INTRODUCTION

Gastric acid related disorders including peptic ulcer disease are common clinical problems. Important advances have occurred over the past 35 years that have improved the understanding and management of these disorders and reduced the need for surgery. Although *H. pylori* plays a crucial role in the pathogenesis and relapse of peptic ulcer disease, effective gastric acid suppression is required in addition to antibiotic therapy for prompt ulcer healing and *H. pylori* eradication. Gastric acid also plays a pivotal role in the development of gastroesophageal reflux disease (GERD). Control of gastric acid therefore remains the cornerstone of effective management of these disorders. Treatment of peptic ulcer disease has evolved from dietary modifications and surgery to acid suppression with antacids, H2 receptor antagonist, proton pump inhibitors and eradication of *H.pylori* infection.

The term peptic ulcer disease refers to spectrum of disorders that include gastric ulcer, pylori channel ulcer, duodenal ulcer and post operative ulcers at or near the site of surgical anastomosis. *H.pylori* has been recognized as the main cause of chronic gastritis, peptic ulcer disease, gastric adenocarcinoma and gastric mucosa associated with lymphoid tissue (MALT lymphoma). Proton pump inhibitors and histamine 2 receptors antagonists are currently the most effective pharmacological treatments for peptic ulcer disease when used in combination with antibiotic.

A number of drugs are now available for treatment of peptic ulcer disease. These drugs are broadly classified into two, those that decrease or counter acid secretion and those that afford cytoprotection by virtue of their effects on mucosal defensive mechanisms. Although these drugs have brought remarkable changes in the ulcer therapy, the efficacy of these drugs is still debatable. Reports on clinical evaluation of these drugs show that there are incidences of relapse and adverse effects and danger to drug interaction during ulcer therapy. A number of novel drugs are also under investigation for reflux peptic ulcer disease. These include transient lower oesophageal sphincter relaxation reducing agents, serotoninergic agents, potassium competitive acid blockers, mucosal protectants, histamine 3 agonists and antiagastin agents.

The search to find a suitable palliative and/or curative agent for the treatment of peptic ulcer disease has also been extended to herbal...
D. Priyadarshini et al. / Journal of Pharmacy Research 2016,10(6),442-449

442-449

The present study aims to verify the claims of *Syzygium alternifolium* as antiulcer agent as mentioned in traditional system of medicine.

MATERIALS AND METHODS

Drugs and chemicals:
The Indomethacin was obtained from Dr. Reddy’s laboratories, India, ethanol from Changshu Yangshuo chemical, China, Ranitidine from Kopran Pharma Ltd., Mumbai and all other chemicals used in this study were obtained from Sd-fine.

Plant material:
The leaves of *Syzygium alternifolium* were collected locally during the month of July. The plant was identified and authenticated by Dr. K. Madhava Chetty, Dept. of Botany, Sri Venkateswara University, Tirupathi. A voucher specimen of the plant is deposited in Indian Pharmaceutical Technology, Sri Padmavathi Mahila Viswavidyalayam, Tirupathi.

Preparation of extract:
Fresh leaves were collected, shade dried and powdered mechanically. About 150gm of the leaf powder was extracted with 1000ml of 95% ethanol by reflux heating over water bath. The extract was concentrated in Vacuo and then air dried at room temperature, weighed and percentage yield was calculated and stored in air tight container in a dessicator until used.

Phytochemical screening:
Preliminary phyto chemical screening of the powdered leaves was performed for the presence of flavanoids, tannins and poly phenols.

Experimental animals:
Albino rats of wistar strain of either sex weighing between 150-200gms were used. They were housed in polypropylene cases at room temperature (25±2 °c) and provided food and water ad libitum.

Acute oral toxicity studies:
Healthy adult male albino rats were fasted overnight with free access to drinking water. They were divided into five groups each consisting of six animals. Group-1 animals were treated with distilled water (2ml/kg/p.o) and Group-2 to Group-5 animals received 500 mg, 1 gm, 2 gm, 4 gm/kg/p.o of ethanolic extract of leaves of *Syzygium alternifolium* respectively.

The animals were observed continuously for 2 hours, then intermittently and at the end of 24 hours. The numbers of the deaths were noted to calculate LD$_{50}$.

Gross behavioral changes:
The animals were observed for behavioural, neurological and autonomic profiles during acute toxicity studies.

