Pharmacodynamic interaction of Boerhaavia diffusa with omeprazole in experimentally induced ulcers in rats

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ABSTRACT

The study evaluates the possible gastro protective of combination therapy of Omeprazole and Boerhaavia diffusa using three different gastric ulcer models. Gastric Ulcers in SD rats were induced by Indomethacin (25mg/kg), pylorus ligation model and stress-induced Ulcer. Various parameters like free acidity and total acidity, ulcer index, ulcer score, pepsin and mucin content, antioxidant parameters like super oxide dismutase and catalase were evaluated. Omeprazole (2mg/kg) was used as the standard drug. Boerhaavia diffusa was administered at two dose levels, 200mg/kg and 400 mg/kg body weight. Statistical analysis was done by ANOVA followed by Dunnet’s Multiple Comparison test. P<0.05 was considerable statistically significant. Oral administration of combination of Omeprazole and Boerhaavia diffusa at 200 and 400 mg/kg produced significant (p<0.01 & p<0.001) decrease in acidity, ulcer index and severity of ulceration in the pylorus ligation model as well as protection against stress and Indomethacin induced ulceraions compared to control. It also shows significant (p<0.001) decrease pepsin content and significant (p<0.001) increase in mucin content compared to control pylorus ligation model. In Indomethacin induced model combination therapy at high level shows significant increase (p<0.001) in antioxidant parameters like SOD and catalase compared to control. The anti ulcer effects of combination of Omeprazole and Boerhaavia diffusa at both the dose levels were significantly higher than that of omeprazole alone. Combination therapy was found to be an effective anti ulcerogenic agent, minimizing any possible side effects. The result of the study suggests that combination therapy causes an inhibitory effect on release of gastric hydrochloric acid and protects gastric mucosal damage.

Introduction

Peptic ulcer disease (PUD) refers to a disruption of the mucosal integrity of the stomach, duodenum, or both, caused by local inflammation, which leads to a well-defined mucosal defect. PUD results from an imbalance between factors promoting mucosal damage (gastric acid, pepsin, H. pylori infection, NSAID use), and those influencing gastro duodenal defense (mucus, bicarbonate secretions, prostaglandins, and mucosal blood flow)[1]. People who take NSAIDs, smoke, cigarettes, excessive intake of alcohol, or use cocaine, high intake of spicy foods or coffee, food poisoning, presence of Helicobacter pylori or secondary due to pathological conditions such as Zollinger-Ellison syndrome are at increased risk of developing PUD. The U.S PUD (Peptic ulcer disease) affects between 3.5 and 7.5 million people with approximately one-half million new cases diagnosed every year. Despite improvements in therapy wide spread use of NSAIDs and low dose aspirin, the economic burden of PUD remains a significant issue. Due to the ineffectiveness as well as the potential side effects of modern drugs, patients are often led to explore complementary or alternative medicines such as herb, and medicinal botanicals in particular[2,3]. However simultaneous administration of herbs and drugs may mimic, magnify or oppose the pharmacological effects of each other[4]. Reports indicate that about 15-20% of individuals on prescription medications also use herbal supplements and less than 40% of patients disclose to their physicians the usage of herbal remedies, even if they experience severe side effects because of the fear of censure or rebuke[5]. The problem is further compounded by the fact of many physicians are themselves not always familiar with the potential for herbal drug interactions [6]. It is imperative to

