ABSTRACT

One side effect of non-steroidal anti-inflammatory drugs is gastric mucosal irritation, possibly causing gastric ulcers. The aim of this study was to evaluate the protective effect of cincau leaves (Cyclea barbata Miers) on aspirin-induced gastric ulcer in Balb/c mice. Twenty five Balb/c mice (20-30 g, 2-3 months old) were randomly divided into 5 groups. Group I-III were given cincau leave infusion at dosages of 2.5 mg/kg BW, 5 mg/kg BW, and 10 mg/kg BW, respectively, while group IV (positive control) received antacid at a dosage of 20 mg/kg BW, and group V (negative control) one milliliter of distilled water. All interventions were given by the oral route, once daily for seven days. On day 7, the mice were given aspirin (600 mg/kg BW) to induce gastric ulcer. After 30 minutes, all mice were sacrified, and their stomachs examined macroscopically for gastric ulcer, characterized by the presence of ulcer(s) and bleeding. Total ulcer scores were analyzed by one-way Anova to compare between-group protective effect of interventions against aspirin-induced gastric ulcer. Results showed that groups treated with cincau leaf infusion at all dosages experienced a gastric ulcer protective effect. There were significant differences (p=0.002) between treatments, compared to the negative control, but no significant differences (p>0.05) when compared to the positive control. Thus cincau leaves (Cyclea barbata Miers) at dosages of 2.5 mg/kg BW, 5 mg/kg BW, and 10 mg/kg BW, had a protective effect against aspirin-induced gastric ulcer in mice. Higher dosages of cincau leaf infusion have a correspondingly higher gastric ulcer protective power.

Keywords: Cyclea barbata Miers, aspirin-induced gastric ulcer, mice

INTRODUCTION

Gastric ulcer is one of the intestinal disorders that occur frequently due to an imbalance between offensive factors (gastric acid secretion) and defensive factors (gastric mucosal defense). Aspirin and other non-steroidal anti-inflammatory drugs (NSAIDs) may induce these ulcers through inhibition of
prostaglandin biosynthesis, reduced gastric blood flow, local irritation, and disruption of local tissue restitution and repair.\(^{(2)}\)

The cincau plant (\textit{Cyclea barbata} Miers) belongs to the \textit{Menispermaceae} family and is traditionally utilized by many Indonesian communities as a refreshing beverage in the form of a gel or infusion. Some communities in Indonesia also use the infusion as medication against cold. Cold is frequently felt to be a digestive disorder, with the complaints of sprue, diarrhea, epigastica fullness, and reflux of gastric acid into the pharynx.\(^{(3)}\)

It is the tannin content of cincau leaves that is believed to be beneficial for abdominal pain. In addition, this plant contains various bis-benzyl-isoquinoline alkaloids, i.e. berbamine, chondocurine, alpha and beta cyclanoline, fangchinoline, homoaaromoline, isochondocurine, isotetrandrine, lemacine, and tetrandrine,\(^{(4)}\) as well as the minerals calcium and phosphorus, and vitamins A and B.\(^{(5)}\)

Cincau leaves contain mucin, thus cincau leaf extract readily turns into a gel, resembling agar.\(^{(5)}\) The major component of cincau gel is the polysaccharide pectin, consisting of water-soluble vegetable fibers that are readily fermented by the colonic microflora.\(^{(6)}\) Because of the pectin content, cincau leaf extract is considered to be a good source of vegetable fiber, the more so as its caloric content is sufficiently low. The high fiber content of cincau leaves leads to ease of defecation in those consuming cincau gel.\(^{(5)}\) The effect of the pectin fibers in cincau leaves may be enhanced by the addition of citric acid, which also increases their antioxidant effect.\(^{(7)}\)

