J Acupunct Meridian Stud 2012;5(2):80—86

Available online at www.sciencedirect.com

Journal of Acupuncture and Meridian Studies

journal homepage: www.jams-kpi.com

RESEARCH ARTICLE

Antiulcer Potential of the Ethanolic Extract of *Aerva Persica* Merrill Root in Rats

Neeru Vasudeva*, Payal Sethi, Surendra Kr. Sharma, Suresh Kumar, Sunil Sharma

Department of Pharmaceutical Sciences, Guru Jambheshwar University of Science and Technology, Hisar, India

Available online Feb 10, 2012

Received: May 10, 2011
Accepted: Sep 9, 2011

KEYWORDS
Aerva persica;
Amaranthaceae;
antiulcer;
antioxidants;
hemorrhagic necrosis

Abstract
The ethanol extract of the roots of *Aerva persica* (Burm f) Merrill (Amaranthaceae) was investigated to determine its antiulcer and *in vivo* antioxidant activities in albino Wistar rats. Ulcers were induced by ethanol and pylorus ligation. The extract was administered at the dose of 200 mg/kg orally, p.o. for 15 consecutive days. The ulcer index of the ethanol extract was found to be significantly reduced compared with control animals. The effect was also assessed by determining the free acidity, pepsin activity, total carbohydrate (TC), and protein content (PK) in control, standard, and test group animals. The *in vivo* antioxidant activity was evaluated by determining the reduced glutathione level (GSH) and malondialdehyde (MDA) level in the tissue homogenates. The results reveal the significant reduction in the level of malondialdehyde and the increase in the level of reduced glutathione in the rats that received the ethanolic extract. Furthermore, histopathological studies have shown that pretreatment with the ethanolic extract of the roots of *A persica* reduces (100%) ethanol- and pylorus ligation-induced hemorrhagic necrosis in rats.

1. Introduction

Peptic ulcer disease (PUD), which includes gastric and duodenal ulcers, is the most prevalent gastrointestinal disorder and requires a well-targeted therapeutic strategy. The pathophysiology of PUD involves an imbalance between offensive (acid, pepsin, and *Helcobacter pylori*) and defensive factors (mucin, prostaglandin, bicarbonate, nitric oxide, and growth factors) [1,2]. The most common sites for ulcers are the stomach and the first few centimeters of the duodenum. Acute peptic ulcers involve tissues down to the depth of the submucosa, and the lesions may be single or multiple. Reasons for the development of ulcers include severe illness, shock, burns, severe emotional disturbance, and postsurgical complications. Chronic peptic ulcers penetrate through the epithelial and

* Corresponding author. Department of Pharmaceutical Sciences, Guru Jambeshwar University of Science and Technology, Hisar, Haryana-125001, India.
E-mail: neeruvasudeva@gmail.com

doi:10.1016/j.jams.2012.01.004

muscle layers of the stomach wall. Complications of peptic ulcers include hemorrhage, perforation, pyloric stenosis, and the development of malignant tumors [3]. Poor digestion and elimination, improper metabolism, mental and physical stress, and difficult to digest food enhance the development of ulcers. A number of drugs are available for the treatment of peptic ulcers, but clinical evaluation of these drugs indicates high incidences of relapse, side effects, and drug interactions. These negative effects are the rationale for the development of new antiulcer drugs and the search for novel molecules in plants, such as *Ocimum sanctum*, *Azadirachta indica*, *Asparagus racemosus*, *Musa sapientum*, *Centella asiatica*, *Bacopa monnieri*, and *Bidens pilosa*, that offer could better protection and decreased relapse [4]. In view of the above, the present study was performed to investigate the antiulcer activity of the ethanolic extract of the roots of *Aerva persica*. The effects of this plant were also confirmed by studying its *in vivo* antioxidant effects; antioxidants, such as malondialdehyde and glutathione, seem to have protective roles against gastric ulcers and carcinomas [3].

2. Materials and methods

2.1. Animals

Albino Wistar rats of either sex, weighing between 150—250 g, were used to determine the antiulcer activity of the ethanolic extract. All animals were acclimatized and maintained under standard laboratory conditions before the start of the experiments. They were fed a normal diet and provided water *ad libitum*. The institutional animal ethics committee of Guru Jambheshwar University of Science and Technology, Hisar, India (Reg. No. 0436) approved the experimental protocol.

