Research Article

Gastroprotective Effect of *Glycyrrhiz aglabra* Linn. on Aspirin Induced Ulcer in Albino Rats

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ABSTRACT

This study was aimed to evaluate the gastroprotective effects of *Glycyrrhiza glabra* Linn. aerial root. *Glycyrrhiza glabra* Linn. aerial root extract (GGR; 150 and 300 mg/kg body weight) was administered orally, once daily for 14 days for prevention from pylorus ligation (PL)-induced ulcers. On 15thday 70mg/kg b.w aspirin administrated One hour after induction the animals were sacrificed. The stomach tissue and blood were collected for further analysis like volume of gastric juice, acid output, and pH value were estimated in PL-induced ulcer model. A significant reduction in lesion index was observed in ulcer-induced animals treated with GGR at different doses when compared with ulcerated rats in all models. A significant decrease occurred in the level of volume of gastric juice, and acid output. Simultaneously the level of gastric wall mucus and pH were increased significantly. These showed dose-dependent action of GGR. The antioxidant enzyme levels of CAT and SOD were decreased while administering GGR at different doses, compared with their control values. Restored the haematological changes. The results of our study showed that *Glycyrrhiza glabra* possess significant gastroprotective activity, probably due to its phytochemicals.

Keywords: Gastroprotective, *Glycyrrhiza glabra,* Antioxidant enzymes, Pylorus ligation.

INTRODUCTION

Ulcers are sore that occur in the upper gastrointestinal tract of the body. An ulcer is an imbalance between aggressive and defensive factors. Acid and pepsin can damage the stomach lining and cause ulceration. On the other hand, the damage comes first from some other cause making the stomach lining susceptible to aggressive factor. Stomach ulcers are also called peptic ulcers. The word peptic refers to pepsin, a stomach enzyme that breaks down protein. A peptic ulcer located in the stomach is called a gastric ulcer.[1]

Gastric ulcers, one of the most widespread disease states, are believed to be due to an imbalance between acid and pepsin along with weakness in the mucosal barrier. Although there are many drugs used for the treatment of gastric ulcers, most of these produce several adverse reactions. Several plants and herbs have also been employed in the treatment of gastrointestinal disorders, including gastric ulcers. Herbal medicines have been used since the dawn of civilization to maintain health and to treat diseases.[2]

Herbal medicines are also in great demand in the developed world for primary health care because of their efficacy, safety and lesser side effects. Flavonoids are well known phytochemicals, which are produced by various plants in large quantities. They have been of interest owing to their observed biological effects *in vitro* free radical scavenging activity of inhibition of cellular proliferation as antibiotic, antiallergic, antidiarrheal, antiulcer, and anti-inflammatory agents.[3] The genus of *glycyrrhiza* consists of about species, of which *Glycyrrhiza glabra* (Family: Fabaceae) is generally recognized as licorice for their sweet taste. It is known as "Athimadhuram" in Tamil.[4] The roots are sweet, refrigerant, emetic in large dose, tonic, mildlaxative, aphrodisiac, haemostatic. They are useful in hyperdipsia, cough, bronchitis, ulceration of urinary tract, pharyngitis, epilepsy, anaemia.[5] The aim of the present study is to evaluate the gastroprotective activity of aqueous root extract of *Glycyrrhiza glabra* Linn. in aspirin induced ulcer in rats.

MATERIALS AND METHODS

Identification and Authentication

Plant sources selected for the present study is *Glycyrrhiza glabra* Linn. aerial roots were collected from in and around Kumbakonam (Thanjavur Dt.), identified and authenticated by Department of Botany, Government Arts College (autonomus), Kumbakonam, Tamil Nadu.

Collection and Preparation of Plant Extract

Glycyrrhiza glabra Linn. root was collected from places in and around Kumbakonam. The plant materials were cleaned and shade dried. When the plants were thoroughly dried, they were coarsely powered using mechanical grinder and the powder was stored in air tight container for further analysis.

200g of *Glycyrrhiza glabra* Linn. Was taken and extracted with water. To one part of the plant material was boiled with six parts of water.

The boiling was continued until the total volume was reduced to one third and it was filtered. The filtrated was evaporated to dryness.

