



## Protective Activity of *Muntingia calabura* Fruits Extract Against Aspirin-Induced Gastric Ulcer in Rats

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Submitted 4 August 2020; Revised 23 December 2020; Accepted 4 June 2021; Published 29 October 2021

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### Abstract

Gastric ulcer can be affected people at any age. One cause of this disease is NSAID usage. The aim of this study is to determine *Muntingia calabura* L. fruit extract activity for prevent ulcer using aspirin-induced gastric ulcer in rat model. Thirty male Wistar rats were divided into six groups: normal (CMC 0.5%), negative control (CMC 0.5%), positive control (omeprazole 20 mg/kg bw), treatment group (ethanol *M. calabura* L. fruits extract): I (100 mg/kg bw), II (200 mg/kg bw), and III (300 mg/kg bw) and given orally once a day for 9 days. On the 10th day, aspirin at a dose of 500 mg/kg bw was given to all groups except the normal group. Four hours after induction, all animals were sacrificed. Preventive effect against stomach ulcer were determined by calculating the index of ulcer and histopathological examination. Ulcer index of *M. calabura* fruits extract at all doses were different than negative group, although not significant ( $p > 0.05$ ). Histopathological examination of *M. calabura* extract at a dose of 300 mg/kg bw showed few inflammatory cells and congestion compared with the other treatment group. In our finding, ethanol *M. calabura* L. fruit extract at a dose 300mg/kg bw obtained the best activity in preventive of gastric ulcer.

**Keywords:** aspirin, gastric ulcer, *Muntingia calabura* fruit

## Efek Protektif Ekstrak Buah *Muntingia calabura* terhadap Ulkus Lambung Akibat Aspirin pada Tikus

### Abstrak

Tukak lambung dapat mempengaruhi masyarakat segala usia. Salah satu penyebab penyakit ini yaitu penggunaan NSAID. Tujuan studi ini yakni untuk menentukan aktivitas buah *Muntingia calabura* L. untuk mencegah tukak menggunakan model tikus tukak lambung yang diinduksi dengan aspirin. Sebanyak 30 tikus jantan Wistar terbagi ke dalam 6 kelompok: normal (CMC 0,5%), kontrol negatif (CMC 0,5%), kontrol positif (omeprazol 20 mg/kg bb), kelompok perlakuan (ekstrak etanol buah *M. calabura* L.): I (100 mg/kg bb), II (200 mg/kg bb), dan III (300 mg/kg bb) dan diberikan per oral sekali sehari selama 9 hari. Pada hari ke-10, aspirin dosis 500 mg/kg bb diberikan pada semua kelompok kecuali kelompok normal. Empat jam setelah induksi, semua hewan uji dikorbankan. Efek pencegahan tukak lambung ditentukan dengan menghitung indeks tukak dan pemeriksaan histopatologi. Indeks tukak ekstrak buah *M. calabura* pada semua dosis berbeda dengan kelompok negatif, walaupun tidak signifikan ( $p > 0,05$ ). Pemeriksaan histopatologi ekstrak *M. calabura* dosis 300 mg/kg bb menunjukkan adanya sel yang mengalami inflamasi dan kongesti yang lebih sedikit dibandingkan pada kelompok perlakuan yang lain. Dalam penemuan kami, ekstrak etanol buah *M. calabura* L. dosis 300 mg/kg bb memberikan aktivitas yang paling baik dalam mencegah tukak lambung.

**Kata Kunci:** aspirin, buah *Muntingia calabura*, tukak lambung

## 1. Introduction

Peptic ulcer disease is suffered by many people in the world. In Indonesia, this disease causes death in 1.7% of the population at all ages.<sup>1</sup> Gastric ulcer, also known as stomach ulcer, is a type of peptic ulcer disease. This disease is marked by abdominal discomfort and pain because there are sores that develop on the lining of the stomach, although about two-third of patients are asymptomatic. Quality of life of patients could be affected when this disease is worsen and complication is developed.<sup>2</sup>

The patophysiology of gastric ulcer disease is often described as imbalance between defensive and aggressive factors. Prostaglandin (PGE<sub>2</sub>), mucus, and bicarbonate production will preserve stomach from ulcer formation. Otherwise, use of non-steroid anti-inflammatory drug (NSAID) and *Helicobacter pylori* infection are reported as major cause of stomach mucosal damage, in addition to acid, pepsin, and bile salts.<sup>3</sup> The incidence of gastric ulcer because of NSAID usage is 20%, and is increasing as getting older.<sup>4</sup>

*Muntingia calabura* L., commonly known as Jamaican cherry, and talok or kersen (Indonesia), can be found easily in warm countries such as Indonesia. People usually consume the fresh fruits and rarely utilize it for traditional medication. Whereas, the fruits exhibited some pharmacological effects such as anti-inflammatory<sup>5</sup>, antibacterial, and antioxidant.<sup>6</sup> For gastroprotective effect, Ibrahim, et al reported that ethanol extract of *M. calabura* leaves could be used as anti-ulcerogenic agent in ethanol-induced rats.<sup>7</sup> In another study by Zakaria, et al (2014), *M. calabura* leaves methanol extract could prevent gastric ulcer in pylorus ligation-induced model.<sup>8</sup> Using the same extract, Balan et al (2014) stated that antiulcer activity shown in ethanol- and indomethacin-induced rats.<sup>9</sup> However, there is little we know about the ability of *M. calabura* fruits for preventive of gastric ulcer. Therefore, we aimed to investigate the effect of ethanol extract of the fruits on aspirin-induced gastric ulcer rat model.

