Original Article

Thai Journal of Pharmaceutical Sciences

Gastroprotective effect of *Phyllanthus reticulatus* Poir. against pylorus ligation-, ethanol-induced, and stress-induced ulcer models in Wistar rats

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Received: May 30, 2021
Accepted: July 26, 2021
Published: March 23, 2022

ABSTRACT

Objective: The objective of the present study is to evaluate the antiulcer property of ethanolic extract of *Phyllanthus reticulatus* Poir. against pylorus ligation-, ethanol-induced, and stress-induced ulcer models in Wistar albino rats. **Materials and Methods:** Three models – pylorus ligation-induced ulcer, ethanol-induced ulcer, and swim stress-induced ulcer – were used to induce ulcer in Wistar rats. The animals were treated with 200 mg/kg and 400 mg/kg p. o of the ethanolic extract of *P. reticulatus* Poir, to estimate the gastroprotective potential. The effect of *P. reticulatus* Poir. on pH of the gastric juice, volume of acid secretion, total and free acidity, ulcer index, and % ulcer protection was assessed to determine the gastroprotective potential. **Results:** A decrease in ulcer index was observed in all three models after treatment with *P. reticulatus* Poir. In pylorus ligation model, the doses of 200 mg/kg and 400 mg/kg exhibited % protection of 88.11 and 91.53, respectively. In ethanol-induced ulcer model, 200 mg/kg and 400 mg/kg displayed % protection of 50.40 and 60.94, respectively. In stress-induced ulcer model, 200 mg/kg and 400 mg/kg displayed % protection of 60.94 and 72.31, respectively. A decrease in aggressive factors and an increase in protective factors were observed during the estimation of biochemical parameters. **Conclusion:** The present study proves that the ethanolic extract of *P. reticulatus* Poir. possesses significant gastroprotective property.

Keywords: Gastric ulcer, gastroprotective, *Phyllanthus reticulatus* Poir., pylorus ligation

INTRODUCTION

The World Health Organization estimates that 80% of human population must depend on plant-based traditional medicines for health care.[1] Phytoconstituents derived from botanicals have been found to be effective in major ailments and are less toxic compared to existing drugs. An imbalance between aggressive and defensive factors of gastric mucosa leads to pathogenesis of ulcer.[2] Chronic stress, consumption of alcohol and tobacco, nonsteroidal anti-inflammatory drugs, and *Helicobacter pylori* are considered to be the major factors that act as aggressive factors in the pathogenesis of gastric ulcer. There is a need to discover newer antiulcer drugs to avoid the potential problem associated with the long-term use of synthetic proton-pump inhibitors.[3]

Phyllanthus reticulatus Poir. is a shrub with smooth or lenticellate branches reaching a maximum height of 10 feet that belongs to the family Euphorbiaceae. The leaves of the plants are traditionally used as diabetic and have also been reported to possess diuretic, astringent, and astringent properties. The leaf extract of *P. reticulatus* Poir. has been reported to possess antimicrobial properties against Gram-negative bacteria and the whole plant extract has been reported to have antioxidant property.[4,5] The major phytoconstituents reported to be present in the leaves of *P. reticulatus* Poir. are lupeol, stigmasterol, scopoletin, friedelin, epifriedelinol, betulin, taraxerone, beta-sitosterol, glochidonol, octacosanol, methyl gallate, ellagic acid, corilagin, methyl brevifolin carboxylate, kaempferol, astragalin, quercetin, and isoquercitin.[5] *P. reticulatus* Poir. has been reported to be effective against Gram-negative bacteria such

as *Escherichia coli*, *Salmonella* Typhi, and *Shigella dysenteriae*.[6] *P. reticulatus* Poir. has also been reported to possess antioxidant properties.[7] The hypothesis of this study is that the leaf extract of *P. reticulatus* Poir. might be effective against gastric ulcer because of its proven antioxidant and antimicrobial property against Gram-negative bacteria. However, it should be highlighted that not all substances effective against Gram-negative bacteria are effective in the treatment of gastric ulcer. Free radicals have long been proven to play a significant role in the pathogenesis of ulcer. Since *P. reticulatus* Poir. also possess antioxidant property, it might be effective in gastric ulcer by negating the deleterious effects of free radicals. The aim of this study is to evaluate the gastroprotective property of *P. reticulatus* Poir. against pylorus ligation-, ethanol-induced, and stress-induced ulcer models in Wistar albino rats.