2.2. Plant materials and preparation of the extract

A persica was collected during the month of July from Hisar, Haryana, India. The plant was taxonomically identified and authenticated by Dr. H.B. Singh, head of the Raw Materials and Herbarium Museum, National Institute of Science Communication and Information Resources (NISCAIR), New Delhi, India. The specimens were deposited in the herbarium section of the Pharmacognosy Division, Department of Pharmaceutical Sciences, Guru Jambheshwar University of Science and Technology, Hisar, India for further reference. The powdered roots were extracted with ethanol (95%) using the continuous hot extraction method for 72 hours [5,6]. The extracts were filtered, concentrated, and dried using a rotary evaporator. The percentage yield of the ethanolic extract of the root was found to be 2.25%. The dried extract was stored at 4 °C until further use. The extract was suspended in 2% weight/volume Tween-20 before administration to the animals.

2.3. Drugs and chemicals

The following chemicals and reagents were procured: ranitidine (Martin Brown, Hisar, India), thiobarbituric acid (Spectrochem, Mumbai, India), reduced glutathione (Himedia, Mumbai, India), 5,5'-dithionitrobenzoic acid (Himedia), bovine serum albumin (Hi-media), trichloroacetic acid (TCA; Qualigens Fine Chemicals, Mumbai, India), potassium chloride (Qualigens Fine Chemicals), sucrose (Qualigens Fine Chemicals), EDTA (S.D. Fine-Chem, Mumbai, India), sodium hydroxide (Spectrochem, Mumbai, India) petroleum ether (40—60 °C), ethanol, phenolphthalein (S.D. Fine-Chem, Mumbai, India), phenol reagent (Ciocalteau-Folins reagent; S.D. Fine-Chem, Mumbai, India), phenol (Qualigens Fine Chemicals), sulfuric acid (Qualigens Fine Chemicals), glucose (Qualigens Fine Chemicals), anhydrous sodium acetate (Qualigens Fine Chemicals), alkaline reagent (S.D. Fine-Chem), phenol red indicator, pyridine (Spectrochem), *n*-butanol (Spectrochem), and Tris buffer (Spectrochem).

2.4. Phytochemical analysis

The ethanolic extract of the roots of *A persica* was subjected to the following tests for phytochemical screening using standard methods [7]: carbohydrates were identified by Molisch's test, proteins were identified by the ninhydrin test, triterpenoids and steroids by the Liebermann-Burchard test, tannins by Braemer's test, alkaloids by Dragendorff's test, saponins by the hemolytic test, glycosides by Legal's test, flavonoids by the Pew test, and fixed oils were identified by the presence of oil stains on filter paper [7].

2.5. Experimental design

The following groups were designed for this antiulcer study. Each group was comprised of six animals.

- Group 1: vehicle (distilled water; DW)
- Group 2: DW + alcohol (control)
- Group 3: DW + pylorus ligation (control)
- Group 4: DW + alcohol + ranitidine (50 mg/kg)
- Group 5: DW + pylorus ligation + ranitidine (50 mg/kg)
- Group 6: DW + alcohol + ethanolic extract (200 mg/kg)
- Group 7: DW + pylorus ligation + ethanolic extract (200 mg/kg)

All animals were deprived of food (but not water) for 24 hours prior to being subjected to ulcerogenic compounds.

2.6. Alcohol-induced ulcer model

Gastric ulcers were induced by the oral administration of absolute alcohol at a dose of 1 mL/200 g-body weight 45 minutes after the oral administration of the ethanolic extract and identified drug to each group of animals [8]. Animals were sacrificed 1 hour after the administration of ethanol. The stomachs were removed, cut along the greater curvature, washed with normal saline (0.9%), and, finally, and ulcer index was scored.

2.7. Pylorus ligation-induced ulcer model

This is the oldest animal model of gastric ulcers, originally developed by Shay in 1945 [9]. Wistar rats weighing

150—200 g were fasted for 24 hours prior to pyloric ligation. Under light ether anesthesia, the abdomen was opened by a small midline incision below the xiphoid process, and the pyloric position of the stomach was slightly lifted and ligated to avoid traction into the pylorus or damage to its blood supply. The stomach was carefully replaced and the abdominal wall was closed by interrupted sutures. The animals were deprived of both food and water the during postoperative period and were sacrificed 6 hours after the operation. The stomachs were dissected, and the contents were drained into tubes and subjected to biochemical analysis to determine the free acidity activity, pepsin activity, and total carbohydrate and protein content [10]. The stomachs were then cut along the greater curvature, the inner surface was examined for ulceration, and the ulcer index was calculated.

