Antiulcerogenic and Toxicological Studies of *Glycyrrhiza glabra* Roots Available in Local Market of Karachi

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Abstract: The aim of the present study is to search antiulcer property and evaluate toxicity of indigenous plant *Glycyrrhiza glabra* roots available in local market of Karachi. The plant was evaluated, toxicologically and pharmacologically, for its antiulcer properties to develop cost effective and safe herbal drug with least side effects. In antiulcerogenic assay *G. glabra* was tested and compared with Cimetidine as positive control and physiological saline as negative control, using standard method. In this study, ethanol induced ulcers were developed in albino rats which were treated for 30 days. The ulcer indices were measured after 24 hours, 15 days and 30 days. The values calculated after 30 days of treatment. Comparison shows that *G. glabra* possesses a very significant antiulcerogenic activity i.e. 77.7% after 15 days and 90% after 30 days of therapy. The results suggest that *G. glabra* could be a good source of alternative medicine for ulcer therapy.

Key Words: *Glycyrrhiza glabra*, toxicity, gastric lesion, ulcer and ulcer index.

INTRODUCTION

Ulcer is a very common disease that affects millions of people in all parts of the world. Throughout the world, the mortality rate directly due to gastric ulcer varies widely, ranging from 3 per 100,000 deaths in Israel to 30 per 100,000 deaths in Japan [1]. In the United States about four million people have active peptic ulcers and about 350,000 new cases are diagnosed each year [2].

Medical treatment of this disease is intended to relieve pain, accelerate healing of the ulcer creator, and prevent complications and recurrences. For these Antacids, Cimetidine, Ranitidine, Famotidine, Anticholinergics, Sucralfate, Prostaglandins, Omeprazole, etc. are used. However, all these synthetic drugs result in a number of side effects, which sometimes become inbearable for the patient [3]. The *"Green wave"* triggered by a growing ecological awareness, has resulted in an increased interest in herbal formulations throughout the world, particularly in the last decade. The efficacy of a number of herbal formulations has been tested by valid phytopharmaceutical techniques and the number of plant-based drugs or health foods has increased steadily. The aim of the present study is to develop low cost antiulcer herbal drug from an indigenous plant *Glycyrrhiza glabra*. *G. glabra* belonging to family Febaceae is commonly known as licorice. The medicinal use of licorice in both western and eastern cultures dates back several thousand years. Its traditional use includes the treatment of peptic ulcers, asthma, pharyngitis and abdominal pain. Licorice is known to exhibit many pharmacological actions including anti-inflammatory, antioxidant, hepatoprotective, expectorant and antitussive activities [4]. Although much of the pharmacology focuses on glycyrrhizin and glycyrrhitinic acid, licorice have many other components, such as flavonoids, that may have significant pharmacological effect. The flavonoids have been reported to possess both anti-inflammatory and antiulcer effects [5].

The plant was evaluated toxicologically and pharmacologically for its antiulcer properties to develop cost effective and safe herbal drug with least side effects.

MATERIALS AND METHODS

Acute Oral Toxicity Test

Plant Material

Glycyrrhiza glabra roots identified by Prof. Dr. Mansoor Ahmad, the Chairman of Pharmacognosy Department, University of Karachi, were purchased from local market. A specimen (NF 035) has been deposited in the Herbarium of the department.

Extraction of Plant Material

5kg of plant material was extracted with ten folds of methanol at 40°C for six consecutive days by the classical method. The extract was lyophilized under 5µm-Hg pressure. All experiments were carried out by using an appropriate amount of lyophilized material.

Selection of Animals

Before proceeding for toxicity studies, animals (Swiss albino rats of both sexes), housed separately, were kept under strict observation for a period of 3 weeks with free access to food and water. Any animal showing sluggish movement or any sign of illness was rejected.

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Toxicity Studies

Glycyrrhiza glabra dissolved in physiological saline was administered in a single dose by means of gavages in graded doses (500mg, 1g and 2.5g) to different groups of animals. Each group comprised of 9 animals (4 males and 5 females). Observations with reference to physico-behavioral changes and mortality rate within 24 hours noted. Animals were further observed for a period of 72 hours for changes in excretion, perspiration, pupil size, behavioral pattern and delayed mortalities. Severity, depth and duration of all these signs and symptoms were found to be highly dose dependent and ameliorate quickly. In higher doses animals took hardly 5-25 minutes to get normalized.

Necroscopic findings were made on autopsied animals and recorded. Autopsy done revealed no gross pathological and physical changes. All vital organs, i. e., heart, liver lungs and kidneys were found to be normal. No mortality was observed during the claimed 72 hours observation period [6,7].