MATERIALS AND METHODS

Collection of Plant

P. reticulatus Poir. plant was collected in and around the district of Erode in Tamil Nadu, India. The plant was identified and authenticated by the Botanical Survey of India, Tamil Nadu Agricultural University, Coimbatore. After the identification of plant, leaves were collected, dried, and ground into a fine powder for extraction. A voucher specimen has been deposited in the herbarium of Tamil Nadu Agricultural University for future reference. The accession number of the specimen is BSI/SRC/5/23/2019-20/Tech-435. This study was approved by the Institutional Animal Ethics Committee (JKKMMRFCP/IAEC/2020/005).

Extraction and Dose

The dried and powdered leaves of *P. reticulatus* Poir. were extracted using petroleum ether in a Soxhlet apparatus until the solvent turned colorless. The marc was then extracted with ethanol to obtain a greenish mass. The extract was suspended in 1% carboxymethylcellulose (CMC) for *in vivo* studies. The dose of the ethanolic extract of *P. reticulatus* Poir. was fixed at 200 and 400 mg/kg based on existing literature.[8]

Evaluation of Anti-Ulcer Activity

Pylorus ligation method

The rats of either sex were divided into four groups of six animals each. The first group served as normal and received 1% CMC. The second group received ranitidine 20 mg/kg/os (p.o.) for 3 days. The third and fourth groups were treated with 200 mg/kg and 400 mg/kg p.o of the ethanolic extract of *P. reticulatus* Poir., respectively. Under ether anesthesia, the abdomen of the rats was opened by a midline incision below the xiphoid process. The pyloric end of the stomach was slightly lifted and ligated without avoiding damage to its blood supply. The stomach was placed back carefully in its original position and the abdominal wall was closed with sutures. The drugs were administered orally 2 h before pyloric ligation and sacrificed 6 h after the operation. The abdomen was opened, the cardiac end of the stomach dissected out, and the contents were drained into a glass tube for the estimation of gastric volume, pH, free acidity, and total acidity. The stomach tissue was examined for ulceration [Table 1]. The ulcer score was divided by a factor of 10 to get the ulcer index. Percentage ulcer protection was calculated using ulcer index values.[9]

$$\frac{\text{Ulcer Index in Control-Ulcer Index in Test}}{\text{Ulcer Index in Control}} \times 100$$

Ethanol-Induced Ulcer

The rats of either sex were divided into four groups of six animals each. Group 1 served as normal control and received 1% CMC, and Group 2 was treated with sucralfate p.o (100 mg/kg). Groups 3 and 4 received 200 and 400 mg/kg of the ethanolic extract of *P. reticulatus* Poir., respectively. One hour after the respective treatment, the animals received 1 ml of absolute ethanol. After 5 h, the animals were sacrificed to score the extent of ulceration.[10]

Swimming Stress-Induced Ulcer

The rats of either sex were divided into four groups of six animals each. Stress ulcers were induced by forcing the animals to swim in a glass cylinder (height 45 cm and diameter 25 cm) containing water (25°C) up to 35 cm for 5 h. Group 1 served as control and received 1% CMC, (p.o). Group 2 received omeprazole p.o (20 mg/kg) as standard. Groups 3 and 4 received ethanolic extract of 200 and 400 mg/kg of *P. reticulatus* Poir. After the drug treatment, animals were forced to swim in water for 5 h and sacrificed later for further analysis.[11]

Biochemical Estimations

The effect of *P. reticulatus* Poir. on biochemical parameters such as gastric volume, pH, free acidity, total acidity, hexoses, hexosamine, and fucose was studied in pylorus ligation model.