Several studies have been conducted on the medicinal properties of cincau leaves, such as antitumor,\(^{(3)}\) antipyretic, antimalarial \(^{(8)}\) and antihypertensive.\(^{(9)}\) The pharmacologic activities of cincau leaves are thought to be caused by the bis-benzyl-isoquinoline alkaloids.\(^{(8,9)}\) Thus far, the effect of cincau leaves on gastric functions has been investigated in a study on gastric hydrochloric acid (HCl) concentration and gastric histopathology after simultaneous administration of an aqueous extract of cincau leaves and aspirin to Wistar rats. The study results indicated that cincau leaves protected the gastric mucosa from the effects of aspirin, as demonstrated by gastric histopathology, but did not affect the gastric HCl concentration.\(^{(10)}\) The present study differs from the abovementioned one in the method of preparation of the cincau leaves (infusion vs gel), and in the observed effects (macroscopic examination and measurement of ulcer index vs microscopic examination and measurement of HCl concentration). On the basis of several studies on the content of active substances in cincau leaves, the present study aims to evaluate the protective effect of cincau leaves against gastric ulcer after administration of aspirin in mice.

**METHODS**

**Research design**

The study was designed as a purely experimental laboratory investigation using controls to evaluate effect of cincau leaves on gastric ulcer.

**Test animals**

This study used 25 Balb/c mice aged 2-3 months and weighing 20-30 grams. The experimental animals were healthy mice (active and without any physical defects), whose condition was monitored before and during the study, and who were handled with reasonable care during the experiments. According to the previous study, with 5 intervention groups, each consisting of 5 test animals (minimum size), the size of the sample was 25, selected by simple random sampling.\(^{(11)}\)

**\textit{Cyclea barbata} Miers**

Cincau leaves (\textit{Cyclea barbata} Miers) were obtained during the period of February-March 2009 in and around the Sleman district. The leaves selected were clean and shiny ones,
as such leaves have a substantially higher content of active substances. The leaf picking was started from the lower stem upwards. The leaves were identified at the Pharmacognosy-Phytochemistry Laboratory, Pharmacy Program, Faculty of Mathematic and Natural Sciences (FMIPA), UII, Yogyakarta, from where the chemicals used in the preparation of the cincau leaf infusion were also obtained. A liquid antacid preparation (Bayer) was used as positive control, and aspirin (Bayer) as inducer of gastric ulcer in the laboratory animals.

**Preparation of cincau leaf infusion**

Cincau leaves were washed, after which 50 grams were weighed off, and 300 mL water was added to cover the leaves. The material was then placed in a waterbath at 80-90°C for 30 minutes. The solution was filtered with filter paper, replaced in the waterbath, and left to evaporate, until only 50 mL water was left.

**Induction of gastric ulcer**

As inducer of gastric ulcer, aspirin was administered orally to the mice. The animals had been fasted previously for 12 hours to empty the stomach of food, and to increase the gastric acid level, thereby facilitating the induction of gastric ulcer upon administration of aspirin. The dosage of aspirin was 600 mg/kg BW, which was modified from previous studies. (12)

**Determination of the dosage of cincau leaf infusion**

The basic dosage of cincau leaf infusion was taken from previous studies to be 2.5 mg/kgBW, but modified for the higher dosages to two and four times the basic dosage, becoming 5 and 10 mg/kgBW, respectively.

**Determination of antacid dosage**

The antacid dosage was calculated from the regular dosage in humans of 50 kg, thus obtaining a dosage of 20 mg/kgBW for the animals weighing 20-30 grams.

**Table 1. Ulcer score (13)**

<table>
<thead>
<tr>
<th>Lesions</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ulcer Length &gt; 10 cm</td>
<td>4</td>
</tr>
<tr>
<td>Length 2-10 cm</td>
<td>2</td>
</tr>
<tr>
<td>Length 1-2 cm</td>
<td>1</td>
</tr>
<tr>
<td>Length &lt; 1 cm</td>
<td>0.5</td>
</tr>
<tr>
<td>Bleeding</td>
<td>2</td>
</tr>
</tbody>
</table>

**Calculation of gastric ulcer protective power**

On macroscopic examination, the signs of gastric ulcer are the presence of an ulcer (or ulcers) and the occurrence of gastric hemorrhage, which were scored as shown in Table 1. (13)

**Data analysis**

The total scores of ulcer(s) and hemorrhage in the various treatment groups were statistically analyzed using one-way Anova (SPSS version 15 for Windows) for significant differences, with p<0.05 being considered significant.

