



Preparation and Evaluation of Floating-Mucoadhesive Alginate Beads as Gastroretentive Drug Delivery System of Antacids

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Abstract: Background: The conventional antacids have a short duration of action, due to gastric emptying effect, it is less effective. Therefore, a gastroretentive of antacids is needed to prolong gastric residence time of antacids.

Purpose: The aim of this study was to prepare floating-mucoadhesive beads containing antacids that could float and adhere in stomach to prolong retention time of antacids and to determine the healing effects of antacids.

Methods : Beads were formulated by using various concentrations of alginate (0.75-1.75%), paraffin liquid, $\text{Al}(\text{OH})_3$ and $\text{Mg}(\text{OH})_2$ that were immersed in 0.15 M CaCl_2 solution. Buffering action of antacids beads to 0.1 N HCl solution was determined on simulated gastric acid secretion. Mucoadhesive of beads was tested by using rats stomach by DuNoy tensiometer and swelling properties in 0.1 N HCl solution was determined based on the increment of bead size. The healing effects of antacids beads on gastric ulcers using male rats induced by 0.6 N HCl solution. Examinations of gastric ulcers were observed macroscopically (number of lesions and lesion index) and microscopically (histopathology).

Results : The diameter of formed beads was about 2.17 mm and containing 10 mg antacids. Beads had no floating lag time and beads could stay floating for more than 12 hours. On buffering action test, beads containing 1.35% alginate could maintain the pH at 3.0 to 3.7 for 9 hours, the mucoadhesive values was 58.73 ± 0.05 dyne and the swelling index was 31.08 ± 7.2 . The healing effects of these beads showed healing for four days, marked with the number of lesions and lesion index were zero and the intact mucosa. On the other hand, rats that received conventional antacids tablet after four days still had lesions and the lesion index was 2.67 ± 1.03 .

Conclusion: Alginate floating-mucoadhesive beads containing antacids is potential to be used as new gastroretentive drug delivery system for antacids.

Key words: Alginate; floating-mucoadhesive; antacids; gastric lesions; healing.

Introduction

Peptic ulcers disease is characterized by epigastric pain, loss of appetite, and weight loss caused by inflamed excavations (ulcers) of the mucosa and underlying tissue of the upper gastrointestinal tract that is exposed to acid-pepsin secretions. The ulcers caused damage to mucous membrane. Mucous membrane normally protects the esophagus, stomach and duodenum from gastric acid and pepsin.¹ The pathogenesis of gastric ulcers due to weak defense of mucosal tissue, is caused by large quantities of gastric acid. During most of the day, the food can stimulate gastric acid secretion also neutralize gastric acid and maintain gastric pH between 3 and 5. When the stomach is empty, approximately 2 to 3 hours after a meal, the gastric pH will

decrease and ulcer patients are likely to experience pain that can be treated by consuming antacids. Acid-mediated pain usually occurs when the pH of the stomach is under 3.²⁻³

Antacids are agents that react with protons in the lumen of the gut. The use of antacids effectively reduce peptic ulcers occurrence when used regularly in large doses which are to raise stomach pH. Aluminum compound also stimulates protective functions of gastric mucosa.⁴ Conventional antacids have a short duration due to short residence time. Conventional antacids are cleared from the empty stomach in 30 minutes due to regular gastric emptying. If antacids were administered while food is in the stomach, the buffering action will last for 2 hours. An additional dose 3 hours after meals will extend buffering time by 1 hour.²⁻³ Ideally, antacids should be rapid in onset and provide continuous neutralization or buffering action. The duration of buffering action is determined largely when antacids is administered.³ Aluminum cause constipation, on the other hand magnesium cause diarrhoea.⁴ This problem can be overcome by combining both of these compounds.⁴

Gastroretentive drug delivery system is an approach to prolong gastric residence time, thereby targeting site-specific drug release in the upper gastrointestinal tract for local effects.⁵ Gastroretentive dosage form can be floating and mucoadhesive system. Floating system has bulk density less than gastric fluids, so it remains buoyant in the stomach without affected by gastric emptying for a prolong period of time.⁶ Bioadhesive system are used as a delivery device within the lumen to enhance drug absorption in a site specific manner.⁶ One of the formulation floating-mucoadhesive beads of clarithromycin for the treatment of *Helicobacter pylori* infection using polymer alginate-HPMC polymer is suitable to treat stomach ulcers.⁷

