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Antiulcer Effect of Gastroretentive Spherical Matrices of Alginate-Chitosan Containing Ranitidine HCl

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Abstract : Background: Ranitidine hydrochloride (RHCl) has short biological half life and poor bioavailability, hence, the treatment of gastric ulcer with RHCl conventional dosage form is less effective. Therefore, it is necessary to prepare and evaluate the anitulcer effect of gastroretentive spherical matrices of Alginate-Chitosan(Alg-Ch) containing RHCl.
Purpose: The purpose of this study was to prepare and evaluate antiulcer effect of gastroretentive spherical matrices of Alg-Ch containing RHCl.
Methods: The gastroretentive spherical matrices of Alg-Ch containing RHCl were prepared by using an Alg:Ch ratio of 1:1. The antiulcer effect was evaluated by two methods, i.e., pylorus ligation-induced ulcer and HCl-induced ulcer methods. In the pylorus ligation induced ulcer method, parameters of gastric secretion (volume, pH, and free and total acidity) were determined. In the HCl-induced ulcer method, one hour after induction, the rats were given gastroretentive spherical matrices of Alg-Ch containing RHCl (once a day) for 3 days. RHCl conventional tablet was used as standard to comparison. Gastric ulcers were evaluated by macroscopic and microscopic observation for both methods.
Results: In the pylorus ligation-induced ulcer method, gastroretentive spherical matrices of Alg-Ch containing RHCl showed significant decrease in gastric juice volume, total acidity and free acidity. While, pH of gastric juice was increased. The number of gastric ulcers and ulcer inhibition in the rats received gastroretentive spherical matrices of Alg-Ch containing RHCl were 0.33 and 89.46% compared to rats received RHCl tablet which were 3.00 and 99.46%. In the HCl-induced ulcer method, the number of gastric ulcer in the rats that received gastroretentive spherical matrices of Alg-Ch containing RHCl was zero with curative ratio of 100% after 3 days treatment, while the number of gastric ulcer in rats that received RHCl conventional tablet was 2,88 with curative ratio of 75.33%. The result of histopathology examination did not show ulcer of the gastric mucosa in the test group rats compared to the rats that received conventional RHCl tablet still had ulcer.
Conclusion: It can be concluded from these results that the antiulcer effect of gastroretentive spherical matrices of Alg-Ch containing RHCl is more effective than conventional tablet of RHCl.
Keywords: Alginate-Chitosan, spherical matrices, ranitidneHCl, antiulcer.

Introduction

Gastric ulcer is a sore on the lining of the stomach. Gastric ulcer disease is a disease of the gastrointestinal tract that are commonly found in people worldwide. Pathogenesis of peptic ulcer is an imbalance between aggressive factors (gastric acid, non steroid antiinflamation drugs, Helicobacter pylori, alcohol) that can damage stomach and defensive factors (mucin, bicarbonate, prostaglandin) which maintain

the integrity of the gastric mucosa.[1]The main goal for protection of the gastric mucosa from gastric acid is pharmacologic control of gastric acid secretion.

Ranitidine, a histamine H2-receptor antagonist, is now well established as a potent inhibitor of gastric acid secretion effective in the treatment and prophylaxis of gastrointestinal ulcers caused by gastric acid secretion. It is used to treat gastric ulcers, duodenal ulcers, Zollinger-Ellison syndrome, and gastroesophageal reflux disease. The biological half-life of RHCl is short (2.5-3 hours). RHCl is absorbed only in the upper part of the small intestine and shows a low bioavailability that is 50%.[2-3] The absorbtion of RHCl will be reduced due to the decomposition and metabolism by microbes in the colon which causes low-bioavailability.[4] Sustained release gastroretentive dosage form of RHCl can remain in gastric region for long time and hence will increase the efficacy of the drug. Recently, several gastroretentive drug delivery approaches being designed and developed, including: high density systems, low density system (floating), mucoadhesive system and swellable systems.[5]