2.8. Drug administration

The test drug (*A persica* at a dose of 200 mg/kg) and the standard drug (50 mg/kg) were orally administered for 15 days to both ulcer models.

2.9. Measurement of the ulcer index

Ulcer scores were calculated viewing the ulcers with a magnifying glass. The following bitrary scoring system was used to grade the incidence and severity of the lesions [11]:

- Shedding of epithelium = 10
- Petechial and frank hemorrhages = 20
- One or two ulcers = 30
- More than two ulcers = 40
- Perforated ulcers = 50

The ulcer index was calculated using these scores as follows:
UI = Us + Up × 10-1; where,
Us = Mean severity of the ulcer score, and
Up = Percentage of animals with ulcer incidence.
The percentage protection index was calculated as follows:
C - T/C × 100; where
C = ulcer index of the control group, and
T = ulcer index of the treated group.

2.10. Biochemical parameters used to investigate gastric juice

Determination of the dissolved mucosubstances was accomplished by determining the total carbohydrate and protein in a 95% ethanol precipitate of the gastric juice. The total carbohydrate and the total carbohydrate:protein (TC:PR) ratio are acceptable as a reliable index of mucus secretion and mucosal resistance [12]. From the gastric juice, the following parameters were determined: free acidity, pepsin activity [13], total carbohydrate [14], and protein content [15].

2.11. Estimation of free acidity

The gastric content was centrifuged at 1000 *g* for 10 minutes. The volume of gastric juice was noted. One mL of the supernatant liquid was pipetted and diluted to 10 mL with distilled water. Then, the total acidity of the gastric juice was estimated by titration using 0.01 N sodium hydroxide and phenolphthalein as the indicator. The result was expressed as the free acid output, which was expressed in terms of mEq/L [13].

2.12. Estimation of pepsin activity

The centrifuged (5000 *g* for 10 minutes) gastric juice (0.1 mL) was added to 1 mL bovine albumin (0.5% w/v in 0.01 N HCl; pH 2) and incubated for 20 minutes at 37 °C. A duplicate background control tube (gastric juice blank), in which 1 mL albumin was replaced with 1 mL of 0.01 N HCl, was simultaneously run. Hydrolysis was stopped by adding 2 mL of 10% TCA. All of the tubes were heated in boiling water for 5 minutes, then cooled. After denaturation of the proteins by heating in a boiling water bath for 5 minutes, the precipitate was removed by centrifugation (9000 *g* for 10 minutes). A total of 1 mL of the supernatant was mixed with 0.4 mL of 2.5 N NaOH and 0.1 ml of the Folin-Ciocalteu reagent, then the volume was adjusted to 10 mL using distilled water. Absorbance was measured at 700 nm. The peptic activity was calculated in terms of micrograms of tyrosine liberated per milliliter of gastric juice [13].

2.13. Estimation of the total carbohydrate content

blank was pipetted out into test tubes containing 0.15 ml gastric juice or blank containing 0.15 mL of distilled water and thoroughly mixed. Five mL of 96% H_2SO_4 was added and mixed slowly. After 10 minutes, the test tubes were shaken, placed in water, and kept at 20 °C for 20 minutes. The optical density of the developed yellow-orange chromophore was read at 482 nm using a UV spectrophotometer. Several concentrations of a standard glucose solution were run in order to prepare a standard curve. Total liberated carbohydrates were expressed in terms of μg/mL-gastric juice. The mucoadhesive activity was expressed as the TC:PR ratio [14].

2.14. Estimation of the protein content

Estimation of the protein content was carried out as described by Lowry [15]. One mL of gastric juice and 9 mL of 95% alcohol were mixed, shaken, and the mixture centrifuged at 3000 *g* for 15 minutes in order to obtain the precipitate. This precipitate was dissolved in 1 mL of 0.1 N NaOH. Then, 0.9 mL of distilled water was added to 0.1 mL of this solution. From this solution, 0.4 mL was placed in another test tube. Four mL of an alkaline reagent was added to this test tube and allowed to react for 10 minutes. Then, 0.4 mL of the phenol reagent was added to this test tube and allowed to react for 10 minutes until color development. Readings were taken against the blank tubes prepared with distilled water. The protein content was obtained by comparing against a standard curve prepared using bovine albumin. The protein concentrations were expressed in terms of μg/mL-gastric juice ± Standard Error Mean (SEM).

