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Gastroprotective effect of *Achyranthes aspera* Linn. leaf on rats

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ABSTRACT

Objective: The study was aimed at evaluating the antiulcer activity of ethanolic extract of *Achyranthes aspera* (EEAA) leaf. **Methods:** The anti-ulcer assays were performed on pylorus ligation and chronic ethanol induced ulcer model. The effects of the EEAA on gastric content volume, pH, free acidity, total acidity and ulcer index were evaluated. **Results:** The percentage of ulcer protection (59.55% and 35.58%) was significantly ($P < 0.001$) higher in the groups treated with the high dose of EEAA (600 mg/kg), it also reduced the volume of gastric juice and total acidity whereas, gastric pH was increased significantly. **Conclusions:** The results of this study show significant gastroprotective activity of EEAA may be due to presence of phyto-constituents like flavanoids, saponins and tannins.

1. Introduction

Gastric hyperacidity and gastroduodenal ulcer is a very common global problem today. It is now generally agreed that gastric lesions develop, when the delicate balance between some gastroprotective and aggressive factors is lost[1,2]. Major aggressive factors are acid, pepsin, *Helicobacter pylori* (*H. pylori*) and bile salts. Defensive factors mainly involve mucus-bicarbonate secretion and prostaglandins[3]. Hypersecretion of gastric acid is a pathological condition, which occurs due to uncontrolled secretion of hydrochloric acid from the parietal cells of the gastric mucosa through the proton pumping H^+K^+ATPase [4]. Proton pump inhibitors (omeprazole, lansoprazole, *etc.*) and histamine H_2-receptor blocker (ranitidine, famotidine, etc.) are extensively used to control increased acid secretion and related disorders caused by stress, NSAID′s and *H. pylori*, but there are reports of adverse effects and relapse in the long run[5,6]. On the contrary, most of the herbal drugs reduces the offensive factors and proved to be safe, clinically effective, better patient tolerance, relatively less expensive and globally competitive[7]. Herbal medicines are generally used in such cases when drugs are to be used for longer periods. Several natural drugs have been reported to poses anti-ulcerogenic activity by virtue of their predominant effect on mucosal defensive factors[8,9].

Achyranthes aspera (*A. aspera*) Linn. belonging to family Amaranthaceae, locally known as chirchita, is an annual, biennial, lower portion perennial erect under shrub or rather stiff herb growing up to 0.3 to 1.0 m in height. It grows throughout the world in tropical and warmer regions. In Unani and Ayurvedic system of medicine, leaves and fruits used as remedy for piles, renal dropsy, pneumonia, cough, kidney stones, skin eruption, snake bite, gonorrhoea and dysentery etc. The plant has antibacterial, antitumor, anti-inflammatory, anti-fertility, abortifacient activity and increases pituitary and uterine weights in ovarectomised rats and reproductive toxicity in male rats[10]. The *A. aspera* has been extensively studied for various pharmacological properties but antiulcer activity of the plant has not been reported so far. The study therefore seeks to access ethanolic extract of the leaves of *A. aspera* with rich presence of flavonoids and tannins, for antiulcer activity in experimental animal model.

2. Materials and methods

2.1. Drugs and chemicals

Ethanol and Diethyl ether (Loba Chemie Pvt. Ltd. Mumbai), omeprazole (Dr Reddys pharmaceutical limited,

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Mumbai) were used in this study. All the dosage form and reagents were prepared freshly before use and all the reagents used were of analytical grade.

2.2. Plant material

The leaves of plant *A. aspera* were collected from fields of Bhopal in the month of Nov. 2009. It was identified and authenticated by Dr. Madhuri Modhak, Department of Botany, M.V.M. College, Bhopal. A voucher specimen (mvm-H/Am- 01) has been deposited at the herbarium of the same institute.

2.3. Extract preparation

The leaves were shade dried at room temperature and and milled into coarse powder. The ethanolic (70 %) extract of *A. aspera* leaves were prepared by using Soxhlet apparatus after defatting with petroleum ether. The extract was filtered through muslin cloth and evaporated under 40 ℃ up to one third of initial volume, remaining solvent was completely evaporated at 40 ℃ using a rotary vacuum evaporator (Superfit, India). The brownish residue (yield 16.74 % w/w) designated as ethanolic extract of *A. aspera* (EEAA) was employed for the experimental studies.