Paste form of the extract obtained was subjected to pre clinical screening.

Phytochemical Analysis

Preliminary phytochemical screening of various extracts was carried out as per the standard method.[6]

Experimental Animals

Healthy adult Wistar strains of albino rats, weighing 100-120g were used as experimental models. Animals were kept in well ventilated cages and fed with standard rat chow pellet obtained from Sai Durga Food and Feeds, Bangalore, India and water *ad libitum.* All the studies were conducted according to the ethical guidelines of CPCSEA after obtaining necessary clearance from the committee (Approval No: SAC / IAEC / BC / 2015/ M.Sc.005).

Induction of Ulcer

Gastric ulcer was induced in group II, III, & IV animals. Animals were starved for 24 hours with access to drinking water *ad libitum.* Animals were given Aspirin (70mg/Kg body weight). One hour after induction the animals were sacrificed. The stomach tissue and blood were collected for further analysis.

Experimental Design

The rats were divided into five groups comprising of four rats each.

Group-I Control

Group-II Disease control (ulcer induced by orally administration of aspirin 70mg/kg b.w on 15th day of experimental period).

Group-III Animals were treated with the aqueous root extract of *Glycyrrhiza glabra* Linn.(150mg/Kg body weight orally) for 14 days. On 15thday 70mg/kg b.w Aspirin administrated.

Group-IV Animals were treated with the aqueous root extract of *Glycyrrhiza glabra* Linn.(300mg/Kg body weight orally) for 14 days. On 15thday 70mg/kg b.w Aspirin administrated.

Group-V Animals treated with standard drug Ranitidine (20mg/Kg body weight orally) for 14 days. On 15thday 70mg/kg b.w Aspirin administrated.

Collection of samples

On completion of the experimental period, animals were anaesthetized with thiopentone sodium (50mg/kg). The blood was collected with or without EDTA as anticoagulant. Blood, plasma and serum were separated for the estimation of various biochemical parameters.

Determination of ulcer index in gastric tissue

Ulcer was induced using Aspirin at the concentration of 70mg/kg body weight. After one hour the animals were sacrificed by cervical decapitation. The stomach was removed and opened along the greater curvature and washed it slowly under the running water. Placed it on the glass slid and observed under microscope (10x) for ulcers. Mean ulcer score for each animal in expressed as ulcer index.

$$Where, X = \frac{Total\ mucosal\ area}{Total\ ulcerated\ area}$$

Biochemical Analysis

Determination of free and total acidity in gastric fluid followed by the method of Varely[22]. Estimation of protein and carbohydrate by the method of Lowry's[7] and Hedge and Hofreiter.[8] Determination of alkaline phosphatase.[9] Assay of catalase, glutathione peroxidise and superoxide dismutase by the method of Maehly and Chance,[10] Rotruck,[11] Misra and Fridovich[12] respectively. Estimation of lipid peroxides.[13] Estimation of reduced glutathione.[14] Determination of haemoglobin.[15] Determination of red blood cells count.[16] Determination of white blood cells count.[15]

Statistical Analysis

The results were expressed as mean ± standard deviation. The data were statistically analyzed by one-way analysis of variance (ANOVA) and P values<0.05 were considered significantly.

RESULTS AND DISCUSSION

Plants have basic nutritional importance by their content of protein, carbohydrate, fats and oils minerals, vitamins and water responsible for growth and development in man and animals. Phytochemical simply means plant chemicals. "Phyto" is the Greek word for plant. Phytochemicals are classified as primary or secondary constituents, depending on their role in plant metabolism. Primary metabolism is important for growth and development of plants include the common sugars, aminoacids, proteins, purines and pyrimidines of nucleic acids, chlrophyll's etc. Secondary metabolism in a plant plays a major role in the survival of the plant in its environment. Attractions of pollinators, natural defense system against predators and diseases, etc., are examples of the roles of secondary metabolite.[17] Preliminary phytochemical screening of the plant powder showed the presence of sterol, Saponin, flavonoids, alkaloid (Table 1).

