A PRELIMINARY STUDY ON GASTRIC ANTIULCER ACTIVITY OF POLYGONUM BARBATUM LINN IN RATS

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Summary

The aim of the present study is to investigate the antiulcer activity of aqueous and methanolic leaf extracts of polygonum barbatum Linn (polygonaceae). Gastric ulcers were induced by Pylorus - ligation model and stress induced model. The effect of single oral dose of the extracts was evaluated at 100 and 200 mg/ kg. It was found that methanolic extract significantly reduces the Gastric volume, Total acidity, free acidity and ulcer index compared to that of control group. A significant effect ($p < 0.001$) at 200 mg/ kg for both the extracts was observed in both the models.

Key Words: Polygonum barbatum, leaf extracts, Anti-ulcer activity.

Short Title: Anti ulcer activity of Polygonum barbatum leaf extracts.

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Introduction

Peptic ulcer is one of the major ailments affecting humans and believed to develop because of an imbalance between aggressive factors such as mucus, bicarbonate, blood flow, epithelial cells restoration and prostaglandin. At present a lot of attention is being focused on herbal medicine as an alternative in the treatment of peptic ulcer. This forms the basic for the antiulcer activity of Polygonum barbatum Linn.

Polygonum barbatum Linn. (Synonym, Polygonum stagninum, Polygonaceae) is a stout, erect annual herb, distributed throughout India, particularly in marshy places. The whole plant is used in traditional medicine to treat ulcers, diarrhea, skin eruptions and abdominal disorders. The decoctions of shoots are used as a stimulating wash for ulcers. The seeds are used as a tonic, purgative and emetic (1, 2).

The genus polygonum comprises of nearly 85 species. Various secondary metabolites like Flavanoids (3), anthroquinones (4), Phenylpropanoids (5) have been reported in various species of polygonum. Various biological activities like Antihypertensive activity of P.perfoliatum (6), Anti inflammatory activity of P.glabrum (7), Anti viral activity of P.tinctorium (8) have been reported for other species of polygonum. But there is no report on the antiulcer activity of this particular species (P. barbatum). Hence we made an attempt to evaluate the antiulcer activity.

Material and Methods

Plant collection and authentication

For our study, the leaves of polygonum barbatum were collected locally from pickup dam of Tiruvannamalai District and were identified by Botanist Dr.P. Jayaraman(Plant Anatomy Research Centre) chennai. A voucher specimen has

been kept in the Department of Pharmacognosy of our institution for further reference.

Preparation of extracts

The leaves were separated from the stalks, washed and shade dried and powdered. The powdered leaves were extracted with Methanol (60 -80º c) for 48 hrs by soxhlet process. The aqueous extract was prepared by maceration process by treating 100g of fresh powder with 500ml of distilled water along with 10ml of chloroform as a preservative .The maceration process was carried for 7 days with occasional stirring. Both the extracts were filtered / condensed and evaporated to dryness under vacuum.

Preliminary phytochemical screening

All the extracts were screened for the presence of various secondary metabolites by adopting standard procedures (9).

Experimental Animals

Adult albino mice (20- 25g), Wistar Rats (150 -200g) were used for the study. All the animals were procured from Sri Venkateshwara Enterprises, Bangalore. They were acclimatized for 5 days with free access to water and food before the commencement of the experiment. The rats and mice were fed with chow diet and water *ad libitum*. The animals were maintained under standard 12-hr light / dark cycle throughout the study. These experiments complied with the guidelines of our animal ethics committee.

Acute Toxicity test

The acute toxicity of both extracts of Polygonum barbatum were determined as per the CPSCEA guideline no 420. The overnight fasted mice were weighed and divided into nine groups of ten each. Group 1 to 8 received dose of Methanolic and aqueous extracts (200mg/kg to 2000 mg/kg) by oral route. After administration of the drugs, the animals were observed continuously for 24 hrs for death due to acute toxicity .The geographic mean of the least dose of the extract that killed the animals and the highest dose that did not kill the animals were taken as the median lethal dose (LD50). At

2000mg/kg body weight no mortality was observed and is considered as the cut off point. Therefore $1/10^{th}$ of this dose was taken as experimental dose for subsequent antiulcer activity study. The extracts were administered at doses of 100 and 200 mg/kg body weight.

Drugs

Ranitidine was procured from Dr. Reddy's lab, Hyderabad. All the solvents like Methanol, chloroform, anesthetic ether, standard silymarin were obtained from Ranbaxy lab, New Delhi.

Anti ulcer activity

The methanolic and aqueous extracts were suspended in 5% w/v Acacia and were tested for the antiulcer activity using two models. These include pylorus ligated model (shay et al, 1945) and stress induced ulcers (Langason et al, 1994). Two dose levels of the extracts were employed in each of the models (100 and 200mg/kg).The animals were divided in to six groups (Group I, Group II, Group III, Group IV, GroupV and Group VI). Each group has six animals.

Group I: Treated with control vehicle (5% acacia)

Group II: Treated with Aqueous extract (100 mg/kg body weight, orally)

Group III: Treated with Aqueous extract (200 mg/kg body weight, orally)

Group IV: Treated with Methanolic extract (100 mg/kg body weight, orally)

Group V: Treated with Methanolic extract (200 mg/kg body weight, orally)

Group VI: Treated with Ranitidine (20 mg/kg body weight, orally)

Pylorus ligation model

Albino Rats of either sex were divided in to six groups of six each. Pregnancy was excluded. The animals were deprived of food for 24 hrs before the commencement of experiment, but water was allowed *ad libitum*. The drugs were given orally 2 hours prior to pylorus ligation method which was carried out according to the technique of shay et al (10).

