Effect of *Centella asiatica Leaves* on Gastric Ulcer in Rats

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Abstract

Background: Gastric ulcer is disruption of gastric mucosal integrity due to lack of smaller defensive factors (e.g. mucus levels) than gastric aggressive factors. *Centella asiatica* is a traditional medicine that contains triterpenoids and flavonoids. The objective of this study was to analyze the effect of ethanol extract of *Centella asiatica* leaves for preventing gastric mucus level reduction of aspirin-induced gastric ulcer model in rats and compared it to misoprostol.
Methods: An experimental laboratory study using 24 rats that were divided into 4 groups. Group I (negative control) received carboxymethy cellulose (CMC) solution, group II (positive control) received CMC and aspirin 450mg/kg, group III (tested group) received Centella asiatica 400 mg/kg and aspirin 450 mg/kg, group IV (compared group) received misoprostol 72µg/kg and aspirin 450mg/kg for three days. Then the rats were laparatomied and their gastric mucus levels were measured. The results were statistically analyzed by Independent T-test.
Results: The mean levels of gastric mucus of group I, II, III, IV, respectively were 0.112; 0.035; 0.537; 0.455 optical densities per gram of tissue. The statistical test suggested a significant difference between the positive control group and tested group. Moreover there is no significant difference between the tested group and compared group.
Conclusions: *Centella asiatica* leaves can prevent gastric mucus level reduction of aspirin-induced gastric ulcer model in rats and has similar effect in gastric mucus level with misoprostol.

Keywords: *Centella asiatica*, gastric mucus, gastric ulcer, misoprostol

Introduction

Gastric ulcer is disruption of gastric mucosal integrityand arises from the smaller level of defensive factors (e.g. mucus levels) than gastric aggressive factors.1This condition can be triggered by the use of aspirin as anti-inflammatory and antiplatelet over a long period of time.

Patients with gastric ulcers often complain of epigastric pain, burning sensation, nausea, and vomiting. The pain arises after eating. This situation led to decreased patient's productivity. Therefore, the patients require treatments which may include modern medicine, such as misoprostol, or traditional medicine. The mechanism of action of misoprostol as a prostaglandin analogue is stimulating the secretion of mucus and bicarbonate, reducing mucosal cell turnover, and increasing mucosal blood flow.[1]

Traditional medicines have been used for treatment from generation to generation based on experiences.[2] The percentage of Indonesian population who use traditional medicine is about 22.26% in 2008.[3] Traditional medicine is medicine derived from plants, animals, minerals, or a mixture of these materials. One of these traditional medicines is *Centella asiatica*.

Centella asiatica is a short-stemmed creeping plant that have been used as a cure for leprosy, syphilis, fever, and plays a role in wound healing, increasing work capacity of neurotransmitters in the brain, and revitalizing body tissues. It grows in tropical and subtropical regions, e.g. Indonesia. *Centella asiatica* has branched tap root, green leaves in a fan-shaped or kidney-shaped, round flowers, and small fruits. The height of *Centella asiatica* is 10-50 cm.[4]

The objective of this study was to analyze the effect of Centella asiatica against gastric