Alginate is an anionic linear copolymer of (1,4) D-mannuronic acid and L-guluronic acid residues arranged in non-regular blockwise pattern, which is a good mucoadhesive agent.⁸ Alginate can be used for the preparation of periodontal drug delivery systems.⁹ The effect of alg chitosan ratio on the swelling, mucoadhesive, and release of ranitidine from spherical matrices of alg-chitosan showed the highest swelling and mucoadhesive is alginate-chitosan ratio 1:1 and the release of ranitidine hydrochloride from spherical matrices followed sustained release type with Higuchi model drug release.¹⁰⁻¹¹ In previous study, gastroretentive drug delivery system for Al(OH)₃ using alginate-chitosan film as the polymer showed that on simulating gastric acid secretion of film could maintain the pH at 3 - 3.4 up to 6 hours and stay in the stomach up to 7 ± 0.5 hours, but the alginate-chitosan could not form good films with Mg(OH)₂.¹²

In the present study, preparation of alginate floating-mucoadhesive beads¹⁹⁻²⁶ containing antacids, buffering action and mucoadhesive properties, and the healing effects on rats stomach lesions will be discussed.

Materials and Methods

Materials

Sodium alginate 500~600 cp was obtained from Wako Pure Chemical Industries, Ltd. Japan. Al(OH)₃, Mg(OH)₂ were obtained from PT. Mutifa, Indonesia. Paraffin liquid, formaldehyde, ethanol, xylol, xylena, haematoxylin, eosin, calcium chloride, hydrochloric acid, and sodium chloride were product of Merck. Antacids conventional tablet (antasida Doen) was the product of Kimia Farma, Indonesia.

Methods

Preparation of floating-mucoadhesive beads containing antacids

The beads were prepared using various formula (Table 1). Sodium alginate was dissolved in distilled water. Paraffin liquidum was added to sodium alginate solution and the mixture was stirred until paraffin liquidum was emulsified. Antacids were added to this mixture and the mixture homogenized. Then, the mixture was dropped to 0.15 M calcium chloride solution using Komagome pipette. The immersion time was 5 minutes. The resultant beads were washed with distilled water and kept drying in cabinet dryer. From 100 ml mixture was obtained 1000 beads and each bead containing 5 mg Al(OH)₃ and 5 mg Mg(OH)₂.

Tabel 1. Composition of alginate floating-mucoadhesive beads containing Al(OH)₃ and Mg(OH)₂.

Formula	Alginate (%)	Al(OH) ₃ (%)	Mg(OH) ₂ (%)	Paraffin (%)
F1	0.75	5	5	10
F2	1.00	5	5	10
F3	1.35	5	5	10
F4	1.50	5	5	10
F5	1.75	5	5	10
F6	1.35	5	5	0

Determination of buffering action of beads

Buffering action of antacids beads to the 0.1 N HCl solution was determined on simulated gastric acid secretion as described in previous study.¹² The secretion of hydrochloric acid from human stomach in normal condition is 1 mEq/h; and the volume of gastric acid in fasting state was 20-30 ml. Based on this condition, the secretion of gastric acid was simulated by dropping 10 ml/h of 0.1 N HCl solution that was conducted by continuously dropping 10 drops/minute by using micro drip infusion 60 drops/ml to 30 ml of 0.1 N HCl solution at 37 ± 0.5°C containing 40 beads, the solution was stirred with a magnetic stirrer and at the certain interval of time the pH solution was measured using a pH meter.

Particle size analysis of beads

The dried beads were randomly selected and the diameter of the beads were determined using a micrometer.

Floating lag time and floating time

The floating lag time and floating time were measured in 100 ml beaker containing 0.1 N HCl solution. Forty beads were added into the beaker and the time required for beads to float and the length of beads can stay floating were determined as floating lag time and floating time.¹³

Determination of the *in vitro* mucoadhesive properties

Mucoadhesive test of bead was measured by DuNoy tensiometer on the rats stomach as described in previous study.¹⁰⁻¹¹ The stomachs tissue of rats were used immediately, the experiment was done at 37°C ± 0.5°C. The stomachs were rinsed with saline solution to replace the stomachs content. Before the measurement the beads and the fresh stomachs were immersed in 0.1 HCl solution for 15 minutes. The arm of tensiometer was lowered until the beads came in proper contact with the stomach tissue and it was kept as for 15 minutes. Then, the knob of tensiometer was moved upward slowly until the beads was completely detached from the stomach tissue. During the experiment the stomachs tissue were wetted by dropping of 0.1 N HCl solution. The numbers that showed in DuNoy tensiometer was used as mucoadhesive strength.