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promote credible research on the safety and efficacy of combined herb drug treatment for variety of ailments. It is believed that although herbs hold promise as therapeutically effective medicaments, appropriate studies should be carried out to confirm their efficacy in the presence of modern medicines[4]. *Boerhaavia diffusa* is also known as punarnava[7]. The plant *Boerhaavia diffusa* is a perennial herb. Medicinal properties of the plant have been utilized since long in the indigenous system of medicine in India[8]. Various types of extracts of different plant parts (especially roots and leaves) of *B. diffusa* have been documented to possess hepatoprotective[9], antiproliferative and antiestrogenic[10], spasmytic[11], antifungal[12], antidiabetic[13], immunomodulatory[14], antiamoebic[15] and anticonvulsant[16] activity. *Boerhaavia diffusa* has been proved to be very effective drug in reducing inflammation[17]. It has been claimed that *Boerhaavia diffusa* has the ability to cure gastric ulcers or protect against its formation in both animals and humans. However, it was also shown that *Boerhaavia diffusa* could not prevent ethanol-induced gastric lesions in rats. The anti-ulcer activities of *Boerhaavia diffusa* has been attributed to several possible mechanisms including its anti-inflammatory properties, healing effects, mucus stimulatory effects and regulation of gastric secretions[18]. *Boerhaavia diffusa* was effectively employed for curing ulcer-induced inflammation in traditional Indian system of medicine; however, till now there is not much scientific demonstration of the claimed uses. Moreover, it is also our interest to determine the possible interaction of *Boerhaavia diffusa* with conventional antulcer drugs such as a proton pump inhibitors as most patients for concurrent administration of traditional remedies with these drugs[18]. Caution should be taken while prescribing drug herb therapy. Hence the current study is designed to determine the effect of *Boerhaavia diffusa* root extract on experimentally induced gastric ulcers in presence and absence of conventional antulcer drug Omeprazole using ethanolic extract of this plant.

### Materials and Methods

This study was performed in Postgraduate Studies and Research Center, Krupanidhi College of Pharmacy, Bangalore, Karnataka, India. Dried root powder of *Boerhaavia diffusa* will be purchased from Bio-gen Extracts Pvt. Ltd., Bangalore, India.

### Experimental Animals

The experiments were performed on Male Sprague Dawley Rats weighing 200-250 g obtained from a inbred colony at the Animal House, Department of Pharmacology Krupanidhi college of Pharmacy Karnataka, Bangalore. These animals were kept in an environment with controlled temperature (25°C), humidity (45-75%) and 12:12 h light: dark cycle, as per the guidelines of Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA). All the rats were provided with normal pellet diet and water ad libitum, prior to the dietary manipulation. The experimental protocol was approved by the the Institutional ethical committee.

### Treatment protocol[11]

The rats will be grouped as follows:
- **Group 1**: Control
- **Group 2**: LBDE (200mg/kg- Low dose of *Boerhaavia diffusa* extract)
- **Group 3**: HBDE (400mg/kg- High dose of *Boerhaavia diffusa* extract)
- **Group 4**: Omeprazole (2mg/kg) (OMPZL)
- **Group 5**: LBDE plus OMPZL
- **Group 6**: HBDE plus OMPZL

### Pylorus ligation induced ulcers (Shay rat 1945)

The animals were fasted for 36 hours before pylorus ligation with water ad libitum by placing them individually in cages to avoid caprophagy and cannibalism. Normal saline (1ml/rat p.o.) was administered twice daily to all the animals. Under light ether anesthesia, the abdomen was opened by midline incision below the sternum. The pyloric portion of the stomach was slightly lifted out and ligated, avoiding damage to its blood supply. The stomach was placed back carefully and the abdominal wall was closed with sutures. The animals were deprived of food and water during the postoperative period and the animal was sacrificed six hours after pylorus ligation by cervical dislocation method[19-21].

The stomach was isolated and the content of the stomach was collected and centrifuged. The volume of the gastric juice was measured and this was used for estimation of free acidity, total acidity, pepsin content and total proteins and mucin content was determined. The stomach was cut open along the greater curvature and the ulcer index, ulcer score was determined[22-24].

### Indomethacin induced ulcers

The gastric ulcers will be induced by administering Indomethacin (5mg/kg.p.o.) for 5 days to the normally fed rats. The animal was treated with the corresponding drug orally for 5 days. On the 4th day animal was kept for fasting for 24 hr. on the 5th day animal was administered Indomethacin 25mg/kg to induce ulcer. Animal was sacrificed after the 6 hrs of the last dose of Indomethacin. Animal was sacrificed by cervical dislocation method. The stomach was removed and gastric juice was collected, they were cut open along the greater curvature and the ulcer index ulcer score was determined. The granular portion of the stomach was taken and used for estimation of mucin content, total proteins, and anti-oxidant factors like super oxide dismutase activity and catalase activity[25,26].

### Stress induced gastric ulcers

The anti ulcer activity of plant extract was investigated by water immersion stress induced model. Rat was fasted for 24 hr. on the test day respective test substances was administered...
orally to the rats and was subjected to swimming for 3 hr in a standard glass cylinder (height 45cm, diameter 25cm with water up to 35cm). After 3hr, rat was sacrificed by cervical dislocation method, stomach was excised, and cut opened along the greater curvature. The ulcer index and ulcer score was determined[27,28].

