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Research Article

ANTI-ULCER ACTIVITY OF NEWLY FORMULATED HERBAL CAPSULE

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ABSTRACT

The anti-ulcer activity of the polyherbal formulation was investigated by ethanol induced gastric ulcer model in wistar rats. In this evaluation the ulcer index was measured using histopathological sections. The formulation with 500mg/kg per oral produced significant inhibition of the gastric lesions in ethanol induced ulcer model with respect to standard 20mg/kg of Omeprazole (P.O) administration. And the dose fixation was made with the help of acute toxicity studies with varying doses in wistar rats. And the result shows that the formulation might be useful in severe gastic ulcer,antiulcerogenic and as well as ulcer healing properties, which might be due to its antisecretory activity. The formulation is non-toxic even at relatively high concentration.

Keywords: Ethanol induced ulcer model, Omeprazole, ulcer index.

INTRODUCTION

Peptic ulcer is one of the major gastro-intestinal disorders, which occur due to an imbalance between the offensive (gastric acid secretion) and defensive (gastric mucosal integrity) factors. It can be either acute or chronic typically a recurrent condition affects upto 10% of the population with sufficient severity to prompt victims to seek medical attention. An estimated 15,000 deaths occur each year as a consequence of peptic ulcer [1]. Ulcers were previously thought to be only due to increase in offensive factors acid and pepsin, but it has been found that acid secretion in either normal or below normal in gastric ulcer patients, and that 40-70% cases of duodenal ulcer patients show acidity within normal range, suggesting that other factors are also involved in ulcerogenesis[2].

Factors implicated in the pathogenesis of gastric ulcer:

- Aggressive factors – increased acid-pepsin secretion, active oxidants, H.pylori, NSAID'S, leukotrienes.
- Defensive factors – impaired mucus secretion, bicarbonate neutralization, blood flow, prostaglandin production.
- Other factors include stress, nutrient deficiency and alcohol.

The treatment of peptic ulcer is directed against either reduction of the aggressive factors or enhancement of defensive mechanism. The ideal aims of treatment of peptic ulcer disease are to relieve pain, heal the ulcer and delay ulcer recurrence.Gastric ulcer remains a major global health problem. A number of drugs including proton pump inhibitors and H_2 receptor antagonists are available for the treatment of peptic ulcer, but clinical evaluation of these drugs has shown incidence of relapse, side effects and drug interactions [3]. This has been the rationale for the development of new antiulcer drugs and search for novel molecules has been extended to herbal drugs that offer better protection and decreases relapses.

Phytogenic agents have traditionally been used by herbalists and indigenous healers for the preventive and treatment of ulcer. A botanical compound with antiulcer activity includes flavonoids, saponins, tannins, gums and mucilage [4]. Gastro protective effect of flavonoid is due toIncrease in mucosal prostaglandin content, Decrease of histamine secretion from mast cells by inhibition of histidine decarboxylase, Inhibition of Helicobacter pylori growth and Free-radical scavengers, which play an important role in ulcerative and erosive lesions of the GIT[5].Gastro-protective effect of Saponin is due toActivation of mucous membrane protective factors and Triterpenoidsaponinregards to the plant for antiulcer activity[5].

Herbal formulation

The herbal formulation developed was contains the following plant part in crude form which was granulated using the technique wet granulation with 15% starch as a granulator and the formula contains *Glycyrrhizia glabra* rhizome part (200mg), *Aegle marmelos* leaf part (150mg), *Hemidesmus indicus* root part (75mg) and *Cuminum cyminum* fruit part (75mg) with varying proportion. Thedevelopedformulation was evaluated (as per WHO guidelines) and was standardunder Ayurvedic pharmacopoeial limits. And the formulation is expected to produce ulcer healing effect and this work was aimed for the same.

MATERIALS AND METHODS

Chemicals and reference drug:

All the chemicals used in this present study were of analytical grade. Omeprazole (reference drug) blocks the enzymes in the wall of the stomach from producing acid. By blocking the enzyme, the production of stomach acid is decreased, thus allowing the stomach to heal [6].

