Original Article

Gastroprotective Effect of Hydro-Alcoholic Extract of *Polygonum bistorta* Lin Root in Indomethacin-Induced Gastric Ulcers in Sprague Dawley Rats

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ABSTRACT

Objective: Gastroprotective effect of hydro-alcoholic extract of *Polygonum bistorta* Linn root (HEPB) was investigated in indomethacin-induced gastric ulcer in Sprague Dawley rats. **Background:** *Polygonum bistorta* has been used as hemostatic drug in Unani system of medicine due to its cold and dry temperament. **Methods:** The rats were grouped into six groups each consisting of five rats. Group-I, group-II, group-III, group-IV, group-V and group-VI rats received 1 mL/kg/day 1% carboxymethyl cellulose (CMC), 1 mL/kg/day 1% CMC, 500 mg/kg/day HEPB, 1000 mg/kg/day HEPB, 20 mg/kg/day ranitidine and 1000 mg/kg/day HEPB per oral (*po*) respectively for 10 days. Further, rats of all groups except group-I and group-VI were administered with 20 mg/kg b.wt indomethacin *po* on eleventh day. Then, rats were sacrificed, stomach was opened, and ulcer index was calculated. Mucus barrier and histopathology was determined. Rest of stomach was homogenized in buffer to evaluate antioxidant parameters thiobarbituric acid, catalase and superoxide dismutase (SOD). **Results:** HEPB in group-III and group-IV significantly ($p < 0.01$) and dose dependently increased the levels of mucus, SOD and catalase while, decreased ulcer index and thiobarbituric acid reactive substances compared to that of ulcer control group-II. Histopathological findings showed that indomethacin treatment caused gastric ulcer while; HEPB treatment protected them from indomethacin-induced ulcer. Ulcer protection potency of HEPB 1000 mg/kg/day in group-IV was comparable to that of 20 mg/kg/day ranitidine in group-V. **Conclusion:** HEPB protected stomach from indomethacin-induced gastric ulcers in rats by prevention of induced muco-oxidative stress. Thus, HEPB possesses gastroprotective effect against indomethacin-induced gastric ulcers in rats.

Key words: *Polygonum bistorta*, Indomethacin, Oxidative stress, Ulcer index, PUD.

INTRODUCTION

Peptic Ulcer Disease (PUD), the most common ulcer of the gastrointestinal tract in the region of duodenum and stomach, is characterized by high acidity which results into the erosion of duodenal and gastric mucosa causing discomfort and severe pain.[1] Probability of its development in the lifetime of a male and female people is about 10% and 4% respectively which is responsible for mortality and morbidity of millions of people throughout the world.[2]

Antiulcer drugs, the drugs used to treat PUD, are mainly targeted to stop the gastric acid secretion. However, it is evidenced now a day that the ideal antiulcer drug should not only have the antisecretory effect but should also have multiple effects such as anti-inflammatory, anti-oxidant and anti-apoptotic activities for an efficient treatment of PUD.[3] The conventional drugs used in its treatment include anticholinergics, antacids, proton pump inhibitors and H_2-receptor antagonists. However, most of these drugs have disadvantages of increased incidence of relapse, drug interactions and undesirable side effects.[4] Herbal drugs have been used

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as a powerful traditional therapeutic agents for the treatment of various diseases including those of gastrointestinal system since a very old time.[5] These are applied to enhance the activity of synthetic medicines, used as drugs supplementation and have also been successfully applied in prophylaxis of hyperacidity in ulcer.[6] Also, more than 80% of the world population in developing countries depends principally on these herbal drugs for their basic healthcare requirements.[6]