The aim of the study was to determine anti ulcer activity of stem bark of *Careya arbore* Roxb. on the Wister strain albino rats. Dried stem bark of *Careya arbore* Roxb. was powdered and this coarse powder was extracted with 70% ethanol by soxhlet extraction method to yield a Ethanol extract of stem bark of *Careya aborea* Roxb. (EECA). The extract was subjected for preliminary phytochemical analysis and was evaluated for anti ulcer activity against various models such as Ethanol induced, cold restraint stress induced and Pylorus ligation induced models. In acute toxicity study, EECA was found to be safe till 3000mg/kg. So the doses of EECA at various concentration of 300 and 600mg/kg body weight was administered orally, twice daily for 5 days for prevention of ulcer from Pylorus ligation, Ethanol and cold restraint stress - induced ulcers. Analytical parameters like

Percentage of Ulcer protection was calculated based on Ulcer index and Gastric juice volume, pH and acidity of gastric juice. Preliminary phytochemical analysis of EECA showed the presence of carbohydrates, glycosides, phytosterols, phenolic compounds, tannins and saponins. The EECA has shown significant activity at both 300mg/kg and 600mg/kg dose level in a dose dependent manner. Phytoconstituents like tannins and saponins may be responsible for anti ulcer activity of EECA.[18]

Effect of plant drug against aspirin induced gastric ulcer was studied. The level of Hb, WBC and RBC were showed in table: 2. Hb and RBC Ulcer is a condition where there is a profound hemorrhage due to the lesion in the gastric mucosa. This was proved from the decreased level of Hb & RBC count obtained for group II animals. Pre-treated with plant showed ulcer prevention and thereby no hemorrhage. Hence there was significantly rise in the level of Hb & RBC count in the plant drug treated groups.

Effect of oral administration of *Glycyrrhiza glabra* Linn. on acid output was presented graphically. From the table 3 it was evident that test drug showed decreased in gastric output. This also proves the antacid effect of plant extract. The gastro protective effect was found to be dose dependent.

Induction of gastric ulcer by aspirin caused a marked in decrease in protein level (table 3). Significant increases in the protein level were noted in plant treated groups. This indicates that *Glycyrrhiza glabra* Linn. helps in pre-treating gastric injury and enhance and regenerating of gastric mucosa.

Induction of gastric ulcer by aspirin caused a marked in increase in carbohydrates level (table 3). This indicates that *Glycyrrhiza glabra* Linn. helps in pretreating gastric injury and enhance and regenerating of gastric mucosa.

The present study was under taken to find out the efficacy of ethanolic extract of *Cocculus hirsutus* against gastric ulcer that developed due to pyloric ligation in rats. Five groups (n=5) of Adult Albino rats were taken. Group-I was the control group. Group-II was given standard drug omeprazole (20mg/kg). Group III and IV were given 100mg/kg (low dose) and 200mg/kg (high dose) of ethanolic extract of *Cocculus hirsutus* respectively. The result indicated a significant reduction in the ulcer index, volume of gastric juice, free acidity and total acidity and increase in pH and protection after treatment with extract. The *in vivo antioxidant* studies were shown that the lipid peroxidation levels of the drug were decreased where as the levels of the nitrite, catalase and reduced glutathione seen to be increased. The result suggests that antioxidant and gastro protective activity of *Cocculus hirsutus*.[19] Animals pretreated with extract of *Glycyrrhiza glabra* Linn. given showed increased activity of ALP (table 3).

The present study revealed that *Glycyrrhiza glabra* Linn. showed ulcer protective effect against aspirin induced gastric ulcer in rats. Activity of SOD, one of the antioxidant enzyme was increased in ulcerated untreated rats (Fig 2). This was evidenced by the increased activity of SOD in plant extract treated group animals in dose dependent manner.

