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Gastroprotective effect of ethanolic extract of *Parmelia perlata* in rats

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Abstract

Gastric ulcer disease (GUD) is one of the major gastrointestinal disorders which occur due to an imbalance between offensive (acid, pepsin and *Helicobacter pylori*) and defensive (mucin, prostaglandin and bicarbonate) factors. **Objective:** The present study was to evaluate the ethanol extract of the *Parmelia perlata* (Huds.) Ach. (family Parmeliaceae) the gastroprotective activity against the cold restraint (CRU), aspirin (AS), alcohol (AL) and pyloric ligation (PL) induced gastric ulcer models in rats. **Materials and methods:** Adult Sprague Dawley rats of either sex, weighing 180-200g were housed in raised bottom mesh cages to prevent coprophagy and were kept in environmentally controlled rooms (25 ± 2°C, 12 hours light and dark cycle). **Results and conclusion:** Potential anti-ulcer activity of the ethanol extract of the *P. perlata* was observed against CRU (50.0%), AS (37.5%), AL (65.41%) and PL (50.00%) induced ulcer models. The reference drug omeprazole (10mg/kg, p.o.) showed 77.40% protection against CRU and 57.08% against AS and 69.42% against PL model. Sucralfate, another reference drug (500 mg/kg, p.o.) showed 62.50% protection in AL induced ulcer model. The ethanol extract of the *P. perlata* reduced free acidity (19.04%), total acidity (14.43%) and upregulated mucin secretion by 14.17% respectively. **Conclusion:** The ethanolic extract of the *P. perlata* was found to possess anti-ulcerogenic activity which might be due to its secretory activity and subsequent strengthening of the defensive mechanism.

Keywords: Protection against Gastric ulcer, *Parmelia perlata*, CRU Ulcer models.

Introduction

Parmelia perlata (Huds.) Ach. (Groot schildmos) Parmeliaceae is mainly found in Himachal Pradesh and West Bengal. Commonly called as Stone Flower. It is also called Chharila. Chharila is a lichen crude drug sold in Indian bazaars and used in Ayurvedic and Unani systems of medicine and it is described by Chandra and Singh.¹ Three lichens can be called Chharila: *Parmotrema Chinense*, *Parmotrema perforatum*, and/or *Everniastrum cirrhatum*. The smoke of Chharila is believed to relieve headaches. When powdered it is applied on wounds, and it is considered to be a good cephalic snuff. Chharila has also been considered useful in dyspepsia, spermatorrhoea, amonorrhoea, calculi, diseases of the blood and heart, stomach disorders, enlarged spleen, bronchitis, bleeding piles, scabies, leprosy, excessive salivation, and soreness of the throat, toothache, and pain in general. When the drug was analyzed, it only contained *Everniastrum cirrhatum* and *Parmotrema perforatum* and about 50% of the Chharila was other lichens which may have just been adulterants: *Leptogium* spp., *Parmelia hyporysalia*, *Ramalina* spp., *Usnea* spp., and *Anaptychia* spp. These lichens are dual

organisms composed of a symbiotic relationship between an alga and a fungus. The fungus, usually an Ascomycete, provides the plant its shape, and the alga provides the ability to photosynthesis. This successful combination is able to produce a more elaborate and durable organism than either partner alone. These are able to colonise inhospitable areas such as bare rock. As pioneer plants, lichens break down the rock surface and, together with decaying material from the lichen, eventually form soil conditions suitable for other plants. Many lichens are epiphytic (able to grow on trees), gaining nutrition from rain running down tree trunks. Lichens are variable in shape; tubular, upright and branching, or flat and leaf-like or forming an amorphous greyish crust.

Gastric ulcer disease (GUD) is one of the major gastrointestinal disorders which occur due to an imbalance between offensive (acid, pepsin and *Helicobacter pylori*) and defensive (mucin, prostaglandin and bicarbonate) factors. Consequently reduction of gastric acid production as well as reinforcement of gastric mucosal protection has been the major therapeutic approaches of gastric ulcer disease.² A number of anti-ulcer drugs including proton pump inhibitors (PPI) and H₂ receptor antagonists are available for the treatment of GUD, but clinical evaluation of these drugs has shown incidence of relapse, side effects and drug interactions. This has been the rationale for the development of new anti-ulcer drugs and thus the search for novel molecules has been extended to medicinal plants that can offer better protection and decrease relapses.

The present study was undertaken to investigate the underlying mechanism of the anti-ulcer property of the ethanol extract of *P. perlata*, responsible for gastro protective effects by which mechanism of action.