2.4. Phytochemical screening

EEAA was subjected to qualitative phytochemical investigation for alkaloids, tannins, phenols, saponins, carbohydrates, steroids and glycosides according to the methods described by Kokate[11].

2.5. Animals

Laboratory bred Wistar albino rats of both sexes (150- 200 g) maintained under standard laboratory conditions at (22 - 2) ℃, relative humidity (50-15) % and photoperiod (12 h dark and light), were used for the experiment. Commercial pellet diet (Hindustan Lever, India) and water were provided *ad libitum*. In order to avoid diurnal variation all the experiments were carried out at same time of the day *i.e.* between 10 a.m. to 5 p.m. The study was approved by the Institutional Animal Ethical Committee (IAEC/RCP/2010/14), following the guidelines of CPSCEA (approved body of Committee for the Purpose of Control and Supervision of Experiments on Animals, Chenni, India) of Radharaman College of Pharmacy, Bhopal (M.P.). Care provided to the animal was as per the 'WHO guidelines for the care and use of animals in scientific research'.

2.6. Acute toxicity studies

Acute oral toxicity of EEAA was determined according to the guidelines of Organization for Economic Co-operation & Development (OECD) following the Up & Down method (OECD guideline No. 425) and Fixed dose method (OECD guideline No. 420). Based on these methods a Limit test was performed to categorize the toxicity class of the compound. The animals (nulliparous and non-pregnant female Wistar albino rats) were fasted overnight with free access to water, weighed and a single dose of the test substance was administered orally. Animals were observed individually during first 30 min, periodically during 48 hours with special attention given during first 4 hours (short-term toxicity) and daily thereafter for total of 14 days (short-term toxicity). LD_{50} was found greater than 2 000 mg/kg, in Limit test, drug substance could be classified in the hazard classification as Class 5, Nontoxic in the Globally Harmonized System[12]. A dose range of 200, 400 and 600 mg/kg was selected for EEAA.

2.7. Anti-Ulcer Activity

2.7.1. Pylorus ligation in rats

Twenty-four hour fasted animals were divided into five groups of 6 rats each. All the drugs were suspended in 2 % carboxyl methyl cellulose (CMC) prepared freshly before administration. Negative control group was treated only with 2 % CMC (0.5 mL/100 g). Control vehicle (Group I), omeprazole (Group II) and EEAA 200, 400 and 600 mg/kg (Group III, IV, and V respectively) were administered orally before one hour of pylorus ligation. Under light ether anaesthesia, the abdomen was opened by a small midline incision at 1 cm below the xiphoid process. Stomach was exposed and a tight knot was applied around the pyloric sphincter. During this process, care was taken to avoid traction to the pylorus or damage to its blood supply. The stomach was placed carefully and abdomen wall closed by interrupted sutures. The animals were deprived of water during the post-operative period. After 4 hrs of pyloric ligation animals were sacrificed by decapitation, abdomen was opened and the stomach was isolated after suturing the lower esophageal end. The stomach was then cut open along the greater curvature, gastric contents were collected in a graduated centrifuge tube. Stomach surface was cleaned with cold normal saline then ulcer index was determined using a hand lens, ulcer was scored and percent protection was calculated[13]

2.7.2. Chronic ethanol induced ulcer in rats

Animals were randomly divided into five groups contains six animals in each to study the effect on chronic ulcer induced by seven days continuous ethanol administration following modified method of Hernández-Muñoz[14]. On the first day of experiment, animals of group I were pretreated with 70 % ethanol (1 mL/ 100 g) and the animals of group II were treated with standard drug i.e. omeprazole (10 mg/kg *p.o.*) along with ethanol. Similarly animals of group III, IV and V were pre-treated with EEAA (200, 400 and 600 mg/kg *p.o.* respectively) along with ethanol. From the next day 20 % (v/v) ethanol were administered to all the animals up to the 7th day of treatment along with continuous seven days once a day treatment schedule. All the animals were sacrificed on 7th day by cervical dislocation after 1 hr of ethanol administration and stomach was incised along the greater curvature and examined[15].

