

## PREPARATION OF MUCOADHESIVE GASTRORETENTIVE DRUG DELIVERY SYSTEM OF ALGINATE BEADS CONTAINING TURMERIC EXTRACT AND ANTI-GASTRIC ULCER ACTIVITY

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Received: 13 September 2018, Revised and Accepted: 13 November 2018

### ABSTRACT

**Objectives:** The objectives of this study were to prepare the mucoadhesive gastroretentive drug delivery system of alginate beads containing turmeric extract and to evaluate its anti-gastric effect.

**Methods:** Turmeric extract was made by macerating turmeric powder in ethanol 96% and evaporated using rotary evaporator at 50°C to obtain concentrated extract. Then, it was prepared alginate beads containing 5% turmeric extract by gelation method. The mucoadhesive properties of alginate beads containing turmeric extract were tested *in vitro* in male rats and the anti-gastric ulcer effect was tested on HCl-induced gastric ulcer in rats.

**Results:** The mean diameter of alginate beads obtained was 1.13±0.19 mm. Bioadhesion test showed that alginate beads containing turmeric extract could be attached on gastric rats mucosa. Anti-gastric ulcer test showed that the healing of gastric lesions in rats was faster using alginate beads containing turmeric extract compared to untreated ones.

**Conclusions:** The results of this study suggest that mucoadhesive drug delivery systems of alginate beads containing turmeric extract are potentially developed as an anti-gastric ulcer.

**Keywords:** Turmeric extract, Alginate beads, Mucoadhesive, Gastroretentive, Anti-gastric ulcer.

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### INTRODUCTION

Turmeric extract obtained from *Curcuma domestica* Val. contains curcumin which is the most effective component and has therapeutic effects such as anti-inflammatory, antibacterial, antifungal, anticancer, antioxidant, anti-human immunodeficiency virus, and antidiabetic. Other research has also demonstrated anti-inflammatory and antioxidant properties of curcumin [1,2]. Treatment of *Helicobacter pylori* with curcumin caused the relief of symptoms in peptic patients [3]. The anti-ulcer effect of curcumin might be due to its properties to decrease gastric acid secretion and enhance the mucosal defensive mechanism through suppression of inducible nitric oxide synthase (iNOS)-mediated inflammation [4]. Another interesting property of turmeric is the antibacterial activity, especially on enteric bacteria [5].

Ulcers are deep lesions penetrating through the gastrointestinal tract mucosa and muscularis mucosa. It is believed that peptic ulcers develop due to an imbalance between aggressive factors (*H. pylori*, nonsteroidal anti-inflammatory drugs [NSAIDs], and gastric acid) and protective factors (mucin, bicarbonate, and prostaglandins) leading to an interruption in the mucosal integrity [6].

Curcumin has low solubility in water, slow absorption rates, and poor absorption in gastrointestinal tract which result in small concentration of curcumin entering the blood circulation and causing low efficacy. Low bioavailability of curcumin (<1%) and degradation in the basic condition of human intestine will limit its clinical application [7,8]. Due to the instability of curcumin in basic condition of intestinal fluid, then it is necessary to prepare curcumin preparation in the gastroretentive drug delivery system to increase the bioavailability of curcumin. Oral drug delivery routes are the most convenient and most commonly used route of administration [9]. Gastroretentive systems can make drug

remain in gastric region and prolong gastric residence time, thereby targeting site-specific drug release in the upper gastrointestinal tract for local or systemic effects. Several approaches of dosage formulations with gastroretentive system have been developed to achieve a controlled release of drug to stay longer in the stomach [10-16].

Mucoadhesive polymers have the ability to adhere to mucose substrate, and thus, the preparation can stay longer in delivery site, giving controlled drug release and an increase of bioavailability. Alginate is a negatively-charged polysaccharide which is non-toxic, biocompatible, and biodegradable, included in anionic bioadhesive polymer, forming hydrogen bond with hydroxyl group in polysaccharide chain in mucus glycoprotein, and its chemical bond was non-covalent [17].

Lamivudine sodium alginate beads which were prepared by ionotropic gelation method showed prolonged drug release (~12 h), and the release was controlled by diffusion from alginate beads that were slow and spread over an extended period of time depending on the drug-polymer ratio [18]. Floating drug delivery system of oil entrapped alginate beads formulation of curcumin showed that it has floating behavior and could release curcumin in a sustained manner for 8 h [19].