The abdomen was cut, opened and Pylorus ligation is done without causing any damage to its blood supply .The stomach was replaced carefully and the abdomen was then closed in two layers with interrupted sutures. The animals were deprived of water during the post-operative period. The animals were sacrificed 6 hours after Pylorus ligation for observation of gastric lesion as described by (11).Table 1.

The gastric juice was collected, centrifuged and its PH and volume were measured. Free and Total acidity estimated titrimetrically with 0.01 NaOH using Methyl orange and Phenolphthalein as indicator.

Pipette 1ml of filtered gastric contents into a small beaker, add 2-3 drops of methyl orange and titrate with 0.01N NaoH, until all trace of the red color disappears and the color is yellowish orange. Note the volume of alkali added. Then add 2-3 drops of phenolphthalein and continue titration until a definite re tings reappears. Again read the burette and so obtain the total volume of alkali added. If a yellow color is obtained on adding methyl orange no free acid is present .Add the phenolphthalein and titrate the combined acid and this gives the total acid value. The significance of the differences between mean values for various treatments was analyzed using the student “t” test. (12). Table 2.

Cold restraint stress induced ulcers

Animals were deprived of food for 12h, then were immobilized in stress cages and placed in a cold room (4-6ºC) for 3h (Vincent et al; 1997).The drug was administered

1h before immobilization. During the cold restraint stress (CRS) procedure, Silymarin (100mg/kg.p.o.) was used as standard instead of omeprazole. The animals were sacrificed by cervical dislocation and scored for intensity as per the method of Szabo. (13). TABLE 3

Statistical analysis

Results were expressed in terms of mean ± SEM. The results were compared to the control values using student's *t*- test, and the difference was regarded as significant at $p< 0.05$

Results and Conclusions

The phytochemical studies revealed the presence of alkaloids, steroids, flavonoids, tannins, glycosides in methanolic extract and carbohydrates, tannins, glycosides in aqueous extract.

The result of the anti ulcer activity showed that Methanolic extract (200mg/kg) produced protection against gastric ulcer induced by pylorus ligation. The methanolic extract of polygonum barbatum (200mg/kg) markedly reduced the ulcer index to 9.7 ± 2.8($p<0.001$) when compared with control (35.4 ± 3.2) and standard (10.5 ± 0.8).The Methanolic extract showed significant anti ulcer activity when compared to aqueous extract.

Table 1

ULCER SCORE

Denuded epithelium	10
Petechial and frank hemorrhages	20
One or two ulcers	30
Multiple ulcers	40
Perforated ulcers	50

Table 2

Effect of Methanolic and aqueous extracts of Polygonum barbatum on various acid secretary parameters against Pylorus- ligated gastric ulcer model.

S.no	Groups	Volume	PH	Total acidity	Free acidity	Ulcer index
1	Control	1.8 ± 0.04	1.3 ± 0.07	98 ± 7.3	77 ± 6.3	35.4 ± 3.2
2	Aqueous extract (100mg/kg)	0.75** ± 0.02	3.9** ± 0.09	41** ± 2.3	31** ± 1.9	13.5** ± 1.2
3	Aqueous extract (200mg/kg)	0.52** ± 0.03	4.2** ± 0.09	30** ± 0.27	19** ± 0.8	10.7** ± 0.8
4	Methanolic extract(100mg/kg)	0.6 ± 0.02**	4.4 ± 0.11**	30 ± 2.9**	18 ± 1.3**	10.6 ± 3.1**
5	Methanolic extract (200mg/kg)	0.53 ± 0.06**	4.6 ± 0.17**	29 ± 3.2**	19 ± 1.8**	9.7 ± 2.8**
6	Ranitidine	0.50** ± 0.02	4.6** ± 0.18	29** ± 1.0	16** ± 0.9	10.5** ± 0.8

All values represent mean ±S.D; n = 6 in each group;
ANOVA ** P<0.0001 VS Control and standard.

In the case of stress induced ulcer model the statistical data conform that the ulcer score was significantly reduced over the increase in doses. Also the methanolic extract (200mg/kg) was found to have better reduction than the Ranitidine group.

Hence it is suggested that the methanolic extract of polygonum barbatum has got significant anti ulcer activity. Recent literature reviews indicated that many flavanoids possess antiulcerogenic and wound healing activity (14). Therefore antiulcer activity of Methanolic extract of Polygonum barbatum may be due to presence of flavanoids and tannins. And hence further works have to be carried out to isolate the active constituents responsible for the activity.

Table 3

Effect of Methanolic and aqueous extracts of Polygonum barbatum on ulcer score for intensity in cold stress induced model.

S.NO	GROUPS	ULCER SCORE
1	Control	2.63 ± 0.17
2	Aqueous extract (100mg/kg)	2.08 ± 0.09
3	Aqueous extract (200mg/kg)	1.54 ± 0.25
4	Methanolic extract (100mg/kg)	1.14 ± 0.15
5	Methanolic extract (200mg/kg)	0.87 ± 0.14
6	Silymarin	1.01 ± 0.17

All values represent S.D; n=6 in each group; ANOVA IS significant ($P<0.0001$) vs. control and standard.

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