Histopathological evaluation of gastro protective effect of *Berberis vulgaris* (Zereshk) seeds against aspirin induced ulcer in albino mice

Wafa Majeed, Bilal Aslam*, Ijaz Javed, Tanweer Khaliq, Faqir Muhammad, Asghar Ali and Ahmed Raza

Institute of Pharmacy, Physiology and Pharmacology, University of Agriculture, Faisalabad, Pakistan

Abstract: The present study was carried out to investigate the antiulcer activity of *Berberis vulgaris* (Zereshk) seeds in albino mice. After acclimatization, animals were divided into six equal groups. Aspirin 150mg/kg was used to induce gastric ulcer in all groups except normal control. Omeprazole 20mg/kg was used as synthetic anti ulcer drug in study. Three dose levels of *B. vulgaris* seed powder 300mg/kg, 600mg/kg and 900 mg/kg were used respectively orally. Histopathological analysis was carried out to evaluate the gastroprotective activity of *B. vulgaris* seed powder. Results of the study showed that in case of aspirin treated mice gastric luminal mucosa villi were decreased in height or were absent. In the glandular region there was connective tissue proliferation and also infiltration of cells. Similar infiltration of cells was present on muscularis mucosa. In esophageal region tumor cells were present. However three dose levels of *B. vulgaris* significantly reduced thetissue proliferation, infiltration of cells and sloughing induced by aspirin. Highest dose of *B. vulgaris* (900mg/kg) showed similar results as synthetic antiulcer drug omeprazole.

Keywords: *Berberis vulgaris*, gastric ulcer, omeprazole, histopathology, gastric luminal mucosa.

INTRODUCTION

Gastric ulcer refers to the sores or destruction in the mucosal lining of stomach, which is mainly caused by imbalance between aggressive and defensive factors (Ramaswamy *et al.,* 2010). These defensive factors include mucus-bicarbonate ions, phospholipids, prostaglandins (PGs), blood flow towards gastric mucosa, cell renewal and antioxidants (Ahmed *et al.,* 2012). Gastric ulcer can be prevented by reducing the gastric acid production and by enhancing the gastric mucosal protection (Hoogerwerf *et al.,* 2001). It is observed that enhanced stimulation of cholinergic neurotransmitters may potentiate the acid secretions from parietal cells, which cause severe gastric damage. A study has demonstrated that ulcer incidence is different in eastern and western countries. Gastric ulcer is more common in eastern countries especially in Asia and prevalence of duodenal ulcer is more in western countries (Sandler, 2002).

Aspirin damages the gastric mucosa by inhibiting the synthesis of prostaglandin's. Two types of cycloxygenase enzyme (COX 1 and COX 2) are present in gastric mucosal membranes, which are responsible for prostaglandins synthesis. Aspirin non-selectively blocks both COX 1 and 2, which results in reduction of PG's synthesis. Aspirin causes the dose dependent reduction of prostaglandins especially of PG E2 and PGI2, which is responsible for gastric erosions and gastric mucosal damage (Sathish *et al.,* 2011). As level of PG's decreases it may leads to poor blood flow towards gastric mucosa. However Secretion of bicarbonate ion, mucosal blood flow and gastric mucus is also decreased along with significant increase in acid and pepsin secretions as a result of reduced levels of prostaglandins by use of NSAIDs (Jaikumar *et al.,* 2010).

Another major cause of ulcer is the formation of free radicals, which are responsible for creating the oxidative stress in body tissues and cells. Lipid peroxidation mechanisms are involved in the formation of free radicals. Studies have demonstrated that there is a significant relationship between formation of free radicals and occurrence of gastric ulcer (Baigent*et al.,* 2009;Patrono*et al.,* 2004). Drugs which are used to treat gastric ulcer include histamine receptor blockers (ranitidine, cimitidine) and proton pump inhibitors (omeprazole, lansoprazole). Now days a tripple therapy regimen is used to treat severe type of gastric erosions which includes two antibiotic and a proton pump inhibitor for a period of 7-14 days (Holtmann and Howden, 2004; Gisbert and Pajares, 2005). Hence, these synthetic antiulcer drugs poses severe side effects, so now focus is diverted towards the use of medicinal plants for the treatment of gastric and peptic ulcer. Different plants have been explored with potent gastroprotective activity due to their antioxidant potentials.