2.7.3. Estimation of pH, gastric volume, total and free acidity

The gastric juice was collected and its volume was measured. The pH of the gastric juice was recorded with digital pH meter. Gastric was centrifuged, diluted (1 mL diluted with 9 mL of D.W.) and clear supernatant was titrated against 0.01N NaOH using Toper's reagent till orange color, corresponds to free acidity and further titrated to pink color

with phenolphthalein, total volume of NaOH corresponds to total acidity[16]. Acidity expressed as:
Vol. of NaOH × Normality × 100 / 0.1 mEq/L/100 g.

2.7.4. Measurement of ulcerative index

The stomach was opened and washed with running tap water, opened stomach was laid on the glass plate and the mucous was exposed, allowing the counting of injuries per square mm. The ulcer index was determined using the formula:
Ulcer Index = 10/X, where X = Total mucosa area/ Total ulcerated area

2.7.5. Assessment of ulcer grading and percentage inhibition

The number of ulcers per stomach were noted and severity of the ulcers were observed microscopically and scoring was done as described by Kulkarni[17], as 0 for normal coloured stomach, 0.5 for red colouration, 1 for spot ulcer, 1.5 for haemorrhagic streaks, 2 for ulcer between > 3 but < 5 mm and 3 for ulcer > 5 mm. Mean ulcer score for each animal was expressed as ulcer index. The percentage healing was calculated as:
Percent inhibition = Ulcer area of control − Ulcer area of treated × 100 / Ulcer area of control

2.7.6. Morphological examination

The stomach was opened and washed with running tap water, placed on a flat glass plate to view the morphological alteration induced by different treatment. Photograph has been taken using digital camera (10 mega pixel 5X zoom).

2.7.7. Statistical analysis

The results are presented as mean ± SEM. The data were analyzed by ANOVA (one-way analysis of variance). The statistical analysis was performed using Tukey-Kramer multiple comparison tests for all parameters. The values were considered significant at the levels of p is less than 0.05.

3. Results

3.1. Phytochemical analysis

The phytochemical screening tests were positive for the presence of alkaloids, carbohydrates, glycosides, flavanoids, steroids and tannins.

3.2. Acute toxicity studies

The parameters for motor activity and other gross effects were normal in all the animals on oral administration of *A. aspera* in 2 000 mg/kg dose. From these observations it is concluded that EEAA is safe even at the higher doses and has no acute toxicity.

3.3. Effect of EEAA on gastric volume and gastric pH

Treatment of EEAA has significantly (P<0.01) reduced the gastric volume and pH in pylorus ligation induced ulcer at the doses of 400 and 600 mg/kg compared with vehicle control group as shown in Table 1. Volume of gastric content and pH was also decreased significantly (P<0.01 − 0.001) by EEAA compared with control group against chronic ethanol induced ulcer at the doses of 400 and 600 mg/kg (Table 2).

3.4. Effect of EEAA on total and free acidity

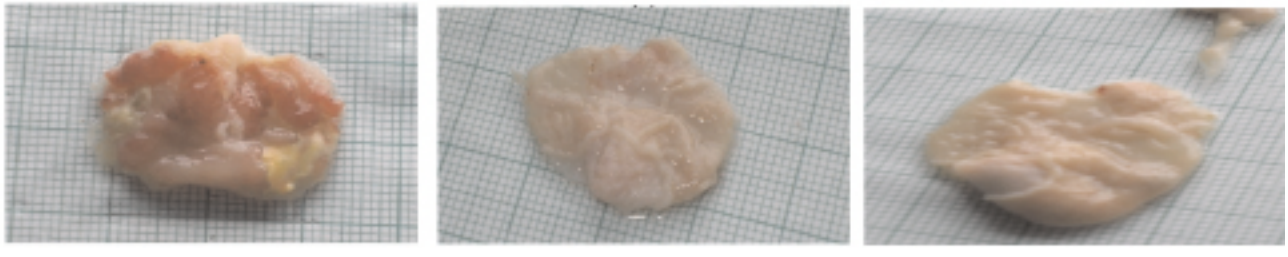
The antisecretory property of *A. aspera* in pylorus ligation model was evident from its significant reduction of total acidity (P < 0.05−0.01) and free acidity (P < 0.01−0.001) at the doses of 400 and 600 mg/kg compared with the control group as shown in Table 1. In chronic ethanol induced ulcer also EEAA showed moderate protection (P < 0.05−0.01)) by decreasing the stomach acidity as shown in Table 2.