The herbal drug *Polygonum bistorta* Linn belonging to family Polygonaceae is known as bistort, knot grass, dragonwort, Adderwort, snake-weed and snake root in English, Bijband in Hindi and Anjbar in Urdu. It contains ascorbic acid and approximately 21% tannin including tannic acid, gallic acid, polygonic acid, phloroglucinol, catechol, phlobaphene, calcium oxalate, starch and essential oil.[7,8] It possesses neuroprotective, hepatoprotective, choleretic, antifungal, antipyretic, antioxidant, antispasmodic, antidiarrheal and several other pharmacological activities.[9-12] Its rhizomes have been used to treat venomous snake bite, epistaxis, hematemesis, haemorrhoidal bleeding, and dysentery with bloody stools, diarrhea, acute gastroenteritis and aphthous ulcer in traditional Chinese medicine since long time.[9-12] It is widely used as hemostatic drug in Unani system of medicine to control bleeding from any part of the body such as liver, chest, lungs, pleura, trachea, bleeding piles and intestinal abrasion as it is considered to be viscous, astringent; and cool and dry in temperament.[13] It strengthens the intestine and stomach being gastrotonic; and is useful in gastric diseases.[13] Sharbat-e-Anjbar a marketed formulation of *P. bistorta* is prescribed for intestinal abrasion.[13] However, there is need to maintain the quality, purity and safety of the Sharbat-e-Anjbar and other marketed formulations of *P. bistorta* by subjecting to scientific validation. Thus, an attempt was made to evaluate the possible gastroprotective effect of hydro-alcoholic extract of *Polygonum bistorta* Linn root (HEPB) in indomethacin-induced gastric ulcer in Sprague Dawley rats.

MATERIALS AND METHODS

Chemical reagents, instruments and equipments

All the chemicals used in the present study were of analytical grade. Alcian blue was procured from SD Fine Chemicals Ltd., ethylene diamine tetraacetic acid (EDTA) from SD Fine Chemicals Ltd, carboxymethyl cellulose (CMC, CDH Mumbai), pyrogallol 98.5% (Himedia chemicals), Folin-Ciocalteu phenol reagent (FCR, Merck), magnesium chloride ($MgCl_2$, SRL), diethyl ether (CDH Mumbai), sucrose (CDH Mumbai), thiobarbituric acid (TBA, Himedia chemicals), sodium acetate (Merck), acetic acid (Rankem Ltd.), ranitidine (GSK), indomethacin (Ipica), centrifuge (Almicro micromeasures and Instrument), UV spectrophotometer (double beam, UV-1700, Shimadzu), electronic balance (Shimadzu AUX 220 Unibloc), micropipette (100-1000µL, Superfit) and refrigerator (Intellocool LG).

Procurement and authentication of the plant material

The plant material of present study was procured from local market Hamdard Dawakhana located at Amina Baad Lucknow and was authenticated by the herbalist, authentication office of Faculty of Pharmacy of Integral University Lucknow as *Polygonum bistorta* Linn root (voucher number: *IU/PHAR/HRB/17/02*).

Preparation of plant extract

Dried root of the herbal drug *P. bistorta* was subjected to a coarse powder with the help of mechanical grinder and was extracted with 70% hydro-alcoholic solvent by Soxhlet extractor. The extract was filtered while hot and concentrated to dryness under reduced temperature (40±1°C) and pressure using rotary evaporator. The extract was stored in refrigerator for further experimental studies.

Evaluation of total phenolic content

Total phenolic content in the extract HEPB was estimated with Folin-Ciocalteu phenol reagent (FCR) using gallic acid calibration curve.[14] The 0.2 mL of HEPB dissolved in 95% ethanol was added to 4 mL of 2% sodium carbonate solution. After 2 min, 0.2 mL of 50% FCR was added to the mixture and then, incubated at 30°C for 30 min. Absorbance was recorded at the wavelength of 718 nm by a UV-spectrophotometer.

Experimental animals

Sprague Dawley rats (125-200 g) were procured from Central Drug Research Institute, Lucknow and kept in animal house at Faculty of Pharmacy, Integral University Lucknow. The rats were grouped, and each group were housed separately in polypropylene cages for acclimatization at a temperature of 23±2°C and relative humidity of 50-60% with a 12 h light/dark cycle one week before and during the commencement of the experimental study. They were kept on standard pellet diet *ad libitum* and drinking water throughout the study period. The protocol of experimental study was approved by Institutional Animal Ethics Committee (IAEC), Faculty of Pharmacy of Integral University (IU) Lucknow (approval number: *IU/IAEC/17/08*).

