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Protective Effects of *Polygonum viviparum* L. Root and its Extracts Against Lipid-peroxidation Induced by Indomethacin in Rats

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Abstract: *Polygonum viviparum*, root has been used in the folkloric medicine for the treatment of a many diseases including gastrointestinal disorders. Powdered roots of *Polygonum viviparum* (PV) and its aqueous (PV-A) and ethanol (PV-E) extracts were evaluated for anti-ulcerogenic efficacy in rats with ulcer lesions in the glandular stomach induced by indomethacin (25 mg kg^{-1}) subcutaneously, to rationalize the folkloric uses. The pretreatment of PV protected the experimental rats against gastric changes caused by indomethacin in a dose-dependent manner. PV-A and PV-E extracts (equivalent to 2.0 g kg^{-1}) of the powder also inhibited the indomethacin-induced effects on gastric juice volume, pH, acid-output and ulcer index. PV, PV-A and PV-E showed highly significantly *in vitro* acid buffering and pepsin binding activities. The data indicates that PV and its extracts protect the gastric mucosa against indomethacin-induced gastric changes, may result from their lipid peroxidation/apoptosis inhibition.

Key words: Anti-ulcer, ulcer index, indomethacin, lipid peroxidation, *Polygonum viviparum*

INTRODUCTION

Polygonum viviparum L. (Alpine Knotweed, Anjabar) [Polygonaceae] has long been used in the conventional medicines for the treatment of various diseases including Gastrointestinal (GIT) disorders[1]. It is a perennial herb, rootstock is woody, stem 1.5 m long, slender, simple and leaves are long-stalked. It is distributed in NWFP, Baluchistan and Kashmir in Pakistan, India, Europe and America[1,2]. The root has been found to be tonic, styptic and useful in affections of the chest and lungs. It has also been used for the treatments of piles, vomiting, biliousness, chronic bronchitis and wounds[3,4]. Root has been reported to be astringent and excellent for the treatment of ulcers[5,6]. Flavonoids like cyanidin, kaempferol, myricetin and quercetin have been reported to be present in leaf[7-9]. The presence of gallic acid and saponins in rhizomes has been shown[10,11].

PV has been considered an effective tool in conventional medicine to treat the gastric disorders e.g. gastric ulcers, which have been caused major health problems throughout the world including Pakistan. Hence, the present study was undertaken to explore the effective cure of gastric ulcers, to serve the ailing humanity and to make use of the indigenous medicinal plants (natural herbal wealth) of Pakistan. The industrial use of natural herbal drugs can uplift the economy of the country by saving the foreign exchange presently being spent for the import of different synthetic drugs for the treatment of peptic ulcer.

MATERIALS AND METHODS

Plant drug and extracts: *Polygonum viviparum*, roots (PV) were purchased locally from herbal dealer in Bahawalpur-Pakistan. The roots of the plant were authenticated and compared with its standard in the herbarium maintained by Department of Botany, University of Agriculture, Faisalabad and a sample was preserved in the Pharmacognosy Laboratory, Department of Pharmacy, Islamia University Bahawalpur. The shade-dried roots were finely powdered to the mesh size 200 and stored in well-closed cellophane bags at 4°C[12,13]. The aqueous extract was prepared by maceration; 1.00 kg of powdered roots were soaked in about 2.00 L of distilled water for 24 h. The extract was decanted; remaining materials was re-soaked in the distilled water twice. The combined extract was dried completely by Rotavapour at 37°C[14]. Ethanolic extract was prepared by same method. All the test substances were suspended in aqueous 2.5% gum tragacanth solution or dissolved in the normal saline solution before their administration[14,15].

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Chemicals: The analytical grade chemicals were used in this study, which were obtained from E. Merck (Darmstadt, FRG), BDH Poole (England) and Sigma Chemical Co. (USA).

Animal used: Healthy adult albino male rats (Sprague-Dawley) weighing 180-230 g each were used in this study. The animals were housed under standard conditions of temperature (23±2°C), humidity (55±15%) and 12 h light (7.00 am to 7.00 pm). The animals were provided a free access to the standard feed (M/S Lever Brothers, Rahim Yar Khan, Pakistan) and water *ad libitum*. The 24 h fasted rats were used in the experiments[16,17]. The use of animals in this study was in accord to the principles and guidelines of the Canadian Council on Animal Care.

Acid buffering activity: PV powder (500 mg) and its equivalent aqueous (PV-A), ethanolic (PV-E) extracts were added to 2.0 mL of distilled water each, which were treated with 10 mL of HCl solution, pH 1.0, separately while 2.0 mL of distilled water was added in the controlled test tubes in addition to above 10 mL of HCl solution. The pH values were measured after 1 and 30 min[18].

