

Healing Effect of Alginate Liquid against HCl-Induced Gastric Mucosal Lesions in Rats

Anayanti Arianto and Hakim Bangun*

Faculty of Pharmacy, University of Sumatera Utara, Jl. Tri Dharma No. 5, Kampus
USU, Medan, Indonesia

Abstract: Background: Hyperacidity of stomach is a common global problem. The pathophysiology of this disorder focuses on the imbalance between destructive and protective factors of gastric mucosa.

Purpose: The purpose of this study was to determine the healing effect of gastric mucosal lesions of alginate liquid compared to sucralfate suspension against HCl-induced in rats.

Methods: Sixty Wistar rats weight 150-200 g were adapted under standard environmental conditions for 2 weeks before used. Rats were fasted for 36 hours before the test, then the rats were orally induced by 1 ml of 1 M HCl. Six animals were killed after 1 hour to confirm the gastric mucosal lesions by macroscopic (number of lesions and lesion index) and microscopic (histopathology) observation. The remaining, 54 rats were divided equally in three groups and each group was divided in three subgroups according to time schedule of treatment (3, 7, and 10 days). First group without treatment (negative control), received 1 ml distilled water only once a day. Second group (standard treatment group), received 1 ml sucralfate suspension once a day. Third group (test treatment group), received 1 ml alginate liquid once a day.

Results: On macroscopic examination, the third group of rats (given alginate liquid) had no gastric lesion and the lesion index was zero compared to second group (received sucralfate suspension) which the number of gastric lesions was 1.67 and ulcer index was 0.01 after 7 days treatment. Microscopic examination, the third group showed the intact mucosa compared to second group after 7 days treatment.

Conclusion: The experimental results indicates that alginate liquid is faster in healing of gastric mucosal lesions than with sucralfate suspension.

Keywords: Gastric lesions, healing, alginate liquid, sucralfate suspension.

Introduction

Peptic ulcer is a lesion that occurs in the mucosa and muscularis mucosa of the gastrointestinal tract. Peptic ulcers are ulcers that occur frequently as gastritis and duodenal ulcer. Ulcers caused by an imbalance between aggressive factors (hydrochloric acid, pepsin, *Helicobacter pylori*, NSAIDs, gastric acid) with protective factors (antioxidant enzymatic, non-enzymatic antioxidants, blood flow, the regeneration of cells, mucin, bicarbonate, prostaglandins), which eventually leads to mucosal damage.¹⁻² Peptic ulcer is a common disease that clinically occurs at any age. There are two types of peptic ulcer which are gastric ulcer, damage the lining of the stomach, and duodenal ulcer, damage the duodenum. Gastric ulcer is one of the widespread diseases.³ Major risk factors that cause gastric ulcer include bacterial infection (*Helicobacter pylori*), certain drugs (NSAIDs), chemicals (HCl/ethanol), gastric cancer and low risk factors include the state of stress, smoking, spicy foods and nutritional deficiencies^[1]. Gastric acid production will increase in a state of stress,

such as the heavy workload, panic and haste. Increase stomach acid levels in the state of stress can irritate gastric mucosa and if this is allowed, after a long time can cause gastritis.

The parietal cells of stomach secrete HCl. The basal acid output is about 1 mEq/hour in normal subjects and 2 to 4 mEq/hour in duodenal ulcer patients.⁴ A strong emotional stimulate can improve the basal acid secretion through the parasympathetic nerves (vagus) and thought to be one of the factors cause peptic ulcers.² Hydrochloric acid causes the change of pepsinogen into pepsin which will lower the barrier function of the stomach. Gastric barrier function loss will result in the destruction of the capillary and venous blood so that there will be bleeding. The high acid also triggers the release of histamine, causing vasodilatation which increases bleeding.²

On average, patients with duodenal ulcers produce more acid than control subjects, particularly at night (basal secretion). Although patients with gastric ulcers have normal or even diminished acid production, ulcers rarely if ever occur in the complete absence of acid. Presumably, a weakened mucosal defense and reduced bicarbonate production contribute to the injury from the relatively lower levels of acid in these patients. *H. pylori* and exogenous agents such as nonsteroidal anti-inflammatory drugs (NSAIDs) interact in complex ways to cause an ulcer.⁵

Currently, increasing the frequency of occurrence of gastric ulcer, development of effective treatment becomes a challenge. Treatment with H₂ receptor antagonist, proton pump inhibitors agents that enhance the mucosal defence and sucralfate has become an alternative in the treatment of ulcers. Sucralfate is commonly used as agent to enhance mucosal defence as gastroprotective agent. But, sucralfate has some side effects. The most common side effect of sucralfate is constipation (about 2%). As some aluminum can be absorbed, sucralfate should be avoided in patients with renal failure who are at risk for aluminum overload. Likewise, aluminum-containing antacids should not be combined with sucralfate in these patients. Sucralfate forms a viscous layer in the stomach that may inhibit absorption of other drugs, including phenytoin, digoxin, cimetidine, ketoconazole, and fluoroquinolone antibiotics.⁵