**Evaluation of antiulcer activity:**
In all the experimental models the animals were divided into 4 groups of 6 animals each. Group-I received saline which served as control, group-II received standard drug (Ranitidine) dose 150 mg/kg, group-III received low dose of drug (1 gm/kg) and group-IV received high dose of drug (2 gm/kg). The treatment schedule is shown in the table 1.

**Table 1: Treatment Schedule for Evaluation of Anti Ulcer Activity**

<table>
<thead>
<tr>
<th>Group</th>
<th>Drug</th>
<th>Dose</th>
<th>Purpose</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Saline</td>
<td>2 ml</td>
<td>To serve as disease control</td>
</tr>
<tr>
<td>II</td>
<td>Ranitidine</td>
<td>150 mg/kg</td>
<td>To serve as standard</td>
</tr>
<tr>
<td>III</td>
<td>EESA</td>
<td>1 gm/kg</td>
<td>To assess the antiulcer activity</td>
</tr>
<tr>
<td>IV</td>
<td>EESA</td>
<td>2 gm/kg</td>
<td>To assess the antiulcer activity</td>
</tr>
</tbody>
</table>

EESA: Ethanolic Extract of *Syzygium alternifolium*

I. Pylorus ligation in rats (shay rat)
A simple and reliable method for production of gastric ulceration in the rat was based on ligature of the pylorus. The ulceration was caused by accumulation of acidic gastric juice in the stomach. Ulcer index and acidity of the gastric content of treated animals are compared with controls. One hour after drug or saline administration under light ether anesthesia the abdomen was opened by small midline incision below the xiphoid process, pyloric portion of the stomach was slightly lifted out and ligated avoiding traction to the pylorus or damage to its blood supply the stomach is replaced carefully and the abdominal wall closed by interrupted sutures. Nineteen hours later, the pylorus ligated rats were sacrificed by ether over dosing and their stomachs were dissected out after ligating the esophagus at cardiac end. Each stomach was cut opened along the greater curvature and the contents were collected into a centrifuge tube, then the mucosa was washed under slow running tap water and the number and size of ulceration was scored as per the method.

The stomach was removed and fixed on a cork plate and the number

The following parameters were measured:

1) Ulcer index
The stomach was removed and fixed on a cork plate and the number
and severity of the ulcers was registered with a stereo-microscope using the following sources, Severity score: 0=normal coloured stomach, 0.5=red colouration, 1=spot ulcer, 1.5=hemorrhagic streaks, 2=ulcer<3 buit*5.3=ulcers>5; Ulcer index was calculated as; U1=UN+US+UP×10⁻¹ Where, U1=ulcer index, UN=average of number of ulcers per animal, US=average of severity score, UP=percentage of animals with ulcer.

2) Determination of total acidity
A known amount of gastric residue was titrated with 0.1N NaOH. Two drops of methyl orange reagent was added which changes to a salmon colour when all the free hydrochloric acid is neutralized. The total acidity was determined by titration using phenolphthalein as indicator.

Reading was taken (ml NaOH) for total acidity.

Y=ml of 0.1N NaOH × 10 Where, Y=total acidity (m Eq/L)

3) Acid volume
The stomach was removed and the contents were drained into a graduated centrifuge tube through a small nick along the greater curvature adjacent to pyloric ligation.

The volume of the juice was measured.

4) pH
1) The contents were drained into a graduated centrifuge tube.
2) The tubes were centrifuged at 3000rpm for 10 minutes and the centrifuged samples were decanted and analysed for pH (Using broad range pH paper).

II. Ethanol induced mucosal damage in rats (cytoprotective activity)
Intragastric application of absolute ethanol is a reproducible method to produce gastric lesions in experimental animals. These lesions can be at least partially inhibited by various drugs, such as prostaglandins. The protective effect against various irritants has been called cytoprotective activity.

The rats were fasted for 48 hours before the experiment but excess water allowed and just two hours before starting the experiment the water also was removed. Thirty minutes after their pretreatment with the drugs as mentioned in table 5. Then the rats were placed vertically in individual restraint cages in water at 22°C for one hour. Then they were removed, dried and injected with 30mg/kg Evans blue via the tail vein, ten minutes later they were sacrificed in ether anaesthesia and their stomachs were removed after ligating both the ends. Formal saline (2% V/V) is then injected into the totally ligated stomachs for storage over night, the next day the stomachs were opened along the greater curvature, washed in warm water and examined under a 3-fold magnifier and the pH of gastric juice, Ulcer index, Severity score parameters were measured as mentioned earlier.