Plant materials

All the plant materials used in this formulation are authenticated by by Prof. P. Jayaraman, Ph. D (National institute of herbal science), West Tambaram,Chennai. The voucher no: PARC/2010/658,659,660 and 669.

Preliminary phytochemical screening:

Qualitative test for the presence of major phytochemical constituents was performed by the standard method [7]. Methods used were given in table 1.

METHODOLOGY

Animal used[12]

Wistar albino rats of either sex weighing about 130-150g were used. In this study animals were grouped in cages and maintained at ambient temperature of 25±5^{0}C with 12 hours light and dark cycle. The animals were fed with pellet diet and water *ad libitum*. All the animals were acclimatized to the laboratory conditions prior to experimentation. The study was conducted after obtaining institutional animal ethical committee clearance.

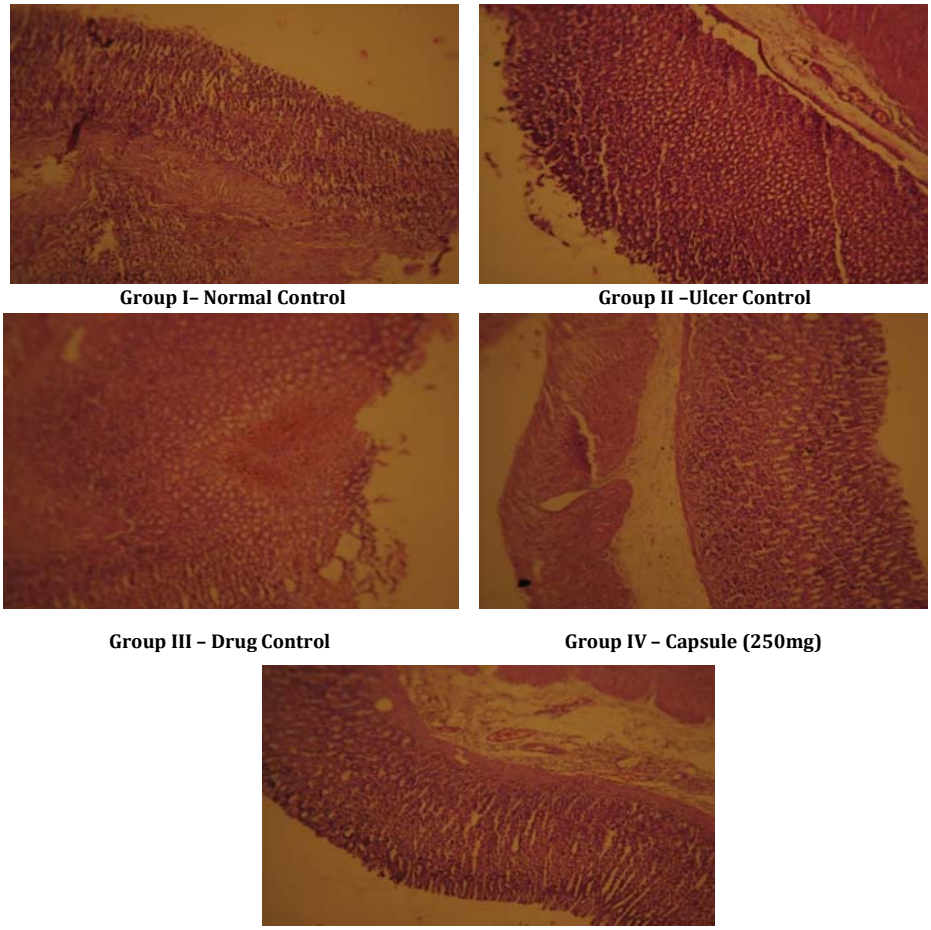
Acute toxicity study:

Preparation of extracts:

Capsule content were dissolved in a suitable solvent for easy administration, in this study distilled water is used as a solvent. The supernatantliquid was injected orally.