Determination of *in vivo* swelling properties

Six rats were fasted for 36 h as described in previous study.⁷ Beads containing antacids was administered to rats orally. Rats were kept fasted until they were sacrificed. Three rats were sacrificed after 12 h and another three rats after 24 h, the stomachs were taken to observed the position of beads in the stomach and the size of beads.

Scanning electron microscopy (SEM)

The surface morphology of the beads was observed using Scanning Electron Microscopy (SEM) TM 3000 (Hitachi).

Antiulcer activity

Thirty male rats weighing 150-200 g were acclimatized under standard condition for one week. Animals had free access to feed and tap water adlibitum. Ethical clearance for experimental experiment

protocol and handling the animals was obtained from Animal Research Ethics Committees/AREC in Faculty of Mathematics and Natural Sciences University of Sumatera Utara prior to the beginning of the project work.

For antiulcer activity experiment the dose of drugs given was adapted with the conversion dose from human dose to rat dose.¹⁴ The rat dose used for $\text{Al}(\text{OH})_3$ and $\text{Mg}(\text{OH})_2$ was 3.6 mg, respectively. For beads preparations, each bead contained 3.6 mg $\text{Al}(\text{OH})_3$ and 3.6 mg $\text{Mg}(\text{OH})_2$. The powder of conventional antacids tablets and the beads were given orally with water using an oral sonde.

Thirty six rats were fasted for 36 hours before the test, then all of rats were orally induced by 1 ml of 0.6 N HCl solution to produce gastric lesions. After 1 hour, 6 rats were sacrificed by chloroform inhalation and the stomachs were observed to determine the condition of gastric lesions. This condition was thought as initial condition of gastric lesion. The remaining rats (24 rats) were divided into 2 groups and each group divided two sub groups, each sub group consisted of 6 rats.

Group I: Given conventional antacids tablet containing 3.6 mg $\text{Al}(\text{OH})_3$ dan 3.6 mg $\text{Mg}(\text{OH})_2$ everyday for 4 days. Six rats were sacrificed after 2 days treatment and another 6 rats were sacrificed after 4 days treatment.

Group II: Given 1 bead containing 3.6 mg $\text{Al}(\text{OH})_3$ and 3.6 mg $\text{Mg}(\text{OH})_2$ everyday for 4 days. Six rats were sacrificed after 2 days treatment and another 6 rats were sacrificed after 4 days treatment.

For macroscopic observation, the number of lesion and the area of lesions were determined. The lesion index was determined based on method as described by Ganguly et al; total of lesions (mm^2) divided by the area of gastric mucosa (mm^2).¹⁵ The length and width of each ulcer in mm were measured by a micrometer. For microscopic observation, the stomachs were washed with 0.9% NaCl solution and immersed in 10% formalin solution then processed for histological staining with haematoxylin-eosin.

Results and Discussion

The shape of beads formed was almost round, the weight and the size increased slightly with the increase of alginate concentrations (F1-F5) as shown in Table 2. The beads containing 5 mg of $\text{Al}(\text{OH})_3$ and 5 mg $\text{Mg}(\text{OH})_2$. The weight and the size of beads without parafin liquidum (F6) was smaller. The beads photo of F3 is shown in Figure 1. For *in vivo* experiments, it was prepared the beads containing 3.6 mg $\text{Al}(\text{OH})_3$ and 3.6 mg $\text{Mg}(\text{OH})_2$ using Formula 3, as shown in Figure 1 (b).

Table 2. Specifications of alg floating-mucoadhesive beads containing $\text{Al}(\text{OH})_3$ and $\text{Mg}(\text{OH})_2$ (n=6).

Formulation	Alginate (%)	Weight average \pm SD (mg)	Diameter average \pm SD (mm)	$\text{Al}(\text{OH})_3$ (mg/beads)	$\text{Mg}(\text{OH})_2$ (mg/beads)
F1	0.75	20.18 \pm 1.17	2.15 \pm 0.01	5	5
F2	1.0	20.67 \pm 1.04	2.16 \pm 0.01	5	5
F3	1.35	21.33 \pm 0.51	2.17 \pm 0.01	5	5
F4	1.50	22.33 \pm 0.51	2.17 \pm 0.02	5	5
F5	1.75	24.17 \pm 1.38	2.19 \pm 0.05	5	5
F6	1.35	19.08 \pm 0.66	2.16 \pm 0.08	5	5



(a)

(b)

Fig. 1. Floating-mucoadhesive alginate beads containing $\text{Al}(\text{OH})_3$ and $\text{Mg}(\text{OH})_2$ (F3). (a) Beads of F3 for *in vitro* evaluation; (b) Beads of F3 for *in vivo* evaluation.