Table 1 Effect of *A Persica* extract on alcohol-induced ulcers.

Sr. No.	Treatment	Dose (mg/kg)	Ulcer index*	% Protection
1.	Control (absolute alcohol)	1 mL/200 gm	34.60 ± 5.80	—
2.	Ranitidine	50 mg/kg	8.70 ± 2.80**	74.85
3.	AP	200 mg/kg	10.23 ± 2.30**	70.43

AP: *Aerva Persica* extract.
* The values of ulcer index are the mean ± SEM of the six animals in each group. Statistical analysis was performed using ANOVA followed by Dunnett's t-test.
** $p < 0.01$ compared with control.

2.15. Preparation of tissue homogenates

The rats were sacrificed by exsanguination through the abdominal aorta while under light ether anesthesia. Four hundred mg of muscle tissue was homogenized in 8 mL of 0.02 M EDTA in a Potter-Elvehjem homogenizer placed in an ice bath. The homogenates were kept in the ice bath until determination of the glutathione and malondialdehyde levels.

2.16. Determination of the reduced glutathione level

Five mL aliquots of the homogenates were mixed in 15 mL test tubes with 4 mL distilled water and 1 mL of 50% TCA. The tubes were intermittently shaken for 10—15 minutes, then centrifuged for 15 minutes at 3000 rpm. Two mL of the filtrate or supernatant was mixed with 4 mL of 0.4 M Tris buffer (pH 8.9), 0.1 mL 5,5'-Dithio-Bis-(2-Nitrobenzoic-Acid) (DTNB) was added, and the samples were shaken. The absorbance was read within 5 minutes of the addition of DTNB at 412 nm against a blank reagent blank without a homogenate [16].

2.17. Determination of the malondialdehyde (MDA) level

After washing the tissues with 0.9% NaCl, the homogenates were prepared in a ratio of 1 g of wet tissue:9 mL of 1.15% KCl using a glass Potter-Elvehjem homogenizer. The reaction mixture contained 0.1 mL of the sample, 0.2 mL of 8.1% sodium dodecyl sulfate, 1.5 mL of 20% acetic acid solution, and 1.5 mL of a 0.8% aqueous solution of thiobarbituric acid (the pH of the 20% acetic acid solution was adjusted with NaOH to be above 3). The mixtures were finally adjusted to 4 mL with distilled water and heated at 95°C for 60 minutes. After cooling with tap water, 1 mL of distilled water and 5 mL of the mixture of *n*-butanol and pyridine (15:1, v/v) were added and the mixtures were vigorously shaken. After centrifugation at 3000 rpm for 15 minutes, the absorbance of the organic layer (upper layer) was measured at 532 nm [17].

2.18. Histopathological procedures

Tissue samples were preserved in 10% buffered formalin and processed for paraffin block preparation. Sections approximately 5-μm thick were cut using an optical rotatory microtome and stained with hematoxylin and eosin [18]. These sections were examined under a microscope for necrosis.

2.19. Statistical analysis

All of the results are expressed as the mean ± SEM. The data of all the groups were analyzed using one-way analysis of variance (ANOVA) followed by the Dunnett's t test using Instat 3.0 (Graph Pad Software Inc., USA). In all of the tests, the criterion for statistical significance was $p < 0.05$.

3. Results and discussion

3.1. Phytochemical analysis

Qualitative phytochemical analysis of the alcoholic extracts of the root showed the presence of carbohydrates, flavonoids, saponins, alkaloids, and tannins. Flavonoids and catechins are secondary metabolites that are present in plants and have attracted the attention of many researchers because of their wide range of biological activities [19].

Table 2 Effect of *A persica* extract on pylorus ligated-induced ulcers.