Pepsin binding activity: The 500 mg powdered PV and its equivalent PV-A and PV-E extracts were added to1 mL of pepsin solution (2 mg mL^{-1}) each in the separate test tubes. Four milliter of 0.2 N HCl buffered with 1 mL of 0.2 N sodium citrate solution to ensure the pH 1.6 was added. One milliter of bovine serum albumin (5 mg mL^{-1}) was added to treat the excess pepsin except control test tubes, preincubation at 37°C for 30 min. The contents were further incubated at 37°C for 30 min following shaking. Remaining protein in each tube was treated with 1.0 mL of Biuret reagent and alkalized by adding 5 mL 0.2 N NaOH solution. Their absorbance were read at 546 nm. The values obtained were expressed as %age binding of pepsin[18,19].

Gastric ulcer-induction: The modified method of Yoshikawa *et al.*[20] was used to produce the gastric ulcers in the experimental rats. The test substances were administered by oral route t.d.s (8.00 am, 3.00 and 10.00 pm) for 14 consecutive days to all the treated group of animals. On 15th day, pylorus of 24 h fasted rats were ligated under a light ether anesthesia. The test drugs were given orally, immediately following pylorus-ligation and indomethacin was injected subcutaneously[20,21].

The normal (untreated) and treated control groups received 3 mL kg^{-1} of 2.5% gum tragacanth vehicle orally[18]. Rats of different treated groups received PV powder 1.0, 1.5 and 2.0 g kg^{-1} while PV-A and PV-E (equivalent to 2.0 g kg^{-1} of powder) orally separately. The treated control and treated animals were administered a single dose of indomethacin 25 mg kg^{-1} subcutaneously (s. c.) on 15th day of treatment.

The operative method of Takeuchi *et al.*[22] was adopted. Briefly the pylorus of animals were located and ligated with silk suture following the opening of their abdominal cavities through the midline incisions in the anesthetized rats. The abdominal wounds were stitched in separate layers and animals were allowed to recover from anesthesia. The drinking water was withheld then and gastric juices were allowed to collect for a period of 4 h. After which rats were killed by the overdose of anesthesia and their abdomens were reopened. The stomachs were removed following the clamping the esophagus. The gastric contents were collected through the esophagus. The stomachs were re-inflated with 3 mL of warm distilled water to wash the gastric mucosa. The combined gastric content of each rat with its washing was centrifuged (4000 rpm for 10 min). About 10 mL of 1% formalin was filled in each stomach following which the stomachs were immersed in 10% formalin for 10 min to fix the inner and outer walls. The stomachs were incised along their greater curvatures and glandular portion of stomachs were examined to locate the gastric ulcers[16,18].

Gastric juice volumes, pH and acid-outputs determinations: According the method of Tanaka *et al.*[23] the volumes and pH of centrifuged gastric secretion were measured by pipette and pH meter, respectively. The acid outputs were calculated by using the following equation[16].

$$EqH^{+}/100\ g/4\ h = 1/\text{antilog pH} \times 1000 \times \text{volume of gastric juice (mL)} \times 100/\text{body weight of animal (g)}$$

Gastric ulcer index determinations: The gastric wounds in the glandular regions were located under a simple microscope. The lengths (mm) of all the elongated black-red lines parallel to the long axis of the stomachs in the mucosa were measured. The ulcer index was calculated by adding the lengths of all the lesions in the glandular region of a stomach[18,23].

Statistical analysis: The data obtained was expressed as means±SEM (Standard Error of Means of six experiments) and analysed statistically by the application of SPSS (Statistical Package for Social Sciences) for Windows version 7.5. The Student's t test was applied and p-values were determined. Differences were considered non-significant at $p>0.05$, significant at $p<0.05$ and highly significant at $p<0.001$[24].

RESULTS AND DISCUSSION

Indomethacin and other NSAIDs (Non-Steroidal Anti-Inflammatory Drugs) have been known to cause the lipid peroxidation[25,26]. It has been found to cause a progressive decrease in the mucosal contents of prostaglandins e.g. PGI_2, PGE_2 and TxA_2 and increase in leukotrienes (LTs) due to selective inhibition of cyclo-oxygenase enzyme leading to inflammation and pain[25,26]. The inhibition of PGE_2 synthesis potentate the secretary effects elicited by histamine. The large amounts of acid and pepsin releases have been reported to cause the gastric ulcer and erosion formation[27,28]. The studies have further shown that indomethacin and many other NSAIDs can cause apoptosis which activate more acid secretion by the stimulatory pathway of histamine[14].

The pretreatments of indomethacin in treated control animals increased the gastric secretion volume and acid out-put while decreased gastric pH highly significantly. Similarly heavy gastric ulcer lesions were formed by indomethacin in the experimental rats (Table 1). These gastric changes have been reported due to its lipid peroxidation/apoptosis activity[29-31].

