



Assessment of Anti-Ulcer Activity of *Rheum emodi* Rhizomes Extract

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ABSTRACT: Purpose: The purpose of the present study was to assess the antioxidant and antiulcer potential of ethanolic extract of rhizomes of *R. emodi* on Pylorus ligation ulcers. Materials and methods: Variable doses of 50 mg/kg and 100 mg/kg of EERE were administered orally by gavage for evaluating their antioxidant and antiulcer effect on pyloric ligation-induced ulcer in rats. Results: Both doses (50 mg/kg/p.o. and 100 mg/kg/p.o.) was found to reduce the ulcer index along with the reduction in volume and total acidity, and an increase in the pH of gastric fluid in pylorus ligated rats. The increase in the levels of superoxide dismutase (SOD), reduced glutathione (GSH), tissue nitrite/nitrate and gastric adhesion mucus content and decrease in lipid peroxidation (MDA) and MPO activity in both the models showed the antioxidant activity of the *R. emodi*. It was also found that EERE at a dose of 100 mg/kg/p.o. was more potent as compared to EERE (50 mg/kg/p.o.). Conclusion: The study validates scientifically the widely claimed use of *R. emodi* as an ethnomedicine to treat ulcers. © 2011 IGJPS. All rights reserved.

KEYWORDS: *Rheum emodi*; Antioxidant; Antiulcer; Pylorus Ligation.

INTRODUCTION

For more than a century, peptic ulcer disease has been a major cause of morbidity and mortality [1]. In clinical practice, Peptic ulcers are a common disorder of the entire gastrointestinal tract (GIT) that occurs mainly in the stomach and the proximal duodenum. Generally ulcers develop due to imbalance between aggressive factors such as hydrochloric acid (HCL), pepsin, refluxed bile, leukotrienes (LTs), reactive oxygen species (ROS) and defensive factors such as mucus-bicarbonate barrier, surface active phospholipids, prostaglandins (PGs), mucosal blood flow, cell renewal and migration [2].

The success of commercially available antiulcer drugs in the treatment of gastric ulcer is usually overshadowed by various side effects [3]. At present, approximately 25% of drugs in modern pharmacopoeia were derived from plants (phytomedicines) and many others were synthetic analogues built on the prototype compounds isolated from plants. Indian folk medicine comprises of numerous prescriptions for therapeutic purposes such a healing of wounds, inflammation, skin infections, leprosy, diarrhea, scabies, venereal diseases, ulcers, snake bite etc [4,5,6].

Rheum emodi is an important medicinal plant, which finds an extensive use in Ayurveda and Unani systems of medicine [7]. *R. emodi* (Indian rhubarb) is commonly known as rewand chini and belongs to family polygonaceae. Interest in the rhizomes of this plant *R. emodi* has been heightened by reports of its traditional uses as antidiabetic, anticancer, hepatoprotective and antiulcer [8]. Keeping these views in mind, aim of current research work was to evaluate the antiulcer potential of *R. emodi* rhizomes.

MATERIALS & METHODS

Collection & Authentication of Plant Material

R. 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 rhizome was then allowed to dry at room temperature and powdered to coarse powder using mortar and pestle. The air dried coarse powder is used for preparation of extract.

Preparation of Extracts

The ethanolic and aqueous extracts were prepared according to the standard procedure of soxhlet extraction. 500 gm of the rhizome was put into the thimble of the soxhlet containing 100ml of 50% of ethanol 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.

Preliminary Phytochemical Screening

Ethanolic extract of *R. emodi* were subjected to preliminary phytochemical screening for the detection of various plants constituents [9].

Animals

Male / female wistar albino rats weighing between 150 to 180 grams, from Animal House, Punjab Agriculture University, Ludhiana, Punjab, India, were divided in ten groups of six animals each. The animals were fed on standard chow diet and water *ad libitum*. They were acclimatized in the animal house of our institute and exposed to natural light and dark cycle. Institutional Animals Ethics Committee (IAEC) approved the experimental protocol and care of animals was taken as per guidelines of CPCSEA, Department of Animal Welfare and Government of India.