ANTI-ULCEROGENIC ASSAY

Animals

A total of 36 Swiss albino rats of both sexes (weighing between 150g to 210g) were used for the experiments. The animals were obtained from the Animal House of PCSIR Labs Complex, Karachi. All animals were healthy, active and alert kept under observation for three weeks prior starting the experiment under standard pathogen free conditions at 26±2°C temperature and 44-56% relative humidity. Animals were provided with standard rodent pellet diet and the food was withdrawn 48h and water 24 hours before the experiment.

Ethanol-Induced Gastric Damage

Rats divided into three groups of 12 animals each (6 males and 6 females) were fasted for 48 hours prior to the experiment. For inducing ulcerogenesis 90% ethanol, 1ml/rat was administered orally [8].

Group 1: Test group treated with methanol extract of *Glycyrrhiza glabra*.

Group 2: Negative control group treated with physiological saline.

Group 3: Positive control group treated with Cimetidine.

Measurement of Gastric Lesions

For obtaining the baseline data of ulcerogenesis, 4 animals (2 males and 2 females) from each group were sacrificed after 24 hours by the inhalation of ether. Their stomachs were taken out and fixed with 1% formalin for three minutes, opened along the greater curvature, gastric contents removed and pH of gastric contents measured. Stomachs were rinsed with physiological saline and the areas of hemorrhagic lesions measured by a dial caliper and the sum of measurements for each animal is referred to as Ulcer Index, i. e., UI [8]. A dose of 500mg/kg body weight was given to the test drug, i. e., *G. glabra* to group 1, while group 2 was treated with physiological saline and group 3 with Cimetidine (100mg/kg). The rats were kept on normal standard diet and water. After 15 days, 4 animals from each group were sacrificed and UI measured. Again after 30 days, 4 animals from each group were sacrificed and UI measured [9].

RESULT & DISCUSSION

Acute Oral Toxicity Test

This test is performed to confirm the safety of oral intake of the test drug. Both the drugs were safe enough in specified doses. In higher doses animals showed minor symptoms of toxicity but to get normalized. Autopsy done revealed no gross pathological and physical changes. All vital organs were found to be normal. Mortality was not observed at all during the claimed observation period of 72-hours.

Anti-Ulcerogenic Assay

Rats of all the three groups were sacrificed at regular intervals to find the ulcer healing effect in different groups. Results are given in following Tables **1-5**. The tables indicate that *G. glabra* showed significant antiulcer activity (77.70% inhibition) after 15 days of therapy and (90.17% inhibition) after 30 days. A comparison is made with the standard allopathic drug Cimetidine which showed 100% inhibition after 30 days of therapy.

Table 1. Baseline Average Data of Animals

S. No.	Group	Mass of Animal (g)	pH of Stomach	Mass of Stomach (g)	U. I.	Inhibition %
1	*G. glabra*	165.00±27.988	2.275±0.250	1.570±0.1489	6.295±1.2555	-
2	Saline	126.25±4.787	2.200±0.2708	1.520±0.0282	5.823±3.066	-
3	Cimetidine	160.00±18.708	2.225±0.2061	1.425±0.0881	4.860±0.740	-

Table 2. Average Data of Animals Sacrificed after 15 Days

S. No.	Group	Mass of Animal (g)	pH of Stomach	Mass of Stomach (g)	U. I.	Inhibition %
1	*G. glabra*	172.00±46.754	2.375±0.2629	1.6825±0.2430	1.215±0.1791	77.70%
2	Saline	143.75±11.086	2.325±0.15	1.8175±0.3140	7.865±1.022	-44.31%
3	Cimetidine	160.00±10.801	2.175±0.2061	1.4575±0.1307	0.61±0.4742	88.26%

Table 3. Average Data of Animals Sacrificed after 30 Days

S. No.	Group	Mass of Animal (g)	pH of Stomach	Mass of Stomach (g)	U. I.	Inhibition %
1	*G. glabra*	195.00±32.4027	2.215±0.2061	1.8500±0.1779	0.536±0.241	90.17%
2	Saline	121.25±15.478	2.125±0.05	1.5875±0.1125	7.945±2.0047	-45.78%
3	Cimetidine	1720.50±21.015	2.15±0.577	1.6575±0.217	0.00	100%

Table 4. Data Showing Comparison of U. I. between *G. glabra,* and Cimetidine

S. No.	Group	Baseline	15 Days	30 Days
1	*G. glabra*	5.570	1.630	1.215
2	Cimetidine	4.860	1.425	0.641

Table 5. Data Showing Comparison of % Inhibition of *G. glabra,* and Cimetidine after 30 Days of Therapy

S. No.	Group	15 Days	30 Days
1	*G. glabra*	77.70%	90.17%
2	Cimetidine	88.26%	100.00%

Statistical Analysis

Results are expressed as mean ± SD. ANOVA single factor, was used to estimate the level of significance for ulcer indices. Result was found significant at <.05 level (F=3.17; Mean ± SD=5.6095±0.818) for the period of 24 hours, at <.01 (F=47.85; Mean ± SD= 1.599±0.497) level for the period of 15 days, and at <.001 (F=81.16; Mean ± SD= 0.588±0.097) level for the period of 30 days (Devore, 1995: Walpole, 1998).