## 2. Materials and methods

### 2.1. Tools and materials

Rotary vacuum evaporator (Buchi R-200), analytical scales (Metler Toledo), omeprazole (Kalbe Farma), aspirin (Bayer), ethanol 96% (Brataco).

### 2.2. Plant material

*Muntingia calabura* L. fruits were picked up from Ngangkruk, Sleman, Indonesia. The plant has been determined by Plant Systematics Lab, Faculty of Biology, Gadjah Mada University, Indonesia. All of stages of ripe fruits were used in this study. The fruits were macerated using ethanol 96% at room temperature for 3x24 h. The filtrate of the extract was evaporated until there are no solvent residual in extract and get yield for 22.33%. Phytochemical properties were analyzed to identify the secondary metabolites that present in the extract of *M. calabura* fruit.

### 2.3. Animals

Three-month-old male Wistar rats were provided from Faculty of Medicine, Gadjah Mada University, Indonesia. The animals were kept under controlled conditions, such as room temperature 25±2°C, dark/light cycle 12/12 h, and free access to food and water. Before experiment, the rats were gone through acclimatization for 7 days. The protocol of the study was approved by Ethical Committee, Faculty of Medicine, Universitas Islam Indonesia (19/Ka.Kom.Et/70/KE/IV/2017).

### 2.4. Methods

Thirty male rats were divided into six groups (n=5 each). Normal and negative control group was received the vehicle (CMC 0.5%) and positive control group was treated with standard drug (omeprazole 20 mg/kg bw). The other groups were administered with ethanol *M. calabura* L. fruits extract in various doses (100; 200; or 300 mg/kg bw). The treatment was given daily for 9 days. On the last day (10th day), all the rats were induced with aspirin at a dose 500 mg/kg bw, but the normal group. Before induction, the rats were fasted for 24 hours. Four hours

after induction, the animals were anesthetized using ketamine and xylazine (100 mg/kg bw:10 mg/kg bw) i.m. then sacrificed by cervical dislocation. Stomach was collected and examined macroscopically to measure the index ulcer. Then stomach was kept in 10% formalin and embedded in parafin. The specimens were sectioned at a thickness of 5µm and stained with hematoxylin and eosin for histopathology examination.

## 2.5. Statistical

### 2.5.1. Ulcer index and percentage of ulcer inhibition<sup>10</sup>

Severity score will be measured as follows: normal (0), red coloration (0.5), spot ulcer (1), hemorrhagic ulcer (2), deep ulcer (3), and perforation (4). Ulcer index was calculated using formula:

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

UI: Ulcer index

UN : average numbers of ulcer

US : average of severity score

UP: percentage animals with ulcer

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

### 2.5.2. Statistical analysis

Statistical evaluation was conducted using one way ANOVA (significances: 95%).

## 3. Result

Phytochemical screening revealed that ethanolic extract of *M. calabura* fruits contained flavonoids, alkaloids, saponins, and terpenoids (Table 1). Table 2 showed that all the treatment groups were able to inhibit gastric ulcer, although not significantly different

from negative control group ( $p > 0.05$ ). These groups had similar ulcer numbers, severity, and index with standard group (omeprazole). Figure 1 depicted that rats given extract of *M. calabura* fruit at a dose 300 mg/kg bw had fewer inflammatory cell than negative control and other treatment groups. The presence of inflammatory cells indicated body's defense mechanism against aspirin administration. Histology examination on negative control groups showed the presences of many inflammatory cells and congestion in muscularis mucosa. Congestion is an increase in the amount of blood in an area due to failure to flow fluid out of the tissue.

## 4. Discussion

Aspirin is one of the most commonly used NSAID drugs for treating mild to moderate pain, such as, in osteoarthritis disease. However high dose aspirin or combination with another NSAID administration is associated with bleeding in the upper gastrointestinal tract.<sup>3</sup> The drug mechanism, corresponding with gastric ulcer, is by inhibiting nonselectively cyclooxygenase (COX) 1 and 2 enzymes. Meanwhile, COX-1 enzyme catalyzes the conversion from arachidonic acid to prostaglandin (PGI1 and PGE2). By inhibiting PGE2 synthesis, gastric mucus secretion and bicarbonate production will be reduced, which mean, defensive factors will be downregulated. Furthermore, NSAID induce reactive oxygen species (ROS) formation and stimulate lipid peroxidation, leukocyte infiltration, and apoptosis, which lead stomach more susceptible forming ulcer.<sup>11</sup> Table 2 shows that negative control group have the highest ulcer index, although not significantly different ( $p > 0.05$ ). Optimizing