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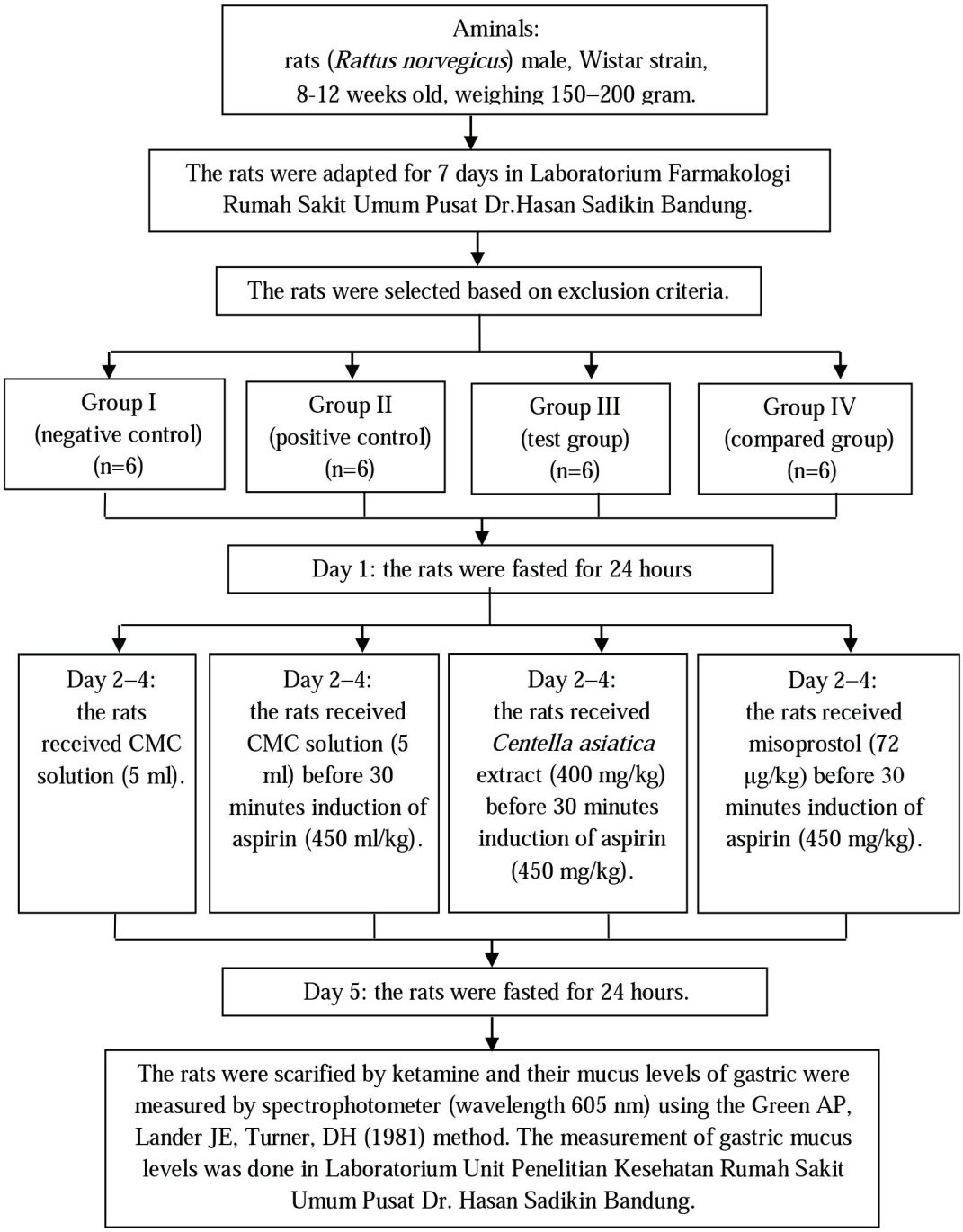

Figure 1 Procedures of The Experiment

ulcers and to compare the effect of *Centella asiatica* with misoprostol.

Methods

An experimental study was carried out, using 24 male wistar strain rats with inclusion criterias as follows: 8-12 weeks old, 150-200 grams weight, active movement, clean hair, no wound on the body, and normal stools. The exclusion criteria are weight lost more than 10% of the initial body weight during the

adaptation and sickness after the adaptation. The rats were obtained from the Laboratory of Sekolah Ilmu Teknologi Hayati Institut Teknologi Bandung and were adapted for seven days in the Pharmacological Laboratory of Dr.Hasan Sadikin General Hospital Bandung to prevent or minimize the stress in a new environment.[5] The leaves of Centella asiatica were obtained from Kebun Percobaan Manoko, Kecamatan Lembang, West Bandung, West Java and extracted in Balai Penelitian Tanaman Rempah dan Obat, Bogor, West Java. The fresh leaves were harvested at the age of 3-6 months. The leaves were sorted and washed to clean from dirt or foreign material and reduce from microbial. Then, the leaves were pulverized. Next, the sample was mixed with ethanol (70%) with a ratio of 1:5 in shaking water bath for 3 hours and allowed to steady for 16 hours. The sample was filtered by filter paper, concentrated in a rotatory evaporator at 500C for 6 hours, and dried with a freezer dryer for 2 days. Twenty kg of wet Centella asiatica plant (all parts) became 836.5 grams of dried leaves and became 96 grams of dried extract. The dose of extract was 400 mg/kg body weight.[6]

