Gastroprotective Activities of *Peperomia Pellucida* L. and *Pachyrhizus Erosus* L. Extracts Combination on Ethanol-Induced Rats

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Research Article

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Abstract

Gastroprotective is an effect caused by the compounds that have the capability of protecting the gastric mucosa. *Peperomia pellucida* plants contain alkaloids, flavonoids, saponins, tannins, and terpenoids, while *Pachyrhizus erosus* contains flavonoids, alkaloids, tannins, and saponins. *Peperomia pellucida* reportedly contains dillapiole compounds with a gastroprotective effect. Moreover, its isolation result from *Pachyrhizus erosus* indicates the presence of dulcitol, gentisic acid, and formononetin, which has antioxidant activity. This study aims to determine the gastroprotective effect of the combination of *Peperomia pellucida* and *Pachyrhizus erosus* extract on rats with gastric ulcer models by looking at the ulcer index, percentage of inhibition, and histopathology. The research method used in this study was by making a combination of *Peperomia pellucida* and *Pachyrhizus erosus* extract. The combined extract was then given to 5 treatment groups. Group I as a negative control, group II as a positive control was given sucralfate, groups III, IV, and V were given a combination of *Peperomia pellucida* and *Pachyrhizus erosus* extract of 100, 200, and 400 mg/kg BW. The treatment was given orally for 14 days, after one hour of treatment on the 14th day, 96% ethanol induction was given orally at a dose of 5 mg/kg BW. The animal dissection was performed 24 hours after the induction. The results from observations showed an increase in body weight before and after the treatment. The ulcer index produced by negative control, positive control in the treatment with doses of 100, 200, and 400 were 4.18; 2.98; 2.42; 2.04; and 1.07. This study showed that the combination of *Peperomia pellucida* and *Pachyrhizus erosus* extract has a gastroprotective effect.

Introduction

Gastritis is an inflammation of the lining of the gastric. If left untreated, gastritis can cause ulcers, gastrointestinal perforation (GP), bleeding in the digestive tract, and anemia (Sipponen and Maaros, 2015). One of the complications from chronic gastritis is gastric ulcers. This condition is caused by the imbalance between the aggressive factors (gastric acid secretion, pepsin, and a bacterial infection caused by *Helicobacter pylori*) and the mucosal defense factors (prostaglandin production, mucus, bicarbonate, and mucosal blood flow) (Widyaningsih and Afdaliah, 2020). The cause of gastric ulcers is often associated with *H. pylori*, psychological stress, and Non-Steroidal Anti-Inflammatory Drugs (NSAIDs). *Helicobacter pylori* are microaerophilic and generally stay under the mucus of the acidic gastric lining.

Drug therapy such as proton pump inhibitors (PPIs) (omeprazole, lansoprazole, pantoprazole), H2-receptor antagonists (cimetidine, ranitidine, famotidine), and antacids are often used to treat gastric ulcers (Longo and Fauci, 2010; Tarigan, 2014). Proton pump inhibitors are chosen as the first choice of therapy because this class of medications has a very strong inhibitory effect on gastric acid secretion, resulting in an 80–90% reduction of the gastric's daily acid production. Proton pump inhibitor (PPIs) is a prodrug that is activated by acid. The activated form of this prodrug binds covalently to the gastric H+, K+-ATPase via a disulfide bond, irreversibly non-activating the pump molecules, therefore stopping the gastric acid secretion. Generally, the use of proton pump inhibitors (PPIs) can cause some side effects

such as nausea, abdominal pain, flatulence, constipation, diarrhea, subacute myopathy, arthralgia, headache, skin rashes, and decreasing in vitamin B12 absorption in chronic use. Other than that, this class of drugs also reacts with warfarin, diazepam, and cyclosporine (Brunton, Lazo, and Parker, 2006).

