Academic Sciences Asian Journal of Pharmaceutical and Clinical Research

Vol 5, Suppl 3, 2012 ISSN - 0974-2441

Research Article

ANTI-ULCER POTENTIAL OF ETHYL CELLULOSE FLOATING MICROSPHERES CONTAINING RANITIDINE HYDROCHLORIDE IN EXPERIMENTAL RODENTS

HITESH KR.[1, 2], HARISH CH. VERMA[1], RAMESH KR. GUPTA[2, 3]*

[1]Department of Pharmaceutics, Babu Banarsi Das National Institute of Technology and Management, Lucknow-226001, Uttar Pradesh, India, [2]Moradabad Educational Trust Group of Institution Faculty of Pharmacy, Moradabad-244001,Uttar Pradesh, India, [3]Pharmacognosy and Ethnopharmacology Division, National Botanical Research Institute, Lucknow-226001, Uttar Pradesh, India.
Email:ram5880@gmail.com

Received:8 June 2012, Revised and Accepted:21 July 2012

ABSTRACT

The aim of this study was to prepare and evaluate the anti-ulcer potential of ethyl cellulose floating microspheres containing Ranitidine hydrochloride. Microspheres were prepared by non-aqueous solvent evaporation method using ethanol/ liquid paraffin system. In vivo antiulcer activity was performed in albino rats in order to investigate the significant effect of floating microspheres for longer period of time. Floating microspheres of Ranitidine hydrochloride showed significant decrease in ulcer index, total acidity and volume of gastric acid secretion and increase the gastric pH in albino rats. The controlled release of drug (Ranitidine hydrochloride) from floating microspheres showed significant results in comparison to the pure form of drug.

Keywords: Anti-ulcer activity, Pylorus ligation, Lipid peroxidation, Ranitidine hydrochloride, Floating microspheres.

INTRODUCTION

Gastric lesions are certainly a major human disorder which affects nearly 5% of the global population. The pathophysiology of peptic ulcer disease has centered on an imbalance between aggressive and protective factors in the stomach.[1] The destructive/aggressive factors may be either endogenous or exogenous in origin. The endogenous damaging factors are hydrochloric acid, pepsin, refluxed bile, leukotrienes and reactive oxygen species (ROS) such as the superoxide anion ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2) and the hydroxyl radical ($^{\cdot}OH$). Oxygen derived free radicals mainly superoxide anion and hydroxyl radical play an important role in the pathogenesis of acute experimental gastric lesion induced by stress, ethanol and NASIDs.[2] The mucosal defense against these aggressive factors include the function of the mucus-bicarbonate barrier, surface active phospholipids, prostaglandin, mucosal blood flow, cell renewal and migration, anti-oxidative enzymes and some growth factors. Today, there are two main approaches for treating peptic ulcer. The first deals with reducing the production of gastric acid and the second with re-enforcing gastric mucosal protection.[3] There has been a rapid progress in the understanding of the pathogenesis of peptic ulcer. Modern approach to this includes proton pump inhibitors, histamine receptor blockers, drugs affecting the mucosal barrier and prostaglandin analog.[4] Ranitidine hydrochloride (RHCl) is a histamine H_2 receptor antagonist. It is widely prescribed in active duodenal ulcers, gastric ulcers, Zollinger-Ellison syndrome, gastro-esophageal reflux disease, and erosive esophagitis[5]. The recommended adult oral dose is 150mg twice daily or 300mg once daily. A conventional dose of 150mg can inhibit the gastric secretion up to five hours only. An alternative dose of 300 mg leads to plasma fluctuation; thus a sustained release dosage form of RHCl is desirable. The short biological half life of drug (2 to 3 hours) also favors development of a sustained release formulation.[6] A traditional oral sustained release formulation releases most of the drug at the colon, thus the drug should have absorption window either in colon or throughout the gastrointestinal tract. Ranitidine is absorbed only in the initial part of the small intestine and has 50% absolute bioavailability. Colonic metabolism of Ranitidine is partly responsible for poor bioavailability of Ranitidine from the colon.[7] These properties of RHCl do not favour the traditional approach to sustain release delivery. The gastro- retentive drug delivery system can be retained in the stomach and assist in improving the oral sustained delivery of drugs that have an absorption window in a particular region of GIT. The principle of buoyant preparation offers a simple and practical approach to achieve increased gastric residence time for the dosage form and sustained drug release.[8] Floating microspheres are one of the multi particulate drug delivery systems and are prepared to obtain prolonged or controlled drug delivery, to improve bioavailability and to target drug to specific sites. Floating microspheres can also offer advantages like limiting fluctuation within therapeutic range, reducing side effects, decreasing dosing frequency, and improving patient compliance.[9] Ethyl cellulose (EC) is a water insoluble polymer in controlled release dosage forms. Although EC is considered insoluble, it can take up water. This is because of its hydrogen bonding capability with water due to the polarity difference between the oxygen atom and the ethyl group of polymer.[9] The aim of this study was to prepare ethyl cellulose floating microspheres containing ranitidine hydrochloride and to evaluate the gastro-protective potential after oral administration.

