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ANTIULCER ACTIVITY OF POLYHERBAL FORMULATION

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ABSTRACT

The lesion of peptic ulcer disease is a disruption in the mucosal layer of the stomach or duodenum. Recent researchers have discovered a number of newer risk factors regulating the development of disease which are not addressed properly by the current clinical therapies. In this study polyherbal formulation was prepared consisting of *Glycyrrhiza glabra*, *Garcinia cambogia*, deglycyrrhizinated licorice extract and *Azadirachta indica*. Suitable extracts depending on effective chemical composition was prepared and studied on four antiulcer models, namely Naproxen induced ulcers, Histamine induced ulcers, Cysteamine induced ulcers and Ethanol induced ulcer models. This polyherbal formulation significantly reduces ulcer index and ulcer area and shows protection index around 80%, the formulation also shows good antioxidant activity. All results show that polyherbal formulation show gastric healing property by multiple mechanisms. These entire drugs target specific etiological and pathological step which take part in development of ulcers.

KEYWORDS: **Peptic Ulcer, offensive factors, *Glycyrrhiza glabra*, *Azadirachta indica* , *Garcinia cambogia*,deglycyrrhizinated licorice extract .**

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INTRODUCTION

The lesion of peptic ulcer disease (PUD) is a disruption in the mucosal layer of the stomach or duodenum[1]. Peptic ulcer is becoming a dreadful disease with an increase in the number of hospital admissions and diseases of civilization. Thus urbanization with changing life-styles like cigarettes and spicy foods and emotional factors like anxiety, stress and strain are instrumental in aggravating peptic ulcers[2]. Current treatment regimens concentrate on eradication of *H.pylori* bacteria, less use of NSAID'S, use of antibiotics, H_2 blockers, proton pump inhibitors and changes in lifestyle and dietary habits[3]. However many serious side effects of the above therapies are seen such as hip bone fracture and resistance[4]. The objective of this study was to evaluate herbs for their potential antiulcer activities and also to see whether these drugs show synergism. Herbal medicines have been in clinical use for centuries in India. Being a time-tested system, it has an edge over other existing systems of health management, especially for dealing with peptic ulcers which involves complex events. *Azadirachta indica* has many chemical constituents that acts against development of ulcers[5], such as decrease in acid and pepsin secretion[6], proton pump inhibition and antioxidant effects[7], Deglycyrrhizinated licorice extract shows antiulcer, antioxidant activity[8], and healing of ulcers[9], Garcinia cambogia extract shows antioxidant and increase in mucosal defensive factors[10,11,12], while licorice extract shows antioxidant and *H.pylori* inhibitory activity[13,14,15], All the above plants were selected for present study as they show best antiulcer potential.

MATERIALS AND METHODS

(i) Composition of herbal formulation

Each gram of herbal formulation (prepared in laboratory) contains aqueous extracts of *Azadirachta indica* L. (Meliaceae ; leaves 150mg), *Glycyrrhiza glabra* L (Fabaceae; rhizomes 150 mg), *Garcinia cambogia*- 500 mg (Clusiaceae; fruit rind), Deglycyyrhizinated Licorice extract (DGL)-200 mg. Formulation was subjected to acute toxicity studies as per OECD guidelines. Animals were divided into four groups consisting of control, standard and two doses of test (300mg/kg, 600mg/kg). Wistar rats weighing 200-250 g were selected for study the protocol for experimentation was approved by the Institutional Animal Ethics Committee Constituted under Committee for the Purpose of Control and Supervision of Experiment on Animals (CPCSEA), India Approval number CPCSEA/07/10. Models used for the study was Naproxen induced ulcer model, Ethanol Induced Ulcer model ,Cysteamine induced duodenal ulcers and Histamine induced ulcer model. Lesions in the stomach were studied according to the following scale: 0 = normal gray colored stomach, 0.5 = pink to red coloration of stomach, 1 = spot ulcer, 1.5 = hemorrhagic streaks, 2 = number of ulcers <5, 3 = number of ulcers >5, 4 = ulcers with bleeding. Ulcer index was calculated by adding the total number of ulcers plus the severity of ulcer.[16]

(ii) Naproxen induced ulcer model

Male wistar rats of 200-230 gm were selected and weighed and marked for identification. All animals were fasted for 24 hrs. Test drug was given in dose of 330mg/kg and 660mg/kg groups respectively. Distilled water (1 mL/ animal) and Omeprazole (30 mg/kg p.o.) was administered to control and standard groups respectively. Naproxen 40mg/kg was administered p.o. after 1 hr of pre-treatment. Animals were sacrificed after 6 hrs of Naproxen treatment and opened along greater curvature to expose inner surface. Inner surface was washed thoroughly with normal saline and scanned for analysis[17]