Test Compound Formulations

The aqueous suspension of ethanolic extract of rhizomes of *R. emodi* (EERE) was prepared in 0.5 % carboxymethylcellulose (CMC) solution in distilled water prior to oral administration to animals. Only freshly prepared solution was used. The vehicle alone served as control.

Chemicals

All the drugs and chemicals were of analytical grade.

Acute Toxicity study

The LD50 of the *R. emodi* was reported to be safe till 1000 mg/kg/i.p. (IJEBA6, 1986). Thus the studies were carried out by using two selected doses of ethanolic extract of *R. emodi* 50 mg/kg and 100 mg/kg. The dose of plant was selected by hit and trial method. No death and side effects were found at both selected doses of plant.

Assessment of Anti-Ulcer Activity

Albino rats of either sex were divided into five groups of six animals each. Animals were fasted for 24 h before the study, but had free access to water. Animals in the disease control group received only distilled water before pylorus ligation. EERE at 50 and 100 mg/kg, (*p. o.*) were given to the animals in the treatment group. Ranitidine (50 mg/kg) was used as a standard. After 1h of drugs treatment, they were anaesthetized with the help of anesthetic ether; the abdomen was opened by a small midline incision below the xiphoid process. Pyloric portion of the stomach was slightly lifted out and ligated according to method of Shay et al. [10] avoiding traction to the pylorus or damage to its blood supply. The stomach was replaced carefully and the abdominal wall was closed by interrupted sutures. Rats were sacrificed by an over dose of anaesthetic ether after four hours of pyloric ligation. The abdomen was opened, cardiac end of the stomach was dissected out and the contents were drained into a glass tube. The volume of the gastric juice was measured and centrifuged at 2000 rpm for 10 min. From the supernatant, aliquots (1 ml of each) were taken for the determination of pH, total and free acidity. Each stomach was examined for lesions in the fore stomach portion and indexed according to severity.

Macroscopic evaluation of stomach

The stomachs were opened along the greater curvature, rinsed with saline to remove gastric contents and blood clots and examined by a 10X magnifier lens to assess the formation of ulcers. The numbers of ulcers were counted by the scoring method of Suzuki et al. [11].

Scoring of ulcer will be made 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

Percent inhibition of ulceration was calculated as below:

$$\% \text{ Inhibition of ulceration} = \frac{(\text{Ulcer Index}_{\text{control}} - \text{Ulcer Index}_{\text{test}})}{(\text{Ulcer Index}_{\text{control}})} \times 100$$

Determination of pH

An aliquot of 1ml gastric juice was diluted with 1ml of distilled water and pH of the solution was measured using pH meter [12].

Determination of total acidity

An aliquot of 1ml gastric juice diluted with 1ml of distilled water was taken into a 50 ml conical flask and two drops of phenolphthalein indicator was added to it and titrated with 0.01N NaOH until a permanent pink colour was observed. The volume of 0.01N NaOH consumed was noted [12]. The total acidity is expressed as mEq/L by the following formula:

$$\text{Acidity} = \text{Vol. of NaOH} \times N \times 100 \text{ mEq/L} / 0.1$$

Assessment of Anti-oxidant activity

The stomach of rats was then weighed and homogenized in chilled phosphate buffer (10 mM, pH 7.4) at a concentration of 10% (w/v). The homogenate was then centrifuged at 10,000 x for 20 min. The clear supernatant was used for the assays of Lipid peroxidation (MDA content), MPO, endogenous antioxidant enzymes (nitrate/nitrite, Superoxide dismutase and reduced glutathione) and for the determination of gastric adhesion mucus content.