Material and Methods

Collection of the Plant material

The plant material was purchased from the local market and was authenticated by the Botany Division of the Lucknow University.

Extraction and Fractionation Procedure

Parmelia perlata (1.0Kg.) was percolated in 95% ethanol at room temperature in glass percolator four times. The combined ethanol extract was filtered and concentrated in a rotavapour below 50°C to a green viscous mass, which was dried under high vacuum to remove last traces of the

solvent. The ethanol extract was evaluated for its antiulcer activity.

Antiulcer Screening

Experimental Animals

Adult Sprague Dawley rats of either sex, weighing 180-200g were housed in raised bottom mesh cages to prevent coprophagy and were kept in environmentally controlled rooms (25 ± 2°C, 12 hours light and dark cycle). Animals were fed with reference laboratory food pellets and water was provided ad libitum. Experimental protocols were approved by our Institutional Ethical Committee following the guidelines of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals) which complies with International norms of INSA (Indian National Science Academy).

Materials

Omeprazole and other chemicals were obtained from M/s. Sigma Chemicals, St Louis, MO, USA. Whereas sucralfate was purchased from Menarini Raunaq pharmaceutical, India.

Treatment Schedule

The ethanol extract of the *Parmelia perlata* (100 mg/kg), reference drugs omeprazole (Omz) (10 mg/kg) and sucralfate (SUC) (500 mg/kg) were prepared in 1% carboxymethyl cellulose (CMC) as suspension and administered orally 45 minute prior to exposure of ulcerogens to the animals at a volume of 1ml/200g of body weight. All animals were deprived of food for 16 h before ulcerogens exposure and were divided into three groups, (n=6).

Group I: Control group of animals were treated with vehicle 1% CMC.

Group II: Graded doses of ethanol extract of *P. perlata* (50,100 and 200 mg/kg p.o.) tested against Cold restraint ulcer (CRU) model to identify the effective dose and selected for further studies in other ulcer models.

Group III: Experimental group was treated with reference anti-ulcer drugs such as omeprazole (Omz) (10 mg/kg, p.o.) in (CRU), aspirin (AS), pyloric ligation (PL) induced gastric ulcer models and sucralfate SUC (500 mg/kg, p.o.) in Alcohol (AL) induced gastric ulcer model.

Anti-ulcer studies

Cold restraint induced gastric ulcer (CRU)

The ethanol extract of the *P. perlata* (100 mg/kg, p.o.) and reference drug omeprazole (10 mg/kg, p.o.) were treated to animals 45 mins before of cold restraint stress. Then all animals were immobilized in restraint cage and kept at 4°C in an environmental chamber.³ Two hours later the animals were sacrificed and stomachs were observed and scored under Magnascope for ulcers.

Aspirin induced gastric ulcer model (AS)

Aspirin at a dose of 150 mg/kg was administered to induce ulcer after 45mins of treatment of the extract (100 mg/kg, p.o.) and reference drug omeprazole (10 mg/kg, p.o.). The animals were sacrificed 5 hours after aspirin treatment and the stomach was dissected out, incised along the lesser curvature and the lesion was scored.⁴

Alcohol induced gastric ulcers in rats (AL)

Gastric ulcer was induced in rats by administering chilled absolute alcohol (1ml/200g, body weight of animals) as described by Roberts *et al.*⁵ The extract (100 mg/kg, p.o.) and reference drug sucralfate (500 mg/kg, p.o.) were administered 45 minutes before alcohol treatment. After 1 hour of alcohol administration, the animals were sacrificed and stomach was cut open along the greater curvature to observe the gastric lesions which appear as hemorrhagic bands along the mucosal ridges of the stomach. The lengths of the lesions were measured using Biovis image analyzer software and summated to give a total lesion score.

Pyloric ligation induced ulcer model (PL)

After 45 minutes of administration of *Parmelia* (100 mg/kg, p.o.) and reference drug omeprazole (10 mg/kg, p.o.), ulcer was induced in rats by pyloric ligation. Under Chloral hydrate anesthesia (300 mg/kg, i.p.), the abdomen was opened and the pyloric end of the stomach was ligated avoiding any damage to the adjacent blood vessels.⁶ Stomach was replaced carefully and the animals were allowed to recover with free access to water. After 4 hours the animals were sacrificed and the stomach was dissected out. Lesions were scored and gastric fluid was collected and centrifuged at 2000 rpm for 10 min. The collected supernatant was used for the estimation of gastric secretion studies and mucin estimation

