

## Gastric Ulcer Healing Effect of Bangle (*Zingiber cassumunar* (Roxb.)) Rhizome Extract in Aspirin-induced Rats Model

Ari Yuniarto<sup>1</sup>, Elis Susilawati<sup>1</sup>, Toni Abdul Rahman<sup>1</sup>, Finna Setiawan<sup>2</sup>, Dadang Juanda<sup>3</sup>  
Pharmacology Research Group, Bandung School of Pharmacy, Jl. Soekarno-Hatta No.754,  
Bandung, Indonesia.

### Abstrak

Dalam sistem gastrointestinal, tukak lambung adalah salah satu penyakit serius yang umum terjadi. Patofisiologi tukak lambung adalah ketidakseimbangan antara faktor pertahanan agresif dan mukosa. Ketidakseimbangan antara faktor pertahanan agresif dan mukosa memiliki kontribusi terhadap perkembangan penyakit tukak lambung. Tujuan dari penelitian ini adalah untuk mengevaluasi efek penyembuhan tukak lambung dari ekstrak rimpang Bangle (*Zingiber cassumunar* (Roxb.)) pada model tikus yang diinduksi aspirin. Aktivitas antiulkus ekstrak rimpang Bangle dievaluasi dengan beberapa parameter yaitu keasaman lambung, jumlah ulkus, diameter ulkus, indeks ulkus, dan rasio penyembuhan. Tingkat dosis ekstrak rimpang Bangle yang digunakan dalam penelitian ini adalah 50 mg / kg dan 100 mg / kg. Histopatologis perut dilakukan dengan menggunakan hematoxylin-eosin yang diwarnai. Hasil penelitian menunjukkan bahwa kelompok yang diberi ekstrak rimpang Bangle mempunyai efek signifikan dalam keasaman lambung, jumlah ulkus, diameter ulkus, indeks ulkus, dan rasio penyembuhan dibandingkan dengan kontrol. Studi histopatologis ulkus lambung menunjukkan regenerasi jaringan dalam kelompok tersebut diobati dengan ekstrak rimpang Bangle dibandingkan dengan kelompok kontrol. Dari penelitian dapat disimpulkan bahwa ekstrak rimpang Bangle (*Zingiber cassumunar* (Roxb.)) memiliki aktivitas anti ulkus potensial pada model tikus yang diinduksi aspirin.

**Kata kunci:** *Zingiber cassumunar* (Roxb.), Ekstrak, Bisul, Aspirin.

### Abstract

In the gastrointestinal system, gastric ulcers are one of the common serious diseases. The pathophysiology of gastric ulcer is an imbalance between aggressive and mucosal defense factor. Imbalance between aggressive and mucosal defense factor have contribution against developed of gastric ulcer disease. The objective of the research was to evaluate gastric ulcer healing effect of Bangle (*Zingiber cassumunar* (Roxb.)) rhizome extract in aspirin-induced rats model. Antiulcer activity of Bangle rhizome extract was evaluated by several parameters involving gastric acidity, number of ulcers, diameters of ulcers, ulcer index, and healing ratio. Doses level of Bangle rhizome extract which used in this study such as 50 mg/kg and 100 mg/kg, respectively. Histopathological of the stomach was performed using hematoxylin-eosin stained. The results of study showed that groups which given Bangle rhizome extract have significant effect in gastric acidity, number of ulcers, diameters of ulcers, ulcer index, and healing ratio compared to the control group. Histopathological study of the stomach ulcers showed tissues regeneration in group were treated by Bangle rhizome extract compared to the control group. It can be concluded that from the experimental study of Bangle (*Zingiber cassumunar* (Roxb.)) rhizome extract have potential antiulcer activity in aspirin-induced rats model.