P. viviparum root powder (PV) and it's aqueous (PV-A) and ethanol (PV-E) extracts showed highly significantly *in vitro* acid-buffering activities. PV increased the pH from 1.0 up to 1.462±0.031, PV-A from 1 up to 1.409±0.028 and PV-E from 1 up to 1.412±0.035 (Table 2). The powdered PV and its extracts showed highly significantly ($p<0.001$) pepsin binding activity *in vitro*. PV showed 33.81±1.58%, while PV-A and PV-E 31.57±1.63% and 27.42±1.49%, pepsin binding capacities, respectively (Table 3).

In addition the PV powder in 1.0-2.0 g kg^{-1} doses attenuated the gastric changes induced by indomethacin. The preventive effects on gastric juice volume, pH, acid-output and ulcer index (i.e. anti-ulcerogenic effect), in the treated animals were caused in a dose-dependent manner by the oral administration of powdered PV. The anti-ulcerogenic effects were highly significantly ($p<0.001$) more with 2.0 g kg^{-1} dose of the powder. PV-A and PV-E equivalent to 2.0 g kg^{-1} of the powder also caused highly significantly ($p<0.001$) attenuation of gastric changes in the treated rats (Table 1).

The inhibitory effects of test agents against indomethacin-induced gastric changes indicate that they may interfere with the lipid peroxidation/apoptosis activity of indomethacin. The data has been pointed out clearly that the antiulcer active principle(s) of PV powder is/are both water and ethanol extractable (Table 1).

Table 1: Effects of *P. viviparum* roots powder and its aqueous and ethanolic extracts on gastric secretion volume, pH, acid-output and ulcer index in rats

	Gastric secretion's			
Treatments	Volume (mL)	pH	Acid output (μEq/100 g/4 h)	Ulcer index (mm)
Normal (untreated control)	2.20±0.06	2.82±0.01	2.82±0.15	2.62±0.26
Indomethacin (25 mg kg^{-1} p.o.) (Treated control)	8.60±0.45**	1.08±0.20**	340.23±14.21**	49.83±2.47**
P. viviparum roots (1.0 g kg^{-1} p.o.) + Indomethacin (25 mg kg^{-1} s.c.)	5.07±0.05**	1.28±0.04	166.30±2.78**	25.82±0.45**
P. viviparum roots (1.5 g kg^{-1} p.o.) + Indomethacin (25 mg kg^{-1} s.c.)	4.37±0.07**	1.68±0.06*	60.87±2.43**	15.86±0.2**
P. viviparum roots (2.0 g kg^{-1} p.o.) + Indomethacin (25 mg kg^{-1} s.c.)	3.83±0.06**	2.69±0.05**	6.01±0.39**	15.45±0.71**
Aqueous extract of *P. viviparum* root (Eq. to 2.0 g kg^{-1} p.o.) + Indomethacin (25 mg kg^{-1} s.c.)	2.77±0.12**	2.25±0.06**	12.35±0.98**	22.53±1.23**
Ethanol extract of *P. viviparum* root (Eq. to 2.0 g kg^{-1} p.o.) + Indomethacin (25 mg kg^{-1} s.c.)	3.10±0.09**	2.90±0.08**	3.61±0.52**	8.43±0.62**

Indomethacin: significant from untreated control (Normal) * $p<0.05$; ** $p<0.001$, Test drugs: Significant from treated control (Indomethacin) * $p<0.05$; ** $p<0.001$, All the other values are NS ($p>0.05$) from treated control (Indomethacin)

Table 2: Acid buffering activities of *P. viviparum* and its extracts (*In vitro*)

Experiments	pH
Control/Standard	1
Plant drug powder (500 mg)	1.462±0.031**
Aqueous extract of powder (equivalent to 500 mg)	1.409±0.028**
Ethanolic extract of powder (equivalent to 500 mg)	1.412±0.035**

Table 3: Pepsin binding capacities of *P. viviparum* and its extracts (*In vitro*)

Experiments	Pepsin binding	% age of pepsin binding
Control/Standard	0.0	0.00
Plant drug powder (500 mg)	676.2±13.69	33.81±1.58
Aqueous extract of powder (equivalent to 500 mg)	631.4±11.58	31.57±1.63
Ethanolic extract of powder (equivalent to 500 mg)	548.3±11.80	27.42±1.49

The reported results have validated the folkloric use of the drug tested for use in the therapy of gastric ulcer disease. In particular, the present studies have pointed out possible gastric anti-secretary and anti-ulcer effects of the PV powder and its extracts. Nevertheless, detailed chemical studies followed by pharmacological investigations and toxicity evaluations are still required to isolate the pure active principle(s) of the PV and to elucidate the precise mechanism(s) of anti-ulcer actions, studies are also needed.

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