Determination of Gastric Volume and pH

The gastric juice was centrifuged for a period of 10 min at 3000 rpm (revolutions per minute). The volume of supernatant was measured and expressed as ml/100 g body weight. The pH of the supernatant was measured using digital pH meter.[12]

Determination of Free Acidity and Total Acidity

A 1 ml of gastric juice was titrated against 0.01 N sodium hydroxide using Topfers reagent as indicator. The point at which disappearance of red color happens corresponds to free acidity. To estimate total acidity, two drops of phenolphthalein were added and titrated until reappearance of a definite red tinge. Acidity was calculated using the formula:[13]

$$\text{Acidity} = \frac{\text{Volume of NaOH} \times \text{Actual Normality of NaOH} \times 100}{0.1} \times 100$$

Preparation of Gastric Mucosa Homogenate

The stomach was opened and washed with normal saline and the gastric mucosa was scraped. The scraped mucosa was suspended in 0.15 M kcl-10 mM potassium phosphate buffer (pH 7.4) and centrifuged at 1000 rpm for 10 min.[14]

Estimation of Total Carbohydrates

Estimation of total hexoses

A mixture of orcinol reagent (3.4 ml) and 0.4 ml of hydrolysate was heated for 15 min. The mixture was then cooled and the intensity of the color was read in a Spectronic-20 absorptiometer set at 540 nm. The total hexoses content was estimated from the standard curve of galactose-mannose and expressed as μg/ml in gastric juice or μg/100 mg gastric mucosa.[15]

Estimation of hexosamine

A mixture containing 0.5 ml of the hydrolyzed fraction and 0.5 ml of acetyl acetone reagent was heated for 20 min. A 1.5 ml of 95% alcohol was added to the above mixture after cooling. A 0.5 ml of Ehrlich's reagent was added and set aside for 30 min for completion of the reaction. Color intensity was measured on the Spectronic-20 absorptiometer set at 530 nm. The hexosamine content was expressed as μg/ml in gastric juice or μg/100 mg of gastric mucosa.[16]

Estimation of fucose

To 0.4 ml of the hydrolysate, 1.8 ml of 6:1 H_2SO_4 was added to the tubes placed in ice-cold water. This mixture was then heated on the boiling water bath for exactly 3 min and then cooled. After 90 min, the absorbance was measured at 396 and 430 nm. True optical density for fucose in the hydrolysate was calculated from the differences in the reading obtained at 396 and 430 nm.[17]

Optical density of the sample was calculated as follows:

$$OD = \frac{(OD_{396} - OD_{430}) - (OD_{396} - OD_{430})}{(OD_{396} - OD_{430})}$$

Estimation of total proteins

A mixture of 1 ml of tissue homogenate and 5 ml of Lowry reagent was mixed well and incubated at 25°C for 10 min. Then, 0.5 ml of Folin–Ciocalteu reagent was added and incubated at 25°C for 30 min. The absorbance of the sample was measured at 750 nm.[18]

RESULTS

Extraction

The yield of *P. reticulatus* Poir. extract using ethanol has a solvent which was 9% w/w.

Antiulcer Activity

Effect of P. reticulatus Poir. on pylorus-ligated (shay) rat model

A significant gastroprotective effect was observed in the animals treated with 200 mg/kg and 400 mg/kg *P. reticulatus* Poir. The doses of 200 mg/kg and 400 mg/kg produced an ulcer index of 2.23 ± 0.044 and 1.66 ± 0.48 and % protection (88.11 and 91.53). A decrease in ulcer index and increase in % ulcer protection were observed [Table 2 and Figure 1].

The figure represents the effect of *Phyllanthus reticulatus* on pylorus ligation at 200 and 400 mg/kg dose compared to control and standard.

Effect of P. reticulatus Poir. on ethanol-induced ulcer model

At 200 mg/kg and 400 mg/kg, *P. reticulatus* Poir. showed a significant gastroprotective effect against ethanol-induced ulcer model. An ulcer protection percentage of 50.40 and 60.94 was observed at 200 and 400 mg/kg of *P. reticulatus* Poir. compared to the standard sucralfate (65.01) [Table 3 and Figure 2].

The figure represents the effect of *P. reticulatus* on ethanol-induced ulcer at 200 and 400 mg/kg dose compared to control and standard.