Buffering action of beads

Buffering action of the beads against the decrease of pH due to the secretion of HCl by parietal cells in the stomach was simulated by addition 0.1 N HCl 10 drops/minutes to the 30 ml 0.1 N HCl solution containing 40 beads. As shown in Figure 3. The beads of F1 maintained the pH above 3.0 about 200 minutes and F2 about 300 minutes. Beads of F3 maintained the pH above 3 about 540 minutes. Beads of F4 maintained the pH above 3 about 300 minutes. Beads of F5 could not be maintained above 3.0. Beads of F6 (without paraffin liquid) did not float and only about 75 minutes maintained the pH above 3.

The beads of F3 only took 10 minutes to arise the pH from 1.2 to 3.7 and gave the longest buffering action (540 min) above 3. Therefore, the beads of F3 was considered the best formula as antacids. The beads did not disintegrate in 0.1N HCl medium. The neutralization of HCl by antacids occurred inside the beads. When the beads contact to 0.1 N HCl solution, then HCl solution penetrated to the beads through the beads pores, then the reaction of antacids with HCl as follows.¹² AlCl₃ and MgCl₂ then diffused out of the beads. The penetration of HCl solution into the beads is influenced by alginate concentration used in the preparation of the beads.

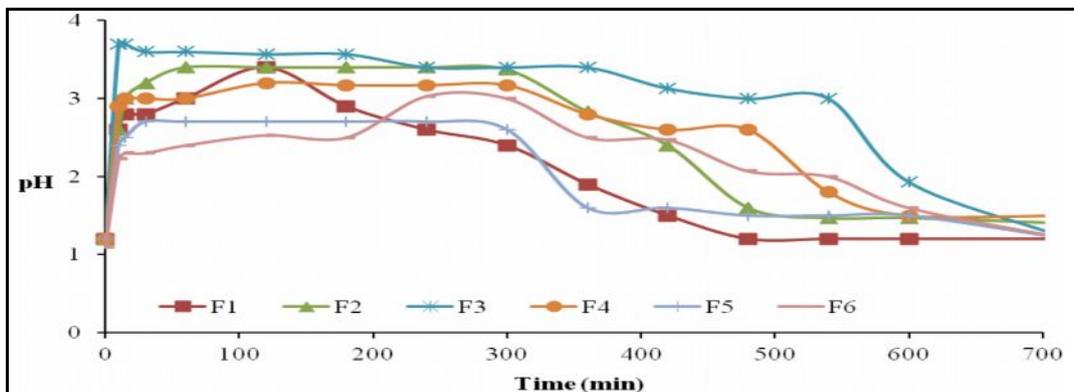
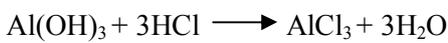


Fig. 2. Buffering action of floating-mucoadhesive beads containing Al(OH)₃ and Mg(OH)₂ against 0.1 N HCl solution on simulated gastric acid secretion at 37°C.

Determination of floating lag time and floating time

The beads of F2-F6 had no floating lag time, this means that when the beads contact with 0.1 N HCl solution the beads directly float. In the other side, beads of F1 did not float. This was due to the low amount of sodium alginate in beads, so that paraffin liquidum was not enough emulsified and caused paraffin liquidum penetrated out from the beads during the beads preparation.

The floating time of beads of F2-F5 was more than 720 minutes, while the floating time of beads of F6 (without paraffin liquidum) is only 1 minute. The floating lag time and the floating of beads in the medium of 0.1 N HCl solution is shown in Table 3 and the photo is shown in Figure 3.

Table 3. Floating lag time and floating time of beads of various formula containing Al(OH)₃ and Mg(OH)₂

No.	Formulation	Floating lag time (min)	Floating time (min)
1	F1	non-floating	non-floating
2	F2	0	>720
3	F3	0	>720
4	F4	0	>720
5	F5	0	>720
6	F6	0	1

