



INVESTIGATIONS INTO THE ANTI-ULCER ACTIVITY OF *RHEUM RIBES* LINN LEAVES EXTRACTS

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ABSTRACT

Methanolic and aqueous extracts of *Rheum ribes* leaves were investigated for gastric protective activity on ethanol plus pylorus ligation induced ulcer models. A number of biochemical parameters such as gastric volume, pH of gastric content, free acidity and total acidity, dissolved mucous substances such as total protein, hexoses, hexosamine, fucose and sialic acid were estimated in 90 % alcoholic precipitate of gastric juice and macroscopically sections were examined on the test and control group animals. The extract at a concentration of 200mg/kg produced a protective effect on ulcer-induced models and was comparable with the standard drug cimetidine. Some of the antioxidant enzyme levels (lipid peroxidation, superoxide dismutase and catalase) were also determined. The present study revealed that the methanolic extract of *Rheum ribes* had ulcer protective activity comparable with standard drugs cimetidine.

Keywords: *Rheum ribes*, ulcer, Ethanol plus pylorus ligation, Biochemical parameters, Methanolic extract

INTRODUCTION

Rheum ribes (Rhubarb) from Polygonaceae, is a species of perennial, stout herbs that are distributed in the temperate and subtropical regions of the world, chiefly in Asian countries ¹. Roots of *Rheum ribes* are used to treat diabetes, hemorrhoids, ulcer, diarrhea, and expectorant activity reported ²⁻⁴. It was investigated the efficacy and safety of a hydroalcoholic extract of *R. ribes* in treatment of mild to moderate major depression disorder and the observations show some anti depressive effects of *Rheum* extracts ⁵.

Peptic ulcer disease is a serious gastrointestinal disorder. The formation of peptic ulcers depends on the presence of acid and peptic activity in gastric juice plus a breakdown in mucosal defenses. There are two major factors that can disrupt the mucosal resistance to injury: non-steroidal anti-inflammatory drugs (NSAIDs) e.g. aspirin and *Helicobacter pylori* infection ⁶. As a matter of fact, many drugs were used to treat this disease but many of them cause adverse effects and recurrent infections frequently occur within a few weeks because of difficulty in eradication of *H. pylori* ⁷. This has been rationale for the development of new antiulcer drugs and search for novel molecule. Drugs of plants origin are gaining popularity and investigating for the various disorders including peptic ulcer. The objective of present study was to evaluate the effectiveness of leaves extract in preventing the formation of gastric ulcer experimentally by ethanol-induced gastric damage in rats. Cimetidine was used as reference drug for comparison.

MATERIALS AND METHODS

Rheum ribes leaves were purchased from local market of Bhiwani (Haryana) in March, 2009. The plant was identified and authenticated by Dr. S. Sharma, Deptt. of Botany, HAU, Hisar. The plant herbarium was stored at Deptt. of Pharmacognosy and Phytochemistry, Chitkara College of Pharmacy, Rajpura, Punjab.

Preparation of extracts

The air-dried *Rheum ribes* leaves were powdered using a mechanical grinder. The dried powdered plant material (500 g) was refluxed with methanol (2L) and the semisolid dark brown mass was concentrated with rotary evaporator, yielding 80.50 g methanol extract (ME). The drug was treated with water (1.5 L), yielding 46.40 g aqueous fraction (AE).

Experimental Animals

The albino Wistar rats of either sex obtained from Chitkara college of Pharmacy, weighing 150-200g were used for the study. Animals were divided into five groups, in each group six animals. They were

maintained in the departmental animal house at 25 ± 2°C and relative humidity 45-55%, respectively for 1 week before and during the experiments. Animals were provided with standard rodent pellet diet (Hindustan Lever) and water *adlibitum*. The experimental protocol was approved by the Institutional Animal Ethical Committee (IAEC) constituted under CPCSEA (Chitkara College of Pharmacy Animal Facility Registration Number: 1181/ab/08/CPCSEA).

Ethanol plus pylorus ligation method (EPPL)

The animals were placed in cages with grating floor to avoid coprophagy and divided into five groups each group six animal viz, Group I receiving 0.5 % acacia gum served as vehicle control, group II ulcer induced group (EPPL), Groups III received aqueous extract at dose of 200 mg/kg and IV received the methanolic extract of *Rheum ribes* leaves (MERR) at dose of 200 mg/ kg respectively by oral route. Group V received Cimetidine 40 mg/kg orally serving as standard drug control for EPPL model. All the extracts and reference drug were suspended in 0.5 % acacia gum for animal administration. Ethanol was administered once daily for 5 days. Cimetidine and the extracts were administered 30 min before each ethanol administration. On day 6 after last dose, the rats were kept for 18 h fasting. Pylorus ligation was done by following the method as described. The animals were deprived of water during the postoperative period. After 4 h, stomachs were dissected out and contents were collected in tubes for estimation of biochemical parameters. Ulcers were scored as described.

