



## GASTROPROTECTIVE AND ANTIOXIDANT ACTIVITY OF *POLYGONUM BARBATUM* AGAINST EXPERIMENTALLY INDUCED GASTRIC ULCER IN WISTAR ALBINO RATS

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### ABSTRACT

The objective of the study is to evaluate the antiulcer and antioxidant activity of ethanolic leaf extract of *Polygonum barbatum* on cold restraint stress induced ulcer in rats. The male Wistar rats were divided into five groups of six animals each. The ethanolic extract was prepared by cold maceration method. Omeprazole (10mg/kg) was used as reference control. Two doses (250 & 500 mg/kg) of ethanolic leaf extract of *Polygonum barbatum* (ELPB) were used in the study. The test drugs were administered once daily for 3 days and cold restraint stress was given by strapping the rats on a wooden plank and keeping them for 2 h at 4–6°C after 18hrs of last dose of test drugs. At the end of the study, the animals were sacrificed by using excess thiopentone sodium and stomach was excised and opened to observe the ulcer index. The stomach tissue was homogenized and the supernatant was used to estimate antioxidant activities. The result showed that, ELPB in both doses of 250 and 500 mg/kg significantly reduced the ulcer index and protected the animals against stress-induced free radical damage as seen from the decrease in LPO and reversal of changes induced by stress on SOD and CAT. From the result it was concluded that, the ethanolic leaf extract of *Polygonum barbatum* exhibited gastroprotective activity which is mediated through its antioxidant property.

**Key words:** *Polygonum barbatum*, Ulcer index, Antioxidant activity, Antiulcer, Lipid peroxidase, Superoxide Dismutase and Catalase.

### INTRODUCTION

Plant-derived substances have recently become of great interest owing to their versatile applications. Medicinal plants are the richest bio-resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs (Ncube *et al.*, 2008). Herbal medicines are

officious and have lesser side effects compared to modern medication and also reduce the recurrence of incidence. A wide range of plants and plants derived products are used in folk medicine for the treatment of ulcer as a prophylactic agent or as curative agent.

One such a plant is *Polygonum barbatum* (Syn: *Polygonum stagninum*) belonging to the family Polygonaceae. It is a stout, annual herb, with erect stem, 90 cm high, distributed throughout the hotter parts of India, particularly wet places, ascending up to an altitude of 1,080 m., and also in Laccadives and the North and South Andamans. Leaves lanceolate or linear lanceolate;

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flowers white or purplish in small spike; nuts shining. The roots are considered astringent and cooling and are reported to be an article of trade in North India. A decoction of the shoots is said to be used as a stimulating wash for ulcers; the juice act as cicatrizant. Powdered leaves are applied to fly infected wounds of goats. The seed possess tonic, purgative and emetic properties and are used in colic (Anonymous 2003, Nadkarni KM, 1996). *Polygonum barbatum* have been used in traditional folk medicine for the treatment and prevention of ulcer. The antiulcer activity of *Polygonum barbatum* (Hitesh Kumar and Mahesh Kumar, 2012) was reported but the exact mechanism was not revealed. Therefore, the purpose of this study is to investigate the antiulcer activity along with its possible mechanism of action.

## MATERIALS & METHODS

### Plant Collection and Extraction

Fresh leaves of *Polygonum barbatum* were collected in the month of October from Wayanad, Kerala. The plant was identified and authenticated by the Scientist D, Botanical Survey of India, Southern Regional Center, Agricultural University, Coimbatore. The voucher (BSI/SRC/A/23/2012-13/Tech.578) specimen was deposited in the herbarium for further reference.

The collected fresh leaves were washed, air dried under shade for 7 days and pulverized into coarse powder. About 1 kg of the powder was extracted with sufficient quantity of 70 % ethanol by cold maceration for 48 hours and filtered (Trease and Evens 2002). The filtrate was dried in rotatory evaporator and the extract (ELPB) stored in desiccators.

### Animals

Male Wistar rats weighing between 150 – 220 gm were used for this study. The animals were obtained from animal house, IRT Perundurai Medical College, Erode, Tamilnadu, India. On arrival, the animals were placed at random and allocated to treatment groups in polypropylene cages with paddy husk as bedding. Animals were housed at a temperature of 24±2°C and relative humidity of 30 – 70 %. A 12:12 light: day cycle was followed. All animals were allowed to free access to water and fed with standard commercial pelleted rat chaw (M/s. Hindustan Lever Ltd, Mumbai). All the experimental procedures and protocols used in this study were reviewed by the Institutional Animal Ethics Committee (688/2/C-CPCSEA) and were in accordance with the Institutional ethical guidelines.

### Cold Restraint Stress Induced Ulcers

The animals were divided into 5 groups each consisting of six rats. Group 1 represented normal control (non-ulcerated) animals received suspension of 0.1% Carboxy Methyl Cellulose (CMC) solution. Group 2

represented as stress control animals received suspension of 0.1%. Group 3 received Omeprazole (10 mg/kg). Groups 4 & 5, received ELPB, in doses of 250 and 500 mg/kg. The drugs were administered orally, once daily for three days. On day 3, for 18 h fasted rats, cold restraint stress was given by strapping the rats on a wooden plank and keeping them for 2 h at 4–6°C (Gupta *et al.*, 1985). The animals were then sacrificed by excess thiopentone sodium. The stomach was taken out and cut open along the greater curvature and observed for ulcers. Ulcer index was determined by following the scoring method of Suzuki *et al.*, 1976.

