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Evaluation of Antiulcer Activity of DGL (Deglycyrrhizinated liquorice)

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Abstract

Peptic ulcer is a disorder of the upper gastrointestinal tract that is caused by gastric acid. There are various chemical agents available for the treatment of peptic ulcers, but claim serious side effects. Herbal drugs are preferred over chemical agents due to their relatively less toxicity, better acceptability, better compatibility with human body, lesser adverse effects, economical, effective and easy availability. The present research has been carried out to investigate antiulcer activity of ethanolic extract of deglycyrrhizinated liquorice (DGL) (250 mg/kg) in ethanol induced, aspirin induced and stress induced gastric ulcers in male wistar rats. Ulcer score, ulcer index and number of ulcers were determined for ethanol induced, aspirin induced and stress induced gastric ulcers. Antiulcer effect of DGL was compared with standard drug omeprazole (20mg/kg). These observations helped us to conclude that ethanolic extract of DGL had significant antiulcer properties.

Keywords-Peptic ulcer, DGL, ethanolic extract, omeprazole, NSAIDs

INTRODUCTION

Some medications like non-steroidal anti-inflammatory drugs (NSAIDS) causes mucosa of stomach or duodenum to break off. This results in ulcers. Various reports indicate that old age group patients are more prone to gastric ulcer. The pathogenesis of peptic ulcer disease includes an imbalance between gastric offensive factors like acid, pepsin secretion, Helicobacter pylori (H.pylori), bile salts, ethanol, some medications like NSAIDS, lipid peroxidation, nitric oxide (NO) and defensive mucosal factors like prostaglandins (PG's), gastric mucus, cellular renovation, blood flow, mucosal cell shedding, glycoproteins, mucin secretion, proliferation and antioxidant enzymes like catalase (CAT), superoxide dismutase (SOD) and glutathione levels[1].

Ulcer disease has become a disease predominantly affecting the older population, with the peak incidence occurring between 55 and 65 years of age. Thirty-five percent of patients diagnosed with gastric ulcers will suffer serious complications. Although mortality rates from peptic ulcer disease are low, the high prevalence and the resulting pain, suffering, and expense are very costly[1].

There are enormous chemical agents available for the treatment of peptic ulcers, but proclaim serious side effects. Herbal drugs have preserved their importance due to relatively less toxicity, better cultural acceptability, better compatibility with human body, lesser adverse effects, economical, effective and easy availability. *Cynodon dactylon, Ocimum sanctum, Glycyrrhiza glabra, Ficus religiosa* are some of the plants which show antiulcer activity[3]. A variety of nutritional strategies and dietary supplements have a positive impact on reducing the symptoms and retarding the development of gastric ulcers. Flavonoids and antioxidants such as anthocyanidins and resveratrol (found in blueberries, cherries, red grapes, and tomatoes) inhibit the growth of *H. pylori*, which can have dramatic implications for gastric ulcer causality[1].

Herbs have also proven helpful with treating gastric ulcers and can be taken fresh or as dried extracts or tinctures. Cranberry (*Vaccinium macrocarpon*), curcumin, enteric coated peppermint (*Mentha piperita*), black pepper (*Piper nigrum*), liquorice (*Glycyrrhiza glabra*), green tea (*Camellia sinensis*) and mastic (*Pistacia lentiscus*) all help to inhibit *H. pylori* growth and protect the stomach against damage from NSAIDs[1,2].

MATERIALS AND METHODS

The drug was procured from Amsar Goa Private Limited. Color, odor, taste, shape, size and texture of the plant material were estimated by visual and sensory evaluation.

Preparation of Deglycyrrhizinated liquorice (DGL) extract

About 50 g of dried powdered roots were moistened with 150 ml of 10% ammonia and then percolated three times with 80% ethanol (3 x 300 ml), each for 24 hrs., at room temperature till exhaustion. The total ethanolic extract was concentrated to a dry mass.

Phytochemical screening of extract

Ethanolic extract of DGL was subjected to qualitative tests like test for carbohydrates, test for starch, test for gums and mucilage, test for protein and amino acid, test for fixed oils and fat, test for flavonoids, test for tannins and phenolic compounds, test for alkaloids, test for glycosides and test for phytosterols for identification of various phytochemical constituents.