Gastric secretion study

Free and total acidity was measured from the collected gastric juice by titrating against 0.01N NaOH, using phenolphthalein as an indicator and expressed in terms of μ equiv./ml.⁷ Mucin level in gastric juice was quantified.⁸

Direct fluorometric assay

Before the fluorometric assay, gastric juice was delipidated by following the protocol described by Wessel. Mucin level in gastric juice was quantified with a fluorometric assay for O-glycosylated glycoprotein as described by Crowther.⁹ In brief, 50 μ l of gastric juice was diluted with 1: 1 (v/v) in Phosphate Buffered Saline buffer (PBS) and methanol 400 μ l was added. Centrifuged at (9000 g for 1 min), Then added 200 μ l of chloroform and 300 μ l of distilled water, thoroughly mixed it, and centrifuged once more. The upper phase was discarded and 300 μ l of methanol was added. After a further centrifugation (9000 g for 2 min), lipid-free proteins were recovered in the pellet. For the fluorometric mucin determination, the pellet was resuspended in 200 μ l of PBS, alkaline reagent 250 μ l (1 ml 0.15 N NaOH and 200 μ l of 0.6 M 2-cyano-acetamide) was added and the mixture was incubated at 100°C for 30 min. Afterwards, 2 ml of 0.6 M borate buffer (pH 8) was added and the fluorescence was measured by varion fluorimeter at 383 nm (excitation 336 nm).

Measurement of ulcer index

Ulcers were formed due to treatment with different ulcerogens observed under Magnascope (5X magnification) and were scored according to the arbitrary scoring system as described.¹⁰ The severity and intensity of the lesions were graded as following:

- (i) Shedding of epithelium = 10;
- (ii) Petchial and frank hemorrhages = 20;
- (iii) One or two ulcers = 30;
- (iv) More than two ulcers = 40;
- (v) Perforated ulcers = 50.

Statistical analysis

All values shown in the figures and tables represent the means \pm S.E.M. Statistical analysis was performed with Prism version 3.0 software using one-way analysis of

variance (ANOVA) followed by Dunnett's multiple comparison test. $P < 0.05$ was considered to be statistically significant.

Results

Anti-ulcer effect of the ethanol extract of the *P. perlata* against cold restraint induced ulcer in rats

Graded doses of extract of the *P. perlata* (50, 100 and 200 mg/kg, p.o.) showed percentage protection of 25.0, 50.0 and 58.5 respectively where reference drug, omeprazole (10 mg/kg, p.o.) showed a percentage protection of 77.4 ($P < 0.01$) in comparison to control against CRU model. From this observation 100 mg/kg dose of *P. perlata* was identified as the effective dose and selected for further studies. The results are graphically represented in figure 1.

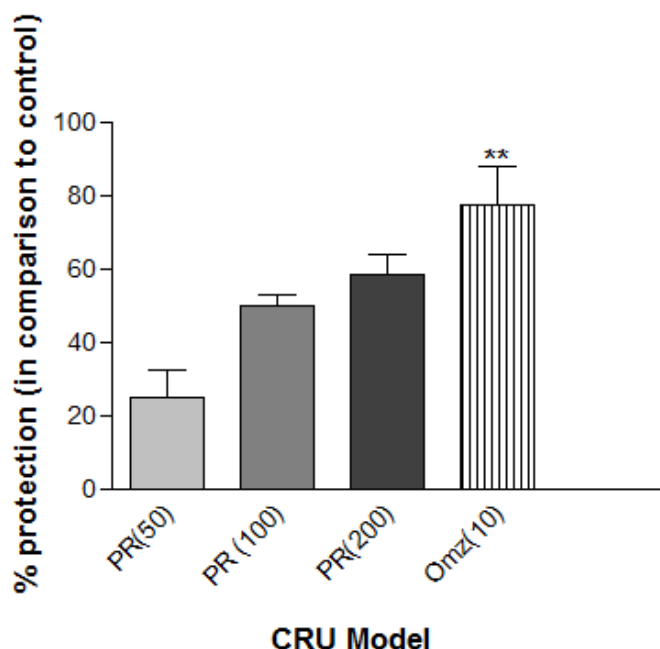


Figure 1: Effect of graded dose of *Parmelia perlata* ethanol extract (PR) and reference drug omeprazole (Omz) on percentage protection of ulcer against cold restraint induced gastric ulcer models in rats. Data expressed as mean % protection \pm S.E.M. Statistical analysis was done by One Way ANOVA followed by Dunnett's Multiple Comparison Test.