Experimental design

Adult Sprague Dawley rats were randomly divided into six groups each consisting of five rats ($n=5$). Animals of group-I (normal control), group-II (toxic control), group-III (extract treated-500), group-IV (extract treated-1000), group-V (standard drug treated) and group-VI (*per se*) received 1 mL/kg/day 1% CMC, 1 mL/kg/day 1% CMC, 500 mg/kg/day HEPB, 1000 mg/kg/day HEPB, 20 mg/kg/day ranitidine and 1000 mg/kg/day HEPB respectively per oral (*po*) for 10 days. Further, 36 h fasted rats of all the groups except group-I and group-VI were administered with 20 mg/kg b.wt of indomethacin *po* on eleventh day. At the end of experiment, rats were sacrificed under thiopental anesthesia. Stomach was cut from gastrointestinal tract, opened along the greater curvature and ulcer index was calculated.[15] After this, the stomachs were weighed and immediately immersed in alcian blue solution for determining the mucus wall thickness.[16] The serum, gastric juice and gastric tissues were collected. After that a small portion of stomach was taken and kept in 10% formalin solution for histopathological study. Rest portion of stomach was homogenized after weighing in phosphate buffer for study of antioxidant parameters such as TBA, catalase and SOD. The homogenized buffer solution and stomach portion for mucus barrier determination was kept in deep freezer at -20°C until further analysis.

Effect of hydro-alcoholic extract of *Polygonum bistorta* Linn root on different ulcer specific variables

Effect of hydro-alcoholic extract of *Polygonum bistorta* Linn root on ulcer index

Stomach was opened and fixed on a cork plate by pin up. Gastric mucosa was examined with a magnifying glass of 10X. Ulcer was counted, quantified and evaluated for the severity where the number 0 denotes to no ulcer, 1 to superficial ulcer, 2 to deep ulcer, 3 to perforation. Ulcer index was calculated using the formula [ulcer index = average number of ulcers per animal + average number of severity score + percentage of animals with ulcers $\times 10^{-1}$].[2]

Effect of hydro-alcoholic extract of *Polygonum bistorta* Linn root on gastric wall mucus

Gastric wall mucus was removed from the stomach, weighed and transferred instantly to 10 mL of 0.1% alcian blue solution as alcian blue stains only the mucus and does not penetrate the mucosal tissue. After 2 h, surplus alcian blue was removed by two successive rinses with 10 mL of 0.25 M sucrose, first for 15 min and second for 45 min. Then, alcian blue complexed with mucus was extracted with 10 mL of 0.5 M $MgCl_2$ solution at 30 min intervals for 2 h. The obtained alcian blue solution was mixed with an equal volume of ether, shaken vigorously and then, centrifuged at 3000 revolutions per minute (rpm) for 10 min. Absorbance of the aqueous layer was recorded at the wavelength of 580 nm using $MgCl_2$ solution as blank. Quantity of alcian blue recovered from each gram of mucus was calculated from the standard calibration curve.[16]

Effect of hydro-alcoholic extract of *Polygonum bistorta* Linn root on thiobarbituric acid reactive substances (TBARS)

One milliliter of suspension from 10% gastric tissue homogenate containing a secondary product of lipid peroxidation i.e., malondialdehyde (MDA), 0.5 mL 30% TCA and 0.5 mL 0.8% TBA were taken in a tube. The tubes were covered with aluminium foil and kept in shaking water bath for 30 min at 80°C. Then, tube was taken out and kept in ice-cold water for another 30 min. Further, it was centrifuged at 3000 rpm for 15 min. The absorbance (A) of the supernatant was observed at the wavelength of 540 nm at room temperature against blank as it contains characteristic chromogenic adduct of MDA, a thiobarbituric acid reactive substances with two molecules of TBA. The amount of MDA present in the sample was calculated to measure extent of lipid peroxidation using the formula [nM of MDA = A × V/ 0.156] Where, V is the final volume of test solution in unit of mL.[2]

Effect of hydro-alcoholic extract of *Polygonum bistorta* Linn root on catalase activity