Berberis vulgaris is a medicinal plant belongs to family *Berberidaceae*. It is commonly known as Zereshk or Kashmal. *B. vulgaris* contains isoquinoline alkaloids which have potential to suppress immune system and also have anti-inflammatory activity. Berberine is used to treat the gastrointestinal disorders and diarrhea. It reduces inflammation, arrhythmias and ion secretions (Mohsen *et*

**Corresponding author:* e-mail: cba933@gmail.com

al., 2011). Antimicrobial, blood pressure lowering, Antiurolithic, antidiabetic, antimalerial and free radical scavenging activities of *B. vulgaris* also have been reported (Khosrokhavar *et al.,* 2010). In order to analyze its therapeutic efficacy gastroprotective activity of *B. vulgar is* has been evaluated in current study. The main objective of the current study was to evaluate the antiulcer and antioxdant potentials of *B. vulgar is* at different dose levels.

MATERIALS AND METHODS

Animals

36 male adult albino mice weighing 30-50g were used in the study. Animals were taken from NIH (National Institute of Health) Islamabad Pakistan. Animals were maintained in well-ventilated room temperature (22+2˚C) and 12/12 period of light and dark at experimental animal room, Department of Physiology and Pharmacology, University of Agriculture, Faisalabad, Pakistan. After acclimatization of one week, experiment was conducted with prior approval by the Directorate of Research and Advanced studies and with the consent of the society of Ethics of Animals, University of Agriculture, Faisalabad, Pakistan. The mice were slaughtered according to the rules laid down by society of Ethics of Animals, University of Agriculture, Faisalabad, Pakistan.

Feed and drugs

The mice were provided normal routine feed till the completion of experiment twice a day and water was available for 24 hours. Aspirin 150mg/kg was used to induce gastric ulcer in all groups except normal control for a period of 7 days (Brzozwski *et al.,* 2000). Omeprazole was used as synthetic anti ulcer drug in study and was administered orally at a dose rate of 20mg/kg (Herbert *et al.,* 2011).

Plant material

Seeds of *B. vulgaris* (zereshk) were collected and identified for the authentication by Department of Botany, University of Agriculture, Faisalabad. The shade-dried seeds were powdered by the use of mechanical grinder, passed through mesh sieve and were stored in airtight container for further experimental use. 5ml distilled water was used to dilute the three different levels of *B. vulgar is* seed powder.

Feeding of drugs

The drugs were administered orally with the help of intragastric tube.

Experimental design

Histopathological evaluation of gastric tissues

Specimens of the gastric walls from each mice were fixed in formal in and processed further through graded ethanol concentration and were embedded in paraffin blocks. Sections of the stomach were made at a thickness of 5 micrometer and stained with hematoxylin and eosin. Then placed a drop of DPX on the stained slide and cover it by putting cover slip on it (Bancroft and Gamble, 2002). After staining with haemotoxylin and eosin stain (Culling, 1974), the stomach sections were examined under a research microscope.

RESULTS

In the current study gastro protective activity of seed powder of *B. vulgar is* was evaluated in male adult albino mice. Thirty-six male adult albino mice of same age and gender were divided into six groups each comprising of six animals. Group 1 treated as normal control, group 2 was on ulcer inducing drug aspirin at dose rate of 150 mg/kg, group 3 was on gastro protective drug omeprazole at dose rate of 20 mg/kg, group 4, 5 and 6 were on three different doses of *B. vulgaris* seed powder 300, 600 and 900 mg/kg respectively. After a period of 7 days animals were fasted for 24 hours and then slaughtered on the 8th day. Stomach of animals were cut open along greater curvature and contents were separated. Gastric tissues were use for histopathological analysis. Results of study revealed that in case of normal control group where animals were not treated with any drug gastric epithelium was intact and there was no sloughing of epithelial cells (fig. 1). However in case of ulcerogenic group where animals were treated only with aspirin there was a reduced height of villi in the luminal mucosa and in some animals even villi were absent. In the glandular region there was connective tissue proliferation and also infiltration of cells. Similar infiltration of cells was present on muscularis mucosa. In esophageal region tumor cells were present. Hence, epithelial cells were also disrupted due to infilteration of leukocytes (figs. 2 & 3).