In this paper will be discussed the preparation, bioadhesion properties, and anti-gastric ulcer activity of mucoadhesive gastroretentive drug delivery system of alginate beads containing turmeric extract.

### MATERIALS AND METHODS

#### Materials

Turmeric (*C. domestica* Val.) rhizome was obtained from Medan Central Market, Indonesia. The plant was authenticated by Herbarium Medanense University of Sumatera Utara, Indonesia. Ethanol 96%,

distilled water, and Tween 80 were obtained from Brataco, and sodium alginate 80–120 cP was obtained from Wako Pure Chemical Industries, Ltd. Japan. Calcium chloride and hydrochloric acid were the products of Merck. All other chemicals were of analytical grade.

### Animals

Healthy male Wistar rats weigh 150–200 g were housed in standard animal house and given standard pellet diets and tap water *ad libitum*. All rats were fasted from all medications at least 2 weeks before the test. All the rats were adhered to the standard operating procedures and approved by the Animal Research Ethics Committee of the Faculty of Mathematics and Natural Sciences, University of Sumatera Utara, beginning of the project work.

### Preparation of turmeric extract

Turmeric powder was macerated with ethanol 96% for 8 days. Then, the macerate was evaporated with a rotary evaporator to obtain concentrated turmeric extract.

### Preparation of alginate beads

Turmeric extract 5%  
 Tween 80 5%  
 Sodium alginate 1.5%  
 Distilled water until 50 ml.

In brief, sodium alginate solution was prepared by adding sodium alginate (1.5% w/v) in distilled water. Turmeric extract was crushed with the addition of ethanol until dissolved, and it was mixed with Tween 80, then added into the alginate solution, and stirred until the mixture became homogeneous. The mixture was dropped through a syringe with 21G size into 0.15 M calcium chloride solution (100 ml) with gentle agitation at room temperature with curing time of 4–5 min [20]. Then, the beads formed were separated from the solution by filtration and rinsed with distilled water. Finally, the beads containing turmeric acid were dried at room temperature and stored in a desiccator.

### Bioadhesion test

Rats gastric mucosa was cleaned and soaked in sterile 0.9% NaCl solution. Rats gastric mucosa was stuck to object glass with the mucosal part faces up. Tilted the object glass at 45°. 20 alginate beads containing turmeric extract were attached to the gastric mucosa, and then it dropped with artificial gastric fluid and was placed in room temperature for 1 min. Mucosa was flowed with artificial gastric fluid at a temperature of 37±0.5°C with a speed of 22 ml/min. The number of beads left in mucosa was then counted after 5 min and 10 min [21,22].

### Induction of gastric lesions by 1N HCl solution and healing of gastric lesions using alginate beads containing turmeric extract

Twenty-seven rats were fasted for 36 h, and then, they were induced by 1 ml 1N HCl solution to produce gastric lesions. After 1 h induced, three rats were sacrificed by ether, then the stomachs were opened and washed with 0.9% NaCl solution, and the lesions were observed macroscopically and microscopically. Lesion index was determined by dividing the total area of lesions (mm<sup>2</sup>) with the area of gastric mucosa (mm<sup>2</sup>). The length and width of each lesion in mm were measured by calipers. The sum of the area of all lesions for each stomach was used in the calculation of the total lesions area [23].

The remaining rats (24 rats) were divided into two groups; each group consisted of 12 rats. Group I: Without treatment (given 2 ml distilled water every day); Group II: Treatment group (given orally 100 mg/kg alginate beads containing turmeric extract every day). The rats were given standard pellet and tap water *ad libitum*. Then, three rats of each group were sacrificed on 2<sup>nd</sup>, 4<sup>th</sup>, 6<sup>th</sup>, and 8<sup>th</sup> days. The gastric mucosa was observed macroscopically for 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.

## RESULTS AND DISCUSSION

### Preparation of alginate beads containing turmeric extract

The use of turmeric extract in this experiment due to turmeric extract contains active ingredient curcumin. The addition of Tween 80 as a surfactant in the formulation was to increase the dissolution of curcumin which is practically insoluble in acidic condition. The alginate mixture containing turmeric extract and the Tween 80 was then dropped into 0.15 M CaCl<sub>2</sub> solution using a syringe with curing time of 4–5 min to form spherical alginate gel beads that entrapped turmeric extract. Alginate is a linear natural polysaccharide consisting of β-D-mannuronic acid and α-L-guluronic acid. Divalent cations, such as calcium ions, can bind to guluronate residues to form “egg box” structures and cause gelation [24,25]. The dried beads obtained were 1.30±0.05 mm in diameter and weigh 29.67±1.36 mg as shown in Fig. 1.