The gastric tissue homogenate homogenized in 50 mM phosphate buffer with a ratio of 1:10 was centrifuged at 10,000 rpm at 4°C in a cooling centrifuge for 20 min. 50 µL supernatant was added to cuvette containing 2.95 mL of 19 mM hydrogen peroxide (H_2O_2) solution. The change in absorbance (ΔA/min) was recorded at 1 min interval for 3 min at the wavelength of 240 nm. Presence of catalase decomposes H_2O_2 leading to a decrease in absorbance. Catalase activity was calculated using the formula [n moles of H_2O_2 consumed/min/mg protein = (ΔA/min × volume of assay) / (0.081× volume of homogenate × mg of protein)].[2]

Effect of hydro-alcoholic extract of *Polygonum bistorta* Linn root on superoxide dismutase activity

One hundred microliter of supernatant was added to tris-HCl buffer and volume was adjusted to 3 mL with the buffer. 25 µL of 24 mM pyrogallol prepared in

10 mM HCl was added and changes in absorbance at 420 nm were recorded at 1 min interval for 3 min. Super oxide anion radical catalyses the auto-oxidation of pyrogallol. The increase in absorbance at 420 nm after the addition of pyrogallol was inhibited by the presence of superoxide dismutase (SOD). 1 unit of SOD is described as the amount of enzyme required to cause 50% inhibition of pyrogallol auto-oxidation per 3 mL of assay mixture and was calculated following the formula [Unit of SOD per mL of sample= $(\Delta A - \Delta B) \times 100 / \Delta A \times 50$], where, ΔA is the difference of absorbance in 1 min in control, ΔB is the difference of absorbance in 1 min in test sample. Data was expressed as SOD units per mg of protein.[2]

Effect of hydro-alcoholic extract of *Polygonum bistorta* Linn root on histopathology of stomach

Small portion of the stomach from different groups of rats was preserved in 10% formalin solution for further examination. Gastric tissues were mounted by embedding in paraffin wax and 6-mm slices were cut. The slices were stained by two dyes eosin and hematoxylin and the prepared slides were observed under light microscope.[2]

Statistical analysis

All the values were expressed as mean ± standard error of mean (SEM). The results were analyzed by one way analysis of variance (ANOVA) followed by Dunnett's t-test. The result values of $p<0.05$ were considered statistically significant.

RESULTS

Total phenolic content in the extract HEPB was estimated to be 4378 µg GAE/ g dried extract of HEPB. The effects of *Polygonum bistorta* Linn root extract on different ulcer specific variables are shown in Table 1.

The mean ulcer index was significantly ($p<0.01$) increased to 3.30±0.10 in ulcer control group-II rats administered with indomethacin 20 mg/kg b.wt as compared to that of 0.00±0.00 in normal control group-I rats administered with 1 mL/kg/day 1% CMC. A significant ($p<0.01$) decrease to 1.68±0.41 and 1.33±0.13 in the mean ulcer index was observed in group-III rats orally treated with HEPB 500 mg/kg b.wt and group-IV rats treated with HEPB 1000 mg/kg b.wt respectively as compared to ulcer control group-II rats administered with indomethacin. A significant ($p<0.01$) decrease to 0.87±0.11 in the mean ulcer index was observed in group-V rats orally treated with ranitidine 20 mg/kg b.wt as compared to ulcer control group-II rats. However, there was no significant ($p>0.05$) change in mean ulcer index in *per se* group-VI rats administered with HEPB 1000 mg/kg/day *po* as compared to normal control group-I rats.

The mean gastric wall mucus thickness was calculated using standard calibration curve for alcian blue. The mean gastric wall mucus was significantly ($p<0.01$) decreased to 1.65±0.24 µg/g of stomach in ulcer control group-II rats administered with indomethacin 20 mg/kg b.wt as compared to that of 3.18±0.17 µg/g of stomach in normal control group-I rats administered with 1 mL/kg/day 1% CMC. A significant ($p<0.01$)

Table 1. Effects of *Polygonum bistorta* Linn root extract on different ulcer specific variables.

