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RESEARCH ARTICLE

EVALUATION OF ANTIULCER ACTIVITY OF POLYHERBAL FORMULATION AGAINST ETHANOL INDUCED GASTRIC ULCER IN ALBINO RATS

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ABSTRACT

Objective: The main objective of the study is to formulate polyherbal formulation which is more effective at a lesser dose.
Methods: In our present study, three plants were extracted and polyherbal formulation was prepared The CMC suspension of Polyherbal formulation was investigated for its anti ulcer activity against ethanol induced ulcer in rats at 100 and 200 mg/kg body weight p.o. Omeprazole was used as standard at a dose of 20 mg/kg. Five groups of adult albino rats were orally pre-treated respectively with CMC solution (ulcer positive control group),Group pre treated with CMC suspension(ulcer negative control group), Omeprazole 20 mg/kg (standard group), 100 mg/kg of Polyherbal formulation(PHF) suspended in CMC suspension(experimental group-1),and 200mg/kg of polyherbal formulation Suspended in CMC suspension(experimental group-2) one hour before oral administration of absolute ethanol to produce gastric mucosal injury. Rats were sacrificed and the ulcer areas of the gastric walls were determined.
Results: Pretreatment of rats with PHF suspension produced protection in the ethanol induced ulceration model as compared to control group. The protection was statistically significant and reduced the severity of ulcer and caused a significant reduction of ulcer index in this model.
Conclusion: The present study indicates that Polyherbal formulation have potential antiulcer activity in the model tested.

Key Words: PHF (Polyherbal formulation), Gastric Ulcer, Ulcer Index, CMC

INTRODUCTION

Peptic ulcer disease (PUD) is a serious gastrointestinal disorder that requires a well targeted therapeutic strategy. A number of drugs including proton pump inhibitors and H2 receptor antagonists are available for the treatment of peptic ulcer, but clinical evaluation by Central Drug Research Institute, of these drugs has shown incidence of relapses, side effects, and drug interactions (Dharmani and Palit, 2006). This has been the rationale for the development of new antiulcer drugs and the search for novel molecules has been extended to herbal drugs that offer better protection and decreased relapse.

Drugs of plant origin are gaining popularity and are being investigated for a number of disorders, including peptic ulcer (Wallace et al., 2000). An indigenous drug possessing fewer side effects is the major thrust area of the present day. The pathophysiology of PUD involves an imbalance between the offensive (acid, pepsin, and H. pylori) and defensive factors (mucin, prostaglandin, bicarbonate, nitric oxide).So, the present work was done to evaluate the anti ulcer activity of polyherbal formulation in both sex albino rats against ethanol induced ulcers.

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MATERIALS AND METHODS

Materials

The plant materials used in this study were purchased from the local market in Narsapur.

Chemicals

- Omeprazole (Dr. Reddys' laboratories)
- Ethanol (Gayathri sugars, Kamareddy)

Animal Maintenance

Both sex of albino rats (150-200g) were procured from the National Institute of Nutrition, Hyderabad. All animal experiments were strictly complied with the approval of institutional animal ethical committee. The animals were kept in polyacrylic cages and maintained under standard housing conditions of temperature (24-27°C) and humidity (60-65%) with 12:12 light: dark cycles. They were acclimatized for seven days. Food was provided in the form of dry pellets and water *ad libitum*[3].

Methods

Acute toxicity studies

Oral administration of graded doses of PHF (50, 100, 250, 500, 1000mg/kg and 2000mg/kg p.o.) to rats did not produce any significant changes in behaviour, breathing, cutaneous

effects, sensory nervous system responses or gastrointestinal effects during the observation period. No mortality was recorded in any group after 24h of administering of the formulation. Mortality was observed with 1000mg/kg and 2000mg/kg after 48 hrs. So, we have selected the doses below 250mg/kg for our study (Litchfield, 1949).

