Antiulcer activity of *Rheum emodi*.

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**ABSTRACT**

**Plan:** The present study was designed to investigate the antioxidant and gastroprotective potential of ethanolic extract of *Rheum emodi*.

**Methodology:** Antioxidant and gastroprotective activity was evaluated using reserpine and ethanol-induced ulcer models. The ethanolic extract of the *Rheum emodi* (EERE) was given by oral route at a dose of 50 mg/kg/p.o. and 100 mg/kg

**Outcome:** Pre-treatment with EERE, dose dependently reduced the ulcer index and lesions with marked attenuation in the level of oxidative stress parameters estimated by TBARS, GSH, SOD and MPO. EERE administration increases the gastric adhesion mucus content in ethanol and reserpine-induced ulcer.

**Keywords:** Antioxidant Activity, gastroprotective activity, ethanolic extract of *Rheum emodi*

1. **INTRODUCTION**

For more than a century, peptic ulcer disease has been a major cause of morbidity and mortality. In clinical practice, Peptic ulcers are a common disorder of the entire gastrointestinal tract (GIT) that occurs due to imbalance between aggressive factors such as hydrochloric acid (HCL), pepsin, refluxed bile, leukotrienes (LTs), reactive oxygen species (ROS) and defensive factors, which include the function of the mucus-bicarbonate barrier, surface active phospholipids, prostaglandins (PGs), mucosal blood flow, cell renewal and migration, non enzymatic and enzymatic antioxidants and some growth factors.

Many synthetic drugs are available in the market for the treatment of ulcers but they are associated with various side effects. Plant kingdom thus emerged as a key target for the development of antiulcer drugs with lesser S/E. *Rheum emodi* is an important medicinal plant, which finds an extensive use in Ayurveda and Unani systems of medicine. *R. emodi* (Indian rhubarb) is commonly known as *Rewand chini* and belongs to family Polygonaceae. The plant is reported to have antibacterial, antifungal, antidiabetic, anticancer, antioxidant, hepatoprotective and immunostimulant activities.

The main constituents of the drug are found to be anthraquinone, tannins and gallic acid. Traditionally in the folk medicine the plant has been used in the treatment of ulcers but no evidence are available that reveal the role of *R. emodi* in augmenting ulcers.

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Hence, we aimed to explore the gastroprotective and antioxidant potential of EERE in ethanol and reserpine-induced ulcers.

2. MATERIALS AND METHODS

2.1 Plant material collection

*Rheum emodi* was collected from the herbal garden of Rayat institute of Pharmacy in the month of June. The plant was and authenticated by Dr. H. B. Singh, Chief Scientist and Head Raw Materials Herbarium &Museum (RHMD), National Institute of Science Communication and Information Resources (NISCAIR) New Delhi. The rhizome part was cut and washed with water. The rhizomes were then allowed to dry at room temperature and powdered to coarse powder using motar and pestle. 500 gm of the rhizome was put into the thimble of the soxhlet containg 100ml of 50% ethyl acetate and and extracted at a temperature of 60 - 90°C for three hours by soxhlet extractor. The plant extract was filtered through various layers of muslin cloth. The extracts were concentrated by distillation method to yield a crude semi-solid mass which was then dried and used.

2.2 Animals

The experimental protocol used in present study was approved by Institutional Animal Ethical Committee (IAEC). Age matched young wistar rats weighing 200-250 g were employed and were acclimatized in the animal house of our institute and exposed to natural light and dark cycle. The animals were fed on standard chow diet and water ad libitum.

2.3 Acute Toxicity study

The LD50 of the *R. emodi* was reported to be safe till 1000 mg/kg/i.p. Thus the studies were carried out by using two selected doses of ethanolic extract of *R. emodi* 50 mg/kg and 100 mg/kg. No death and side effects were found at both selected doses of plant.

2.4 Antiulcer activity

Antiulcer activity was assessed using 2 models: Ethanol-induced and Reserpine-induced ulcers. Animals were treated with omeprazole in ethanol induced model and Ranitidine in reserpine-induced model. At the end of each experiment, the animals were sacrificed by cervical dislocation; the stomach was removed and its gastric content was collected. Then stomach was opened along the greater curvature. The stomachs were stretched on a corkboard and the ulcer index was obtained according to scoring method of Suzuki as follows: Score 1: maximal diameter of 1mm; Score 2: maximal diameter of 1-2mm Score 3: maximal diameter of 2-3mm;Score 4: maximal diameter of 3-4mm;Score 5: maximal diameter of 4-5mm;Score 10: an ulcer over 5mm in diameter; Score 25: a perforated ulcer.