MATERIALS AND METHODS

Preparation of microspheres

Ranitidine hydrochloride floating microspheres were prepared by non-aqueous solvent evaporation technique.[10] Different amounts of polymer (150, 300, 450, 600, and 750 mg) was dissolved in 25 ml of ethanol by using a magnetic stirrer (Popular India Limited, Mumbai). Powdered Ranitidine hydrochloride (150 mg) was dispersed in polymer solution. The resulting dispersion was then poured into a vessel of 1000 ml containing the mixture of 270 ml liquid paraffin and 30 ml n-hexane with stirring. Span 20 was added drop by drop into vessel during stirring. A mechanical stirrer with a blade (6 cm) diameter (Prompt, F.H.P Motor, Uttam Electrical Industries, and Varanasi) was used. Stirring was continued for an hour, until ethanol evaporated completely.

Experimental Animals

Albino wistar rats of both sexes weighing between 150-250 g were used. The experimental protocol was approved from Institutional Animal Ethics Committee (BBDNITM/IAEC/Clear/02/2008). Animals were housed under standard conditions of temperature (24 ± 2°C) and relative humidity (30-70%) with a 12:12 light: dark cycle. Animals were provided with standard rodent pellet diet (Dayal, India) and the food was withdrawn 18-24 h before the experiment though water was allowed *ad libitum*.

Pharmacological Evaluation

Cold-restraint stress (CRS)-induced ulcers

Rats were deprived of food, but not water, for about 18 h before the experiment. Reduced glutathione (RG) 150 mg/kg was injected *i.p.* twice: once before 20 h and another 1 h prior to subjecting the animals to cold-restraint stress and reported to exert its antioxidant

defense mechanism.[2] On day six, the experimental rats were immobilized by strapping the fore and hind limbs on a wooden plank and kept for 2 h, at temperature of 4-6◦ C.[11] Two hours later, the animals were sacrificed by cervical dislocation and ulcers index were examined. Control group of animals received suspension of 1% carboxymethyl cellulose in distilled water (10 ml/kg). The total severity of the ulcers was determined by recording the severity of each ulcer after histological confirmation as follows.[12] 0, no ulcer; +, pin point ulcer and histological changes limited to superficial layers of mucosa and no congestion; ++, ulcer size less than 1mm and half of the mucosal thickness showed necrotic changes; +++, ulcer size 1-2mm with more than two-thirds of the mucosal thickness destroyed with marked necrosis and congestion, muscularis remaining unaffected; ++++, ulcer either more than 2mm in size or perforated with complete destruction of the mucosa with necrosis and hemorrhage, muscularis still remaining unaffected. The pooled group ulcer score was then calculated according to the method of Sanyal et al. [12]

Estimation of lipid peroxidation (LPO)

