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Gastroprotective effects of combination of hot water extracts of turmeric (*Curcuma domestica* L.), cardamom pods (*Amomum compactum* S.) and sembung leaf (*Blumea balsamifera* DC.) against aspirin-induced gastric ulcer model in rats

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PEER REVIEW

Peer reviewer

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Comments

The manuscript shows comprehensive data concerning the effect of combination of 3 herbals for gastro protection. The anatomy and histological data support the effect of the herbal combination clearly on gastro protection.

Details on Page S504

ABSTRACT

Objective: To investigate the protective effect of the combination of turmeric (*Curcuma domestica*), cardamom pods (*Amomum compactum*) and sembung leaf (*Blumea balsamifera*) on gastric mucosa in aspirin-induced gastric ulcer model rats.

Methods: Thirty male Wistar rats weighing 150–200 g were divided into 6 groups. Four groups were administered with the hot water extracts combination consisted of cardamom pods 36.6 mg/200 g body weight and sembung leaf 91.5 mg/200 g body weight (fixed doses). The herbal extracts combination were also consisted of turmeric in various doses *i.e.* 10 mg/200 g body weight in the second group, 30 mg/200 g body weight in the first and third groups, and 50 mg/200 g body weight in the fourth group. The fifth group rats received sucralfate 72 mg/200 g body weight. Ten minutes after receiving herbal extracts combinations or sucralfate, the rats were induced with aspirin 90 mg/200 g body weight except the first group. Another group (sixth group) only received aspirin without any protective agent. All treatments were administered orally for seven days. The number and area of the gastric ulcers were counted and measured macroscopically. Score of mucosal damage and the number of eosinophils as well as the number of mast cells were observed in paraffin sections stained with hematoxylin eosin and toluidine blue, respectively.

Results: The groups receiving herbal infuse combination exhibited less number and smaller area of gastric ulcers as well as smaller score of mucosal damage in comparison to those of aspirin group ($P < 0.05$). The number of mast cells and eosinophil of herbal groups were also smaller than that of aspirin group.

Conclusions: The herbal extracts combination of turmeric (*Curcuma domestica*), cardamom pods (*Amomum compactum*) and sembung leaf (*Blumea balsamifera*) has potential gastroprotective effects.

KEYWORDS

Hot water extract, *Curcuma domestica*, *Amomum compactum*, *Blumea balsamifera*, Gastroprotective

1. Introduction

Gastric ulcer is a gastrointestinal disorder which can be induced by infection, smoking, stress and nonsteroidal

anti-inflammatory drugs (NSAIDs). Gastric ulcers can induce inflammatory reaction. Inflammatory reaction involves the number of mast cells and eosinophils. Sucralfate is widely used for prevention of gastric ulcers caused by NSAIDs.

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However, the use of these drugs have several side effects^[1].

Indonesia has the second largest biodiversity in the world after Brazil including medicinal plants. Some Indonesian medicinal plant empirically used for treating gastric ulcer are turmeric [*Curcuma domestica* (*C. domestica*)], cardamom pods [*Amomum compactum* (*A. compactum*)] and sembung leaf [*Blumea balsamifera* (*B. balsamifera*)]. These plants are widely found in some areas of Indonesia. *C. domestica* is a plant that contains curcumin. *C. domestica* has beneficial effect to increase mucin secretion and then acts as gastroprotectant^[2]. Reportedly, the *C. domestica* extract reduced gastric acid secretion and protected against the lesion formation on gastric mucosa. Its gastroprotective effect involves the blocking process on H₂ histamine receptors^[3]. *A. compactum* exhibited activity to reduce the gastric motility^[4]. Its extracts were also reported to inhibit the gastric lesions induced by aspirin, ethanol and pylorus ligation. Empirically, *B. balsamifera* is mentioned in Medicine Plant of Myanmar^[5], indicated for treating gastric ulcer. Based on these facts, this study was aimed to investigate the protective effect of combination of hot water extracts of *C. domestica*, *A. compactum* and *B. balsamifera* against aspirin-induced gastric ulcer model in rats.

2. Materials and methods

2.1. Materials

Turmeric (*C. domestica*), sembung leaves (*B. balsamifera*) and cardamom pods (*A. compactum*) were collected during June 2012 from Tawangmangu, Karanganyar, Central Java Indonesia. The dried powder of these herbals were extracted by infusion method using hot water as a solvent. Sucralfate, a cytoprotective agent indicated for peptic ulcers, was obtained from PT Kalbe Farma Tbk Indonesia. Aspirin (inducer for gastric ulcers), hematoxylin, eosin, lithium carbonate, Canadian balsam, toluidine blue were purchased from Sigma Chemical, St Louis, MO.