Histopathological evaluation in synthetic antiulcer drug (Omeprazole) treated group showed that there were no pathological changes in epithelial cells of animals and gastric epithelium was intact (fig. 4). Three different levels of *B. vulgaris* were used in study to evaluate the gastro protective activity of *B. vulgar is* at different levels. In animals treated with lowest dose level (300mg/kg) of seed powder of *B. vulgar is* mild sloughing of epithelial cells was observed in some animals while epithelial cells of some animals in the same group were normal (fig. 5). Results also showed that in animals treated with *B. vulgar is* at a dose level of 600mg/kg there was no sloughing, hemorrhage and disruption of cells of gastric mucosa (fig. 6). However from the results it was obvious that in animals treated with highest dose of *B. vulgar is* (900mg/kg) there were no pathological changes, edema, necrosis and sloughing, hence the epithelium of gastric mucosa was intact (fig. 7).

Results of the study revealed that *B. vulgaris* in the tested dosages showed no signs and symptoms of toxicity and

the results of the study also have pointed out that the test plant showed gastro protective activity similar as synthetic antiulcer drug omeprazole which is mostly widely used as first line therapy in the treatment of gastric ulcer.

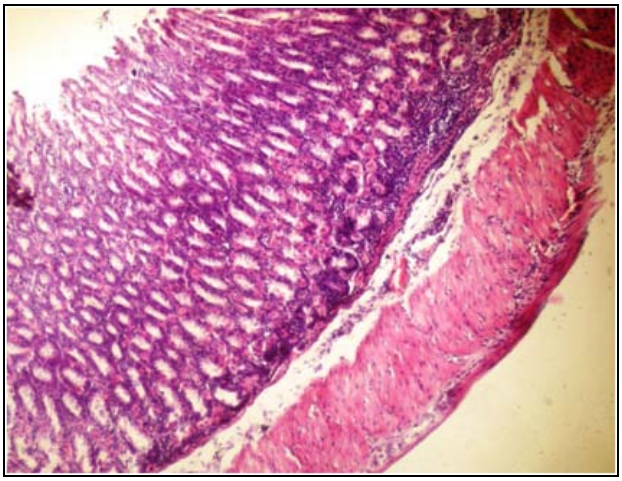

Fig. 1: Photomicrograph of stomach of mice showing normal epithelium (H&E staining 200X)

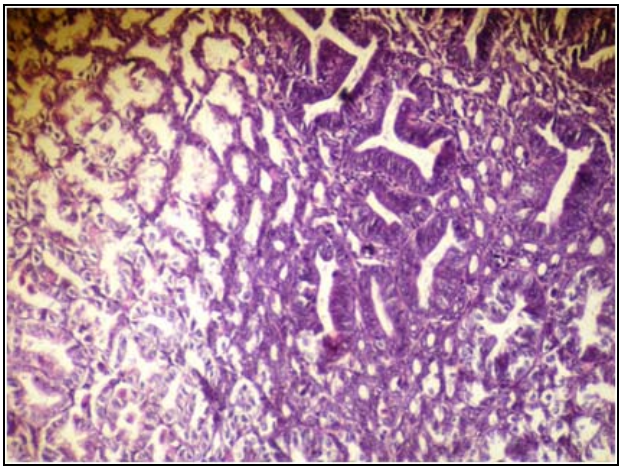

Fig. 2: Photomicrograph of stomach of mice showing connective tissue proliferation in the glandular region (H&E staining 200X).

