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Anti-Ulcerogenic Effects of *Salmalia Malabarica* in Gastric Ulceration – Pilot Study

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A – research concept and design; **B** – collection and/or assembly of data; **C** – data analysis and interpretation; **D** – writing the article; **E** – critical revision of the article; **F** – final approval of article; **G** – other

Abstract

Background. According to an estimation of the WHO, almost 80% of people globally are treated by traditional medicine.
Objectives. We evaluated the anti-ulcerogenic potential of *Salmalia malabarica* extract in rats using aspirin-, alcohol- and pylorus ligation-induced ulcer models.
Material and Methods. Two different doses (200 and 400 mg/kg body weight) of *Salmalia malabarica* extract was administered intraperitoneally (*i.p.*) to all 3 ulcer-induced models for 5 consecutive days. The anti-ulcerogenic potential in rats treated with 2 doses of *Salmalia malabarica* extract and omeprazole (20 mg/kg, *i.p.*) was determined and compared to the control groups.
Results. *Salmalia malabarica* extract showed a significant decrease in ulcer index as compared to the control group in a dose-dependent manner. *Salmalia malabarica* extract also showed protection of 66.22% and 74.54% in asprin-, 73.79% and 78.14% in alcohol- and 68.94% and 78.84% in pylorus ligation-induced ulcers. However, omeprazole showed protection of 84.73%, 85.5% and 86.12% in aspirin-, alcohol- and pylorus ligation-induced ulcers, respectively. Furthermore, *Salmalia malabarica* extract significantly decreased the volume of gastric juice, free and total acidity, whereas it increased gastric pH when directly compared to the control group.
Conclusions. Conclusively, *Salmalia malabarica* possesses anti-ulcerogenic, antisecretory, and cytoprotective potential and can be used as a supplement for the treatment of gastric ulcers in a dose dependent manner (**Adv Clin Exp Med 2015, 24, 4, 595–605**).

Key words: *Salmalia malabarica*, ulcer index, cytoprotective mechanism, peptic ulcer.

Peptic ulcer disease is a term used for both stomach and duodenal ulcer. In clinical practice, peptic ulcer disease is most commonly known as a gastrointestinal disease which is interlinked with the acute and chronic inflammation of the gastric and duodenal epithelium [1]. According to the pathophysiology, peptic ulcer disease appears as a result of an imbalance between offensive and defensive factors. The major offensive factors that potentiate a peptic ulcer are acid, pepsin, and *Helicobacter pylori*, whereas the defensive factors which antagonize the harmful effects of the offensive factors are mucin, prostaglandin, bicarbonate, nitric oxide and growth factors [2]. Other factors that may influence the potentiation for peptic ulcer are improper digestion, metabolism and elimination of food. Mental and physical stress might also participate in the development of peptic ulcers. Chronic users of non-steroidal anti-inflammatory drugs (NSAID'S) also represent the pathogenesis of peptic ulcer disease. Alcohol consumption is also a major risk factor and contributes in the formation of gastric ulcers [3, 4].

Peptic ulcer is most commonly known as a necrotic injury destroying the whole mucosal depth near acid-producing areas of the gastrointestinal

tract [5], which ruins the balance between the defensive and offensive agents. Therapy for peptic ulcer disease has been broadly classified into 2 major types. The first type of these drugs causes a decrease in acid or pepsin secretion such as H2-blockers, M1-blockers and proton pump inhibitors. The second class of these drugs causes an increase in mucosal defensive factors [6]. Ninety eight percent of all gastrointestinal ulcers are most likely to be found in the duodenum and stomach at an approximate ratio of 4 : 1 [7]. There are remarkable findings that have suggested boiling water, absolute ethanol, 25% sodium chloride, 0.2 N sodium hydroxide and 0.6 N hydrochloric acid are necrotizing agents participating in ulcerogenesis [8].

The traditional systems for the treatment of different diseases *via* medicinal plants have gained strong acceptance. Today, natural products are the foremost symbol of safety for the treatment of various diseases as compared to synthetic drugs [9–16]. Different parts of *Salmalia malabarica* [*Malvaceae* (*Bombacaceae*)] have been proven traditionally and scientifically for the treatment of several ailments. The gum of *Salmalia malabarica* has been traditionally used mainly in the treatment of haemoptysis of pulmonary tuberculosis, influenza and menorrhagia. It is also known for various other properties such as astringent, antiangiogenic, expectorant, analgesic, emetic, stimulant, anti-inflammatory, anti-hypertensive and antioxidant properties [17]. It has also been used in bladder disorders, calculus, leucorrhoea, tuberculosis and to cure conjunctivitis [18]. *Salmalia malabarica* has been proven effective in the treatment of acne and skin eruption problems [19]. Antibacterial and antifungal characteristics are also reported to be found in *Salmalia malabarica*. The methanol extract of *Salmalia malabarica* has shown a potent antibacterial activity [20]. Moreover, hypoglycemic and hypolipidemic effects were also studied in an n-hexane extract of sepal of *Salmalia malabarica* [21]. However, other effects of *Salmalia malabarica* still need to be elucidated.

The purpose of our present study was to investigate the anti-ulcerogenic and ulcer healing potential of a methanol extract of *Salmalia malabarica* using three experimental models i.e. aspirin-, alcohol- and pylorus ligation-induced gastric ulceration using albino Wistar rats.