Groups / Ulcer specific variables	Treatment schedule (n=5)	Ulcer index scores	Mucus (µg/g of stomach)	TBARS (nmol/mg protein)	Catalase (nmol of H2O2 consumed/ min/mg protein)	SOD (units/mg protein)
I (Normal Control)	1% CMC; 1 mL/kg, *po*	0.00±0.00	3.18±0.17	1.41±0.07	0.058±0.003	0.65±0.02
II (Ulcer Control)	1% CMC; 1 mL/kg *po* + indomethacin; 20 mg/kg *po*	3.30±0.10**	1.65±0.24**	4.33±0.16**	0.008±0.002**	0.08±0.01**
III (Extract treated-500)	HEPB; 500 mg/kg *po* + indomethacin; 20 mg/kg *po*	1.68±0.41#	2.97±0.14#	3.43±0.13#	0.048±0.004#	0.47±0.02#
IV (Extract treated-1000)	HEPB; 1000 mg/kg *po* + indomethacin; 20 mg/kg *po*	1.33±0.13#	3.41±0.14#	2.62±0.11#	0.053±0.003#	0.53±0.02#
V (standard drug treated)	Ranitidine; 20 mg/kg *po* + indomethacin; 20 mg/kg *po*	0.87±0.11#	2.35±0.13#	2.74±0.08#	0.039±0.004#	0.62±0.01#
VI (Per se)	HEPB *per se*; 1000 mg/kg *po*	0.50±0.22*	3.39±0.21*	1.92±0.09*	0.049±0.003*	0.65±0.01*

All values were expressed as mean±SEM, ($n=5$). The comparisons were done by ANOVA followed by Dunnett's 't' test. * indicates $p>0.05$, ** $p<0.01$ as compared to normal control group-I and # $p<0.01$ as compared to ulcer control group-II.

increase to 1.65±0.24 and 2.97±0.14 µg/g of stomach in the mean gastric wall mucus was observed in group-III rats orally treated with HEPB 500 mg/kg b.wt and group-IV rats treated with HEPB 1000 mg/kg b.wt respectively as compared to ulcer control group-II rats administered with indomethacin. A significant (p<0.01) increase to 3.41±0.14 µg/g of stomach in the mean gastric wall mucus was observed in group-V rats orally treated with ranitidine 20 mg/kg b.wt as compared to ulcer control group-II rats. However, there was no significant (p>0.05) change in mean gastric wall mucus in *per se* group-VI rats administered with HEPB 1000 mg/kg/day *po* as compared to normal control group-I rats.

The thiobarbituric acid reactive substances (TBARS) was significantly (p<0.01) increased to 4.33±0.16 nmol/mg protein in ulcer control group-II rats administered with indomethacin 20 mg/kg b.wt as compared to that of 1.41±0.07 nmol/mg protein in normal control group-I rats administered with 1 mL/kg/day 1% CMC. A significant (p<0.01) decrease to 3.43±0.13 and 2.62±0.11 nmol/mg protein in the TBARS was observed in group-III rats orally treated with HEPB 500 mg/kg b.wt and group-IV rats treated with HEPB 1000 mg/kg b.wt respectively as compared to ulcer control group-II rats administered with indomethacin. A significant (p<0.01) decrease to 2.74±0.08 nmol/mg protein in the TBARS was observed in group-V rats orally treated with ranitidine 20 mg/kg b.wt as compared to ulcer control group-II rats. However, there was no significant (p>0.05) change in TBARS in *per se* group-VI rats administered with HEPB 1000 mg/kg/day *po* as compared to normal control group-I rats.

The catalase activity was significantly (p<0.01) decreased to 0.008±0.002 nmol of H_2O_2 consumed/min/mg protein in ulcer control group-II rats administered with indomethacin 20 mg/kg b.wt as compared to that of 0.058±0.003 nmol of H_2O_2 consumed/min/mg protein in normal control group-I rats administered with 1 mL/kg/day 1% CMC. A significant (p<0.01) increase to 0.048±0.004 and 0.053±0.003 nmol of H_2O_2 consumed/min/mg protein in the catalase activity was observed in group-III rats orally treated with HEPB 500 mg/kg b.wt and group-IV rats treated with HEPB 1000 mg/kg b.wt respectively as compared to ulcer control group-II rats administered with indomethacin. A significant (p<0.01) increase to 0.039±0.004 nmol of H_2O_2 consumed/min/mg protein in the catalase activity was observed in group-V rats orally treated with ranitidine 20 mg/kg b.wt as compared to ulcer control group-II rats. However, there was no significant (p>0.05) change in catalase activity in *per se* group-VI rats administered with HEPB 1000 mg/kg/day *po* as compared to normal control group-I rats.