Alginate is a polymer derived from brown algae (Phaeophyceae). Alginate is an anionic copolymer consisting of residue β -D-mannuronic acid and α -L- guluronic in bond 1,4.⁶ Alginate has been known to be non-toxic, cause no allergies, biodegradable and biocompatible.⁷ Alginate can be used for the preparation of gastroretentive and periodontal drug delivery systems.⁸⁻¹¹ Beside, alginate can be utilized in various biomedical applications, including wound healing, cartilage repair, bone and tissue regeneration.¹² Sodium alginate also has a protective effect to the stomach.¹³

Based on the protective effect of alginate to the stomach, we studied the usage of sodium alginate for healing rats gastric lesions that was induced by excessive hydrochloric acid. The alginate was used in liquid preparation.

Materials and Methods

Materials

Sodium alginate 300-400 cP (Wako Pure Chemical Industries, Ltd Japan), nipagin HCl, liquid paraffin, xylol, xylene, hematosillin, and eosin were the product of Merck. Canada balsam (Entellan), sucralfate suspension (Combiphar), and formaldehyde obtained from P.T. Rudang. Sucralfate suspension product of Combiphar, Indonesia).

Animals

Sixty healthy male of Wistar rats weigh 150-200 g were maintained in standard animal house, given standard pellet diets and tap water ad libitum. All rats were fasted from all medications at least 2 weeks before treatment was given.

Preparation of liquid

A certain amount of alginate was dissolved in distilled water with the addition of preservative and simplex syrup.

Induction of gastric lesion by 1 M HCl solution and healing of gastric lesion using alginate liquid

All rats were fasted for 36 hours, then all of rats were induced by 1 ml of 1 M HCl solution to produce gastric lesion. After 1 hour of induction, six rats were sacrificed by ether, then the stomach was opened and washed with 0.9% NaCl solution, and the lesions were observed macroscopically and microscopically (histopatology).

The remaining rats (54 rats) were divided into 3 groups, each group consisting of 6 rats. Group I: Without treatment (given 1 ml distilled water instead of alginate liquid or sucralfate suspension); Group II: Standard treatment group (given orally 1 ml sucralfate suspension every day); Group III: Test treatment group (given orally 1 ml alginate liquid every day).

The group without treatment were given orally 1 ml distilled water instead of sucralfate suspension or alginate liquid. Alginate liquid and sucralfate suspension were given once a day. The rats were given standard pellet and tap water adlibitum. Then, the stomachs of rats were opened and washed with 0.9% NaCl solution. Then, rats were sacrificed using ether inhalation on the third, seventh, and tenth days. The gastric mucosa were observed macroscopically about the number of lesions, length and width of lesions. For microscopic observation, the gastric mucosa was immersed in 10% formalin for histopathology processed with hematoxylin Eosin staining and observed using a microscope.

Measurement of lesion index

Lesion index was determined based on method reported by Ganguly and Bhatnagar; total area of lesions (mm^2) divided by the area of gastric mucosa (mm^2).¹⁴ The length and width of each lesion in mm were measured by a calipers. The sum of the area of all lesions for each stomach was used in the calculation of the total lesions area.