Material and Methods

Plant Material

The fresh bark of *Salmalia malabarica* was collected in March 2013 from nearby areas of Layyah from the Punjab province of Pakistan. The bark was cut into small pieces and dried. The plants, i.e. bark, was grounded to a fine powder by Wiley mill (standard model 4). The plant was authenticated by the Botany Department, University of the Punjab, Lahore, Pakistan and the voucher specimen no. of *Salmalia malabarica*, LAH-3112013 ML, was preserved.

Chemicals

Omeprazole (OMZ) was purchased from Getz Pharma Pvt. Ltd., Karachi, Pakistan. Aspirin was purchased from Highnoon Laboratories Pvt. Ltd., Lahore, Pakistan. Methanol (95%) was obtained from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals used in this study were analytical grade and used without further modification.

Animals

Healthy adult male albino Wistar rats (130––180 g) were used for the anti-ulcerogenic study. The animals were purchased from the animal house of the University of Agriculture, Faisalabad, Pakistan. The animals were acclimatized with standard environmental conditions of a 12 h light: dark cycle, temperature of 25 ± 2° and relative humidity of 65 ± 10%. They were provided with a standard rodent pellet diet. Access to food and water was provided *ad libitum*. The food was withdrawn 18–24 h and water was withheld 2 h before the start of the experimental procedure.

Ethical Considerations

The experimental animals were used under the guidelines of the Institute of Laboratory Animals Resource, Commission on Life Sciences, National Research Council and approved by the Ethical Committee of Government College University Faisalabad, Faisalabad, Pakistan.

Preparation of Crude Extract of *Salmalia Malabarica*

The powdered bark of *Salmalia malabarica* was extracted by cold maceration in methanol (95%) in airtight containers with continuous shaking. After 7 days of continuous soaking and shaking, the extracts were separated by filtration using Whatman No.1 filter paper. The extracts were made concentrated by rotary vacuum evaporator at 40°C. The contents were air dried to get a solid mass. The weights of the dried extracts were then measured. The final crude extracts of *Salmalia malabarica* obtained were stored in airtight amber-colored bottles at 4°C till further analysis.

Phytochemical Analysis and Determination of LD50 in Rats

Salmalia malabarica were subjected to the preliminary screening of phytochemical compounds using various test as described previously with some modifications [22, 23]. Briefly, alkaloids were detected using the Mayer's test. Tannins were detected using a gelatin test. Flavonoids were detected using an alkaline reagent test. Proteins and amino acids were detected using a xanthoproteic test and ninhydrin test.

We investigated the acute toxicity of *Salmalia malabar*ica extract in order to select the suitable dose for an investigation of the anti-ulcerogenic potential of *Salmalia malabarica* on Wistar rats. We administered a single *i.p.* dose of methanol extract of *Salmalia malabarica* with the dose range of 0.1––20 g/kg. Following the treatment period, the animals were observed for up to 72 h and all the observational changes and death were recorded during the whole observation period. The % of death in each group was calculated and LD50 was determined as described previously [24], with some modifications.

Treatment Schedule

The standard dose of plant extract *Salmalia malabarica* was diluted at a dose of 200 mg/kg (SM-200) and 400 mg/kg body weight of the rats (SM-400). The treatment therapy was continued for 5 consecutive days in all groups of experimental animals. The extracts of *Salmalia malabarica* were administered intraperitoneally according to the weight of the animal. Omeprazole (OMZ) was used as a standard anti-ulcer drug. OMZ was given at a dose of 20 mg/kg PO in 5 mL of normal saline.

Evaluation of Anti-Ulcerogenic Potential of Extract of *Salmalia Malabarica*

Aspirin-Induced Ulcer

Healthy albino Wistar rats were divided into 5 groups (5 × 7). Group I was treated as placebo, group II served as a negative control treated with aspirin, group III was treated with the standard drug OMZ, group IV with the 200 mg/kg body weight of extract of *Salmalia malabarica* (SM-200) and group V with 400 mg/kg body weight of extract of *Salmalia malabarica (*SM-400). The animals were fasted for 24 h and water was withdrawn 2 h prior to the surgical procedures. After 45 min of the administration of OMZ and extracts of SM-200 and SM-400, aspirin was administered at a dose of 20 mg/mL of body weight. The animals were killed by cervical dislocation after 4 h and the stomach was cut along the greater curvature following the procedure as described previously [25]. The ulcers were measured with the help of a microscope.

Alcohol-Induced Ulcer

Healthy albino Wistar rats were divided into 5 groups with 7 mice in each group. Group I was treated as placebo, group II served as a negative control treated with ethanol, group III with the standard drug OMZ, group IV with SM-200 and group V with SM-400. The animals were fasted for 24 h and water was withheld 2 h before the surgical procedures. Absolute ethanol (96%) was given PO at a dose of 1 mg/200 gm/kg body weight of rats after 45 min of the administration of the methanol SM extracts and OMZ treatment [26]. The animals were euthanized by cervical dislocation after 1 h of ethanol administration. The stomach was taken out and incised along the greater curvature and pinned on a soft board. The ulcer was scored microscopically for the measurement of gastric lesions.

