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The Gastroprotective Activity of Ethanol Extract of *Curcuma domestica* Val. on Mice Induced Ethanol - HCl

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Abstract

Curcuma domestica Val. (CD) is one of plant used as traditional medicine in around the world. One of the benefit of CD is to treat peptic ulcers. The aim of preset study to prove that the ethanol extract of CD has gastroprotective activity. This study used 25 male mice were divided into 5 groups. Group I as a control group were given suspension of 0.5% Na-CMC, groups II, III and IV were each given ethanol extract of CD suspense with 0.5% Na-CMC at a dose of 50 mg/kg; 100 mg/kg; 200 mg/kg respectively, and group V was given ranitidine 10 mg/kg. All groups were treated orally for 6 days, then fasted for 24 hours, on the seventh day all mice induced 3M HCl - ethanol 60% (1:1) 0.2 ml/25 gBW. One hour later, mice were sacrificed by cervical dislocation, then dissected and taken gastric. Then performed an ulcer scoring and the curative ratio is calculated later in the histopathologic test. Data were analyzed using Kolmogorov-Smirnov test and the Levene test and ANOVA followed by LSD test level of 95%. The results showed there was no significant difference between the control with dose 50 mg/kgBW ($p>0.05$) and showed significant difference between control with dose 100 mg/kgBW ($p<0.05$),dose of 200 mg/kgBW ($p<0,05$), and ranitidine 10 mg/kgBW ($p<0.05$). *Curative ratio* percentage dose of 100 mg/kgBW; 200 mg/kgBW, and ranitidine are 38.89%, 61.11% and 66.67 % respectively. The conclusion of this research, the ethanol extract of *Curcuma domestica* Val at the dose 100 mg/kgBW and 200 mg/kgBW showed have gastroprotective activity.

Keywords: Gastroprotective, *Curcuma domestica* Val., ethanol-HCl, Gastric ulcer

INTRODUCTION

Currently, peptic ulcers become a disease that affects many people and may caused of death. Gastric ulcer is one form of peptic ulcer characterized by the mucosal layer damage. The imbalance between aggressive and protective factors is the onset of peptic ulcers. Hyper secretion of gastric acid as a factor in aggressive is a pathological condition that occurs due to secretion of HCl uncontrolled from the parietal cells of the gastric mucosa, while damage to the mucus layer that serves as a protective factor on the surface of the gastric mucosa can worsen the situation (Saputri *et al.*, 2008).

Many conditions cause an imbalance of both factors. Excessive reaction to certain foods, beverages containing caffeine and alcohol, stress, stimulation of the parasympathetic and histamine stimulates parietal cells to produce HCl. The use of drugs such as non-steroidal anti-inflammatory (NSAID) is closely related to the occurrence of gastric bleeding through the irritation of the epithelial cells directly and digestive tract mucosal prostaglandin synthesis. The existence of *Helicobacter pylori* can remove toxins that disrupt the mucosal defenses and increase the release of gastrin (Saputri *et al.*, 2008).

Curcuma domestica (CD) or *Curcuma longa* or turmeric, has long been used as traditional medicine for example to resolve inflammation, diarrhea, abdominal pain, jaundice, gastritis and peptic ulcers. From the research results, CD extract shows anti-inflammatory, antibacterial, antioxidant, antiulcer, and gastroprotective (Atmaja, 2008).

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Curcumin has yellow phenolic groups derived from the rhizome of C. *longa*. In the crude extract of C. *longa* rhizomes are 70-76% curcumin, 16% desmethoxycurcumin and 8% bisdemethoxycurcumin (Kohli *et al.*, 2005).

MATERIALS AND METHOD

Extraction of CD

CD rhizomes are cleaned peeled, chopped into small pieces, dried in an oven at a temperature of 50°C for 24 hours, and then blended to obtain the powder. Ethanol extract of CD was made by maceration. CD has added 96% ethanol and stirred with an electric mixer at a scale speed of 70 rpm for 3 hours and allowed to stand for 24 hours, sealed with plastic then filtered and evaporated over a water bath with the temperature of 60°C.