Biochemical estimation

In EPPL induced ulcer models the following were estimated by procedures described, gastric volume, pH of gastric content, free acidity and total acidity. Dissolved muco substances such as total protein, hexoses, hexosamine, fucose, sialic acid were estimated in 90 % alcoholic, precipitate of gastric juice and expressed as µg/ml. ⁸.

Statistical analysis

The data are expressed as mean ± SEM. Statistical comparisons were performed by one-way ANOVA followed by Dunnet's 't' test. The results were considered statistically significant if the p-values were less than 0.05.

RESULTS

Estimation of acid secretry parameters such as pH, gastric volume, free acidity and total acidity was increased significantly in the ethanol administered group. Administration of MERR a significant (p <0.01) reduction in all the parameters and the results were

comparable with the standard drug Cimetidine 40 mg/kg (Table 1). Determination of the concentrations of several muco proteins such as total protein, total hexoses, hexosamine, fucose and sialic acid revealed a decrease in ulcer induced group. The extract at 200 mg/kg increased the level of the muco proteins significantly and comparably with the standard drug ($p < 0.01$) (Table 2). The ulcer scores obtained in ulcer induced group of EPPL an increased score. Administration of the extract showed a significant decrease in EPPL models ($p < 0.05$ and $p < 0.01$) (Table 3). Effect of MERR on level of SOD, LPO and CAT.

The histopathological sections of the drug treated group EPPL had shown a reduction in ulcer focus and a hyperplastic gastric mucosa with regenerating mucosal epithelium (Fig 1, 2, 3 and 4).

DISCUSSION

MERR showed significant dose-dependent ulcer protective effect against ethanol plus pylorus ligation induced gastric ulcers. Ulcers are caused due to imbalances between offensive and defensive mucosal factors and hence the effects of MERR can be explained based on these factors. Mucin is a viscous glycoprotein with physicochemical properties producing relatively resistant acid barrier⁹. It makes up the major part of the mucus, an important pre-epithelial factor that acts as a first line of defence against ulcerogens. Increase in mucin can be due to increased levels of individual

mucopolysaccharide like sialic acid and total hexoses. The increase in mucosal defence may also be due to decrease in cell exfoliation¹⁰. Hence, the protection afforded by MERR in EPPL induced ulcers may be predominantly due to strengthening mucosal defense. The ability of MERR to protect stomach against ulcerogens by neutralizing intra gastric acidity can as well lead it to classify as a cytoprotective agent. Prostaglandins have often been quoted as a model cytoprotective agent, although this has been disputed. To ascertain this effect, the activity of MERR was studied on Ethanol plus pylorus ligation model, where ethanol is known to further aggravate mucosal damage caused by pylorus ligation¹¹. It was found that MERR was effective in this model, suggesting that the activity of MERR may also involve other defensive factors apart from PG synthesis. The role of the free radicals in gastric ulcerations is well-documented. MERR significantly reduced lipid peroxidation in rat gastric mucosa. *R. ribes* has been reported to possess antioxidant activity. SOD scavenges the super oxide radical O_2^- , one of the reactive oxygen species (ROS) responsible for lipid peroxidation. CAT and other peroxidases further reduce H_2O_2 .

The antioxidant activity of plant is already reported in literature. Thus the ulcer protective activity of MERR may also be due to its antioxidant effect. Hence, it can be suggested that MERR have anti-ulcer potential in rats and further in future, isolate the phytoconstituents which responsible for antiulcer activity may be studied⁸.

Table 1: Effect of MERR on pH, gastric volume, free acidity and total acidity

Treatment group	pH	Gastric volume (mL/100g)	free acidity (m Eq/L/100g)	Total acidity (m q/L/100g)
Control	3.791 ± 0.1352	1.671 ± 0.1441	62.822 ± 0.2798	45.511 ± 0.4083
EPPL	1.223 ± 0.0736 ^{a**}	6.045 ± 0.1152 ^{a***}	72.304 ± 0.5827 ^{a**}	80.103 ± 0.2068 ^a
Aqueous extract <i>R. Ribes</i>	3.172 ± 0.1012 ^{b**}	4.733 ± 0.3575 ^{b**}	58.463 ± 0.7307 ^{b**}	57.322 ± 0.1536 ^{b**}
Methanolic extract <i>Rheum ribes</i>	3.497 ± 0.8924 ^{b**}	3.542 ± 0.1612 ^{b**}	47.597 ± 0.4161 ^{b**}	53.014 ± 0.4081 ^{b**}
Cimetidine	3.862 ± 0.1352 ^{b**}	3.014 ± 0.1659 ^{b**}	44.061 ± 0.2382 ^{b**}	07.223 ± 0.2518 ^{b**}