Procedure:

Four groups (n = 6) of wistar albino rats were used. Animals from all groups were fasted overnight and administered (p. o) with single dose (5, 50,300 and 2000 mg kg^{-1}) of the extract. Changes in the behaviour of animals were observed for 24 h after extract administration. For any signs of toxicity and mortality, animals were observed for 14 days.



Group I– Normal Control **Group II –Ulcer Control**

Group III – Drug Control **Group IV – Capsule (250mg)**

Group V– Capsule(500mg)

Fig. 1: Anti-ulcer activity (Histopathological observation)

Table 1: Phytochemical screening

Group of compounds	Method used for identification
Alkaloids	Dragondorff's, Mayer's and
Flavonoids	Wagner's reagent[8]
Tannins	Shinoda test[9]
dichromate	Ferric chloride and potassium solutions[8]
Steroids	Salkowski test [10]
Carbohydrates	Molisch and Barfoedtest [11]
Aminoacids	Ninhydrine test [11]

Table 3: Anti-ulcer activity of herbal formulation

GroupDose (mg/kg)	Gastricvolume	Ulcer area (mm^2)	Protection (%)
Contr 1ml	0.8ml	0.00±0.0	-
Ulcer control 1ml/200g	2.9ml	669.90±96.65*	-
Drug control 20mg/kg	1.9ml	115.14±22.01 *	66.67%
Formulation 250mg/kg	2.5ml	575.70±52.27*	50%
Formulation 500mg/kg	1.8ml	499.95±47.05*	63%

Values are expressed as mean ± SE. Data wasanalyzed using one way ANOVA F – multi comparison test. Values in a column followed by different letters are significantly different ($p<0.001$).Values in a column with an asterix(*) are significantly different from the ulcer control ($p<0.001$) with F value 106.36.* The mean difference is significant at the 0.05 level.

Table 2: Changes in the animal behaviour after administration of 2000 mg kg-1 dose

Activity	2hrs	3hrs	5hrs	7hrs	12hrs	24hrs
Respiration	-	-	-	-	-	-
Writhing	-	-	-	-	-	-
Tremor	-	-	-	-	-	-
Convulsion	-	-	-	-	-	-
Salivaiton	-	-	-	-	-	-

Activity	2hrs	3hrs	5hrs	7hrs	12hrs	24hrs
Diahhorea	-	-	-	-	-	-
Mortality	-	-	-	-	-	-
Hind limb paralysis	-	-	-	-	-	-
Sedation	-	-	-	-	-	-
Skin irritation	-	-	-	-	-	-
Eye irritation	-	-	-	-	-	-
CNS depression	-	-	-	-	-	-

+: Indicates that change was observed.

–: Indicates that there was no Change.

Ethanol induced gastric ulcer model

- The ulcer was induced by administrating ethanol.
- All the animals were fasted for 36 hours before administration of ethanol.

Grouping of animals

Animals were divided into five groups of six animals each.
Group I (normal control group) – receives distilled water orally.
Group II (ulcer control group) – receives 1ml/200g of 90% ethanol orally.
Group III (drug control group) – omeprazole 20mg/kg orally.
Group IV (test group) – receives 250mg/kg of capsule content.
Group V (test group) – receives 500mg/kg of capsule content.

Procedure

The gastric ulcer was induced in the rats by administrating absolute ethanol (90%) of 1ml/200g (body weight of animal) through oral. 45 minute prior to the ulcer induction the test drug and the standard drugs were administrated through the same route. They were kept is specially constructed cages to prevent corpophages during and after the experiment. Then the animals were anaesthetized 1hour latter with ketamine Hcl in a dose of 22-24mg/body weight through Intra Muscular route and the stomach was incised along the greater curvature and ulceration will be scored [13, 14].

P% = (UA ulcer control-UA treatment)/UA ulcercontrol × 100

Where P% - percentage of ulcer protection.