Biochemical Estimations

Superoxide dismutase was determined by the method of Wang et al. [13]. Reduced glutathione was estimated by the method of Jollow et al [14]. Lipid peroxidation or malondialdehyde formation was estimated by the method of (Ohkawa et al [15]. Nitrate level was determined by the method of Green et al [16]. Gastric adhesion mucus content was determined by the method of Corne et al [17]. Assay of Gastric mucosal myeloperoxidase activity was estimated by Qiu et al [18]. Total proteins were determined by the method of Biuret by using protein estimation kit.

Assesment of integrity of stomach using Histopathological studies

The stomach was excised 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 haematoxylin (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).

Statistical analysis

The results were analysed statistically using one way analysis of variance (ANOVA) followed by tukey test. A probability value of $p < 0.05$ was considered to be statistically significant.

RESULTS & DISCUSSION

Study of antiulcer and antioxidant activity using pylorus ligation model

It was observed that in disease control, the ulcer index was 93.50 ± 6.93 and the maximum numbers of ulcers were of the score 4 and 5, and a number of perforated ulcers (score 25) were also observed. EERE (50mg/kg/p.o. and 100 mg/kg/p.o.) was found to produce significant decrease in ulcer index. All the ulcers were of scores 1 and 2 and no perforated ulcers were observed.

In control rats, pylorus ligation for 4 h resulted in accumulation of 2.1 ± 0.3 ml of gastric secretion with pH 2.11 ± 0.254 and a total acid output of 103.2 ± 9.24 mEq/L/100 gm. The volume of gastric secretion in the rats treated with 50 mg/kg/p.o. and 100 mg/kg/p.o. of EERE significantly reduced to 1.67 ± 0.14 ml and 0.87 ± 0.07 ml respectively. A marked increase in the pH of gastric content was observed with EERE at a dose of 100 mg/kg (4.25 ± 0.305). However, EERE at lower dose (50 mg/kg/p.o.) produced a non significant effect on pH of gastric content. A significant decrease in total acid output was observed in the rats treated with EERE (50 mg/kg/p.o. and 100 mg/kg/p.o.) (Table 1).

Table 1. Effect of *R. emodi* on the various gastric parameters of pylorus-ligated rats

Parameters	Pylorus Ligation Control	Ranitidine Treated	<i>Rheum emodi</i> Treated	
			50mg/kg	100 mg/kg
Ulcer Index (mm ²)	93.50 ± 6.93	7.5 ± 2.36 ^{***} (91.98%)	56.83 ± 8.85 ^{**} (39.22%)	12.67 ± 2.81 ^{***} (86.45%)
Volume of Gastric Fluid (ml)	2.1 ± 0.3	0.8 ± 0.095 ^{***}	1.67 ± 0.14 ^{**}	0.87 ± 0.07 ^{***}
pH of Gastric fluid	2.11 ± 0.254	4.32 ± 0.35 ^{***}	2.63 ± 0.34 ^{ns}	4.25 ± 0.305 ^{***}
Total Acidity (m Eq/l/100g)	103.2 ± 9.24	16.5 ± 3.7 ^{***}	71.33 ± 7.20 [*]	25.67 ± 6.86 ^{***}

Values are expressed as mean+SEM. Control group was compared with normal control. *Rheum emodi* treated groups were compared with disease control. *p>0.05 ; ** p>0.01 ; ***p>0.001 ; ns = non significant; Values in parenthesis indicate the % reduction in ulcer index in relation to the control group.

As compared to normal rats, pylorus-ligation was found to increase lipid peroxidation and MPO activity. A significant decrease in SOD, reduced glutathione, gastric adhesion mucus content and nitrate/nitrite levels in the disease control has observed. Administration of EERE (50 mg/kg/p.o. and 100 mg/kg/p.o.) brought about a significant reduction in lipid peroxidation and MPO activity and an increase in the activities of antioxidant enzymes namely, SOD and GSH. An increase in the level of tissue nitrate/nitrite has also observed. A similar observation was done with gastric wall mucus. The treatment of rats with EERE (50mg/kg/p.o. and 100 mg/kg/p.o.) significantly increased the alcian blue binding capacity of gastric wall mucus as compared to disease control (Table 2).