Alg and Ch are natural polymers that are biocompatible, biodegradable, non toxic and mucoadhesive.[6-7]Alg has been used in the preparation of periodental drug delivery systems.[8]Alg-ch film has been used to prepare gastroretentive drug delivery system of antacid.[9]The spherical of Alg-Ch matrices posses the highest swelling degree and mucoadhesive force, and give extended drug release in simulated gastric fluid (SGF) compare to Alg, Ch, and Calcium Alg-Ch matrices.[10] The study of the effect of Alg-Ch ratio on the swelling, mucoadhesive and drug release from Alg-Ch spherical matrices found that Al-Ch (1:1) matrices shows the highest swelling and mucoadhesive properties, and give the RHCl release follows a sustained release type (more than 10 hours) in the medium of SGF.[11]The cationic amino groups of chitosan interact electrostatically with the anionic carboxylate groups of Alg to form polyelectrolyte complexes in the form of mucoadhesive matrix of Alg-Ch to achieve a prolonged gastric residence time of RHCl. Prolonged gastric retention increases duration of drug release, reduces drug waste, and could be advantageous for local action in the upper part of the small intestine e.g. treatment of gastric ulcer. Therefore, in this research we studied further antiulcer effect of gastroretentive spherical matrices of Alg-Ch containing RHCl in rats.

Materials and Methods

Materials

Sodium Alg300-400 cP (Wako Pure Chemical Industries, Ltd Japan),Ch (Funacoshi, Ltd Japan) RHCl (Peddadevulpalli, India), HCl, liquid paraffin, xylol, xylene, hematosillin, and eosin were the product of Merck. Canada balsam (Entellan), and formaldehyde obtained from P.T. Rudang, sodium chloride (PT Merk). Rantin Tablet (Kalbe Farma),

Animals

Rattus-norvegicus rats weighing 180-200 g were maintained in standard animal house, given standard pellet diets and tap water adlibitum. The rats were fasted from all medications at least 2 weeks before the treatment was given. All the rats were adhered to the Standard Operation Procedures and approved by the Animal Research Ethics Commitees of FMIPA University of Sumatera Utara.

Preparation of gastroretentive spherical matrices of alginate-chitosan containing RHCl

The gastroretentive spherical matrices of Alg-Ch containing RHCl were prepared with Alg-Ch ratio of 1:1 followed the previous procedure.[11]Alg incorporated with Ch and RHCl in a mortar with the addition of starch mucilage as binding agent to form a compact mass and then divided into ten parts and each part was molded to be spherical matrix

Evaluation of antiulcer effect on pylorus ligation-induced ulcer

Gastric ulceration was induced in the rats by pylorus ligation. After 36 hours fasting, the rats were divided into 4 groups, each group consisting of six rats.

Group I (Normal control group**) :** Received distilled water only

Group II (Ulcer control group) : Pylorus ligation

Group III (Standard Group) : Received conventional tablet of RHCl (13.5 mg/ kg p.o) + pylorus ligation

Group IV (Test Group) : Received gastroretentive spherical matrices of Alg-Ch containing RHCl (13.5 mg/kg p.o) + pylorus ligation.

After thirty minutes of treatment, pyloric ligation was done by ligating the pylorus end of stomach of rats under ketamine (1 ml/kg) i.p. as anesthesia. Ligation was done without causing any damage to the blood supply of the stomach.[12-13]The stomach was replaced carefully and the abdomen was closed with interrupted sutures. Nineteen hours later, each rat was sacrificed and stomach was dissected out. Contents of stomach was drained into a centrifuge tube and centrifuged at 3000 rpm for 10 min and subjected to analysis for volume, pH, free and total acidity. The emptied stomach was opened along the greater curvature, rinsed with saline, ulcer lessions were counted using a magnifying glass. The ulcer index (UI) and percentage of inhibition of ulcer were determined.

