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Research Article

ANTI-ULCER EVALUATION OF *POLYGONUM BARBATUM LINN* LEAF EXTRACT

QUEEN ROSARY SHEELA X[A*], AROCKIASAMY P[B], JEBA SINGH D[C], ILAVARASAN R[D], ALEX RAMANI V[A]

[a]PG & Research Department of Chemistry, St.Joseph's College, Trichy, Tamil Nadu, [b]PG & Research Department of Chemistry, Auxilium College, Vellore, Tamil Nadu, [c]Department of Pharmacology, Mohamed Sathak A.J. College of Pharmacy, Chennai, [d]Captain Srinivasa Murthi Research Institute for Ayurveda and Siddha Drug Development, Chennai. Email: sheela910@gmail.com

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ABSTRACT

Polygonum barbatum Linn (Family: Polygonaceae), annual herb with multifarious medicinal properties. The present study was undertaken to evaluate the antiulcer activity of *Polygonum barbatum* leaf extract (PBLE).The PBLE was administrated orally at two different doses of 200 and 400 mg/kg body weight. The antiulcer activity was evaluated by aspirin+ pylorus ligation and aspirin induced ulcer models. Parameters like ulcer score, acidity, volume and pH of gastric secretion were studied. The healing of aspirin + pylorus ligation and aspirin induced ulcer was increased by both the doses of the extract. In aspirin + pylorus ligation ulcer rats, the extract showed significant ($P<0.05$) decrease in ulcer index, acidity, volume and increase in pH of the gastric content. The extract also reduced the ulcer index in aspirin induced ulcer.

Keywords: *Polygonum barbatum*, Antiulcer activity, Aspirin + pylorus ligation, Aspirin induced ulcer.

INTRODUCTION

During the past decade, the therapeutic use of herbal medicine is gaining considerable momentum in the world. The use of herbal medicine due to toxicity and side effects of allopathic medicines has led to sudden increase in the number of herbal drug manufactures. Herbal medicines as the major remedy in traditional system of medicine have been used in medical practices since antiquity[1]. The clinically useful drug against pain and inflammation exhibits many adverse effects; this leads to considerable interest in search of safer drug for these conditions[2].

Peptic ulcer is one of the major gastro-intestinal disorders which occur due to an imbalance between offensive and defensive factor. Major offensive factors are acid, pepsin, Helicobacter pylori and bile salts. Defensive factors mainly involve mucus-bicarbonate secretion and prostaglandins. Consequently reduction of gastric acid production as well as re-inforcement of gastric mucosal production has been the major approaches for therapy of peptic ulcer disease[3].

Polygonum barbatum Linn (Family Polygonaceae) a stout, annual herb, with erect stem, distributed throughout the hotter parts of India, particularly in wet places. The seeds are employed in Malabar and Canara to relieve the griping pains of colic. In Patna, the roots are used as an astringent and cooling remedy. In China, decoction of the leaves and stalks are said to be used as a stimulating wash for ulcers[4]

MATERIAL AND METHODS

Plant material

The leaves of *Polygonum barbatum* were collected along the beds of Cauvery river, near Trichy, Tamil Nadu in February 2008. The plant was identified and authenticated by Dr.G.V.S.Moorthy, Joint Director, Botanical Survey of India (BIS), Agriculture University campus, Coimbatore, India. The voucher specimen number was BIS/SC/5/23/08-09/Tech-1614, and the specimen was deposited at herbarium.

Preparation of plant extract

Fresh leaves were collected, shade dried and powdered mechanically. About 100 g of the powder were extracted with 1000 ml of 70% ethanol by hot percolation method using soxhlet extractor for 4 h. The extract obtained was evaporated at 45°C to get a semisolid mass. The yield of alcoholic extract was found to be 30%. This extract was used for further studies.

Preliminary phytochemical screening

The major secondary metabolites classes such as alkaloids, flavanoids, saponins, phenols and terpenoids were screened according to the common phytochemical methods described by Harborne[5].

Animals

The inbred wistar albino rats of either sex weighing between 150-200 g were used. Pregnancy was excluded. They were housed in standard cages at room temperature 24°C ± 2°C and were feed with *ad libitum* and water. The animals were deprived of food for 24 h before experimentation, but had free access to water. The study was conducted after obtaining Institutional Animals Ethics Committee clearance (IAEC) bearing the number 991/C/06/ CPCSEA.