Methods for Biochemical estimations like free acidity, total acidity, mucin content, total proteins and pepsin secretion in gastric juice [22-25]

Collection of Gastric juice

Gastric juice was collected from pylorus-ligated rats as mentioned earlier. The gastric juice collected was centrifuged for 1000 rpm for 10 minutes and the volume of gastric juice was measured. This gastric juice was used for biochemical estimations as follows.

Determination of free acidity and total acidity

Gastric juice (1ml) was pipetted out and was diluted to 10 ml with distilled water, to this 2-3 drops of Topfer’s reagent was added and titrated with 0.01 N sodium hydroxide until it turns to orange colour (end point). The volume of alkali added was noted. This volume corresponds to free acidity. Then 2 to 3 drops of phenolphthalein solution was added and titration was continued until a definite red tinge reappears. The volume of alkali added was noted which corresponds to total acidity[22-23].

Acidity = volume of NaOH x Normality of NaOH x 100 mEq/litre/0.1

Estimation of pepsin

Pepsic activity was determined by a modification of the method of Anson (1938). For estimation of pepsin, placed 4 test tubes (1) and (2) containing 5ml of substrate, (3) And (4) containing 10ml of TCA. The gastric juice was mixed with an equal volume of 0.01M hydrochloric acid, warmed to 370 C, 1ml of this mixture was added to each of test tubes of (1) and (4). Incubated for 15 min at the end mixed tube (1) with (3). Allow to stand for about four minutes. (1) + (3) give test and (2) + (4) give blank. The mixture was filtered. To 2ml of the filtrate, 10 ml of NaOH was added. Then 1 ml of phenol reagent was added and mixed by gentle rotation. After 30 minutes, the absorbance was measured at 680 nm. The difference between test and blank gives the measures of pepsic activity[24].

Estimation of total proteins

The method is based on the formation of colored complex of proteins on addition of Folin Ciocalteau reagent that can be measured by colorimetric method at 610 nm using Bovine serum albumin as the standard. To 0.1 ml of gastric juice, 0.9 ml of 90 % alcohol was added and centrifuged at 3000 rpm for 10 minutes. The supernatant was discarded and precipitate was dissolved in 1 ml of 0.1N NaOH. 0.05 ml of the above solution was taken in a test tube, to which 4 ml of freshly prepared alkaline mixture was added and allowed to stand for 10 minutes. To the above reaction mixture 0.4 ml of phenol reagent was added and allowed to stand for further 10 minutes for the reaction to complete. The absorbance was measured at 610 nm using distilled water as blank. 0.1 ml of standard was taken in a test tube and processed as mentioned in step 2&3. The amount of protein was calculated using the formula: Protein = O.D of sample x concentration of standard (mg/ml) / O.D of standard [29].

Estimation of mucin

After the collection of gastric juice, the glandular portion of the stomach was excised and opened down the lesser curvature. The weight of the tissue is noted. The everted stomachs were soaked for 2 hrs in 10 ml of 0.1% Alcian blue 8GX dissolved in 0.16 M sucrose solution buffered with 0.05 M sodium acetate. Uncomplexed dye was removed by two successive washes with 10 ml of 0.25 M of sucrose for 15 minutes and 45 minutes. Dye complexed with mucus was diluted by immersion in 10 ml aliquots of 0.5 M magnesium chloride for two hours. The resultant solution was vigorously shaken with an equal volume of diethyl ether and the emulsion was then centrifuged at 3000 rpm for 10 min. The absorbancy of the aqueous layer was measured against a buffer blank at 580 nm. The quantity of blue dye recovered per gram of wet glandular tissue was then calculated from a standard curve[25].

Anti oxidant enzyme determination in stomach homogenate

Superoxide dismutase and Catalase are the endogenous enzymes that counteract the free generated radical during gastric damage. Estimation of these enzymes has been reported for the assessment of gastric function. In the present study, levels of these anti oxidant enzymes were estimated in stomach homogenates of animals treated with Indomethacin using standard method. The enzyme levels were expressed in terms of units/mg of proteins.