Determination of free and total acidity

Gastric juice was titrated with 0,01 N NaOH solution by using methyl red reagent until red color disappeared and the color of solution became yellow. The volume of alkali added were noted. This volume corresponds to free acidity. Two drops of phenolphthalein solution were added and titration was continued until a permanent pink color was observed. The total volume of alkali added corresponds to total acidity. Acidity was calculated using following formula.[13]

$$\text{Acidity} = \frac{Volume\ of\ NaOH\ x\ Normality\ of\ NaOH\ x\ 100}{0.1} mEq/L$$

Macroscopic evaluation of stomach

The emptied stomach was opened along the greater curvature, rinsed with saline, ulcers were counted. The ulcer index (UI) and the percentage of inhibition against ulceration were measure using the expressions:[14]

Ulcer index (U.I) = [Ulcerated area/total stomach area] x 100

% Ulcer inhibition = [U.I. in control – U.I. in test] / U.I in control x 100

Histopathological evaluation of gastric ulcers

The gastric tissue samples were fixed in 10% formalin. The processed tissues were embedded in paraffin blocks and about 5-µm thick sections were cut using an microtome. These sections wete stained with hematoxylin and eosin and observed using a microscope.

Evaluation of antiulcer effect on HCl-induced ulcer

The rats were fasted for 36 hours before treatment. Gastric ulceration was induced in the rats by administration of 1 ml HCl 0.6 N.[15]

The rats were divided into 4 groups, each group consisting of six rats.

Group I (Normal Control) : Received distilled water only

Group II (Negative control) : Received 1 ml HCl 0.6 N

Group III (Standard Group) : Received 1 ml HCl 0.6 N + tablet of RHCl (13.5 mg/ kg p.o) once a day for 3 days

Group IV (Test Group) : Received 1 ml HCl 0.6 N + gastroretentive spherical matrices of Alg-Ch containing RHCl (13.5 mg/kg p.o) once a day for 3 days

The rats were sacrificed one day after the last administration. The stomach was removed, fixed with formalin and incised along the greater curvature. Subsequently, the curative ratio (%) were measured using the following formula:[16]

$$\text{Curative ratio} = \frac{Control\ (ulcer\ index) - Test\ (ulcer\ index)}{Control\ (ulcer\ index)}\ x\ 100\ \%$$

Results and Discussion

Preparation of gastroretentive spherical matrices of Alg-Ch containing RHCl

The gastroretentive spherical matrices of Alg-Ch (1:1) containing RHCl were prepared. Each matrix contained RHCl 2,7 mg for rat with body weight in 200 gram (13,5 mg/kg). The mean diameter and weight of matrices were 1.57 ± 0.21 mm and 10.5 ± 0.44 mg. The photograph of gastroretentive spherical matrices of Alg-Ch containing RHClis shown in Figure 1.

Figure 1. Photograph of gastroretentive spherical matrices of Alg-Ch containing RHCl that were given to the rats.

Evaluation of antiulcer effect of gastroretentive spherical matrices of Alg-Ch containing RHCl on pylorus ligation induced ulcer model

The pyloric ligation-induced gastric ulcer method was particularly used to study the antiulcer effect on gastric secretion. The ligation of the pyloric end of the stomach causes accumulation of gastric acid in the stomach that produces ulcers.RHCl reduce secretion of gastric acid, thus effective in reducing development of gastric ulcers. From this study, oral administration of RHCl tablet (standard group) and gastroretentive spherical matrices of Alg-Ch containing RHCl (test group) showed protection against gastric ulcers in the experimental rats, it was indicated by reducing of acidity, ulceration, and raised of gastric pH. These parameters were significant different with pylorus ligation control group ($p<0.05$) as listed in Table 1.

Table 1. Effect of gastroretentive spherical matrices of Alg-Ch containing RHCl on gastric juice, acidity, and pH in pylorus ligated rats (n=6).

Rats group	Volume of Gastric juice (mL)	pH	Free acidity (mEq/L)	Total acidity (mEq/L)
Normal control control	a 0,34 ± 0,08	a 3,67 ± 0,26	a 15,33 ± 3,27	a 28,67 ± 2,07
Ulcer control	b 5,95 ± 0,42	b 2,47 ± 0,16	b 82,08 ± 4,25	b 119,30 ± 3,11
Standard group (RHCl tablet 13.5 mg/kg)	c 4,27 ± 0,46	c 4,20 ± 0,29	c 47,08 ± 3,32	c 85,51 ± 1,70
Test group (Gastro retentive of	d 3,52 ± 0,41	c 5,15 ± 0,67	d 24,47 ± 3,64	d 34,85 ± 1,84

Different superscript letters in the same columm denotes significant difference at $p<0.05$

Table 1 indicates that standard and test groups having an antisecretory effect. But, the antisecretory effect of spherical of Alg-Ch matrices containing RHCl was stronger than RH conventional tablet. The ulcer index inthe rats received gastroretentive spherical matrices of Alg-Ch containing RHClwas lower than rats received RHCl tablet as showed in Table 2.