3.5. Effect of EEAA on ulcer index and ulcer grading

The observations of positive control (omeprazole) group showed (0.75 ± 0.13) ulcer index in pylorus ligation induced gastric ulcerations whereas vehicle control group had (4.45 ± 0.29) scoring. Ulcer index was extreme significantly (P < 0.001) reduced in pyloric ligated rats treated with EEAA at 600 mg/kg compared to the control group as shown in Table 1. These parameters were also significantly (P < 0.01) reduced in chronic ethanol induced ulcer at 600 mg/kg dose. Pretreatment with test extracts reduced the ulceration in a dose dependant manner. The extent of gastroprotection showed by the test extracts (600 mg/kg) was 59.55 % and 35.58 % respectively against pyloric ligation and ethanol induced ulceration compared to 83.14 % and 50.25 % of standard drug omeprazole respectively (Table 1 and 2).

3.6. Morphological examination

Microscopic observation of pylorus-ligation and chronic ethanol induced ulcerated rat stomach, showed ulcerated stomach wall, mucosal epithelium and glands with inflammatory exudates. Protection against these morphological changes was observed as apparent epithelialization, loss of inflammation, redness and haemorrhagic strikes, and reduced size of ulcer crater in EEAA pretreated rats (Figure 1).



(A) (B) (C)

Figure 1. Photograph of gastric mucosal layer.
Stomach mucosa of (A): control animal; (B): omeprazole treated; (C): EEAA treated (600 mg/kg)

Table 1

Effect of *A. aspera* ethanolic extract on biochemical parameters in pylorus-ligation induced ulcer of rat.

Treatment (mg/kg)	Gastric pH	Gastric Volume (mL/100 g)	Free acidity (Meq/L/100 g)	Total acidity (Meq/L/100 g)	Ulcer index	Ulcer grading	% Inhibition
Vehicle	1.85±0.25	2.98±0.08	23.08±0.93	34.91±1.90	4.45±0.29	2.08±0.20	–
Omeprazole (10)	4.60±0.53[c]	1.38±0.22[c]	7.18±0.21[c]	18.40±0.70[c]	0.75±0.13[c]	0.34±0.16[c]	83.14
EEAA (200)	1.93±0.37[a]	2.76±0.06	19.91±0.40[a]	27.05±2.02[a]	3.81±0.14[a]	2.08±0.15	14.38
EEAA (400)	2.98±0.17[b]	2.25±0.11[b]	14.95±0.44[b]	24.71±0.61[a]	2.98±0.13[a]	1.60±0.10[b]	33.03
EEAA (600)	3.80±0.12[b]	1.60±0.16[b]	10.13±0.42[c]	20.10±0.63[b]	1.80±0.09[c]	0.58±0.15[c]	59.55

Values are expressed as Mean±S.E.M of six rat in each treatment group where, [a]$P < 0.05$, [b]$P < 0.01$ and [c]$P < 0.001$ *vs*. control.

Table 2

Effect of *A. aspera* ethanolic extract on biochemical parameter in chronic ethanol induced ulcer on rat

Treatment (mg/kg)	Gastric pH	Gastric Volume (mL/100 g)	Free acidity (Meq/L/ 100 g)	Total acidity (Meq/L/ 100 g)	Ulcer index	Ulcer grading	% Inhibition
Vehicle	1.98±0.04	4.33±0.10	4.93±0.16	12.67±0.38	7.70±0.21	2.33±0.10	–
Omeprazole (10)	4.61±0.10[c]	1.83±0.07[b]	1.00±0.08[c]	3.35±0.17[c]	3.83±0.14[c]	0.15±0.08[c]	50.25
EEAA (200)	2.81±0.23[a]	4.02±0.84	4.21±0.23	11.70±0.40	7.01±0.25	2.04±0.18	8.96
EEAA (400)	2.91±0.10[b]	3.36±0.11[a]	3.56±0.16[a]	8.25±0.33[a]	5.63±0.27[a]	1.83±0.10[a]	26.88
EEAA (600)	3.60±0.17[c]	2.91±0.06[b]	2.26±0.16[b]	6.13±0.38[b]	4.96±0.13[b]	1.16±0.24[b]	35.58

Values are expressed as Mean±S.E.M of six rat in each treatment group where, [a]$P<0.05$, [b]$P<0.01$ and [c]$P<0.001$ *vs*. control.