2.2. Animals

Thirty male Wistar rats (*Rattus norvegicus*) aging 2–3 months and weighing 100–250 g were obtained from Laboratory of Pharmacology and Toxicology, Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmacy, Universitas Gadjah Mada, Indonesia. The animal handling protocols of this study referred to the guidelines of the animal care of the Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmacy, Universitas Gadjah Mada, Indonesia.

The rats were housed under constant temperature (23–27 °C) with constant relative humidity (50%–70%) on an automatically controlled light (12 h light/dark). All rats were fed with standard rodent chow and water *ad libitum*. The animals were acclimatized and quarantined for at least one week before the experiment.

2.3. Experimental design

The rats were randomly divided into six groups consisting of five rats. Four groups were administered with the combination of *A. compactum* 36.6 mg/200 g body weight (fixed dose), *B. balsamifera* 91.5 mg/200 g body weight (fixed dose) and *C. domestica* (various doses depending on the group). First and third group rats received the the combination containing *C. domestica* 30 mg/200 g body weight, whereas second and fourth groups received the the combination containing *C. domestica* 10 and 50 mg/200 g body weight, respectively. Fifth group rats received sucralfate 72 mg/200 g body weight. Ten minutes after receiving herbal extract combination or sucralfate, the rats were administered with aspirin 90 mg/200 g body weight except the first group. Another group (Group 6) only received aspirin without any protective agent (negative control). All treatments were administered orally for 7 d. The number and area of the gastric ulcers were counted and measured macroscopically. Score of mucosal damage and the number of eosinophils as well as the number of mast cells were microscopically observed in paraffin sections stained with hematoxylin eosin and toluidine blue, respectively.

2.4. Histological observation

In the eighth day, after 10 h fasting the animals were sacrificed, and stomach of each animal was opened along the greater curvature. Specimens of the gastric tissue were fixed in 10% buffered formalin and were processed in the paraffin tissue-processing machine. Sections of the stomach were sectioned at 5 µm and stained with hematoxylin and eosin for histological evaluation^[6]. The size of lesions were measured using a light microscope and graded according to the scoring system as described by Djam'an^[7]. Paraffin sections were stained with toluidine blue to count the number of mast cells.

3. Results

3.1. Number and size of lesions and score of mucosal damage in the gastric mucosa

In the study, treatment of high dose of aspirin could cause ulceration represented by the presence of lesions on the gastric mucosa (Group 6). After treatment of the herbal extract combination, in aspirin-induced gastric ulcer rats, the quantity of lesions on gastric mucosa decreased. Based on the observation, there were many smaller area of gastric ulcer as well as smaller score of mucosal damage in the herbal extract combination treated groups with comparison to the sixth group (negative control) (Figure 1 and 2). The effect of these herbal extract combination were dose-dependent. In line with this result, sucralfate, a cytoprotective agent indicated for peptic ulcers, also succeeded to protect the mucosal damage.

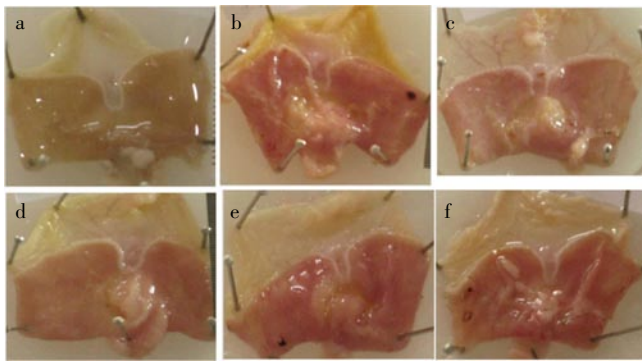


Figure 1. Macroscopic observation on gastric mucosa after aspirin induction. Infuse and sucralfate were administered for 7 d. Group 1: herbal extract combination only (a); group 2–4 : herbal extract combination low–dose, medium–dose, high–dose with aspirin induction (b–d); group 5: sucralfate 72 mg/200 g body weight with aspirin induction (e); group 6: aspirin induction without any drug treatment (f). Lesion areas were observed in group 2 and 6 (a and f).

