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RESEARCH ARTICLE

Evaluation of Anti-Ulcer Activity of *Polygonum Barbatum* Linn. (Whole Plant)

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ABSTRACT

Polygonum barbatum (Polygonaceae) is a plant, reported for its variety of ethnic medicinal uses. Hence we have planned to screen antiulcer activity of whole plant with the alcoholic and aqueous extracts. Whole plant was successively extracted with alcohol and water was subjected for phytochemical screening to identify different phytoconstituents. Ld50 studies for both (alcoholic and aqueous) extracts were conducted upto the dose level of 2 g /kg by following OECD up and down method of guidelines No.425. Anti-ulcer activity was evaluated in various animal models like Pylorus ligation, Ethanol Induced gastric mucosal damage ulcer modelsin rats. Preliminary phytochemical studies revealed the presence of saponins, sterols, mucilage, glycosides, alkaloids, steroidal saponins in both the alcoholic and aqueous extracts of *P. barbatum*. No mortality was observed with any of the 2 extracts up to the maximum dose level of 2 g/kg. Further alcoholic and aqueous extracts at 200 and 400 mg/kg, p.o but not with 100 mg/kg p.o doses significantly ($P < 0.01$) reduced the ulcer score, ulcer number, ulcer index, free acidity and total acidity in Pylorus ligation, Ethanol Induced gastric mucosal damage ulcer models in rats.The present study revealed the antiulcer activity of whole plant extracts of *P. barbatum and* the activities are due to the presence of phytochemical constituents such as saponins, sterols, mucilage, glycoside, alkaloids, steroidal saponins as these phytochemical constituents were already reported for the above mentioned effects.

KEYWORDS: *P. barbatum*, Pylorous ligation, Ulcer index

INTRODUCTION:

Gastric ulcers the most wide state disease and are a very common global problem today. Peptic ulcer is a lesion of the gastric/duodenal mucosa occurs at a site where the mucosal epithelium is exposed to acid and pepsin. Peptic ulcers occur due to imbalance between the offensive (gastric acid secretion) and defensive (gastric mucosal integrity) factors [1]. The aggressive and protective factors in the stomach are acid pepsin secretion, mucosal barrier, blood flow, cellular regeneration, prostaglandins and epidermal growth factors. Sometimes the gastric mucosa is continuosly exposed to potentially injurious agents such as pepsin, bile acids, food ingradients, bacterial products and drugs [2]. Factors such as stress, smoking, nutritional deficiency and ingestion of NSAID'S all can increase the incidence of gastric ulcers. It is reported that prolonged anxiety, emotional stress, haemorrhagic surgical shock, burns and trauma are known to cause severe gastric irritation [3].

MATERIALS AND METHODS:

PREPARATION OF EXTRACT:

P.barbatum whole plant were collected from Sri Venkateswara University Campus, Tirupati in January 2012 and authenticated by Dr. K. Madhav Chetty, Assistant Professor Dept. of Botany Sri Venkateswara University, Tirupati, Andhra Pradesh. One kg. of the air dried whole plant were blended to a fine powder and extracted with Ethanol and Water for 6 days (144hours). The extract was concentrated using a rotavapor.

PHYTOCHEMICAL SCREENING:

The extract and its fraction were tested by the librman Burchard, Ferric chlorides, Magnesium tracings, Vanilin sulphuric Acid and Mayer's, Wagner's and Dragendroff's tested to determine the presence of sterols phenolic compound, Flavonoids, saponins and alkaloids respectively[4,5].

ANIMALS:

Wistar rats (200 – 250g) of both sexed were used for the studies. The rats were obtained from VNS Institute of Pharmacy animal house, Bhopal (M.P) (CPCSEA Reg. No 778/03/C/CPCSEA). The animals were housed in cages under standard laboratory conditions (12:12 hours light/ dark cycle at 25 + 20 C). They had free access to standard commercial diet and water. The animals were divided into groups of six. The ethical guidelines for the investigation of animals used in experiments were followed in all tests [6].

ANTI-ULCER ACTIVITY:

Pylorus ligation induced ulcer model:

The ulcer protective effect of AOE and ETOH were studied as per the method of Shay et al 1945. The accumulation of acidic gastric juice in the stomach causes ulceration and in

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this method several parameters were estimated. Albino rats weighing between (200-250 gm were divided into 8 groups of 6 rats in each. They were fasted in individual cages with measures taken to avoid coprophagy for 24 h prior to the experiment with free access to water. Group A was served as normal control given with vehicle only. Group B with standard drug, groups C, D, E and F were treated with medium and high doses of AOE and ETOH respectively. The various groups were treated with vehicle/drug/ extracts 30 min prior to pylorus ligation and the details of the protocol was given below: Group A: Normal animals treated with vehicle only; Group B: Standard Omeprazole (10 mg/kg p.o); Group D: Low dose of AOE (100 mg/kg); Group E: High dose of AOE (200 mg/kg); Group F: Low dose of ETOH (100 mg/kg); Group H: High dose of ETOH (200 mg/kg) [7].