The fundic part of the cold-restraint stress (CRS)-induced ulcer stomach was homogenized (5%) in ice-cold 0.9% NaCl with a Potter-Elvehjem glass homogenizer for 30 s. The homogenate was centrifuged at 800 × g for 10 min and the supernatant was again centrifuged at 12,000 × g for 15 min and the obtained mitochondrial fraction was used for the following estimations.[2] A volume of the homogenate (0.20 ml) was transferred to a vial and was mixed with 0.2 ml of a 8.1% (w/v) sodium dodecyl sulfate solution, 1.50 ml of a 20% acetic acid solution (adjusted to pH 3.5 with NaOH) and 1.50 ml of a 0.8% (w/v) solution of thiobarbituric acid (TBA) and the final volume was adjusted to 4.0 ml with distilled water. Each vial was tightly capped and heated in a boiling water bath for 60 min. The vials were then cooled under running water. Equal volumes of tissue blank or test samples and 10% trichloroacetic acid were transferred into a centrifuge tube and centrifuged at 1000×g for 10 min. The absorbance of the supernatant fraction was measured at 532 nm (Beckman DU 650 spectrometer). Control experiment was processed using the same experimental procedure except the TBA solution was replaced with distilled water.[13] 1,1,3,3 -Tetraethoxypropan was used as standard for calibration of the curve and is expressed as nanomoles per milligram protein.

Assay of antioxidant enzymes

The fundic stomach was homogenized (5%) and mitochondrial fraction was prepared as described above. Decomposition of H_2O_2 in presence of catalase (CAT) was followed at 240 nm.[14] One unit (U) of catalase was defined as the amount of enzyme required to decompose 1µmol of H_2O_2 per minute, at 25 ◦C and pH 7.0. Results are expressed as units of CAT activity per milligram of protein. Superoxide dismutase (SOD) activity was estimated by the inhibition of nicotinamide adenine dinucleotide (reduced)-phenazine methosulphate-nitrobluetetrazolium reaction system as described by Nishikimi et al.[15] as adapted by Kakkar et al.[16] One unit of the enzyme is equivalent to 50% inhibition in the formazan formation in 1 min at room temperature (25±2 ◦C) and the results have been expressed as units of SOD activity per milligram of protein.

Gastric secretion in pylorus-ligated rats

Gastric secretion content, pH and total acidity were measured according to the method of Shay et al.[17] One hour after oral administration of RHCL (50 and 100mg/kg) or Rabeprazole (20 mg/kg) or vehicle (10ml/kg mixture of 3% (w/v) acacia and tragacanth in distilled water); the animals were subjected to pylorus ligation under thiopental sodium anesthesia. The animals were sacrificed with an over dose of thiopental sodium after 4h of pyloric ligation. The abdomen was opened, cardiac end of the stomach was dissected out and the contents were drained into a glass tube. The volume of the gastric juice was measured and centrifuged at 2000 rpm for 10min. From the supernatant, aliquots (1ml of each) were taken for the determination of pH, total and free acidity. Total acidity was determined using titrimetry.[18]

Acetic acid-induced chronic ulcer

Induction of chronic gastric lesions was studied according to the methods of Okabe et al.[19] A solution of 0.06 ml 50% acetic acid was instilled into the glass tube of 6mm in diameter and allowed to remain 60 s on the anterior serosal surface of the glandular portion of stomach 1 cm away from the pyloric end under anesthesia. After removal of the acid solution, the abdomen was closed in two layers and animals were caged and fed normally. RHCL was given in the dose of 50 and 100 mg/kg on day 1, orally, twice daily, 4 h after the application of acetic acid and continued up to 5th and 10th days after induction of ulcer and the animals were sacrificed after 18 h of the last dose of drug on 6th and 11th day of experiment respectively to assess the ulcer size and healing. Ulcer index was calculated based upon the product of length and width (square millimeters per rat) of ulcers (Table 3).