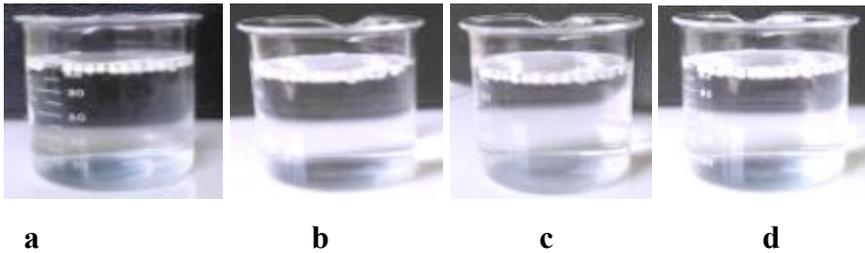


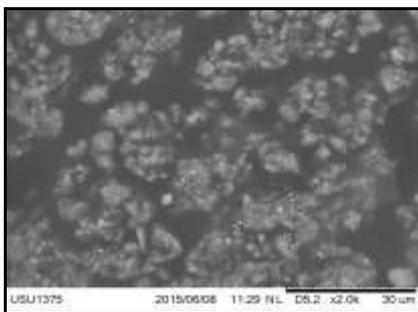
Fig. 3. Floating-mucoadhesive alginate beads containing $\text{Al}(\text{OH})_3$ and $\text{Mg}(\text{OH})_2$ (F3) in the medium of 0.1N HCl solution. (a) initial (0 min); (b) after 240 min; (c) after 480 min; (d) after 720 min.

In vitro mucoadhesive properties

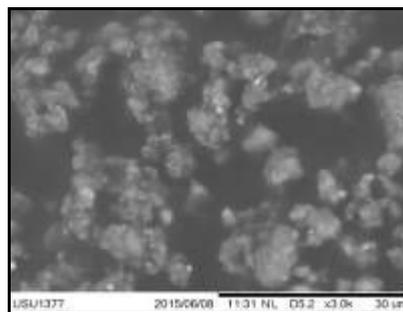
The mucoadhesive strength of F3 obtained was 58.73 dyne. The mucus lining of the stomach is rich in mucin, which contain an oligosaccharide chain with terminal sialic acid. According to Chikering *et al.*,¹⁶ polyanions, especially polymer bearing carboxylic groups and high charge density, serve as powerful “ligands” for mucin and are called mucoadhesive polymers, for example alginate.

Scanning electron microscopy (SEM)

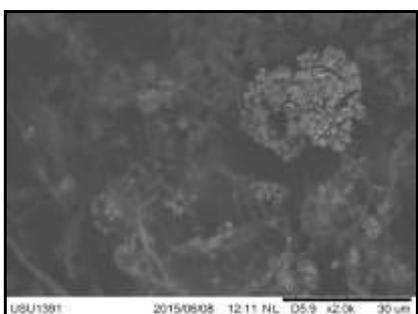
Morphological characterization beads of F3 containing $\text{Al}(\text{OH})_3$ and $\text{Mg}(\text{OH})_2$ is shown in Figure 4. Before drug release experiment (initial) there were a lot of antacids particles on the surface of beads (Figure 4 (a)), but after drug release experiment there were a few antacids particles on the surface of beads. (Figure 4 (b)).



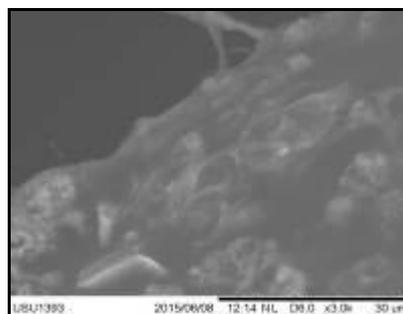
(a) 2000x



(a) 3000x



(b) 2000x



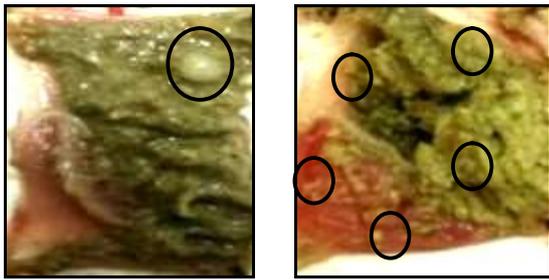
(b) 3000x

Fig. 4. SEM photomicrographs of F3 beads containing $\text{Al}(\text{OH})_3$ and $\text{Mg}(\text{OH})_2$. (a) initial and (b) after drugs release experiment.

In vivo gastroretentive properties of beads

Floating-mucoadhesive alginate beads containing antacids were still remained in the stomach for 12-24 h (Fig. 5), where the beads swelled and then eroded in stomach. The initial weight of beads was 8 mg and diameter was 2.08 ± 0.02 , but after 12 h the weight increased to 11 mg and diameter to 2.83 ± 0.12 . But, after

24 h the beads seen already disintegrated to some particles and no bead was found in intact condition. The average of bead weight decrease to 0.06 mg and the average diameter decreased to 1.47 mm.



(a) (b)
Fig. 5. The floating-mucoadhesive alginate beads containing Al(OH)₃ and Mg(OH)₂ (F3) adhered to the stomach of rat. (a) After 12 h and (b) after 24 h.