Table 2. Effect of gastroretentivespherical matrices of Alg-Ch containing RHCl on ulcer index and inhibition of ulcer in pylorus ligated rats (n=6)

Rats group	Ulcer index	Inhibition (%)
Normal control	0.00	0.00
Ulcer control	a 1.86 ± 1.46	0.00
Standard (RHCl tablet 13.5 mg/kg)	b 0.26 ± 0.19	a 89.46 ± 6.36
IV Test (Gastroretentive of Alg-Ch matrices	c 0.01 ± 0.02	b 99.46 ± 1.32

Different superscript letters in the same columm denotes significant difference at $p<0.05$

Macroscopic observation of rat stomach

The alimentary canal comprises of four concentric layers: mucosa, submucosa, muscularisexterna, and serosa. The mucosa of the fundic stomach is composed of the usual three components: (1) an epithelium lining the lumen; (2) an underlying lining connective tissue, the lamina propria; and the smooth muscle layers forming the muscularis mucosae.[17].Figure 2shows a rats normal stomach(A), gastric mucosa (B), and histological of partially gastric mucosa (C) that was observed in this experiment.

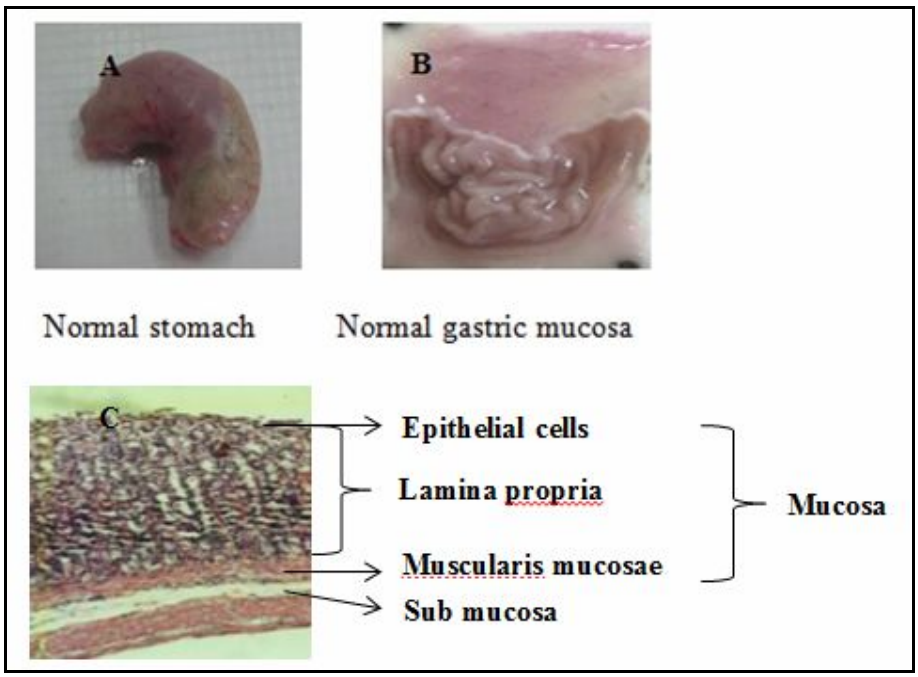

Figure 2. Normal stomach of rat A.Normalstomach B. Normal gastric mucosa C. Histological of normal gastric mucosa

The macroscopic observation of pylorus ligation induced ulcer method experiment was shown in Fig.3. The gastric mucosa of normal control group showed intach mucosa (Fig.3A). The gastric mucosa of ulcer control (3B) and and standard group (Fig.3C) showed gastric ulcers, but the test group that received gastroretentive matrices of Alg-Ch containing RHCl (Fig 3.D) demonstrated only one rat of six rats showed gastric ulcer.