Drugs and Chemicals

Aspirin was obtained from German Remedies Ltd., Mumbai, India and ranitidine from Dr. Reddys Lab, Hydrabed, India. All other chemicals used in this study were of analytical grade.

Acute Oral Toxicity test

The procedure was followed by using OECD guidelines (Organization of Economic Cooperation and Development) 423 (Acute toxic class method). The acute toxic class method is a stepwise procedure with three animals of a single sex per step. Depending on the mortality and/or moribund status of the animals, on the average 2-4 steps may be necessary to allow judgment on the acute toxicity of the test substance. This procedure results in the use of a minimal number of animals while allowing for acceptable data-band scientific conclusion.

The method used defined does (5, 50, 300, 2000 mg/kg body weight) and the results allow a substance to be ranked and classified according to the Globally Harmonized System (GHS) for the classification of chemical, which cause acute toxicity[6].

Fixing dose of the extract

LD_{50} was done as per OECD guidelines for fixing the dose for biological evaluation. LD_{50} of the extract as per OECD guidelines falls under class four values with no signs of acute toxicity at 2000 mg/kg. The biological evaluation was carried out at doses of 200 and 400 mg/kg body weight.

Pharmacological evaluation

Aspirin + pylorus ligation- induced ulcer model

PBLE, aspirin and standard ranitidine were prepared in 1% Sodium Carboxyl Methyl Cellulose (SCMC) suspension as vehicle and administrator orally once daily. The animals were divided into five groups, consisting of six in each. Group I received 1% SCMC 10 ml/kg body weight. Group II received aspirin 200 mg/kg body

weight. Group III and IV received PBLE at dose of 200 and 400 mg/kg body weight respectively for seven days. Group V received ranitidine 50 mg/kg body weight for seven days. From day's five to seven, animals of all groups received aspirin orally as an aqueous suspension at a dose of 200 mg/kg body weight, 2 h after[7-8]. Animal in all the groups were fasted for 36 h after the respective assigned treatment and were anaesthetized with anesthetic ether. The abdomen was opened by a small midline incision below the xiphoid process and pylorus portion of the stomach was lifted out and ligated without causing any damage to the blood supply of the stomach[9]. Precaution was sutured with interrupted sutures. Four hours after pylorous ligation, the rats were sacrificed and the stomach was removed. The gastric contents were collected, centrifuged and the volume of the supernatant was determined[10]. The pH of the gastric juice was directly measured by using a pH meter. Free and total acidity were determined by titrating with 0.01N NaOH using Topfer's reagent and phenolphthalein as an indicator[11]. The stomach was then incised along the greater curvature and observed for ulcers. The number of ulcers was counted using a magnifying glass. The following arbitrary scoring system[12] was used to grade the incidence and severity of lesions.0: normal colored stomach, 0.5: red coloration, 1: spot ulcers, 1.5: haemorrhagic streak, 2: ulcers, 3: perforation.

Mean ulcer score for each animal was expressed as ulcer index. The percentage of ulcer inhibition was determined as follows:

Inhibition of ulcer (%) = Control mean ulcer index - Test mean ulcer index/ Control mean ulcer index x100

Aspirin induced ulcer model

In aspirin induced ulcer experiment animals are divided into five groups, each consisting of six rats. I group received 1% SCMC 10 ml/kg body weight, II group received aspirin 200 mg/kg. III and IV groups received PBLE in a dose of 200 and 400 mg/kg respectively. Ranitidine, in the dose of 50 mg/kg was being administrated intraperitoneally for group V as a reference standard drug. After 15 days of treatment, animals were fasted for 24 h; aqueous suspension of aspirin was administrated, after 30 min of PBLE and ranitidine treatment. The animals were sacrificed 4 h later using anaesthetic ether and open the abdominal cavity and stomach was excised. The excised stomach was opened along the greater curvature and cleaned the interior by normal saline and examined for the degree of ulceration[13].

Histopathological evaluation

The gastric tissue samples were fixed in neutral buffered formalin for 24 h. Section of tissue from stomachs was examined histopathogically to study the ulcerogenic or antiulcergenic activity of *Polygonum barbatum*. The tissues were fixed in 10% buffered formalin and were processed using a tissues processor. The processed tissues were embedded in paraffin blocks and about 5 µm thick sections were cut using a rotary microtome. These sections were stained with hematoxylin and eosin using routine procedures. The slides were examined microscopically for pathomorphological changes such as congestion, haemorrhage, oedema and erosions using an arbitrary scale for the assessment of severity of these changes.

