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Gastroprotective Effects of the Combination of *Chromolaena odorata* L. and *Pachyrhizus erosus* L. Extracts on Rats with Gastric Ulcer Model

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ABSTRACT

Gastroprotective effects are caused by compounds that can protect the gastric mucosa. Gastroprotective activity provided by plants is due to the presence of a group of secondary metabolite compounds found in the plants. The types of secondary metabolite compounds are flavonoids, tannins, alkaloids and saponins. *Chromolaena odorata* L. contains tannins, phenols, flavonoids, saponins and steroids, while *Pachyrhizus erosus* L. is known to contain flavonoids and saponins. This study aimed to determine the gasprotective activity of the combination of *Chromolaena odorata* L. and *Pachyrhizus erosus* L. extracts in ethanol-induced rats by observing the parameters of the number of peptic ulcers, protection ratio, and images of gastric histopathology. The research method was to make a combination of *Chromolaena odorata* L. and *Pachyrhizus erosus* L. extracts which were given to six treatment groups: normal group without treatment; negative control group; positive control group given sucralfate; and three groups given a combination treatment of *Chromolaena odorata* L. and *Pachyrhizus erosus* L. extract with a dose of 100, 200, and 400 mg/kgBW. Treatment was done by oral administration for 14 days. On Day 14, after one hour of treatment, 96% ethanol induction was given orally at a dose of 2 ml/200 gBW except for the normal group. The ulcer index produced by negative control, positive control, the treatment with doses of 100, 200, and 400 were 4.18; 2.98; 2.49; 1.64; and 0.78, respectively. The combination of *Chromolaena odorata* L. and *Pachyrhizus erosus* L. extracts can prevent gastric damage in rats caused by ethanol induction.

INTRODUCTION

Gastric ulcers are a disease caused by a disorder of the upper gastrointestinal tract due to an excessive secretion of acid and pepsin by the gastric mucosa (Avunduk, 2008). Gastric ulcers are characterized by mucosal integrity disorder which then develop into local or widespread lesions caused by active inflammation. The pathophysiology of gastric ulcers is caused by an imbalance between aggressive factors, *Helicobacter pylori* (*H. pylori*) and non-steroidal anti-inflammatory medicines, and local mucosal defensive factors, such as mucus, blood flow, and prostaglandins (Dharmani, 2003). Ethanol is known to have a local effect on gastric tissues. The longer the

consumption of ethanol, the more gastric cells that are damaged. The damage of the mucosa barrier due to alcohol can cause acute and chronic gastritis. Excessive alcohol consumption can also lead to the release of the superficial mucosal epithelium (erosion). Severe forms of erosion are a major cause of acute gastrointestinal bleeding (Goodman, 2008; Pan, 2008).

Gastroprotective effects are caused by compounds that can protect the gastric mucosa. Gastroprotective effect mechanisms can occur by reducing pepsin secretion, gastric acid, and increasing endogenous prostaglandins and reducing leukotrienes (LTs) levels. In addition to inhibiting the growth of *H. pylori* which is a bacterium that causes peptic ulcers, gastroprotective compounds inhibit the H+/K+ ATPase enzyme, thereby reducing gastric acid secretion (Goel *et al.*, 2002).

Chromolaena odorata L. leaves contain several main compounds such as tannins, phenols, flavonoids, saponins and steroids (Ifora *et al.,* 2017). *Chromolaena odorata* L. leaves that were combined with *Ocimum gratissimum* showed gastroprotective activity (Agbor, 2019). The *Pachyrhizus erosus L.* tubers are known to contain flavonoids and saponins (Lukitaningsih, 2009). The administration of *Pachyrhizus erosus L.* juice can reduce the number of ulcers and improve gastric histopathology due to ethanol induction (Pertiwi, 2019).

Studies on the gastroprotective activity of *Chromolaena odorata* L. and *Pachyrhizus erosus* L. have been conducted separetely so it is necessary to do research on the gastroprotective activity of the combined extracts. With this purpose, this study was conducted on ethanol-induced mice because both types of leaves are known to contain important compounds that protect the stomach.

METHODS

Materials

The materials used in this research were *Chromolaena odorata* L.*, Pachyrhizus erosus* L., rats, feeds, Sucralfate, 0.9% NaCl, ethanol, liquid paraffin, CMC-Na, aquadest, and Hematoxylin and Eosin dyes.