The potential use of *Peperomia pellucida* and *Pachyrhizus erosus* plants as gastroprotective agents is based on the metabolites contained in the plants, which are flavonoids, tannins, and saponins. Using the mechanism of prostaglandin increase in the gastric mucus, flavonoids can protect the gastric mucus. Flavonoids can also reduce the secretion of histamine from mast cells by inhibiting histidine decarboxylase (Ebadi, 2007). Meanwhile, saponins activate the gastroprotective activities through fibronectin increase. After that, the fibrin clots that are formed will act as the base of the tissue re-epithelialization process (Indraswary, 2011). In this case, *Pachyrhizus erosus* have been known to be containing flavonoids and saponins (Lukitaningsih, 2009). Therefore, the extract of *Pachyrhizus erosus* can reduce ulcers and repair the gastric's histopathology caused by ethanol exposure. In a study by Rojas-Martínez et al. (2013) it is reported that dillapiolese is the most active gastroprotective agent of *Peperomia pellucida*. Some compounds, such as dulcitol, gentisic acid, formononetin, kaicasaponin III, p-coumaric acid, and vitexin, are known to be present in *Pachyrhizus erosus* but are not tested/studied in the context of biological activity *Pachyrhizus erosus* is known to have important biological activities such as antidiabetics, anticancer, antioxidants, and antiinflammation (Jaiswal et al., 2021).

Thus, according to the background presented, it is hoped that the *Peperomia pellucida* and *Pachyrhizus erosus* extracts combination can be used to reduce the ulcers index, increase protection ratio, and refine cells on the gastric's histopathology.

Materials And Method

Materials

The materials used in this study were *Peperomia pellucida*, *Pachyrhizus erosus*, rats, pellets, sucralfate, 0.9% NaCl, 96% ethanol, aquadest, and hematoxylin and eosin as coloring substances.

The tools used were as follows: baskets, glass beakers, stirrers, stoves, porcelain cups, measuring flasks, volumetric flasks, dropper pipettes, volume pipettes, propipettes, scissors, buckets, analytical scales, a rotary evaporator, Büchner funnels, suction flasks, filter papers, a water bath, chambers, microtomes, and an oven. In the treatment of tested animals, injection syringes with the volume of 3.0 ml and 1.0 ml (Terumo) were used. Flakons, capillary tubes, *eppendorf*, surgical instruments, glass objects, and digital optical microscopes were also used in this research.

Methods

Extraction Process

200 g of *Peperomia pellucida* and *Pachyrhizus erosus* were macerated in 2 L of 96% ethanol in a closed container. The container was saved and kept away from direct sunlight for 48 hours and stirred

occasionally during the process. At the end of the extraction, the mixture was separated from its marc by filtration. Using a *Rotary Evaporator*, the ethanol extract was evaporated until a thick mixture was formed.

Preparation of Combined Plant Extract

The plants extracts combination were made for 100 mg/kgBW, 200 mg/kgBW, and 400 mg/kgBW doses. 1% solution was made using 0.5 g of each *Peperomia pellucida* and *Pachyrhizus erosus* dissolved in Na-CMC until it was brought to a final volume of 100 ml. 2% solution of the 200 mg/kgBW dose was made from 1 g of the plants extracts dissolved in Na-CMC until it was brought to a final volume of 100 ml. While 4% solution was made from 2 g of the plants extract dissolved in Na-CMC until it was brought to a final volume of 100 ml.

Animal Testing

The animal used to test the plants extracts combination was male Wistar rats aged 3-4 months weighing 150-200 g. The rats then were divided into 6 groups: the healthy group, which are the no-treatment rats; the negative control group, involving rats which only being induced with ethanol; the positive control group, as the comparison group involving rats with 400 mg/kgBW dose of sucralfate; and last group, involving rats with 100, 200, and 400 mg/kgBW doses of *Peperomia pellucida* and *Pachyrhizus erosus* extracts combination administered orally about 2 ml/200 gBW. The treatment lasted for 12 days. An hour after the treatment was done, the rats were induced with a 5 g/kgBW dose of 96% ethanol. After that, the rats were fasted for 24 hours before the stomaches were taken out in surgery.

Data Analysis

Ulcer index data and protection ratio of all test groups were analyzed using SPSS 16.0 for Windows software. The statistical test used was Kruskal Wallis to test the average comparison of data for each group, followed by Mann Whitney to see significant differences between groups ($p < 0.05$). The combination treatment group of suruhan and yam had a gastroprotective effect if the ulcer index was lower and significantly different ($p < 0.05$) compared to the negative control.

