Gastroprotective activities of *Peperomia pellucida* L. and *Pachyrhizus erosus* L. extracts combination on ethanol-induced rats

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**Abstract**

Gastroprotective is an effect caused by the compounds that have the capability of protecting the gastric mucosa. *Peperomia pellucida* L. plants contain alkaloids, flavonoids, saponins, tannins, and terpenoids, while *Pachyrhizus erosus* L. contains flavonoids, alkaloids, tannins, and saponins. *Peperomia pellucida* L. reportedly contains dillapiole compounds with a gastroprotective effect. Moreover, its isolation result from *Pachyrhizus erosus* L. 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* L. and *Pachyrhizus erosus* L. 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* L. and *Pachyrhizus erosus* L. extract. The combined extract was then given to five 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* L. and *Pachyrhizus erosus* L. extract of 100, 200, and 400 mg/kg BW. The treatment was given orally for 14 days, after 1 h 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 h 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* L. and *Pachyrhizus erosus* L. extract has a gastroprotective effect.

**Keywords** *Peperomia pellucida* L. · *Pachyrhizus erosus* L. · Gastric ulcer · Gastroprotective

**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 Afidaliah 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 2001). 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 et al. 2006).

The potential use of *Peperomia pellucida* L. and *Pachyrhizus erosus* L. 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* L. have been known to be containing flavonoids and saponins (Lukitaningsih 2009). Therefore, the extract of *Pachyrhizus erosus* L. 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 dillapiole is the most active gastroprotective agent of *Peperomia pellucida* L. Some compounds, such as dulcitol, gentisic acid, formononetin, kaikasaponin III, p-coumaric acid, and vitexin, are known to be present in *Pachyrhizus erosus* L. but are not tested/studied in the context of biological activity *Pachyrhizus erosus* L. is known to have important biological activities such as antiadibetics, anticancer, antioxidants, and antiinflammation (Jaiswal et al. 2021).

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

**Materials and methods**

**Materials**

The materials used in this study were *Peperomia pellucida* L., *Pachyrhizus erosus* L., 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 tube, surgical instruments, glass objects, and digital optical microscopes were also used in this research.

**Methods**

**Extraction process**

Two hundred (200 g) of *Peperomia pellucida* L. and *Pachyrhizus erosus* L. were macerated in 2 L of 96% ethanol in a closed container. The container was saved and kept away from direct sunlight for 48 h 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* L. and *Pachyrhizus erosus* L. 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, as the comparison 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* L. and *Pachyrhizus erosus* L. 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 h before the stomachs 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* L. 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 1.

*Pachyrhizus erosus* L. known to have gastroprotective activity based on research conducted by Pertiwi and Saputra (2019) where the administration of tuber juice *Pachyrhizus erosus* L. could reduce the number of 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):

\[
\text{% Protection ratio} = 100\% \times \frac{\text{IU tested group}}{\text{IU ulcer control}}
\]

Table 1 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.
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 Fig. 1.

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 2. 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 3.

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 Fig. 2.
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...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/kg BW 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/kg BW 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.

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**Author contributions** RP: contributed to the main study’s conception and design. Material preparation was performed by SPYS and AGS. RP, AH, NKW, TP, DN, and RHW: collected and analyzed data. The first draft of the manuscript was written by RP, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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**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.

**Declarations**

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

**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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