Results and Discussions

Macroscopic and microscopic observation of normal stomach of rats

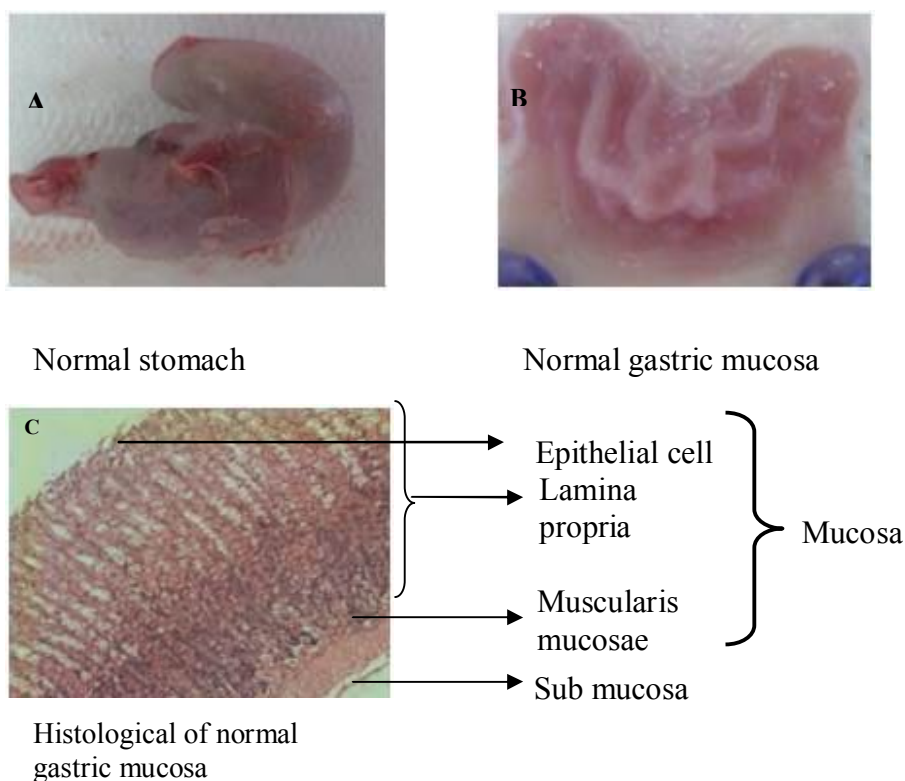


Figure 1. Normal stomach of rats A : Normal stomach; B, Normal gastric mucosa; and C, histological of normal gastric mucosa.

The alimentary canal comprises of four concentric layers: mucosa, submucosa, muscularis externa, 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.¹⁵ Figure 1 shows a rat's normal stomach (A), gastric mucosa (B), and histological of partially gastric mucosa (C).

Induction of gastric mucosa lesions

The initial condition of rats used on the study of healing effect of alginate liquid compared to sucralfate suspension was considered the condition of rats after induction with 1 ml 1 M HCl solution. Gastric mucosa has a special barrier against noxious agents, such as aspirin, ethanol, bile, indomethacin, or hydrochloric acid. As a barrier, sodium bicarbonate is secreted from the surface of epithelial cells and incorporated into mucus layer might play role in the mucosal barrier against gastric acid and other noxious substances that are ingested. When the barrier is broken by noxious agent the gastric mucosa then allows a back diffusion of gastric acid into the mucosal cells, leading to mucosal damage.

The induction of fasted rats with 1M HCl solution caused swelling of the rats' stomach due to the accumulation of gas (Figure 2A). This gas is thought come from the result of the reaction between hydrochloric acid and sodium carbonate that is secreted from the surface of epithelial cells. Hydrochloric acid caused damage of mucosal barrier. The damages of mucosal barrier cause the back diffusion of hydrochloric acid and lead the destruction of the capillary and venous blood so that resulting stomach bleeding. High acid also trigger the release of histamine causing vasodilation that increases bleeding.² Furthermore, hydrochloric acid will change pepsinogen to pepsin which will lower the barrier function of the stomach. The effect of the administration of 1 ml 1 M HCl solution is shown in Figure 2 (B and C).