(iii) Histamine Induced Ulcer model

Male wistar rats of 200-230 gm were selected and weighed and marked for identification. All animals were fasted for 24 hrs. The

Prophylactic dose of 330mg/kg and 660mg/kg was given to test groups. Distilled water (1 mL/ animal) and Ranitidine (100 mg/kg p.o.) was administered to control and standard groups respectively. Histamine 300 mg/kg was administered p.o. after 1 hr of pre-treatment. Animals were sacrificed after 6 hrs of Histamine treatment. Stomach was isolated and opened along greater curvature to expose inner surface. Inner surface was washed thoroughly with normal saline and scanned for analysis[18]

(iv) Ethanol Induced Ulcer model

Male wistar rats of 200-230 g were selected and weighed and marked for identification. All animals were fasted for 24 hrs. Test group received 330 mg/kg and 660 mg/kg of the test drug. Distilled water (1 mL/ animal) and Sucralfate (100 mg/kg p.o.) was administered to control and standard groups respectively. Ethanol 8 ml/kg was administered p.o. after 1 hr of pre-treatment. Animals were sacrificed after 6 hrs of Ethanol treatment. Stomachs were isolated and opened along greater curvature to expose inner surface. Inner surface was washed thoroughly with normal saline and scanned for analysis[19]

(v) Cysteamine induced duodenal ulcers

Male wistar rats of 200-230 g were selected and weighed and marked for identification. All animals were fasted for 24 hrs. Test group was treated with 330mg/kg and 660mg/kg of the test drug. Distilled water (1 mL/ animal) and Cimetidine (100 mg/kg p.o.) was administered to control and standard groups respectively. Cysteamine 400 mg/kg was administered p.o. after 1 hr of pre-treatment. Animals were sacrificed after 24 hrs of Cysteamine treatment. Stomachs were isolated and opened along greater curvature to expose inner surface. Inner surface was washed thoroughly with normal saline and scanned for analysis[20]

Determination of acidity[21]

$$\text{Acidity} = \frac{\text{volume of NaOH x Normality of NaOH x 100}}{0.1} \text{ mEq/L}$$

Determination of Percentage Protection

$$\%\text{Protection} = \frac{\text{Control mean ulcer index --- Test mean ulcer index}}{\text{Control mean ulcer index}} \text{ X100}$$

RESULTS

Table1

Effect of Test preparation on ulcer area and ulcer index in Ethanol induced ulcers

Parameters	Control	Sucralfate(100mg/kg)	Test(300mg/kg)	Test (660mg/kg)
Ulcer area	178.4±5.337	57.83±3.156	160.5±2.912	98.29±3.843
Ulcer index	29.74	9.638	26.75	16.38

Table 2

Effect of Test preparation on ulcer area and ulcer index in Histamine induced ulcers

Parameters	Control	Ranitidine(100mg/kg)	NGL(330mg/kg)	NGL(660mg/kg)
Ulcer area	19.41±0.6570	7.220±0.4322	16.88±0.6087	11.74±0.4661
Ulcer index	3.236±0.1095	1.203±0.07203	2.814±0.1014	1.957±0.07768

Table 3

Effect of Test preparation on ulcer area and ulcer index in Naproxen induced ulcers.

Parameters	Control	Omeprazole (30mg/kg)	NGL(330mg/kg)	NGL(660mg/kg)
Ulcer area	10.72±0.3421	4.900±0.2067	9.408±0.2623	6.505±0.2953
Ulcer index	1.787±0.05702	0.8167±0.03445	1.568±0.04371	1.084±0.04921

Table 4

Effect of Test preparation on ulcer area and ulcer index in Cysteamine induced ulcers

Parameters	Control	Cimetidine(100mg/kg)	NGL(330mg/kg)	NGL(660mg/kg)
Ulcer area	22.95±1.767	9.728±0.7808	17.66±0.7074	11.58±0.6341
Ulcer index	3.824±0.2945	1.621±0.1301	2.944±0.1179	1.930±0.1057