Histological evaluation

The gastric ulcers were induced in rats by administrating 100% EtOH (1 ml/200 g, 1 h)[20] and the ulcerated portions from stomachs of EtOH induced ulcers group were cut out with a scalpel and level fixed for 4 h in 4% buffered paraformaldehyde, then dehydrated gradually in ethanol and embedded in ^{paraffin} using xylene as intermediate solvent. Serial sections were obtained by cutting the block in a plane perpendicular to the mucosal surface with a microtome. Coded gastric sections were stained with haematoxylin and eosin before light microscope evaluation.

Statistical analysis

The values were represented as mean ± S.E.M. for six rats. Analysis of variance (ANOVA) test was followed by individual comparison by Newman-Keuls test using Prism Pad software for the determination of level of significance. The p-values of <0.05 being considered significant for the experiment.

RESULTS

Effect of RHCL on Cold-restraint stress (CRS)-induced ulcers

In ulcerogen treated animals were shown extensive gastric lesions in the stomach. The effect various doses of RHCL were studied on ulcer index and anti-oxidant enzymes in CRS intoxicated animal. The results in (Table 1) showed clear significant percentage change in the levels of LPO in CRS intoxicated rats as 37.14 (P< 0.001) compared to control group. Treatment with RHCL at the doses of 50 and 100 mg/kg significantly prevented this heave in levels and the percentage protection in LPO were 18.75 (P< 0.01) and 39.58 (P< 0.001) respectively. The SOD content had significantly diminished while the CAT level significantly increased in RHCL treated groups as compared to CRS treated group. The percentage changed of SOD and CAT in CRS intoxicated group were as 97.97 (P< 0.001), and 44.71 (P< 0.001) respectively. The percentage protection in SOD as 10.11 (p<0.01), 24.54(p<0.001) and while in CAT 28.41 (p<0.001), 51.03 (P < 0.001) at the doses levels 50 and 100 mg/kg, respectively. In different doses level of RHCL, 100 mg/kg has shown maximum protection which was almost comparable to those of the normal control and reduced glutathione.

Table 1: Effect of RHCL on cold restraint stress (CRS)-induced gastric ulcers in rats

Groups	UI	LPO	CAT	SOD
Control	0.0±0.0	0.35±0.01	33.1±2.0	88.9±2.0
CRS	21.05±3.1^a	0.48±0.02^a	18.3±1.5^a	176.0±4.1^a
RHCL-50	13.05±2.8^c	0.39±0.02^b	23.5±1.3^a	158.2±3.4^b
RHCL-100	8.02±3.21^a	0.29±0.01^a	27.64±1.7^a	132.8±2.8^a
R G-150	4.08±2.4^a	0.21±0.01^a	30.01±1.9^a	122.9±2.3^a

Effect of RHCL on Gastric secretion in pylorus-ligated rats

Gastric secretion measurements of pylorus-ligated rats showed that RHCL significantly decreased the gastric content, pH, total and acidity at doses of 50 mg/kg and100 mg/kg. Rabeprazole (20mg/kg), the reference compound used also showed significant reduction of all these secretory parameters**.** The results in showed clear significant change in the levels of gastric content in pylorus-ligated (Table 2) rats as compared to RHCL and standard group. Treatment with RHCL at the doses of 50 and 100 mg/kg significantly prevented this heave in levels in gastric content and total acidity were 5.0 (P< 0.01), 8.3 (P< 0.001) and total acidity 22.77 (P< 0.01),

45.89 (P< 0.001) respectively. The pH of gastric content had significantly increased in RHCL treated and standard groups whereas in control group pH of gastric content significantly decrease. The percentage increment in pH level as 20.77 (p<0.01) and 39.43 (P< 0.001) at the doses levels 50 and 100 mg/kg, respectively. The standard drug Rabeprazole showed greatest percentage increment in pH level as 73.23 (p<0.001). In different doses level of RHCL, 100 mg/kg has shown maximum protection which was almost comparable to those of the standard.

Table 2: Effect of RHCL on gastric content, pH and total acidity in pylorus ligation induced ulceration in rats.

