

ANTIOXIDANT AND GASTRIC ULCER HEALING EFFECT OF *ORTHOSIPHON STAMINEUS* (BENTH.) LEAVES EXTRACT IN ASPIRIN-INDUCED RATSARI YUNIARTO^{1*}, ELIS SUSILAWATI¹, ISMI KHAIRUNNISA¹, DADANG JUANDA², FINNA SETIAWAN³

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Received: 25 October 2016, Revised and Accepted: 02 November 2016

ABSTRACT

Objective: In the gastrointestinal system, gastric ulcer is one of the common serious problems in human life and gives contribution against morbidity and mortality incidence. The pathophysiology of gastric ulcer is an imbalance between aggressive factor and mucosal integrity factor. Increase of aggressive factors and decrease of mucosal integrity factors have potential against developed of gastric ulcer disease. The objective of the research was to evaluate antioxidant and gastric ulcer healing effect of *Orthosiphon stamineus* (Benth.) leaves extract in aspirin-induced rats.

Methods: *In vivo* antiulcer activity of *Orthosiphon* leaves extract was evaluated through several parameters involves gastric acidity, number of ulcers, diameters of ulcers, ulcer index (UI), and healing ratio. Dose level of *Orthosiphon* leaves extract which used in this study such as 250 and 500 mg/kg, respectively. Antioxidant activity of *Orthosiphon* leaves extract was evaluated by 1,1-diphenyl-2-picrylhydrazyl hydrate (DPPH) method. Histopathological of the stomach was performed using hematoxylin-eosin stained.

Results: The results of the study showed that groups which given *Orthosiphon* leaves extract have significantly different for gastric ulcer healing compared to the control group and were supported by histopathological analysis. The *Orthosiphon* leaves extract also showed maximum scavenging activity at a concentration of 100 µg/ml (58.86% inhibition) and minimum at 50 µg/ml (29.60% inhibition) with inhibitory concentration 50% (IC₅₀) 84.54 µg/ml when compared to ascorbic acid as the standard with IC₅₀ 5.08 µg/ml by DPPH method.

Conclusion: It can be concluded that from the experimental study of *O. stamineus* (Benth.) leaves extract has potential antiulcer activity in aspirin-induced rats model and antioxidant effect using DPPH method. Stomach tissues regeneration in gastric ulcer model might be affected by the improvement of antioxidant status.

Keywords: *Orthosiphon stamineus* (Benth.), Extract, Ulcer, Aspirin, 1,1-diphenyl-2-picrylhydrazyl hydrate.

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INTRODUCTION

Gastric ulcer disease affects more than 5-10% people in their life [1]. Non-steroid anti-inflammatory drugs (NSAIDs), *Helicobacter pylori* infection, and stress factor are common causes of gastric ulcer disease and play a role in the pathophysiology of gastric and duodenal ulcer. Gastric ulcer is believed to be due to an imbalance between aggressive and mucosal integrity factors. Increase of aggressive factors (gastric acid and pepsin secretion) and decrease of mucosal integrity factors (inhibition of prostaglandin, decrease of bicarbonate concentration, and diminished of blood flow in gastric) have potential against developed of gastric ulcer [2-4].

Gastrointestinal toxicity associated with the use of aspirin (acetylsalicylic acid) and other non-selective NSAID against upper gastrointestinal is well documented. The long-term effect of aspirin and non-selective NSAID group administration can influence gastric mucosal integrity. These agents have been implicated in the pathophysiology of gastric and duodenal ulcer. Aspirin has two mechanisms to influence gastric damaged. First mechanism by direct irritation in gastric mucosal and second mechanism by inhibition of cyclooxygenase enzyme [5].

The goals of treating gastric ulcer disease are to reduce pain, ulcer healing, and prevent ulcer recurrence. Long-term administration of antiulcer drugs showed that these drugs give contribution against several side effects [6,7]. In this case, target of the current study is to find a suitable treatment for gastric ulcer from natural product

sources. Several researches using natural product sources to treat gastric ulcer in experimental animal has been shown best results. The mechanism of action of natural product on tissue regeneration in gastric ulcer condition is not clear although several hypotheses have been proposed.

Orthosiphon stamineus (Benth.) can be found in Southeast Asia region involves Indonesia, Vietnam, Thailand, Malaysia, and Australia. *O. stamineus* (Benth.) (Family *Lamiaceae*), locally known as "Kumis Kucing" in Indonesia, has been used in traditional medicine for the treatment of several conditions such as arthritis disorders, diabetes mellitus, hypertension, renal disorders, and antipyretic [8,9]. Several researches explained that *O. stamineus* (Benth.) leaves also have antioxidant activity. This effect 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 *O. stamineus* (Benth.) leaves extract in aspirin-induced rats model and its antioxidant activity using 1,1-diphenyl-2-picrylhydrazyl hydrate (DPPH) method.