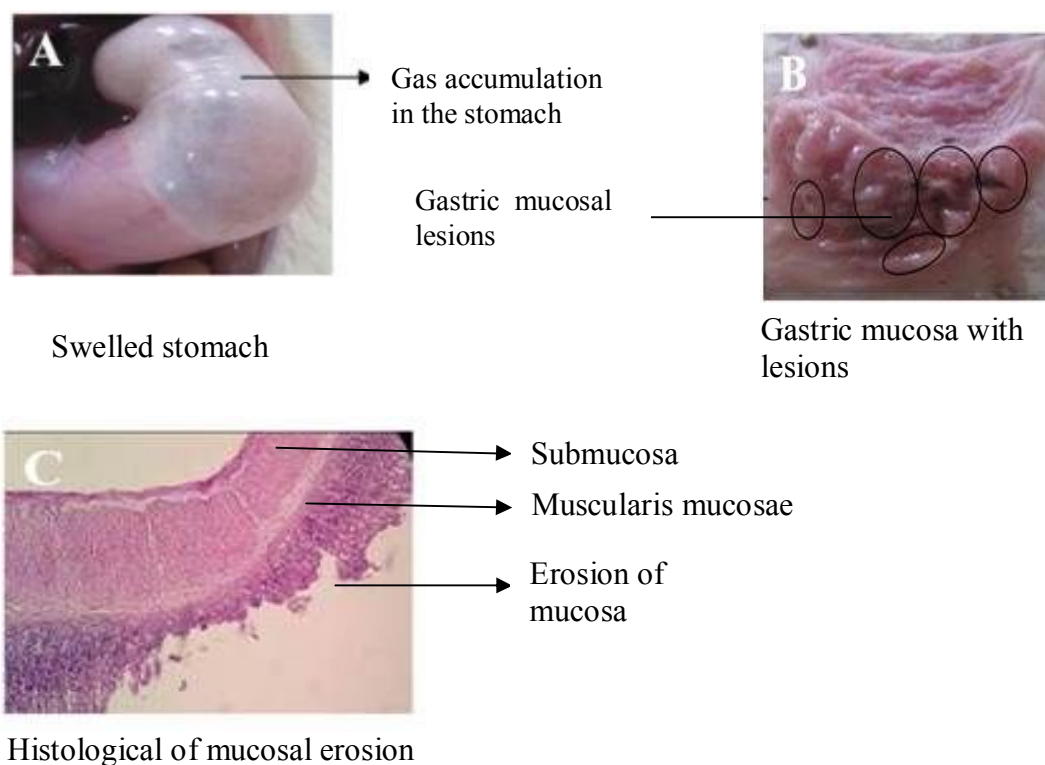


Figure 2: Effect of 1 ml 1 M HCl solution on the rats' stomach

A: Swelled of stomach due to gas accumulation; B: Gastric mucosa with lesions, and C: Histological of mucosal erosion

Beside the swelling of stomach, the gastric mucosa lesions were formed after the induction with 1 ml 1 M of HCl solution. The average number of lesions and lesion index were 4.50 ± 2.02 and 0.61 ± 0.89 , respectively (Table 1).

Figure 2 (C) shows the histological section of rats stomach after induction with 1 ml 0.6 M HCl solution. The microscopically observation showed that the administration of 1 ml 1M HCl solution caused the erosion of rats' mucosa.

Macroscopic observation of rats stomach after three days treatment

Figure 3 shows the gastric mucosa of rats that received preparation for three days. There were still lesions, either treated with alginate liquid or sucralfate suspension. But, the number of lesions were higher on without treatment.

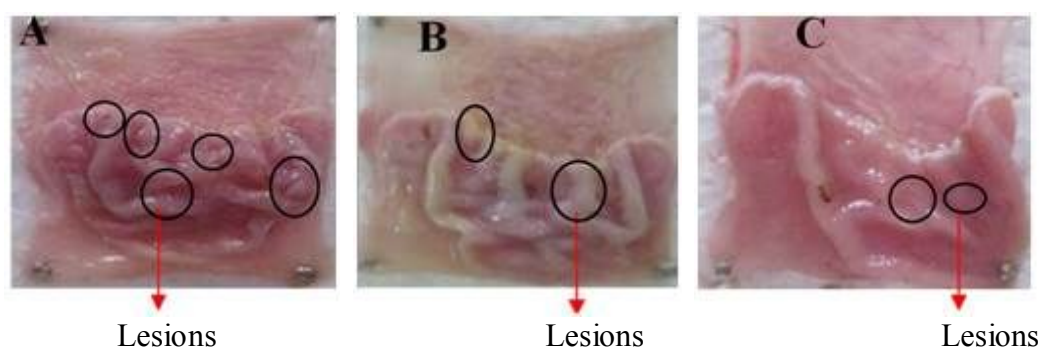


Figure 3. Gastric mucosa of rat after 3 days treatment. A: Without treatment, B: Given sucralfate suspension, and C: Given alginate liquid

Microscopic observation of rats' stomach after three days treatment

In addition to macroscopic observation, it was also conducted the microscopic or histological observations. Figure 4 shows hystological section of without treatment, given sucralfate suspension, and given alginate liquid. All of rats showed erosion of mucosa, but erosion of mucosa was deeper in rats without treatment.

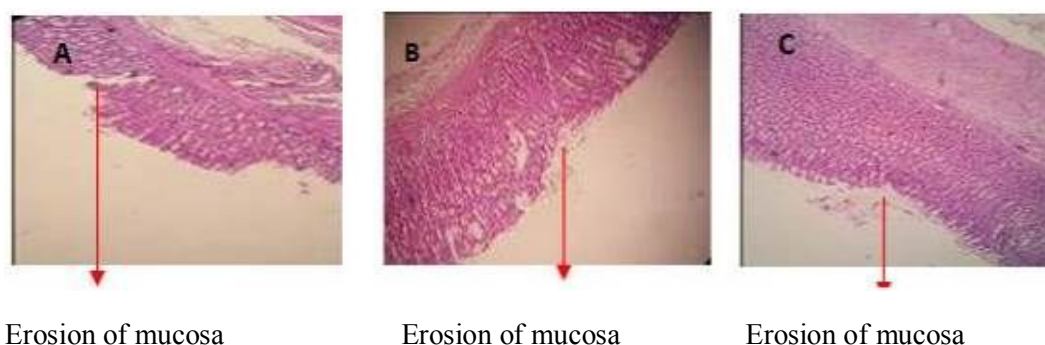


Figure 4. Histological section of rats gastric mucosa (10x10) after 3 days treatment. A: Without treatment; B: Given sucralfate suspension; C: Given alginate liquid.