Misoprostol was used as a comparative drug and obtained from PT Novell Pharmaceutical Laboratories. Misoprostol as a prostaglandin analogue has mechanisms of action for stimulating the secretion of mucus and bicarbonate, reducing mucosal cell turnover, and increasing mucosal blood flow.[1] The dose of misoprostol was 72 µg/kg body weight. In this study, Aspirin was used to create a gastric ulcer condition in the rats and was obtained from PT Bayer Indonesia. Aspirin belongs to a class of Nonsteroidal Anti-inflammatory Drugs (NSAIDs). Mechanism of action of NSAIDs is to inhibit synthesis of prostaglandin. This mechanism can harm mucosal defense and repair, as well as stimulating mucosal damage. The NSAIDs are weak acids. The NSAIDs will remain as non-ionized lipophilic form if NSAIDs are in acidic environment. In that condition, NSAIDs will cross through the lipid membranes of epithelial cells and be trapped in the cell. If the drugs are trapped inside the cell, then the cell will be damaged.[1]The dose of aspirin was 450 mg/kg body weight. The doses used in this study were the results from conversion using table experimental animals and humans.[7] Centella asiatica extracts, aspirin, and misoprostol were dissolved in 5 ml CMC solution and given orally to the rats.[8]

The rats were divided into 4 groups. Group I (negative control) received carboxymethy cellulose (CMC) solution, group II (positive control) received CMC and aspirin 450mg/kg, group III (tested group) received Centella asiatica 400 mg/kg and aspirin 450 mg/kg, group IV (compared group) received misoprostol 72µg/kg and aspirin 450mg/kg during three days. The function of group I was to determine the normal gastric mucus level in rats. After 3 days, all the rats were scarified by ketamine and were laparatomied. Their gastric mucus levels were collected and measured by spectrophotometer (wavelength 605 nm) using the Green AP, Lander JE, Turner, DH (1981) method. The measurement of gastric mucus levels was done in the Laboratory of Unit Penelitian Kesehatan Rumah Sakit Umum Pusat Dr. Hasan Sadikin Bandung. The results were analyzed using Independent T-test. The test in this study used Confidence interval (CI) 95% (α= 5%).

The procedures were based on the research of M. Sancar et. al. (2009).[9] The measurement of mucus levels were based on the Green AP, Lander JE, TurnerDH (1981) method.[10] (Figure 1)

Table 1 Gastric Mucus Levels of the Gastric Ulcer Induced Rats

Group	Gastric Mucus Level (optical density units per gram of tissue)			
	Group I	Group II	Group III	Group IV
1	0.186	0.047	0.585	0.528
2	0.050	0.028	0.720	0.370
3	0.070	0.061	0.652	0.573
4	0.140	0.015	0.339	0.361
5	0.134	0.011	0.411	0.556
6	0.092	0.048	0.513	0.342
Mean(SD)	0.112(0.051)	0.035(0.020)	0.537(0.145)	0.455(0.108)

Table 2 Effect of Aspirin in Reducing Gastric Mucus Levels in Rats

	n	Mean (SD)	Mean Difference (CI 95%)	P*
Group I	6	0.112 (0.051)	0.077 (0.024-0.130)	0.006
Group II	6	(0.035 0.020)		

Results

The results of measurement of rat gastric mucus levels can be seen in Table 1.

There was a significant difference in the mean of mucus level of gastric between group I and II (Table 2). This result states that induction of aspirin (450 mg/kg) can decrease mucus level and create a gastric ulcer model in rats.