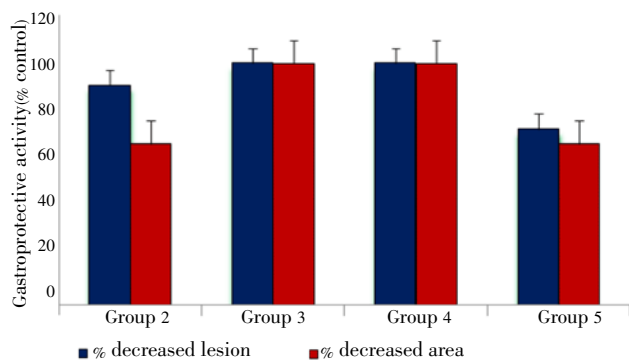


Figure 2. Gastroprotective activities of each group in aspirin-induced gastric ulcer model rats regarding to the parameters of number and area of lesions.

Histological observation revealed that treatment of aspirin markedly caused gastric ulcers and mucosa erosions (Figure 3). Treatment of herbal extract combination or sucralfate reduced the evidence of gastric ulcers and mucosal erosions. Figure 2 and 4 showed a comparison among the gastroprotective effect of all treatments macroscopically and microscopically. The effect of moderate and high doses of the herbal extract combination were more potent than that of sucralfate regarding to the parameters of number, size and score of mucosal damage.

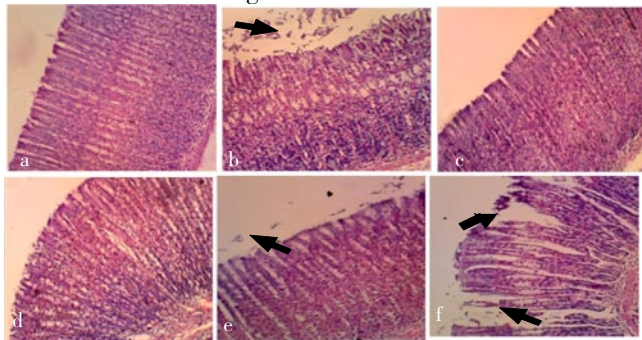


Figure 3. Hematoxylin and eosin–stained sections of gastric mucosal tissues for gastric ulcer and mucosal erosion observation.

Herbal extract combination and sucralfate were administered for 7 d. Group 1: herbal extract combination only (a); group 2–4: herbal extract combination low–dose, medium–dose, high–dose with aspirin induction (b–d); group 5: sucralfate 72 mg /200 g body weight with aspirin induction (e); group 6: aspirin induction without any drug treatment (f). Magnification 100X. Mucosal damage is showed by black arrow.

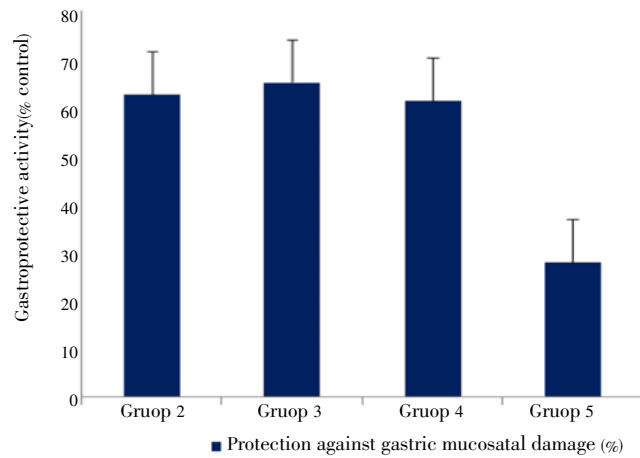


Figure 4. Gastroprotective activities of each group in aspirin-induced gastric ulcer model rats regarding to the parameters of gastric mucosal damage.

3.2. Number of mast cells and eosinophils in the mucosa and sub-mucosa

Treatment of high dose of aspirin (Group 6) also increased the number of mast cells in sub mucosal layer (Figure 5).