**RESULTS**

Among the 25 mice (n=25) used in the study, one mouse in group I did not live to completion of the study, as it died through an error in using the stomach tube, thereby leaving only 24 animals for analysis. Mean total ulcer scores of the experimental animals are shown in Table 2 and Figure 1.

**DISCUSSION**

Aspirin is an anti-inflammatory drug known for its gastric toxicity, which is frequently characterized by gastric ulcers and hemorrhage. (14) Therefore this drug is frequently used as a model in studies on in vivo cytoprotective activity of new substances or compounds. (1-2, 15-18) Administration of NSAIDs, including aspirin and indomethacine, inhibits the biosynthesis of prosta glandins, particularly of PGE2 and PGI2, which are protective factors...
(mucosal resistance factors) against irritation of the stomach by gastric acid. Inhibition of prostaglandins results in early damage to the mucosal, parietal, and endothelial cells, thus leading to the formation of an ulcer. Moreover, the occurrence of an ulcer is also mediated by free radicals from the conversion of hydroxyperoxyl into hydroxyl fatty acids, causing destruction of the cells. These hydroxyperoxyl compounds in turn are produced by the degranulation of mast cells and the complete lipid peroxidation accompanying cellular damage.

Figure 1 shows that the mice in the groups receiving cincau leaf infusion and antacid had lower total ulcer scores, in comparison with the negative controls receiving distilled water. This indicates that cincau leaf infusion at the three dosage levels administered simultaneously with antacids, was capable of reducing the occurrence of gastric ulcer due to oral aspirin administration. From these data it is also apparent that with increasing dosage of cincau leaf infusion, there is a proportional increase in gastric ulcer protective effect, with the highest dosage of 10 mg/kg BW giving the highest protective power.

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Total ulcer score of mice no.</th>
<th>Mean ± SD</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infusion of cincau leaves at dose 2.5 mg/kgBW</td>
<td>3 4 2 4 -</td>
<td>3.25 ± 0.96</td>
<td></td>
</tr>
<tr>
<td>Infusion of cincau leaves at dose 5 mg/kgBW</td>
<td>3 1 4 2 2.5</td>
<td>2.5 ± 1.12</td>
<td>0.002</td>
</tr>
<tr>
<td>Infusion of cincau leaves at dose 10 mg/kgBW</td>
<td>0.5 0 1 1 1</td>
<td>0.7 ± 0.45</td>
<td></td>
</tr>
<tr>
<td>Antacid at dose 20 mg/kgBW</td>
<td>0 1 0.5 1 0.5</td>
<td>0.6 ± 0.42</td>
<td></td>
</tr>
<tr>
<td>Aquadest</td>
<td>25 6 9 8 6.5</td>
<td>10.9 ± 7.97</td>
<td></td>
</tr>
</tbody>
</table>

Figure 1. Mean of total ulcer score by treatment groups in mice.

Group I: Infusion of cincau leaves at dose 2.5 mg/kgBW; Group II: Infusion of cincau leaves at dose 5 mg/kgBW; Group III: Infusion of cincau leaves at dose 10 mg/kgBW; Group IV: Antacid at dose 20 mg/kgBW; Group V: Aquadest
The results of one-way Anova indicated that there were significant differences in ulcer protection at all dosage levels of cincau leaf infusion, compared with negative controls (p=0.002). However, the three dosages of cincau leaf infusion did not show significant differences with antacid (p>0.05). From the lack of significant differences with antacid it may be concluded that cincau leaf infusion at the dosages of 2.5 mg/kg BW; 5 mg/kg BW; and 10 mg/kg BW, has a gastric ulcer protective power equivalent to an antacid dosage of 20 mg/kg BW.