Preparation of Polyherbal Formulation

Leaves of *Azadirachta indica* were collected, dried, powdered and extracted by Soxhlet extractor using distilled water as the solvent. Crude extract was collected and dried. The rhizomes of *Zingiber officinale* and *Curcuma longa* were collected, dried in shade for 15 days and to ensure complete dryness plant rhizomes were kept in hot air oven at 45˚C for 10 minutes. Then rhizomes were subjected to size reduction to make coarse powder and passed through 40-mesh sieve and stored in an airtight container for further use. The dried and powdered rhizomes were subjected to hot extraction in Soxhlet apparatus with methanol and ethanol respectively (Bandyopadhyay et al., 2004; Raji et al., 2004; Aziz and Qamer, 1990). From the data available (Purohit and Richa, 2013; Lee et al., 1987) about the research on above plants, the therapeutic dose of three plants is in the ratio of 1: 1.42: 2.51 of *Zingiber officinale, Curcuma longa, Azadirachta indica* respectively . So we have prepared our PHF in the said ratio.

Evaluation of anti ulcer activity (Rafatullah et al., 1990; Nagle et al., 2012)

The albino rats of either sex weighing between 180 –200 gm were divided into five groups of 6 animals each and fasted for 24 hrs with water *ad libitum* prior to experiment (Thirunavukkarasu et al., 2003).

Group1- Positive Control (1%CMC)
Group2- Negative Control (1% CMC + Ethanol)
Group3- Standard (Omeprazole 20mg/kg + Ethanol)
Group4- Experimental group (PHF100mg/kg +Ethanol)
Group5- Experimental group (PHF 200mg/kg +Ethanol)

Here, for the first group which is positive control, 1%CMC is administered orally. For the Group-2 animals, which is negative control 1%CMC is given and after 60 min 2ml/kg of 100% Ethanol is administered orally. For the Group-3 animals, Standard i.e., Omeprazole 20mg/kg is given (Berenguer et al., 2006) and after 60min Ethanol is administered orally (Gupta, 2009). For the experimental Groups, PHF of 100mg/kg and 200mg/kg is suspended in 1%CMC and is administered orally. After an hour, 100% Ethanol is administered 2ml/kg p.o. After an hour of administration all the animals were sacrificed by cervical dislocation (Sanchez et al., 2002; Borella et al., 1989; Franzone et al., 1988; Herling and Weidmann, 1994; Hollander et al., 1985; Lindberg et al., 1990; Long et al., 1983; Masuda, ?; Robert et al., 1979; Starrett et al., 1989; Szabo et al., 1981).

RESULTS

Peptic ulcers are caused when the natural balances between the aggressive factors of acid, pepsin, defensive mechanisms of mucus, bicarbonate, mucosal turnover and blood supply (mucosal barrier) are disturbed .It is reported that acid and pepsin are relatively less important as causative agents and that a defect in the defensive mechanism of gastric mucosa is the first step towards ulcer formation. Although in most cases, the etiology of ulcer is unknown, it is generally accepted that it is the result of an imbalance between aggressive factors and maintenance of the mucosal integrity through the endogenous defense mechanism). It is known that gastric lesions produced by ethanol administration appeared as multiple hemorrhagic red bands of different sizes along the glandular stomach. Ethanol is commonly used for inducing ulcers in experimental rats and leads to intense gastric mucosal damage. Studies suggest that the ethanol damage to the gastrointestinal mucosa starts with micro vascular injury, namely disruption of the vascular endothelium resulting in increased vascular permeability, oedema formation and epithelial lifting. Ethanol produces necrotic lesions in the gastric mucosa by its direct toxic effect, reducing the secretion of bicarbonates and production of mucus.