Preparation of tissue homogenate

Rats were sacrificed using anesthesia and the stomach was dissected out. The glandular portion of the stomach was perfused with cold normal saline. 250 mg of stomach tissue was sliced and was homogenated in 5 ml of 0.25% sucrose in phosphate buffer pH 7.4 and the homogenate was ceentrifused at 3000 rpm for 10 min. the supernatant was used for estimation of super oxide dismustase and glutathione. 250 mg of tissue was sliced and was homogenated in 5 ml of cold 0.15 M KCl and the tissue homogenate was centrifused at 800 rpm for 10 min. the supernatant was collected used for estimation of proteins and catalase.
**Estimation of superoxide dismutase**

Estimation of SOD is based on the detection of $O_2^-$ be auto-oxidation of hydroxylamine hydrochloride producing nitrite, which is measured colorimetrically at 560 nm. The value of SOD was calculated in term of units defined as the amount of SOD that inhibits the reduction of nitroblue tetrazolium (NBT) by 50 %. NBT is reduced by auto-oxidation of hydroxylamine and nitrite is produced in presence of EDTA, which can be detected colorimetrically.

100 µl of stomach homogenate in 0.2 M sucrose in 0.25 M phosphate buffer of pH 7.4 was taken in a test tube to which 1ml of sodium carbonate solution, 0.4 ml of NBT and 0.2 ml of EDTA was added and zero minute reading was taken at 560 nm. The reaction was initiated by adding 0.4 ml of 1 mM hydroxylamine hydrochloride to the above test tube. The reaction mixture was incubated at 25°C for 5 minutes during which the NBT gets reduced and was measured at 560 nm. A parallel control without stomach homogenate was also treated in similar manner as test. One enzymatic unit of SOD is the amount in the form of proteins present in 100 µl of serum required to inhibit the reduction of 24 mM NBT by 50% and is expressed as units /mg of protein. The concentration of the enzyme is calculated using the formula [30].

**Estimation of catalase**

The rate of decomposition of $H_2O_2$ was measured spectrophotometrically at 240 nm. Catalase catalyzes following reaction:

$$2H_2O_2 \rightarrow 2H_2O + O_2$$

$$ROOH + AH_2 \rightarrow H_2O + ROH + A$$

Catalase was estimated by determining the decomposition of $H_2O_2$ at 240 nm in an assay mixture containing phosphate buffer (0.25 M, pH 7). 100 µl of stomach homogenate in 0.15 M KCl buffer was added to test tube containing 1/9 ml of 0.25 M phosphate buffer at pH 7 and absorbance was measured at 240 nm. To the above reaction mixture 1ml of hydrogen peroxide was added and the sample was allowed to stand for 1 minute and the absorbance was measured at 240 nm using phosphate buffer as blank solution.

One international unit of catalase utilized is that amount which catalyzes the decomposition of 1mM hydrogen peroxide per minute at room temperature and is expressed in terms of units/mg of protein [31].

The catalase was calculated using the following formula;

$$\text{Units/mg} = \frac{A_{240}/\min \times 1000}{43.6 \times \text{mg of enzyme/ml reaction mixture}}$$

**Histopathological Studies**

Sections of tissue from stomachs were examined histopathologically to study the antiulcerogenic activity of *boerhaavia diffusa*. The gastric samples were fixed in 10% buffered formalin for 24 hr and afterwards processed using a tissue processor. The processed tissues were embedded in paraffin blocks and about 5-µm thick sections were cut using a rotary microtome. These sections were stained with hematoxylin and eosin. The slides were then examined microscopically for pathomorphological changes such as congestion, hemorrhage, edema and erosions.

**Statistical analysis**

The statistical significance was assessed using one-way analysis of variance (ANOVA). For comparing nonparametric ulcer scores, ANOVA followed by tukey test was used. The values are expressed as mean ± SEM and $p<0.05$ was considered significant.
Effect of *Boerhaavia diffusa* on ethanol induced and cold restraint stress induced ulcer