**Keyword :** *Zingiber cassumunar* (Roxb.), Extract, Ulcer, Aspirin.

## Introduction

Gastric ulcer disease influences more than 10% people in their life and any ages<sup>1-2</sup>. Use of Non-steroid anti-inflammatory drugs (NSAID), *Helicobacter pylori* invasion in gastric lining, and stress condition are common causes of gastric ulcer disease and have important role in the pathophysiology of gastric and duodenal ulcer. Pathophysiology of gastric ulcer is imbalance between aggressive and mucosal defense factor. Increase of aggressive factors (gastric acid and pepsin) and decrease of mucosal defense factors (prostaglandin inhibition, decrease of bicarbonate, blood supply, mucus film, and epithelial barrier) have contribution against developed of gastric ulcer<sup>3-6</sup>.

Gastrointestinal disorders associated with Aspirin and non-selective NSAID administration against portion of upper gastrointestinal are well documented. Long-term impact of Aspirin and non-selective NSAID administration give contribution against gastric mucosal defense. These agents have been implicated in the pathophysiology of gastric and duodenal ulcer. So, the objective of treatment for gastric ulcer such as reducing pain condition, ulcer healing, and prevent ulcer recurrence, although long term administration of synthetic drugs showed that these drugs give contribution against several side effects<sup>7-9</sup>.

Target of the current study are to find a suitable treatment for gastric ulcer as potentially drug candidate based natural product. Several research using natural product sources to treat gastric ulcer in animal model have been shown promising results.

*Zingiber cassumunar* Roxb. (family *Zingiberaceae*), locally known as “Bangle” in Indonesia, “Kunyit Bolai” in Malaysia, “Plai” in Thailand, and “Curry Turmeric” in England. *Zingiber cassumunar* Roxb. is one of the most widely used as traditional medicine in South East Asia, especially Indonesia. Bangle have been used as folk

medicine for the treatment of several diseases involves rheumatoid arthritis, wounds, respiratory disorders, cough, colds, nausea, carminative, and a mild laxative<sup>10</sup>. In Indonesia, Bangle used for relieve colic condition in children<sup>10</sup>. Use of Bangle rhizome to treat gastric ulcer have reported. In current study, we try to evaluate potency of Bangle rhizome extract through in vivo experimental gastric ulcer model. Effect of Bangle rhizome extract would be expected could improve tissues damaged in gastric ulcer condition. Therefore, the aim of the present study is to evaluate gastric ulcer healing effect of *Zingiber cassumunar* (Roxb.) rhizome extract in aspirin-induced rats model.

## Materials and Method

### Experimental material

All reagents in this study were of analytical grade. Aspirin® and Sucralfate were purchased from Kimia Farma Pharmacy, Bandung, Indonesia.

### Plant material and identification

Bangle (*Zingiber cassumunar* (Roxb.)) were collected from Manoko, Lembang, West Java, Indonesia. Furthermore, Bangle rhizome identification was performed at Herbarium of Jatinangor, Laboratory of Plant Taxonomy, Department of Biology, Padjajaran University, Indonesia.

### Preparation of Bangle rhizome extract

Bangle rhizome which obtained from Manoko, were performed by several stages. Bangle rhizome were dried and then were powdered. The powder of Bangle rhizome was extracted by maceration method and evaporated by Rotary Evaporator. Bangle rhizome extract was used for in vivo study.

### Phytochemical screening of Bangle rhizome extract

Phytochemical screening of Bangle rhizome extract were performed to evaluate the presence of chemical compounds such as alkaloids, flavonoids, saponins, tannins,

quinones, and steroids/triterpenoids.

#### Experimental animal

Healthy adult male wistar rats with weight 200-250g were used for the in vivo study. Rats were placed at controlled room with easy access to get food and water (ad libitum). 12 hours before the study, rats were transferred to the Laboratory of Pharmacology, Bandung School of Pharmacy and were fasted, given only water. The in vivo experiment study was performed after approval by the Health Research Ethics Committee, Faculty of Medicine, Padjajaran University.