Sr. No.	Treatment	Ulcer index***	% Protection	Free acidity (mEq/L)	Pepsin activity (U/mL)	Total carbohydrates (TC) (μg/mL)	Protein Content (PR) (μg/mL)	TC/PR ratio
1.	Control	41.0 ± 4.3	—	189.5 ± 12.11	41.1 ± 3.6	523.6 ± 35.76	421.56 ± 44.78	1.24 ± 0.19
2.	AP (200 mg/kg)	16.4 ± 3.0**	36.88	117.6 ± 10.1**	27.76 ± 4.2*	652.70 ± 21.43**	316.18 ± 16.69*	2.06 ± 0.17**
3.	Ranitidine (50 mg/kg)	10.7 ± 2.1**	62.95	82.3 ± 8.9**	13.8 ± 2.4**	594.9 ± 18.45**	377.78 ± 17.29**	1.57 ± 0.12**

AP: *Aerva Persica* extract.
***The values of the ulcer index are the mean ± SEM of the six animals in each group. Statistical analysis was performed using ANOVA followed by Dunnett's t test.
*$p < 0.05$, **$p < 0.01$ compared with the control.

Table 3 Effect of the alcoholic extract of *A persica* on biochemical (i.e., antioxidant) parameters in rats.

Parameters	Control	Standard	AP (200 mg/kg)
Malondialdehyde levels (nmol/mg of protein)	0.58 ± 0.03	0.22 ± 0.07*	0.29 ± 0.03*
Reduced glutathione	78.00 ± 4.80	107.80 ± 1.70*	104.80 ± 8.10*

Results are the mean ± SEM.
*$p < 0.01$ compared with the control.
AP: *Aerva Persica* extract.

There are many studies on the antiulcerogenic properties of flavonoids [20,21]. Tannins, saponins, and flavonoids are known to affect the integrity of mucous membranes [22]. Tannins, with their protein precipitating and vasoconstrictive effects, prevent the development of ulcers. Flavonoids are free radical scavengers that are known to play an important role in ulcerative and erosive lesions of the gastrointestinal tract [23]. The antiulcer activities of the ethanolic extract of this root could be attributed to its flavonoids and tannins.

Qualitative phytochemical analysis of the ethanolic extract of the roots of *A persica*.

Sr. No.	Phytoconstituents	Ethanolic root extract*
1.	Carbohydrates	+
2.	Proteins	−
3.	Sterols	−
4.	Alkaloids	+
5.	Tannins	+
6.	Saponins	+
7.	Flavonoids	+
8.	Fixed oils	−

*−/+ indicates the absence or presence of the phytoconstituent, respectively.

3.2. Alcohol-induced ulcer model

Table 1 shows the ulcer index and percent protection from ulcers in the alcohol-induced ulcer model. The extract of the plant showed significant protection from ulcers (70.43%) at a dose of 200 mg/kg ($p < 0.01$) compared with the controls. The standard drug, ranitidine, also showed a significant protective effect against ulcers (74.85%) at a dose of 50 mg/kg when compared with the control groups ($p < 0.01$).

3.3. Pylorus ligation-induced ulcer model

The ulcer index and percent protection against ulcers in the pylorus-induced ulcer model are shown in Table 2. The extract of the plant showed significant protection against ulcers (36.88%) at a dose of 200 mg/kg ($p < 0.01$) when compared with the control animals. The standard drug, ranitidine, also showed significant protective effects against ulcers (62.95%) at a dose of 50 mg/kg when compared with the control groups ($p < 0.01$). Free acidity and pepsin activity were also significantly decreased at a dose of 200 mg/kg of the extract, as shown in Table 2, when compared with the control animals. The TC:PR content ratios were significantly increased when compared with the control groups.

The results indicate that the ethanolic extract of the roots has potent protective effects against induced ulcers in animal models. A significant decrease in the ulcer index ($p < 0.01$) was observed following treatment with the