Score 1: maximal diameter of 1 mm.

Score 2: maximal diameter of 1–2 mm.

Score 3: maximal diameter of 2–3 mm.

Score 4: maximal diameter of 3–4 mm.

Score 5: maximal diameter of 4–5 mm.

Score 10: an ulcer over 5mm in diameter.

Score 25: a perforated ulcer.

### Estimation of free radical generations:

The fundus of stomach was used for estimation of free radical generation. The fundic part of the stomach was homogenized (5%) in ice cold 0.9% saline with a glass homogenizer. The homogenate was then centrifuged at 800 x/g for 10 min followed by centrifugation of the supernatant at 2000 x/g for 5min and the obtained mitochondrial fraction was used for the following estimations (Goel *et al.*, 2001).

**Lipid peroxidase (LPO) activity:** LPO product malondialdehyde (MDA) was estimated using 1,1,3,3-tetraethoxypropane as the standard and is expressed as nmol/mg protein (Ohkawa *et al.*, 1979).

**Superoxide dismutase (SOD) activity:** SOD was estimated by following the procedure of (Kakkar *et al.*, 1984). The inhibition of reduction of nitro blue tetrazolium (NBT) to blue colored formazan in presence of phenazine metha sulphate (PMS) and NADH was measured at 560 nm using n-butanol as blank. One unit (U) of enzyme activity was defined as the amount of enzyme that inhibits rate of reaction by 50% in 1 min under the defined assay conditions and the results have been expressed as U/mg protein.

**Catalase (CAT) activity:** Decomposition of H<sub>2</sub>O<sub>2</sub> in presence of catalase was followed at 240 nm (Beers and Sizer, 1952). One unit of (U) CAT was defined as the amount of enzyme required to decompose 1 mmol of H<sub>2</sub>O<sub>2</sub>/min, at 25°C and pH 7.0. Results are expressed as U/mg protein.

### Statistical Analysis

The values were expressed as mean ± SEM. The

statistical analysis was carried out by one way analysis of variance (ANOVA) followed by Dunnett's 't' – test using graph pad version I. *P* values <0.05 were considered significant.

The Effect of ELPB on ulcer index, LPO, SOD and CAT in cold restraint stress ulcer was shown in Table 1. Severe Hypothermic and Immobilization stress produced considerable ulcer in rats. The ulcers were in the form of haemorrhagic mucosal lesions in the stomach, which were confined to the rugae of glandular segment. The parameters studied included ulcer index and % protection of ulcer by ELPB. ELPB at the doses of 250 and 500 mg/kg resulted in a significant (*P*<0.001)

reduction in ulcer index (  $21.67 \pm 1.61$  and  $11.36 \pm 1.02$  respectively) and % protection of ulcer (72.28 and 85.47 respectively) when compared with the control group. Omeprazole the standard drug showed significant anti-ulcer activity in this model.

Stress significantly increased lipid per oxidation in the gastric mucosa with concomitant increase in SOD and decrease in CAT. ELPB in both doses of 250 and 500 mg/kg significantly protected the animals against stress-induced free radical damage as seen from the decrease in LPO and reversal of changes induced by stress on SOD and CAT.

**Table 1. Effect of ethanolic leaf extract of *Polygonum barbatum* on ulcer index, LPO, SOD and CAT in cold restraint stress ulcer model in rats**

Drug Treatment	Ulcer Index	% Protection	LPO (nmols of MDA/mg of protein)	SOD (U/mg of protein)	CAT (U/mg of protein)
Group 1 Normal Control 0.1% CMC 1ml/kg	--	--	$141.63 \pm 7.60$	$96.87 \pm 7.45$	$59.32 \pm 4.72$
Group 2 Stress Control 0.1% CMC 1ml/kg	$78.17 \pm 5.51$	--	$251.79 \pm 10.41$	$153.61 \pm 8.63$	$21.77 \pm 2.02$
Group 3 Omeprazole (10 mg /kg)	$6.32 \pm 0.51^{***}$	91.91	$149.33 \pm 8.92^{***}$	$99.74 \pm 6.17^{***}$	$51.48 \pm 1.29^{***}$
Group 4 ELPB 250mg/kg	$21.67 \pm 1.61^{***}$	72.28	$172.23 \pm 5.72^{***}$	$121.45 \pm 8.94^*$	$35.65 \pm 2.46^*$
Group 5 ELPB 500mg/kg	$11.36 \pm 1.02^{***}$	85.47	$162.03 \pm 5.52^{***}$	$109.03 \pm 7.77^{**}$	$42.49 \pm 3.28^{***}$

Values are presented as mean  $\pm$  SEM (n = 6) <sup>\*\*\*</sup>*P*<0.001, <sup>\*\*</sup>*P*<0.01 and <sup>\*</sup>*P*<0.05 Vs control

## CONCLUSION

The antiulcer activity of ethanolic leaf extract of *Polygonum barbatum* was studied in cold and restraint stress induced ulcer in rats in order to evaluate its mode of action. From the result it was concluded that ELPB

exhibited gastroprotective activity by its antioxidant property. Further study may be focused on isolation, separation and characterization of active principle responsible for its antiulcer property of this plant may bring a new potential gastroprotective drug.

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