Table 2. Effect of *R. emodi* on the various Biochemical parameters of pylorus-ligated rats

Parameters	Normal Control	Disease Control	Ranitidine Treated	<i>Rheum emodi</i> Treated	
				50mg/kg	100 mg/kg
SOD (unit/mg protein)	8.52 ± 0.84	1.65 ± 0.28 ^{***}	7.88 ± 0.64 ^{***}	5.47 ± 0.33 ^{**}	8.24 ± 0.84 ^{***}
Reduced glutathione (µmol/mg protein)	0.75 ± 0.029	0.24 ± 0.033 ^{***}	0.77 ± 0.039 ^{***}	0.49 ± 0.034 ^{***}	0.77 ± 0.047 ^{***}
Nitrate (µmol/mg protein)	10.06 ± 0.39	2.65 ± 0.49 ^{***}	8.86 ± 0.48 ^{***}	7.05 ± 0.58 ^{***}	8.72 ± 0.47 ^{***}
Lipid peroxidation (nmoles/mg protein)	3.92 ± 0.61	17.91 ± 1.83 ^{***}	4.84 ± 0.69 ^{***}	10.82 ± 1.38 ^{**}	4.87 ± 0.69 ^{***}
MPO (µ/g of protein)	12.95 ± 0.70	21.38 ± 1.20 ^{***}	14.32 ± 0.80 ^{***}	17.97 ± 0.49 [*]	13.17 ± 0.70 ^{***}
Gastric adhesion mucus content (µg/g wet glandular tissue)	220 ± 8.29	158.2 ± 11.25 ^{***}	211 ± 5.85 ^{***}	192.8 ± 2.22 [*]	215.2 ± 6.16 ^{***}

Values are expressed as mean+SEM. Control group was compared with normal control. *Rheum emodi* treated groups were compared with disease control. *p>0.05 ; ** p>0.01 ; ***p>0.001

Histological evaluation of gastric lesions induced by Pylorus ligation

Histological observation of pyloric ligation-induced gastric lesions in disease control showed comparatively extensive damage to the gastric mucosa, oedema and leucocytes infiltration of the submucosal layer as compared with normal control (Figure 1 and 2). Rats that received pretreatment with EERE (50 mg/kg/p.o. and 100 mg/kg/p.o.) had comparatively better protection of the gastric mucosa as seen by reduction in ulcer area, reduced or absence of submucosal oedema and leucocytes infiltration (Figures 3 and 4). The EERE has been shown to exert the cytoprotective effects in a dose-dependent manner.