### Bioadhesion test

Table 1 shows the bioadhesion test results of alginate beads containing 5% turmeric extract. Initially, the beads in mucosa were 20 beads, after 5 min flowed with 0.1N HCl solution, the average beads remained in rats mucosa were 17 beads (87%), and after 10 min, average beads remained in rats mucosa were 16 (81%). These results indicate the mucoadhesive properties of alginate beads.

Mucoadhesive polymers are composed of polar functional groups such as the hydroxyl (-OH), carboxyl (-COOH), amide (-NH<sub>2</sub>), and sulfate (-SO<sub>4</sub>) groups which can interact with the mucin glycoprotein [26]. Alginate contains the -OH and (-COOH) groups causing it to have mucoadhesive properties, so it can be used as a gastroretentive pharmaceutical excipient that attached to the gastric mucosa. Previous study was also reported the bioadhesive properties of alginate beads [12,13].

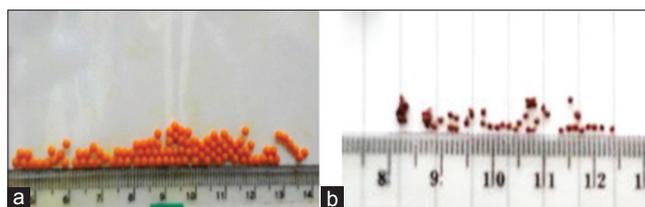


Fig. 1: Photograph of alginate beads containing turmeric extract. Before (a) and after (b) drying

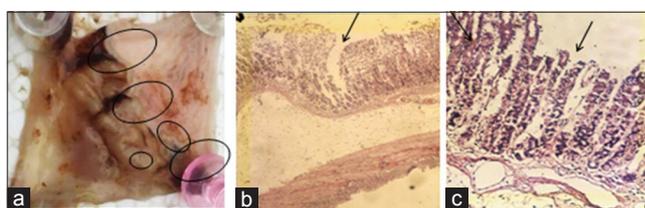


Fig. 2: Macroscopic (a) and microscopic (b and c) pictures of rat stomach after induction with 1N HCl solution. Circles show the gastric lesions. Magnification: b: ×10, c: ×100. Arrows show the erosion of gastric mucosa tissue

Table 1: Data of bioadhesion test of alginate beads containing turmeric extract

Experiment	Number of beads left in mucosa		
	Initial	5 min	10 min
1	20	15	15
2	20	18	15
3	20	19	19
Mean		17	16
Mean percentage		87	81

**Induction of gastric mucosa lesion using 1N HCl solution**

Fig. 2 shows the control group of rats lesion after 1 h induced by 1N HCl solution. The erosion formed is shown by the circle image. The condition of these rats stomach (number of lesions and value of ulcer index) as shown in Table 2 is considered as initial condition of rats after induction with 1N HCl solution. Damage and erosion of epithelial cells that are deep enough (shown by circle image) on the surface of the gastric mucosa are due to direct contact with HCl solution. Local irritation leads to tissue damage and causes back diffusion of stomach acid to the mucosa [27]. The etiology of gastric ulcers is due to an imbalance between aggressive factor (*H. pylori*, NSAID, and gastric acid) and protective factors (mucin, bicarbonate, and prostaglandin), leading to the disruption of the mucosal integrity [6]. Peptic ulcer occurs when aggressive effects of acid-pepsin outweigh the protective effects of gastric mucosa [28].

Fig. 2 shows the macroscopic and microscopic pictures of rats gastric mucosa 1 h after induction with 1N HCl solution.

**Treatment of gastric lesions with alginate beads containing turmeric extract**

*Macroscopic observation of rats stomach*

The macroscopic observation of rats stomach was done by counting the number of gastric lesions and the area of lesion and then calculated the lesion index. The number of lesions in both the groups is shown in Table 2, while the average lesion index in both groups is shown in Table 3. Fig. 3 shows the macroscopic pictures of rats mucosa that were not treated (upper) and that were treated with alginate beads containing turmeric extract during 8 days. The more days of treatment, the less of the number of lesions and the lower the lesion index value. Reduction of the number of lesions and lesion index value was faster in rats given alginate beads containing turmeric extract compared to rats that were not given beads. This means that alginate beads containing turmeric extract have anti-gastric ulcer activity.