**Table 1.** Secondary metabolites of ethanol *M. calabura* L. fruits extract

Secondary metabolites	Results
Flavonoid	+
Alkaloid	+
Saponin	+
Tannin	-
Terpenoid	+

+: detected; -: not detected

**Table 2.** Ulcer index and ulcer inhibition percentage within various treatment groups

Groups	Number of ulcer	Severity of ulcers	Ulcer Index	% ulcer inhibition
Normal control	0.00±0.00	0.00±0.00	0.01	-
Negative control	2.25±2.87	0.63±0.48	5.29	-
Positive control	0.25±0.50	0.25±0.50	2.55	52%
Treatment I	0.25±0.50	0.25±0.50	2.55	52%
Treatment II	0.25±0.50	0.25±0.50	2.55	52%
Treatment III	0.25±0.50	0.25±0.50	2.55	52%

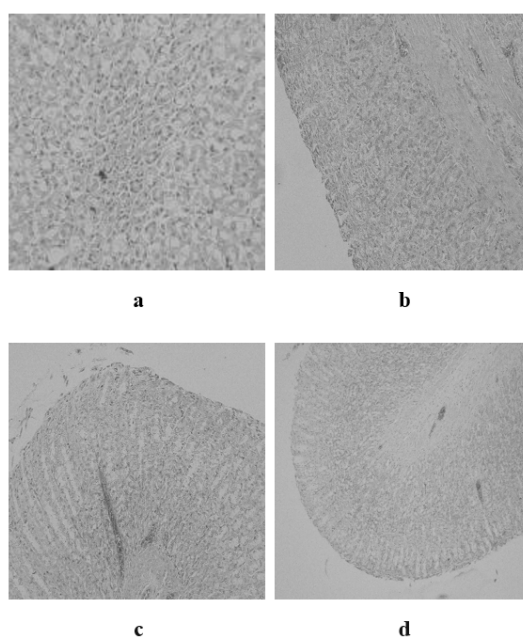
Groups: normal control (CMC 0.5%); negative control (CMC 0.5%); positive control (omeprazole 20 mg/kg); treatment I (ethanol *M. calabura* fruits extract 100 mg/kg bw); treatment II (ethanol *M. calabura* fruits extract 200 mg/kg bw); treatment III (ethanol *M. calabura* fruits extract 300 mg/kg bw).

of aspirin dosage, combining with other ulcerogenic, or replacing with other NSAID may be considered for gastric ulcer inducer in the future study.

Based on the result of ulcer index, percentage of ulcer inhibition, and stomach histology, ethanol *Muntingia calabura* fruits extract at a dose 300 mg/kg gave the best activity in prevention of gastric ulcer. The treatment had similar efficacy with standard drug, omeprazole. Whereas, long term usage of proton pump inhibitor is associated with gastric cancer in ECL-cells. Research by Lin, et al, revealed that ethanol *M. calabura* fruits extract could suppress production of nitric oxide (NO). Meanwhile, NO could modulate

gastric mucosa integrity, control acid, alkaline, and mucus production, and gastric mucosa blood flow.<sup>12</sup> Although the extract could prevent gastric ulcer, the results were not significantly different than negative group ( $p>0.05$ ). This may be due to the insufficient dose of the extract used in this study.

The gastroprotective mechanism by *M. calabura* fruits may involve anti-secretory, antioxidant, and antiinflammatory effects because of saponins and flavonoids. Flavonoids will stimulate production of gastric mucus and also scavenge for ROS and free radicals. Furthermore, flavonoids are able to inhibit pepsinogen formation and reduce acid secretion. The saponins could promote



**Figure 1.** Representative result of stomach histopathology in various treatment groups: (a) negative control (CMC 0.5%), (b) treatment I (ethanol *M. calabura* fruits extract 100 mg/kg bw), (c) treatment II (ethanol *M. calabura* fruits extract 200 mg/kg bw), and (d) treatment III (ethanol *M. calabura* fruits extract 300 mg/kg bw)



mucus secretion.<sup>8</sup>

According to Lin, et al. study, ethanol *M. calabura* fruits extract contained four flavonoids, consisted of epicatechin, rutin, diosmin, and luteolin, and eleven phenolic acids, such as gallic acid, gentisic acid, vanillic acid, p-hydroxybenzoic acid, caffeic acid, ferulic acid, p-coumaric acid, syringic acid, sinapic acid, rosmarinic acid, p-anisic acid.<sup>12</sup> Rutin showed ability to prohibit gastric ulcer formation in some studies. The mechanism may be involving endogenous platelet-activating factor (PAF). Rutin also exerted gastroprotective effect, through antioxidant activity.<sup>8</sup> Dubey, et al., claimed that rutin could inhibit gastric proton pump.<sup>13</sup> Therefore, the presence of secondary metabolites in the *M. calabura* fruits may protect against stomach lesion formation. This study may provide data about use of *M. calabura* L. fruit and could develop the plant for another use or fractionate/isolate it to get specific compound in the future study.

## 5. Conclusion

Ethanol extract of *Muntingia calabura* L. fruits at a dose 300mg/kg bw could inhibit gastric ulcer production induced by aspirin, although not significantly different than negative control group ( $p > 0.05$ ).

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