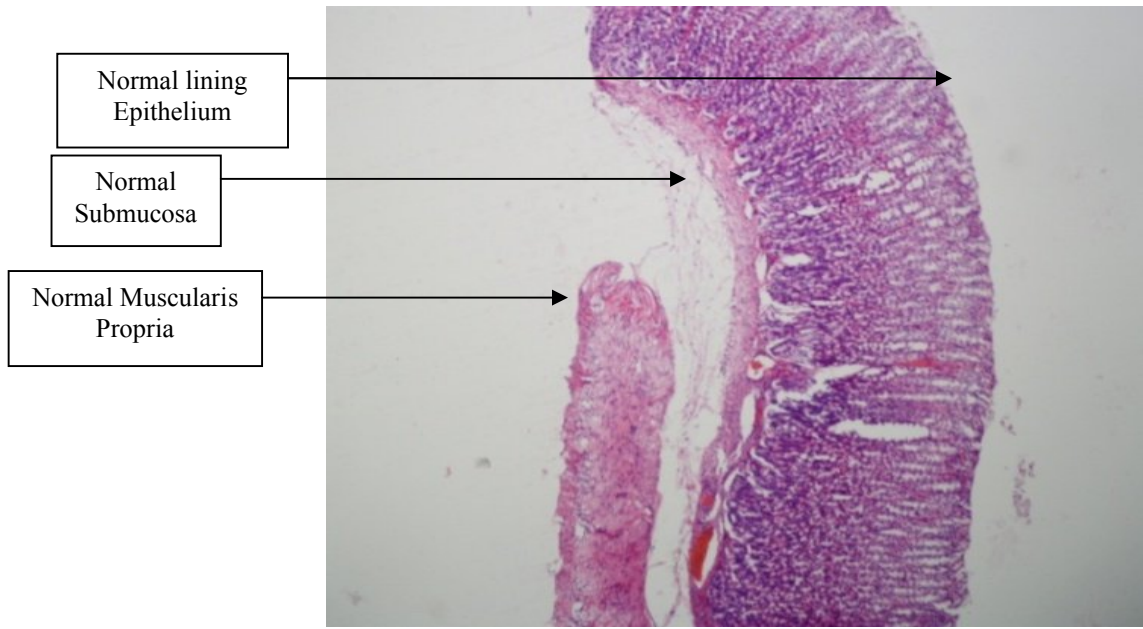


Figure 1. Histological section of gastric mucosa of normal control rat. There is normal epithelial lining along with normal mucosa and submucosa (H&E stain, 100x).

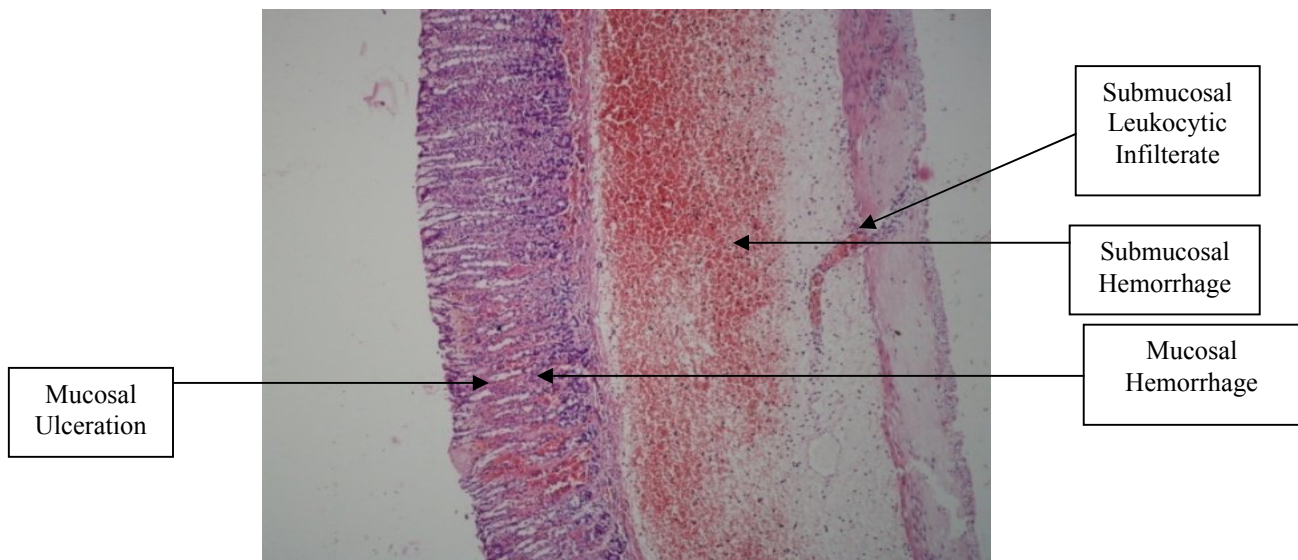


Figure 2. Histological section of gastric mucosa in a pylorus-ligation control rat. There is severe disruption to the surface epithelium, and edema of the submucosal layer with leucocytes infiltration (H&E stain, 100x).