#### Aspirin-induced rats model

Experimental rats were divided into five groups, each group consisting of five rats. Group I or normal group received 0.5% Carboxymethylcellulose-Na (CMC-Na), group II or control group received Aspirin (500 mg/kg). Group III received the standard drug (Sucralfate 90 mg/kg), group IV and V received Bangle rhizome extract at the doses 50 mg/kg and 100 mg/kg, respectively. Group II to group V received Aspirin 500 mg/kg. Gastric ulcer model on rats was induced by Aspirin administration 500 mg/kg for 2 days. After induction period for 2 days, rats were sacrificed, the rats stomach were incised along the greater curvature.

The gastric juice was collected, centrifuged at 2000 rpm/min for 10 min. Measurement of acidity level through titration method. Furthermore, the severity of ulcers was observed and scored to determine ulcer index (UI). Severity score based on Gupta et al.<sup>11</sup> (0=normal, 0.5=redness, 1=spot ulcer, 2=hemorrhagic ulcer, 3=deep ulcer, 4=perforation). Ulcer index was determined by the following formula of Vogel<sup>12</sup>:

$$UI = UN + US + UP \times 10^{-1}$$

UN = Average of number of ulcers per animal

US = Average of severity score

UP = Percentage of animals with ulcers

The percentage of ulcer healing was determined as: Ulcer healing (%) = (UI control-UI test group)/UI control group x 100%.

#### Histopathological examination

At the last of study, rats were sacrificed. The stomach were collected and washed with normal saline solution. Rats stomach was kept to 10% formalin solution for 24 hours in bottle and dehydrated using alcohol, were embedded in paraffin wax, and cleaned with xylene and alcohol. Furthermore, the washed tissues were treated using Hematoxylin-Eosin. The objective of histopathological examination is to observe pathology condition in gastric tissues which caused by Aspirin administration.

#### Statistical Analysis

Statistical analysis of the results of antiulcer effect were performed using One-way analysis of variance (ANOVA) method, coupled with the post-hoc Dunnett's test. A value of  $p < 0.05$  was used to denote statistical significance. All data were expressed as mean  $\pm$  standard deviation of the mean (SD) for each group.

## Results

### Phytochemical screening of Bangle rhizome extract

The phytochemical screening of Bangle rhizome extract showed the presence of alkaloids, flavonoids, saponin, tannin, quinone, and steroids/triterpenoids compounds. These phytochemical compounds might be responsible against antiulcer activity in Aspirin-induced rats model.

### Aspirin-induced rats model

As shown in table 1, the standard drug (Sucralfate 90 mg/kg), Bangle rhizome extract 50 mg/kg, and Bangle rhizome extract 100 mg/kg given ulcer healing significantly different compared to the

Table 1 Effect of Bangle rhizome extract on ulcer healing

Group	Gastric pH	Number of ulcers	Diameters of ulcers (mm)	UI groups	Ulcer healing (%)
Normal group	2.37±0.26	0	0	0	-
Control group	1.5±1.35*	8.3±2.62	3.75±0.62	22.05	0
Sucralfate 90 mg/kg	2.63±0.26	1.33±1.18*	2.33±0.47*	13.66	38.05
Bangle Rhizome Extract 50 mg/kg	2.43±0.12	4.33±1.69	2.43±0.12*	16.76	24
Bangle Rhizome Extract 100 mg/kg	2.5±0.14	1.83±1.03*	2.39±0.28*	14.22	35.52

control group in number of ulcers and diameters of ulcers.

#### Histopathological examination

Histopathological examination was used to support in vivo experimental data. Based on the results, both Bangle rhizome extract at the dose 50 mg/kg and 100 mg/kg effectively to improve stomach tissue damaged.

#### Discussions

As shown in table 1, the standard drug (Sucralfate 90 mg/kg), Bangle rhizome

extract 50 mg/kg, and Bangle rhizome extract 100 mg/kg given ulcer healing significantly different compared to the control group in number of ulcers and diameters of ulcers. Bangle rhizome extract with dose 50 mg/kg and dose 100 mg/kg effectively to affect diameters of ulcers and ulcer index (UI) such as 2.43±0.12 (16.76) and 2.39±0.28 (14.22), respectively. The ulcer index, diameters of ulcers, and number of ulcers were found to be significantly increased in the control group (were induced by Aspirin). This result is consistent with histopathological examination as supportive data, where