The superoxide dismutase (SOD) activity was significantly (p<0.01) decreased to 0.08±0.01 units/mg protein in ulcer control group-II rats administered with indomethacin 20 mg/kg b.wt as compared to that of 0.65±0.02 units/mg protein in normal control group-I rats administered with 1 mL/kg/day 1% CMC. A significant (p<0.01) increase to 0.47±0.02 and 0.53±0.02 units/mg protein in the SOD activity was observed in group-III rats orally treated with HEPB 500 mg/kg b.wt and group-IV rats treated with HEPB 1000 mg/kg b.wt respectively as compared to ulcer control group-II rats administered with indomethacin. A significant (p<0.01) increase to 0.62±0.01 units/mg protein in the SOD activity was observed in group-V rats orally treated with ranitidine 20 mg/kg b.wt as compared to ulcer control group-II rats. However, there was no significant (p>0.05) change in SOD activity in *per se* group-VI rats administered with HEPB 1000 mg/kg/day *po* as compared to normal control group-I rats.

Effects of hydro-alcoholic extract of *Polygonum bistorta* Linn root on gross anatomy and histopathology of stomach have been shown in **Figure 1** and **Figure 2.** Intact mucosal lining of flattened epithelial cells and compactly arranged mucosal glands consisting of cells with abundant eosinophilic cytoplasm, vesicular nuclei and nucleoli were seen in normal control group-I rats. Administration of indomethacin 20 mg/kg b.wt in group-II rats induced severe lesion and deep ulcers showing hemorrhagic areas, denudated lining epithelium, degenerated cellular changes, interstitial and intracellular cell oedema, and degenerated glandular epithelial cell changes.

Administration of HEPB 500 mg/kg b.wt in group-III rats protected the stomach to some extent from gastric

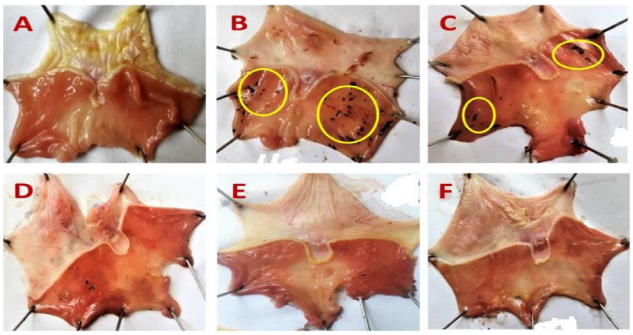

Figure 1: Effect of hydro-alcoholic extract of *Polygonum bistorta* Linn root on gross structure of stomach. [A]. Normal control group-I. [B]. Ulcer control group-II. [C]. Exctract treated-500 group-III. [D]. Exctract treated-1000 group-IV. [E]. Standerd drug treated group-V. [F]. *Per se* group-VI (*yellow circle denoting gastric ulcer*).

