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The effect of *Raphanus sativus* and *Pachyrhizus erosus* juice combination on the ethanol-induced gastric of mice

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ABSTRACT

Gastric ulcers can be caused by active inflammation due to impaired mucosal integrity, which causes local or widespread lesions. Gastric ulcers can occur due to the use of ethanol, which has a local effect on the gastric. Therefore, the continuous consumption of ethanol can damage gastric cells. *Raphanus sativus* and *Pachyrhizus erosus* contain flavonoids, which are well-known to reduce stomach acid as a curative agent. The objective of this study is to see the effect of *Raphanus sativus* and *Pachyrhizus erosus* juice as a preventive agent on alcohol-induced gastric ulcers in mice (*Mus musculus*). A combination of *Raphanus sativus* and *Pachyrhizus erosus* was given at a dose of 100, 300, and 600 mg/kgBW. Specifically, *Raphanus sativus* was given at a dose of 300 mg/kgBW, *Pachyrhizus erosus* at a dose of 300 mg/kgBW, and positive control was included using sucralfate. The treatment was carried out for 12 days, and 1 hour after the last day of treatment, 20 ml/kgBW of ethanol induction was given 24 hours after the animals were dissected. The results showed that the index value of gastric ulcers in the normal group, negative control, and the combination of *Raphanus sativus* and *Pachyrhizus erosus* juice given orally at a dose of 100, 300, and 600 mg/kgBW (*Raphanus sativus* at a dose of 300 mg/kgBW, *Pachyrhizus erosus* at a dose of 300 mg/kgBW, and sucralfate as positive control) were 0; 4.00; 1.00; 1.33; 2.33; 2.67; 0 and 2.00. The results of the histopathological analysis also showed improvement in the gastric of mice fed with *Pachyrhizus erosus* juice. Therefore, it is inferred that treatment using *Raphanus sativus* and *Pachyrhizus erosus* juice can reduce the number of ulcers, increase ratio protection, and repair the cells in gastric histopathology. Additionally, giving a single dose of *Pachyrhizus erosus* juice results in a more significant gastric improvement.

Keywords: *Raphanus sativus*, *Pachyrhizus erosus*, gastric ulcer, gastroprotective

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INTRODUCTION

Disease due to disorders in the upper gastrointestinal tract caused by the presence of pepsin and excessive acid secretion by the gastric mucosa is known as peptic ulcers. Gastric ulcers are characterized by impaired mucosal integrity that subsequently causes localized or widespread lesions as a result of active inflammation. The pathophysiology of gastric ulcers is caused by the imbalance of aggressive factors, such as *Helicobacter pylori*, pepsin, hydrochloric acid, and non-steroidal anti-inflammatory drugs, as well as the local mucosal defensive factors, blood flow, prostaglandins, and mucus (Dharmani et al., 2012).

Ethanol is known to have a local effect on the stomach. The longer the ethanol is consumed, the more stomach cells will be damaged. The damage of the gastric mucosal barrier due to alcohol can cause acute and chronic gastritis. Excessive alcohol consumption can also cause superficial mucosal epithelial detachment (erosion). The severe form of erosion is a crucial cause of acute gastrointestinal bleeding (Goodman, 2008; Pan et al., 2008).

Medicinal plants that can be used in treating peptic ulcers are *Lobak Putih* or White Radish (*Raphanus sativus)* and *Bengkuang* or Jicama (*Pachyrhizus erosus)*. *Raphanus sativus* contains flavonoid compounds, such as kaempferol, glycosides, peroxidase, and antioxidants (Beevi et al., 2012). *Pachyrhizus erosus* is included in the Fabaceae family, which is widely reported to contain phytoestrogens and may also contain flavonoids. *Pachyrhizus erosus* is presumed to have a potential source of antioxidants (Lukitaningsih, 2014). Furthermore, flavonoids have anti-ulcer and anti-inflammatory effects through several mechanisms in their substances, namely inhibiting K^+ / H^+ ATPase, decreasing HCl secretion, increasing PGE2 and COX-1 synthesis, and inhibiting the growth of *Helicobacter pylori* and antioxidants (Narayana et al., 2001). The ulcer research model shows that flavonoids can protect the gastric mucosa from damage. In addition, quercetin has an anti-secretory mechanism of action. Garcino, rutin, and quercetin compounds have antioxidant properties with mechanisms such as the inhibition of free radicals, transition of metal ions chelatin, inhibition of oxidizing enzymes, and reduction of lipid peroxidation (De Lira Mota et al., 2009).

Based on previous studies, *Pachyrhizus erosus* tuber juices are proven to reduce the number of ulcers formed due to ethanol's exposure. Gastric repair is indicated by a histopathological picture of the stomach, which shows a significant improvement in the distribution of *Pachyrhizus erosus* juice (Pertiwi and Saputra, 2019).