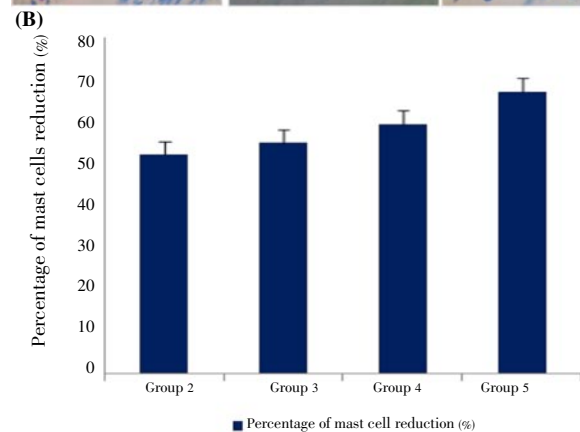
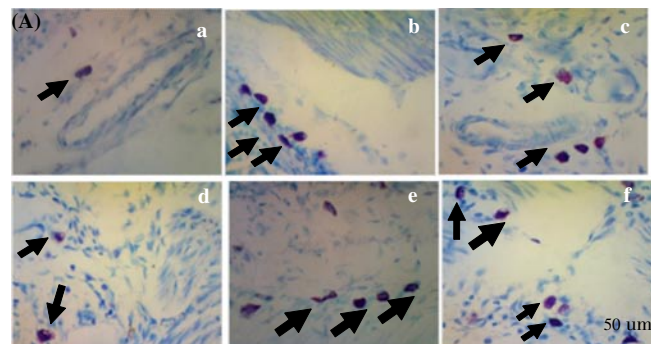


Figure 5. (A) Toluodine blue–stained sections of gastric mucosal tissues for gastric ulcer and mucosal erosion observation.

Herbal extract combination and sucralfate were administered for 7 d. Group 1: herbal extract combination only (a); group 2–4: herbal extract combination low–dose, medium–dose, high–dose with aspirin induction (b–d); group 5: sucralfate 72 mg /200 g body weight with aspirin induction (e); group 6 : aspirin induction without any drug treatment (f). Magnification 100X. Mast cell is showed by black arrow.

(B) Gastroprotective activities of each group in aspirin-induced gastric ulcer model rats regarding to the parameters of mast cells reduction number.

Mast cells are multifunctional immune cells that express high–affinity immunoglobulin E receptors, and release potent inflammatory mediators including histamine, leukotrienes, serotonin, and proteases such as trypsin[8]. In

line with this results, the aspirin treatment also stimulated eosinophil mobilization into sub-mucosa and mucosal tissues (Table 1). The higher number of eosinophils mobilizations showed an inflammatory process in gastric mucosa. Treatment of herbal extract combination could prevent the increase of mast cells number as well as eosinophil mobilization into sub-mucosa and mucosal tissues. In this study, sucralfate also succeeded to avoid the increase of mast cells number, and eosinophil mobilization. In the study, regarding to eosinophil mobilization the effects of high dose of the herbal extract combination was higher than that of sucralfate (Figure 6).

Table 1

Number of mast cells and eosinophils in the gastric mucosa and sub-mucosa of each treatment group and the control.

Group	Number of sub mucosal mast cells/ $10^2 \mu\text{m}^2$	Number of eosinophils/ $10^2 \mu\text{m}^2$	
		Sub mucosa	Mucosa
Group 1: herbal extract combination only	0.45±0.07	0.08±0.16	2.30±2.06
Group 2: low dose herbal extract combination (+aspirin)	0.69±0.33	0.86±0.21	3.10±1.84
Group 3: moderate dose herbal extract combination (+aspirin)	0.65±0.22	0.84±0.85	2.68±0.84
Group 4: high dose herbal extract combination (+aspirin)	0.58±0.07	0.54±1.28	2.37±1.28
Group 5: sucralfate (+aspirin)	0.47±0.44	0.95±1.53	3.37±0.81
Group 6: aspirin only (negative control)	1.44±0.85	2.07±2.37	4.77±1.71

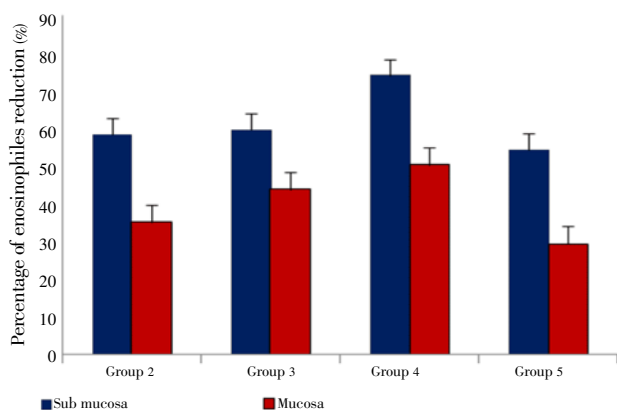


Figure 6. Percentage of eosinophiles reduction of each group in aspirin-induced gastric ulcer model rats.