Pylorus Ligation-Induced Ulcer

Healthy albino Wistar rats were divided into 4 equal groups each containing 7 animals. Group I was considered as positive control which received normal saline. Group II was treated with the standard dose of OMZ. Group III was treated with 200 mg/kg of extract of *Salmalia malabarica* (SM-200) and group IV was treated 400 mg/kg of extract of *Salmalia malabarica* (SM-400). On the 5th day of treatment, pylorus was ligated after 24 h of starvation. Pyloric ligation was done after 45 min of administration of extract of *Salmalia malabarica* and standard drug OMZ. The animals were anesthetized with light Chloroform. The abdomen was cut and opened by a mid-line incision below the xiphoid process. The stomach was slightly lifted up and the pylorus was ligated with a silk surgical suture with great care to avoid ligation of blood vessels. The stomach was replaced in the abdominal cavity and the wall was sutured with silk surgical threads. The animals recovered from anesthesia after a period of 4 h and were allowed to stay alive. The animals were deprived of food and water after the surgical procedure. At the end of 4 h, the animals were euthanized again by cervical dislocation. The abdominal wall was removed and the stomach was dissected out of the abdominal cavity. The stomach was cut along the greater curvature and gastric juice was collected in presterilized tubes for the analysis of gastric contents. The tubes were centrifuged at 2000 rpm for 15 min. The severity of the ulcer was scored microscopically.

Measurement of Ulcer Index and Percantage of Protection from Ulcer

We examined the opened stomach of each rat in order to calculate the ulcer index (UI) by giving a scoring number [2]. The ulcer score was estimated by viewing the ulcers with a magnifying glass. The UI was calculated as follows where "X" was the ratio of total mucosal area to that with ulcerated area. The percentage of protection was calculated using the following formulaas described previously [2].

Gastric Secretion Study

The centrifuged gastric volume was measured from measuring the cylinder after transferring the supernatant liquid from centrifuge tubes [27]. The supernatant fluid was taken from the centrifuged tubes. 1 mL of gastric juice was taken in a 100 mL conical flask and diluted with 10 mL of distilled water. Two–three drops of Topfer's reagent was poured into the conical flask and was titrated against 0.01 N NaOH until the color of the solution turned to yellowish orange and all traces of red color disappeared. The volume of NaOH was noted to measure the pH of the gastric acid, volume of the gastric secretion and free acidity following the method as described previously [28]. For the determination of total acidity, 2–3 drops of phenolphthalein indicator was poured into the conical flask and again titrated against NaOH, a clear red tinge reappeared. The volume of alkali added was noted and the total volume used determined the total acidity. Total acidity was calculated following the method as described previously [29].

Histopathology of Ulcer Models

The rats were made unconscious by overdose of chloroform. The rats were euthanized by cervical dislocation with the help of a presterilized sharp blade. Tissues from the gastric walls were separated and fixed in 10% buffered formalin overnight and processed in a paraffin tissue processing machine. Sections of the stomach were made at a thickness of 5 μ and stained with haematoxylin and eosin (H & E) for histological evaluation. Histochemical alterations were observed in these tissues and images were taken using Olympus color video camera with Histolab software (Biocom).

Statistical Analysis

The results were expressed as mean ± SEM. The data of all groups was analyzed using a one-way analysis of variance (ANOVA), Student-Neuman-Keuls multiple comparison tests or Student's *t*-test using Graph Pad Prism 5 (Graph Pad, Software Inc., USA). The criteria for statistically significant difference between the treated group and that of the control group were adjusted at $p < 0.05$.

Results

Phytochemical Analysis and Determination of LD50 in Rats

Preliminary phytochemical analysis of *Salmalia malabarica* revealed the presence of a rich amount of phytochemicals such as alkaloids, tannins, flavonoids, proteins and amino acids. The extract of *Salmalia malabarica* was found to be non-toxic when administered *i.p.* to Wistar rats with dose range of 0.1–20 g/kg and the LD50 value was found to be safe at the highest dose of *Salmalia malabarica.*

Anti-Ulcer Effect of *Salmalia Malabarica*

Effects of *Salmalia Malabarica* on Aspirin-Induced Ulcer

In the aspirin-induced ulcer model, the placebo group did not show any kind of ulcer index. The rat group treated with aspirin alone showed an elevated value of the mean ulcer index at its significant level of 6.1 ± 0.872 (Fig. 1). The OMZ-treated group showed the lesser values of the ulcer index (2.7 ± 0.734) when directly compared with that

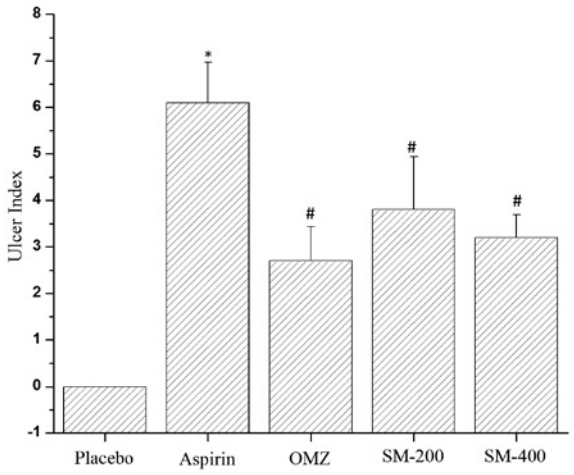

Fig. 1. Aspirin-induced ulcer model. Ulcer index of extract of *Salmalia malabarica* on ulcer index in aspirin-induced model. The graph was plotted against placebo, aspirin, OMZ, and groups treated with 2 doses of *Salmalia malabarica* extract. Placebo group showed 0 ulcer index in aspirin-induced ulcer model. *; significant difference at $p < 0.05$ when compared to placebo control group, #; significant difference at $p < 0.01$ when compared to group treated with aspirin

