Original Article

Comparative Evaluation of Gastro-protective Effect of Aqueous & Ethanolic Extract of Glycyrrhiza Glabra or Licuorice (Jastimodhu) Root in Ibuprofen Induced Gastric Ulcer in Experimental Rats

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Abstract

Introduction: *Peptic ulcer develops when aggressive factors overcome the protective mechanisms. Although numbers of anti-ulcer drugs are available, in traditional system of medicine a number of herbal preparations have been used for the treatment of peptic ulcer.*

Objective: *This study carried ant to investigate the comparative gastro-protective effect of aqueous & ethanolic extract of Glycyrrhiza Glabra.*

Material & Method: *The study was conducted in the department of Pharmacology of Dhaka Medical College from July 2014 to June 2015. The anti-ulcer activity of Glycyrrhiza Glabra or licuorice root was investigated by Ibuprofen induced ulcer in rats. The rats were provided with aqueous & ethanolic extract of Glycyrrhiza Glabra or licuorice root (500mg /kg body wt) orally by gastric tube for 5 days. Then Ibuprofen (200 mg/kg body wt) was given to all groups after 24 hour fasting on the 7th day orally by gastric tube. After 4 hour of administration of ibuprofen, all rats were sacrificed. Stomach will be dissected out and collected for morphological and histopathological examination.*

Result: *Rats pretreated with ethanolic extract of Glycyrrhizin Glabra showed significant decrease in stomach damage than rats pretreated with aqueous extract both macroscopically and microscopically as compared to control. This study indicates both extract of Glycyrrhiza Glabra have potential gastro-protective activity.*

Conclusion: *The present study is to compare the gastro-protective effect of aqueous and ethanolic extract of Glycyrrhiza Glabra or Licuorice or Jastimodhu root.*

Keywords: *Gastro-protective, Glycyrrhiza Glabra (Jastimodhu).*

Received: *02.11.2020*

Accepted: *10.08.2021*

Introduction

Peptic ulcer is defined as disruption of the mucosal integrity of stomach and/or duodenum leading to a local defect or excavation due to active inflammation. Upper gastrointestinal integrity is dependent upon the delicate balance between naturally occurring protective factor as mucus or prostaglandins and damaging factor as hydrochloric acid present in the digestive juices. An imbalance causes peptic ulcer formation and destruction of gastrointestinal tract mucosal lining.The ulcer irritates surrounding nerves and causes considerable amount of pain[1, 2] .

There are three important cause of peptic ulcer disease: NSAIDS, Helicobacter pylori infection and acid hyper-secretory states such as Zollinger Ellison Syndrome. Today there are two main approaches for treating peptic ulcer.The first deals with reducing the production of gastric acid and the second with reinforcing gastric mucosal protection. Although a number of antiulcer drugs such as H_2 receptor antagonist, proton pump inhibitors and cytoprotectants are available, these entire drug have side effect and limitation[3, 4, 5].

Liquorice derived from the sweet root of various species of *Glycyrrhiza*, a genus which contains about fourteen species, natives of warmer temperate countries in both the New and Old Worlds, ten of them having roots more or less sweet, but most of them not sufficiently so to be of use. Liquorice is one of the most commonly used herbs in Western herbal medicine. Liquorice has been used in medicine for more than 4000 years. The earliest record of its use in medicine is found in 'Code Humnubari' (2100 BC). It was also one of the important plants mentioned in Assyrian herbal (2000BC). Hippocrates (400 BC) mentioned its use as a remedy of ulcers and quenching of thirst.The drug was also mentioned by Theophrastus and Dioscorides. In traditional Siddha system of medicine, liquorice is used as a demulcent, expectorant, anti-tussive, laxative and sweetener [6, 7, 8, 9].

The present study was designed to investigate the comparative gastro-protective effect of aqueous & ethanolic extract of *Glycyrrhiza glabra.*

Materials and methods

The experimental study was carried out in the Department of Pharmacology of Dhaka Medical College from July 2014 to June 2015.

Plant material: The plant *Glycyrrhiza Glabra* or Licuorice root were purchased from BCSIR laboratory & authenticated by National Herbarium, Mirpur, Dhaka.

Preparation of aqueous & ethanolicextract: Preparation of both extracts was performed in the department of Chemistry of Dhaka University, Dhaka.

Procedure: Aqueous extract of Liquorice was prepared using 80 gram of powdered plant in conical flask with 300 ml of distilled water and Ethanolic extract soaked in 95% ethanol.

Animals: A total number of 42 healthy Wister albino rats of both sex weighting 150-200 grams were selected for study and collected from ICDDR'B Dhaka.

Ibuprofen: Ibuprofen was obtained from local market as they are available as drugs.The dose of Ibuprofen was calculated as 200 mg/kg body weight then calculated amounts was dissolved in distilled water and was administered orally at a volume of 1ml/40mg body wt.[10]

Animal Experimentation: The experiment was divided into two parts: Experiment-I and Experiment-II.