Therefore, this study conducted a test on the gastroprotective effect of *Raphanus sativus* and *Pachyrhizus erosus* juices on the stomach of ethanol-induced rats. *Raphanus sativus* and *Pachyrhizus erosus* contain flavonoid compounds that have an important role in the gastroprotective activity. The combination of *Raphanus sativus* and *Pachyrhizus erosus* is expected to provide significant gastric repair results compare to the use of only one juice.

MATERIALS AND METHOD

Materials

The materials used in this study were white radish, feed, sucralfate, 0.9% NaCl, 96% ethanol, aquadest, and hematoxylin and eosin as coloring substances.

Preparation sample

The preparation was conducted by preparing fresh *Raphanus sativus* and *Pachyrhizus erosus*. The skin of the two ingredients was removed, then washed using running water to remove the dirt. *Raphanus sativus* and *Pachyrhizus erosus* that had been cleaned were mashed using a grater, and the grated results were then squeezed and filtered. Assembling the test preparation was carried out every day before the treatment.

Experimental animals

The experimental animals used were male mice. The mice used in this research were Swiss Webster mice with an average weight of around 20 grams. Furthermore, this research has been

approved by the committee of Ethical Health Research Ethics, Faculty of Medicine and Health Sciences at the University of Bengkulu, and has been registered with the number 215/UN30.14.9/LT/2020. A total of 40 mice were divided into 8 groups. For 12 days, Group I (normal group) was only given food and drink, Group II (negative control group) was given food and drink, and Group III (positive control group) was given sucralfate at a dose of 130 mg/kgBW. Meanwhile, Groups IV, V, and VI were given a combination of *Raphanus sativus* and *Pachyrhizus erosus* at a dose of 100, 300, and 600 mg/kgBW. Group VI was given *Raphanus sativus* at a dose of 300 mg/kgBW, and group VIII was given *Pachyrhizus erosus* at a dose of 300 mg/kgBW. The treatment was carried out for 12 days by giving each dose orally. After one hour of treatment on the 12th day, 96% ethanol induction was given orally at a dose of 20 mL/kgBW, except for group I. Following the giving of ethanol induction, the animals were treated to be in a fasting condition. Animal dissection was performed after 24 hours of ethanol induction.

Macroscopic observation of gastric ulcers

To see the number and size of the lesions/ulcers formed, the gastric was isolated after the surgery. The observation was carried out by opening the gastric of the mice, which was then deeply observed from 5 points of view. The gastric was dissected on the largest curve (major curvature) and cleaned with 0.9% NaCl. Then, the gastric was spread on a flat surface to observe the ulcers formed (Gusdinar et al., 2009). The scores, according to Szabo et al., (1985), are presented in Table 1.

Table 1. Modified scoring of ulcer severity by Szabo *et al* (1985)

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

Description: Hyperemia is a condition of dilated blood vessels with an excessive filling of blood granules. Erosion is the release of superficial mucosal epithelium. Hemorrhage (bleeding) is blood droplets leaving the blood vessels and scattered between the tissues. Petechiae are bleeding spots with the size of 0.1-0.2 cm. Ecchymoses are bleeding spots with the size of 0.2-3.0 cm. Purpura is a bleeding spot with the size of > 3 cm (Szabo et al., 1985)

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 formula 1 (Saptarini and Suryasaputra, 2011).

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$$\%\text{Protection Ratio} = 100\% - \frac{\text{IU tested group}}{\text{IU Ulcer control}} \text{ x } 100\% \quad (1)$$

Histopathological examination

The gastric was placed in gauze, dehydrated, and soaked in a graded volume of ethanol solution, with the percentages of 70%, 80%, 90%, 100%, 100%, and 100%, respectively, for 60 minutes at room temperature. The next process was clearing the gastric using xylol at room temperature for 15 minutes; this process was repeated three times. After the clearing process, the infiltration process was conducted

with liquid paraffin for 3 shifting times, in which each shifting was carried out for 60 minutes in a 60ºC degree incubator. Subsequently, the gastric was immersed in liquid paraffin and cooled down at room temperature until it turned into a paraffin block.

Furthermore, the process of embedding and cutting was carried out using a horizontal microtome with a thickness of 3µ. Toluidine blue painting was carried out using the following procedure: paraffin was removed using xylol, then put in 100%, 95%, and 70% ethanol, respectively, for 5 minutes, and continued with putting it in distilled water. Toluidine blue painting was conducted for 40-60 minutes in a 60° C oven, which was then put in 70%, 95%, and 100% ethanol. After the Canada balsam was added to it, it was covered with a glass deck. Two incisions were taken from each of the experimental animals, in which each incision was observed with a microscope from 5 points of view.

Data Analysis

The percentage of the protection was analyzed using SPSS. The analysis for the histopathological image of the gastric was conducted by observing it under a microscope. Gastric slice preparation analysis is carried out by observing the specific changes that occur in the gastric (Maslachah et al., 2008).