Effect of cysteamine induced ulcers

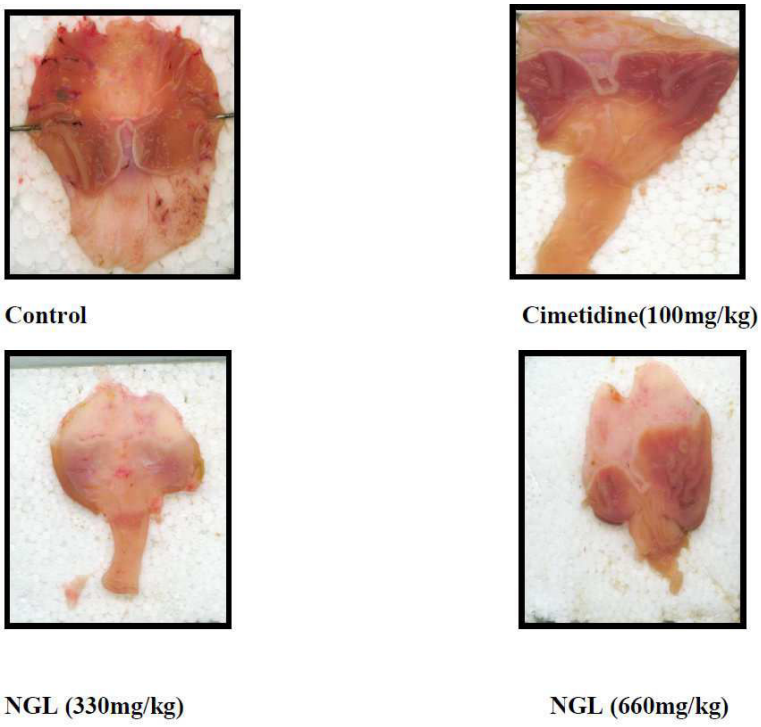
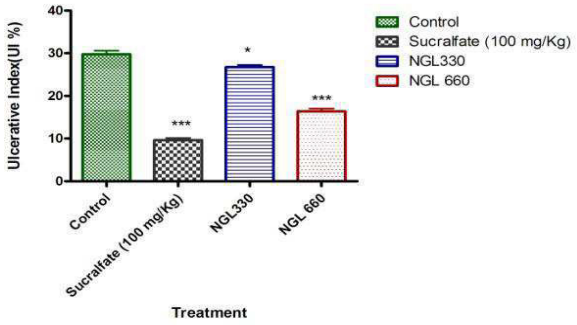


Figure 1

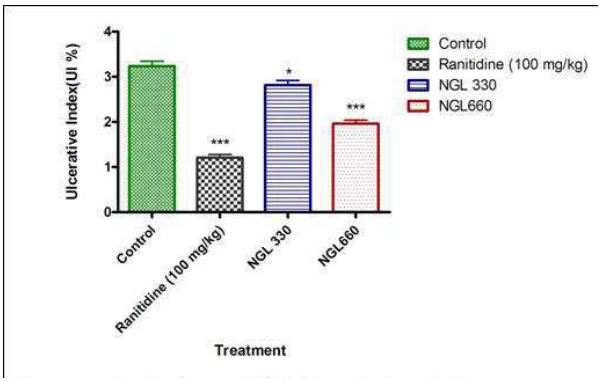
First picture shows isolated stomach of rat that was under control group, second picture shows isolated stomach of rat in test group. Test stomach shows absence of lesions and redness. Similarly isolated stomachs of all rats in different tests were studied.

Graph 1
Effect of Test preparation on ulcer index in Ethanol induced ulcers



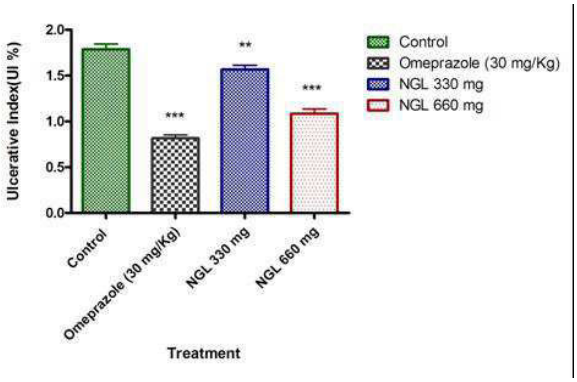
Data was analysed by one way ANOVA followed by Dunnet's Test (*P<0.05,**P<0.01,P<0.001)***

Graph 2
Effect of Test preparation on ulcer index in Histamine induced ulcers



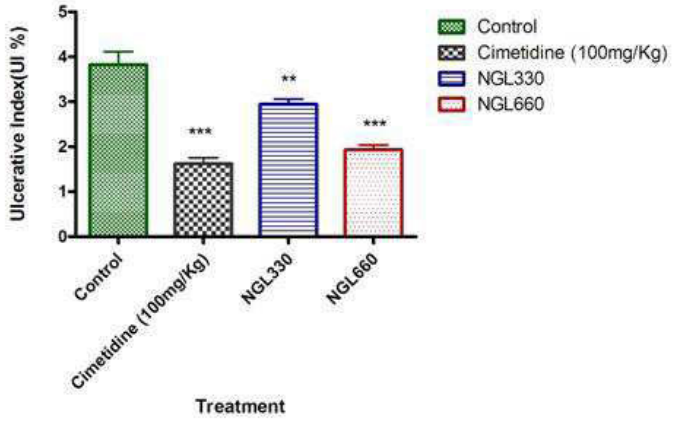
Data was analysed by one way ANOVA followed by Dunnet's Test (*P<0.05,**P<0.01,P<0.001)***