Effect of P. reticulatus Poir. on swim stress-induced ulcer model

At 200 mg/kg and 400 mg/kg, *P. reticulatus* Poir. showed a significant gastroprotective effect against swim stress-induced ulcer model. An ulcer protection percentage of 60.94 and

Table 1: Scoring of ulcers

Ulcer scoring point	Size and severity of ulcers
0	Normal colored stomach
0.5	Red coloration
1	Spot ulceration
1.5	Hemorrhagic streak
2	Ulcers≥3 mm but≤5 mm
3	Ulcers>5 mm

Table 2: Effect of *Phyllanthus reticulatus* Poir. on ulcer index in pylorus-ligated (shay) rat model

S. No.	Treatment	Ulcer index	Percent ulcer protection
1.	Control (1% CMC) (1 ml/kg)	18.75±0.150	-
2.	Ranitidine (20 mg/kg)	1.68±0.040**	91.04
3.	*P. reticulatus* Poir. (200 mg/kg)	2.23±0.044**	88.11
4.	*P. reticulatus* Poir. (400 mg/kg)	1.66±0.048**	91.53

Values represent mean±SEM, n=6. Data were analyzed by one-way analysis of variation followed by Dunnett's test. ***P<0.001, **P<0.01, *P<0.05 and ns: Non-significant. Standard and test were compared with the control group

Table 3: Effect of *Phyllanthus reticulatus* Poir. on ethanol-induced ulcer model

S. No.	Treatment	ulcer index	Percent ulcer protection
1	Control (1% CMC) (1 ml/kg)	13.36±0.35	-
2	Sucralfate 100 mg/kg	4.67±0.22**	65.01
3	*P. reticulatus* Poir. (200 mg/kg)	6.63±0.21**	50.40
4	*P. reticulatus* Poir. (400 mg/kg)	5.22±0.13**	60.94

Values represent mean±SEM, n=6. Data were analyzed by one-way analysis of variation followed by Dunnett's test. ***P<0.001, **P<0.01, *P<0.05 and ns: Non-significant. Standard and test were compared with the control group

72.31 was observed at 200 and 400 mg/kg of *P. reticulatus* Poir. compared to the standard omeprazole (72.31) [Table 4 and Figure 3].

The figure represents the effect of *P. reticulatus* on stress-induced ulcers at 200 and 400 mg/kg dose compared to control and standard.

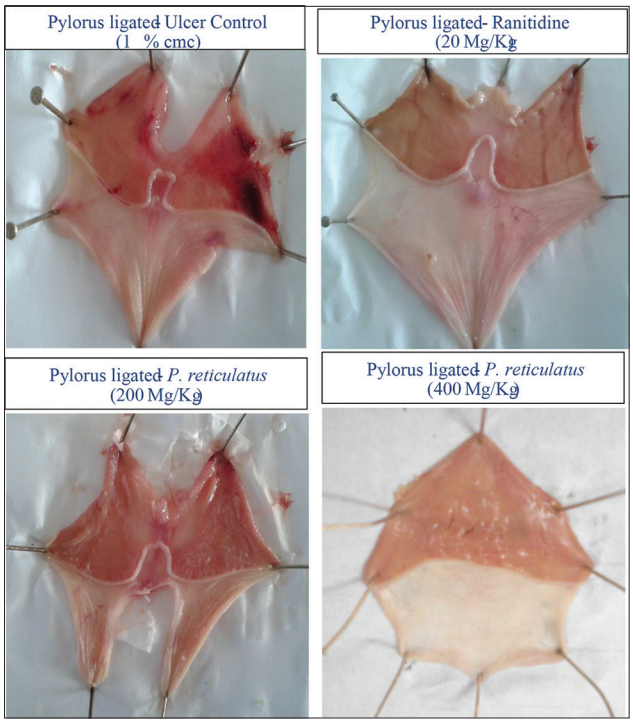

Figure 1: Effect of *Phyllanthus reticulatus* on pylorus ligation-induced ulcers

Table 4: The effect of *Phyllanthus reticulatus* Poir. on swim stress-induced ulcers

S. No.	Treatment	Ulcer index	Percent ulcer protection
1.	Control (1% CMC) (1 ml/kg)	13.34±0.23	-
2.	Omeprazole (20 mg/kg)	3.04±0.05**	77.18
3.	*P. reticulatus* Poir. (200 mg/kg)	5.21±0.10**	60.94
4.	*P. reticulatus* Poir. (400 mg/kg)	3.69±0.12**	72.31

Values represent mean±SEM, n=6. Data were analyzed by one-way analysis of variation followed by Dunnett's test. ***P<0.001, **P<0.01, *P<0.05 and ns: Non-significant. Standard and test were compared with the control group

Effect of P. reticulatus Poir. on gastric volume, pH, free acidity, and total acidity

A significant effect was observed at 200 and 400 mg/kg of *P. reticulatus* Poir. on acid parameters compared to control. Acid volume, total acidity, and free acidity were decreased and pH of the gastric juice was increased in animals treated with test drugs [Table 5].