2.4.1. Antiulcer and antioxidant activity using Ethanol-induced ulcers

The animals were divided into five groups, each group consisting of six rats. Group 1 represented the normal control group, which received 5 ml/kg body weight of vehicle (0.5% carboxy methyl cellulose, p.o.). Groups II represented disease control in which 1 ml of ethanol eas administered to rats Group III was treated with omeperazole (20mg/kg, p.o). Group IV and V are treated with ethanol extract of rhizomes of *R.emodi (EERE)* orally at the doses of 50 and 100 mg/kg body weight. Ethanol (1ml) was administered after one hour for inducing ulcers.
Animals were sacrificed after 1h following the administration of absolute ethanol. Further, gastroprotective effect was too evaluated by estimating gastric mucus adhesion content.

2.4.2. Antiulcer and Antioxidant activity using Reserpine-induced ulcers

Overnight fasted rats were divided into 5 groups each comprising of 6 animals. Group I served as normal control. Group II served as reserpine control. Group II served as Ranitidine as standard control (50 mg/kg). Group III and IV served as treatment group in which rats were treated with EERE (50 mg/kg and 100mg/kg) orally. After one hour, Reserpine (10 mg/kg; i.p) was administered to all overnight fasted rats according to the method of Gupta. Animals were sacrificed after 20 hours following administration of reserpine and are employed for various estimations.

2.4.3. Study of antioxidant activity

The stomach was then weighed and homogenized in phosphate buffer (pH 7.4) at a concentration of 10% (w/v). The homogenates were centrifuged at 10,000 x g for 20 min. The clear supernatant was used for the assays of lipid peroxidation (TBARS), myeloperoxidase activity (MPO), endogenous antioxidant enzymes (superoxide dismutase and reduced glutathione (GSH)).

2.4.5. Determination of gastric adhesion mucus content

The glandular segments from stomachs which had been opened along their greater curvature were removed and weighed. Each segment was transferred immediately to 10 ml of 0.1% w/v Alcian blue solution (in 0.16 M sucrose solution, buffered with 0.05 M sodium acetate pH 5). After immersion for 2 h, excess dye was removed by two successive rinses with 10 ml of 0.25 M sucrose, first for 15 and then for 45 min. Dye complexed with the gastric wall mucus was extracted with 10 ml of 0.5 M magnesium chloride (MgCl2) by shaking intermittently for 1 min at 30 min intervals for 2 h. Four milliliters of blue extract were then shaken vigorously with an equal volume of diethyl ether. The resulting emulsion was centrifuged at 3600 rpm for 10 min and the absorbance of the aqueous layer was recorded at 580 nm. The quantity of Alcian blue extracted per gram of net glandular tissue was then calculated.

2.4.6. Assessment of integrity of stomach using Histopathological studies

The stomach was then dehydrated and immediately immersed in 10% buffered formalin. They were then dehydrated in the graded concentrations of ethanol, immersed in xylene, and then embedded in paraffin. From the paraffin blocks, 4-mm thin sections were cut, and staining is done using with haemotoxylin (0.6% w/v) for 15 min followed by counterstaining with eosin (1% w/v) for 2 min. They were then examined using light microscopy to analyze integrity of stomach, using an image analysis program (NIH Scion image analyzer).

2.4.7. Statistical analysis

All the results were expressed as Mean ± SEM. Statistical comparisons were made between drug treated groups and disease control rats. Data of biochemical parameters were analyzed using one way analysis of variance (ANOVA) followed by Tukey’s multiple range test. The p-value <0.05 was considered to be statistically significant.
3. RESULTS

3.1. Study of gastroprotective effect of Ethanolic extract of Rheum emodi on ethanol and reserpine-induced ulcers

A significant increase in the Ulcer index was (125.5 ± 9.13 and 53.17 ± 7.25) observed in ethanol and reserpine-administered rats respectively.

The maximum numbers of ulcers were of the score 4 and 5, and a number of perforated ulcers (score 25) were also observed. Treatment with EERE in ethanol and reserpine was found to produce significant decrease in ulcer index 8.667 ± 2 and 13.83 ± 2.07 at a dose of 100mg/kg with ulcer protection of 93% and 86.51% in a dose dependent manner respectively. Ranitidine and omeperazole produce significant (P<0.01) reduction in ulcer score with ulcer protection of 84.19% and 85.3% when compared with 100mg EERE. Further, diseased group (ethanol and reserpine) showed a marked attenuation in the release of mucin (P<0.001). Pretreatment with EERE showed a significant increase in the mucin content at a dose of 100mg/kg (p<0.001) in both the models.