Chemicals

All the analytical grade chemicals were purchased from spectrochem. Deglycyrrhizinated liquorice was purchased from Amsar Goa Pvt. Ltd.

Experimental animals

Animals were procured from Bharat Serum and Vaccines Pvt. Ltd. Healthy Wistar male rats aged 2 – 3 months, weighing 180 – 250 gm were selected for the study. The protocol for the evaluation of antidiabetic activity and toxicity study was approved by Institutional Animal Ethical Committee. The protocol number is

CUSCP/IAEC/24/2014. The animals were housed in polypropylene cages, maintained under standard conditions (12h / 12h light and dark) at 25 ± 3° C and 35 – 60% humidity. They were fed with standard rat pellet and water *ad libitum.*

Acute toxicity study

OECD guidelines 423 were followed to carry out acute toxicity study at dose level of 2000mg/kg. This study was carried out by administering the test solutions orally to rats, at the dose level of 2000 mg/kg for 14 days, to check whether the test solution has any toxic effects. Signs and symptoms of toxicity were observed for next 48 hrs. No toxicity or death was observed in the experimental rats when they were subjected to toxicity study.

Induction of ulcers

Ethanol induced gastric ulcers

Rats were fasted for 18 hours and then were administered orally with the drug, standard and normal saline solution. One hour later rats were administered 90% ethanol (5mg/kg) orally. The rats were anesthetized using pentobarbitone and then euthanized by CO_2, 1 hour after ethanol treatment. The stomach of each rat was excised and opened along the greater curvature. The gastric content of each rat was collected in a graduated tube. The stomach was rinsed with saline solution. Ulcer area on the surface of each stomach was examined.

Aspirin induced gastric ulcers

Rats were fasted for 18 hours and then were administered orally with test drug, standard and normal saline solution. One hour after the treatment, all the rats received aspirin (300mg/kg) to induce gastric ulcers. After 5 hours, rats were anesthetized using pentobarbitone and then euthanized by CO_2. The stomach of each rat was excised and open along the greater curvature. Ulcer area on the surface of the stomach was examined.

Stress induced ulcers

Stress ulcers were induced by forced swimming in glass cylinder containing water to the height of 5 cm and maintained at 25°C for 3 hours. Rats were fasted for 18 hours prior to induction of ulcers. After the treatment with test drug, standard and normal saline solution, rats were allowed to swim in water for 3 hours. Rats were anesthetized using pentobarbitone and then euthanized by CO_2. The stomach of each rat was excised and open along the greater curvature. The gastric content of each stomach was collected in a graduated tube. Ulcer area on the surface of the stomach was examined.

Experimental design

The animals were divided into six groups of 6 animals each (table no.1).

Statistical data analysis

Statistical analysis was carried out using instat software. All the results were expressed as mean ± SEM. Post hoc Dunnett's test was used to determine statistical significance of the results obtained.

Histopathological studies

At the end of the study, animals were sacrificed and stomach was isolated for histopathological studies.

RESULTS AND DISCUSSION

Starch, gums and mucilage, proteins and amino acid, flavonoids, tannins and phenolic compounds and glycosides were found to present in the extract.

Table no. 1. Groups of animals for antiulcer study

	No. of Animals	Name of drug
Group I(normal control)	06	Nil
Group II (negative control)		
Group II-A	06	Ethanol
Group II-B	06	Aspirin
Group II-C	06	Stress induced ulcers
Group IV	06	Omeprazole

Table no. 2. Ulcer score and ulcer index

Groups	Ulcer score (mean ± SEM)	No. of ulcers (mean ± SEM)	Ulcer index (mean ± SEM)
Normal control	-	-	-
Negative control	5.55 ± 0.24	389.1 ± 13.7	5.00 ± 0.1
Standard	0.34 ± 0.2**	2.66 ± 0.4**	0.03 ± 0.005**
Ethanol induced ulcers + DGL extract	4.56 ± 0.23**	20.67 ± 3.5**	0.45 ± 0.03**
Aspirin induced ulcers + DGL extract	4.12 ± 0.3**	25.45 ± 3.9**	0.67 ± 0.02**
Stress induced ulcers + DGL extract	3.67 ± 0.21**	16.45 ± 3.4**	0.23 ± 0.002**

a) N = 6, values expressed as mean ± SEM, **p<0.01, Dunnett's test compared to negative control