Effect of P. reticulatus Poir. on total hexose, hexosamine, fucose, and total protein

The result shows a significant increases in total hexoses present in gastric juice of *P. reticulatus* Poir. treated animals. The concentration of total hexoses at the dose of 200 mg/kg and 400 mg/kg group was found to be 320.96 ± 0.3.49 µg/ml and 351.11 ± 4.21 µg/ml which show significant increase when compared to the control group (247.43± 4.29 µg/ml). The concentration of hexosamine at the dose of 200 mg/kg and 400 mg/kg group was found to be 423.53 ± 4.41 µg/ml

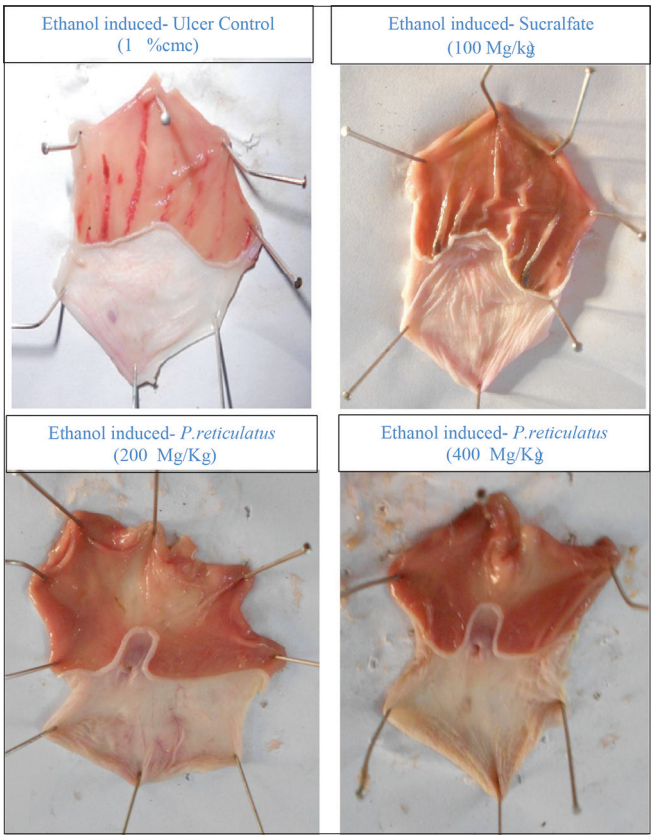

Figure 2: Effect of *Phyllanthus reticulatus* Poir. on ethanol-induced ulcer

Table 5: Effect of *Phyllanthus reticulatus* Poir. on gastric volume, pH, free acidity, and total acidity

S. No.	Treatment	Gastric Vol. (ml)	pH	Free acidity (µeq/ml/100 g)	Total acidity (µeq/ml/100 g)
1	Control	5.20±0.16	2.31±0.16	35.97±0.97	49.45±0.66
2	Ranitidine	2.18±0.13**	4.52±0.21**	15.86±0.95**	24.32±0.56**
3	Test (200 mg/kg)	3.79±0.21**	3.47±0.14**	23.67±0.80**	33.11±0.69**
4	Test (400 mg/kg)	2.56±0.19**	4.532±0.23**	20.28±0.66**	28.55±0.61**

Values represent mean±SEM, n=6. Data were analyzed by one-way analysis of variation followed by Dunnett's test. ***P<0.001, **P<0.01, *P<0.05 and ns: Non-significant. Standard and test were compared with the control group

and 451.02 ± 6.67 µg/ml. It shows a significant increase in hexosamine content when compared to the control group (287.88 ± 5.35 µg/ml). The concentration of fucose at the dose of 200 mg/kg and 400 mg/kg group was found to be 126.71 ± 3.94 µg/ml and 168.62 ± 2.84 µg/ml. This shows a significant increase of fucose content when compared to the control group (86.65 ± 3.58) µg/ml. The concentration of protein at the dose of 200 mg/kg and 400 mg/kg group was found to be 522.64 ± 2.33 µg/ml and 542.70 ± 3.36 µg/ml. It shows the significant increase of protein content when compared to the control group (146.25 ± 2.55 µg/ml) [Table 6].