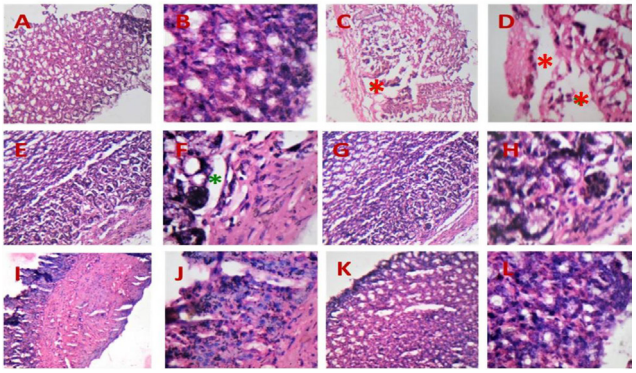

Figure 2: Effect of hydro-alcoholic extract of *Polygonum bistorta* Linn root on histopathological structure of stomach. [A-B]. Normal control group-I at 10X and 40X. [C-D]. Ulcer control group-II at 10X and 40X. [E-F]. Extract treated-500 group-III at 10X and 40X. [G-H] Extract treated-1000 group-IV at 10X and 40X. [I-J]. Standard drug treated group-V at 10X and 40X. [K-L]. *Per se* group-VI at 10X and 40X. (* *indicating cell damage*).

ulcers induced with indomethacin in rats with few lesion and superficial ulcers while, administration of HEPB 1000 mg/kg b.wt in group-IV rats or ranitidine 20 mg/kg b.wt in group-V rats more significantly protected the stomach from gastric ulcers induced with indomethacin in rats without any lesion and superficial ulcers. They showed intact mucosal lining of flattened epithelial cells, compactly arranged mucosal glands where glands were separated by thin strands of fibro-connective tissue, thick and intact basement membrane, and occasional blood vessels. Administration of HEPB 1000 mg/kg b.wt only in *per se* group-VI rats showed no changes compare to normal control group-I.

DISCUSSION

Peptic Ulcer Disease is the most common ulcer of the gastrointestinal tract in the region of duodenum and stomach.[1] Its occurrence is mostly due to an imbalance in mucus content in duodenum and stomach of gastro-intestinal tract.[1] Excessive intake of nonsteroidal anti-inflammatory drugs (NSAIDs) favors its development via mucosal damage through the generation of reactive oxygen species (ROS).[17] Indomethacin is an NSAID and causes mucosal damage.[18] Hence, indomethacin was used in the present study to induce gastric ulceration in rats.

There was no significant ($p>0.05$) change in the mean ulcer index, gastric wall mucus, TBARS, SOD and catalase in *per se* group-VI treated with 1000 mg/kg *po* of HEPB as compared to normal control group-I rats. It suggested that HEPB in doses upto 1000 mg/kg *po* is free from any acute toxicity. Hence, present doses selected for the study of possible gastroprotective effect of hydro-alcoholic extract of *Polygonum bistorta* Linn root (HEPB) in indomethacin-induced gastric ulcer in Sprague Dawley rats were 500 mg/kg b.wt and 1000 mg/kg b.wt.[2]

The present study showed that normal control group-I, which was fed only with the basal di*et al*ong with CMC, produced normal levels of mean ulcer index, gastric wall mucus, TBARS, SOD and catalase. The present study also showed that ulcer control group-II rats, which were administered with indomethacin (20 mg/kg *po*), resulted in significant ($p<0.01$) increase in levels of mean ulcer index and TBARS while significant decrease in levels of mean gastric wall mucus, SOD and catalase as compared to that of normal control group-I rats.

Extent of ulcer index is the indicator of intensity of stress or ulcerogenic potential of any drug.[19] A decrease in mean ulcer index is observed in test drug treated rats as compared to the indomethacin treated rats.[2] The present study showed that mean ulcer index was significantly decreased in extract treated-500 group-III, extract treated-1000 group-IV and standard drug treated group-V as compared to that elevated in ulcer control group-II rats.

The gastric mucosal integrity depends on several factors including mucus. Therefore, the main guidelines for the treatment are aimed to increased production of factors responsible for protecting the gastric mucosa, thus avoiding damage to the epithelium.[1] The present study showed that mean gastric wall mucus was significantly increased in extract treated-500 group-III, extract treated-1000 group-IV and standard drug treated group-V as compared to that decreased in ulcer control group-II rats.

The primary products of lipid peroxidation damage by ROS are a complex mixture of peroxides which breakdown to produce carbonyl compounds, malondialdehyde (MDA) which forms a characteristic chromogenic adduct with two molecules of TBA.[2] The present study showed that mean TBARS level was significantly decreased in extract treated-500 group-III, extract treated-1000 group-IV and standard drug treated group-V as compared to that elevated in ulcer control group-II rats.