Group	Dose (Kg^{-1})	Gastric content	pH of Gastric content	Total acidity
Control	10	2.4 0±0.03	2.84 ±0.13	4886.2±7.5
RHCL-50	50	2.28 ± 0.02^b	3.43±0.15^b	4721.3±5.6^b
RHCL-100	100	2.20 ±0.02^a	3.96±0.16^a	3793.8±5.8^a
Rabeprazole	20	2.01± 0.02^a	4.92±0.19^a	2643.8±4.6^a

Effect of RHCL on acetic acid-induced chronic ulcers in rats

In chronic ulcers induced by 50% acetic acid, RHCL reduced ulcer index significantly as 29.25% (p<0.05), 52.51% (P< 0.01) on 5th days treatment while on 10th day treatment, RHCL reduced ulcer index as 32.60% (p<0.05) and 75.36% (P< 0.001) at the doses levels 50 and 100 mg/kg, respectively (Table 3).

Table 3: Effect of RHCL on (twice daily for 5 and 10 days) on 50% acetic acid-induced chronic ulcers in rats.

Group	Five days treated		Ten days treated	
	UI	Ulcer inhibition (%)	UI	Ulcer inhibition (%)
Control	21.5±2.2	-	13.8±1.4	-
RHCL-50	15.21±1.7^c	29.25	9.3±1.2^c	32.60
RHCL-100	10.21±1.3^b	52.51	3.4±0.6^a	75.36

Values are mean ± SEM for six rats.
^cp <0.05, ^bp <0.01 and ^ap <0.001 compared to respective control group.

Histopathological evaluation

The results on histological evaluation reveled that EtOH provoked a deep alteration of glandular epithelium and a loss of the histological structure. The lesion was characterized by abundant granulation tissue, intense inflammation and infiltration of leukocytes. Pretreatment with RHCL prevented the hemorrhage, edema, necrosis, erosions and deep ulceration induced by the EtOH (Fig 1.)

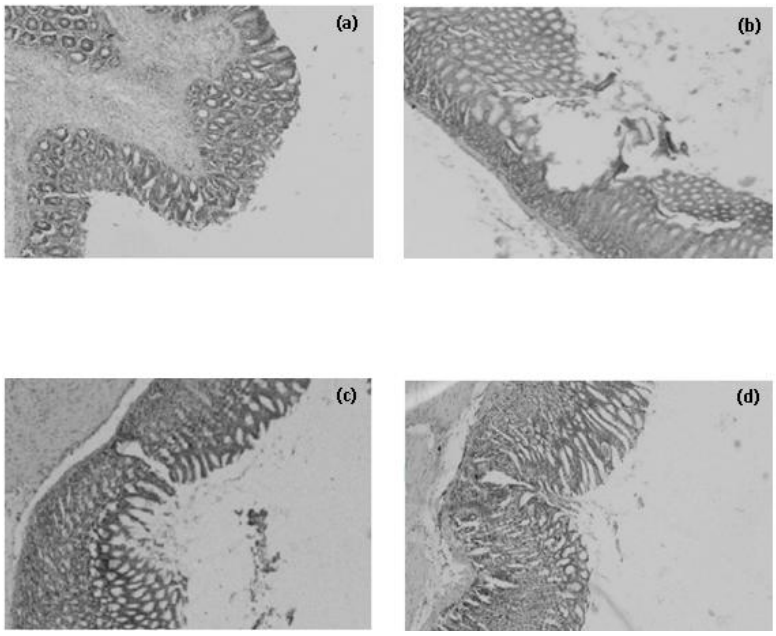

Figure 1:Histopathological evaluation of floating microspheres containing Ranitidine hydrochloride (RHCL) on ethanol (EtOH) induced gastric lesion in rats (Hematoxylin and eosin10x). (a) Normal mucosal wall. (b) Section of ulcerated stomach of ethanol induced ulcer rats showing hemorrhagic lesion in mucosal layer, edema of submucosal layer and necrosis of surface mucous cells of gastric mucosa. (c) The stomach wall of RHCL (50 mg/kg) pretreated rats showing regeneration of mucosal lesions and epithelization are apparent even after exposure of EtOH less infiltration and edema. (d) Section of stomach wall of RHCL (100 mg/kg) pretreated rats showed near to normal cytoarchitecture of gastric mucosa.