After the administration of the beads, in the stomach in the presence of gastric fluid, curcumin is released from the beads. The anti-ulcer effect of curcumin is due to its properties to decrease the gastric acid secretion and increased the mucosal defense mechanisms through inflammatory suppression mediated by NO synthase enzyme [4]. Wound healing is a process involving growth factor of peptides where transforming growth factor (TGF)-beta is one of the most important. NO is also an important factor of healing, and its production is controlled by iNOS. Curcumin

significantly accelerates wound healing due to an increased expression of TGF-beta 1 and TGF-beta tllrc and increased iNOS levels [29]. Other properties of curcumin are antibacterial activity, especially in enteric bacteria [5].

*Microscopic observation of rats stomachs*

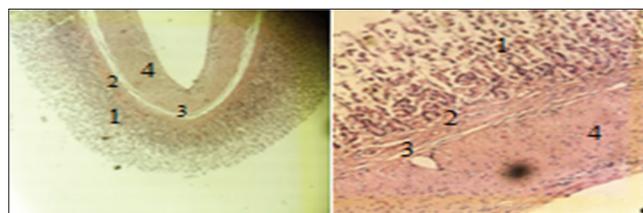
Fig. 4 shows the microscopic pictures of a normal rat stomach, and it is a representative of a normal condition of rats gastric mucosa

**Table 2: Average number of lesions of each group (n=3)**

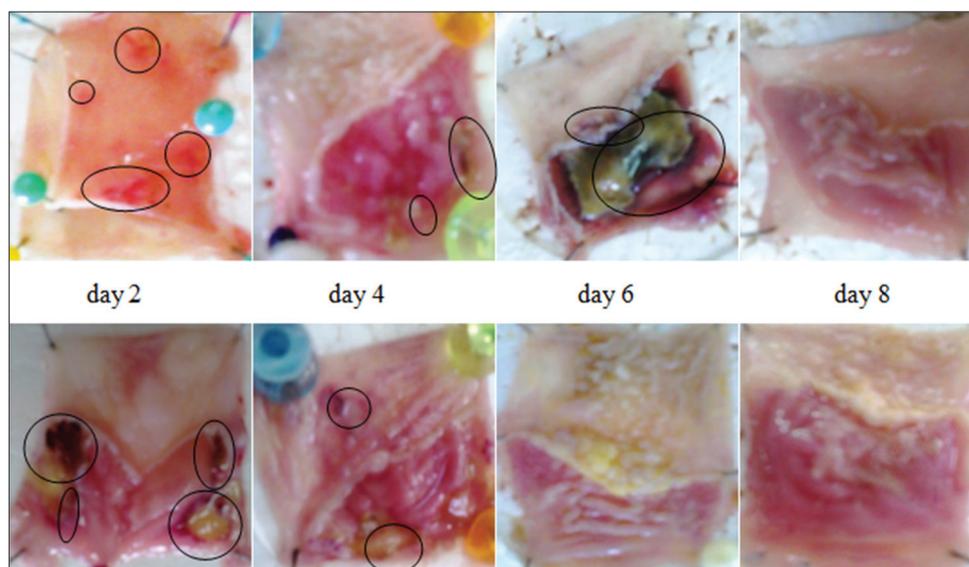
Days	Without treatment	Given alginate beads containing turmeric extract
0	8.67±1.15	
2	8.00±1.00	6.67±1.52
4	13.67±1.52	3.67±1.52
6	3.33±3.51	1.00±1.73
8	1.33±2.30	0

**Table 3: Average lesion index of each group (n=3)**

Days	Without treatment	Given alginate beads containing turmeric extract
0	0.0947±0.0378	
2	0.1078±0.0227	0.0460±0.0380
4	0.0190±0.0084	0.0174±0.0204
6	0.0984±0.1623	0.0001±0.0001
8	0.0035±0.0061	0



**Fig. 4: Microscopic pictures of a normal rat stomach. Magnification left (x40) and right (x100). (1) Mucosal layer, (2) mucosal muscular layer, (3) submucosal layer, (4) tunic muscular layer**



**Fig. 3: Macroscopic pictures of representative of rats stomach during 8 days. Without treatment (upper) and with treatment with alginate beads containing turmeric extract (under)**