Experimental design-I

This part of the experiment was carried out to demonstrate the effect of ibuprofen on gastric tissue on normal rat. It was comprised of 24 rats which were divided into 4 groups each having 6 rats. These are labeled as Group-A, Group-B and Group- C.

Group A: This group served as control group was received normal diet and 1ml of distilled water (5ml/kg body wt) for 7 day orally by gastric tube.

Group B: Gastric damage experimental group was received 1 ml distilled water (5ml/kg body wt) for 5 day and 200 mg/kg of Ibuprofen on 7th day after 24 hours fasting.

Group C: This group was provided with 1 ml of distilled water (5ml/kg body wt) and aqueous extract of *Glycyrrhiza Glabra* (500mg/kg body wt) orally by gastric tube for 7 day.

Group D: This group was provided with 1 ml of distilled water (5ml/kg body wt.) and ethanolic extract of *Glycyrrhiza Glabra* (500mg/kg body wt.) orally by gastric tube for 7 day.

Experimental design-II

This was comprised of 18 rats. They were divided into 3 groups each containing 6 rats labeled as Group-E, Group-F, and Group-G.This part of experiment was carried out to demonstrate the effect of pretreatment of aqueous extract & ethanolic extract on ibuprofen induced gastric ulcer in experimental rats.

Group E: Gastric damage control group was received normal diet, distilled water for 5 day.

Group F: They were served with aqueous extract of *Glycyrrhiza Glabra* (500mg/kg body wt) orally by gastric tube for 5 day.

Group G: They were served with ethanolic extract of *Glycyrrhiza Glabra* (500mg/kg body wt) orally by gastric tube for 5 day.

Then Ibuprofen (200 mg/kg body wt) was given to all groups after 24 hour fasting on the 7th day orally by gastric tube.

After 4 hour all rat were sacrificed, stomach were dissected out and collected for morphological and histopathological examination.

Collection of the stomachs

Abdomen was opened by "T" incision which is a vertical incision from xiphoid process to the upper part of symphysis pubis and a transverse incision extending 2 cm laterally on each side from top to vertical line.The stomach was separated from the rest of the small intestine.The stomach was opened along the greater curvature and gently rinsed under running tap water and were spread on paraffin plate. Lesion in the glandular part of the stomach was observed with the help of dissecting microscope grossly (10x) with a square grid eye piece to access the formation of ulcer.

Parameter studied

Morphological parameter:

1. Number of lesion per rat in each group.
2. Individual lesion length and breadth in mm for each group.
3. Individual lesion area in square mm for each group.
4. Mean ulcer index (sum of length of all lesions in each stomach) in mm for each group.
5. Percentage inhibition of licuorice extract.

Histological parameter:

Degrees of damage were determined depending on the extent of involvement of lesion in stomach. Gross & microscopic examination and measurement of the morphological lesion: The mucosa was washed in running Tap water. Then the whole mucosa of the stomach of each rat were examined very carefully with the help of dissecting microscope (x20) with the aid of square grid eye piece (1 mm square). The ulcer were counted with the help of hand lens (5 times magnification power) and visible big lesions were measured with the help of hand lens and mm scale. Oculo-micrometer was used for examination of mucosal surface and the measurement of small lesion length, breadth & area. The graduation of oculo-micrometer was standardized by comparing those with the marking of the stage micrometer following way-

When one magnification was used:

10 graduation of oculo-micrometer equal to1mm of stage micrometer

Or 1 graduation of oculo-micrometer equal to 0.1 mm of stage micrometer

Similarly with two (x2), (x4), (x10) magnification,1 graduation of oculo-micrometer was equal to 0.05,0.025 respectively. The maximum length, breadth and area of each lesion were measured and the mean lesion area and mean ulcer index per rat in each group was calculated and used in lesion index for each group.

The ulcer index or lesion index (UI) were determined as the sum of length of all gastric lesions in mm for each stomach and the inhibition percentage were expressed by the following formula

Inhibition percentage (%I) = $[(UI_{control} - UI_{treated}) \div UI_{control}] \times 100$.

Data analysis and Result

All relevant information of each rat was recorded in a predesigned data collection sheet. Collected data was tabulated and statistical analysis was done by appropriate significance test (Unpaired student's't' test).

Result and Observation:

Table I: Showing the effect of Ibuprofen on mean number of lesions, mean lesion length, mean lesions breadth, mean lesions area and lesions index in each group in experiment-I

Groups	Mean number of lesions	Mean lesions length	Mean lesions breadth	Mean lesions area	Lesion index
Group A N=6	0	0	0	0	0
Group B N=6	5.33 ± 0.81**	5.56 ± 0.61**	1.99±0.23**	11.07± 1.55**	26.32 ± 3.89
Group C N=6	0	0	0	0	0
Group D N=6	0	0	0	0	0

Table-II: Comparison of Pretreatment of aqueous extract of Glycyrrhizaglabra (Group F) and ethanolic extract of glycyrrhizaglabra (Group G) on mean number of lesions, mean lesions length, mean lesions breadth, mean lesion area and lesions index on each group in experiment-II

