PROTECTIVE EFFECTS OF A POLY HERBAL FORMULATION AGAINST ASPIRIN INDUCED ULCERS IN WISTAR RATS

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ABSTRACT

The poly herbal formulation has been used traditional in folk medicine due to its anti-septic, astringent and anti-fungal property. The formulations are rich in tannins and have been proved experimentally to possess antibacterial, wound healing and antitumorogenic effects. The present investigation was designed to determine the gastro protective effect of Polyherbal Formulation (PHF) at a dose of 150mg/kg in a model of aspirin induced ulcers in rats and to study the mechanism involved using H2 receptor blocker Ranitidine as comparison. In treated groups of animals the in vivo antioxidant levels such as SOD, CAT, and Glutathione levels were increased and LPO levels was decrease and found more or less equal to the normal values. The histopathological examinations of the stomach of the ulcerated animal’s show severe erosion of gastric mucosa, submucosa edema and neutrophil infiltration. These results suggest that the gastroprotective effects of PHF in this experimental model through antioxidant and cytoprotective activity.

Keywords: PHF, NSAID induced gastric ulcer, Antioxidant, Antitumorogenic Action

INTRODUCTION

Peptic ulcer disease encompassing gastric and duodenal ulcer is the most prevalent gastrointestinal disorder. Three out of 1000 individuals have peptic ulcer every year and an estimated 15,000 deaths occur each year as a result of Peptic ulcer disease. Ulcer therapy faces a major drawback in modern days due to the unpredictable side effects at long term uses at commercially available drugs. As it affects 5% at the global population. Peptic ulcer is due to imbalance between aggressive factors and local mucosal defenses. Depending on site of formation these are classified as esophageal ulcers, gastric ulcers, duodenal ulcers. Gastric and duodenal ulcers are common pathologies that may be induced by a variety of factors such as stress, smoking, nutritional deficiencies and nosocomic agents, including non-steroidal anti-inflammatory drugs (NSAID’s). NSAID’s are worldwide used for the treatment of pain, rheumatic and cardiovasulares diseases, and more recently for the prevention of colon cancer and Alzheimer’s disease. Aspirin is a potent non-steroidal anti-inflammatory drug (NSAID’s) used for the treatment of rheumatoid arthritis and related diseases. Ulcers associated with the use of NSAID’s remain a major clinical problem, which has not been solved through the introduction of selective inhibitors of COX-2. The aspirin induced gastric damage cell owed by a multistage pathogenetic event in which reactive oxygen species (ROS), vascular permeability, luminal contents, neutrophils, gastric motility and microcirculation all play a role in the development of inflammation and ulcers.

Plants have been proved to be powerful therapeutic agents for the treatment of various lumen suffering, including attherosclerosis, cancer, ulcer etc. Due to the lack of side effects compared to synthetic drugs, approximately 60% of the world’s population relies almost entirely on plants for medication and natural products have long been recognized as an important source of therapeutically effective medicine. In traditional Indian medicine, several plants have been used to treat gastrointestinal disorders; including gastric ulcer and the phytochemical analysis of these plants have yielded a number of compounds with gastro-protective activity.

The common synthetic drugs used in the treatment of ulcer are H2 receptor blockers, proton pump inhibitors, acid Neutralizers (Antacids) and drugs affecting the mucosal barrier. Single drug is not sufficient to treat the symptoms associated with the ulcer. Thus the need of combination therapy emerged. Thus a poly herbal formulation is used in the treatment of the peptic ulcer.

The aim of this study was to assess the gastro protective effect of PHF in comparison with Ranitidine in a model of NSAID’s induced gastric ulcer in rats and to elicit the underlying mechanisms. For this purpose, we studied the role of the PHF in oxidative stress by measuring changes in Glutathione peroxidase (GPx) and superoxide dismutase (SOD) activity. In addition, the inhibition of lipid peroxidation (LPO) was assessed in vitro in rat liver membranes.