4. Discussion

In the present study we have assessed the gastroprotective effects of *A. aspera* on gastric ulcer models, to ascertain its ulcer healing and antisecretory property. Various mechanisms are involved in the pathogenesis of pyloric-ligation induced peptic ulcer and ethanol induced ulcer. Out of which hyper acid secretion and accumulation are thought to be the most important factor.

Role of free radicals and oxidative stress in gastric ulceration is well documented. The radicals promote lipid per oxidation and membrane damage by cross- linking proteins, lipids, and nucleic acids[18]. Antioxidants reported to play a significant role in the protection of gastric mucosa against various necrotic agents[19]. Administration of antioxidants inhibits ethanol-induced gastric injury in rat[20]. Ethanol is involved in the formation of oxygen free radicals generated extra- and/or intracellularly. Chronic low dose ethanol-induced mucosal injury, decreases levels of mucosal glutathione and induces lipid peroxidase that persists even during the recovery period (after discontinuation of ethanol), could be attributed to a post-injury proliferative response of the gastric mucosa. Furthermore, disturbances in gastric secretion, damage to the gastric mucosa, alterations in permeability, gastric mucus depletion and free-radical production are observed after the administration of chronic ethanol[14]. These data suggest that antioxidant compounds could be active in this experimental model, producing antiulcerogenic effects.

Ethanol-induced damage, characterized with the presence of haemorrhagic lesions, was prevented by *A. aspera* given before ethanol exposure along with decrease in ulcer index and grading. Regeneration of gastric surface cells occurs as a result of migration of epithelial cells from the gastric pits, this would suggest that beneficial agents not only provide protection to the gastric mucosa but may also allow mucosal reconstitution[21]. Some antioxidants and scavenging agents for free radicals have been shown to prevent experimental short-term gastric mucosal damage[22]. EEAA has been reported to contain flavonoids[23], saponins and tannins. Anti-ulcer activity of this plant could be linked to the flavonoids since flavonoids are reported to protect the mucosa preventing the formation of lesions induced by various necrotic agents[24]. It is well known that many flavonoids display anti-secretory and cytoprotective properties in different experimental models of gastric ulcer[25]. Flavonoids possess anti-oxidant properties in addition to strengthening the mucosal defence system through stimulation of gastric mucus secretion[26] and flavonoids can scavenge the reactive oxygen species (super-oxide anions) and free radicals produced by ethanol. Flavonoids also protect ulcer development by improving microcirculation and increasing capillary resistance, in turn, increasing gastric defensive factors[27]. Tannins being astringent may precipitate microproteins in the site of ulcer thus preventing absorption of toxic substances forming a protective layer and resisting the mucous layer against the attack of proteolytic enzymes. Tannins could prevent ulcer development with their protein precipitating and vesoconstrictory effects[28].

In this study, a significant decrease in gastric fluid volume, and acid output with an elevation in gastric pH, observed after oral administration of EEAA, indicates its antisecretory potency. Digestive effect of the accumulated gastric juice is believed to be responsible for producing ulcers in the pyloric ligated rats[29]. Reflex or neurogenic effect in addition to acid secretion has also been suggested to play an important role in the formation of gastric ulcer in this mode[30]. Antisecretory activity of EEAA in this model is evident from its significant reduction in acid secretary parameters viz. total acidity and free acidity. Moreover, these results showed that the antiulcer activity of this plant was not only related to a local neutralization of gastric content but also it has effective systemic effect.