*Statistically significant at $**P < 0.01$, in comparison to control. $n = 6$ in each group.

Effect of the ethanol extract of the *P. perlata* against aspirin induced ulcer in rats

P. perlata (100 mg/kg, p.o.) was tested against aspirin (150 mg/kg, p.o.) induced ulcer model and showed 37.81% protection while reference drug omeprazole (10 mg/kg,

p.o.) showed 57.08% protection in comparison to control as shown in figure 2.

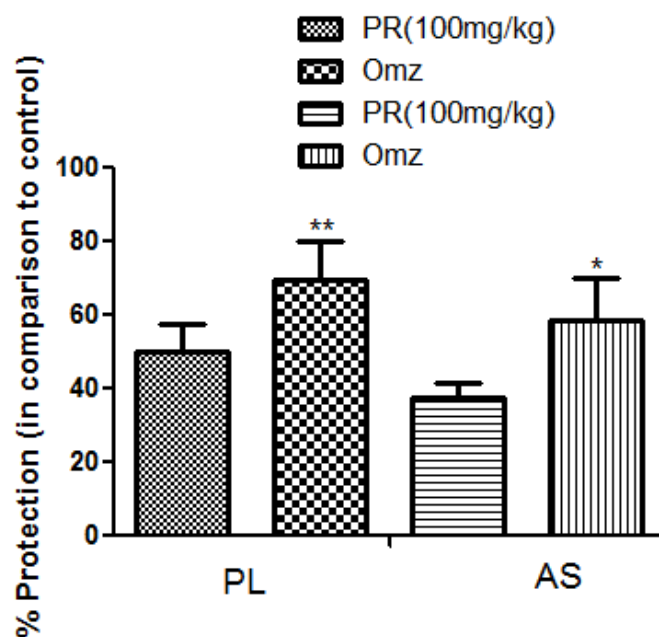


Figure 2: Effect of *Parmelia perlata* ethanol extract (PR) and reference drug omeprazole (Omz) on percentage protection of ulcer against pyloric ligation (PL) and aspirin (AS) induced gastric ulcer models in rats. Data expressed as mean % protection \pm S.E.M. Statistical analysis was done by One Way ANOVA followed by Dunnett's Multiple Comparison Test.

*Statistically significant at $*P < 0.05$ and $**P < 0.01$, in comparison to control. $n = 6$ in each group.

Effect of the ethanol extract of the *P. perlata* against alcohol induced ulcer

Anti-ulcer activity of the ethanol extract of the *P. perlata* against ethanol induced ulcer having 65.41% protection ($P < 0.01$), whereas the reference drug, sucralfate (500 mg/kg, p.o.), showed 62.72% protection ($P < 0.05$) as depicted in figure 3.

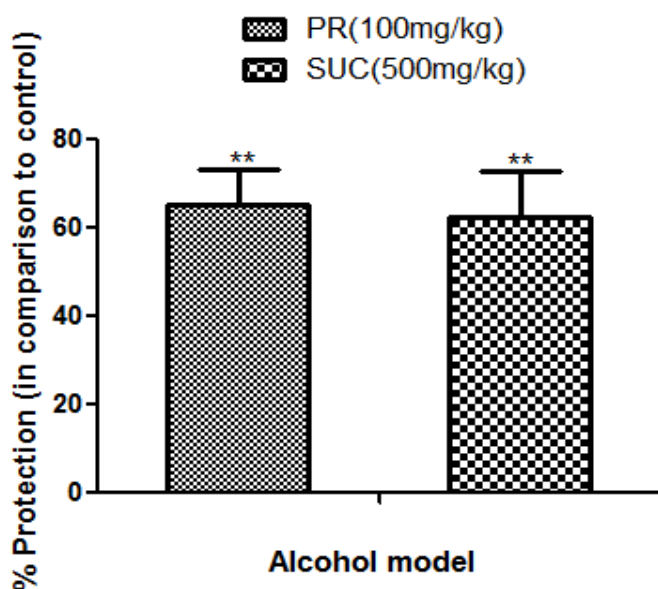


Figure 3: Effect of Parmelia (PR) and reference drug sucralfate (SUC) on percentage protection of ulcer against alcohol induced gastric ulcer model in rats. Data expressed as mean % protection \pm S.E.M. Statistical analysis was done by One Way ANOVA followed by Dunnett's Multiple Comparison Test.

*Statistically significant at $**P < 0.01$, in comparison to control. $n = 6$ in each group.