Table 4. Characteristics of alginate floating-mucoadhesive beads of F3 containing Al(OH)₃ and Mg(OH)₂ after 12-24 h in the rats stomach (n=6)

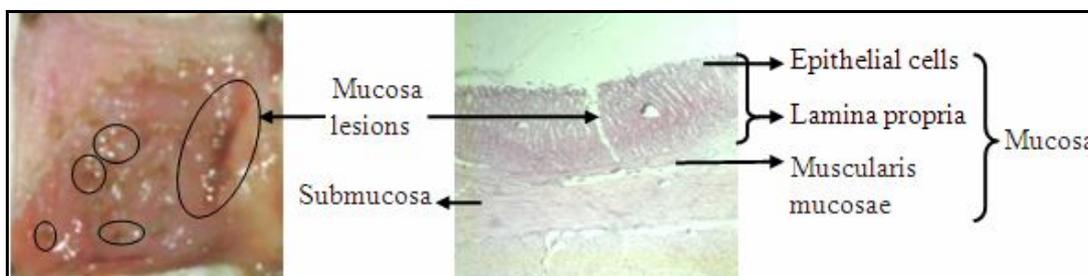
Beads specifications	Initial	After 12 hours	After 24 hours
Weight (mg)	0.08 ± 0.00	0.011 ± 0.00	0.06 ± 0.00
Diameter (mm)	2.08 ± 0.02	2.83 ± 0.12	1.47 ± 1.27

***In vivo* antiulcer activity**

The mucosa of the stomach is composed of the epithelium cells, the connective tissue lamina propria, and the muscularis mucosae.¹⁷ Gastric mucosa has a special barrier against hydrochloric acid. When the barrier is damage by hydrochloric acid the gastric mucosa then allows a back diffusion of gastric acid into the mucosal cells, leading to mucosal damage. In this experiment the gastric ulcers was induced by 1 ml 0.6 N HCl solution. Hydrochloric acid production activates the proenzyme pepsinogen to become the active proteolytic enzyme pepsin, due to pepsin requires a low pH for its activity, the presence of HCl also provides the necessary acidic conditions (pH 1 to pH 2).¹⁸

Initial condition of gastric ulcer of rats (induced by 1 ml 0.6 N HCl solution)

The initial condition of rats after induction with 1 ml 0.6 N HCl solution was thought as initial condition of gastric ulcer that will be treated with floating-mucoadhesive beads containing antacids. After the induction, all the rats showed that the erosion of mucosa. The result of macroscopic showed the average number of lesions was five lesions (Fig. 6a) and microscopically showed the damage of mucosa (Fig. 6b).



(a) (b)
Fig. 6. Initial condition of rats stomach (induced by 1 ml 0.6 N HCl solution). (a) Macroscopic and (b) Microscopic (Histopathology) observations.

Two days treatment

Treatment with conventional antacid tablet

After two days treatment with conventional antacid tablet The result of macroscopic showed the number of lesions are four lesions on conventional antacids tablet treatment (Fig.7a) and microscopic shows there were still mucosal lesions of rats that treatment with conventional antacids tablet (Fig. 7b).

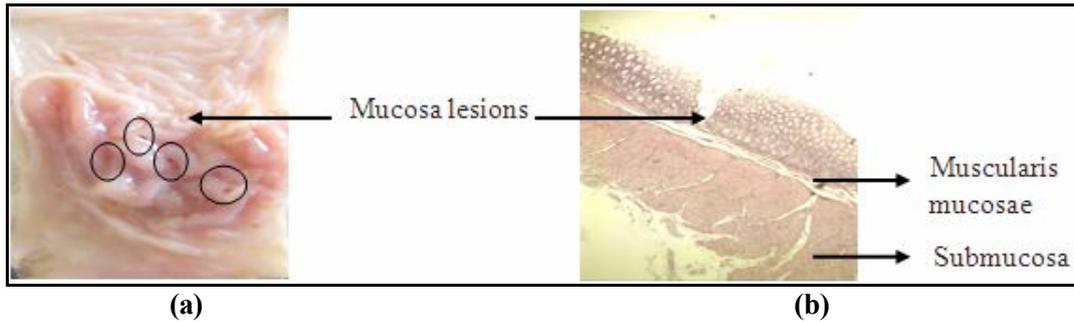


Fig. 7. Gastric mucosa of rats after 2 days treatment with conventional antacids tablet. (a) Macroscopic and (b) Microscopic (Histopathology).