Macroscopic observation of rats' stomach after 7 days treatment

Figure 5 shows the gastric mucosa of rats after 7 days treatment. Rats that were treated with alginate liquid showed no lesion of stomach, but those were treated with sucralfate suspension still showed lesions of

stomach. In without treatment rats were found more number of lesions. Therefore, it can be concluded that the administration of alginate liquid was faster in healing gastric lesions than sucralfate suspension.

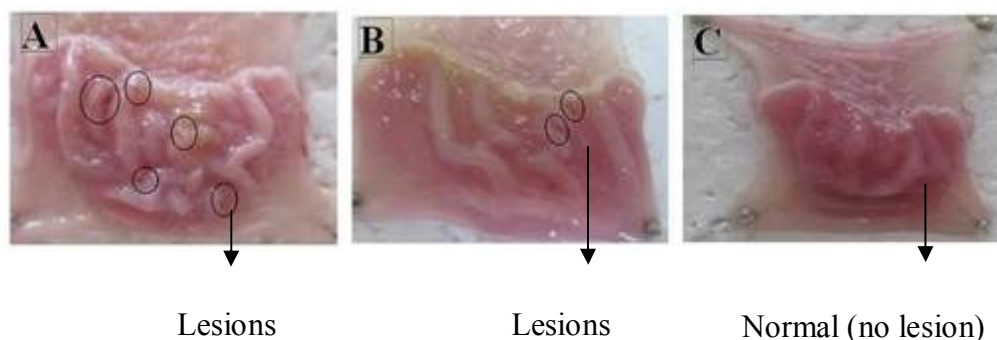


Figure 5. Gastric mucosa of rats after 7 days treatment. A: Without treatment, B: Given sucralfate suspension, and C: Given alginate liquid

Microscopic observation of rats' stomach after 7 days treatment

Figure 6 shows the histological section of rats' stomach after 7 days treatment. The cohesion between epithelial cells was damaged and the epithelial cells were eroded in the rats without treatment (Fig. 6A). Like wise, rats that were treated with sucralfate suspension still showed the erosion of epithelial cells (Fig. 6B). However, that rats treated with alginate liquid showed good cohesion between the epithelial cells, no erosion of epithelial cells, and showed the intact mucosa after 7 days of treatment (Fig. 6C). From the result of microscopic observation we can conclude that alginate liquid can heal gastric lesion after 7 days treatment.

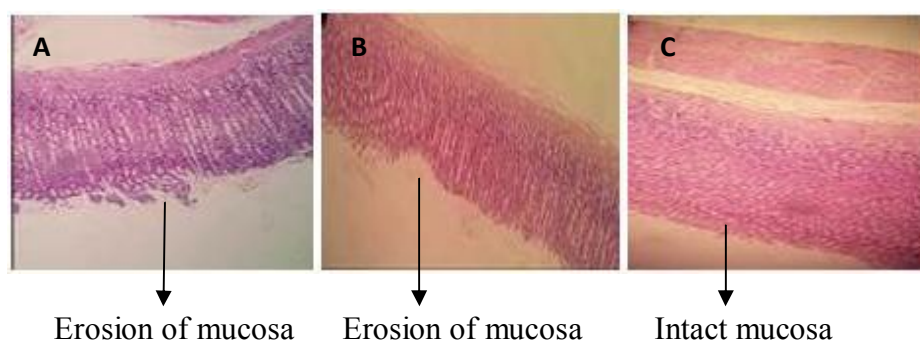


Figure 6. Histological section of rats gastric mucosa (10x10) after 7 days treatment. A: Without treatment ; B: Given sucralfate suspension, and C: Given alginate liquid

Macroscopic observation of rats' stomach after 10 days treatment

The macroscopic observation of rats' stomach after 10 days treatment shows in Figure 7. After 10 days treatment, it showed that there were lesions in stomach of untreated rats (Fig. 7A). However, there was no lesion found in rats that were treated with sucralfate suspension (Fig. 7B), like rats treated with alginate liquid (Fig. 7C). From the result of macroscopic observation, we can conclude that alginate liquid can heal gastric ulcer after 7 days, while sucralfate suspension after 10 days treatment.