It is well established that various antisecretory agent, such as H_2-receptor antagonist and proton pump inhibitor prevent gastric lesions. Famotidine treatment of animals subjected to chronic mucosal damage accelerated cellular proliferation, leading to a histological and functional improvement of the gastric mucosa in rats[31]. Omeprazole, a proton pump inhibitor has been reported to possess

antioxidant, anti-inflammatory and cytoprotective effect, which is responsible for its anti ulcerogenic activity[32,33]. Hence, in the present study omeprazole was taken as a standard drug to compare the antiulcer activity of *A. aspera* featuring its phytoconstituents having antioxidant and antisecretory activity. It can be concluded that *A. aspera* can suppress chronic gastric damage induced by administration of ethanol and pyloric acid accumulation by virtue of its antioxidant phytoconstituents like flavonoid and tannin. The results indicates that *A. aspera* exerts cytoprotective effect in addition to its gastric antisecretory activity that could be due to the presence of flavonoids and tannins responsible for its protective effect by maintaining an efficient gastric mucosal microvascular supply[34]. The study outcome needs further phytopharmacological and mechanistic correlation to identify bioactive constituents.

Conflict of interest statement

We declare that we have no conflict of interest.

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References

[1] Tripathi KD. *Essentials of medical pharmacology*. 4th ed. New Delhi: Jaypee Brothers Medical Publishers; 1999,p.628.
[2] Goel RK, Bhattacharya SK. Gastroduodenal mucosal defence and mucosal protective agents. *Indian J Exp Biol* 1991; **29**: 701-714.
[3] Hoogerwerf WA, Pasricha PJ. Agents used for the control of gastric acidity and treatment of peptic ulcers and gastrooesopahgeal reflux diseases. In: Hardman JG, Limbrid LE, Goodman CS, Gilman BT. (eds.) *Goodman and Gilman's: The pharmacological basis of therapeutics*. 10th ed. New York: McGraw-Hill; 2001,p.1005-1019.
[4] Sachs G, Shin JM, Briring C, Wallmark B, Hersey S. The pharmacology of the gastric acid pump: the H^+K^+ATPase. *Annu Rev Pharmacol Toxicol* 1995; **35**: 277-305.
[5] Martelli A, Mattlioli F, Mereto E, Brambilla CG, Sini D, Bergamaschi R, et al. Evaluation of omeprazole genotoxicity in a battery of *in vitro* and *in vivo* assays. *Toxicology* 1998; **30**: 19-41.
[6] Wolfef MM, Sachs G. Acid suppression: optimizing therapy for gastroduodenal ulcer healing, gastrooesophageal disease and stress-related erosive syndrome. *Gastroenterology* 2000; **118**: S9-S31.
[7] Goel RK, Sairam K. Antiulcer drugs from indigenous sources with emphasis on *Musa sapientum, Tamrabhasna, Asparagus racemosus* and *Zingiber officinale*. *Ind J Pharmacol* 2002; **34**: 100-110.
[8] Sairam K, Rao CV, Goel RK. Effect of *Centella asiatica* Linn. on physical and chemical factors induced gastric ulceration and secretion. *Indian J Exp Biol* 2001; **39**: 137.
[9] Sairam K, Rao CV, Goel RK. Effect of *Convolvulus pluricaulis* Chois on gastric ulceration and secretion in rats. *Indian J Exp Biol* 2001; **39**: 350.
[10] Barua CC, Talukdar A, Begum SA, Lahon LC, Sharma DK, Pathak DC, et al. Antinociceptive activity of methanolic extract of leaves of *A. aspera* Linn. in animal models of nociception. *Indian J Exp Biol* 2010; **48**: 817-821.
[11] Kokate CK, Purohit CK, Gokhale SB. *Pharmacognosy*. Pune:Nirali Prakashan; 1996,p. 510-512.
[12] Diener W, Mischke U, Kayser D, Schlede E. The evaluation of the OECD modified version of acute toxicity class method (oral). *Arch Toxicol* 1995; **69**: 729-734.
[13] Rao CV, Verma AR, Vijayakumar, Rastogi S. Gastroprotective effect of standardized extract of *Ficus glomerata* on experimental gastric ulcers in rats. *J Ethnopharmacol* 2008; **115**: 323-326.