4. Discussion

In the study, aspirin was used for inducing the gastric ulcers. The incidence of upper gastrointestinal damage in patients taking long-term aspirin has been investigated. The mechanism of action of NSAIDs can be subdivided into local action and systemic action, and several mechanisms have been reported in a previous review. The pathogenesis related to inhibition of cyclooxygenase (COX)-1 includes reduced mucosal flow, reduced mucus and bicarbonate secretion, and impaired platelet aggregation. The pathogenic mechanisms involved in inhibition of COX-2 are reduction of angiogenesis and increase of leukocyte adherence. The pathogenesis related to direct epithelial damage involves acid back diffusion and impaired platelet aggregation. Aspirin is a more potent inhibitor of COX-1 than that of COX-2[9].

These gastric mucosal damages were also accompanied

by granulocyte infiltration. Mucosal cells synthesize proinflammatory chemotactic as inflammatory mediators that can attract more eosinophils due to the present of mucosal-damaging agents[10]. In the study, several lesions were also observed in the gastric mucosal lining. Aspirin, one of NSAID that has been widely used for several clinical indications, causes damage of gastrointestinal mucosa, induces stress ulcer and exacerbates the previous gastric ulcer. Interaction process between NSAID and stress may cause lesion of stomach mucosa[11].

Sucralfate, a cytoprotective agent indicated for peptic ulcers, was used as a comparison for herbal extract combination. Sucralfate could protect the mucosal damage as well as avoid the increase of mast cells number and eosinophil mobilization. However, binding of sucralfate to aspirin is not considered to be a factor regarding to its protective effect against aspirin-induced gastric ulcer. Sucralfate acts by stimulating an increase of mucosal prostaglandin E2 synthesis, secretion of bicarbonate and mucus secretion[12].

In the study, the herbal extract combination was administered in rats in absence of aspirin. The experiments aimed to observe the possibility of herbal infuse to influence the gastric mucosa. In the study, there was no any significant evidence of gastric mucosal damage or irritation. However, *C. domestica* may cause mild irritation[13]. Its main compounds, namely, curcuminoids, can cause mild gastric mucosal irritation if consumed for long term and in an empty stomach. In presence of aspirin, the infuse decreased the gastric ulcer regarding to reduction of lesion quality.

Previously, *C. domestica* was reported to exhibit a potential gastroprotective effect with several mechanisms, such as blockade of histamine H₂ receptor and inhibition of gastric acid secretion[3]. In addition, it was reported that the protective effect of *C. domestica* on gastric mucosa was related to inhibition on acid secretion and its vasodilator effect[14]. The main compound of *A. compactum* and *B. balsamifera* are flavonoids. Reportedly, these compounds exhibited a gastroprotective effect regarding to their antioxidative effect[15]. Flavonoids exhibited a protective effects by reducing the severity of ulcers and increasing the amount of gastric mucus glycoprotein, through inhibition on prostaglandin stimulation, production of mucus and their antioxidative effect[16].

In addition, flavonoids showed a protective effects against ethanol-induced gastric ulcer, through its ability to reduce the levels of malondialdehyde, an indicator of lipid peroxidation, in the stomach homogenates[17]. Among the various pathological conditions caused by an imbalance between oxidative damage and antioxidant defense system, lipid peroxidation is known to be an example of oxidative damage that affects cell membranes.

As a conclusion, combination of hot water extracts of *C. domestica*, *A. compactum* and *B. Balsamifera* was effective to protect the gastric mucosa and decrease the number of mast cells and eosinophils in aspirin-induced gastric ulcer model in rats.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

Gastric ulcer is a gastrointestinal disorder which can be induced by infection, smoking, stress and NSAIDs. Sucralfate is widely used for prevention of gastric ulcers caused by NSAIDs. However, the use of these drugs have several side effects.

Research frontiers

The research work describes the effect of combination of *C. domestica*, *A. compactum* and *B. balsamifera* on aspirin-induced gastric ulcer and compared it with sucralfate.

Related reports

Aspirin and other NSAIDs are known to induce gastric ulcer in prolong use. There should be agent that can protect gastric region against the damage from NSAIDs and aspirin. Exploration of herbal medicines for gastroprotective agents is a rational approach.

Innovations and breakthroughs

Combination of three kind of herbals *C. domestica*, *A. compactum* and *B. balsamifera* for gastroprotective effect has not been reported elsewhere. This gives insight that herbal combination can be used for aspirin-induced gastric ulcer.

Applications

This combination has been used empirically for gastric disorder. With this research report, it can be suggested to use this kind of combination to protect from gastric injury due to NSAIDs consumption in prolong time.

Peer review

The manuscript shows comprehensive data concerning the effect of combination of 3 herbals for gastro protection. The anatomy and histological data support the effect of the herbal combination clearly on gastro protection.

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