DISCUSSION

The aim of the present study is to evaluate the potential beneficial and protective role of the ethanolic extract of *P. reticulatus* Poir. in pylorus-ligated, ethanol-induced, and stress-induced ulcers in Wistar rats. Pyloric ligation model induces gastric ulceration through histamine-2 receptors (H2R). This triggers the secretion of acid into the stomach through activation of cyclic adenosine monophosphate/protein kinase pathway. H2R is the primary target of antiulcer drugs, and H2R antagonists have been shown to inhibit gastric acid secretion in many animal models. Hence, ranitidine was used as a standard in this model to study the effect of *P. reticulatus* Poir. against gastric ulcer.[19] The ligation of the pylorus end of the stomach leads to accumulation of aggressive factors such as gastric acid and pepsin in the stomach, leading to pathogenesis of gastric ulcer. The accumulation of acid and pepsin leads to disruption of protective barriers potentiating the damaging effects of aggressive factors such as acid and pepsin.[20] The doses of 200 mg/kg and 400 mg/kg produced an ulcer index of 2.23 ± 0.044 and 1.66 ± 0.48 and % protection (88.11 and 91.53). This shows a decrease in ulcer index and increase in % ulcer protection. The ethanolic extract of *P. reticulatus* Poir. showed a protective effect in the pylorus-ligated model and this could be due to protective effects of phytoconstituents such as phenols, flavonoids, and sterols present in the extract.

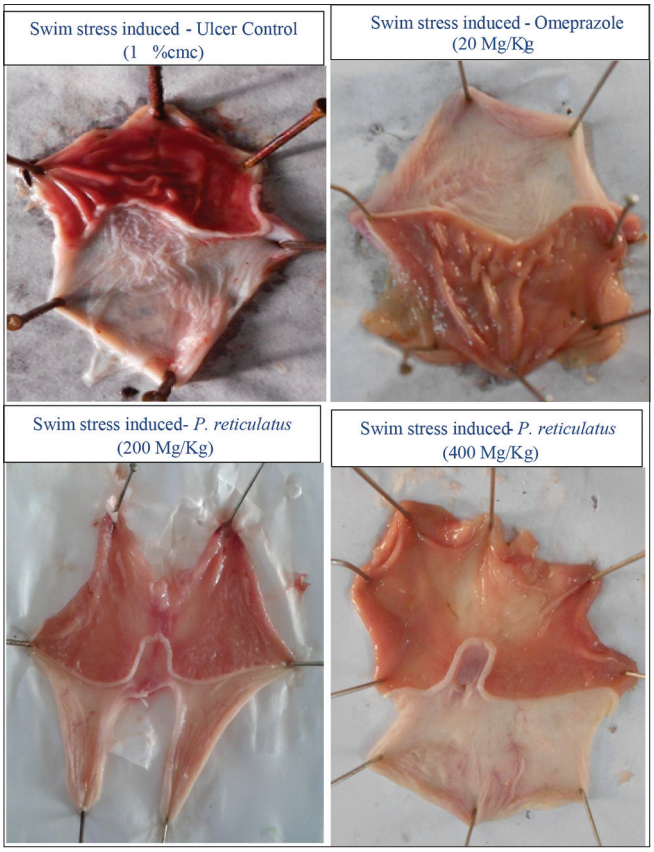

Figure 3: Effect of *Phyllanthus reticulatus* Poir. on swim stress-induced ulcer model