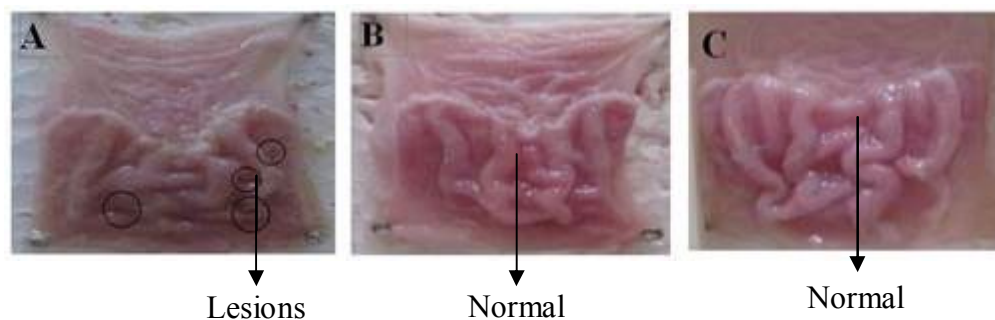


Figure 7. Gastric mucosa of rats after 10 days treatment. A: Without treatment, B: Given sucralfate suspension, and C: Given alginate liquid

Microscopic observation of rats' stomach after 10 days treatment

The histological section of rats gastric mucosa shows in Figure 8. There was still erosion on mucosa of rats without treatment (Fig. 8A). However, there was no erosion on mucosa in rats' gastric mucosa that were treated with sucralfate suspension (Fig. 8B) like rats which were treated with alginate liquid (Fig. 8C). After 10 days treatment with sucralfate suspension.

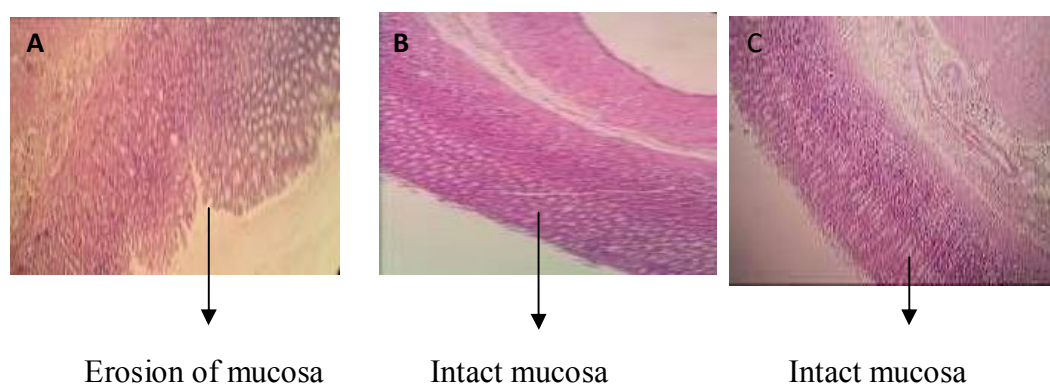


Figure 8. Histological section of rats gastric mucosa (10x10) after 10 days treatment. A: Untreated, B: Given sucralfate suspension, and C: Given alginate liquid

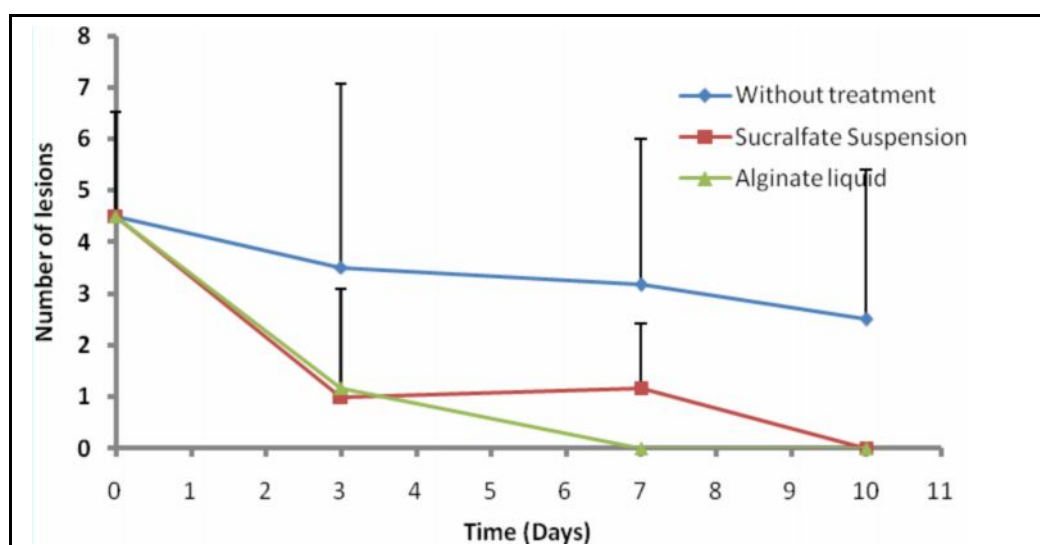
Comparison of healing effect of alginate liquid on gastric mucosal lesions based on number of lesions and lesion index

Number of lesions

Table 1 lists the average number of lesions before and after treatment with sucralfate suspension and alginate liquid and the graph is shown in Figure 9. The initial average number of lesions was 4.50 ± 2.02 . The number of lesions decreased with the longer time of treatment. After 7 days treatment, there was no lesion observed in the rats that were treated with alginate liquid. However, in the rats that were treated with sucralfate suspension, there were still lesions in the stomachs. The rats that were treated with sucralfate suspension was found no lesion after 10 days treatment. The rats without treatment haven't healed after 10 days, it was observed as many as 2.50 ± 2.90 lesions in the stomach.