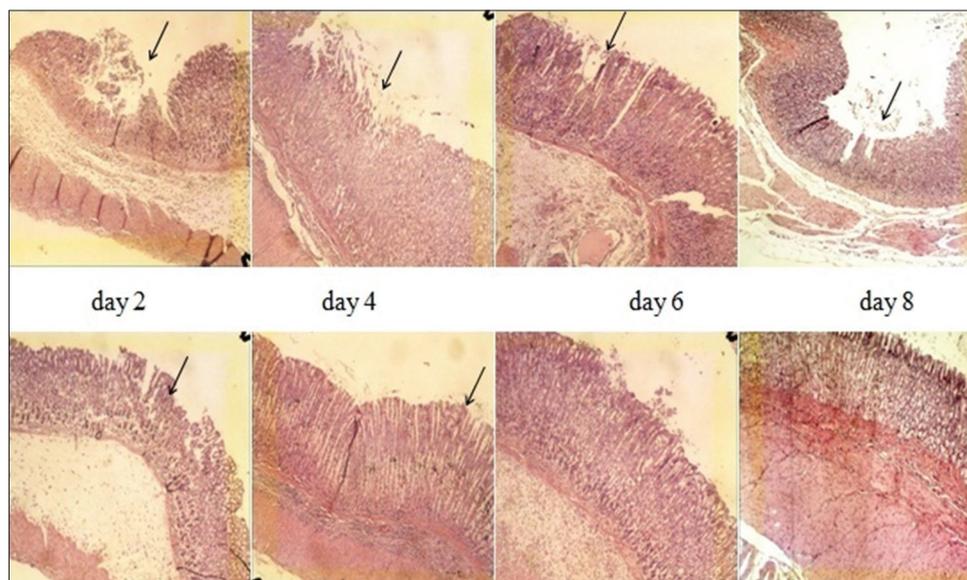


Fig. 5: Microscopic picture of representative rats stomachs. Without treatment (upper) and with treatment with alginate beads containing turmeric extract (under) (×40). Arrows show the erosion of tissue

Table 4: Conditions of rats gastric mucosa after induction with 1N HCl solution during 8 days of treatment compared to rats without treatment. Each subgroup consisted of three rats

Group of rats	Days				
	0	2	4	6	8
Treatment group	x	x x x	x x x	x o o	x o o
Without treatment group	x	x x x	x x x	x o x	x x o

x: Not yet healed; o: Healed

Fig. 5 shows the comparison gastric lesions between group without treatment and group with treatment with alginate beads containing turmeric extract. Microscopic observation shows that, at day 2, there are still damage and erosion of gastric mucosa in both groups. At day 4, there are differences, in which the epithelial cells of a group without treatment show the erosion in all three rats tested, whereas in the group with treatment, the epithelial cells begin to approach normal condition. The microscopic observation at day 6 shows that the group without treatment still has erosion while group with treatment shows all in normal epithelial cells. Microscopic observation at day 8 shows that the group of without treatment there are still erosion of gastric mucosa, while group with treatment with alginate beads containing turmeric extract shows all in normal gastric mucosa.

Healing peptic ulcers require rearrangement of epithelial structures and the connective tissues, including the lining of the vessels and muscles. Several growth factors have been involved in this process, because of their ability to regulate important cell functions, such as cell proliferation, migration, differentiation, secretion, and extracellular matrix degradation, where everything are important during tissue healing [30].

Table 4 summarizes the condition of rats gastric mucosa after induction with 1N HCl solution during treatment with alginate beads containing turmeric extract for 8 days compared to rats without treatment. This evaluation was done based on the results of macroscopic and microscopic observations. At day 4, two of three treated rats group were healed. However, on non-treated group, only one of three of rats tested was healed. At day 6, all of three treated rats group was healed. However, on rats of non-treated group, only one rat was healed. Even, at day 8, one rat was not recovery from three rats tested. Thus, it can be concluded that alginate beads containing turmeric extract have an

anti-gastric ulcer effect. Therefore, in general, it can be concluded that alginate beads containing turmeric extract are effective as anti-gastric ulcer.

CONCLUSIONS

Mucoadhesive gastroretentive alginate beads containing turmeric extract are successfully prepared and showed that this product has a mucoadhesive properties and it heals the stomach lesions faster than that without treatment.

ACKNOWLEDGMENT

This work was supported by the University of Sumatera Utara grant 2018 through the scheme of TALENTA.

AUTHORS' CONTRIBUTIONS

Hakim Bangun planned and designed the study, Fadlina Aulia conducted the study, Anayanti Arianto supported the conduction of study, and Marline Nainggolan supported the preparation of turmeric extract preparation.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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