During pharmacological studies, firstly, the acute oral toxicity test was performed to evaluate the safe dose of test drug. The result indicates that the extract of *G. glabra* is safe up to a dose of 2.5g.Autopsy was done and all vital organs i.e. heart, liver, lungs and kidneys were observed. They were found to be normal showing no symptom of toxicity. No mortality was observed during the claimed 72-hour observation period.

Secondly, ulcer was developed in experimental animals and then treated with the test and standard drugs to find out ulcer healing effects. Table **1** shows the ulcer indices just 24 hours after the development of ulcer, which is treated as baseline data, which were found to be 5.570±1.1479 for

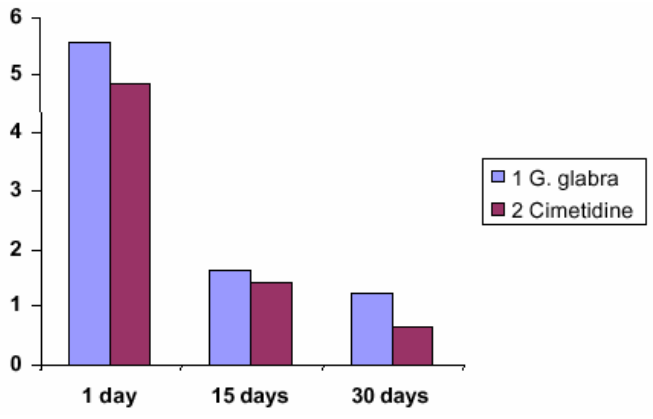

Fig. (1). Comparison of U.I. between *G. glabra and* Cimetidine.

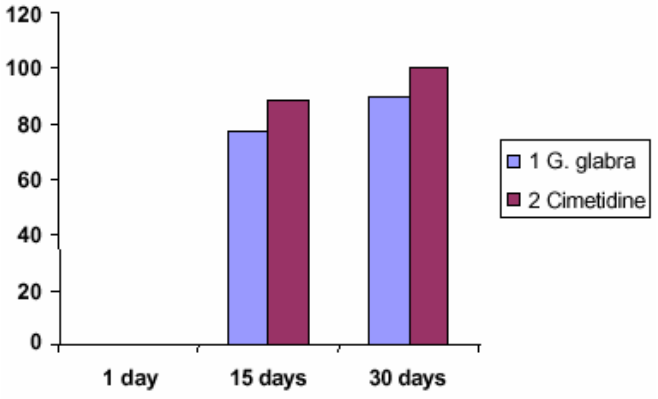

Fig. (2). Comparison of % inhibition between *G. glabra* and Cimetidine.

G. glabra, 5.823±0.3066 for physiological saline and 4.860± 0.740 for Cimetidine. The animals were then treated with respected drugs and the values obtained after 15 days of treatment were 1.215±0.1791 for *G. glabra*, 7.865±1.022 for physiological saline and 0.641±0.4742 for Cimetidine (Table **2**). For these values %inhibition were 77.7% for *G. glabra*, -44.31% for physiological saline and 88.26% for Cimetidine (Table **5**). Final values were calculated after 30 days of treatment which comes out to be 0.536±0.241 i.e. 90.17% inhibition for *G. glabra*, 7.945±2.0047 i.e. -45.78% inhibition for physiological saline and 0.00 i.e. 100% inhibition for Cimetidine (Table **3-5**). Comparison shows that *G. glabra* possesses a very significant antiulcerogenic activity i.e. 77.7% after 15 days and 90% after 30 days of therapy. The results suggest that *G. glabra* could be a good source of alternative medicine for ulcer therapy.

CONCLUSION

In this study the antiulcerogenic properties of *G. glabra* or licorice available in local market of Karachi were thoroughly searched and compared with standard antiulcer drug Cimetidine. Comparison shows that *G. glabra* possesses a very significant antiulcerogenic activity i.e. 77.7% after 15 days and 90% after 30 days of therapy. The results suggest that *G. glabra* available in local market meets the standard and could be a good source of alternative medicine for ulcer therapy.

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