RESULTS:
Plant material-qualitative chemical tests
The preliminary phytochemical screening of EESA showed presence of flavonoids and tannins.

Pharmacological studies
a) Acute toxicity studies
The EESA was found to be safe since no animal died even at the
maximum single dose of 4gm/kg body weight when administered orally.

b) Gross behavioral changes
The animals did not show any gross behavioral changes.

c) Antiulcer studies

1) Effect of the EESA on pylorus ligation induced gastric ulceration
In pylorus ligated rats, oral administration of Syzygium alternifolium showed significant (p<0.001) reduction in ulcer index as compared to control group. Activity of EESA preventing in the formation of lesions corresponded with that of ranitidine. Volume of gastric contents was significantly (p<0.001) by ethanolic EESA when compared to control group pH was increased significantly by administration of EESA (p<0.001) when compared to control group (table 2). Antiulcer activity of the EESA was equipotent with Ranitidine.

Table 2: Effect of EESA on various parameters in pylorus ligation model.

<table>
<thead>
<tr>
<th>Drug and Dose</th>
<th>pH</th>
<th>Total Acidity mEq/lt</th>
<th>Free Acidity mEq/lt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline 2ml</td>
<td>2.23±0.12</td>
<td>103.3±1.30</td>
<td>87.3±0.52</td>
</tr>
<tr>
<td>Ranitidine</td>
<td>5.83±0.18*</td>
<td>40.0±0.54*</td>
<td>20.2±0.04*</td>
</tr>
<tr>
<td>150mg/kg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EESA 1gm/kg</td>
<td>4.38±0.15*</td>
<td>53.83±1.60*</td>
<td>46.7±0.16*</td>
</tr>
<tr>
<td>EESA 2gm/kg</td>
<td>5.73±0.08*</td>
<td>40.17±0.54*</td>
<td>21.5±0.05*</td>
</tr>
</tbody>
</table>

II) Effect of the EESA on ethanol induced Gastric ulceration
In control animals, oral administration of absolute ethanol produced characteristic lesions in the glandular portion of rat stomachs. These necrotizing agents produced severe gastric damage visible from the outside of the stomach as dark lines. Upon opening the stomach, elongated bands of thick, black and dark red lesions were found in the mucosa. In animals pre-treated with EESA significantly (p<0.001) inhibition of gastric ulceration was observed when compared to control group (Fig. 1.). EESA caused significant (p<0.001) reduction in ulcer index and severity score when compared to control. pH was increased significantly by EESA was found to be less compared to ranitidine (Fig. 2.).

III) Effect of the EESA on Indomethacin induced gastric ulceration
Indomethacin treatment resulted in the production of gastric lesion, mainly in the glandular segments of the stomach. EESA caused significant (p<0.001) reduction in ulcer index and severity score when compared to control. pH was increased significantly by EESA was found to be less compared to ranitidine (Fig. 2.).

IV) Effect of the EESA on stress induced gastric ulceration
A significant (p<0.001) gastric protective action was observed on treatment with EESA. This was evidenced by reduction in ulcer index and severity score when compared to control group. pH was increased significantly by EESA (p<0.001) when compared to control group (table 4). Activity of the EESA in preventing the formation of lesions was comparable to that of Ranitidine (Fig. 3.).
DISCUSSION:

Peptic ulcer is one of the common diseases in human population. Its incidence is increasing due to rapid development and civilization constraints. Peptic ulcer is erosion in the lining of the stomach or duodenum (the first part of the small intestine). The word peptic refers to pepsin, a stomach enzyme that breaks down proteins. The ulcer is mainly considered as an imbalance between aggressive factors (acid, pepsin, H. pylori and NSAIDS) and local defense factors. Integrity of gastroduodenal mucosa is maintained between these aggressive and defensive factors. The major complications of peptic ulcer are bleeding, perforations, penetrations, obstruction and malignancy.

The management of peptic ulcer has undergone many strides over past few years and number of drugs is now available for treatment. Many of these drugs are assumed to ultimately balance the aggressive factors and defensive factors. They suffer mainly from incidences of relapses, adverse effects and Danger of drug interactions during the ulcer therapy. Hence the search for an ideal antiulcer drug continues and has also been extended to herbal drugs in search for new and novel molecules that can afford better protection and decrease the incidences of relapse.