Data are mean ± SEM, n=6, Statistical significance * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, a - Group I vs. Group II; b - Group II vs. Groups III, IV and V

Table 2: Effect of MERR on gastric juice mucoprotein (µg/mL) in ELP rats

Treatment group	Protein	Total hexoses	Hexosamine	Fucose	Sialic acid
Control	264.92 ± 3.330	421.36 ± 5.678	207.27 ± 5.870	96.324 ± 1.656	72.983 ± 1.179
EPPL	473.36 ± 3.265 ^{a***}	195.51 ± 5.750 ^{a***}	110.21 ± 4.026 ^{a***}	6.347 ± 0.961 ^{a***}	21.161 ± 1.520 ^{a***}
Aqueous extract <i>R.</i>	399.38 ± 4.867 ^{b**}	329.52 ± 8.399 ^{b**}	162.19 ± 6.036 ^{b**}	81.423 ± 0.702 ^{b**}	48.322 ± 1.400 ^{b**}
MeOH extract	373.14 ± 11.91 ^{b**}	7.656 ± 6.085 ^{b**}	171.30 ± 4.973 ^{b**}	85.716 ± 1.073 ^{b**}	56.276 ± 0.869 ^{b**}
Cimetidine	329.83 ± 6.006 ^{b**}	403.14 ± 6.256 ^{b**}	175.53 ± 4.699 ^{b**}	116.14 ± 1.564 ^{b**}	64.096 ± 0.775 ^{b**}

Data are mean ± SEM, n=6, Statistical significance * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, a - Group I vs. Group II; b - Group II vs. Groups III, IV and V

Table 3: Effect of *Rheum ribes* leaves extracts on induction of gross lesions in the ethanol plus pylorus ligation method

Group	Treatment	No. of rats	Dose orally	Ulcer index (Mean ± S.E.M)	Inhibition (%)
RR1	Control (0.5% Acacia gum)	6	200 mg/ kg ⁻¹	0	0
RR2	Absolute ethanol-HCl (ulcer control)	6	200 mg/ kg ⁻¹	59.742 ± 1.655	0
RR3	Aqueous extract (AE)	6	200 mg/ kg ⁻¹	49.157 ± 1.275	30.42
RR4	Methanolic extract (ME)	6	200 mg/ kg ⁻¹	28.959 ± 1.391	71.06
RR5	Cimetidine	6	40 mg/ kg ⁻¹	25.907 ± 1.485	76.19

* $p < 0.05$ significant from (Absolute ethanol-HCl) ulcer control; ** $p < 0.05$ significant from aqueous extract

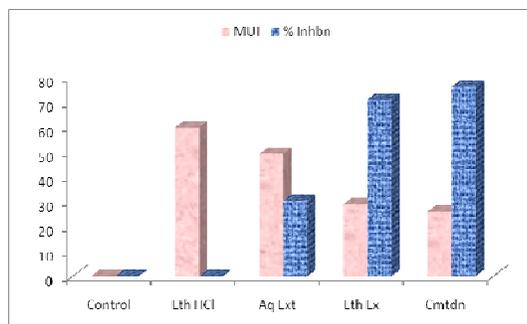




Fig. 1: Control group

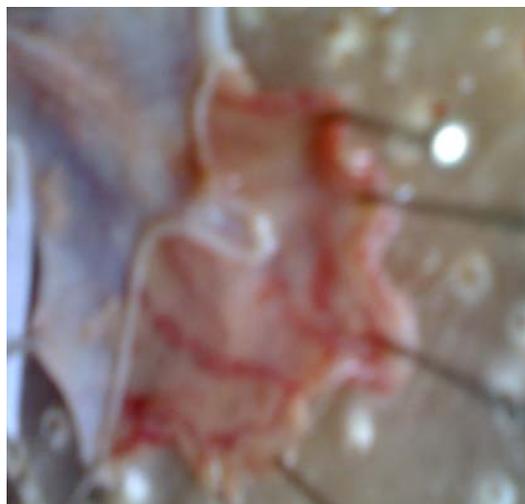


Fig. 2: Aq. extract treated group



Fig. 3: Methanol extract treated



Fig. 4: Cimetidine treated group

CONCLUSION

The present study reveals that the methanolic extract of *Rheum ribes* leaves shows ulcer protective effect in ethanol plus pylorus ligation method. Hence, it can be suggested that MERR have anti-ulcer potential in rats and further in future, isolate the phytoconstituents which were responsible for anti-ulcer activity may be studied.

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