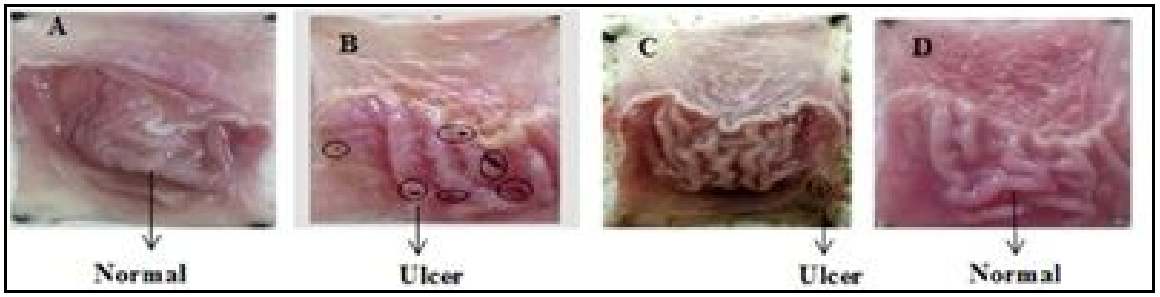

Figure 3.Macroscopical appearance ofrats stomach in pylorus ligation induced ulcer model. A: Normal Control, B:Ulcer Control, C: Given RHCl tablet), D: Given gastroretentive spherical matrices of RHCl.

The results of this study indicated that oral administration of gastroretentive matrices of Alg-Ch containing RHCl and RHCl conventional tablet prevented gastric ulcer caused by pylorus ligation, but the ulcer index of test group was lower than control and standard groups (Fig.4).

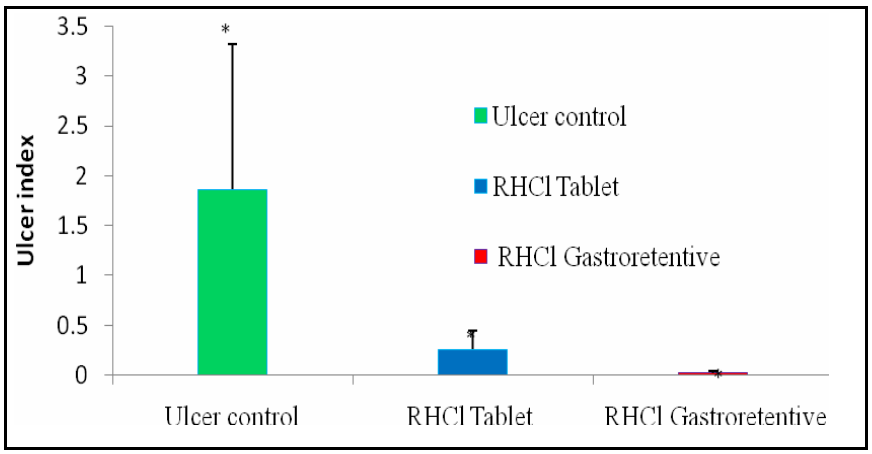

Figure4. Effect of gastroretentive spherical matrices of RHClon ulcer Index (pylorus ligation-induced ulcer method) Results are mean ± SD determined by Kruskal Wallis followed by Mann Whitney's test: *P < 0.05.

The percentage of inhibition of gastric ulcer using gastroretentive matrices of Alg-Ch containing RHCl showed higher and significant different ($p<0.05$) with conventional tablet of RHCl. These results is shown in Fig 5.