Treatment to Animal Testing

The experiment was performed according to the method of Mizui and Douteuchi (1983), with some modifications. Before the experimental, the mice were maintained under the constant condition and had free access to rodent food and tap water and keep under 12 h light/dark. After acclimatization to the laboratory conditions for 1 week, 25 male mice were divided into five groups. Each group consisted five mices. The body weight of mice 20-30 grams , 6-8 weeks aged. Each groups received one of the following treatments for 6 days. Control group were administered vehicle 0,5 % Na-CMC ; ethanol extract of CD dose 50;100;200 mg/kg groups, and ranitidine dose 10 mg/kg . Each group was treated for 6 days at relatively the same hour. Then fasting for 24 hours, on the seventh day all mice induced 3M HCl - ethanol 60% (1: 1) are orally 0.2 ml / 25 grams mice to induce the gastric ulcer. One hour later, mice were sacrificed by cervical dislocation, then dissected and taken gastric. Gastric was cleaned with 0.9% NaCl physiological fluid to removed gastric content, and then put in a 5% formalin solution for 10 minutes. Then performed an ulcer scoring and curative ratio and test.

Table I. Scoring severity ulcers with modification (Manaiyan *et al.*, 2008)

Observation	Ulcer score
No ulcer	0
Erythema mucus	1
Mild bleeding	2
Moderate bleeding	3
Severe bleeding	4

Description:
Erythema : mucosal reddish color
Mild bleeding : 41-60 % bleeding
Moderate bleeding : 61-80 % bleeding
Severe bleeding : 81-100 % bleeding

Curative Ratio Calculations (Darbar, 2010)

$$Curative\ Ratio = \frac{\text{ulcus score of control} - \text{ulcus score of sample}}{\text{ulcus score of control}} \times 100\ \%$$

The Data were analyzed using Kolmogorov-Smirnov test and the Levene test and ANOVA followed by LSD test level of 95%.

Histopathology of Mice Gastric

Mice were sacrificed and gastric were separated, washed repeatedly with normal saline and stored in 10% formalin until further use . The gastric tissues processed by passing through multiple changes of dehydrating using ethanol and clearing solvent, xylene. After processing, the specimens were embedded in paraffin and 5 µm thick paraffin sections were sliced and stained with hematoxylin and eosin (H&E), tested under binocular light microscope and photographs were taken.

RESULTS AND DISCUSSION

Gastro Protector Activity

Table II showed the ethanol extract of CD dose 50 mg/kg significant not different with control group, while dose 100 and 200 mg/kg of ethanol extract of CD obviously different significant compare with control group.

Table 2. The average score ulcers ± SD and curative ratio in male mice induced by ethanol-HCl each group.

Groups	Dose (mg/kg)	Ulcus Score	*Curative Ratio* (%)
Control	-	3,6 ± 0,55	-
Ethanol extract of CD	50	3,0 ± 0,71	16,67
	100	2,2 ± 0,45*	38,89
	200	1,4 ± 0,89*	61,11
Ranitidine	10	1,2 ± 0,45*	66,67

note : * p < 0,05, different significant compare control group

The result of this study clearly demonstrate that oral administration of ethanol extract of CD induced ethanol-HCl significantly reduced the severity of gastric ulcers in mice also showed dose dependent in gastroprotector activity. The gastroprotective activity of CD lower than ranitidine. The imbalance between aggressive and protective factor is the onset of peptic ulcers. Hyper secretion of gastric acid as a factor in aggressive is a pathological condition that occurs due to secretion of HCl uncontrolled from the parietal cells of the gastric mucosa, while damage to the mucus layer that serves as a protective factor on the surface of the gastric mucosa can worsen the situation (Saputri *et al.*, 2008)..

The extract ethanol of CD dose 50 mg/kg showed significant not different with control group, while dose 100 and 200 mg/kg of ethanol extract of CD obviously different significant compare with control group.