DISCUSSION

The present study showed that the Ranitidine hydrochloride (RHCL) floating microspheres possess gastroprotective activity as evidenced by its significant inhibition in the formation of ulcers induced by various physical and chemical agents. Stress plays an important role in aetiopathology of gastro-duodenal ulceration. Stress-induced ulcers are probably medicated by histamine release with enhancement in acid secretion, reduction in mucous production[21], increase in gastric motility, vagal over activity mast cell degranulation[22] decreased gastric mucosal blood flow and decreased prostaglandin synthesis[23] are involved in genesis of stress induced ulcers. Stress-induced ulcers also involve damage by reactive oxygen species (ROS) apart from acid and pepsin related factors.[24] Free radicals affect lipids by initiating peroxidation. Superoxide anion radical ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($OH^{\bullet}$) are important ROS causing tissue damage[25] and lipid peroxide level is an indicator for the generation of ROS in the tissue. The experimental data stated that the CRS aggravated the ulcer severity, and lipid peroxidation as compared to unstressed rats. The higher lipid peroxidation and SOD levels indicated increased production of $O_2^{\bullet-}$ within the tissue as elevated $O_2^{\bullet-}$ level was thought to increase the concentration of cellular radical level. These radicals functioned in concert to induce cell degeneration via peroxidation of membrane lipids, breaking of DNA strands and denaturing cellular proteins.[26] This effect was significantly reversed by prior administration of RHCL providing a close relationship between free radical scavenging activity. SOD scavenges the superoxide radical; one of the ROS responsible for lipid peroxidation. This reaction leads to increase in the peroxyl radical H_2O_2 and its accumulation due to decreased CAT level, which is also capable of producing more oxidative damage.[27] RHCL also inhibited the oxidation of reduced glutathione in a dose-

dependent manner. The importance of thiols especially of cysteine and glutathione to lymphocyte function has been known for many years. GSH is a non-enzymic mode of defense against the free radicals. Henke stated that the central nervous system played an important role in stress ulceration and regulation of plasma corticosterone.[28] The antioxidant mechanism plays an important role in the treatment of peptic ulcers. Oka et al. developed an in vivo experimental ulcer model in order to assess the impact of antioxidant mechanism on antiulcerogenic activity[19] and RHCL almost completely protected gastric ulceration by scavenging the free radicals that involved in the endocrinological (plasma corticosterone) response. Ethanol-induced gastric lesions are due to superficial damage to mucosal cells caused by the direct action of ethanol and gastric acid is not involved in the formation of such lesions.[29] The resulting gastric mucosal damage may be partly due to free radical formation.[30] Ethanol is metabolized in the body to cause increased production of O^{2-} within the tissues, and simultaneously this increased the cellular free radical concentration. These free radicals caused breaking of DNA strands and protein denaturation[31] oral pretreatment with RHCL before EtOH administration prevented depletion of gastric wall mucus. Pylorus ligation induces gastric ulcers due to accumulation of gastric secretion in the stomach.[17] Pylorus ligation-induced ulcers are due to autodigestion of the gastric mucosa and break down of the gastric mucosal barrier.[32] The RHCL significantly decreased the total acidity and gastric content indicating that it has both gastric antisecretory and gastric cytoprotective effects in a dose dependent manner. Gastric ulcer is often a chronic disease and it may persist for 10–20 years characterized by repeated episodes of healing and re-exacerbations. The healing of chronic gastric ulcer induced by acetic acid closely resembles to the healing of peptic ulcers.[19] Acetic acid-induced ulcer better resembles clinical ulcers in location, chronicity, severity and servers as the most reliable model to study healing process.[33] RHCL significantly healed the penetrating ulcers induced by acetic acid after 5 and 10 days treatment in dose dependent manner.