There was a significant difference in the mean of mucus level of gastric between group II and III (Table 3). This result state that Centella asiatica extract can prevent a declining of mucus levels of aspirin-induced gastric ulcer model in rats.

There was no significant difference in the mean of mucus level of gastric between group III and IV (Table 4). This result state that Centella asiatica extract has similar effect in preventing gastric mucus level reduction of aspirin-induced gastric ulcer model in rats with misoprostol.

Discussions

The gastric mucus is produced by epithelial cells on the surface of gastroduodenal organ. The mucus consists of water (95%), mucin, and lipid. The functions of gastric mucus are lubricate to protect mucous from mechanical damage, protect gastric lining from self-digestion from pepsin (without disturbing the activity of pepsin) and from acid by neutralizing HCl (without disturbing the function of HCl in the lumen).[1,11]

This study showed that rats which received *Centella asiatica* extract (400 mg/kg) prior to administration of aspirin (450 mg/kg) for three consecutive days had mean mucus level 0.537 optical density per gram of tissue. It demonstrated that *Centella asiatica* extract can prevent declining of mucus levels of aspirin-induced gastric ulcer model in rats ($p<0.05$).

Centella asiatica consists of triterpenoids and flavonoids. Triterpenoid is the most common component in *Centella asiatica*.[4] Centella asiatica has been proved to have roles as wound healing, anti-inflammatory, and antioxidant. A research of Shukla *et al*. stated that asiaticoside derived from *Centella asiatica* had a role in wound healing in vitro and in vivo. It was proved by increasing tensile strength, collagen, fibroblast proliferation, and wound epithelization in guinea pig and rat. Its role in wound healing was also proved from the increasing angiogenesis in vitro.[12] The research also stated that saponin, asiatic acid, madecassoside derived from *Centella asiatica* had the effect of wound healing by inducing the production of collagen type I to stimulate the wound healing process.[4]

Normal phases of wound healing consist of hemostasis, inflammation, proliferation, and remodeling. *Centella asiatica* has a role in the proliferative phase. This phase is dominated by granulation tissue formation and epithelization. In this phase there will be releasing of macrophage and growth factor from platelets to stimulate migration and activating of fibroblast. Then, fibroblast will produce an important substance for wound healing. One of the substances is collagen.[13]

Research of Frederico *et al*. stated that there is a positive correlation between the flavonoids received from *Centella asiatica* and antioxidant. *Centella asiatica* can absorb or neutralize free radicals. Free radicals can damage proteins, DNA, and lipids in cell membrane.[14] Research of Huang *et al*. stated that asiatic acid derived from *Centella asiatica* had the effect as anti-inflammatory. Anti-inflammatory response can be seen from declining malondyaldehyde and nitric oxide levels. Both of them are produced by impact of free radicals that attack plasma

Table 3 Effect of Centella asiatica and Aspirin-induced Gastric Ulcer in Gastric Mucus Level

	n	Mean (SD)	Mean Difference (CI 95%)	p*
Group II	6	0.035 (0.020)	0.502 (0.350-0.653)	<0.001
Group III	6	0.537 (0.145)		

Table 4 Effect of Centella asiatica and Misoprostol in Gastric Mucus Level Induced rats

	n	Mean (SD)	Mean Difference (CI 95%)	p*
Group III	6	0.537 ± 0.145	0.081 (0.083-0.246)	0.147
Group IV	6	0.455 ± 0.108		

membrane. Anti-inflammatory response can also be seen by increasing antioxidant enzyme, e.g. catalase, superoxide dismutase (SOD), and glutathione peroxidase in liver tissue.[15] A research of Somchit et al. stated that asiatic acid and madecassic acid derived from *Centella asiatica* had the effect as analgesic and anti-inflammatory.[16]

Moreover, this study showed that Centella asiatica (400 mg/kg) had similar effect with misoprostol in preventing gastric mucus levels reduction of aspirin-induced gastric ulcer model in rats ($p \geq 0.05$). The conclusion of this study is Centella asiatica leaves can prevent gastric mucus level reduction of aspirin-induced gastric ulcer model in rats.

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