[14] Hernández-Muñoz R, Montiel-Ruíz C, Vázquez-Martínez O. Gastric mucosal cell proliferation in ethanol-induced chronic mucosal injury is related to oxidative stress and lipid peroxidation in rats. *Lab Invest* 2000; **80**: 1161-1169.
[15] Roy SP, Shirode D, Patel T, Prabhu K, Setty SR, Rajendra SV. Antiulcer activity of 70 % ethanolic extract of bark of *Madhuca longifolia*. *Indian J Nat Prod* 2008; **24**(4): 8-10.
[16] Hawk A. *Hawk's physiological chemistry*. 14th ed. New York: McGraw-Hill Book Co.; 1965,p.483-485.
[17] Kulkarni SK. *Handbook of experimental pharmacology*. New Delhi: Vallabh Prakashan; 2002,p.148.
[18] Kumar MM, Joshi MC, Prabha T, Dorababu M, Goel RK. Effect of plantain banana on gastric ulceration in NIDDM rats: Role of gastric mucosal glycoprotein's, cell proliferation, antioxidants and free radicals. *Indian J Exp Biol* 2006; **44**: 292-299.
[19] Trivedi NP, Rawal UM. Hepatoprotective and antioxidant property of *Andrographis paniculata* Nees in BHC induced liver damage in mice. *Indian J Exp Biol* 2001; **39**: 41-46.
[20] Ligumsky M, Sestieri M, Okon F, Ginsburg I. Antioxidants inhibit ethanol-induced gastric injury in the rat. Role of manganese, glycin and carotene. *Scandanavian J Gastroenterol* 1995; **30**: 854-860.
[21] Black BA, Morris GP, Wallace JL. Effects of acid on the basal lamina of the rat stomach and duodenum. *Virchows Arch B Cell Pathol Incl Mol Pathol* 1985; **50**: 109-118.
[22] Yoshikawa T, Ichikawa H, Naito Y, Kondo M. Role of leukotrienes in gastric mucosal injury induced by ischemia-reperfusion in the rats. *J Clin Gastroenterol* 1992; **14**: 568-570.
[23] Akowuah GA, Sadikun A, Mariam A. Flavonoid identification and hypoglycemic studies of butanol fraction from *Gynura procumbens*. *Pharma Biol* 2002; **40**: 405-410.
[24] Saurez J, Herreta MD, Marhuenda E. Hesperidin and neohesperidin dihydrochalcone on different experimental models of induced gastric ulcer. *Phytother Res* 1996;**10**: 616-618.
[25] Zayachkivska OS, Konturek SJ, Drozdowicz D, Konturek PC, Brzozowski T, Ghegotsky MR. Gastroprotective effects of flavonoids in plant extracts. *J Physiol Pharmacol* 2005; **56**: 219-231.
[26] Martin MJ, Marhuenda E, Perez-Guerrero C, Franco JM. Antiulcer effect of naringin on gastric lesion induced by ethanol in rats. *Pharmacology* 1994; **49**: 144-150.
[27] Gonzaler FG, Di Stasi LC. Anti-ulcerogenic and analgesic activities of the leaves of Wilbrandia ebracteata in mice. *Phytomedicine* 2002; **9**(2): 125-134.
[28] Aguwa CN. Gastrointestinal effects of the extracts of *Rhigiocarya racemifera* (Menispermaceae). *Gen Pharmacol* 1985; **16**(4): 387-390.
[29] Brodie DA. The mechanism of gastric hyperacidity produced by pyloric ligation in the rat. *Am J Dig Dis* 1966; **11**: 231-241.
[30] Goswami S, Jain S, Santani D. Antiulcer activity of cromakalim, against experimentally induced gastric and duodenal ulcers in rats and guinea pigs. *J Pharm Pharmacol* 1997; **49**: 195-199.
[31] Hernández-Muñoz R, Montiel-Ruíz F. Reversion by histamine H2-receptor antagonists of plasma membrane alterations in ethanol-induced gastritis. *Dig Dis Sci* 1996; **41**: 2156-2165.
[32] Sood S, Muthuraman A. Activity of tacrolimus: An immunosuppressant, in pyloric ligation induced peptic ulcer in rat. *J Pharm Soc Japan* 2009; **129**(12): 1523-1528.
[33] Eissele R, Roakoph B, Koop H, Alder G, Arnold R. Proliferation of endocrine cells in the rat stomach caused by drug-induced achlorhydria. *Gastroenterology* 1991; **101**: 70-76.
[34] Alvarez A, Pomar F, Sevilla MA, Montero MJ. Gastric antisecretory and antiulcer activities of ethanolic extract of *Bidens pilosa* L. var. radiate Schult. *Bip J Ethnopharmacol* 1999; **67**(3): 333-340.