Histopathological evaluation

The gastric tissue samples were fixed in neutral buffered formalin for 24 hours. Sections of tissue from the stomach were examined histopathologically to study the ulcerogenic and/ or anti-ulcerogenic activity. The tissues were fixed in 10% buffered formalin and were processed using a tissue processor. The processed tissues were embedded in paraffin blocks and about 5-μm thick sectionswere cut using a rotary microtome. These sections were stained with hematoxylin and eosin using routine procedures. The slides were examined microscopically for Pathomorphological changes such as congestion, haemorrhage, oedema and erosions using an arbitrary scale for the assessment of severity of these changes[15]

RESULTS

Phytochemical screening

Phytochemical analyses of the capsule content revealed the presence of alkaloids, flavonoids, saponins, terpenoids, flavonoids, tannins, carbohydrates and proteins.

Acute toxicity study

Single dose (5, 50, 300 and 2000 mg kg^{-1}) of capsule content was administered to wistar rats showed no death up to 14 days study period. Even at the highest dose (2000 mg kg^{-1}), there were no physical sings of toxicity as evidenced by normal breathing and the absence of tremors, convulsions, diarrhoeas, salivation and paralysis in the treated animals (Table 3). Even the CNS depression, skin irritation, sedation were noticed up to 3 h after administration of 2000 mg kg^{-1}extract showed nil effect. From the above observations it reveals that the oral LD50 of capsule content is greater than 2000 mg kg^{-1}in rats. Observation of animals over the next 14 days showed no adverse effect of treatment.

Anti-ulcer (ulcer-preventive) activity

Dose-dependent protection from ethanol-induced ulceration. When compared to the ulcer control animals, extract treatment provided more or less 50 and 63% protection respectively at 250 and 500 mg kg^{-1} doses. Although omeprazole, the reference drug used in the study at 20 mg kg^{-1} dose provided the animals with the highest ulcer protection (66.67%) with regeneration of epithelium, the effect of 500 mg kg^{-1} dose of extract was comparable (63%) with the reference drug regeneration of epithelium congestion and oedema of sub mucosa occurs. Regeneration of epithelium occurs with minimal ulcer protection (50%) with 250mg kg^{-1}.

Gross pathological studies of the stomachs removedfrom animals that were not pre-treated with eitheromeprazole or capsule content showed complete ulceration. However, a preventive effect against ulceration (in terms of ulcer area) was noticed in animals pre treated with omeprazole and 500 mg kg^{-1}capsule content.Histopathological observation is given in the fig 1.

DISCUSSION

Ulcer has long been recognized as one of the most important gastrointestinal problem. With the ever growing interest in herbal medicine, this formulation have been screened and reported to be useful in treating and managing ulcer. *Glycyrrihiza glabra* have potent anti-ulcer activity [16]. In spite of its use in the traditional medicine against various ailments, this formulation as far not used and screened for anti-ulcer activity with this new combination for the first time here.

The result of the present study have been shown that this formulation possess gastro protective activity, as evidenced by its significant inhibition in the formation of ulcers induced by ethanol (Table 3).The protective effect was confirmed by histo pathological examination showing prevention of mucosal lesions and sub-mucosal oedema. As flavonoids been identified in the formulation, we believe that the anti-ulcer activity of this preparation is probably due to the antioxidant activity of the preparation. Antioxidant activities of flavonoids have been well documented in the literature. Moreover, flavonoids have been reported for their anti-ulcerogenic activity and gastro protection already.[17, 18]

Acute toxicity study have revealed that the formulation show slight CNS depression for a few hours after treatment at the dose of 2000mg/kg(Table 2). However, there was no sign of toxicity or mortality up to 14days indicates that the formulation is relatively safe. Any substance that is not toxic at 2000mg/kg is considered relatively safe. [19, 20]

CONCLUSION

From this study, it is clear that the formulation have significant anti-ulcer activity in experimental animal models. Andit shows muco-protective activity and gastric antisecretary activitywhen compared with that of reference drug (omeprazole). And the extract is non-toxic even at relatively higherconcentrations. The anti-ulcer activity is probably due to the presence of flavanoids. Further studies are being carried out to characterize and explore the biological activity of the compounds present in the extract.

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