Extract preparation

Chromolaena odorata L. and *Pachyrhizus erosus* L. were cleaned of dirt by washing under running water and drying. Then, the dried *Chromolaena odorata* L. and *Pachyrhizus erosus* L. were mashed using a blender. Leaf powder of *Chromolaena odorata* L. and *Pachyrhizus erosus* L. tubers were each macerated using 96% ethanol for 48 hours. The ethanol extract obtained was then collected, and the liquid filter was evaporated until a thick extract was obtained using a rotary evaporator. Preparation of the *Chromolaena odorata* L. and *Pachyrhizus erosus* L. combined extract solution was done using CMC-Na 0.5% as the suspending agent for treatment in a ratio of 1:1.

Laboratory animal acclimatization

Prior to the treatment, the mice were acclimatized in laboratory conditions for one week with adequate feeding and drinking**.**

Laboratory animals

This research has been approved by the Health Research Ethics Committee Faculty of Medicine and Health Science, University of Bengkulu under approval number 240/UN30.14.9/LT/ 2021. The laboratory animals used were male mice which were divided into 6 groups, each consisting of 5 mice. The normal group was only given food and drink without induction, the negative control group was given food and drink, the positive control group was given sucralfate, and the combination groups were given *Chromolaena odorata* L. and *Pachyrhizus erosus* L. extract (EMB), each with a dose of 100 mg/kgBW, 200 mg/kgBW, and 400 mg/kgBW. The treatment is administered orally for 14 days with a volume of 2 ml/200 gBW. After an hour of treatment on day 14, ethanol induction was administered orally at a dose of 5 g/kgBW except for the normal group. After 24 hours of ethanol induction, the animals were euthanized and dissected.

Macroscopic observation of gastric ulcer

After surgery, macroscopic observations on the gastric were done to determine the number and size of the lesions/ulcers that formed on the gastric mucosa. The gastric was opened by dissecting the largest curve (major curvature), cleaned with 0.9% NaCl solution and then stretched on a flat surface so that ulcer observation could be made (Gusdinar *et al.*, 2009).

Table 1. Ulcer severity scoring

Gastric Cross-Section		Scoring
Normal		0
Hyperemia		1
Hemorrhage	Petechiae	2
	Ecchymoses	3
	Purpura	4
Erosion		5

Observations of ulcers were done with a scoring based on methods which had been modified (Table 1). Hypereremia is a condition where blood vessels are dilated and filled with blood granules in excess. Erosion is the detachment of the superficial mucosal epithelium. Bleeding (hemorrhage) is a drop of blood that exits the blood vessels and spreads among the tissues. Petechiae are bleeding spots of 0.1-0.2 cm. Ecchymoses are bleeding spots of 0.2-3.0 cm. Purpura is a bleeding spot of >3 cm (Pertiwi *et al.,* 2021).

The average total score for each treatment groups was considered as the ulcer index (UI), which was then compared with the control groups. The protection ability or protection ratio of a materials against ulcers was calculated based on Saptarini *et al.* (2011) with the following formula:

$$\% \text{ Protection Ratio } = 100\% - [\frac{\text{UI test group}}{\text{UI ulcer control}} \text{ x } 100\%]$$

Gastric Histopathology Evaluation

The gastric was put in gauze, dehydrated and immersed in a 70%, 80%, 90%, and 100% ethanol solution for 60 minutes at room temperature. The next process was cleaning using xylol for 15 minutes at room temperature which was done three times. After that, the infiltration process with liquid paraffin was done 3 times, each for 60 minutes in the incubator at 60ºC. The tissue was then immersed in liquid paraffin and cooled at room temperature so that it became a paraffin block.

Furthermore, the embedding and cutting was done horizontally by a microtome with a thickness of 3µm. Then, the Toluidine Blue staining was done by the following procedure: paraffin was removed with xylol and put into 100%, 95% and 70% ethanol for 5 minutes respectively before being added in distilled water. The Toluidin Blue staining was conducted for 40-60 minutes in an oven temperature of 60°C. After that, dripping of ethanol from high to low concentration (100%, 95%, and 70%) was done for 3 minutes. After being given Canada Balsam, the tissue was covered with a glass deck.

Data analysis

The percent data of gastric ulcer index and protection ratio obtained were statistically analyzed by Kolmogorov-Smirnov and Levene tests. Statistical analysis was started with a normality test using the Kolmogorov-Smirnov test to determine whether the data were normally distributed or not. Furthermore, homogeneity test was conducted using the Levene test to determine the homogeneity variance. If the data were normally distributed and the variance was homogeneous, then it was tested using one-way ANOVA, each with 95% confidence interval (CI). Histopathological analysis of the gastric was conducted through observations under a microscope. The analysis of gastric slices was done by observing the specific changes that occurred in the gastric (Maslachah *et al*., 2008).