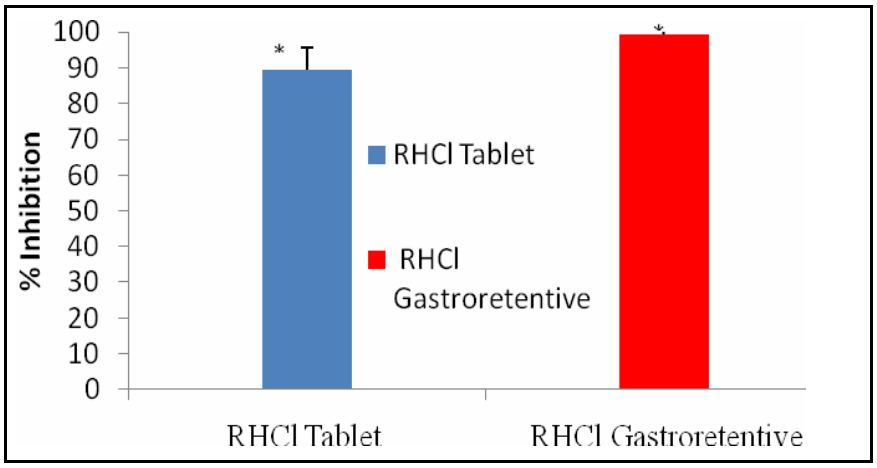

Figure5. Effect of RHCl tablet and gastroretentivespherical matrices of RHClon Inhibition of ulcer induced by pylorus ligation.

Results are mean ± SD determined by T test: *P<0,05

Microscopic (histopathology) observation of rat stomach

The histopathological stomach tissue of normal control rat is shown in Fig. 6A. The histopathogical the stomach tissue of test group of rats that received gastroretentive spherical matrices containing RHCl (Fig.6D) showed intact mucosa or did not show erosion of the gastric mucosa like the stomach tissue of normal control, but stomach tissue of ulcer control group (Fig.6B) and standard group (Fig.6C) showed erosion of gastric mucosa.

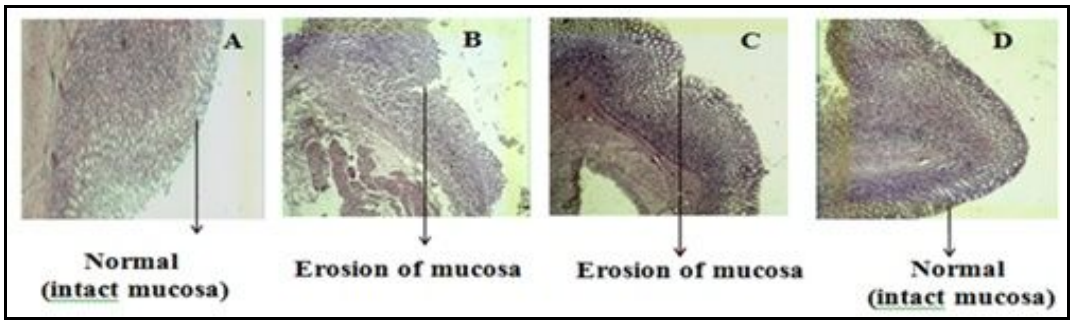

Figure 6. Histopathology of rats gastric mucosa inpylorus ligation-induced ulcer method (Hematoxyllin and Eosin). A: Normal control, B: Ulcer control, C: Given RHCl tablet, D: Given gastroretentive spherical matrices of RHCl.

Evaluation of antiulcer effect of gastroretentive spherical matrices of alginate-chitosan containing RHCl on HCl induced ulcer model

The induction of fasted rats with 0.6 N HCl solution caused damage of gastric mucosal barrier. The damages of gastric mucosal barrier caused the back diffusion of HCl and lead to destruction of capillary and venous blood so that resulting stomach bleeding. Hydrochloric acid also change pepsinogen to pepsin which will lower the barrier function ofgastric mucosa.[18] The results of this study found that treatment with RHCl tablet and gastroretentive spherical matrices of Alg-Ch containing RHCl reduced ulcer index more effective than control group ($p<0.05$), but Alg-Ch spherical matrices containing RHCl was more effective than RHCl conventional tablet (Table 3). The ulcer index was zero after 3 days treatment but using conventional tablet it was 0.44 (Fig.7).

Curative ratio of gastric ulcer in rats that received gastroretentive spherical matrices containing RHCl was 100%, while the rats that received RHCl tablet it was 75.33 % (Fig 8). Thus, the ulcer healing of the rats that are givengastroretentive spherical matrices containing RHCl is faster than rats that are given RHCl tablet.