Table 1. Effect of methanol extract of *Salmalia Malabarica* in 3 experimental induced ulcers (n = 5)

Sr.#	Treatments	Percent protection			
		dose administered	aspirin-induced ulcer (%)	alcohol-induced ulcer (%)	pyloric ligation--induced ulcer (%)
1	Control (placebo)	–	–	–	–
2	Control (normal saline)	1 mL/kg (*i.p.*)	–	–	–
3	Omeprazole	20 mg/kg (*i.p.*)	84.73	85.5	86.12
4	SM-200	200 mg/kg (*i.p.*)	66.22	63.79	66.94
5	SM-400	400 mg/kg (*i.p.*)	74.54	78.14	79.84

OMZ – omeprazole, SM – *Salmalia malabarica*, *i.p.* – intraperitoneal.

of the aspirin-treated group. The other 2 groups, treated with SM-200 and SM-400, showed values of the ulcer index of 3.8 ± 1.140 and 3.2 ± 0.489, respectively, as shown in Fig. 1A. The percentage of protection of the OMZ-treated groups was highest, at 84.73%, whereas the rat groups treated with SM-200 and SM-400 maintained the % protection of 66.22% to 74.54% (Table 1). The mean ulcer index obtained from the rat groups treated with SM-200 and SM-400 also showed a significant difference between the values of the ulcer index of the aspirin-treated group.

Effects of *Salmalia Malabarica* on Alcohol-Induced Ulcer

In alcohol-induced ulcer, the rat group treated with alcohol alone showed the highest ulcer index, of 5.8 ± 0.492, whereas the rat group treated with OMZ showed the least value of ulcer index, at 2 ± 0.694, when directly compared to that of the alcohol-treated rat group (Fig. 2). The OMZ--treated groups also showed the highest percentage of protection against alcohol-induced ulcer (Table 1). The effect of *Salmalia malabarica* extract at the dose of SM-200 showed the least significant effect against ulcer protection (3.7 ± 0.832 vs. 5.8 ± 0.492 when compared to the alcohol-treated group, and 3.7 ± 0.832 vs. 2.6 ± 0.694 when compared to the OMZ-treated group), with a percentage of protection of 78.79%, whereas the *Salmalia malabarica* extract at the dose of SM-400 showed a highly significant effect in gastric protection against ulceration caused by alcohol (2.9 ± 1.092 vs. 5.8 ± 0.492) when directly compared to that of the OMZ-treated and/or SM-200-treated groups, respectively (Fig. 1B). The percentage of protection against ulcer in the SM-400-treated group was significantly higher than that in the SM-200-treated group (Table 1).

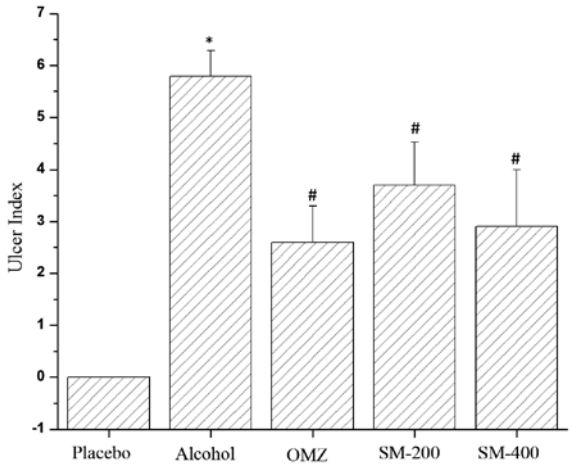

Fig. 2. Alcohol-induced model. Ulcer index of extract of *Salmalia malabarica* on ulcer index in alcohol--induced model. The graph was plotted against placebo, alcohol, OMZ, and groups treated with 2 doses of *Salmalia malabarica* extract. Placebo group showed 0 ulcer index in alcohol-induced ulcer model. *; significant difference at $p < 0.05$ when compared to placebo control group, #; significant difference at $p < 0.01$ when compared to group treated with alcohol

Effects of *Salmalia Malabarica* on Pylorus Ligation-Induced Ulcer

In pyloric ligation-induced ulcer, group I served as a control group that only received normal saline, and showed a significant mean ulcer index with the highest value of 6.2 ± 0.583. Group II was treated with OMZ, which showed the lowest value of mean ulcer index (2.1 ± 0.400 vs. 6.2 ± 0.583) when compared to the mean ulcer index value of the control group (Fig. 1C), with a significant value of %protection at the level of 66.12% (Table 1). Group III and group IV were treated with *Salmalia malabarica* extracts of SM at 2 different doses. The SM-400 showed the lowest value of mean ulcer index when directly compared to that of the control