Both the doses of *Boerhaavia diffusa* showed a significant reduction in ulcer index when compared to control. Omeprazole showed more reduction in ulcer index than *Boerhaavia diffusa* in ethanol induced and cold restraint stress induced ulcer models respectively.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Ulcer index</th>
<th>Ulcer score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.556 ± 0.03</td>
<td>0.730 ± 0.18</td>
</tr>
<tr>
<td>LBDE</td>
<td>1.671 ± 0.02*</td>
<td>0.424 ± 0.71***</td>
</tr>
<tr>
<td>HBDE</td>
<td>2.686 ± 0.02**</td>
<td>0.174 ± 0.68***</td>
</tr>
<tr>
<td>OMPZL</td>
<td>2.303 ± 0.03***</td>
<td>0.080 ± 0.48***</td>
</tr>
<tr>
<td>LBDE+OMPZL</td>
<td>0.353 ± 0.03***</td>
<td>4.303 ± 0.48***</td>
</tr>
<tr>
<td>HBDE+OMPZL</td>
<td>0.303 ± 0.01***</td>
<td>2.303 ± 0.56***</td>
</tr>
</tbody>
</table>

Effect of *Boerhaavia diffusa* extracts & Omeprazole on pylorus ligation induced gastric ulcers

Both the doses of *Boerhaavia diffusa* showed a significant reduction in ulcer index when compared to control. Omeprazole showed more reduction in ulcer index than *Boerhaavia diffusa*. Effect on free acidity, total acidity, ulcer index, ulcer score, mucin content, and pepsin content and total proteins in pylorus ligated rats.

**Discussion**

The present study was done to evaluate the interaction of *Boerhaavia diffusa* with Omeprazole in experimentally induced ulcers.
The gastric ulcer was induced by different models viz; Indomethacin induced ulcer, pylorus ligation induced ulcers and water immersion stress induced ulcers model.

Indomethacin and NSAIDs produces erosions and ulcers in the G.I.T of experimental animals. A layer of mucus that apparently forms a barrier covers the gastric mucosa. The gastric mucus production is stimulated by prostaglandins. The administration of Indomethacin results in the production of gastric mucosal damage mainly in the glandular portion of the stomach. Indomethacin is known as a prominent inhibitor of prostaglandin synthesis that in turn damages the mucosal barrier; the damage in the mucosal barrier causes the permeation of sodium ions from the mucosa in to the lumen[32].

The cytoprotective agent prevents the ulcers induced by Indomethacin. The plant extract and the combination with omeprazole were effective in reducing ulcer index and ulcer score. This showed the cytoprotective activity of B.diffusa. B.diffusa was also effective in increasing gastric mucus content and activities of endogenous antioxidant enzymes such as SOD and catalase. Hence it can be suggested that cytoprotective effect in Indomethacin induced gastric ulcers may be due to both increase in cytoprotective mucus secretion and antioxidant property of Boerhaavia diffusa. The gastric anti secretory effect was evaluated in pylorus ligated rats. The ligation of pyloric end of the stomach causes accumulation of gastric acid and pepsin in the stomach leading to development of ulcers. Boerhaavia diffusa roots extract and Omeprazole reduced the secretion of gastric cytoprotective factor mucin.

Stress induced ulcers are probably mediated by histamine release with enhancement in acid secretion and a reduction in mucus production. There is also an increase in gastrointestinal motility, which causes folds in the gastrointestinal tract that comes in contact with leading to induction of gastric ulcers and stress also brings central nervous system into play. Agents that decrease the G.I motility and that have central actions are helpful in reducing the ulcers due to stress full conditions. The extract of Boerhaavia diffusa roots and Omeprazole was effective in reducing the ulcers induced due to stress. This suggests that some of the constituents present in the extract of Boerhaavia diffusa roots may have central actions, which are helpful in the reducing the gastric ulcers or the reduction may be due to local effect on gastric motility or gastric secretion[33].

Conclusion

The Boerhaavia diffusa root extract and its interaction with Omeprazole showed effective decrease in the development of gastric ulcer secretion in pylorus ligation induced gastric ulcers, indomethacin induced gastric ulcers; stress induced gastric ulcers and duodenal ulcers induced in rat. The extract was also found to be effective in increases the healing of gastric ulcer, the high dose and interaction of Boerhaavia diffusa with Omeprazole shows more effective in healing. The anti ulcer effect of Boerhaavia diffusa root extract is due to reductions in both gastric acid secretion and gastric action.

Acknowledgement

The authors are sincerely thankful to Green Chem, Bangalore, India, for providing the gift sample of Boerhaavia diffusa extract.

Conflict of interest statement

We declare that we have no conflict of interest.

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