EXPERIMENTAL PROCEDURE:

Under light ether anesthesia, the abdomen was opened and the pylorus ligation performed and then sutured. 4 h after pylorus ligation all the animals were sacrificed with excess of anaesthetic ether and the stomach of each rat was dissected out. Gastric juice collected into centrifuge tubes was centrifuged at 1000 rpm for 10min and volume was noted. The pH of the gastric juice was recorded by pH meter. The gastric content was subjected for analysis of free and total acidity. The stomachs were washed under running tap water and then focused under microscope to note the ulcers in the glandular portion. The number of ulcers per stomach was scored microscopically with the help of (10x) hand lens and the scoring is done as per standard procedure. Mean ulcer score for each animal is expressed as Ulcer Index. The percentage ulcer protection was calculated using the formula [8].

Percentage ulcer protection = Ut / Uc X 100

Where Ut = Ulcer index of treated group and Uc = Ulcer index of the control group

ETHANOL INDUCED GASTRIC MUCOSAL DAMAGE:

Animals were fasted for about 16 hrs. before the experiment, but were allowed free access to water. One ml of absolute alcohol was administered orally to the rats. In the treatment groups the rats were given the drug 1 hr prior to the administration of ethanol. After 2 hrs of ethanol treatment, animals were sacrificed; stomach was removed and cut along the greater curvature and examined for lesions. Severity was determined by measuring ulcer index [9].

MEASUREMENT OF ULCER INDEX:

The stomach was washed with saline and the lesion was examined under a 10 X dissecting microscope, ulcer index of each animal was calculated by adding the values and their mean values were determined by the following scoring system [10].

- Normal coloured stomach - 0
- Red coloured stomach -0.5
- Spot ulceration -1
- Hemorrhagic streak -1.5
- Ulcers -2
- Perforations -3

STATISTICAL ANALYSIS:

Results were analyzed by student's t-test. Minimum level of significance was fixed at $p<0.05$.

RESULTS:

ACUTE TOXICITY STUDY:

Before the study of Anti ulcer activity preliminary toxicity studies of the compound was carried out. The compound failed to cause any mortality when administered up to a dose of 2000 mg/kg body weight orally [11]. Antiulcer activity by pylorous ligation method ETOH treated animal has showed significant reduction in ulcer index. ETOH 400 mg/kg and Omperazole treated showed significant reduction ($P<0.01$) in gastric volume, total and free acidity and increase in GI Ph and when compared with the control group [12] (Table-1).

S. No	Groups	Gastric Volume	Ph	Total acidity	Free acidity
1.	Control	4.51±0.19	1.56±0.094	105.16±3.06	83.16±3.21
2.	Standard	2.23±0.18**	3.50±0.21**	32.33±2.84**	24.33±1.70**
3.	AOE 200 mg/kg	4.05±0.11^{ns}	1.77±0.04^{ns}	45.16±2.94**	38.83±2.96**
4.	AOE 400 mg/kg	3.43±0.21**	2.12±0.37^{ns}	41.33±2.83**	35.66±2.97**
5.	ETOH 200 mg/kg	2.71±0.17**	2.32±0.21^{ns}	46.33±3.26**	41±3.12**
6.	ETOH 400 mg/kg	2.38±0.28**	2.71±0.25**	43.15±3.13**	38.13±3.00**

Table No. 1: Effects of Aqueous (AOE) and Ethanolic (ETOH) extracts of *Polygonum barbatum* against Pylorus ligation induced gastric ulcers in Rats.