Exposure to ethanol increases the extension of cellular damage in a dose-dependent way. Oxidative stress plays an important role in the pathogenesis of various diseases including gastric ulcer, with antioxidants being reported to play a significant role in the protection of gastric mucosa against various necrotic agents. Antioxidants could help to protect cells from damage caused by oxidative stress while enhancing the body's defense systems against degenerative diseases. Administration of antioxidants inhibits ethanol-induced gastric injury in rat. It is speculated that the gastro protective effect exerted by polyherbal formulation could be attributed to its antioxidant property of these compounds. Moreover, further insight into the precise mechanism of action is essential to exploit the complete potency of polyherbal formulation and increase its usage in contemporary medicine. Pretreatment of rats with polyherbal formulation produced protection in the ethanol induced ulceration model as compared to control group. The protection was statistically significant and reduced the severity of ulcer and caused a significant reduction of ulcer index in this model. However the ulcer protection is less when compared to standard.

Calculation of ulcer index and Percentage ulcer inhibition

Ulcer index has been calculated by adding the total number of ulcers per stomach and the total severity of ulcers per stomach (Witt et al., 1985).

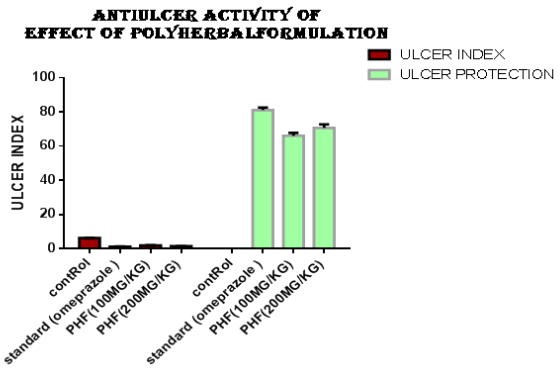
- 0: normal colored stomach.
- 0.5: red coloration.
- 1: spot ulcers.
- 1.5: haemorrhagic streak.
- 2: ulcers.
- 3: perforation.

Mean ulcer score for each animal will be expressed as ulcer index. The percentage of ulcer protection was determined as follows:-

% Protective = Control mean ulcer index - Test mean ulcer index x 100

Control mean ulcer index

The results obtained were represented graphically.



Graph 1. Percentage of Ulcer Index and Ulcer Protection

Table 1 - Mean ± SEM of ulcerative index obtained with oral doses of 100mg/kg and 200 mg/kg of PHF, control (1% CMC) and Omeprazole (20 mg/kg) induced by ethanol model. ANOVA and Dunnet's test were utilized for comparisions, (*p< 0.01), (n=6).

Table 1. Determination of Ulcer Protection and Ulcer index

[Agonist M]	ULCER INDEX			ULCER PROTECTION		
Control	6.0	6.2	6.8	0	0	0
Standard (Omeprazole)	1.1	1.5	1.3	79.	80.	84.
PHF(100mg/kg)	1.8	2.0	2.2	63.	66.	69.
PHF(200mg/kg)	1.6	1.4	1.7	67.	71.	74.

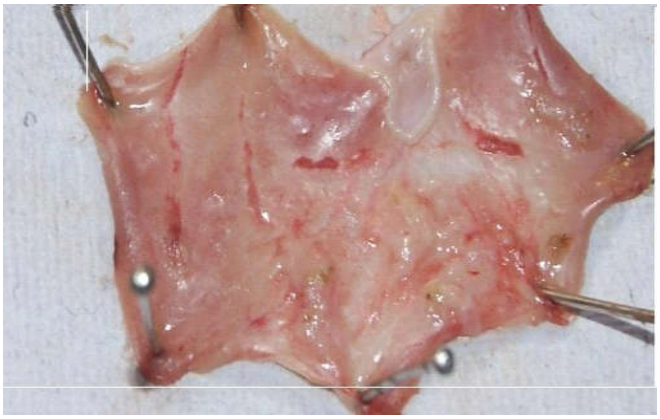


Fig. 1. Control Group treated with 1%CMC

Gross appearance of the gastric mucosa in a rat pretreated with 5ml/kg of 1%CMC suspension (negative control). Severe red coloration, spot ulcer and severe hemorrhagic streaks were observed.