Table 1. Number of gastric mucosa lesions of rats in each groups after certain days of treatment.

Day	Mean number of lesions (n = 6) ($\bar{X} \pm SD$)		
	Without treatment	Standard treatment group (Given sucralfate suspension)	Test treatment group (Given alginate liquid)
0	4.50 ± 2.02	4.50 ± 2.02	4.50 ± 2.02
3	3.50 ± 3.56	1.00 ± 2.10	1.17 ± 1.92
7	3.17 ± 2.84	1.17 ± 1.24	0 ± 0.00
10	2.50 ± 2.90	0 ± 0.00	0 ± 0.00

**Figure 9. The comparison of the healing effect between alginate liquid, sucralfate suspension, and without treatment based on number of lesions (n=6).**

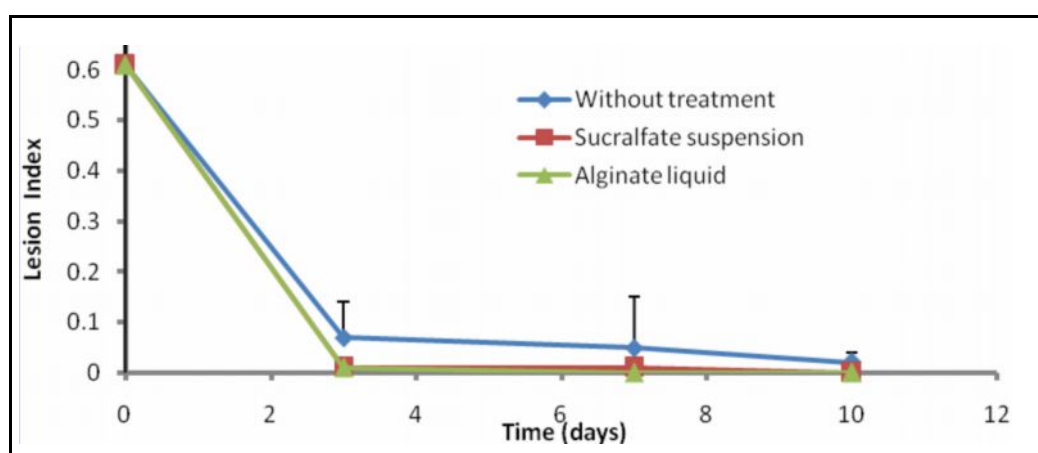
Lesion index

The value of lesion index without and after the treatment using sucralfate suspension and alginate liquid is listed in Table 2 and the graph is shown in Figure 10. The lesion index is the ratio of the total area of lesions and total area of mucosa. After the treatment, the value of lesion index decreased. The decrease of lesion index was faster with the treatment using alginate liquid, where the lesion index became zero after 7 days of treatment. But, the treatment with sucralfate suspension, the lesion index became zero after 10 days, while without treatment the ulcer index rats was 0.02 ± 0.02 after 10 days.

In healing experiment, the doses of alginate liquid and sucralfate suspension once a day each 1 ml. But, the concentration of alginate was lower than the concentration of sucralfate. The concentration of sodium alginate in alginate liquid was 10 mg/ml, while the concentration of sucralfate in sucralfate suspension was 100 mg/ml. Sucralfate has mucoprotective effect, in an acid environment ($pH < 4$) sucralfate undergoes extensive cross-linking to produce a viscous, sticky polymer that adheres to epithelial cells and ulcer craters for up to 6 hours after a single dose. This provides physical protection against irritant substances such as excessive stomach acid.⁵ In the stomach, in the presence gastric acid, sodium alginate was changed to alginic acid gel. This alginic acid gel has a mucoadhesive properties.⁸⁻⁹ Therefore, alginic acid adhered to the surface of gastric mucosa and acted as a mucoprotective agent by providing a physical barrier of hydrochloric acid and pepsin contact to the gastric mucosa, thereby improve the healing effect of lesion or ulcer. The healing of lesions was faster by alginate liquid than sucralfate was thought to be due to alginate increased the hexosamine levels, which are glycoprotein constituting of gastric mucus,¹⁶ in addition of protective effect of alginate.