Results And Discussion

Peperomia pellucida L. is suspected to have gastro protector activity based on the results of previous research by Roslida and Aini (2009) which showed that *Peperomia pellucida* L. ethanol extract is able to provide a gastro protector effect with an effective dose of 100 mg/KgBW. The gastro protector activity of the messenger is produced by each metabolite compound with a different mechanism. Phytochemical screening of *Peperomia pellucida* L. showed that this plant contains secondary metabolites in the form of flavonoids, tannins, saponins, triterpenoids and steroids (Rachmawati and Rantelino, 2018). A previous study has found that dillapiole is the most active gastroprotective agent of *Peperomia pellucida*. However, the gastroprotective mechanism shown in dillapiole needs further study since the gastroprotective mechanism by dillapiole is not associated with endogenous nitric oxide or

prostaglandins (Rojas-Martínez *et al.,* 2013). Phytochemical test results extracted from *Peperomia pellucida* L. can be seen in Table 2.

Pachyrhizus erosus L. known to have gastroprotector activity based on research conducted by Pertiwi and Saputra (2019) where the administration of tuber juice *Pachyrhizus erosus* L. can reduce the number of ulcers that form and improve gastric histopathology due to ethanol exposure. Giving tuber juice *Pachyrhizus erosus* L.with a dose of 300 mg/kgBW gave a better gastroprotective effect than the juice of yam tubers combined with *Raphanus sativus L*.juice with the same dose (Pertiwi *et al*., 2021). *Pachyrhizus erosus* is known to contain flavonoids and saponins (Lukitaningsih, 2009). The isolation of *Pachyrhizus erosus* indicates the presence of the daidzein compounds; daidzein-7-O-β-glucopyranose, 5-hydroxy-daidzein-7-O-β-glucopyranose, and 8,9-furanyl-pterocarpan-3-. These compounds contain antioxidant activity and maybe one of the essential reasons for most of their biological properties, such as anti-diabetic, anticancer, immune modulation, and prevention of gastric ulcers reported from extract *Pachyrhizus erosus* (Jaiswal *et al.,* 2021). Phytochemical test results extracted from *Pachyrhizus erosus* L. can be seen in Table 2.

Flavonoids have anti-ulcer and anti-inflammatory effects through several mechanisms of inhibition of K+ /H+ ATPase, decreased secretion of HCl, increased synthesis of PGE2 and COX-1 and inhibition of H. pylori growth, and antioxidants (Kalogeromitros *et al.* 2008). The mechanism of action of alkaloids as another gastroprotective is by accelerating wound healing and increasing gastric mucus production after injury due to inducing agents (Tan *et al.,* 2002). Tannins are also known to have styptic properties, namely the ability to react with proteins in the gastric mucosal tissue layer. Its ability is useful to coat the outermost layer of the mucosa which makes it less permeable and more resistant to ulcers or irritation (Souza *et al.,* 2012). Saponins provide gastroprotective activity through an increase in fibronectin, then the fibrin clot formed will be the basis for the reepithelialization process in the tissue. Therefore, if fibrin clots form quickly, fibroblasts will immediately proliferate to the wound area to immediately restore tissue (Indraswary, 2011).

Observations on the gastric anatomy of rats were carried out in each treatment group, the normal group did not show any ulcers, while the negative and positive control groups showed various characteristics of gastric ulcers such as hyperemia, hemorrhage petechiae, hemorrhage ecchymoses, hemorrhage purpura, or erosion (loss of gastric wall tissue). The ulcer index (IU) is calculated based on the comparison between the total score and the number of animals in each group. The mean total score of each group's treatment was stated as an ulcer index or gastric ulcer index, which was then compared with the negative control group. The protective ability or protection ratio of the material towards the ulcer was calculated using the following formula by Saptarini and Suryasaputra (2011):

$$\%\text{Protection Ratio} = 100\% - \frac{\text{IU tested group}}{\text{IU Ulcer control}} \text{ x } 100\%$$

In the treatment group, the extract combination of *Peperomia pellucida* L. and *Pachyrhizus erosus* L. doses of 100 and 200 mg/kgBW showed hyperemia, hemorrhage petechiae, and hemorrhage

ecchymoses, whereas at a dose of 400 mg/kgBW only hyperemia and hemorrhage petechiae were found. Anatomical images of the gastric of rat can be seen in Figure 2.