MATERIALS AND METHODS

Plant material

Poly herbal formulation (PHF) (dry powder, cream color) was purchased from Chemiloids (manufactures and exporters of herbal extracts; Vijayawada, Andhra Pradesh, India). It contain each 5 ml different plant material like Glycyrrhiza glabra-70 mg, Terminalia chebula-75 mg, Zingiber officinale-50 mg, Cassia senna-60 mg, Operculina turpethum-50 mg, Asparagus rhacemosus-55 mg, Aloe barbadensis-200 mg. Herb-to-product ratio was 6:1 and the extract was stored at 0 – 4 °C and dissolved in water just before use.

Chemicals

Epinephrine, DTNB (Sigma chemical Co., St Louis MO, USA), Thiobarbituric acid (TBA) and Trichlosoacetic acid, Hydrogen peroxide (SD fine Chemicals Ltd). Sodium hydrogen phosphate, Potassium dihydrogen phosphate, Sodium hydrogen phosphate, Tris buffer, all other reagents used for analytical grade.

Animals

Male Wistar rats weighing (100±25g, 8 to 9 weeks old) were procured from Sri Raghavendra Enterprises (Bangalore, India) and acclimatized for 7 days before dietary manipulation. Animals were maintained at standard conditions of temperature and relative humidity, with a 12-h light/dark cycle. Water and commercial rat feed were provided ad libitum. The current work was carried out with a prior permission from our institutional animal ethical committee (Regd. No. 470 / 01 /a/CPSEA, dt. 24 Aug 2001). Animals were randomly assigned into four groups of each six animals.

Acute toxicity studies

Acute oral toxicity study was performed as per OECD-423 guidelines (acute toxic class method). Wistar rats (n=6) of either sex selected by random sampling technique were used for acute toxicity study. The animals were kept fasting for overnight providing only water, after which the formulations were administered orally at the dose...
level of PHF with 1500 mg/kg, 1800 mg/kg body weight by gastric intubations and observed for 24 hours. If mortality was observed in 2 out of 3 animals then the dose administered was assigned as toxic dose. If mortality was observed in one animal, then the same dose was repeated again to confirm the toxic dose. The mortality and morbidity was observed after 24 hours.

Dose selection
The dose selected for the extracts were about 1/10th of the maximum tolerated safe dose found from acute toxicity studies.

PHF dose 150 mg/kg body weight
Aspirin 200 mg/kg body weight
Ranitidine 50 mg/kg body weight

Experimental design
Group-I: Received normal diet (Normal Group).
Group-II: (Ulcer control) were received normal diet and drink tap water.
Group-III: (Test control) were received normal diet and drink tap water and poly herbal formulation with a dose 150 mg/kg body weight, administered for 7 days.
Group-IV: (Standard drug) were received normal diet and drink tap water and Ranitidine with a dose 50 mg/kg body weight, administered for 7 days.

On the day of the experiment III and IV received PHF and Ranitidine respectively, 30 minutes prior to the administration of aspirin (200 mg/kg b.w) and after four hours animals were killed.