The pathogenesis of peptic ulcer is multifactorial. Various animal models have been proposed to induce peptic ulcer in experimental animals by numerous investigators from time to time, which include shay rat model, cytoprotective models, anti secretory models that tries to mimic peptic ulcer conditions in humans. But there has been only a limited success in developing an ideal animal model of peptic ulcer that can include all the factors leading to ulcer development. Hence the selection of experimental models for screening antiulcer compounds must meet the following criteria.

1. They should be simple, reproducible and allow for easy quantification of results.
2. They should make use of a variety of animal species.
3. They should induce characteristic ulceration in specific locations (stomach and duodenum)
4. They should involve different mechanisms by which ulceration is produced.
5. The ulcers induced should not spontaneously heal during the observation period.

A large number of animals are necessary to generate dependable and reproducible data when evaluating antiulcer activity of new compounds. This factor restricts the choice of animals especially to the rats. To some extent guinea pigs, cats, dogs have also been used to a very limited measure in order to minimize the special variation by many investigators.

The rat stomach shows an obvious division into two parts. The upper two fifth non secretory portion (lumen) is translucent and thinner than the lower three fifth glandular secretory portion which is analogous to the body of the stomach in man both anatomically and functionally. The rat being omnivorous resembles man nutritionally. It is advisable to use adult rats of either sex for the antiulcer studies.

In the present study Ranitidine (ZANTAC) was used as standard drug. It is H2 receptors antagonist. It acts by inhibiting acid production by reversibly competing with histamine for binding to H2 receptors on the basolateral membrane of parietal cells.

The present study has been done for the investigation of antiulcer effect of EESA in pylorus ligation model, ethanol-induced, Indomethacin and stress induced models of gastric ulceration.

In pylorus ligation model, digestive effect of the accumulated gastric juice is believed to be responsible for producing ulcers, in addition gastric acid secretion reflex or neurogenic effect has also been suggested to play an important role in the formation of gastric ulcers. The causes of gastric ulcer after pyloric ligation are also believed to be due to stress induced increase in gastric hydrochloric acid secretion and or stasis of acid. The volume of secretion is also an important factor in the formation of ulcer due to exposure of the unprotected lumen of the stomach to the accumulating acid. Gastric acid secretion is mainly under vagal control and over activity of vagus also contributes to ulcer formation in the pylorus ligation model. Vagal stimulation increases acetylcholine that acts directly on the muscarinic receptors on parietal cells and secretes...
hydrochloric acid through a calcium dependent pathway. In the earlier studies have shown that the protective effect of Verapamil and Nifedipine against peptic ulcers in pylorus ligation model may partly be due to inhibition of this calcium dependent pathway. The gastric antisecretory effect of Amlodipine was also proposed due to a similar mechanism.

The EESA has significantly increased the gastric pH and also exhibited a significant decrease in both total acidity and also volume of gastric content in pylorus ligation model. The reduction in acid output, peptic activity and increase in mucin secretion were the major mechanisms behind the protection shown in the pylorus ligation model by various plants like Rhamnus procumbens and Datura fastuosa. So the plant Syzygium alternifolium may also be exhibiting potential antisecretory effect by similar mechanisms. Anyhow the exact mechanism need to be further investigated.

Ethanol induced gastric lesions occur probably due to the stasis in gastric blood flow produced because of vascular congestion and mucosal capillary necrosis which contribute to the development of haemorrhagic and necrotic aspect of tissue injury. It is also reported to produce pathogenicity by damage to gastric mucosa, alteration in permeability, gastric mucus depletion and free radical production, ethanol increases superoxide anion and hydroxyl radical production and lipid peroxidation in the gastric mucosa.

Ethanol induced gastric damage may be due to a direct action on the gastric epithelium causing lipid peroxidation. Ethanol treatment induces intracellular oxidative stress and produces mitochondrial permeability transition and mitochondrial depolarization such as glutathione may have a significant protective action against ethanol in gastric mucosal cells.

Ethanol is involved in the formation of free radicals generated extracellularly and or intracellularly. Because intragastric administration of superoxide dismutase was able to protect the gastric mucosa against the damaging effect of ethanol. This would suggest the involvement of superoxide free radicals in the pathogenesis of ethanol induced gastric mucosal damage.