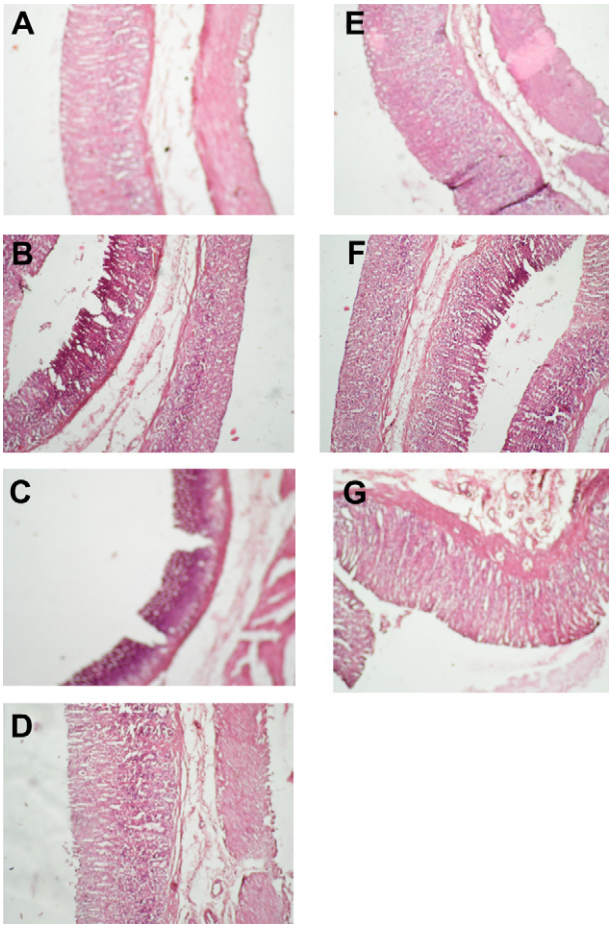

Figure 1 Histopathology of stomach. (A) Normal group showing its normal appearance. (B) Control group treated with 1 mL of absolute alcohol. (C) Control group 2: pylorus ligation-induced ulcer. (D) Alcohol-induced ulcer: treated with 50 mg/kg ranitidine. (E) Pylorus ligation-induced ulcer: treated with 50 mg/kg ranitidine. (F) Alcohol-induced ulcer: treated with the extract of the root. (G) Pylorus ligation-induced ulcer: treated with the ethanol extract of the root.

ethanolic extract of the roots of *A persica* at dose of 200 mg/kg. The extract also significantly increased the glycoprotein content of the mucosal cells, as seen by the increase in the TC:PC ratio of the gastric mucosa.

4. Biochemical parameters

The alcoholic extract of the roots significantly reduced malondialdehyde levels ($p < 0.01$) and increased glutathione levels in tissue homogenates (Table 3). Glutathione is an important constituent of the intracellular protective mechanism against a number of noxious stimuli, including oxidative stress. Intracellular glutathione also seems to be responsible for protecting gastric cell against ethanol-induced injuries. The excessive generation of oxygen radicals in the extracellular space and depletion of glutathione in conjunction with the inhibition of glutathione peroxidase activity are responsible for oxidative tissue damage of the gastric mucosa after the administration of ethanol, as suggested by various studies [24,25]. In our study, decreased glutathione concentrations were observed in the control groups, whereas the rats that were pretreated with the ethanol extract of the root of *A persica* showed a significant increase in the glutathione level, suggesting that these extracts prevent the depletion of nonprotein sulfhydryl groups caused by ethanol treatment. The increase in the malondialdehyde level in the control rats is probably due to the ability of ethanol to produce oxygen-free radicals. Pretreatment with the extracts provided protective effects to the mucosal membrane against ethanol.

4.1. Histopathological investigation

The histopathological investigation of the gastric mucosa (Fig. 1) of the rats revealed that the ethanol treatment caused hemorrhagic necrosis. Pretreatment with the ethanol extract of the roots of *A persica* reduced ethanol-induced hemorrhagic necrosis in the rat stomach in both models.

5. Conclusion

The results of our study indicate that the ethanolic extract of *A persica* produces significant cytoprotective effects against alcohol- and pylorus ligation-induced ulcers ($p < 0.01$). Thus, the ethanolic extracts of *A persica* roots can be used as a new source for antiulcer drugs.

6. Relevance

Currently, non steroidal anti-inflammatory drugs (NSAIDs) are commonly used as analgesics and anti-inflammatories, which may cause ulcers. Various other factors, like alcoholism, also cause ulcer. In these situations, we need drugs that have less severe side effects and are highly potent. In the light of these facts, the present study is highly relevant and addresses the utility of the roots of *A persica* as an antiulcer agent.