DISCUSSION

Gastric ulcer is an inflammatory disease, which is caused by the homeostatic imbalance between defensive and aggressive factors. Now a days NSAIDS are major cause of gastric ulcer due to their inhibitory effects on both cycloxygenase 1 and 2 enzymes, which are responsible for the synthesis of prostaglandins. Prostaglandins act as a defensive factor for stomach mucosa by protecting it from excessive effects of acid secreted form parietal cells of stomach (Burke *et al.,* 2006). *Helicobacter pylorus* is a bacterium, which is also responsible for gastric ulcer. *H. pylorus* causes enhanced release of inflammatory mediators and increases the cell mass of parietal cells resulting in an increased secretion of gastric acids from parietal cells of gastric mucosa (Suleiman *et al.,* 2007). Proton pump inhibitors (omeprazole and lansoprazole) are most commonly used to treat gastric ulcer (Sachs *et al.,* 2006). Omeprazole was used as synthetic antiulcer drug in the above study. It reduced the acid secretions by blocking the hydrogen potassium ATPase pump (Abdulla *et al.,* 2010; Rajeshkumar *et al.,* 2002).

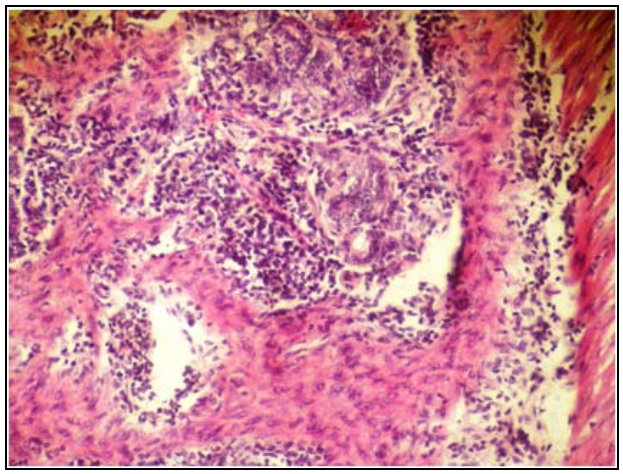

Fig. 3: Photomicrograph of stomach of mice showing cellular infiltration (H&E staining 200X).

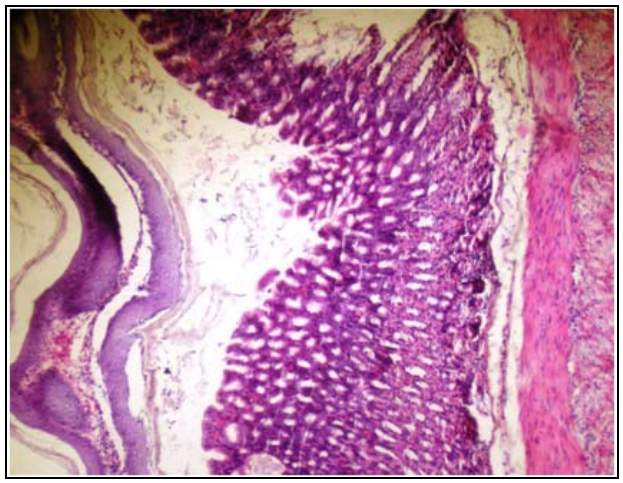

Fig. 4: Photomicrograph of stomach of mice of omeprazole showing normal epithelium (H&E staining 200X).

Antiulcer drugs pose severe side effects on withdrawal e.g. relapse, tolerance, nausea and vomiting have been reported in different studies (Choudhary *et al.,* 2001). In connection with severe side effects of synthetic antiulcer drugs focus is now diverted towards the use of medicinal plants as antiulcer agents. Earlier studies have demonstrated that the medicinal plants have gastro protective activity due to the presence of antioxidants, however it is also suggested that antiulcer activity of medicinal plants may be endorsed to their essential components such as flavonoids, phenols, terpenes and

fatty acid (Fiore *et al.,* 2005; Hasanein, 2011). In the present study antiulcer activity of seed powder of *B. vulgaris* was evaluated against aspirin induced gastric toxicity in male adult albino mice by performing histopathological studies. However our study has also reinforced the idea that oxidative stress is enhanced in gastric ulcer.