Table 1: Effect of PR and reference drug omeprazole Omz on free acidity, total acidity and mucin contents in pyloric ligation model ($n = 6$ in each group)

Treatment	Free acid $\mu\text{equiv./ml}$	Total acid $\mu\text{equiv./ml}$	Mucin $\mu\text{g/ml}$
Control	60.50 ± 5.106	108.23 ± 6.970	1083.02 ± 179.56
PR (100 mg/kg)	48.98 ± 5.634	92.61 ± 8.310	1261.94 ± 156.40
Omz (10 mg/kg)	$33.81 \pm 4.762^{**}$	$58.37 \pm 8.361^{**}$	$1459.73 \pm 102.40^*$

Discussion

Natural products have gained powerful attention due to its effective roles in chemo-therapeutic agents. The plants were used in traditional system of medicine or folklore for the treatment or amelioration of the incidence of gastric ulcers.¹¹ The anti-ulcer activity of *P. perlata* has been studied against various models of experimentally induced gastric ulcer in order to evaluate its mechanism of action involved in the prevention of ulcer formation. The finding receives an impetus by considering the fact that the ethanol extract of the *P. perlata* showed anti-ulcerogenic activity in all the models, each of which induced ulcer through a different mechanism.

Anti-ulcer effect of the ethanol extract of the *P. perlata* against pyloric ligation induced ulcer in rats

The ethanol extract of the *P. perlata* at (100 mg/kg, p.o.) posses anti ulcer activity against pyloric ligation induced ulcer in rats where it showed protection of 50.00% ($P < 0.01$) and reference drug omeprazole (10 mg/kg, p.o.) showed 69.42% ($P < 0.01$) protection (figure 2).

Effect of the ethanol extract of the *P. perlata* on gastric secretion

The antisecretory effect of Parmelia was evaluated by estimating free and total acidity of gastric juice and by estimating the activity of mucin as shown in Table1. Parmelia has reduced free acidity (19.04%), total acidity (14.43%) which was comparable with reference drug omeprazole (44.11%, $P < 0.01$) and (46.06%, $P < 0.01$) respectively. It is up regulated mucin secretion by 14.17% whereas omeprazole increased mucin secretion by 25.80% ($P < 0.05$) in comparison to control.

Gastric ulcer is postulated to develop when there is a disbalance of aggressive and defensive factors either because of the increased secretion of acid or pepsin or because of impairment of mucosal resistance.

A dose dependent anti-ulcer study of the ethanol extract of the *P. perlata* in CRU model was performed. CRU is a well-accepted model for the induction of gastric ulcers, in which peripheral sympathetic activation and increased acid secretion play important roles.¹² The ethanol extract of the *P. perlata* exhibited significant protection in a dose dependent manner in the CRU model; In addition, the ethanol extract of the *P. perlata* exerted a protective effect against ethanol-induced gastric lesions in contrast to reference drug, sucralfate.

Graded doses of the ethanol extract of the *P. perlata* exerted anti-ulcer effect in the CRU model, offering maximum protection at dose of 100 mg/kg. Hence, 100 mg/kg dose was considered to be the optimum dose for evaluation in further studies. The ethanol extract of the *P. perlata* was highly effective in decreasing the hemorrhagic lesions induced by ethanol in contrast to reference drug, sucralfate, reflecting its cytoprotective activity.

Furthermore, gastric acid is an important factor for the genesis of ulceration in pyloric-ligated model. In this model, auto-digestion of mucosa by gastric acid and pepsin results in the development of ulcers.¹³ The ethanol extract of the *P. perlata* reduced free and total acidity in this model, which suggests its anti-secretory potency.

The cytoprotective ability of the ethanol extract of the *P. perlata* was evident with increase in mucin content in pyloric ligation model and protection against ethanol induced ulcer model in comparison with the reference drugs. To further substantiate the cytoprotective potency of the ethanol extract of the *P. perlata*, its effect against NSAIDs induced ulcer model was explored. Studies suggest that NSAIDs induces ulcers due to their effect on cyclooxygenase enzyme leading to reduced prostaglandin production and increase in acid secretion.¹³ The ethanol extract of the *P. perlata* significantly reduced ulcer incidence, which further supports cytoprotective effect of the ethanol extract of the *P. perlata* which may be mediated by prostaglandins.

Conclusion

Our study is the first of its kind to show significant anti-secretory and cytoprotective effect of the ethanol extract of the *P. perlata*. Thus, the ethanol extract of the *P. perlata* may emerge as a more potent therapeutic agent in treating gastric ulcer incidences.

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Conflict of Interest

No conflict between the authors.

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