Table 2. Mean lesion index of each group

Day	Mean lesion index (n = 6) (X ± SD)		
	Without treatment	Standard group (Given sucralfate suspension)	Test treatment group (Given alginate liquid)
0	0.61 ± 0.89	0.61 ± 0.89	0.61 ± 0.89
3	0.07 ± 0.07	0.01 ± 0.02	0.01 ± 0.01
7	0.05 ± 0.10	0.01 ± 0.01	0 ± 0.00
10	0.02 ± 0.02	0 ± 0.00	0 ± 0.00

**Figure 10. The comparison of healing effect between alginate liquid, sucralfate suspension, and without treatment based on value of lesion index (n=6).**

Conclusion

Based on the results, it can be concluded that alginate liquid is potential to be used for healing gastric lesions or ulcers.

Acknowledgment

This research was supported by Directorate General of Higher Education of Indonesia through the scheme of competitive research (Hibah bersaing) 2014.

References

1. Sunil K., Amandeep K., Robin S., and Ramica S., Peptic Ulcer: A Review on Etiologi and Pathogenesis. *International Research Journal of Pharmacy*, 2012, 3(6), 34-38.
2. Price S.A. and Wilson L.M., *Pathophysiology: Clinical Concepts of Diseases Processes*, Third Edition, New York: McGraw-Hill Book Company, 1986, p. 242-258.
3. Alkofahi A. and Atta A.H. Pharmacological screening of the antiulcerogenic effects of some Jordanian medicinal plants in rats, *Journal Ethnopharmacol*, 1999, 65, 341-345.
4. Perigard C.J., *Clinical Analysis, Gastric Analysis*. In: Remington: *The Science and Practice of Pharmacy*, Volume I, Editor: Gennaro A., London: Lippincott Williams & Wilkins. 2000, p. 580-581; 1227.
5. Wallace J.L and Sharkey K.A., Pharmacotherapy of gastric acidity, peptic ulcers, and gastroesophageal reflux disease, In: Brunton L.L, Chabner B.A., Knollmann B.J, editors. *Goodman & Gilman's The Pharmacological Basis of Therapeutics*. 12th ed. New York: Mc Graww Hill Medical, 2011, p. 1309-1321.
6. Haug A., Larsen B. and Smidsrod O., *Acta Chem. Scand.*, 1966, 20, 183.

7. Smidsrod O. and Braek-Skjak G., Alginate as immobilization matrix for cells, Tibtech-March. 1990, 71-78.
8. Arianto A., Bangun H., Harahap U., Ilyas S., The comparison of swelling, mucoadhesive, and release of ranitidine from spherical matrices of alginate, chitosan, alginate-chitosan, and calcium alginate-chitosan, Journal of PharmTech Research. 2014, 6(7), 2054-2063.
9. Arianto A., Bangun H., Harahap U., Ilyas S., Effect of alginate chitosan ratio on the swelling, mucoadhesive, and release of ranitidine from spherical matrices of alginate-chitosan, Journal of PharmTech Research, 2015, 8(4), 653-665.
10. Mariadi, Bangun H., and Karsono, Formulation and in vitro evaluation of gastroretentive drug delivery system of antacids using alginate-chitosan films, International Journal of PharmTech Research, 2015, 8(9), 1-12.
11. Aryana, Sinurat D., Ervina I., and Bangun H., Formulation of alginate based metronidazole periodontal gel, Asian Journal of Pharmaceutical and Clinical research, 2014, 7 (1), 224-227.
12. Sun J and Huaping T., Alginate-based biomaterial for regenerative medicine applications, Journal Materials, 2013, 6, 1285-1309.
13. Daigo K., Wada Y., Yamada C., Yamaji M., Okuda S., Okada M., and Miyazato T., Pharmacological studies of sodium aginate. I. Protective effects of sodium alginate on mucous membranes of upper-gastrointestinaal tract, Yakugaku Zasshi, 1981, 101(5), 452-457.
14. Ganguly A.K. and Bhatnagar O.P., Effect of bilateral adrenalotomy on production of restraint ulcers in stomach of albino rats. Canadian Journal of Physiology and Pharmacology, 1973, 51, 748-750.
15. Gartner L. P. and Hiatt J. L., Color Texbook Histology, Third Edition, Phyladelpia: Saunders Elsevier, 2007, p. 381-403.
16. Daigo K., Yamaji M., Yamada C., Matsutani K., Yagi M., Nakajima Y., Curative effect of sodium alginate on experimental hydrochloric acid and tetra-gastrin induced ulcer, Japanese Pharmacol Ther., 1982; 10, 281-289.