However no significant effect was obtained at a dose of 50mg/kg dose (Table 1 and 2).

3.2. Antioxidant potential of R. emodi in ethanol and reserpine-induced ulcers

Ethanol and reserpine control group was found to increase lipid peroxidation estimated by TBARS with attenuation in the level of SOD and GSH, thus leading to marked oxidative stress. Treatment with EERE when administered at a dose of 100mg/kg showed marked attenuation in the level of TBARS with significant increase in the level of SOD and GSH in a dose dependent manner. Further, Ranitidine and omeperazone too have a marked effect by significantly attenuating (p<0.001) the oxidative stress.

3.3. Effect of EERE on MPO activity.

Ethanol and reserpine administered rats showed (p<0.001) marked increase in the level of MPO. However, treatment with 100 mg/kg showed (p<0.001) marked attenuation in the level of MPO in a dose dependent manner.

3.4. Effect OF EERE on histopathogy of stomach

Stomach of rats administered with reserpine and ethanol showed severe mucosal injury along with marked sub mucosal oedema and leukocyte infiltration as compared with normal control (Figure 1 and 2). However, pre-treatment with EERE (50 mg/kg/p.o. and 100 mg/kg/p.o.) showed comparatively better protection of the gastric mucosa indicated by reduced or absence of sub mucosal edema and leucocytes infiltration in a dose-dependent manner.

4. DISCUSSION

Rhubarb (Rheum emodi, F. Polygonaceae) has been traditionally used to treat pathological ailments like fevers, ulcers, bacterial infections, fungal infections, jaundice and liver disorders \(^{21}\). For this reason, the antiulcer activity of the leaves extract was evaluated using reserpine and ethanol-induced ulcer models. Phytochemical screening shows a number of anthraquinone derivatives, tannins, oxalic acid, others that has been reported to have antioxidant potential \(^{7,22}\).
Scientific literature demonstrates that tannins are involved in the anti-ulcer activities of several medicinal plants. Further, several researches too indicated that Rhubarb increased secretion of several immune associated substances of the mucous membrane in normal intestine, indicating a possibility to abate the injury of intestine.

Both Ethanol and reserpine are involved in the progression of ulcer. Generation of free radicals has been found to be involved in the mechanism of acute and chronic ulceration in the gastric mucosa. Ethanol has been reported to stimulate the formation of leukotriene C4 (LTC4), histamine and reactive oxygen species that has been responsible to smash up the gastric mucosa. Exact mechanism of Reserpine-induced ulcers is not clear but reports indicated that reserpine has been documented to increase free radical generation and PG production. Further, Mucus secreted by the goblet cell plays an important role by forming a protective covering thereby preventing mucosal layer from any type of physical damage.

Administration of reserpine and ethanol both resulted in severe damage to goblet cells resulting in decrease in mucus. Moreover, reserpine and ethanol resulted in increased ulcer index, and ulcers were of 4 and 5 score with perforations. In addition, rats administered reserpine and ethanol there was increased generation of ROS estimated by TBARS with attenuation in the level of Glutathione and superoxide. Results too depicted that in both the models there was increase level of MPO, indicator of neutrophil infiltration with attenuation in the level of NO.

However, treatment with Rheum emodi significantly (p<0.001) attenuated the ulcer index with ulcer protection of in ethanol and reserpine models respectively with subsequent increasing gastric adhesion mucus content that indicated its antiulcer and gastroprotective potential. Further, treatment with EERE significantly reduced the generation of ROS indicating antioxidant potential of R. emodi. In this study we too revealed the anti-inflammatory role of R.emodi that is depicted from our results by attenuating the level of MPO, increasing NO level and reduction in gastric lesion and leucocyte infiltration.