	Group F	Group G	P value
Mean number of lesions (± SD)	3.0± 1.26	3.16± 0.75	>0.05
Mean lesion length(± SD)	2.75 ±0.77	2.38 ± 0.93	>0.05
Mean lesion breadth(± SD)	0.48 ±0.33	0.65 ± 0.18	>0.05
Mean lesion area (± SD)	2.32 ± 0.99	1.51 ± 0.74	>0.05
Mean lesion index (± SD)	5.74 ±2.16	6.61 ± 1.42	>0.05

Table III: Showing the effect of pretreatment of aqueous &ethanolic extract of G Glabraon % Inhibition in Ibuprofen treated rats in each group in experiment II

Groups	Number of rats	% Inhibition
Group E	6	
Group F	6	75.68
Group G	6	83.16

Discussion

The peptic ulcer results from an imbalance between aggressive factors and the maintenance of mucosal integrity through the endogenous defense mechanism.

In present study, Ibuprofen was used as agent to induce stomach ulcer in rats. The Ibuprofen is what is called a non selective cyclooxygenase inhibitor which means it inhibits all types of cyclooxygenase, not just the ones that produce inflammatory mediators especially PGI_2 and PGE_2. Ibuprofen inhibit prostaglandin involved in the blood supply to the stomach as well as blood supply to the kidney. Study Showed that oral administration of Ibuprofen (200 mg/kg) to fasted rats produced gastric mucosal damage and pretreated with *Momordica Dioica extract* could effectively and dose dependently prevent this gastricdamage.[10] Dephour*et al* showed oral administration of Ibuprofen to fasted rats produced gastric damage and pretreated with oral administration of *Glycyrrhiza Glabra* root extract could effectively and dose-dependently prevent such damage[11].

Abid *et al* in another study showed that oral administration of Ibuprofen produce lesion of gastric mucosa and pretreated with *Glycyrrhiza Glabra* extract before the administration of Ibuprofen could effectively and dose dependent prevent the formation of such lesion[12].

Glycyrrhiza Glabra extract stimulates gastric mucus production enhances the rate of incorporation of various sugars into gastric mucosal glycoproteins promotes mucosal cell proliferation inhibits mucosal cell exfoliation, inhibits prostaglandin degradation increases the release of PGE2 and reduces the formation of thromboxane B2 and regulates DNA and protein synthesis rates in gastric mucosa. More recently nitric oxide has been claimed to contribute to the anti-ulcer effect of carbenoxolone[13, 14, 15].

Reports suggest that reactive oxygen free radical species (ROS) play an important role in the pathophysiological processes of gastric ulcer. Recently, much attention has been focused on the role of ROS, including O2-, OH- and H_2O_2 in mediating tissue damage. Preventive endogenous antioxidants, such as SOD and catalase enzymes are the first line of defense against ROS. Reduced glutathione is a major scavenger of free radicals in the cytoplasm and an important inhibitor of free radical mediated lipid peroxidation. The presence of some antioxidant phytoconstituents might have protected the gastric mucosa from free radical induced damage. Some of which include; gallic acid. This supports the fact that antioxidants reduce oxidative damage in tissues. The root of Glycyrrhiza species is one of the richest sources of biological active compounds such as phenolic and flavanoid compounds. The experiment also showed the highest percent inhibition occurred in Group-G (Pretreated with ethanolic extract of Glycyrrhiza Glabra) followed by group F (Pretreated with aqueous extract of Glycyrrhiza Glabra. Licorice flavonoids were found to have exceptionally strong antioxidant effects that were over 100 times stronger than that of vitamin E. A dose of 2.58 mg/ml licorice flavonoids was found to scavenge more free radicals than 258 mg/ml of vitamin E (20.6% vs. 11.2%). The flavonoids (which are a part of the phenolic compounds) were found to be 2.3 and 6.8 mg/100 mg dry extract in aqueous and ethanolic extract

of licorice roots respectively. The ethanolic extract shows high anti-oxidative activity as compared with aqueous extract. The aqueous and ethanolic extracts of licorice roots show high reducing power ability comparing with their abilities as chelating agents. Study suggests that licorice extract can be used as a potential source of natural antioxidant[16, 17, 18].

H. pylorie affects the gastric and duodenal mucous layer because this organism produces proteases that degrade the protective mucous layer. Moreover, H. pylori infection decreases the production of epidermal growth factor, which normally promotes healing of gastric and duodenal mucosa.Extracts of licorice have been shown to inhibit the adhesion of *H. pylori* to gastric mucosa, as well as the growth of antibiotic resistant strains, suggesting multiple mechanisms of action for its anti-ulcer[19].

Conclusion

In Conclusion this study established that *Glycyrrhiza Glabra* or Licuorice (Jastimodhu) has gastro-protective ability following consumption of Ibuprofen. For human consumption, further pharmacological test needs to be conducted to determine appropriate dose for human and to uncover any adverse effects which may arise from *Glycyrrhiza Glabra* (Jastimodhu).

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