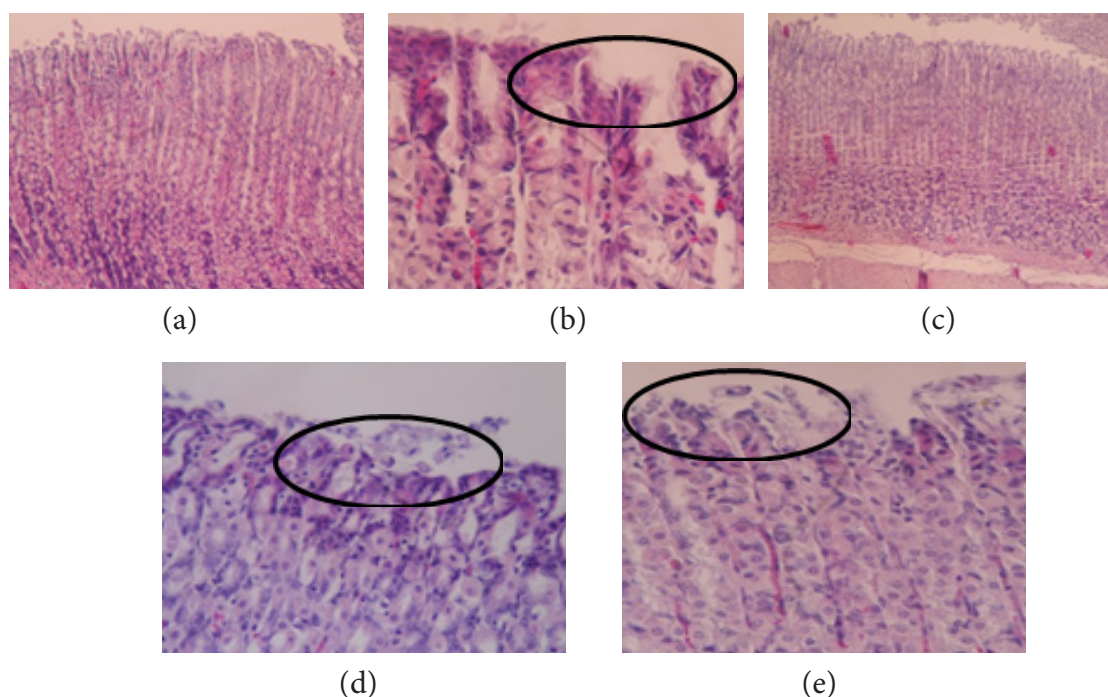


Figure 1 (a) Normal group, (b) Control group, (c) Sucralfate 90 mg/kg (d) Bangle rhizome extract 50 mg/kg (e) Bangle rhizome extract 100 mg/kg.

stomach tissue and inflammation area were reduced in groups given treatments with Bangle rhizome extract 50 mg/kg and 100 mg/kg compared to the control group.

It has been observed from histopathological results, showed that both Bangle rhizome extract 50 mg/kg and 100 mg/kg effectively to improve stomach tissues damaged. Bangle rhizome extract at the dose 50 mg/kg could reduce necrosis, edema, and inflammation very well compared with the dose 100 mg/kg for 7 days treatment. Sucralfate-treated rats didn't show severe damaged in stomach lining. Furthermore, Control group which administered by Aspirin 500mg/kg showed the presence of inflammatory condition, epithelial desquamation, edema, and deep perforation for 2 days induction. Necrosis, inflammation, and diminished of mucosal integrity factors have ability to activating several inflammatory mediators and macrophage which contributed to oxidative stress<sup>13</sup>. It can be concluded that, both Bangle rhizome extract 50 mg/kg and 100 mg/kg has ability for improving stomach tissue damaged.