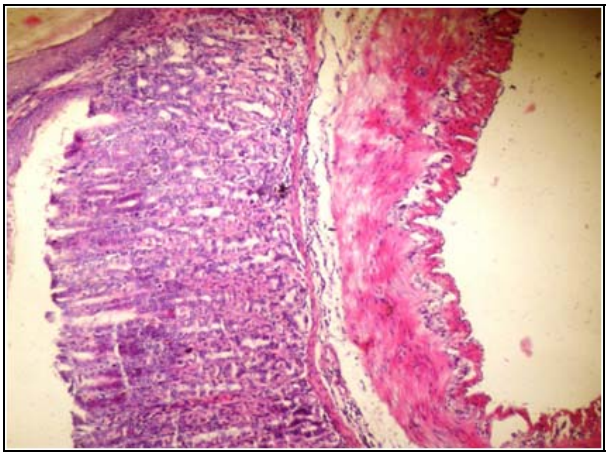

Fig. 5: Photomicrograph of stomach of mice of *B. vulgaris* (300mg/kg) showing mild epithelium sloughing (H&E staining 200X).

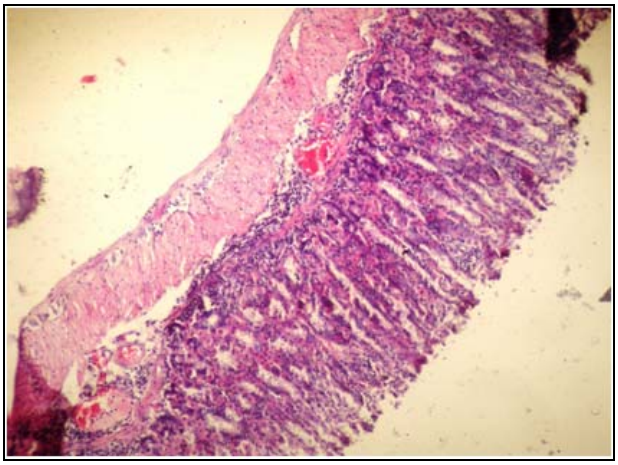

Fig. 6: Photomicrograph of stomach of mice of *B. vulgaris* (600mg/kg) showing intact epithelium (H&E staining 200X)

Earlier studies on aspirin induced gastric ulcer also showed that aspirin causes the enhanced formation of nitric oxide, which leads to gastric epithelial damage. Nitric oxide also acts as defensive factor but in presence of aspirin the amount of nitric oxide (NO) increases which can cause gastric damage (Kontureck *et al.,* 2006). Aspirin causes the inflammation of gastric mucosa, which leads to leukocyte infiltration by the activation of interleukin pathway, which causes excessive entry of leukocytes inside the gastric epithelial linings. This enhanced level of leukocytes inside the gastric mucosa is responsible for inflammation of gastric linings (Jainu *et al*., 2006; Odashima *et al.,* 2006). Leukocytes enhance the lipid per oxidation and generate the reactive oxygen species, which damage the gastric mucosa. Some studies have demonstrated that aspirin causes aggravation of hypoxic conditions in epithelial lining of gastric mucosa, which is responsible for enhanced formation of reactive oxygen species, which ultimately leads to severe gastric damage (Saravanan, 2011). *B. vulgaris* reduced the inflammation by blocking the interleukin pathway and decreasing the formation of interleukins (Zhou and Mineshita, 2000; Kawashima *et al.,* 2004; Cannon *et al.,* 2006). Studies also have been suggested that isoquinoline alkaloid berberine present in *B. vulgaris* reduces the leukocyte infiltration (Mohsen *et al.,* 2011).

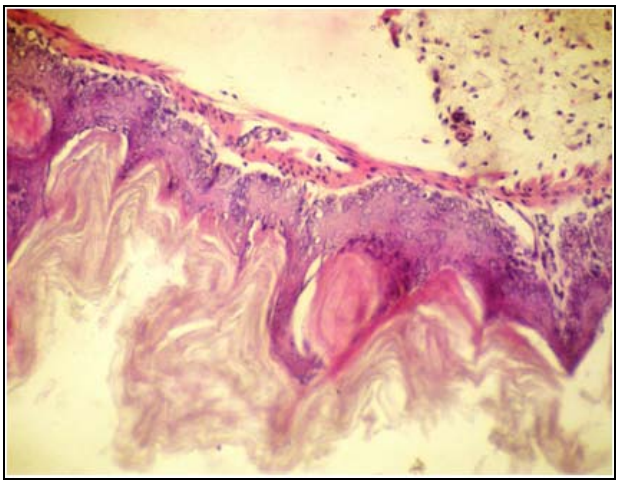

Fig. 7: Photomicrograph of stomach of mice of *B. vulgaris* (900mg/kg) showing intact epithelium (H&E staining 200X).