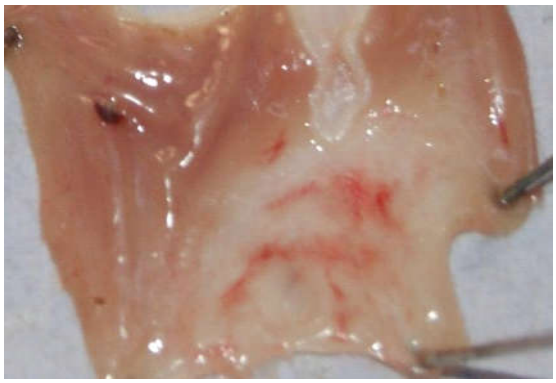


Fig. 2. Response of the animal treated with 200mg/Kg of PHF

Gross appearance of the gastric mucosa in a rat treated with polyherbal formulation (200mg/kg).On treatment with CMC suspension of Polyherbal formulation less red coloration and hemorrhagic streaks were observed when compared to control and flattening of gastric mucosa is seen.

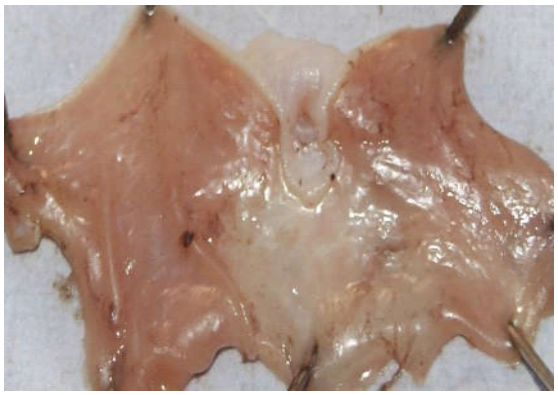


Fig. 3. Animal treated with 20mg/Kg of Omeprazole

Gross appearance of the gastric mucosa in a rat pretreated with Omeprazole (20mg/kg). No red coloration and no hemorrhagic streaks when compared to control.

Statistical Calculations

The data expressed are mean ± standard error of mean (SEM). All statistical comparisons between the groups are made by means of One Way Analysis of Variance (ANOVA) with post hoc Dunnett's test using Graph pad Prism 5 software. The p value less than 0.01 is regarded as significant.

DISCUSSION

This study revealed a significant anti-ulcer effect of a Polyherbal formulation containing *Azadirachta indica, Curcuma longa* and *zingiber officinalis* in experimental models of gastric lesion induced by ethanol. Ethanol-acid causes more severe gastric mucosal ulceration. The ulcers are caused either by a direct effect of the ethanol-acid solution on the gastric epithelium, or are modulated indirectly by the release of vasoactive products from mast cells, resulting in the release of mediators such as histamine.

Endogenous histamine formation and its release from mast cells in the gastric mucosa also have been implicated in the pathogenesis of gastric ulcers produced by acute stress. The allopathic drugs of ulcer inhibit the acid secretion, protect the mucosa, and inhibit the Helicobacter pylori [27, 28] .In our experiments, PHF prevented acute, gastric mucosal injury induced by ethanol-acid. The protective action was produced at 100mg/kg and 200 mg/kg and the activity was more at the dose 200mg/kg. The percentage protection was found to be significant when compared with the standard drug (Omeprazole). Polyherbal formulation has shown synergistic effect compared to the available data of therapeutic doses of individual extracts. The specific mechanisms underlying this action is unknown. However, as a first step, the individual extracts should be fractioned and further studied. The formulation did show a significant, cytoprotective effect against the gastric lesions induced by necrotizing agents, which suggests a direct, protective effect on the gastric mucosa. The mechanism underlying the protective action of the extract against ethanol induced gastric lesions is unclear. Further studies using more specific methods are required to explore the compounds responsible for the protective effect, and the mechanism of this activity. Chronic toxicity studies are also in progress.

Conclusion

Polyherbal formulation could significantly protect the gastric mucosa against ethanol induce injury. The percentage protection was found to be significant when compared with standard drug Omeprazole. Poluherbal formulation has shown synergistic effect compared to the available data of therapeutic doses of individual extracts

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