Observation of gastric ulcers was carried out by scoring each cross-section of the gastric using the Szabo method *et al* 1985 which has been modified. To avoid subjectivity to the results, the scoring is done by 3 observers. The results of observations of gastric ulcers in rats can be seen in Table 3. After obtaining the gastric ulcer index value, the value of the protection ratio was calculated. The results of the protection ratio can be seen in Table 4.

The histopathological observations of rats gastric were aimed to see the description of gastric tissue from damage by gastric ulcer-inducing compounds and to see the repair of gastric tissue after administration of a combination of extracts. *Peperomia pellucida* L. and *Pachyrhizus erosus* L.. The results of the histopathological picture of the gastric can be seen in Figure 3.

In the histopathological picture, the normal group showed a normal gastric histopathological picture and no abnormalities or changes in tissue structure were found. In the negative control group, there was a lot of tissue damage which was indicated by the disappearance of lesions on the mucosa, the presence of ulcers, bleeding, and hemorrhage, while in the positive control group, there was tissue damage with the presence of ulcers, bleeding, and lesions on the mucosa but only in some parts of the tissue. The treatment group were given extract *Peperomia pellucida* L. and *Pachyrhizus erosus* L. doses of 100, 200, and 400 mg/kgBW showed significant gastric improvement with increasing doses. This was shown in the combination of extract at a dose of 100 there were still ulcers and lesions, but at doses of 200 and 400 mg/kgBW there was an improvement in gastric cells and no more lesions on the mucosa were found.

Conclusion

Administration of a combination of extracts *Peperomia pellucida* L. and *Pachyrhizus erosus* L in the gastric of rats induced by ethanol showed gastro protector activity with the improvement of gastric damage as seen from the parameters of the number of gastric ulcers, protection ratio, and gastric histopathology.

Statements & Declarations

We, the undersigned authors of the manuscript entitled "**Gastroprotective Activities of** *Peperomia pellucida* **L. and** *Pachyrhizus erosus* **L. Extracts Combination On Ethanol-Induced Rats**. Authors: Reza Pertiwi, Sal Prima Yudha S, Agung Giri Samudra, Aanisah Hanuun, Noval Kurnia Wati, Tesa Pebiani, Doni Notriawan, and Risky Hadi Wibowo.

Hereby declare the funding, competing interests, author contributions, data availability, ethics approval, consent to participate, and consent to publish, in order to publish the article in the journal Inflammopharmacology.

1. Funding

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2. Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

3. Author Contributions

Reza Pertiwi contributed to the main study's conception and design. Material preparation was permormed by Sal Prima Yudha S and Agung Giri Samudra. Reza Pertiwi, Aanisah Hanuun, Noval Kurnia Wati, Tesa Pebiani, Doni Notriawan, and Risky Hadi Wibowo collected and analyzed data.The first draft of the manuscript was written by Reza Pertiwi, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

4. Data Availability

To avoid plagiarism, the datasets generated during and/or analysed during the current study are not publicly available but are available from the corresponding author on reasonable request.

In addition to the above, this manuscript reports the results of research involving animals, so that there are the following declarations:

Ethics approval

This study was performed in line with the principles of the International Association for the Study of Pain (IASP) Guidelines for the Use of Animals in Research. Approval was granted by the Ethics Committee of the University of Bengkulu (18 Oct 2021/No: 240/UN30.14.9/LT/2021).

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Tables

Table 1 is not available with this version

Table 2. Phytochemical Test Results of *Peperomia pellucida* L. and *Pachyrhizus erosus* L. Extract

Phytochemical Test	*Peperomia pellucida* L. Extract	*Pachyrhizus erosus* L Extract
Flavonoids	+	+
Tannins	+	-
Alkaloids	+	+
Saponins	+	+
Steroids/Triterpenoids	+	+

Description: + indicates the test results contain the compound

Table 3. Average Gastric Ulcer Index in 96% Ethanol Induced Rat

Group	Dosage (mg/kgBW)	Average ± SD
Normal	-	0 ± 0^{b}
Negative Control	-	4.18 ± 0.84^{a}
Positive control		2.98 ± 0.63^{a}
ESB Combination	100	2.42 ± 0.97^{ab}
ESB Combination	200	2.04 ± 0.14^{ab}
ESB Combination	400	1.07 ± 1.04^{b}