Aspirin (ASP)-induced ulcer
Aspirin was suspended in 0.5% carboxymethyl cellulose in water and administered orally in the dose of 200 mg/kg body weight to 36 hrs fasted rats according to the method of Hemmati et al (1973) . After aspirin administration of four hours the stomachs were excised and cut along the greater curvature, washed carefully with 5.0 ml of 0.9% NaCl and ulcers were scored by person unaware of the experimental protocol in the glandular portion of the stomach. Ulcer index has been calculated by adding the total number of ulcers per stomach and the total severity of ulcers per stomach. The total severity of ulcers was determined according to the method of Hemmati et al (1973) . After aspirin administration of four hours the animals were sacrificed and the stomach was then excised and cut along the greater curvature, washed carefully with 5.0 ml of 0.9% NaCl and ulcers were scored by person unaware of the experimental protocol in the glandular portion of the stomach. Ulcer index has been calculated by adding the total number of ulcers per stomach and the total severity of ulcers per stomach. The total severity of ulcers was calculated according to the method of the severity of each ulcer after histological confirmation as follows: 0, no ulcer; +, pin point ulcer and histological changes limited to superficial layers of mucosa and no congestion; ++, ulcer size less than 1mm and half of the mucosal thickness showed necrotic changes; +++, ulcer size 1–2mm with more than two-thirds of the mucosal thickness destroyed with marked necrosis and congestion, muscular is remaining unaffected; ++++, ulcer either more than 2mm in size or perforated with complete destruction of the mucosa with necrosis and hemorrhage, muscular is still remaining unaffected. The pooled group ulcer score was then calculated according to the method of the severity of each ulcer after histological confirmation as follows: 0, no ulcer; +, pin point ulcer and histological changes limited to superficial layers of mucosa and no congestion; ++, ulcer size less than 1mm and half of the mucosal thickness showed necrotic changes; ++++, ulcer size 1–2mm with more than two-thirds of the mucosal thickness destroyed with marked necrosis and congestion, muscular is remaining unaffected; ++++, ulcer either more than 2mm in size or perforated with complete destruction of the mucosa with necrosis and hemorrhage, muscular is still remaining unaffected. The pooled group ulcer score was then calculated according to the method of the severity of each ulcer after histological confirmation as follows: 0, no ulcer; +, pin point ulcer and histological changes limited to superficial layers of mucosa and no congestion; ++, ulcer size less than 1mm and half of the mucosal thickness showed necrotic changes; ++++, ulcer size 1–2mm with more than two-thirds of the mucosal thickness destroyed with marked necrosis and congestion, muscular is remaining unaffected; ++++, ulcer either more than 2mm in size or perforated with complete destruction of the mucosa with necrosis and hemorrhage, muscular is still remaining unaffected. The pooled group ulcer score was then calculated according to the method of

Stomach tissues preparation
In this study, the superoxide dismutase (SOD), catalase (CAT), and the levels of total glutathione (GSH) and LPO in rat stomach tissues were determined. The stomachs were homogenized on ice using an ultra-turraks homogenizer for 15 min. Homogenates were filtered and centrifuged using a refrigerated centrifuge at 4°C. These supernatants were then used for the determination of the enzymatic activities. All assays were carried out at room temperature in triplicate.

Biochemical analysis
SOD activity was measured according to the method described by Sun et al (1988). CAT activity was measured using Aebi’s (1984). Reduced GSH in the gastric mucosa was measured by Ellman’s reaction using 5,5'-dithio-bis-2-nitrobenzoic acid as described previously. LPO was determined by estimating Malondialdehyde produced using the thiobarbituric acid test. Histopathology
After the termination of experiments, animals were sacrificed, the stomach was excised and cut open through the ventral suture and a small portion was fixed in 10% formalin solution immediately after sacrifice, which was passed through ascending grades of alcohol, cleared in xylene and impregnated and embedded in paraffin-dehydrated specimens. Three to four micrometer sections were made using a microtome and after staining with haematoxyline and eosine, the sections were molded in DPX and observed under light microscope.

Statistical analysis
All the data expressed as Mean ± SEM. Statistical significance between more than two groups was tested using one way ANOVA followed by the Turkey test using computer based fitting programme (Prism, Graph pad). Statistical significant was determined at P<0.05.

RESULTS
Ulcer index
The rat pretreated with PHF (150 mg/kg) produced significant (P<0.01) decrease in ulcer index compared to respective NSAIDs treated group. Ranitidine also show similar effects but was more effective compared with PHF. (Table 1)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Ulcer index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Nil</td>
</tr>
<tr>
<td>Induced control</td>
<td>3.2 ± 0.84*</td>
</tr>
<tr>
<td>PHF</td>
<td>2.4 ± 0.55*</td>
</tr>
<tr>
<td>Ranitidine</td>
<td>1.2 ± 0.45*</td>
</tr>
</tbody>
</table>