The anti-ulcer activity of the rhizomes of *R. emodi* was evaluated by employing pylorus ligation ulcer model. This model represents some of the most common causes of gastric ulcer in humans. Many factors and mechanisms are implicated in the ulcerogenesis and gastric mucosal damage induced by pylorus ligation. Pylorus ligation -induced ulcers occur as a result of discernible increase in acid-pepsin accumulation due to pylorus obstruction and subsequent mucosal digestion. Further, literature review reveals that in pylorus ligation model, the interference of gastric blood circulation is too responsible for induction of ulcers [19]. Gastric acid and pepsin are important factors for the formation of ulcers in pylorus ligated rats [10]. In addition to this, increase oxidative stress is also responsible in the progression of ulceration [20]. This contention is supported by results obtained in our study that there is marked increase in lipid peroxidation along with significant attenuation in the level of various antioxidants like GSH and SOD. Pylorus ligation also caused a significant depletion of gastric adhesion mucus content along with increase MPO activity characterized by leukocyte infiltration.

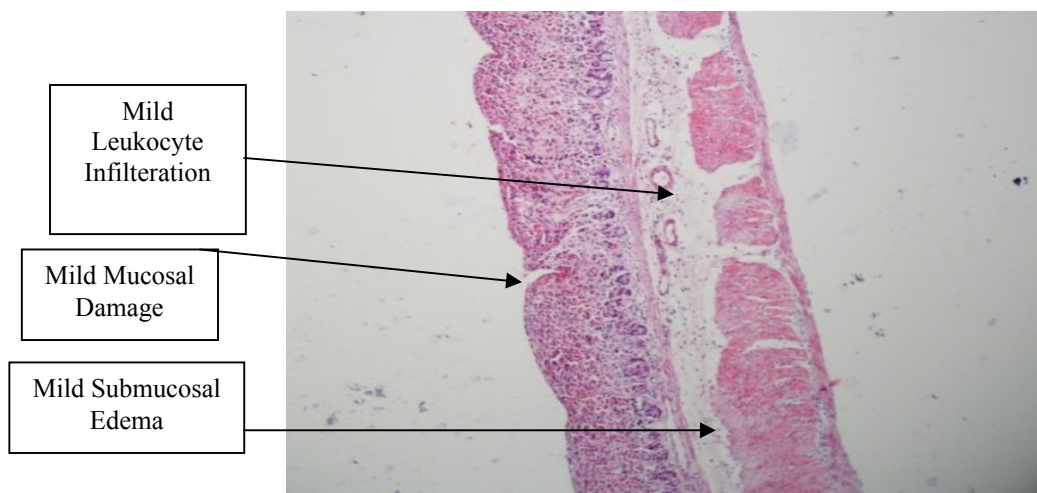


Figure 3. Histological section of gastric mucosa in a rat pre-treated with EERE (50 mg/kg/p.o.) There is mild disruption to the surface epithelium with mild edema and leucocytes infiltration of the submucosal layer (H and E stain 100x).