Values are mean ± SEM, n=6, * $p< 0.05$, ** $p< 0.01$

ETHANOL INDUCED GASTRIC MUCOSAL DAMAGE:

In control animal oral administration of absolute ethanol produced characteristic lesion in the glandular

portion of rat stomach. ETOH has shown significant inhibition of ulcer at the doses of 200 mg/kg and 400 mg/kg respectively in comparison to control. Ethanol induced mucosal damage was significantly and dose dependently reduced by pre-treatment of the animal with ETOH [13]. (Table-2)

S. No	Groups	Pyloric Ligation Method		Ethanol induced Model	
		Ulcer Index	% Protection	Ulcer Index	% Protection
1.	Control	13.33±0.6280	-	28.75±1.716	-
2.	Standard (Omeprazole 20 mg/kg)	3.66±0.6009**	66.66	9.16±1.531**	68.139
3.	AOE 200 mg/kg	9.33±0.6412**	23.54	21±1.176**	26.95
4.	AOE 400 mg/kg	6.83±0.4944	33.94	17.75±1.124**	38.26
5.	ETOH 200 mg/kg	6.16±0.6412**	35.19	18.41±0.9075**	35.96
6.	ETOH 400 mg/kg	5.66±0.4410**	44.34	12.33±0.9888**	57.10

Table No. 2: Ulcer Index and % Protection of Aqueous and Ethanolic extract in Pylorous ligation and Ethanol induced gastric ulcers in Rats.
Values are mean ± SEM, n=6, * p< 0.05, ** p< 0.01

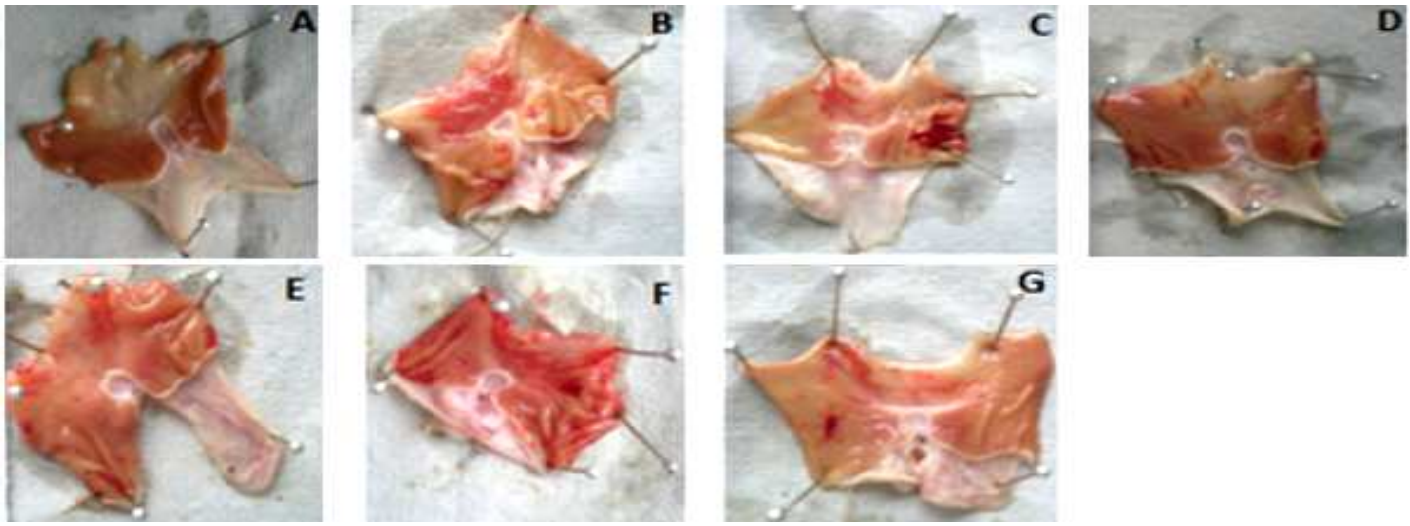

Figure No. 1. Photograph Showing Ethanol induced mucosal damaged model A) Normal, B) Standard, C) Ulcer control, D) AOE 200 mg/kg, E) AOE 400 mg/kg, F) ETOH 200 mg/kg, G) ETOH 400 mg/kg

DISCUSSION:

In this work we have studied anti-ulcerogenic activity of *P. barbatum* whole plant extracts in models including Antiulcer activity by pylorous ligation method and ethanol induced gastric mucosal damage, where ulcerogens produce ulcer is either due to the effect on acid secretion or on cytoprotection [14].

The different constituents like flavonoids, tannins, sterols, phenolic compounds, saponins and alkaloids [15].

Similarly both the extracts were evaluated for their anti-ulcer activity in pylorus ligation, ethanol induced ulcer models in rats. Both the extracts produced a significant ($p<0.01$) anti-ulcer activity but similar to the above experiment a relatively better anti-ulcer activity was recorded with alcoholic extract [16].

CONCLUSION:

The above findings justify the Anti ulcer properties of Whole plant extracts that is comparable with standard drug [17].

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