Statistical analysis

The antiulcer activity was analyzed by using one way analysis of variance (ANOVA) followed by Dunnet's *t*-test, $P < 0.05$ was considered as significant.

RESULTS

Preliminary phytochemical screening

Phytochemical screening of the PBLE showed the presence of steroids, triterpenoids, phenols, flavanoids, alkaloids, tannins, lignans and glycosides.

Acute toxicity test

In acute toxicity studies, it was found that the animals were safe up to a maximum dose of 2000 mg/kg body weight. There were no changes in normal behaviour pattern and no signs and symptoms of toxicity and mortality were observed. The biological evaluation was carried out at doses of 200 and 400 mg/kg body weight.

Aspirin+ pylorus ligation- induced ulcer model

Aspirin+ pylorus ligated animals showed a significant (P<0.05) increase in the ulcer index and acid secretory parameters when compared with these of vehicle treated group. Administration of PBLE produced significant (P<0.05) decrease in ulcer index in a dose dependent manner. When compared to the aspirin treated groups, the extract also significantly reduced the gastric volume, total and free acidity, and increased the pH of the gastric fluid, proving its anti secretory activity. PBLE at a dose of 200 and 400 mg/kg body weight showed protection index of 55.63% and 69.42% respectively, where as standard ranitidine showed protection index of 72.45% (Table 1).

Aspirin induced ulcer model

Both doses of PBLE showed a significant (P<0.05) reduction in the ulcer index when compared with the control group. PBLE at a dose of 200 and 400 mg/kg body weight showed protection index of 61.4% and 72.96% respectively. Standard ranitidine also significant (P<0.05) reduced ulcer index of aspirin-induced gastric ulcers 74.27% (Table 2).

Table 1: Effect of *Polygonum barbatum* leaf extract (PBLE) on aspirin + pylorus ligation induced gastric ulcer in rats

Groups	Treatment	Dose (mg/kg)	Gastric volume (ml/100g)	pH	Free acidity (meq./l/100g)	Total acidity (meq./l/100g)	Ulcer index	% inhibition
I	1 % SCMC	-	2.410± 0.005	4.613± 0.008	12.50± 0.428	23.67± 0.333	0	100
II	Aspirin	200	4.830± 0.008^{a***}	1.145± 0.016^{a***}	76.50± 0.428^{a***}	97.50± 0.428 ^{a***}	30.25± 0.478^{a***}	-
III	PBLE	200	1.828± 0.007^{b***}	4.243± 0.007^{b***}	25.00± 0.365^{b***}	39.33± 0.421^{b***}	13.42± 0.238^{b***}	55.63
IV	PBLE	400	1.405± 0.008^{b***}	4.432± 0.004^{b***}	20.00± 0.365^{b***}	32.17± 0.307^{b***}	9.250± 0.170^{b***}	69.42
V	Ranitidine	50	1.107± 0.008^{b***}	4.532± 0.024^{b***}	18.67± 0.494^{b***}	31.17± 0.477^{b***}	8.333± 0.210^{b***}	72.45

Each value is the mean ± SEM of six determinations. P< 0.05 when compared to aspirin treated group, (One -way ANOVA followed by Dunnet's test)

Table 2: Effect of *Polygonum barbatum* leaf extract (PBLE) on aspirin induced gastric ulcer in rats

Groups	Treatment	Dose (mg/kg)	Ulcer index	% inhibition
I	1 % SCMC	-	0	100
II	Aspirin	200	31.75± 1.116^{a***}	-
III	PBLE	200	12.25 ± 0.214^{b***}	61.4
IV	PBLE	400	8.583 ± 0.396^{b***}	72.96
V	Ranitidine	50	8.167 ± 0.247^{b***}	74.27

Each value is the mean ± SEM of six determinations. P< 0.05 when compared to aspirin treated group, (One -way ANOVA followed by Dunnet's test)

Histopathological evaluation

Histopathological changes on aspirin + pylorus ligation ulcer model showed that mucosal ulcer with mono nuclear cell infiltration and loss of epithelial layer (Fig. 1). PBLE (400 mg/kg) treated rats showed no ulceration but gastric epithelium was not completely restored (Fig. 2). PBLE (400 mg/kg) treated rats showed no ulceration in the mucosa (Fig. 3). Glands are regular with complete restoration of epithelial layer. Ranitidine treated groups showed no ulceration and epithelial layer are regular (Fig. 4).