Table 3. Antiulcer effect of gastroretentivespherical matrices of containing RHCl based on ulcer index and curative ratio.

Group	Ulcer Index	Curative ratio
I Normal Control	0.00	0.00
II Negative Control	a 1.77 ± 0.43	0.00
III RHCl tablet (13,5 mg/kg)	b 0.44 ± 0.15	a 75.33 ± 8.71
IV Gastroretentif spherical matrices of RHCl (13,5 mg/kg)	0.00	100.00 ± 0.00

Results are mean ± SD determined by Kruskal Wallis followed by Mann Whitney'stest. Different superscript letters in the same columm denotes significant difference at $p<0.05$.

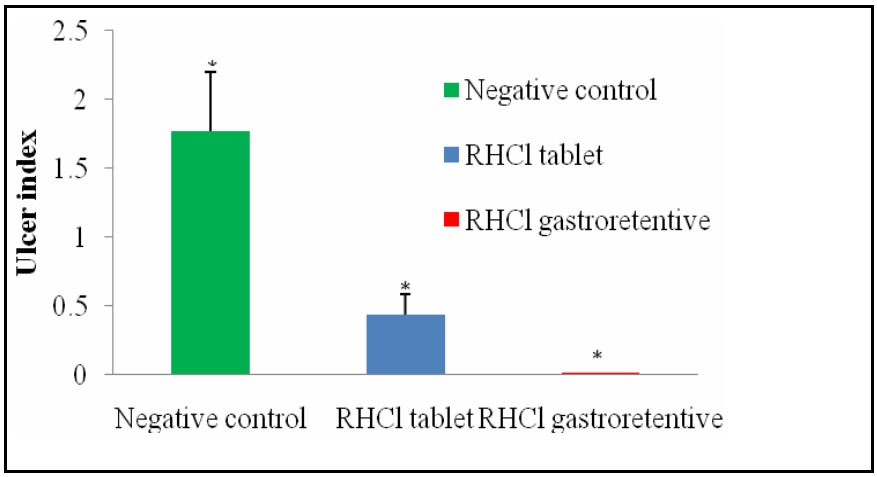

Figure7.Effect of gastroretentive spherical matrices of RHCl on ulcer index (HCl induced-ulcer method).Results are mean ± SD determined by Kruska Wallis followed by Mann Whitney' test: *P < 0.05.

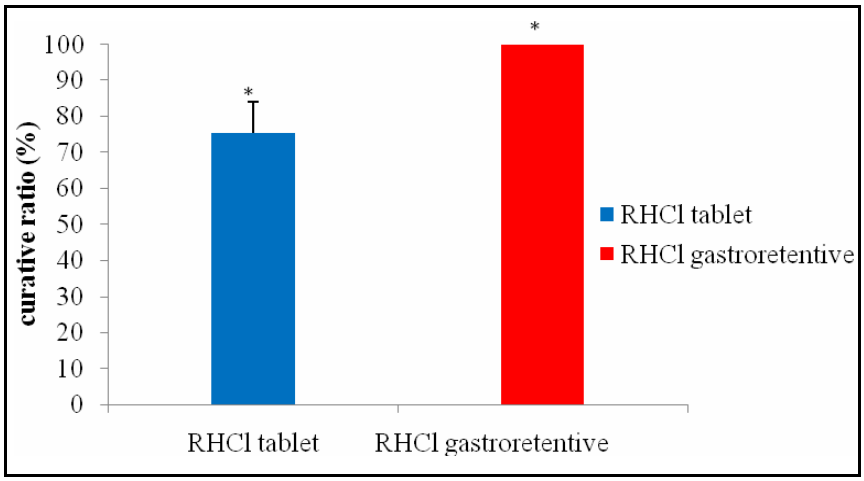

Figure 8. Effect of RHCl tablet and gastroretentive spherical matrices of RHCl on curative ratio in HCl-induced ulcer method Results are mean ± SD determined by T test: *P < 0.05.

Macroscopic and microscopic observation of rats stomach after 3 days of treatment.