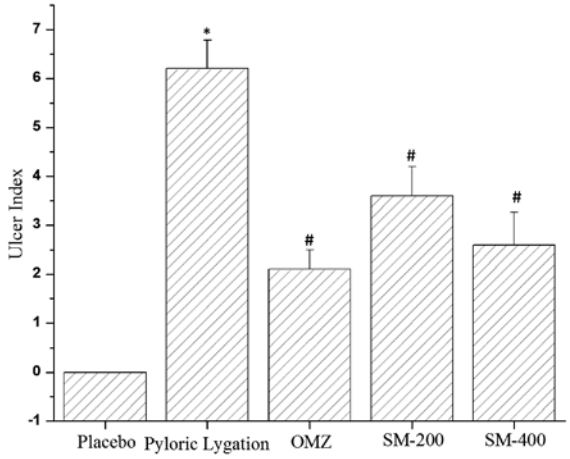

Fig. 3. Pyloric ligation-induced ulcer. Ulcer index of extract of *Salmalia malabarica* on ulcer index in pyloric ligation-induced ulcer. The graph was plotted against placebo, OMZ, and groups treated with 2 doses of *Salmalia malabarica* extract. Placebo group showed 0 ulcer index in pyloric ligation ulcer model. *; significant difference at $p < 0.05$ when compared to control group, #; significant difference at $p < 0.01$ when compared to group induced by pyloric ligation

group (2.6 ± 0.663 vs. 6.2 ± 0.583). However, the group treated with SM-200 extract of *Salmalia malabarica* showed a mean ulcer index value of 3.6 ± 0.600. A non-significant difference was also observed between the mean ulcer values of the rat group treated with SM-400 extract of *Salmalia malabarica* and the OMZ-treated rat group (Fig. 3). We also measured the percentage of protection against ulcer and found that the rat group treated with SM-400 extract of *Salmalia malabarica* showed maximum percentage of protection against ulcer when directly compared to the group treated with SM-200 extract of *Salmalia malabarica* (Table 1).

Effect of *Salmalia Malabarica* on Gastric Secretion

At the end of the treatment, we also measured the effects of *Salmalia malabarica* on the secretion of gastric contents in pyloric ligation-induced ulcer. We measured the volume of gastric juice, the free acidity, total acidity and pH of the gastric secretion in all rat groups of pyloric ligation-induced ulcer. The control group, treated with normal saline, showed a significantly high volume of gastric juice (6.86 ± 0.272) when directly compared to that of the other 3 groups (Fig. 4A). Similarly, the rat group treated with SM-400 extract of *Salmalia malabarica* showed the lowest value of gastric juice when directly compared to that of the control group (2.88 ± 0.287 vs. 6.86 ± 0.272). The OMZ-treated groups also showed a decreased secretion of gastric juice as compared to both the control group (2.16 ± 0.333 vs. 6.86 ± 0.272) and the rat group treated with SM-200 extract of *Salmalia malabarica* (2.16 ± 0.333 vs. 3.52 ± 0.655).

In pyloric ligation-induced ulcer, we measured free acidity in all rat groups. Due to the occurrence of ulcer, the control groups showed a significantly higher value of free acidity when directly compared to the OMZ-treated group and the rat groups treated with extract of *Salmalia malabarica* SM-200 and SM-400 (Fig. 4B). The value of free acidity in the rat group treated with extract of *Salmalia malabarica* SM-400 showed a non-significant difference when directly compared to that of the OMZ-treated group (Fig. 4B). We also measured the total acidity in all rat groups. The control group followed the same pattern for total acidity as it followed in the case of free acidity (Fig. 2C). However, the value of total acidity was significantly very high as compared to the OMZ-treated group and/or rat groups treated with the extracts of *Salmalia malabarica* (Fig. 4C).

We also measured the pH of the gastric contents of the pyloric ligation-induced ulcer model, where the control group revealed the lowest value of pH when directly compared to the OMZ-treated and/or extracts of *Salmalia malabarica* rat groups (Fig. 4D). The extract of *Salmalia malabarica* with a dose of SM-400 increased the value of pH significantly very highly ($p < 0.001$) as compared to the extract of *Salmalia malabarica* with a dose of SM-200.

Histopathology of Ulcer Models

At the end of the treatment period, we also assessed the histopathological alterations in ulcer-induced models treated with extract of *Salmalia malabarica* (Fig. 5). The specimens of the gastric walls from each rat were fixed in 10% buffered formalin overnight and processed in a paraffin tissue processing machine. Sections of the stomach were made at a thickness of 5 μ and stained with haematoxylin and eosin (H & E) for histological evaluation. In the pyloric ligation-induced ulcer model, the rat groups exhibited damaged mucosal epithelium, proliferated fibroblasts, inflammatory exudates and the infiltration of leucocytes, due to which necrosis occurred (Fig. 5A). Extract of *Salmalia malabarica* showed a significant protection against these histopathological changes in pyloric ligation-induced ulcer with apparent epithelializations and regeneration of mucosa (Fig. 5A-D).