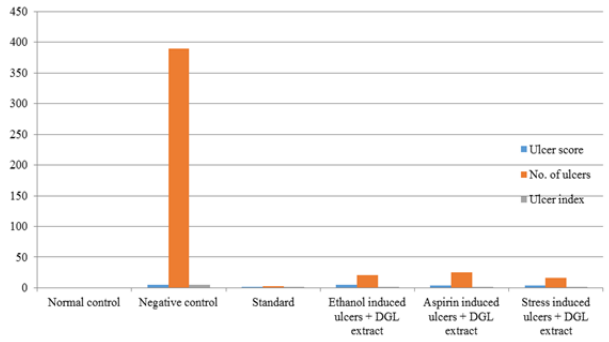


Fig. 1: Effect of DGL extract on ulcer score, number of ulcers and ulcer index

Ulcer score, ulcer index and number of ulcers

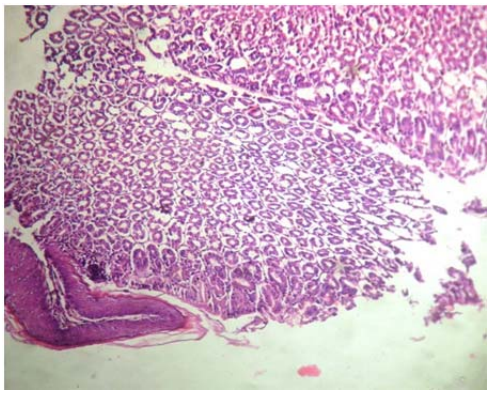
Oral administration of ethanol and aspirin produced deep gastric ulceration. Ulceration was significantly reduced in DGL extract treatment group animals. Ulcer score, ulcer index and number of ulcers was found to be statistically significant when compared to negative control. When treated with DGL extract, the ulcer score was found to be 4.56 ± 0.23 for ethanol induced gastric ulcers. 4.12 ± 0.3 for aspirin induces gastric ulcers and 3.67 ± 0.21 for stress induced gastric ulcers. Negative control group had ulcer index of 5.00 ± 0.1, which was significantly reduced to 0.45 ± 0.03, 0.67 ± 0.02 and 0.23 ± 0.002 for ethanol induced, aspirin induced and stress induced ulcers respectively. Number of ulcers were significantly reduced to 20.67 ± 3.5, 25.45 ± 3.9 and 16.45 ± 3.4 for ethanol induced, aspirin induced and stress induced ulcers respectively (table no.2 and fig.1)

Histopathological studies

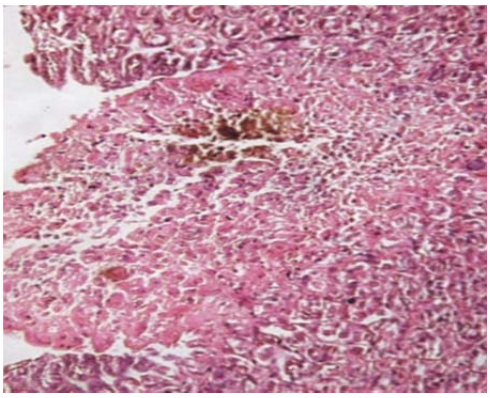
After the treatment with drug, aspirin induced ulcers in rats (fig.2.4) and ethanol induced ulcers in rats (fig.2.6) treated with DGL extract showed mildly multifocal ulcers as compared to the negative control group (fig.2.3). Whereas, stress induced ulcer group animals treated with DGL extracts showed minimally multifocal ulcers as compared to aspirin and ethanol induced ulcer groups treated with DGL extract (fig.2.5). Severity of ulcers is mild in rats in case of aspirin induced and ethanol induced ulcers group as compared to that of the negative control group, where the severity of ulcer is mild to moderate in stress induced ulcers (table no.3). Grade of inflammation is more for negative control group as compared to the standard treatment group and DGL extract treated groups (table no.3). Hence, from the above study, it can be concluded that DGL extracts can lower the frequency of ulcers, reduce the severity and inflammation of ulcers.