Treatment with floating-mucoadhesive alginate beads containing antacids

The result of macroscopic showed the number of lesions are three lesions on conventional antacids tablet treatment (Fig.8a) and microscopic showed there were still mucosal lesions of rats that treatment with conventional antacids tablet (Fig. 8b).

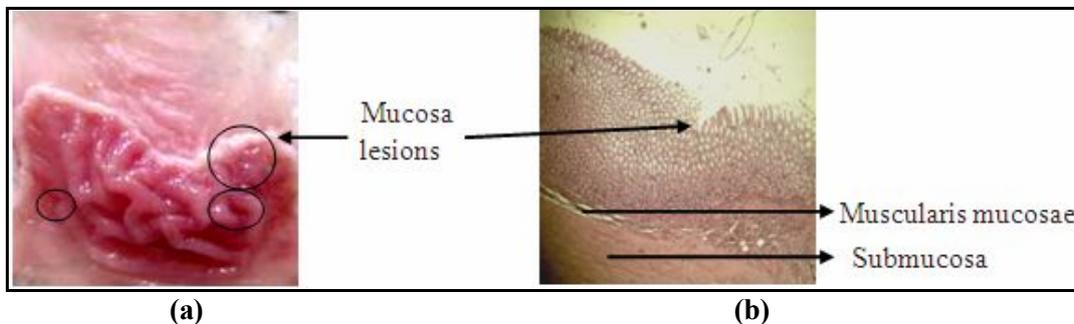


Fig. 8. Gastric mucosa of rats after 2 days treatment with floating-mucoadhesive alginate beads containing antacids. (a) Macroscopic and (b) Microscopic (Histopathology).

Four days treatment

Treatment with conventional antacids tablet

The result of macroscopic showed the number of lesions are four lesions on conventional antacids tablet treatment (Fig.9a) and microscopic showed there were still mucosal lesions of rats that treatment with conventional antacids tablet (Fig. 9b).

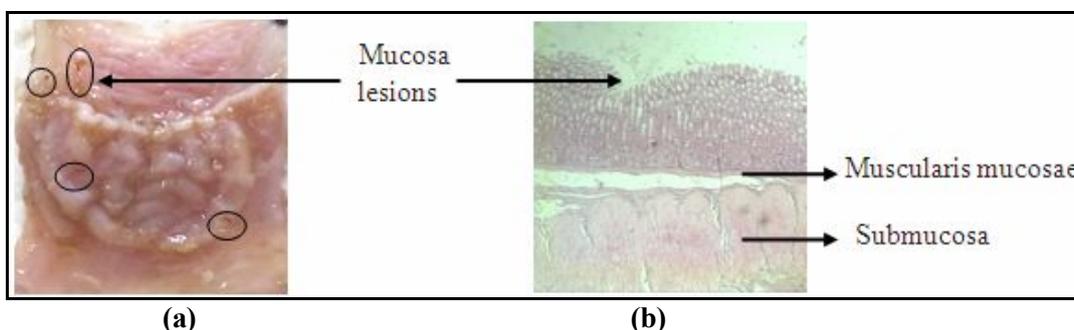


Fig. 9. Gastric mucosa of rats after 4 days treatment with conventional antacids tablet. (a) Macroscopic and (b) Microscopic (Histopathology).

Treatment with floating-mucoadhesive alginate beads containing antacids

The result of macroscopic showed the intact mucosa on beads treatment (Fig.10a) and microscopic showed intact mucosa with alg floating-mucoadhesive beads of F3 containing Al(OH)₃ and Mg(OH)₂ treatment (Fig. 10b).

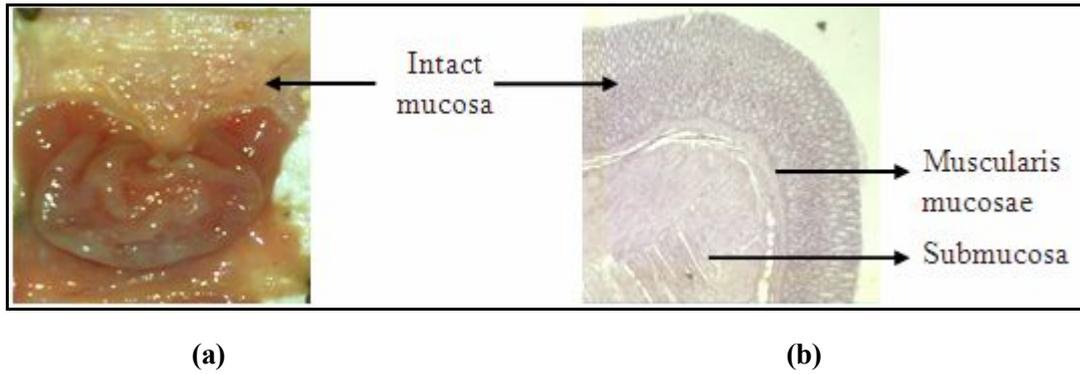


Fig. 10. Gastric mucosa of rats after treatment 4 days treatment with floating-mucoadhesive alginate beads of F3 containing antacids. (a) Macroscopic and (b) Microscopic (Histopathology) observations.