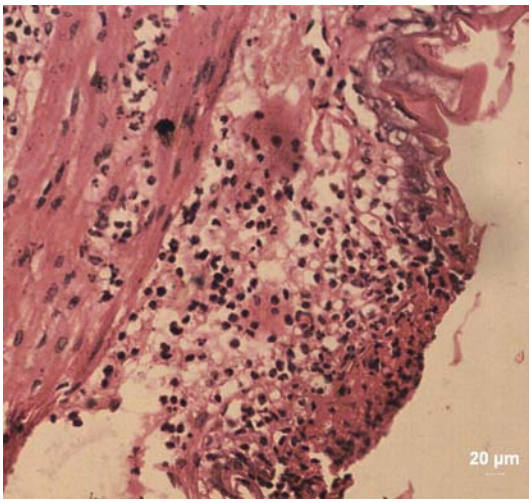

Fig. 1: Pylorus ligated +Aspirin treated

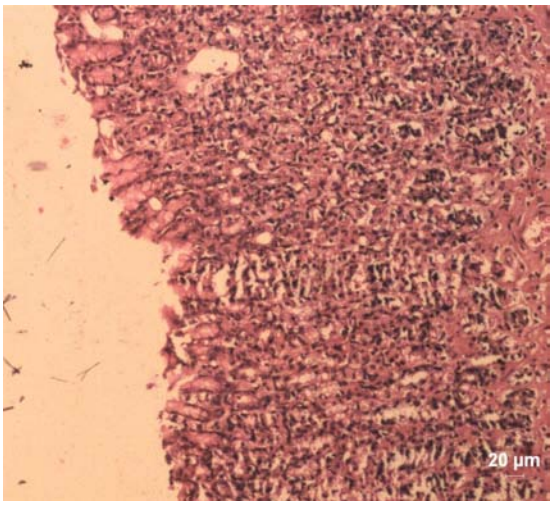

Fig. 2: Pylorus ligated + 200mg/kg PBLE

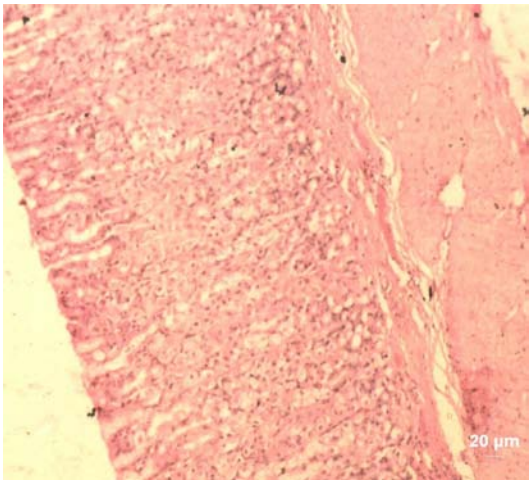

Fig. 3: Pylorus ligated + 400mg/kg PBLE

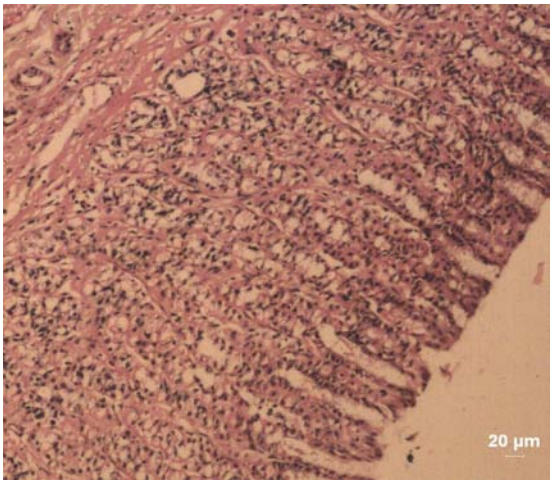

Fig. 4: Pylorus ligated +Ranitidine treated

DISCUSSION

The results of the present study reveals that PBLE at the dose of 400 mg/kg produced a better protection against gastric ulcer when compared to aspirin treated groups and the effect was similar to ranitidine treated group.

The phytochemical screening also reveals the presence of flavonoids and tannins, various flavonoids has been reported for its antiulcerogenic activity with good level of gastric protection[14]. So the possible mechanism of antiulcer activity of PBLE may be due to its flavonoid and tannin contents present in the plants.

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