In the present study oral administration of EESA markedly reduced ethanol induced gastric mucosal damage. The cytoprotection offered here may be due to the significant antisecretory property of Syzygium alternifolium can be attributed partially to decrease in acid secretion and enhancement of mucin activity. However more investigations need to be done to elucidate the exact mechanism of protection.

Indomethacin was used in the present study to mimic ulcers produced by NSAIDs. Indomethacin is a non selective cyclooxygenase inhibitor that causes more gastric damage when compared to the other NSAIDS. Indomethacin was shown to reduce synthesis of gastroprotective PGE2 which is necessary to maintain blood flow to gastric mucosa and also play an important role in mucus production. Prostaglandins are known to play an important role in maintaining mucosal integrity. An increase in certain endogenous prostaglandins can enhance gastric mucosal integrity. An increase in certain endogenous prostaglandins can enhance gastric mucosal resistance to ulcerogenic agents. The mechanisms involved in the action of prostaglandins are multiple including stimulation of mucus and bicarbonate output.

In the present study the EESA has significantly reduced the ulcer index in rats that received Indomethacin. This might be due to the enhanced growth of gastric mucosa, increased prostaglandin synthesis and also altered mucosal permeability. Anyhow in the present study direct effect on prostaglandin biosynthesis has not been evaluated.

Stress-induced ulcers are probably mediated by the release of several biogenic amines and metabolites of arachidonic acid by lipoxygenase with enhancement of acid secretion and reduction of mucus production. Disturbances of gastric mucosal microcirculation and abnormal motility also have been considered to be the pathogenic mechanisms responsible for stress-induced gastric mucosal lesions. Stress can also produce consistent disturbance in acid secretion, bile and pancreatic juice reflux which are important factors leading to ulcer formation. Emotional stress was reported to decrease both quality and quantity of mucus of the ribosomal peptides which cause loss of integrity of the mucosal membrane.

On administration of EESA a significant inhibition in ulcer was observed. The ulcerative index was significantly lower in the Syzygium alternifolium treated groups when compared to the control in stress induced model. In this study EESA significantly protected the gastric mucosa against injury induced by stress. These results indicate that Syzygium alternifolium may enhance gastric mucosal defensive factors.

In the present study it may be concluded that the antiulcer activity and significant antioxidant property of EESA may be due to the presence of flavanoids. The exact mechanism underlying the antisecretory and gastro protector activity of Syzygium alternifolium against pylorus ligation, ethanol induced, stress induced and Indomethacin induced models are unclear. Further studies using...
more specific methods are required to explore the active compounds responsible for the protective effect and also to establish the mechanism of this activity. Chronic toxicity studies may also be rewarding.

CONCLUSION:
Peptic ulcer disease is a chronic inflammatory condition involving a group of disorders characterised by ulceration in the region of upper gastrointestinal tract where parietal cells secrete pepsin and hydrochloric acid.

In the indigenous system of medicine, the plant Syzygium alternifolium is claimed to be useful in diabetes, stomach ache, gastric ulcers, rheumatic pains and bacillary dysentery. There are no systemic pharmacological studies on this plant to verify its anti-ulcer activity. Hence in the present study ethanolic extract of the leaves of Syzygium alternifolium was evaluated for its anti-ulcer activity.

The EESA was evaluated for LD₅₀, gross behavioural changes, anti-ulcer activities. The anti-ulcer activity was evaluated by pylorus ligation in rats (shay rat model), stress ulcers by cold water immersion, Indomethacin induced ulcers and ethanol-induced mucosal damage models. The parameters monitored in the present study are ulcer index, total acidity, pH and severity score.

The EESA was safe orally and exhibited no gross behavioural changes. The EESA showed anti-secretory and cytoprotective effect as a significant decrease in ulcer index, acid volume, total acidity, free acidity and significant increase in gastric pH were observed.

The results obtained in this study proved the efficacy of the leaf extract of Syzygium alternifolium as anti-ulcer agent, supporting the claim made in the indigenous system of medicine.

REFERENCES:

31. Hernandez Munoz, R., Montiel Ruiz, C. and Vazquez Martinez, O. Gastric mucosal cell proliferation in ethanol induced chronic mucosal injury is related to oxidative stress and lipid peroxidation in rats, laboratory investigation.,2000, 8: 1161-1169.

Source of support: Nil, Conflict of interest: None Declared