Description: ESB = Combination of *Peperomia pellucida* L. and *Pachyrhizus erosus* L. extract

[a]sig<0.05 there is a significant difference with normal group

[b]sig<0.05 there is a significant difference with negative group

SD: Standard Deviation

Table 4. Protection Ratio In Ethanol Induced Rat 96%

Group	Dosage (mg/kgBW)	Protection Ratio ± SD (%)
Normal	-	100 ± 0
Positive control		28.76 ± 15.09[a]
ESB Combination	100	42.05 ± 23.31[a]
ESB Combination	200	51.09 ± 3.28[a]
ESB Combination	400	74.48 ± 24.84

Description: ESB = Combination of *Peperomia pellucida* L. and *Pachyrhizus erosus* L.Extract

[a]sig<0.05 there is a significant difference with normal group

SD: Standard Deviation

Figures

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Figure 1

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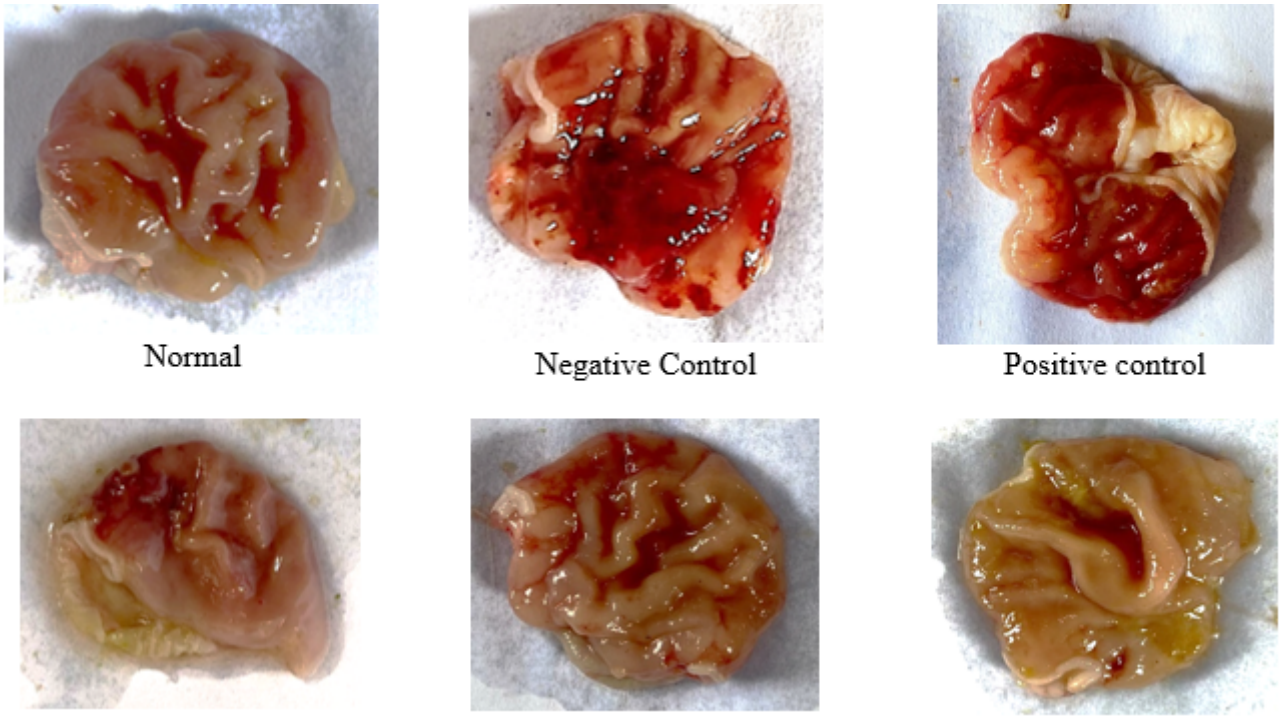

Figure 2

Gastric Macroscopic View Rat after treatment

Figure 3

Histopathological Description of the Gastric of Rats after treatment