To evaluate the role of reactive oxygen species in the pathogenesis of acute ethanol-induced gastric mucosal lesions and the effect of *Nigella sativa* L oil (NS) and its constituent thymoquinone (TQ) in an experimental model. Male Wistar albino rats were assigned into 4 groups. Control group was given physiologic saline orally (10 mL/kg body weight) as the vehicle (gavage); ethanol group was administrated 1 mL (per rat) absolute alcohol by gavage; the third and fourth groups were given NS and TQ respectively 1 h prior to alcohol intake. One hour after ethanol administration, stomach tissues were excised for macroscopic examination and biochemical analysis. NS and TQ could protect gastric mucosa against the injurious effect of absolute alcohol and promote ulcer healing as evidenced from the ulcer index (UI) values. NS prevented alcohol-induced increase in thiobarbituric acid-reactive substances (TBARS), an index of lipid peroxidation. NS also increased gastric glutathione content (GSH), enzymatic activities of gastric superoxide dismutase (SOD) and glutathione-S-transferase (GST). Likewise, TQ protected against the ulcerating effect of alcohol and mitigated most of the biochemical adverse effects induced by alcohol in gastric mucosa, but to a lesser extent than NS. Neither NS nor TQ affected catalase activity in gastric tissue.[20]

The present study revealed that *Glycyrrhiza glabra* Linn. showed ulcer protective effect against aspirin induced gastric ulcer in rats. Activity of glutathione peroxidase, one of the antioxidant enzymes was decreased in ulcerated untreated rats (Fig 1). This was evidenced by the decreased glutathione peroxidase activity of in plant extract treated group animals in dose dependent manner. Animals pretreated with extract of *Glycyrrhiza glabra* Linn. given showed decreased activity of catalase (Fig 3).

The present study was aimed to evaluate gastroprotective and antioxidant activities of ethanolic extract of *Ceropegia juncea (L.)* Taub in rats. Effects of various doses (100, 200, 300 and 400 mg/kg p.o.) of Ceropegia juncea leaf ethanolic extract (CJEE) were studied in pylorus-ligation and ethanol-induced gastric mucosal injury in rat. The effect of CJEE on free radical induced lipid peroxidation determined by malondialdehyde estimation method. Amount of antioxidant enzymes (viz. superoxide dismutase (SOD), Catalase (CAT), reduced glutathione (GSH)) along with various membrane bound enzymes in tissue homogenate was also determined using previously described methods. Treatment with CJEE showed significant reduction in ulcer index ($P<0.01$) in both the models along with the reduction in volume and total acidity, and an increase in gastric juice pH. The animals treated with different doses of CJEE showed an increase in the levels of SOD, CAT, GSH and membrane bound enzymes like Ca^{2+} ATpase, Mg^{2+}

ATpase, Na^{2+} ATpase and decrease in lipid peroxidation in both the models suggest its antioxidant activity of CJEE. These effects of CJEE suggest its gastroprotective activity, which can be attributed to its antioxidant properties. Further, the polypenolics of the plant may be held responsible for these effects, which has been found active against various ulcerogenic agents in previous reports.[21]

The present study concluded that *Glycyrrhiza glabra* Linn. resulted antiulcer activity against experimentally induced gastric ulcer in rats. Group II animals which have not been pre-treated with plant extract had decreased level of lipid peroxide. The extract showed inhibition of lipid peroxidation. Generation of manoindialdehyde and related substance from lipid that react with thiobarbituric acid was found to be inhibited by extract in an dose dependent manner (Fig 5).

The present study indicated that *Glycyrrhiza glabra* Linn. resulted antiulcer activity against experimentally induced gastric ulcer in rats. Group II animals which have not been pre-treated with plant extract had decreased level of reduced glutathione. GSH, an major non protein thiol in living organisms play a central role in coordinating the body's antioxidant defense process. Treatment with test drug resulted in increased level of total tissue sulfhydryl compound to the untreated ulcerated rats (Fig 4). Excessive peroxidation caused increased GSH consumption. The antioxidant activity of the herbal extract would have prevented excessive peroxidation.

In the present study, we investigated the gastroprotective activity of aqueous root extract of *Glycyrrhiza glabra* Linn. (AREGG) in aspirin induced animal model of ulcer. Various serum markers such as protein and carbohydrates. The antioxidant parameters such as SOD, ALP, GPx, LPO, reduced glutathione, glutathione peroxidase and catalase were showed to assess the gastroprotective activity (Table 3; Fig 1 – 3). These parameters were assessed after treatment with AREGG in two different dose (150 and 300mg/kg b.w for 15 days) and compared them with toxicant agent aspirin 70mg/kg b.w/ for 15 days.