METHODS**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

O. stamineus (Benth.) leaves were collected from the Centre of Research and Development of Medicinal Plants and Traditional Medicine, Central Java, Indonesia. *Orthosiphon* leaves identification was performed at Herbarium of Jatinangor, Laboratory of Plant Taxonomy, Department of Biology, Padjadjaran University, Indonesia.

Preparation of extract

O. stamineus (Benth.) leaves which obtained from the Centre of Research and Development of Medicinal Plants and Traditional Medicine were processed through several stages. *O. stamineus* (Benth.) leaves were dried and then were powdered. The powder of *Orthosiphon* leaves was extracted by maceration method and evaporated by Rotary Evaporator. *Orthosiphon* leaves extract was used for further studies.

Phytochemical screening of extract

Phytochemical screening of *Orthosiphon* leaves extract was performed to evaluate the presence of phytoconstituents such as alkaloids, flavonoids, saponins, tannins, quinones, and steroids/triterpenoids.

Experimental animal

Healthy adult male Wistar rats (200-250 g) were used for the experiment. Rats were maintained at controlled room with free access to food and water. 12 hrs before the experiment, rats were transferred to the Laboratory of Pharmacology Bandung School of Pharmacy and were fasted, given only water *ad libitum*. The experiment study was performed after approval by the Health Research Ethics Committee, Faculty of Medicine, Padjadjaran University (No.316./UN6.C1.3.2/KEPK/PN/2016).

Aspirin-induced rats model

Experimental animals were divided into five groups, each group consisting of five rats. Group I or normal group received 0.5% carboxymethyl cellulose-Na and Group II or control group received aspirin (500 mg/kg). Group III received the standard drug and Group IV and V received *Orthosiphon* leaves extract at the doses 250 and 500 mg/kg, respectively (Group II to Group V received aspirin 500 mg/kg). Gastric ulcer model on experimental rats was induced by administering aspirin 500 mg/kg for 2 days. After induction period, rats were sacrificed, the rats' stomach was incised along the greater curvature. Sucralfate 90 mg/kg was used as the standard drug for this experiment.

The gastric juice was collected, centrifuged at 2000 rpm/min for 10 minutes. Measurement of acidity was through titration method. Furthermore, the severity of ulcers was observed and scored to determine UI. Severity score based on Gupta *et al.* [10] (0=Normal, 0.5=Redness, 1=Spot ulcer, 2=Hemorrhagic ulcer, 3=Deep ulcer, and 4=Perforation). UI was determined by the following formula of Vogel [11]:

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

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 × 100%.

Histopathological examination

At the final of study, rats were sacrificed. The stomach was collected and washed with normal saline solution. Stomach was kept in 10% formalin

solution for 24 hrs and dehydrated using alcohol, was embedded in paraffin wax, and cleaned with xylene and alcohol. Furthermore, the washed tissues were treated using hematoxylin-eosin dye. The purpose of the examination is to observe pathology condition in gastric tissues.

Antioxidant assay of *Orthosiphon* leaves extract

The antioxidant activity of the *Orthosiphon* extract was determined using DPPH method. Radical scavenging activity of *O. stamineus* (Benth.) leaves extract against DPPH was determined by spectrophotometry at 515 nm. Ascorbic acid was used as the standard. The absorbance was carried out in triplicates. Percentage inhibition was calculated by the formula:

$$A_0 - A_1/A_0 \times 100\%$$

Where, A_0 was the absorbance of control, and A_1 was the absorbance of test or standard sample.

Statistical analysis

Statistical analysis of the results of antiulcer effect was performed using one-way analysis of variance 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±standard deviation for each group.

RESULTS AND DISCUSSION

Phytochemical screening of extract

The phytochemical screening of *Orthosiphon* leaves extract showed the presence of alkaloids, flavonoids, saponin, tannin, quinone, and steroids/triterpenoids compounds. These 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), *Orthosiphon* leaves extract 250 mg/kg, and *Orthosiphon* leaves extract 500 mg/kg given ulcer healing significantly different compared to the control group in a number of ulcers. *Orthosiphon* leaves extract with dose 250 and dose 500 mg/kg efficiently affecting diameters of ulcers and UI such as 2.94 ± 0.08 (15.11) and 2.67 ± 0.47 (14.17), respectively. The UI, diameters of ulcers, and number of ulcers were found to be significantly increased in the control group. It can be concluded that both *Orthosiphon* leaves extract 250 and 500 mg/kg have the ability to improve stomach tissue damaged. This result is consistent with histopathological examination, where stomach tissue and inflammation area were reduced in groups given treatments with *Orthosiphon* leaves extract 250 and 500 mg/kg compared to the control group.