HCl caused severe damage to the gastric mucosa, while ethanol produces lesions necrosis with action necrosis directly reducing factors such defensive secretion of bicarbonate and mucus production (Alrashdi *et al.*, 2012), and increased acid secretion (Lima *et al.*, 2009). Ranitidine is an H2 receptor antagonist role in reducing gastric acid secretion by inhibiting the binding of histamine H2 receptor selectively (Aziz, 2002). CD extract showed selective H2R antagonist effects and competitive on the mechanism for the reduction of gastric ulcers (Kim et al., 2005). Curcumin is one of the best-known examples of cytoprotector. The cytoprotective effect is due to its ability to maintain the network and cellular integrity in the face of mucosal damage by maintaining prostaglandin secretion, inhibits acid secretion, stimulate mucus and bicarbonate secretion and increases cell turnover and mucosal blood flow (Mohamed, 2010).

Histopathology Gastric Mice

Based on the result analysis of histopathological images (fig.1-5), the control group there were gastritis and hemorrhage, also the dose groups of 50 mg/kgBW are gastritis, hemorrhage, the dose group of 100 mg/kgBW are gastritis, the dose group of 200 mg/kgBW are gastritis and hemorrhage, whereas in the ranitidine group there were gastritis and edema. Result didn't show difference all the group. The weakness in the histopathological test sample is used only a fraction of its organs, so that can't be observed from the whole gastric mucosa.

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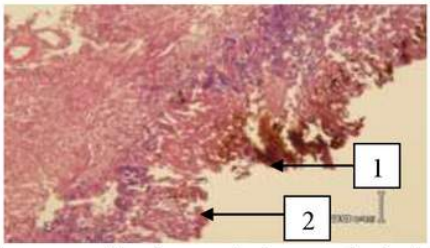

Figure 1. Histopathologic images gastric ulcer control group of mice induced with ethanol-HCl HE staining, magnification 400X. appearance (1) hemorrhage, (2) gastritis.

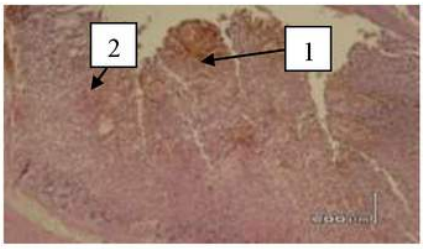

Figure 2. Histopathologic images gastric ulcer ethanol extract if CD group dose 50 mg/kgBW induced ethanol-HCl with HE staining, magnification 400X. appearance (1) hemorrhage, (2) gastritis.

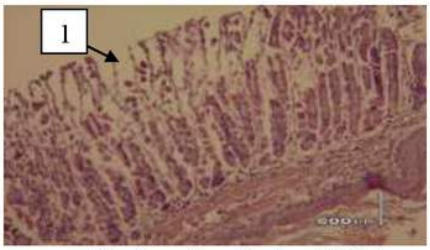

Figure 3. Histopathologic images gastric ulcer ethanol extract if CD group dose 100 mg/kgBW induced ethanol-HCl with HE staining, magnification 400X. appearance (1) gastritis.

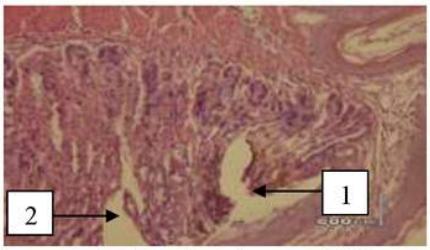

Figure 4.Histopathologic images gastric ulcer ethanol extract if CD group dose 200 mg/kgBW induced ethanol-HCl with HE staining, magnification 400X. appearance (1) hemorrhage, (2) gastritis.

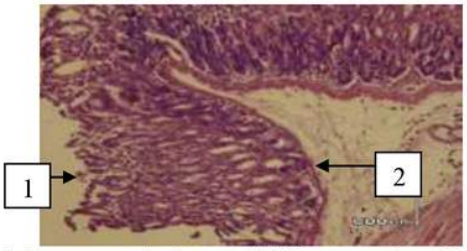

Figure 5. Histopathologic images peptic ulcers ranitidine group dose 10 mg/kgBW induced ethanol-HCl with HE staining, magnification 400X. appearance (1) gastritis, (2) edema.

Based on macroscopic qualitative observations on the score ulcers and curative ratio, showed that the ethanol extract of *Curcuma domestica* at 100 mg/kgBW and 200 mg/kgBW possessed gastroprotective activity in ethanol-HCl induced mice.

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