Table 1. Effect of R. emodi on various parameters of Ethanol-induced Rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Normal Control</th>
<th>Disease Control</th>
<th>Omeprazole Treated</th>
<th>Rheum emodi Treated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>125.5 ± 9.13***</td>
<td>19.83 ± 2.93***</td>
<td>44 ± 7.07***</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(84.19%)</td>
<td>(64.94%)</td>
<td>(93%)</td>
</tr>
<tr>
<td>Ulcer Index</td>
<td></td>
<td>5.03 ± 0.31***</td>
<td>4.42 ± 0.42*</td>
<td>6.00 ± 0.25***</td>
</tr>
<tr>
<td>SOD (unit/mg protein)</td>
<td>8.51 ± 0.84</td>
<td>2.15 ± 0.30***</td>
<td>0.66 ± 0.04***</td>
<td>0.47 ± 0.062**</td>
</tr>
<tr>
<td>Reduced glutathione</td>
<td>0.88 ± 0.064</td>
<td>0.208 ± 0.038***</td>
<td>0.66 ± 0.04***</td>
<td>0.47 ± 0.062**</td>
</tr>
<tr>
<td>(µmol/mg protein)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nitrate (µmol/mg protein)</td>
<td>10.07 ± 0.39</td>
<td>3.48 ± 0.325***</td>
<td>9.86 ± 0.259***</td>
<td>9.39 ± 0.696***</td>
</tr>
<tr>
<td>Lipid peroxidation (nmol/mg protein)</td>
<td>3.92 ± 0.61</td>
<td>12.79 ± 1.29***</td>
<td>4.33 ± 0.92***</td>
<td>7.9 ± 0.789**</td>
</tr>
<tr>
<td>MPO (µg of protein)</td>
<td>12.95 ± 0.59</td>
<td>22.93 ± 1.33***</td>
<td>16.65 ± 1.29**</td>
<td>17.88 ± 0.696**</td>
</tr>
<tr>
<td>Gastric adhesion mucus content (µg/g wet glandular tissue)</td>
<td>220 ± 8.29</td>
<td>163.7 ± 6.05***</td>
<td>200 ± 4.50***</td>
<td>195.3 ± 3.43***</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM. Control group was compared with normal control. Rheum emodi treated groups were compared with disease control (Ethanol control). *p>0.05; **p>0.001; ***p>0.001; ns= non significant. Values in parenthesis indicate the % reduction in ulcer index in relation to the control group.
Table 2. Effect of *R. emodi* on various parameters of Reserpine-induced Rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Normal Control</th>
<th>Disease Control</th>
<th>Ranitidine Treated</th>
<th>Rheum emodi Treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ulcer Index</td>
<td>-</td>
<td>53.17 ± 7.25***</td>
<td>7.83 ± 1.4***</td>
<td>26.33 ± 2.29**</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(85.3%)</td>
<td></td>
<td>(86.51%)</td>
</tr>
<tr>
<td>SOD (unit/mg protein)</td>
<td>8.52 ± 0.84</td>
<td>2.5 ± 0.43***</td>
<td>6.83 ± 0.85***</td>
<td>4.46 ± 0.21**</td>
</tr>
<tr>
<td>Reduced glutathione (μmol/mg protein)</td>
<td>0.75 ± 0.029</td>
<td>0.27 ± 0.08***</td>
<td>0.62 ± 0.05***</td>
<td>0.56 ± 0.03**</td>
</tr>
<tr>
<td>Nitrate (μmol/mg protein)</td>
<td>10.06 ± 0.39</td>
<td>2.54 ± 0.33***</td>
<td>8.3 ± 0.64***</td>
<td>5.95 ± 0.43**</td>
</tr>
<tr>
<td>Lipid peroxidation (nmoles/mg protein)</td>
<td>3.92 ± 0.61</td>
<td>11.07 ± 1.10***</td>
<td>4.56 ± 0.82***</td>
<td>6.72 ± 0.59**</td>
</tr>
<tr>
<td>MPO (μg of protein)</td>
<td>12.95 ± 0.59</td>
<td>22.57 ± 1.27***</td>
<td>14.5 ± 1.21***</td>
<td>17.6 ± 0.75**</td>
</tr>
<tr>
<td>Gastric adhesion mucus content (μg/g wet glandular tissue)</td>
<td>220 ± 8.29</td>
<td>159.5 ± 10.2***</td>
<td>211.6 ± 8.06**</td>
<td>161.5 ± 4.40**</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM. Control group was compared with normal control. Rheum emodi treated groups were compared with disease control (Reserpine control). *p > 0.05; ** p > 0.001; ***p > 0.001; ns = non significant. Values in parenthesis indicate the % reduction in ulcer index in relation to the control group.

*Fig.1* Effect of *Rheum emodi* on gastric mucosal injury integrity in reserpine-induced ulceration.
5. CONCLUSION

The above findings thus suggest the gastroprotective and antiulcer potential of *Rheum emodi*. These findings support the traditional use of EERE for controlling ulcer.

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REFERENCES

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