The phytochemical analysis revealed that the presence of tannin, flavonoids, terpenoids, alkaloids, saponins, glycosides in aqueous extracts. The amount of protein and carbohydrates was determined in aqueous extract of *Glycyrrhiza glabra* Linn. and the concentration was found to be increased. The drug materials exhibited different shades of brown & green fluorescence, which indicates the nature of chromopore present in the formation. The immediate cause of gastric ulcer is disturbance in the protection of stomach mucosa against gastric acid.

The gastroprotective effect of *Glycyrrhiza glabra* Linn. extract may be related to an antacid and antioxidant effect. The gastro protective effect of the drug may be due to the phytoconstituents present in AREGG lessened the negative effects of aspirin induced ulcer possibly by inhibiting free radical mediated process. Based on improvement in serum marker levels, level of antioxidant enzyme and presence of important phytoconstituents.

The study concludes that the AREGG possess gastroprotective activity and thus supports the traditional claim of the same under the light of modern science.

Further investigation of these promising protective effects of AREGG against aspirin induced ulcer may have a considerable impact on developing clinically feasible strategies to treat patients with ulcer.

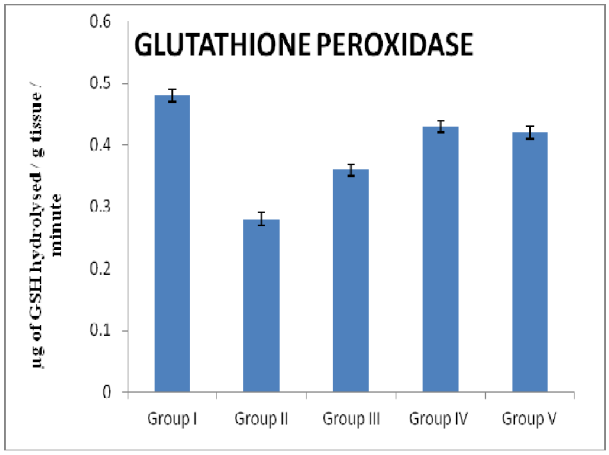

Figure 1: Effect of aqueous extract *Glycyrrhiza glabra* on glutathione peroxidase activity of experimental animals.

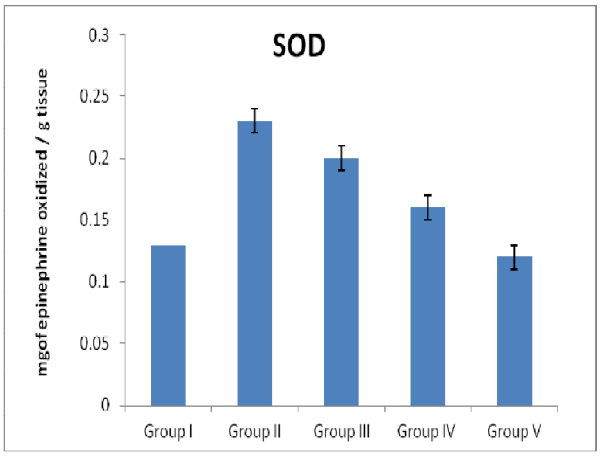

Figure 2: Effect of aqueous extract *Glycyrrhiza glabra* on SOD activity of experimental animals.

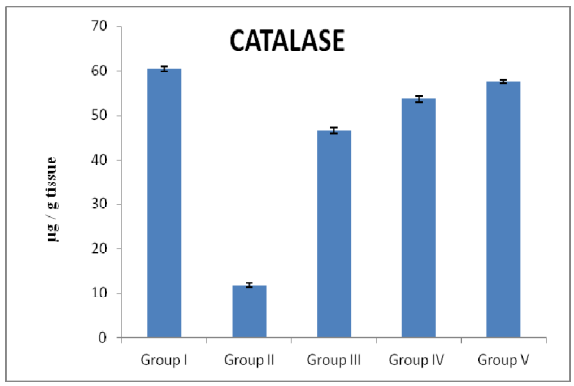

Figure 3: Effect of aqueous extract *Glycyrrhiza glabra* on catalase activity of experimental animals.