Ethanol-induced gastric damage is caused possibly through leukotrienes production and also involvement of 5-lipoxygenase in the formation of ulcer lesion. Prostaglandins also play a role in ethanol-induced ulcer. It is well known that ethanol-induced gastric lesions are not inhibited by antisecretory agents like ranitidine but are inhibited by agents who enhance mucosal defense factors such as sucralfate. Hence, sucralfate was used as a standard in ethanol-induced ulcer model.[9] Ethanol penetrates the mucosa of the gastrointestinal tract because of its ability to solubilize the protective mucous and expose the mucosa to the damaging actions of HCl and pepsin. Further, alcohol stimulates the secretion of acid and decreases blood flow leading to microvascular injuries, through disruption of the vascular endothelium and facilitating vascular permeability.[21,22] Ethanol also triggers imbalances in cellular antioxidant processes. In ethanol-induced ulcer model, 200 mg/kg and 400 mg/kg of the ethanolic extract of *P. reticulatus* Poir. produced an ulcer index of 6.63 ± 0.21 and 5.22 ± 0.13 and % protection (50.40 and 60.94). Stress-induced ulcers manifest as single or multiple mucosal defects. The ulcers are produced due to enhanced release of histamine, which, in turn, leads an increased release of acid, a decline in mucus production, pancreatic juice reflux, and poor flow of gastric blood. In stress-induced ulcer model, 200 mg/kg and 400 mg/kg ethanolic extract of *P. reticulatus* Poir. produced an ulcer index of 5.21 ± 0.10 and 3.69 ± 0.12 and % protection (60.94 and 72.31).

Gastric acid is an important factor for the genesis of ulceration in pylorus-ligated rats.[23] The effects of the ethanolic

Table 6: Effect of *Phyllanthus reticulatus* Poir. on total hexose, hexosamine, fucose, and total protein

S. No	Treatment	Total hexoses (µg/ml)	Hexosamine (µg/ml)	Fucose (µg/ml)	Total protein (µg/ml)
1	Control	247.43±4.29	287.88±5.35	86.65±3.58	146.25±2.55
2	Ranitidine	376.95±4.72**	461.37±6.56**	181.76±3.48**	553.42±5.58**
3	Test (200 mg/kg)	320.96±3.49**	423.53±4.41**	126.71±3.94**	522.64±2.33**
4	Test (400 mg/kg)	351.11±4.21**	451.02±6.67**	168.62±2.84**	542.70±3.36**

Values represent mean±SEM, $n=6$. Data were analyzed by one-way analysis of variation followed by Dunnett's test. ***$P<0.001$, **$P<0.01$, *$P<0.05$ and ns: Non-significant. Standard and test were compared with the control group

leaf extract of *P. reticulatus* Poir. on acid parameters showed significant ($P < 0.01$) at 200 and 400 mg/kg doses compared to ulcer control animals. The volume of acid secretion, total acidity, and free acidity was decreased and pH of the gastric juice was increased in animals of extract 200 mg/kg group and 400 mg/kg group compared to ulcer control group. The current data clearly demonstrate that ethanolic leaf extract of *P. reticulatus* Poir. dose dependently decreases the gastric acid, which clearly exhibits the antisecretory activity of *P. reticulatus* Poir.

Mucus serves as first line of defense against aggressive factors.[24] The concentration of total hexoses at the dose of 200 mg/kg and 400 mg/kg group was found to be 320.96 ± 0.3.49 µg/ml and 351.11 ± 4.21 µg/ml which show significant increase when compared to the ranitidine group (376.95 ± 4.72 µg/ml). The concentration of hexosamine at the dose of 200 mg/kg and 400 mg/kg group was found to be 423.53 ± 4.41 µg/ml and 451.02 ± 6.67 µg/ml. It shows a significant increase in hexosamine content when compared to the ranitidine group (461.37 ± 6.56 µg/ml). The concentration of fucose at the dose of 200 mg/kg and 400 mg/kg group was found to be 126.71 ± 3.94 µg/ml and 168.62 ± 2.84 µg/ml. This shows a significant increase of fucose content when compared to the ranitidine-treated group (181.76 ± 3.48 µg/ml). *P. reticulatus* Poir. significantly increased the secretion of mucus as observed from the increase in mucopolysaccharides. This finding is further strengthened by the observation of decreased protein leakage into the gastric juice. This effect was due to increase in the production of mucopolysaccharides that are responsible for viscous and gel-forming tendency of the mucus. Therefore, it is logical that an increased production of mucus might have contributed to the gastro protective role of *P. reticulatus* Poir.

CONCLUSION

The findings of the present study show that the ethanolic extract of *P. reticulatus* Poir. has gastroprotective effect against pylorus ligation-, ethanol-induced, and swim stress-induced ulcer models in rats. However, further studies are required to identify the active constituents responsible for the gastroprotective effect of *P. reticulatus* Poir. plant.

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