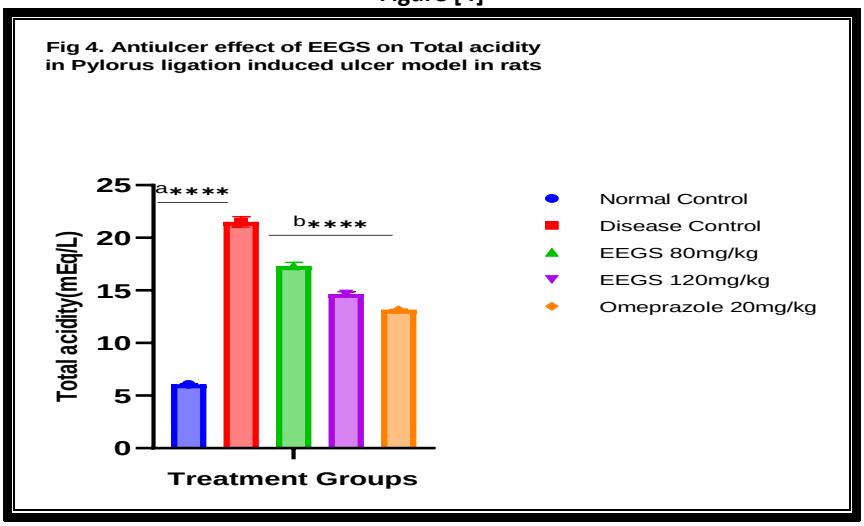

Figure [5]

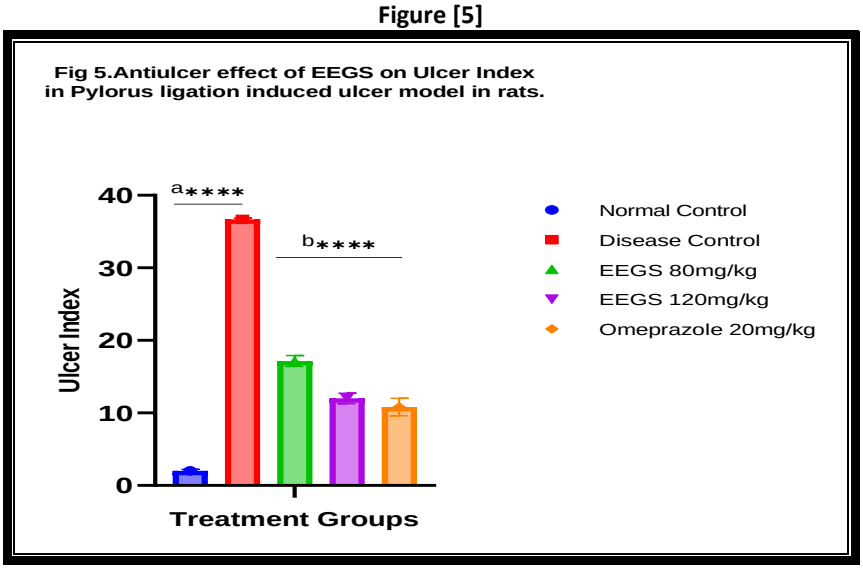

Figure [6]

Fig [6]. Effect of Ethanolic extract of *Glorios superba Linn* on Pylorus Ligated Ulcer Model in Rats

(a) Normal Control (b) Disease Control

(c) Omeprazole (d) EEGS(80mg/kg)

(e)EEGS 120mg/kg

In this figure [6], we can see that deep ulcer and hemorrhagic streaks were observed in the disease control (a) and some hemorrhagic streaks and minor spots were also observed in the lower dose of the extract (d). As we go in advance from lower dose to higher (e) and in standard (c), it showed that significant ulcer formations were not observed.

CONCLUSION:

The present study showed that the ethanolic extract of tubers of *Gloriosa superba Linn* has significant antiulcer activity which upholds the traditional claim of the experimental plant. Isolation and structural elucidation of active compounds in the alcoholic fraction of the extract and in vitro activity against H. pylori should be tested. The antiulcer activity along with its safety profile could make the tubers of *G.superba* a good candidate for the treatment of Peptic Ulcer Disease in humans.

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