References

1. Valle DL. Peptic ulcer diseases and related disorders, *Harrison's Principles of Internal Medicine*. 16th ed. New York: McGraw-Hill Medical Publishing Division; 2005. 1746—1762.
2. Hoogerwerf WA, Pasricha PJ. Agents used for control of gastric acidity and treatment of peptic ulcers and gastroesophageal reflux disease. In: *Goodmann and Gilmann's Pharmacological Basis of Therapeutics*. 10th ed. New York: McGraw-Hill Medical Publishing Division; 2001. p. 1005—1019.
3. Waugh A, Grant A. *Ross and Wilson Anatomy and Physiology in Health and Illness*. 10th ed. Spain: Churchill Livingstone: Elsevier Ltd; 2006. p. 320—321.
4. Gautam P. Exploring Indian medicinal plants for antiulcer activity. *Indian J Pharmacol*. 2006;38(2):95—99.
5. Vasudeva N, Sharma SK. Post coital antifertility activity of Hibiscus rasasinensis roots. *Evid Based Complement Alternat Med*. 2009;5(1):91—94.
6. Kalra P, Sharma S, Suman, Kumar S. Antiulcer effect of the methanolic extract of Tamarindus indica seeds in different experimental models. *J Pharm Bioall Sci*. 2011;3:236—241.
7. Harbone JP. *Phytochemical methods, a guide to modern technique of plant analysi*. London: Chapmann and hall; 1973. 1—271.
8. Suleyman H, Demirezer LO, Kuruuzum-Uz A. Effects of *Rumex patientia* root fractions on indomethacin and ethanol induced gastric damage in rats. *Pharmazie*. 2004;59:147—149.
9. Shay H, Komarov SA, Fels SS, Meranze D, Gruenstein M, Siplet H. A simple method for the uniform production of gastric ulceration in the rat. *Gastroenterology*. 1945;48:43—61.
10. Parmar NS, Desai JK. A review of current methodology for the evaluation of gastric and duodenal antiulcer agents. *Indian J Pharmacol*. 1993;25:120.
11. Srivastava SK, Nath C, Gupta MB, Vrat S, Sinha JN, Gupta GP. Protection against gastric ulcers by Verapamil. *Pharmacol Res*. 1991;23:81—85.
12. Sanyal AK, Mitra PK, Goel RK. A modified method to estimate dissolved mucosubstances in gastric juices. *Ind J Exp Biol*. 1983;21:78—80.
13. Prino G, Paglialunga S, Nardi G, Lietti A. Inhibition of experimentally-induced gastric ulcers in the rat by a new sulfated glycopeptide. *Eur J Pharmacol*. 1971;15:119—126.
14. Nair BR. Investigations on the neuron of south Indian scorpion hetrometrus scaber (dissertation). Trivendrum (Kerala): University of Kerala, 1976.
15. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the folin-phenol reagent. *J Biol Chem*. 1951;193:265—275.
16. Sedlak J, Lindsay RH. Estimation of total, protein-bound and nonprotein sulfhydryl groups in tissue with Ellman's reagent. *Anal Biochem*. 1968;25:192—205.
17. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem*. 1979;95: 351—358.
18. Young B, Heath JW. *Wheater's Functional Histology*: A Text and Colour Atlas, 4th ed. New York: Harcourt Publishers Ltd; 2000; 251—253.
19. Harborne JB. *Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis*. 2nd ed. London: Chapman and Hall Ltd; 1984. 54—5.
20. Gracioso JS, Vilegas W, Hiruma-Lima CA, Souza Brito AR. Effects of tea from *Turnera ulmifolia* L. on mouse gastric mucosa support the Turneraceae as a new source of anti-ulcerogenic drugs. *Biol Pharm Bull*. 2002;25:487—491.
21. Gonazlez FG, Di Stasi LC. Antiulcerogenic and analgesic activities of the leaves of *Wilbrandia ebracteata* in mice. *Phytomed*. 2002;9:125—134.

22. Nwafor PA, Okwuasaba FK, Onoruvwe OO. Contraceptive and non-estrogenic effects of methanolic extract of *Asparagus pubescens* root in experimental animals. *J Ethnopharmacol*. 1998;62:117—122.
23. Konan NA, Bacchi EM. Antiulcerogenic effect and acute toxicity of a hydroethanolic extract from the cashew (*Anacardium occidentale* L.) leaves. *J Ethnopharmacol*. 2007;112:237—242.
24. Banerjee RK. Nonsteroidal anti-inflammatory drugs inhibit gastric peroxidase activity. *Biochim Biophys Acta*. 1990;1034: 275—280.
25. Yoshikawa T, Naito Y, Kishi A, Tomii T, Kaneko T, Iinuma S, et al. Role of active oxygen, lipid peroxidation and anti-oxidants in the pathogenesis of gastric mucosal injury induced by indomethacin in rats. *Gut*. 1993;34:732—737.