The results of the present study supports those of a previous study, in which cincau leaf press liquid was protective against gastric ulcer, being capable of improving the histopathological picture of aspirin-induced gastric ulcer.(10) The present study supports the previous study results, as with a different method of preparation, cincau leaves are still capable of exerting gastroprotective activity against ulcers.

Although this study was able to provide evidence that the cincau plant (Cyclea barbata Miers) possesses protective activity against gastric ulcer, the active compounds involved in exerting this protective effect are not known with certainty. One of the suggestions relate to the antioxidant effects of the flavonoids, saponins, and tannins, that are known to be present in the cincau leaves.(3,5) Antioxidant compounds have been demonstrated to have cytoprotective effects, thus being capable of protecting cells from various kinds of damage and possibly of functioning as anti-ulcer drugs.(21-23) Antioxidants act by inhibiting lipid peroxidation and by scavenging free radicals, thereby preventing the occurrence of gastric ulcer.(24)

Several studies demonstrated that flavonoids from various plants are reportedly capable of preventing the occurrence of gastric ulcer. This may take place through an increase in the amounts of neutral glycoproteins and in prostaglandin concentrations, and inhibition of histamine secretion from mast cells by inhibition of histidine decarboxylase,(25) thus reducing stimulation of H2 receptors, (26) or by secretion of prostaglandin-like compounds.(27) Another possible mechanism of action for inhibiting ulcer occurrences is by decreasing pepsin secretion and activity,(28) in view of the fact that one of the major pathogenetic mechanisms of ulcer formation is by hypersecretion of gastric juice and by pepsin hyperactivity.(29) However, elucidation of the exact mechanism underlying the protective effect of cincau leaves awaits further studies.

One of the flavonoids in the cincau plant are the bisbenzylisoquinolines (including tetrandrine), which is thought to play a role in the mechanism of ulcer prevention. Bisbenzylisoquinoline compounds from various plants possess anti-inflammatory, anti-allergic, antioxidant, antifibrogenetic, and immunomodulating effects. The alkaloid tetrandrine from various plants also has anti-inflammatory properties against a number of lung infections.(30) However, whether or not the bisbenzylisoquinolines (including tetrandrine) from the cincau plant possess these effects, is a subject for further study.

In addition to flavonoids, other compounds playing a role in ulcer protection are the saponins, which have hemolytic, expectorant, immunostimulant, and anti-inflammatory properties.(31) Another member of the Menispermaceae is Cyclea peltata, which also has a high saponin content. (32) It is thought that the anti-inflammatory effects of saponins reduces the risk of ulcers, by increasing defensive factors of gastric mucosa and stopping the inflammatory process resulting from induction by aspirin (indicated by absence of edema in the gastric mucosa of mice receiving cincau leaf infusion). The protective effect of saponins against gastric ulcer may also be mediated by the formation of a protective mucus layer on the gastric mucosa and by selective inhibition of PGF2α.(25)
One study reported that cincau leaves are safe for consumption by the community, as it was demonstrated that cincau leaf ethanolic extract at a dosage of up to 30,000 mg/kg BW did not induce significant toxic effects, in other words, the LD$_{50}$ of cincau leaf extract (Cyclea barbata Miers) in mice is greater than 30,000 mg/kg BW.\(^{(33)}\) There are several limitations to this study, as there were no observations on the effect of cincau leaf infusion in ulcer healing, active compounds playing a role in prevention of gastric ulcer were not isolated, and the specific mechanism of action of the gastroprotective effect was not studied.

**CONCLUSIONS**

Infusions of cincau leaves (Cyclea barbata Miers) have protective activity against the occurrence of gastric ulcers in mice induced by aspirin at a dosage of 20 mg/kg BW, from an initial dosage of 2.5 mg/kg BW. Higher dosages of cincau leaf infusion are correlated with a correspondingly higher gastric ulcer protective power. Further studies are needed on the active compounds in cincau leaves responsible for protective activity against gastric ulcer.

**ACKNOWLEDGEMENT**

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**DAFTAR PUSTAKA**