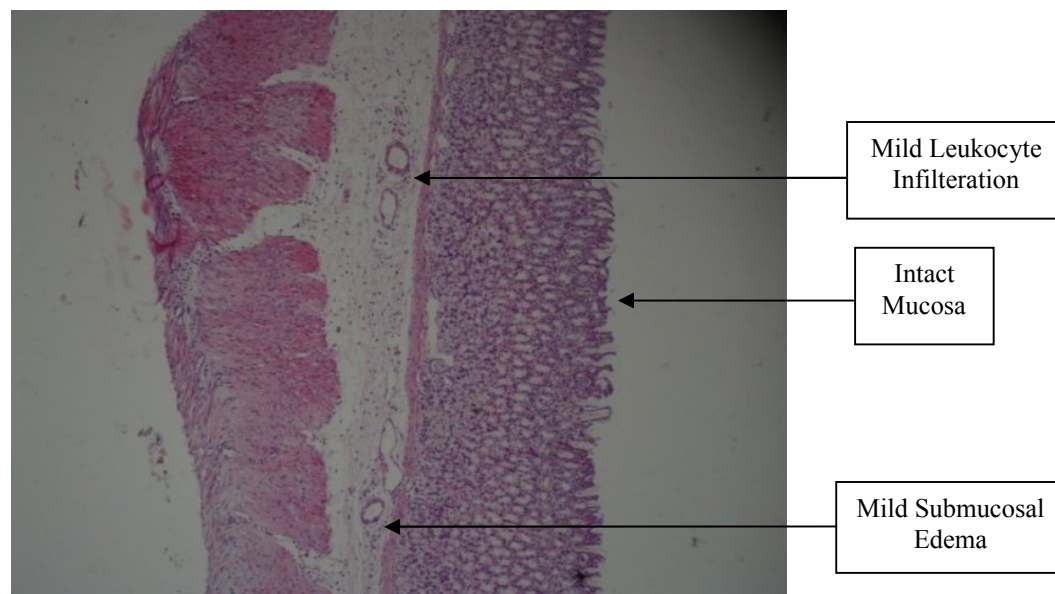


Figure 4. Histological section of gastric mucosa in a rat pre-treated with EERE (100 mg/kg/p.o.) There is no disruption to the surface epithelium with mild edema and mild leucocytes infiltration of the submucosal layer (H and E stain 100x).

It is evident from the result obtained in our study that the ethanolic extract of *R. emodi* rhizomes (50mg/kg and 100 mg/kg) significantly inhibit gastric ulceration in pylorus ligated rats. The anti-ulcer activity of *R. emodi* extract in pylorus ligation model is evident from its significant reduction in gastric volume, total acidity, free acidity and increase in pH of gastric juice. It is suggested that EERE can suppress gastric damage induced by aggressive factors. The ulceration in pyloric-ligated rats is generally caused by increased acid and peptic activity.

Also EERE (50 mg/kg and 100 mg/kg) produced a significant increase in the level of various antioxidants with subsequent decrease in the level of oxidative stress parameters, which provides its role as antioxidant. EERE significantly restore the level of NO and also restore the depletion of gastric adhesion mucus content.

Pylorus ligation has been reported to causes severe hemorrhagic necrosis [21]. Results of Histopathological studies have shown that pre-treatment with the EERE (50 mg/kg/*p.o.* and 100 mg/kg/*p.o.*) reduced pylorus ligation-induced hemorrhagic necrosis in rats and also reduced submucosal oedema and leukocyte infiltration. Histopathology of reserpine administered stomach showed severe mucosal injury along with oedema in submucosal layer. Pre-treatment with EERE (50 mg/kg/*p.o.* and 100 mg/kg/ *p.o.*) reduced the effects and maintained the mucosal integrity in a dose dependent manner. Preliminary phytochemical studies revealed the presence of anthraquinones and tannins. So, the possible mechanism of antiulcer action of EERE may be due to its tannins content.

CONCLUSION

It may be concluded that EERE (50 mg/kg/*p.o.* and 100 mg/kg/ *p.o.*) exerts gastroprotective and antioxidant effect as it reduces the oxidative stress and consequently improves the integrity of gastric mucosa and enhances the generation of nitric oxide and mucus in experimentally-induced gastric ulcers. It was also concluded that EERE at a dose of 100 mg/kg was more potent than 50 mg/kg.

ACKNOWLEDGEMENT

Authors would like to express their gratitude towards the management of Rayat institute of Pharmacy for providing research facilities to execute this research plan.

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Indo Global Journal of Pharmaceutical Sciences(ISSN 2249 1023 ; CODEN-IGJPAI) indexed and abstracted in EMBASE(Elsevier), SCIRUS(Elsevier), Chemical Abstract Services(CAS), American Chemical Society(ACS), Index Copernicus, EBSCO, DOAJ, Google Scholar and many more. For further details, visit <http://iglobaljournal.com>