In current study histopathological studies were carried out to evaluate the gastroprotective efficacy of *B. vulgaris* seeds. Results suggested that aspirin severely damaged the gastric mucosa by disrupting the gastric epithelial linings. It reduced the height of villi inside the luminal mucosa. In the glandular region there was connective tissue proliferation and also infiltration of cells was observed. However similar infiltration of cells was present on muscularis mucosa. In esophageal region tumor cells were also present. Administration of synthetic gastroprotective drug omeprazole significantly reduced the gastric damage and cellular infiltration. However administration of test plant (*B. vulgaris*) along with aspirin significantly reduced the gastric sloughing, infiltration, edema, hemorrhage and tissue proliferation at three different doses 300, 600 and 900mg/kg respectively. Hence *B. vulgaris* at 900mg/kg showed more effective results as synthetic antiulcer drug omeprazole. The above-mentioned results are in accordance with previous studies (Ahmed *et al.,* 2012).

Proposed mechanism for antiulcer activity of *B. vulgaris* is may be the presence of phenolic contents, which

Table 1: Feeding and drug administration schedule in mice during the experimental period of 0-7 days

Group1: Normal control on normal routine feed.	Routine diet 0-7 days.
Group 2: Treated with ulcer inducing drug Tablet Disprin® (aspirin) 150 mg/kg orally.	Routine diet + Aspirin 0-7 days.
Group 3: Treated with synthetic antiulcer drug Cap Omega® (omeprazole) 20 mg/kg orally.	Routine diet + Cap Omega® (omeprazole) + Aspirin 0-7 days.
Group 4: Treated with *B. vulgaris* seed powder 300 mg/kg orally.	Routine diet + *B. vulgaris* seed powder in 5 ml distilled water + Aspirin 0-7 days.
Group 5: Treated with *B. vulgaris* seed powder 600 mg/kg orally.	Routine diet + *B. vulgaris* seed powder in 5 ml distilled water + Aspirin 0-7days.
Group 6: Treated with *B. vulgaris* seed powder 900 mg/kg orally.	Routine diet + *B. vulgaris* seed powder in 5 ml distilled water + Aspirin 0-7 days.

possesses potent antioxidant and antihistaminergic activity (Mohsen *et al.,* 2011; Zovko *et al.,* 2010). As histamine is an inflammatory mediator which causes dilation of blood vessels. It also acts on T-cells and releases Interleukins. *B. vulgaris* shows anti-inflammatory activity by blocking the interleukin pathway (Cannon *et al.,* 2006). A possible reason for gastro protective activity of *B. vulgaris* is the presence of antioxidants specially vitamin C, flavonoids, isoquinoline alkaloids and other phenolic constituents present in the seeds of *B. vulgaris* (Mohsen *et al.,* 2011). Antibacterial activity of *B. vulgaris* has been evaluated against *helicobactor pylori* (Singh *et al.,* 2010). Hence anticholinergic potential of *B. vulgaris* may also attribute to its antiulcer activity. However no study has yet been conducted about the mechanisms involved in antiulcer activity of *B. vulgaris*. On the other hand, comprehensive therapeutic and chemical investigations are still obligatory for further understanding of mechanisms involved in gastro protective activity of *B. vulgaris*.

CONCLUSION

Gastro protective activity of *B. vulgar is* seed powder was tested in aspirin induced gastro toxicity in male adult albino mice. It was concluded that *B. vulgaris* significantly reduced the gastric sloughing and infiltration induced by aspirin. Therefore, it is documented that seed powder of *B. vulgaris* is efficacious in reducing the gastric toxicity mainly due to its antioxidant potential.