Table no. 3. Effect of DGL extract on ulcer induced rat model

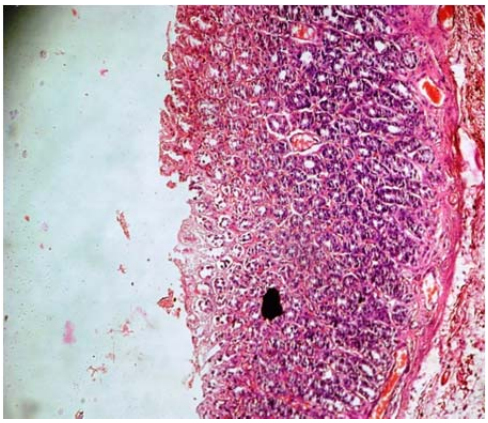
Sample particulars	Frequency of ulcers	Severity of ulcers	Grade of inflammation
Normal control	Nil	---	0
Negative control	Moderate multifocal	Mild to moderate	2 - 3
Standard	Minimally multifocal	---	0
Aspirin Induced + Drug	Mildly multifocal	Mild	1 - 2
Stress Induced + Drug	Minimally multifocal	---	0
Ethanol Induced + Drug	Mildly multifocal	Mild	1 - 2



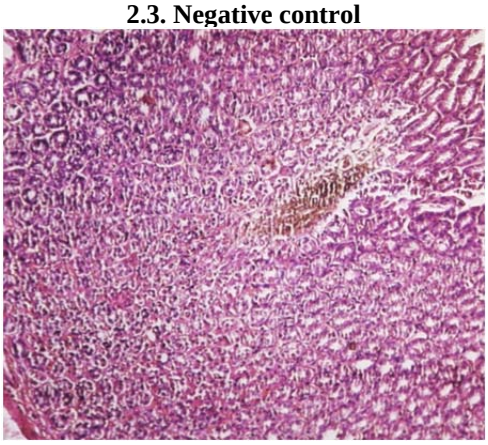
2.1. Normal control



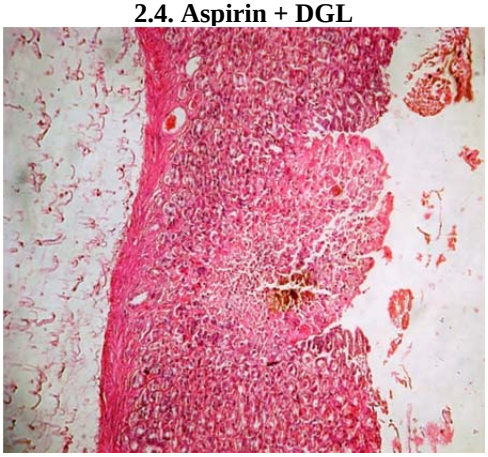
2.2. Standard drug (Omeprazole)



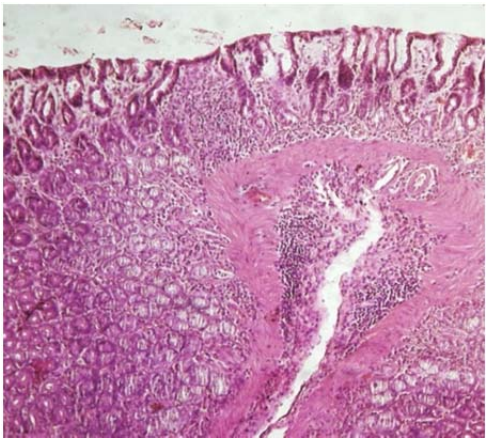
2.3. Negative control



2.4. Aspirin + DGL



2.5. Stress induced ulcers + DGL



2.6. Ethanol + DGL

Fig. 2: Histopathological examination of stomach of rats

CONCLUSION

The phytochemical investigation of ethanolic extract of DGL revealed the presence of starch, gums and mucilage, proteins and amino acid, flavonoids, tannins and glycosides. Antiulcer activity of DGL was evaluated using three models i.e. ethanol induced ulcer model, aspirin induced ulcer model and stress induced ulcer model. There was a statistically significant reduction in ulcer score, ulcer index and number of ulcers indicating promising antiulcer activity.

From the above study, it can be concluded that DGL extract was found to lower the frequency of ulcers, reduce the severity and inflammation of ulcers in all the three ulcer models.

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