Discussion

According to the World Health Organization (WHO), 80% of the world's population relies on traditional medicines for the treatment of different

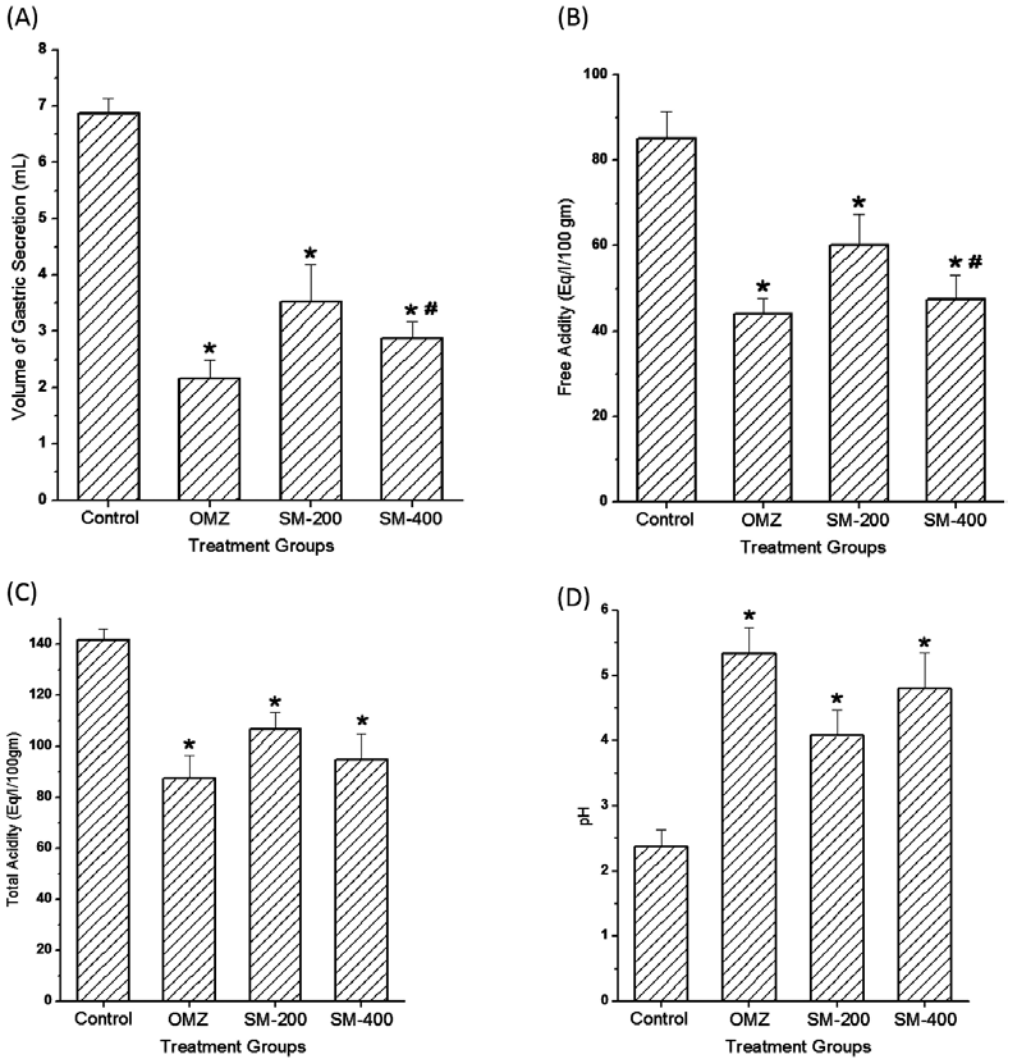

Fig. 4. Measurement of gastric contents in pyloric ligation-induced ulcer model. Effects of methanol extract of *Salmalia malabarica* on volume of gastric secretion (A), on total acidity (B), on free acidity (C) and on pH of gastric contents (D) in pylorus ligation-induced ulcer model. *Significant difference at $p < 0.05$ when compared to control group, # significant difference at $p < 0.05$ when compared to group treated with SM-200

diseases and their primary health care needs [30]. Methanol extract of *Salmalia malabarica* has also shown pronounced characteristics of antibacterial and antifungal activities. The present study elucidated the effect of methanol extract of *Salmalia malabarica* at 2 doses, i.e. SM-200 and SM-400, on ulcer induction by aspirin-, alcohol- and pylorus ligation-induced ulcers.

Preliminary phytochemical analysis of *Salmalia malabarica* showed rich custody of phytochemicals such as tannins, fatty acids, amino acids, flavonoids and alkaloids. Several studies have proved that the phytoconstituents such as saponins, tannins, terpenoids and flavonoids have been reported as gastroprotective agents [31–34]. As *Salmalia malabarica* showed a rich amount of these phytoconstituents, we focused on the anti-ulcerogenic potential using three ulcer-induced experimental models in rats.