REFERENCES

Abdulla MA, AL-Bayaty FH, Younis LT and Hassan MI (2010). Anti-ulcer activity of Centellaasiatica leaf extract against ethanol-induced gastric mucosal injury in rats. *J. Med. Plan. Res.,* **4**: 1253-1259.

Ahmed S, Al Rashdi SMS and Asykin N (2012). Mechanisms of gastroprotective effects of ethanolic leaf extract of *jasminumsambac* against HCl/ethanol-induced gastric mucosal injury in rats. *J. Evid. Bas. Compl. Alte. Med.,* **5**: 1-24.

Ahmed SA, Salama MS, Alkiyumi S, Abdulla MA, Hadi AH, wahab SI, Taha M and Asykin N (2012). Mechanisms of gastro protective effects of ethanolic leaf extract of Jasminumsambac against HCl/ethanol-induced gastric mucosal injury in rats. *J. Evid. Bas. Compl. Alte. Med.,* **2**: 1-15.

Baigent C, Blackwell L and Collins R (2009). Aspirin in the primary and secondary prevention of vascular disease: Collaborative meta analysis of individual participant data from randomized trials. *Lanc.,* **373**: 1849-1860.

Bancroft JD and Gamble M (2002). Theory and practice of histological techniques. 5th edition, Livingstone Publications, Churchill, Edinburgh, pp.172-175.

Brzozowski T, Konturek SJ and Kwiecien S (2000). Role of gastric acid secretion in progression of acute gastric erosion induced by ischemia reperfusion into gastric ulcer. *Eur. J. Pharmac.*, **398**: 147-158.

Burke A, Smyth E and Fitzgerald GA (2006). Analgesic-antipyretic agents. *In*: Goodman and Gilman, Pharmacological Bases of Therapeutics, 11th ed., McGraw Co., New York, pp.671-715.

Cannon JG (2006). Cytokines and Eicosanoids. *In*: Modern Nutrition in Health and Disease, 10th edition, Philadelphia Lippincott Williams and Wilkins, pp.655-669.

Choudhary AM, Roberts I, Nagar A, Tabrez S and Gupta T (2001). Helicobacter pylori related gastric outlet obstruction. *J. Clin. Gastroent.,* **32**: 272-273.

Culling CF (1994). Hand Book of Histopathological and Histochemical Techniques. 3rd edition, Butterworth, London. pp.671-681.

Fiore C, Eisenhut M, Ragazzi E, Zanchin G and Armanini D (2005). Ahistory of the therapeutic use of licorice in Europe. *J.Ethnopharmac.,* **99**: 317-324.

Gisbert JP and Pajares JM (2005). Systematic review and meta-analysis: Is 1-week proton pump inhibitor-based triple therapy sufficient to heal peptic ulcer. *Aliment. Pharmacol. Ther.,* **21**: 795-804.

Hasanein P (2011). Glabridin as a major active isoflavan from Glycyrrhizaglabra (licorice) reverse learning and memory deficits in diabetic rats. *Act. Phys. Hung.,* **98**: 221-230.

Herbert M, Jackson C, Ekpo M, Okopedi E and Anah V (2011). Gastroprotective effects of ethanolic leaf extract of Musa paradisiaca in rats. *J. Chem. Pharmac. Res.*, **3**: 322-327.

Holtmann G and Howden CW (2004). Management of peptic ulcer bleeding-the role of proton pump inhibitors and *Helicobacter pylori* eradication. A review. *Suppl.*, **1**: 66-70.

Hoogerwerf WA and Pasricha PJ (2001). Agents used for control of gastric acidity and treatment of peptic ulcers and gastro esophageal reflux disease. *In*: Goodman and Gilman, The Pharmacological Basis of Therapeutics, 10th edition, McGraw-Hill, New York. Pp.1005-1019.

Jaikumar S, Ramaswamy S, Asokan BR, Mohan T and Gnanavel M (2010). Anti ulcer activity of methanolic extract of Jatrophacurcas (Linn.) on aspirin-induced gastric lesions in wistar strain rats. *Res. j. pharm. Biol. Chem. Sci.*, **1**: 886-897.