Histopathological examination

It has been observed from histopathological results showed that both *Orthosiphon* leaves extract 250 and 500 mg/kg have the ability to improve stomach tissues damaged. *Orthosiphon* leaves extract at the dose 500 mg/kg could reduce necrosis and inflammation very well compared with the dose 250 mg/kg for 7 days treatment. Sucralfate-treated rats did not show severe damage in stomach. Furthermore, control group which administered by aspirin 500 mg/kg showed the presence of inflammatory condition and deep perforation for 2 days induction (Fig. 1).

Table 1: Effect of *Orthosiphon* leaves 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	1.83±0.44	8.3±3.29	3.75±0.74	22.05	0
Sucralfate 90 mg/kg	2.63±0.26	1.33±1.18*	2.33±0.47	13.66*	38.05
<i>Orthosiphon</i> leaves extract 250 mg/kg	2.53±0.18	2.17±1.43*	2.94±0.08	15.11*	31.47
<i>Orthosiphon</i> leaves extract 500 mg/kg	2.27±0.23	1.5±1.08*	2.67±0.47	14.17*	35.74

*Significantly different compared to control group ($p < 0.05$). UI: Ulcer index

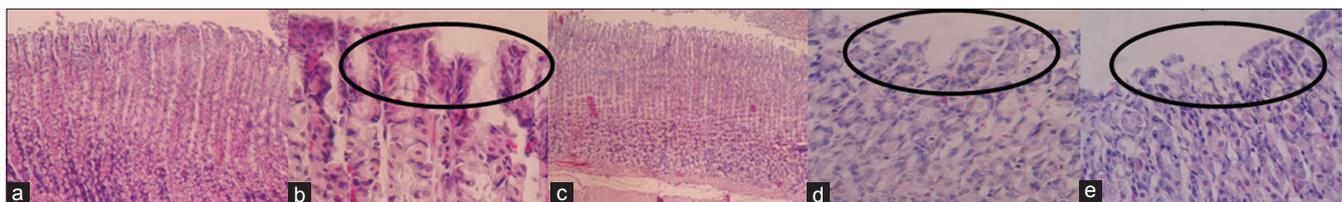


Fig. 1: (a) Normal group, (b) control group, (c) sucralfate 90 mg/kg (d) *Orthosiphon* leaves extract 250 mg/kg, (e) *Orthosiphon* leaves extract 500 mg/kg

Antioxidant activity of *Orthosiphon* leaves extract

The free radical scavenging activity of *Orthosiphon* leaves extract was studied by its ability to reduce the DPPH, a stable free radical. *Orthosiphon* leaves extract showed maximum scavenging activity at a concentration of 100 µg/ml (58.86% inhibition) and minimum at 50 µg/ml (29.60% inhibition) with inhibitory concentration 50% (IC₅₀) 84.54 µg/ml when compared to ascorbic acid as the standard with IC₅₀ 5.089 µg/ml. Based on the result, *Orthosiphon* leaves extract has best antioxidant activity with IC₅₀ 84.54 µg/ml. The purpose of antioxidant activity assay in the present study is to find a correlation between antioxidant status and stomach tissues regeneration. Necrosis, inflammation, and diminished of mucosal integrity factors have the ability to activating several inflammatory mediators and macrophage which contributed to oxidative stress [12].

The ability of *Orthosiphon* leaves extract in gastric ulcer healing is depends on its antioxidant activity. The antioxidant activity of *Orthosiphon* leaves extract is suggested because the presence of flavonoids compound. This mechanism might be through decrease of free radical formation. Other compounds involve alkaloids, saponin, tannin, quinone, and steroids/ triterpenoids in *Orthosiphon* leaves extract also supporting stomach tissues regeneration through unknown mechanism.

CONCLUSION

Based on the experimental study showed that *O. stamineus* (Benth.) leaves extract has potentially antiulcer activity in aspirin-induced rats and best antioxidant activity using DPPH method. Stomach tissue regeneration in gastric ulcer model might be affected by improvement of antioxidant status.

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.

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