Initially, an aspirin-induced ulcer model was used for the study of antiulcer activity. This method is a well-substantiated ulcer model because it has been used by various researchers [5, 31, 35–40]. Aspirin is used for the induction of ulcer. It is an over the counter NSAID used as an analgesic and anti-inflammatory drug. Aspirin mostly damages gastrointestinal mucosa, causing suppression of the biosynthesis of prostaglandins and altering mucosal permeability [41]. NSAIDs are weakly acidic in nature and remain in a unionized lipophilic form in the acidic environment of the stomach and exert topical irritant effects on the linings of GIT. NSAIDs can move across the lipid membranes of epithelial cells and cause cell injury by trapping intracellularly in an ionized form. Aspirin causes an increase in the pH of the stomach and the supply of HCO3 ions is also stopped from the blood stream, this may contribute to the accumulation

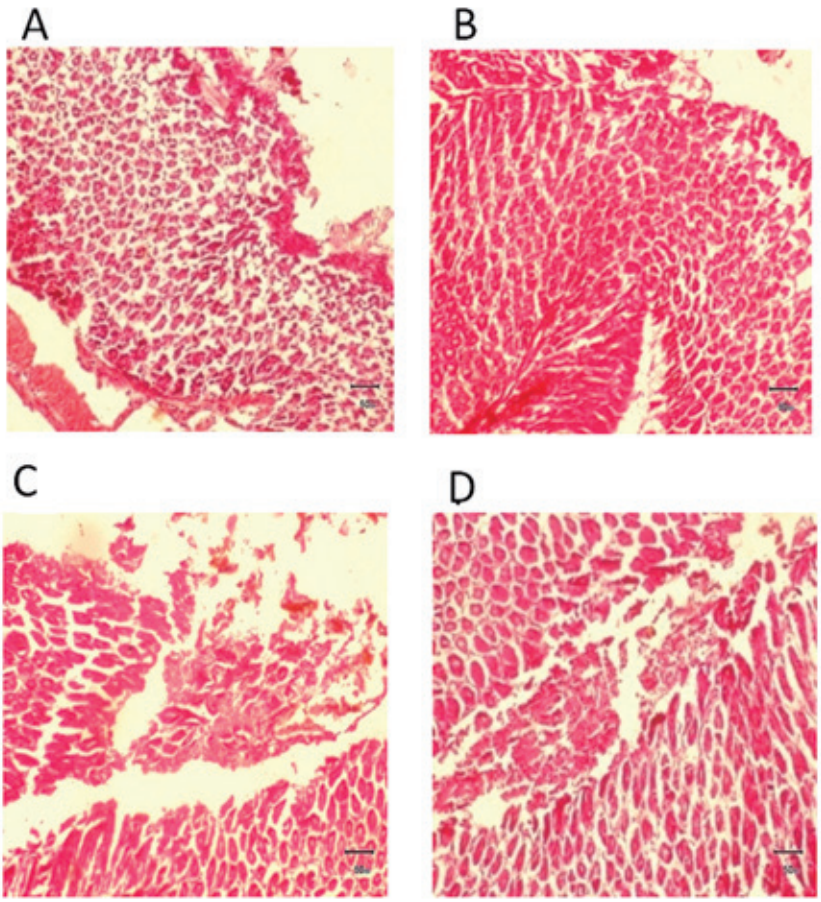

Fig. 5. Histochemical evaluation of mucosal epithelium. Photomicrograph of pyloric ligation-induced rats showing hemorrhagic lesions of gastric mucosa with heavy infiltration of necrotic tissue (A). Rats treated with OMZ showed normal cytoarchitecture of gastric mucosa with no pathological changes (B). There is less evidence of hemorrhagic lesions, less infiltration and edema in the gastric mucosa of rats treated with extract of *Salmalia malabarica* with a dose of SM-200 (C) and SM-400 (D) in pyloric ligation-induced ulcer model

of acid in GIT [20]. Hence NSAIDs correspond to the initial stages of destruction of the mucosal cell membrane, parietal cells and endothelial cells [42]. Prostaglandins play a crucial role in the maintenance of gastroduodenal mucosal integrity and repairing of GIT [43]. NSAIDs also cause disturbance in prostaglandin synthesis and thus can damage mucosal defense. Arachidonic acid originates from membrane phospholipids by the enzyme phospholipase A2. The metabolism of arachidonic acid to prostaglandins is catalyzed by the cyclooxygenase (COX) pathway [44]. COX-1 and COX-2 are 2 isoforms having similarity in their amino acid sequences but have different roles in the pathophysiological cascade of events [45]. Aspirin is a potent COX blocker which also decreases gastroduodenal bicarbonate secretion [46]. Ulcers induced by NSAIDs resulted in the production of excess amounts of acid leading to pyloric obstruction and mucosal necrosis. Although various factors are involved for the induction of ulcerogenesis, the most important is an imbalance between offensive and defensive factors. Methanol extract of *Salmalia malabarica* significantly decreased the ulcer index in aspirin-induced ulcer and increased the percentage of protection up to 74.54%, as shown in Fig. 1 and Table 1. Methanol extract of *Salmalia malabarica* showed its anti-ulcerogenic effects in a dose-dependent manner. A high dose of extract of *Salmalia malabarica* showed significant ulcer healing effects when compared to the low dose of *Salmalia malabarica* extract (Fig. 1). We also found that a high dose of *Salmalia malabarica* extract showed a non-significant difference in ulcer healing effect when compared to the standard dose of OMZ. The methanol extract of *Salmalia malabarica* showed a significant potential of protection against ulceration. This effect may be due to the presence of phytoconstituents, especially tannins, which help prevent the corrosion of the gastric wall from excessive acid and promotes the healing effect. Tannins are powerful astringent compounds that are phenolic in nature and have an ability to bind with the proteins and leads to denaturation of protons [20]. Tannins act by their action on protein precipitation and vasoconstriction, and also form a protective layer that protects the mucosa from ulcerogens [34].

