INTRODUCTION

Reactive oxygen species (ROS) have thus been regarded as highly toxic agents responsible for a wide variety of tissue damage. Recently interest has been focused on the role of ROS in gastroduodenal pathogenesis related to gastric hypersecretion and gastroduodenal mucosal damage. ROS has been implicated in gastric mucosal damage by non-steroidal anti-inflammatory drug (1). The lipids content of mucus gel, along with its renewable quality, appear to play a major role in the inherent resistance of the mucosa to injury (2). While in gastric disease condition, imbalance in lipid metabolism occurs, leading to disturbance of the mucosal integrity.

Recently attention has been focused towards the polyherbal formulations, which are traditionally used as potential therapeutic agents in the prevention and management of gastrointestinal disease. Due to side effects of the available therapeutic agents, attempts were and are being made to look for the natural products
of plants for the treatment of gastric ulcer (3). The present work has been undertaken to study the effect of ambrex, an amber based formulation on experimental gastric ulceration and their possible mechanism of antiulcer action.

The herbal formulation consists of six medicinal plants namely, Withania somnifera (100 mg), Pon amber (37.5 mg), Cycas circinalis (62.5 mg), Shalamisri (25 mg) and Roomi mastagi (25 mg). Amber is used as a medicine used by the sick to recover health and increase resistance to disease, stamina and endurance of athletes and for tremendous boost to their performance by increasing the energy in the human body (4). The plant herbs present in ambrex are mentioned in ayurvedic texts as a remedy for peptic ulcer (5). In addition, ambrex has been reported to possess free radical scavenging activity (6). With this information, ambrex has been evaluated to find out the antiulcer efficacy against aspirin induced lesions in rats.

METHODS

Chemicals

Aspirin was purchased from SRL, India. Ambrex (amber based formulation) was obtained as a gift from Care and Cure Ltd., Anna Salai, Chennai. All other chemicals used were of analytical grade.

Animals

Male albino rats weight 150–200 g were purchased from Tamil Nadu University of Veterinary and Animal Sciences, India. The animals were housed in polypropylene cages maintained in controlled temperature and light cycle with food and water ad libitum. The experiments were initiated only after the approval of the Institutes of Animal Ethics Committee.

Experimental study design

The rats were divided into 4 groups of 6 animals each as follows: Group I animals served as control. Group II animals received a single dose of aspirin (200 mg/kg) orally. Group III animals received aspirin 200 mg/kg orally on the 15th day of ambrex pretreatment. (A dose dependent study with ambrex revealed that it provides gastroprotection at doses ranging 10–40 mg/kg body weight of animal. 40 mg/µg of ambrex was found to be effective antiulcer dose, showed maximum protection).

Preparation of gastric mucosal homogenate

The stomach was removed and kept in ice-cold phosphate buffer (pH 7.2). It was cut along greater curvature and the scrapped mucosa was weighed and homogenized in ice-cold phosphate buffer. The homogenate was used for the assay of lipid peroxidation (7), protein carbonyl content (8), conjugated dienes (9), glutathione (GSH) content (10), superoxide, dismutase (SOD) activity (11), catalase (CAT) activity (12), glutathione peroxidase (Gpx) activity (13), total cholesterol (14), phospholipids (15), triglycerides (16) and free fatty acids (FFA) (17) were determined. Protein was estimated by the method of Lowry et al. (18).
**Statistical analysis**

The results were presented as the mean ± SD. Student's ‘t’ test was used to analyse statistical significance. P values less than 0.05 were considered significant.

**RESULTS**

Ambrex reduced lipid peroxidation, protein carbonyl content and conjugated dienes as compared to aspirin control. GSH content was found to decrease in gastric mucosa of aspirin administered rats when compared with the group I controls. The decreased content of GSH was restored to near normal in ambrex pretreated animals (Table I). Aspirin caused significant alteration in SOD, CAT and GPX activity, the important antioxidant enzymes of the mucosa. Ambrex caused an increase in the concentration of SOD, CAT and GPX activity when compared to aspirin administered groups (Table II). In gastric mucosa, significant alteration in lipid levels was observed in aspirin induced rats (Fig. 1). Pretreatment with ambrex significantly abated the levels of phospholipids and triglycerides when compared to aspirin control group.