Jainu M, KV Mohan and CSS Devi (2006). Gastrorotective effect of Cissus quadrangularis extract in rats with experimentally induced ulcer. *Ind. J. Med. Res.*, **123**: 799-806.

Kawashima K, Nomura A, Makino T, Saito K and Kano Y (2004). Pharmacological properties of traditional medicine: Effect of hang-shashin and the combinations of its herbal constituents on rat experimental colitis. *Bio. Pharmace. Bull.*, **27**: 1599-1603.

Khosrokhavar R, Ahmadiani A and Shamsa F (2010). Antihistaminic and anticholinergic activity of methanolic extract of barberry fruit (*Berberis vulgaris*) in the guinea-pig ileum. *J. Med. Plan.*, **9**: 99-105.

Konturek PC, Kania J, Hahn EG and Konturek JW (2006). Ascorbic acid attenuates aspirin-induced gastric damage: role of inducible nitric oxide synthase. *J. Phy. Pharm.*, **57**: 125-136.

Mohsen M, Ghannadi A, Mahzouni P and Shirazi EJ (2011). Comparative study of *Berberis vulgaris* fruit extract and berberine chloride effects on acetic acid induced colitis in rats. *Iranian J. Pharmace. Res.*, **10**: 97-104.

Odashima M, Otaka M, Jin M, Komatsu K, WadaI and Horikawa Y (2006). Attenuation of gastric mucosal inflammation induced by aspirin through activation of adenosine receptor in rats. *W. J. Gastroent.*, **12**: 568-573.

Patrono C, Bachmann F and Baigant C (2004). Expert consensus document on the use of antiplatelet agents. The task force on the use of antiplatelet agents in patients with atherosclerotic cardiovascular disease of the Euroupean society of cardiology. *Eur. Heart J.*, **25**: 166-181.

Rajeshkumar NV, KL Joy, G Kuttan, RS Ramsewak, MG Nair and RKuttan (2002). Anticarcinogenic activity of Phyllanthusamarus extract. *J. Ethnopharmaco.*, **81**: 17-22.

Ramaswamy S, Sengottuvelu S, Sherief H, Jaikumar S, Saravanan R, Prasadkumar C and Sivakumar T (2010). Gastroprotective activity of ethanolic extract of trachyspermumammi fruit. *Inter. J. Pharma. Bio. Sci.*, **1**: 1.

Raskin JB (1999). Gastrointestinal effects of NSAID therapy. *Am. J. Med.*, **106**: 3-12.

Sachs G, Shin JM and Howden CW (2006). The clinical pharmacology of proton pump inhibitors. *Alim. Pharmaco. Therap.*, **23**: 2-8.

Sandler RS (2002). The burden of selected digestive diseases in the United States. *J. Gastroent.*, **122**: 1500-1511.

Saravanan J, Venkatesh P, Soumya V, Hariprasath K, Prasad RH and Kumar KTM (2011). Antiulcer and antioxidant activity of methanolic leaf extract of Soymidafebrifuga. *J. Phar. Res.*, **5**: 528-531.

Sathish R, S Alokand and K Natarajan (2011). Antiulcer and antioxidant activity of ethanolic extract of *Passiflorafoetida* L. *Ind. J. Pharmaco.*, **43**: 336-339.

Singh A, Duggal S, Kaur N and Singh J (2010). Berberine: Alkaloid with wide spectrum of pharmacological activities. *J. Nat. Pro.*, **3**: 64-75.

Suleiman MM, Romanus IO and Yusuf S (2007). Gastroprotective effect of the crude methanol extract of Terminaliaavicennioides in rats. *Vet. Arch.*, **77**: 345-354.

Zhou H and Mineshita S (2000). The effect of berberine chloride on experimental colitis in rats *in vivo* and *in vitro*. *J. Pharm. Exp. Therap.*, **294**: 822-829.

Zovko M, KremerD, Karlovic K and Kosalec I (2010). Evaluation of antioxidant activities and phenolic content of *Berberis vulgaris* and *Berberis croaticahorvat. Food Chem. Toxi.*, **48**: 2176-2180.