Alcohol causes necrotic damage and enhances the process of lipid peroxidation in response to reactive oxygen species (ROS) generation. These oxygen free radicals lead to gastric injury visible as red streaks of lesions [47]. ROS plays an important role in acute and chronic ulceration, and superoxide anion and hydroperoxy free radicals are produced in the metabolism of alcohol [48]. Alcohol induces

gastric lesions by augmentation of aggressive factors while weakening the mucosal protective factors [49]. Alcohol also acts through the destruction of cells and the epithelium layer by increasing lipid peroxidation linked with the generation of oxygen free radicals [50]. The alcohol-induced ulcer model has long been used to investigate the ulcer healing effects of various medicinal plants [1, 2, 25]. Keeping in mind the destructive role of alcohol in ulcerogenesis, we also used the alcohol-induced ulcer model to investigate the cytoprotective effects of *Salmalia malabarica* extract at 2 dosage levels, SM-200 mg/kg and SM-400 mg/kg. The extract showed significant potential to protect peptic ulcer induced by ethanol. *Salmalia malabarica* extract showed a % protection against ulcer from 63.79% to 78.14% in a dose dependent manner (Table 1). *Salmalia malabarica* extract also significantly decreased the value of the mean ulcer index when directly compared to that of the alcohol-induced ulcer group (Fig. 2). The high dose of *Salmalia malabarica* extract (SM-400) also showed the same effects against ulcer as observed in the OMZ-treated group. The phytoconstituents of *Salmalia malabarica* also contain flavonoids that are efficient free radical scavengers, and also reduce the secretion of histamine from mast cells [51] which, in turn, may protect the gastric mucosa from ulcerogenesis.

Various factors are known to be involved in the generation of ROS, which play an important role in the pathogenesis of ulcerative mucosal lesions. Flavonoids are usually attributed with their antioxidant activity, which might be involved in gastroprotection during ulcerogenesis. In our preliminary phytochemical analysis, we found that *Salmalia malabarica* also contains flavonoids. Ligation-induced ulcer of the pyloric end of the duodenum is an important method for the measurement of mean ulcer index in ulcerogenesis. It causes the accumulation of gastric juice in the stomach and this may result in an increase in total acid output, which is the root cause of ulcer [49]. Pyloric ligation-induced ulcers are suggested to be caused by autodigestion of mucosa by the gastric juice that leads to the loss of integrity of the mucosal barrier by excessive acid-pepsin secretion [35]. The major cause of gastric ulceration in the ligation of the pyloric end model may be the stress-inducing secretion of HCl in excess amounts from the parietal cells, which ultimately leads to gastric ulcer. Extract of *Salmalia malabarica* in the pyloric ligation-induced model reduced the mean ulcer index in a dose dependent manner (Fig. 3) producing a percentage of protection index of 68.94% and 79.84% at doses of 200 mg/kg and 400 mg/kg body weight, respectively. We also noticed that *Salmalia malabarica* extract showed a significant reduction of ulcer index in pyloric ligation-induced ulcer as compared to aspirin- and/or alcohol-induced ulcer models (Fig. 1) whereas, a non-significant difference was observed in the % protection of extract of *Salmalia malabarica* in all 3 ulcer-induced rat models (Table 1).

We also measured the effects of *Salmalia malabarica* extract on gastric contents in a pyloric ligation-induced ulcer model. In this model, *Salmalia malabarica* extract showed a significant reduction in the volume of gastric juice, free acidity, total acidity and pH when directly compared to the control rat group treated with normal saline (Fig. 4). The results in this model clearly suggest the cytoprotective ability of *Salmalia malabarica* showing its effects on the offensive and defensive factors. The enhanced synthesis of cytoprotective prostaglandins might protect the epithelial cells from the ulcerogens and increase the healing ability of mucosa. Thereby, we also investigated the histopathological alterations in the pyloric ligation-induced ulcer model. The gastric mucosa of rats in the pyloric ligation-induced ulcer model revealed that pyloric ligation resulted in hemorrhagic necrosis of the gastric mucosa in rats. However, pretreatment of these rats with extract of *Salmalia malabarica* reduced the pyloric ligation-induced haemorrhagic necrosis of the rat stomach which was similar to that of the OMZ-treated rats. Our investigations are in validation with the anti-ulcerogenic activity of the extract of *Salmalia malabarica* observed under the studies on pharmacological evaluation. Further work is also required to elucidate the actual mechanism involved in the anti-ulcerogenic activity of *Salmalia malabarica.*

In our present study, the dose was selected keeping in mind the method of allometric dose translation which is based upon normalization of body surface area [52].

Here in our present study, we have reported for the first time the anti-ulcerogenic activity of *Salmalia malabarica.* On the basis of our findings from the present investigation, we conclude that the extract of *Salmalia malabarica* has the potential to cure ulcerogenesis and gastric mucosal lesions. Extract of *Salmalia malabarica* also has a potential to decrease the gastric contents such as gastric juice, and free and total acidity by increasing the pH of the gastric contents. Our findings have revealed that *Salmalia malabarica* possesses anti-ulcerogenic potential and can be used as an adjuvant for the treatment of gastric ulcers. Further investigations are still required to elucidate the actual mechanism of the anti-ulcerogenic effect of *Salmalia malabarica* and identify its active constituents that are responsible for anti-ulcerogenic potentials.

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