The results of macroscopic observation of gastric mucosa of normal control rat did not indicate the presence of ulcers. After 3 days treatment the gastric mucosa of negative control and standard rats group that were given RHCl tablet still showed ulcers. But, the gastric mucosa of test rats group that were given gastroretentive spherical matrices of alginate-chitosan contained RHCl did not show ulcers as shown in Figure 9. The histological section of gastric mucosa of normal control did not show erosion. The results of microscopic observation of the negative control and standard control rats group showed erosion of gastric mucosa. However, the gastric mucosa of rats that were given gastroretentive spherical matrices of RHCl did not show erosion as shown in Figure 10. This is due to the gastroretentive spherical matrices of alginate-chitosan containing RHCl intimate contact to stomach mucosa (mucoadhesive) and release the drugs slowly for longer time (sustained release) and consequently increase the efficacy of drug and resulting the ulcer healing was faster compared to RHCl convensional tablet.

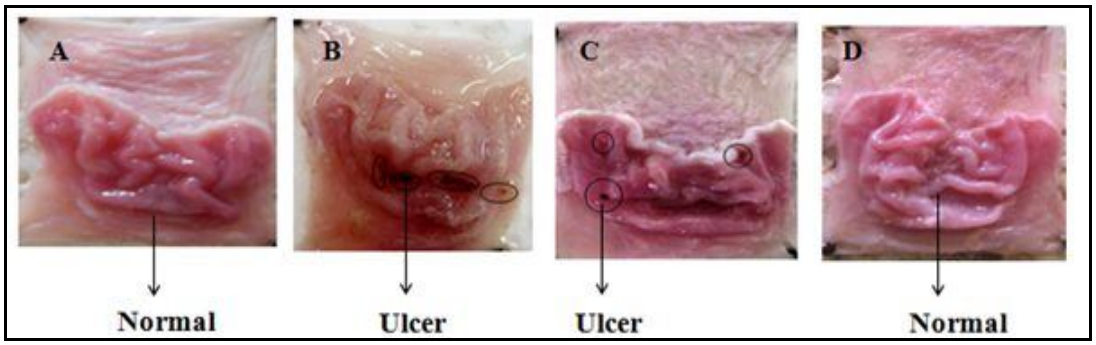

Figure9.Macroscopical appearance of gastric mucosa inHCl-induced ulcer method. A: Normal control, B: Negative control, C: Given RHCl tablet, D: Given gastroretentive spherical matrices containing RHCl

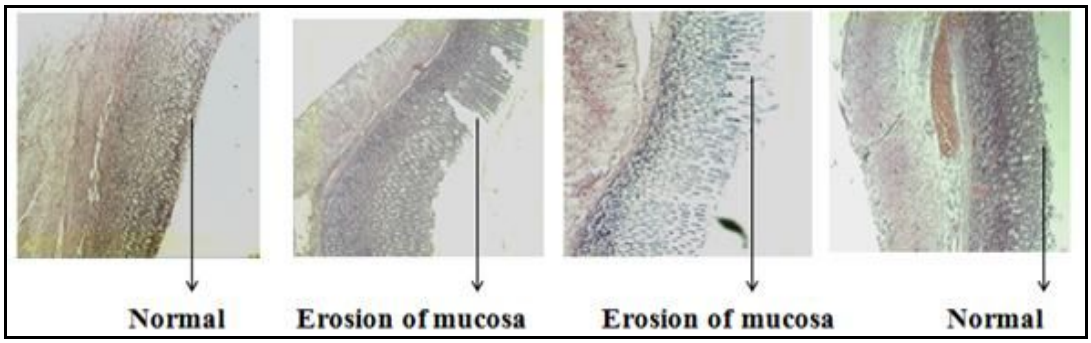

Figure10.Histopathology of gastric mucosa inHCl-induced ulcer method (Hematoxyllin and Eosin x 10) A: Normal control, B: Negative control, C: Given RHCl tablet, D: Given gastroretentive spherical matrices containing RHCl.

Conclusion

Based on the results, it can be concluded that gastroretentive Alg-Ch spherical matrices containing RHCl is more effective than RHCl conventional tablet in healing the gastric ulcer. Even though, it is needed further study in human experiment.

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