CONCLUSION

In conclusion, result showed that the floating microspheres containing Ranitidine hydrochloride (RHCL) at a dose of 100mg/kg possesses the more significant anti-ulcer activity against stress induced, pylorus ligation, and ethanol induced gastric ulcers and acetic acid-induced chronic ulcer as compared to 50mg/kg dose of RHCL. Thus gastroretentive floating microspheres of Ranitidine hydrochloride supposed to remain in the stomach for longer period of time and give controlled release. These formulations can reduce dosing frequency, decrease side effects and improve patient compliance. In vivo antiulcer activity also supports the above statement showing significant reduction in gastric ulceration. This activity thus lends pharmacological credence to the suggested use of floating microspheres containing Ranitidine hydrochloride in the treatment and management of ulcer.

ACKNOWLEDGEMENTS

The authors would like to gratefully acknowledge the Director of Babu Banarsi Das National Institute of Technology and NBRI for providing necessary facilities for this research work.

REFERENCES

1. Rao CV, Sairam K, Goel RK. Experimental evaluation of *Bocopa monniera* on rat gastric ulceration and secretion. Indian J Physiol Pharmacol 2000; 44: 435-141.
2. Das D, Banerjee RK. Effect of stress on the antioxidant enzymes and gastric ulceration. Mol. Cell Biochem 1993; 125: 115-125.
3. Valle DL. Peptic ulcer diseases and related disorders. Vol. 16; eds. Harrison's Principles of Internal Medicine, Braunwald E, Fauci AS, Kasper DL, Hauser SL, Longo DL, Jameson JL. McGraw-Hill, New York, 2005. p.1746–1762.
4. Manonmani S, Viswanathan VP, Subramanian S, Govindasamy S. Biochemical studies on the antiulcerogenic activity of cauvery 100, an ayurvedic formulation in experimental ulcers. Indian J Pharmacol 1995; 27: 101–105.
5. Indian Pharmacopoeia. Controller of Publication, Govt. of India, 7th ed, New Delhi, 1996; 2: p. 659.
6. Brijesh S. Dave, Avani F. Amin, Madhabhai M. Patel. Gastro retentive drug delivery system of ranitidine hydrochloride formulation and in vitro evaluation, AAPS Pharm sci tech 2004; 5: 1-6.
7. The Merk Index. An Encyclopedia of Chemicals, Drug and Biologicals. Merck Research Laboratories, Division of Merck & Co. INC. Whitehouse Station, NJ. USA, 12th ed,1996; p. 8110.
8. Ibrahim M. El-Bagory, Ehab AH. Effect of sphere size, polymer to drug ratio and plasticizer concentration of the release of theophyllin from ethyl cellulose microspheres. Saudi Pharm J 2007; 15: 213-217.
9. Emeje MO, Kunle OO. Compaction characteristics of ethyl cellulose in presence of some channeling agent: technical note. AAPS Pharma Sci Teh 2006; 7: 58.
10. Patel A, Ray S, Thakur RS. *In-vitro* evaluation and optimization of controlled release floating drug delivery system of metformin hydrochloride. DARU 2006; 14: 57-64.
11. Gupta, MB, Nath R, Gupta GP, Bhargava KP. A study of the antiulcer activity of diazepam and other tranquillo sedatives in albino rats. Clin Exp Pharmacol 1985; 12: 61–63.
12. Sanyal AK, Pandey BL, Goel RK. The effect of a traditional preparation of copper, tamrabhasma, on experimental ulcers and gastric secretion. J Ethnopharmacol 1982; 5: 79-89.