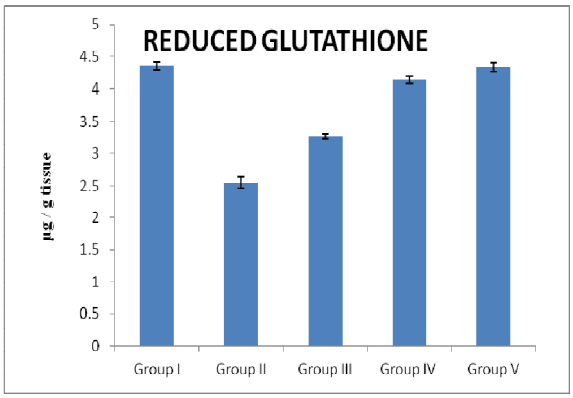

Figure 4: Effect of aqueous extract *Glycyrrhiza glabra* on reduced glutathione activity of experimental animals.

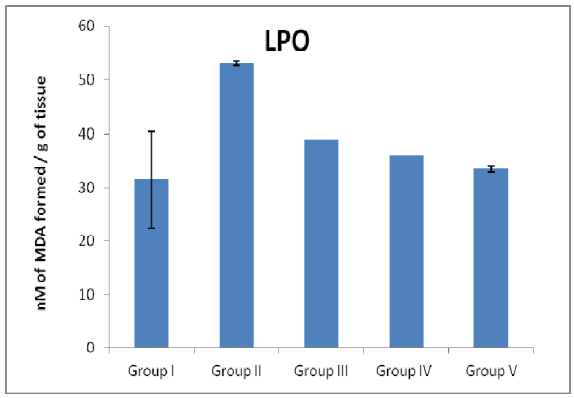

Figure 5: Effect of aqueous extract *Glycyrrhiza glabra* on LPO activity of experimental animals.

Table 1: Phytochemical analysis of various extracts of *Glycyrrhiza glabra* Linn.

S.No	Test	Observation	
		Plant powder	Extract
1	Terpenoids	-	-
2	Flavonids	+	+
3	Steroids	+	+
4	Glycosidase	-	-
5	Alkaloids	+	+
6	Quinine	+	+
7	Phenol	-	-
8	Tannins	-	-
9	Saponins	+	+
10	Coumarin	+	+
11	Lignin	-	-

\+ Presence - Absence

Table 2: Haematological assay, Ulcer score and Index of experimental rats

Groups/Parameters	RBC (10^6/mm^3)	WBC (Cells/mm^3)	Hb (g %)	Ulcer Score	Ulcer Index
I	6.98±0.09	4832.50±67.50	12.78±0.09	----	---
II	3.30±0.20	15850.00±17.80	8.45±0.06	0.9 ± 0.063	2.1 ± 0.047
III	4.43±0.06	8740.00±83.77	11.83±0.09	0.6 ± 0.042	0.5 ± 0.035
IV	5.80±0.04	5667.50±42.89	12.51±0.02	0.5 ± 0.035	0.4 ± 0.028
V	6.75±0.10	5550.00±8.16	12.61±0.04	0.4 ± 0.028	0.3 ± 0.021

Table 3: Effect of aqueous extract *Glycyrrhiza glabra* on biochemical parameters of experimental animals.

Groups/ Parameters	Free Acidity (mEq/l)	Total Acidity (mEq/l)	Serum Protein (g/dl)	Tissue protein (mg/g)	Carbohydrate (mg/dl)	ALP (U/L)
I	0.7 ± 0.021	0.6 ±0.042	7.3 ± 0.511	0.18 ±0.012	226 ± 15.4	41 ± 3.21
II	50 ± 3.5	58 ±4.06	4 ± 0.28	0.08 ±0.005	682 ±47.6	72 ±6.35
III	18 ± 2.6	20 ±14	6 ± 0.42	0.13 ±0.009	474 ±32.9	64 ± 5.23
IV	15 ±0.05	15 ± 1.05	6.5 ± 0.455	0.15 ±0.010	353 ±24.5	48 ±4.12
V	10 ±0.7	25 ± 1.75	7 ± 0.49	0.2 ±0.014	304 ±21	46 ± 3.86

Values are expressed as Mean ± SD for six rats

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