**Fig. 1:** Effect of ambrex on lipid profile in aspirin induced rats.
Values are expressed as mean ± SD.

\( ^a P < 0.001 \) as compared to group I; \( ^b P < 0.001 \) as compared to group II.

**TABLE I**: Effect of ambrex on lipid peroxidation, protein carbonyl content and glutathione level of the gastric mucosa.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipid peroxidation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MDA content (nmol/mg protein)</td>
<td>0.42±0.03</td>
<td>0.68±0.4(^a)</td>
<td>0.51±0.03(^b)</td>
</tr>
<tr>
<td>Protein carbonyl content (nmol/mg protein)</td>
<td>1.56±0.09</td>
<td>2.78±0.17(^a)</td>
<td>1.75±0.11(^b)</td>
</tr>
<tr>
<td>Glutathione content (nmol/mg)</td>
<td>56.8±4.1</td>
<td>37.2±3.1(^a)</td>
<td>49.7±3.8(^b)</td>
</tr>
<tr>
<td>Conjugated dienes (µmol/100 g tissue)</td>
<td>0.37±0.03</td>
<td>0.65±0.06(^a)</td>
<td>0.42±0.04(^b)</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD.

\( ^a P < 0.001 \) as compared to group I; \( ^b P < 0.001 \) as compared to group II.

**TABLE II**: Levels of antioxidant status in gastric mucosa of control and experimental groups.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Superoxide dismutase (units/mg protein)</td>
<td>4.75±0.20</td>
<td>2.56±0.17(^a)</td>
<td>4.51±0.17(^b)</td>
</tr>
<tr>
<td>Catalase activity (µmoles of H(_2)O(_2)/mg of protein)</td>
<td>4.02±0.43</td>
<td>2.16±0.19(^a)</td>
<td>4.27±0.49(^b)</td>
</tr>
<tr>
<td>Glutathione peroxidase (nmole of GSH/mg of protein)</td>
<td>212±16.2</td>
<td>145±12.0(^a)</td>
<td>203±13.3(^b)</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD.

\( ^a P < 0.001 \) as compared to group I; \( ^b P < 0.001 \) as compared to group II.
DISCUSSION

The role of ROS in ulcer generation by various factors has recently attracted the attention of many investigators. Lipid peroxidation leads to loss of membrane fluidity, ion transport and membrane integrity of the surface epithelial cell and helps to generate gastric lesions (19). Aspirin caused significant increase in lipid peroxidation and protein carbonyl content with significant decrease in the mucosal glutathione level, indicating that lesions were due to oxidative damage caused by ROS. Pretreatment with ambrex prevented these alterations. In stress condition, ulcer is developed mainly due to oxidative damage by OH− generated from derangements of the antioxidant enzymes. Severe depletion of GSH affects the synthesis of 2 major cellular polymers, i.e. proteins ad DNA. Elevation of GSH status in gastric mucosal cells can be achieved by ambrex after aspirin administration.

Any compound, a natural or synthetic with antioxidant properties might contribute towards the partial or total alleviation of this type of damage. Several antioxidants of plant origin have been identified and used as protective agents against oxidative stress (20). In recent study, researchers revealed that Withania somnifera possess significant and potent antioxidant activity in rats (21). Withania somnifera is one of the herbal plant in polyherbal formulation, which might play a very important role in the antioxidant property of ambrex.

Stimulation of lipid influence lipid metabolism in a biological system. The lipids content apparently determines the degree of resistance of mucin to peptic degradation and contribute significantly to mucus viscosity, hydrophobicity and impedance to hydrogen ion diffusion (22). Aspirin reduces phospholipid concentrations, which leads to altered surface hydrophobicity and weakened gastric mucosal barrier. These lipids are also known to exert the greatest impact on the physiochemical characteristics of mucus. The results presented in this report demonstrated that intragastric administration of ambrex not only possess antioxidant property but also increase the lipid composition in the gastric mucosal surface when compared to the respective controls and there by providing antiulcerogenic efficiency.

Further study on this line might prove importance in the development of new and improved therapies for the treatment and prevention of peptic ulcers.

REFERENCES