Graph 3
Effect of Test preparation on ulcer index in Naproxen induced ulcers



Data was analysed by one way ANOVA followed by Dunnet's Test (*P<0.05,**P<0.01,P<0.001)***

Graph 4
Effect of Test preparation on ulcer index in Cysteamine induced ulcers



Data was analysed by one way ANOVA followed by Dunnet's Test (*P<0.05,**P<0.01,P<0.001)***

DISCUSSION AND CONCLUSION

In the present thesis, the pathophysiological basis of peptic ulcer has been considered to find out the best treatment. Hence, according to various causes, the appropriate drugs have been selected. The activities of these drugs have been reported. For the present study, four different indigenous plants were selected namely, *Azadirachta indica (*Neem) *Glycyrrhiza glabra* (Licorice) *Garcinia cambogia* (Vrikshamla) and Deglycyrrhizinated licorice (DGL) Ulcers are thought to occur due to an imbalance between offensive factors like acid and pepsin and defensive factors like mucin secretion, cell proliferation, prostaglandins etc (Dorababu et al., 2006). Unlike the other marketed herbal formulations that contain astringent, tannins and mucoprotective agents; present formulation contains drugs that target etiological and pathological basis of ulcer development, thus aimed to provide overall and complete healing of ulcers. Neem leaf has a role in the treatment of many disorders. Aqueous extract contains flavonoids like rutin, quercetin and phytosterols (stigmasterols, campasterol) that possess anti-ulcer activities. The aqueous Neem leaf extract is found to possess anti-secretory activities by acting on H^+-K^+ Pump and Histamine receptors. (Maity et al., 2009; Dorababu et al., 2006) Deglycyrrhizinated licorice extract is licorice extract without glycyrrhizin; therefore no mineralocorticoid activity is seen. DGL decreases acid secretion, ulcer area, ulcer index and total acidity, all these are highly desirable properties of anti-ulcerogenic agent. DGL shows antioxidant and cytoprotective properties as it possess prostaglandin like substances (Mukherjee et al., 2010).

Licorice derived flavonoids are found to be an exceptionally strong antioxidant and effects were found to be 100 times stronger than that of vitamin E (Fukai et al., 2002; Gordan et al.,1995) . Glabridin, liquiritin, liquiritigenin, isoliquiritigenin and dimethylsiloxane are the compounds with established anti-*H*.pylori activity. Licorice also prolongs the life span of cells in the stomach and has an anti-pepsin effect (Aly et al., 2005). *Garcinia cambogia* inhibits vagus nerve stimulation, thereby reducing HCl output and acidity; it also shows cytoprotective properties (Mahendran et al., 2002) Hydroxycitric acid present in extract controls acid output and Garcinol shows antioxidant property (Padhye et al ., 2009) In the present study formulation

was tested in four different antiulcer models namely, ethanol, histamine, naproxen and cysteamine induced ulcers. In all the model formulation showed decrease in ulcer area and ulcer index at 660 mg/kg and 330 mg/kg, however effect at 660mg/kg was more pronounced. All the extracts also showed an effective *in-vitro* antioxidant activity, thus extracts prevent against increased lipid peroxidation during ulceration. Decrease in above mentioned parameters marks essential requirements of anti-ulcer formulation. Formulation shows good results in naproxen induced ulcers followed by cysteamine, histamine and finally ethanol induced ulcers. This suggests that test formulation may act by inhibiting COX pathway and promoting the synthesis of prostaglandins that acts as mucoprotective, however this need to be confirmed from further studies.

Present allopathic treatment strategies involve various side-effects and drug interactions; moreover there are very few drugs in the market that heal ulcer. Thus there is need to develop a potent herbal antiulcer formulation without interactions and that would heal ulcer. Although formulation was found to be less effective than Omeprazole, Ranitidine and Cimetidine but the overall comparison would be adequate after refinement of extracts. Also formulation is a mixture of four herbs therefore the results cannot be conclusive without herb-herb drug interaction data, which we aim to explore in the future. *H.pylori* activity needs to be studied for this formulation as it could not be carried out in present study due to non-availability of the strain and storage conditions of the laboratory. Future aspects will be to explore ulcer healing properties of this formulation by carrying out chronic long term study, establishment of herb-herb interaction data, clinical trials and formulation in the effective dosage form.

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