RESULTS AND DISCUSSION

Based on the research results, the plant *Chromolaena odorata* L. showed activity as a gastroprotector. This finding is due to the presence of secondary metabolite compounds in the *Chromolaena odorata* L. plant. The results of the phytochemical screening test of the *Chromolaena odorata* L. plant showed that it contained secondary metabolites, namely flavonoids, alkaloids, tannins, saponins, and steroids (Munte *et al.*, 2016; Frastika *et al.*, 2017; Sirinthipaporn *et al.*, 2017). According to research conducted by Pertiwi *et al*. (2021), the tubers juice of *Pachyrhizus erosus* L. at a dose of 300 mg/kg BW gave a better gastroprotective effect than the juice of *Pachyrhizus erosus* L. tubers combined with the juice of *Raphanus sativus* L. at the same dose.

Each secondary metabolite compound has a different mechanism in its role as a gastroprotector. Flavonoids which act as gastroprotectors through their antioxidant activity are able to become gastric cytoprotective agents with various mechanisms, stabilizing the membrane and affecting several processes of intermediate metabolism and lipid peroxidation by increasing the activity of the enzyme Superoxide Dismutase (SOD) and the content of prostaglandins in the gastric mucosa (Islamiah *et al.*, 2017). Alkaloid compounds as gastroprotectors work by reducing gastric acid secretion, increasing mucus and alkaline secretion, and improving gastric mucosal blood flow to help heal and prevent gastric ulcers against irritant agents/factors. Tannins are able to precipitate microproteins at the ulcer site to form a thin protective layer to prevent the attack of proteolytic enzyme irritant factors. Saponins provide gastroprotective activity through an increase in fibronectin, and the formed fibrin clot will be the basis for the reepithelialization process in the tissue (Indraswary, 2011). While the gastroprotective nature of steroids through their antibacterial activity causes leakage of liposomes, they interact with cell phospholipid membranes which causes a decrease in membrane integrity and changes in cell

membrane morphology, causing cell brittleness and lysis (Lake *et al*., 2019).

Observations of the gastric anatomy of rats in the negative and positive control groups showed various characteristics of gastric ulcers such as hyperemia, hemorrhage petechiae, hemorrhage ecchymoses, hemorrhage purpura, and erosion (loss of gastric wall tissue). In the treatment groups of combined extracts of *Chromolaena odorata L.* and *Pachyrhizus erosus L. with* doses of 100 and 200 mg/kg BW showed hyperemia, hemorrhage petechiae, and hemorrhage ecchymoses, while in the group with a dose of 400 mg/kgBW there were no gastric ulcer characteristics. The results of the anatomical appearance of the rat's gastric for each treatment group can be seen in Figure 1.

Gastric ulcers observation was conducted by scoring each cross-section of the gastric using the modified method described by Szabo *et al.* (1985) in Pertiwi *et al.* (2021). To avoid subjectivity to the results, the scoring was conducted by three expert observers. The results of the observation of gastric ulcers in the test rats can be seen in Table 3. After the gastric ulcer index value was obtained, the value of the protection ratio was calculated. The results of the protection ratio can be seen in Table 4.