13. Jamall IS, Smith JC. Effects of cadmium on glutathione peroxidase, superoxidase dismutase and lipid peroxidation in the rat heart: a possible mechanism of cadmium cardiotoxicity. Toxicol Appl Pharmacol 1985; 80: 33-42.
14. Aebi H. Catalase. In: Bergmeyer H.U. (Ed.), Methods in Enzymatic Analysis. Academic Press Inc, New York, vol., 3: 1974; p. 673-686.
15. Nishikimi M, Rao NA, Yagi K. The occurrence of superoxide anion in the reaction of reduced PMS and molecular oxygen. Biochem Bioph Res Comm 1972; 46: 849–854.
16. Kakkar P, Das B, Viswanathan PN. Modified spectrophotometric assay of SOD. Indian J Biochem Bioph 1984; 2: 130-132.
17. Shay H, Komarov SA, Fele SS, Meranze D, Gruenstein H, Siplet H. Asimple method for uniform production of gastric ulceration in rat. Gastroenterology 1945; 5: 43–46.
18. Trease GE, Evans WC.Text Book of Pharmacognosy, 13th ed, 1992; p. 202–205.
19. Okabe S, Roth JLA, Pfeiffer CJ. Differential healing periods of the acetic acid ulcer model in rats and cats. Experientia 1971; 27: 146–148.
20. Hollander D, Taranawski A, Krause WJ, Gergely H. Protective effect of sucralfate against alcohol induced gastric mucosal injury in the rat, Macroscopic, histologic, ultrastructural, and functional time sequence analysis. Gastroenterology 1985; 88: 366-374.
21. Rao CV, Verma AR, Vijayakumar M, Rastogi S. Gastroprotective effect of standardized extract of *Ficus glomerata* fruit on experimental gastric ulcers in rats. J Ethnopharmacol 2008; 115: 323-326.
22. Cho CH, Ogle CW. Cholinergic-mediated gastric mast cell degranulation with subsequent histamine H1- and H2-receptor activation in stress ulceration in rats. Eur J Pharmacol 1979; 55: 23-33.
23. Rao CV, Maiti RN, Goel RK. Effect of mild irritant on gastric mucosal offensive and defensive factors. Indian J Physiol Pharmacol 1999; 44: 185-191.
24. Miller TA. Mechanisms of stress-related mucosal damage. Am J Med 1987; 83: 8-14.
25. Fridovich I. Biological effects of superoxide radical. Arch Biochem Biophy 1986; 247: 1-11.
26. Halliwell B, Gutteridge JMC. Oxygen free radicals and the nervous system. Trends Neurosci 1985; 8: 22-26.
27. Boyd SC, Sasame HA, Boyd MR. Gastric glutathione depletion and acute ulcerogenesis by diethylmaleate given subcutaneously to rats. Life Sci 1981; 28: 2987-2992.
28. Henke PG.The hypothalamus-amygdala axis and experiment gastric ulcers. Neurosci Biobehav Rev 1979; 3: 75-78.
29. Oka S, Ogino K, Hobara T, Yoshimura H, Okazaki Y, Takemoto T, Ishiyaoa H, Imaizumi T, Yamasaki K, Kanbe T. Role of active oxygen species in diethyldithiocarbamate-induced gastric ulcer in the rat. Experientia 1990; 46: 281–283.

30. Miller TA, Henagan JM. Indomethacin decreases resistance of gastric barrier to disruption by alcohol. Digest Dis Sci 1984; 29: 141-149.
31. Oates PJ, Hakkinen JP. Studies on the mechanism of ethanol-induced gastric damage in rats. Gastroenterology 1988; 94: 10-21.
32. Al-Bekairi AM, Qureshi S, Ahmed MM, Afzal M, Shah AH. A study of uric acid pretreatment for the protection of rat gastric mucosa against toxic damage. Food Chem Toxicol 1992; 30, 525–552.
33. Sairam K, Rao CV, Dora BM, Agrawal VK, Goel RK. Antiulcerogenic activity of methanolic extract of *Emblica officinalis*. J Ethnopharmacol 2002; 82: 1–9.33. Okabe S, Pfeiffer CJ. Chronicity of acetic acid ulcer in the rat stomach. Dig Dis 1972; 7: 619–629.