Values are mean ± SEM for six rats. *p<0.01 compared to control group

SOD, Catalase, GSH
The results revealed that at 150 mg/kg dose level, the PHF significantly increased SOD, GSH (p<0.05) and Catalase (0.01) levels compared to respective NSAID treated group. But Ranitidine more significant (0.001) effect than PHF compared to NSAID treated group. There was NSAID treated group significantly (0.001) decrease in the SOD, Catalase and GSH levels compared to normal treated group. (Fig 1, 2,3)

LPO (MDA)
The results revealed that at 150 mg/kg dose level, the PHF significantly (p<0.01) decreased LPO levels compared to respective NSAID treated group. But Ranitidine more significant (p<0.001) effect than PHF compared to NSAID treated group. There was NSAID treated group significantly (p<0.001) decrease in the MDA levels compared to normal treated group. (Fig 4)
DISCUSSIONS

The present study on Antioxidant and cytoprotective was carried out on the rats of either sex Wistar strain. The project was aimed to study the Antioxidative stress and protective role of polyherbal formulation in ulcer. Biochemical parameter of oxidative stress was analyzed from stomach homogenate, and ulcer index study was carried out to confirm the biological changes.

NSAIDs are well known gastric mucosal barrier breakers. Synthetic NSAIDs like aspirin cause mucosal damage by interfering with prostaglandin synthesis, increasing acid secretion and back diffusion of H+ ions15.

Free radicals affect lipids by initiating peroxidation. Superoxide (O2−), hydrogen peroxide (H2O2) and hydroxyl radical (OH•) are important ROS causing tissue damage15; and lipid peroxide level is an indicator for the generation of ROS in the tissue. Superoxides produced by peroxidases in the tissues might damage the membranes and stomach tissues by increasing LPO16. Similarly, our results showed that there was a significant increase in LPO levels in rat stomach tissues treated with Aspirin. However, the administration of doses of PHF significantly decreased the levels of LPO.

Previous studies have shown that the administration of ethanol and NSAIDs decreased the levels of SOD, CAT, GPx, and GSH in tissues17, 18. Similarly, in the present study, the levels of SOD, CAT, and GSH in rat stomach tissues were significantly reduced by the administration Aspirin, and the pretreatment with PHF at dose of 150 mg/kg resulted in a significant increase in the levels of SOD, CAT, and GSH (Fig.1, 2, 3).
However, another study showed that the administration of Aspirin significantly reduced the gastric GSH level compared to normal rats, and treatment with the test drug resulted in an increased level of total tissue sulfhydryl as compared to the untreated ulcerated rats. GSH, a major non-protein thiol in living organisms, plays a central role in coordinating the body's antioxidant defense process. Pretreatment with PHF resulted in increased level of total tissue sulfhydryl compared to the control rats.

Polyherbal formulation containing extract of several herbs. Ethanolic as well as alcoholic extract of phenolic content of Zingiber officinale has been reported nephroprotective and antioxidant activity21, 22. Terminalia chebula possess Renoprotective and antioxidant activity23. Glycyrrhiza glabra reported antioxidant activity and also cytoprotective24. Aloe barbadensis show preventing oxidative damage to cells25. Operculina turpethum protect the oxidative stress26 and Cassia senna reported antioxidant activity28. Asparagus racemosus has been reported ulcer protected activity and antioxidant activity29, 30.

(A) Normal control

(B) Disease control (Aspirin 200mg/kg)

(C) Test (PHF 150 mg/kg)

(D) Standard (Ranitidine 50mg/kg)

CONCLUSION
The present studies find out the role of antioxidant and cytoprotective activity of poly herbal formulation. Administration of NSAID increasing ulcer index. It also increases the levels of biomarkers of oxidative stress in the stomach. The treatment with polyherbal formulation significantly reduced ulcer index count in NSID induced ulcer rats. Polyherbal formulation treatment reduced elevated levels of biomarkers of oxidative stress. Hence from the present study we can conclude that the poly herbal formulation may be useful in the management of Oxidative stress and ulcer.

REFERENCES
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