Other research explored that bangle rhizome have antioxidant activity<sup>14</sup>. The ability of Bangle rhizome extract in gastric ulcer healing might be is depends on its antioxidant activity. The antioxidant activity of Bangle rhizome extract is suggested because the presence of flavonoids and curcuminoids compound<sup>10</sup>. This mechanism might be through decrease of free radical formation in inflammation area. Other compounds involving alkaloids, saponin, tannin, quinone, and steroids/triterpenoids were found in Bangle rhizome extract also supporting stomach tissues regeneration through unknown mechanism.

### Conclusion

Based on the experimental study showed that Bangle (*Zingiber cassumunar* (Roxb.)) rhizome extract has potentially antiulcer activity in Aspirin-induced rats model.

### Acknowledgment

This research was supported by Internal Research Grant from the Centre of Research and Community Services (P3M), Bandung School of Pharmacy, West Java, Indonesia.

### Conflict of Interest

There is no conflict of interest to disclose.

### References

- [1] Adnyana IK, Sigit JI, Kusumawardhani LA. Gastric ulcer healing effect of wild honey and its combination with turmeric (*Curcuma domestica* Val.) rhizome on male wistar rats. *Journal of Chinese Pharmaceutical Sciences*. 2014, 23(12): 844-849.
- [2] Ewadh M, Al-bayati N, Ijam A. Role of leukotriene in gastric ulcer induced by acetylsalicylic acid in male rabbits: gastroprotection by montelukast. *Advances in Life Science and Technology*. 2015, 28: 7-14.
- [3] Midha G, Arora R, Parveen S. Study different experiment models used in peptic ulcers. *International Journal of Recent Advances in Pharmaceutical Research*. 2015, 5(2): 31-37.
- [4] Rodriguez LAG and Hernandez-diaz S. Risk of uncomplicated peptic ulcer among users of aspirin and nonaspirin anti-inflammatory drugs. *Am J Epidemiol*. 2004, 159: 23-31.
- [5] Quan C and Talley NJ. Management of peptic ulcer diseases not related to *Helicobacter pylori* or NSAIDs. *Am J Gastroenterol*. 2002, 97(12): 2950-61.
- [6] Matsui H, Shimokawa O, Kaneko T, Nagano Y, Rai K, Hyodo I. The Pathophysiology of non-steroidal anti-inflammatory drug (NSAID)-induced mucosal injuries in stomach and small intestine. *J Clin Biochem Nutr*. 2011, 48(2): 107-11.
- [7] Adinortey MB, Ansah C, Galyuon I, Nyarko A. In vivo models used for evaluation of potential

- antigastroduodenal ulcer agents. *Ulcers*. 2013: 1-12.
- [8] McCarthy DM. Adverse effects of proton pump inhibitor drugs: clues and conclusion. *Curr Opin Gastroenterol*. 2010,26(6): 624-31.
- [9] Singh KD, Chetia D, Junejo JA. Antiulcer and in vitro antioxidant activity of *Allium hookeri*: an ethnomedicinal plant of Manipur. *Asian J Pharm Clin Res*. 2015,8(5): 130-35.
- [10] Singh CB, Manglembi N, Swapana N, Chanu SB. Ethnobotany, phytochemistry, and pharmacology of *Zingiber cassumunar* Roxb. *Journal of Pharmacognosy and Phytochemistry*. 2015. 4(1): 1-4.
- [11] Gupta J, Dinesh K, Ankit G. Evaluation of gastric anti-ulcer activity of methanolic extract of *Cayratia trifolia* in experimental animals. *Asian Pac J Trop Dis*. 2012, 99-102.
- [12] Vogel HG. Drug discovery and evaluation: Pharmacological assays. Springer-Verlag, Berlin. 1997, 1235-40.
- [13] Bhattacharyya A, Chattopadhyay R, Mitra S, Crowe SE. Oxidative stress: An essential factor in the pathogenesis of gastrointestinal mucosal diseases. *Physiological Reviews*, 2014, 94(2):329-54.
- [14] Koparde AA, Magdum CS, Kadam S. Studies on antioxidant activity of *Zingiber cassumunar* Roxb. by DPPH radical scavenging method. *Asian Journal of Biochemical and Pharmaceutical Research*. 2016. 6(3): 2231-2560.