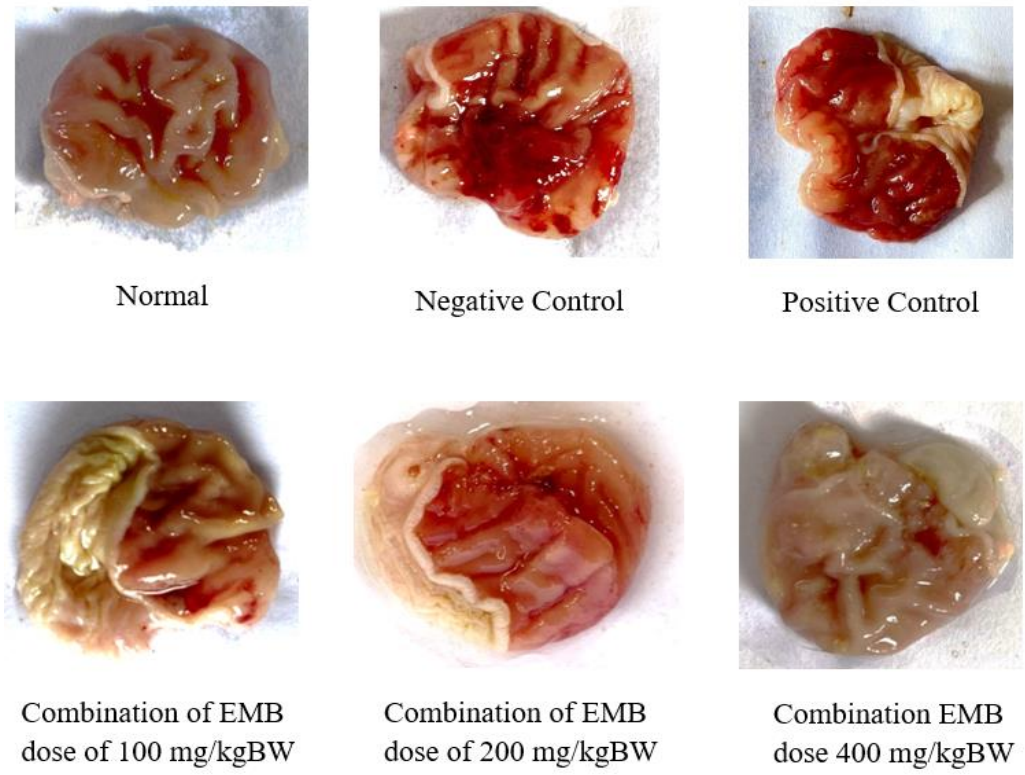

Figure 1. Macroscopic overview of the rat's gastric after treatment. Notes: EMB = Combination of *Chromolaena odorata* L. and *Pachyrhizus erosus* L. Extract.

Table 3. Mean of Gastric Ulcer Index in Mice Induced by Ethanol 96%

Group	Dose (mg/kgBB)	Average ± SD
Normal	-	0 ± 0[b]
Negative Control	-	4.18 ± 0.84[a]
Positive Control		2.98 ± 0.63[a]
EMB Combination	100	2.49 ± 0.46[a,b]
EMB Combination	200	1.64 ± 1.17[a,b]
EMB Combination	400	0.78 ± 0.33[b]

SD: standard deviation;
[a]sig<0.05 there is a significant difference with normal group.
[b]sig<0.05 there is a significant difference with negative group.

Table 4. Protection Ratio in 96% Ethanol Induced Mice

Group	Dose (mg/kgBB)	Protection Ratio (%)± SD
Normal	-	100 ± 0
Positive Control		28.72 ± 15.10[a]
EMB Combination	100	40.43 ± 11.08[a]
EMB Combination	200	60.64 ± 28.08[a]
EMB Combination	400	81.38 ± 7.85

SD: standard deviation;
[a]sig<0.05 there is a significant difference with normal group.

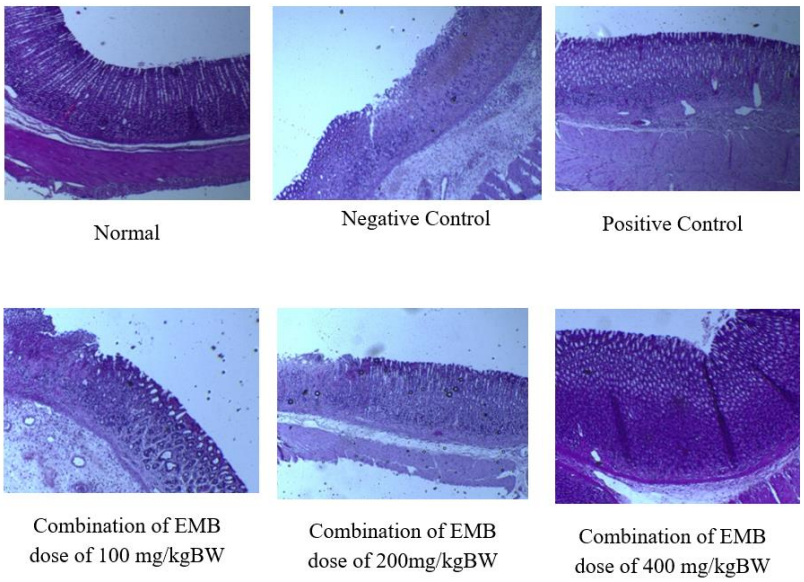

Figure 2. Histopathological overview of the rat's gastric

In the histopathological image (Figure 2), the negative control group showed tissue damage which was characterized by the disappearance of lesions on the mucosa and the presence of ulcers, bleeding, and hemorrhage. Meanwhile, the positive control group showed tissue damage with ulcers, bleeding, and mucosal lesions but only on some parts of the network. The treatment groups that were given the extracts of *Chromolaena odorata* L. and *Pachyrhizus erosus* L. at a dose of 100, 200, and 400 mg/kgBW showed significance differences as protective agents with increasing doses. This was shown in the combination of extracts at doses of 100 and 200 mg/kgBW, which still had ulcers and lesions, while in the combination of extracts at a dose of 400 mg/kgBW, there was an improvement in gastric cells and no mucosal lesions were found. literature which determined 3 (three) significance classifications of effect size (Sullivan *et al.*, 2012).

CONCLUSION

The combination of leaf extract of *Chromolaena odorata* L. and tuber of *Pachyrhizus erosus* L. can prevent gastric damage in rats caused by ethanol induction.

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