In some cases, ROS production is increasing for a defensive purpose in response to certain external stimuli, harmful diet and human disease. Normally these produced ROS are neutralized by endogenous antioxidant cellular system such as catalase and SOD. However, oxidative stress status will occur when ROS production accumulated and exceeded over the cellular antioxidant system. Consequently, oxidative stress may cause induction and

the aggravation of gastric ulcer.[20] Catalase and SOD are essential to the body in order to discharge the harmful ROS from the cellular environment.[21] The present study showed that mean SOD and catalase levels were significantly increased in extract treated-500 group-III, extract treated-1000 group-IV and standard drug treated group-V as compared to that decreased in ulcer control group-II rats. HEPB is free from toxic substances and is a safe source of antioxidants.[21] These results suggested the possible involvement of endogenous antioxidants in the experimental effect of HEPB in gastric ulcer.

The gastroprotective effect of HEPB could be due to the presence of phenolics present in the extract which was found to be 4378 µg GAE/ g dried extract of HEPB as phenolics possess antioxidant potential.[9]

The histopathological result of our study revealed that treatment with HEPB resulted in maintaining the integrity of gastric mucosa by decreasing the histopathological changes.

CONCLUSION

Oral treatment with hydro-alcoholic extract of *Polygonum bistorta* Linn root protected stomach from indomethacin-induced ulcers in Sprague Dawley rats by prevention of induced muco-oxidative stress. Thus, *Polygonum bistorta* Linn root possesses gastroprotective effect against indomethacin-induced gastric ulcers in rats.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

HEPB: Hydro-Alcoholic Extract of *Polygonum bistorta* Linn root; **CMC:** Carboxymethyl Cellulose; **SOD:** Superoxide Dismutase; **PUD:** Peptic Ulcer Disease, **TBA:** Thiobarbituric Acid, **MDA:** Malondialdehyde, **TCA:** Trichloroacetic Acid, **GAE:** Gallic Acid Equivalent, **NSAIDs:** Nonsteroidal Anti-Inflammatory Drugs, **ROS:** Reactive Oxygen Species.

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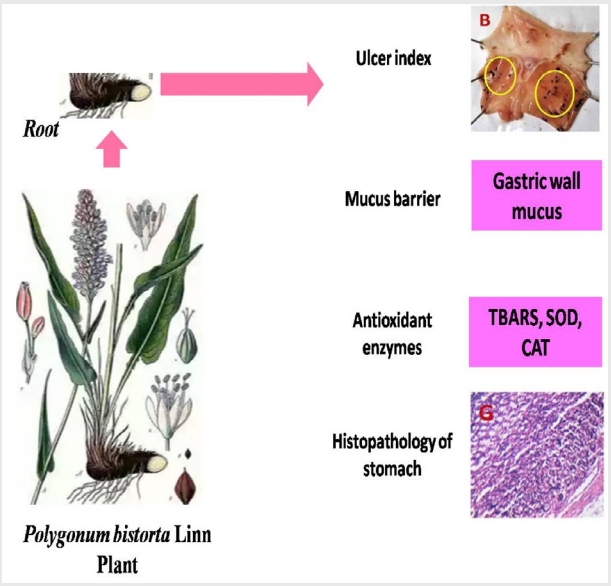
PICTORIAL ABSTRACT



SUMMARY

- *Polygonum bistorta* has been used as hemostatic drug in Unani system of medicine due to its cold and dry temperament. Thus, gastroprotective effect of hydro-alcoholic extract of *Polygonum bistorta* Linn root (HEPB) was investigated in indomethacin-induced gastric ulcer in Sprague Dawley rats.
- HEPB in group-III and group-IV significantly ($p < 0.01$) and dose dependently increased the levels of mucus, SOD and catalase while, decreased ulcer index and thiobarbituric acid reactive substances compared to that of ulcer control group-II. Ulcer protection potency of HEPB 1000 mg/kg/day in group-IV was comparable to that of 20 mg/kg/day ranitidine in group-V. HEPB protected stomach from indomethacin-induced gastric ulcers in rats by prevention of induced muco-oxidative stress.

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