Furthermore, the comparison of the antiulcer activity between conventional antacids tablet and floating-mucoadhesive beads containing antacids is showed in Figure 11 and Figure 12. After the treatment with floating-mucoadhesive beads, the number of lesions decreased, initially was 3.67 ± 1.5 , after 2 days it was 2.17 ± 0.75 , and after 4 days treatment it was zero (no lesion); the value of lesion index initially was 0.019 ± 0.016 , after 2 days it was 0.005 ± 0.003 , and after days treatment it was zero. On the other hand, the treatment using conventional antacids tablet, the value of number of lesions initially 3.67 ± 1.5 , after 2 days it was 3.17 ± 1.32 , and after 4 days treatment it was 2.67 ± 1.03 ; the value of lesions index initially was 0.019, after 2 days it was 0.006 ± 0.004 , and after 4 days treatment it was 0.005 ± 0.002 . This results shows that floating-mucoadhesive beads containing antacids is faster to treat gastric lesions than conventional antacids tablets. This results is due to floating-mucoadhesive antacids beads had pH buffering effect and stayed longer in the stomach as described previously (Figure 2 and 5). In *in vitro* evaluation, the beads of formula 3 could maintain the pH 3.0-3.7 for 540 min and *in vivo* experiment showed that the bead adhered to stomach mucosa for 12-24 h. The adhesion of beads to stomach mucosa occurred if the beads is non-floating due to the amount of water is little in the stomach.

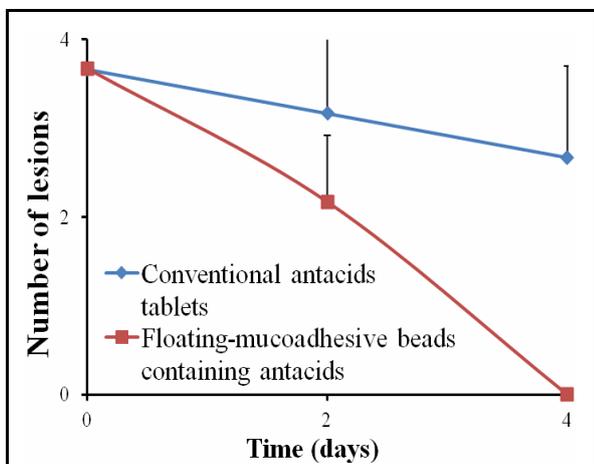


Fig. 11. The comparison of number of lesions between conventional antacids tablet with floating-mucoadhesive beads during the treatment.

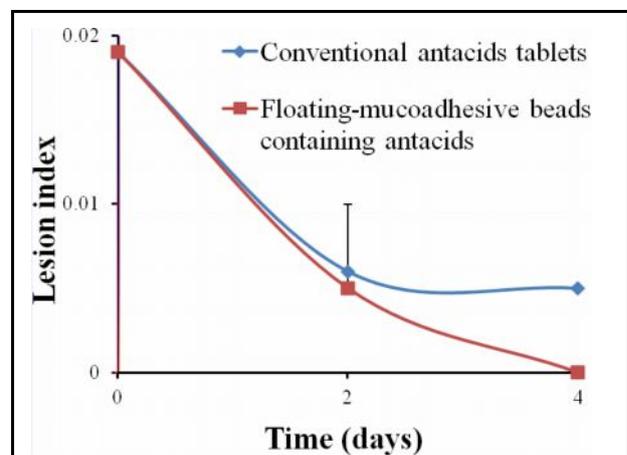


Fig. 12. The comparison of lesion index between conventional antacids tablet with floating-mucoadhesive beads during the treatment.

Conclusions

Alginate floating-mucoadhesive beads containing $Al(OH)_3$ and $Mg(OH)_2$ could stay float for more than 720 min in medium of 0.1N HCl solution. The beads has also mucoadhesive and biodegradable properties. The antiulcer activity of beads is stronger than conventional tablets which containing the same amount of antacids. Therefore, these beads are potential to be used as a gastroretentive system of antacids.

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