RESULT AND DISCUSSION

Raphanus sativus contain flavonoid compounds, such as kaempferol, glycosides, peroxidase, and antioxidants (Beevi et al., 2012). *Pachyrhizus erosus* to the Fabaceae family that is widely known to contain phytoestrogens and may contain flavonoids. Moreover, *Pachyrhizus erosus* is thought to have a potential source of antioxidants (Lukitaningsih, 2014). Flavonoids have anti-ulcer and anti-inflammatory effects through several mechanisms of the substances they contain, namely through the inhibition of K+ /H+ ATPase, decrease in HCl secretion, increase in PGE2 and COX-1 synthesis, and inhibition of *Helicobacter pylori* growth and antioxidants (Narayana et al., 2001). The qualitative test of *Raphanus sativus* and *Pachyrhizus erosus* can be seen in Table 2.

Table 2. Qualitative test of *Raphanus sativus* and *Pachyrhizus erosus*

Phytochemical Test	*Raphanus sativus*	*Pachyrhizus erosus*
Flavonoid	+	+
Tanin	-	+
Alkaloid	+	+
Saponin	+	+

The gastroprotective effect for the impact of giving *Raphanus sativus* and *Pachyrhizus erosus* tubers was observed by looking at the anatomy and histopathology of the mice's gastric. The results of the gastric anatomy of mice treated with *Raphanus sativus* and *Pachyrhizus erosus* on the mice's macroscopic gastric anatomy can be seen in Figure 1, and the gastric ulcer index of alcohol-induced mice treated with *Raphanus sativus* and *Pachyrhizus erosus* can be seen in Table 3.

Normal

Negative Control

Positive Control

RP dose 100 mg/kgBW

RP dose 300 mg/kgBW

RP dose 600 mg/kgBW

Raphanus sativus dose 300 mg/kgBW

Pachyrhizus erosus dose 300 mg/kgBW

Figure 1. Macroscopic overview of mice's gastric after *Raphanus sativus* and *Pachyrhizus erosus* Tubers were given (RP: *Raphanus sativus* and *Pachyrhizus erosus*; : hemorrhage ecchymoses; : hyperemia; and : hemorrhage Purpura)

Table 3. The gastric ulcer index of alcohol-induced mice treated with *Raphanus sativus and Pachyrhizus erosus* juices

Groups	Dose (mg/kgBW)	Mean ± SD
Normal	-	0
Negative Control	-	4.00 ± 0.82[a]
Positive Control (Sucralfate)	130	2.00 ± 0.81[a,b]
Raphanus sativus + *Pachyrhizus erosus* juice	100	1.00 ± 0.41[b]
Raphanus sativus + *Pachyrhizus erosus* juice	300	1.33 ± 0.47[b]
Raphanus sativus + *Pachyrhizus erosus* juice	600	2.33 ± 0.47[a,b]
Raphanus sativus Juice	300	2.67 ± 0.24[a,b]
Pachyrhizus erosus juice	300	0[b]

[a] sig<0.05 there is a significant difference with the normal group.
[b] sig<0.05 there is a significant difference between treatments.
SD: Standard Deviation

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.

Table 4. The protection ratio of alcohol-induced mice treated with *Raphanus sativus and Pachyrhizus erosus* juice

Groups	Dose (mg/kgBW)	Protection Ratio (%) ± SD
Normal	-	100 ± 0
Positive Control (Sucralfate)	130	50.00 ± 20.41[a,b]
Raphanus sativus + *Pachyrhizus erosus* juice	100	75.00 ± 10.21[b]
Raphanus sativus + *Pachyrhizus erosus* juice	300	66.67 ± 11.79[b]
Raphanus sativus + *Pachyrhizus erosus* juice	600	41.67 ± 11.79[a,b]
Raphanus sativus Juice	300	33.33 ± 5.89[a,b]
Pachyrhizus erosus juice	300	100 ± 0[b]

[a] sig<0,05 there is a significant difference with the normal group.
[b] sig<0,05 there is a significant difference between treatments
SD: Standard Deviation

The results show that the largest protection ratio was produced by giving only a single dose of *Pachyrhizus erosus* juice. According to previous studies, *Pachyrhizus erosus* tuber juices are proven to reduce the number of ulcers caused by ethanol's exposure. Gastric repair is indicated by a histopathological picture of the stomach, which shows a significant improvement in the distribution of *Pachyrhizus erosus* juice (Pertiwi and Saputra, 2019). Another study showed that there was a decrease in gastric lesions with treatment using radish *Raphanus sativus* on experimental gastric ulcer models at a dose of 20 mL/kgBW (Alqasoumi et al., 2008). Treatment of extracts of *Raphanus sativus* leaf is reported to have gastroprotective activity with a significant decrease in ulcer index, free acidity, and total acidity (Devaraj et al., 2011).

Gastric histopathology observation of mice was also carried out to support the research results. In addition, histopathological observations also aim to see a picture of gastric tissue from the damage caused by gastric ulcer-inducing compounds and to see gastric tissue repair after giving *Raphanus sativus* and *Pachyrhizus erosus* juices. Following are the histological preparations of the mice's gastric, which will be analyzed after observation using a microscope. The results of gastric histopathology observations of mice can be seen in Figure 2.

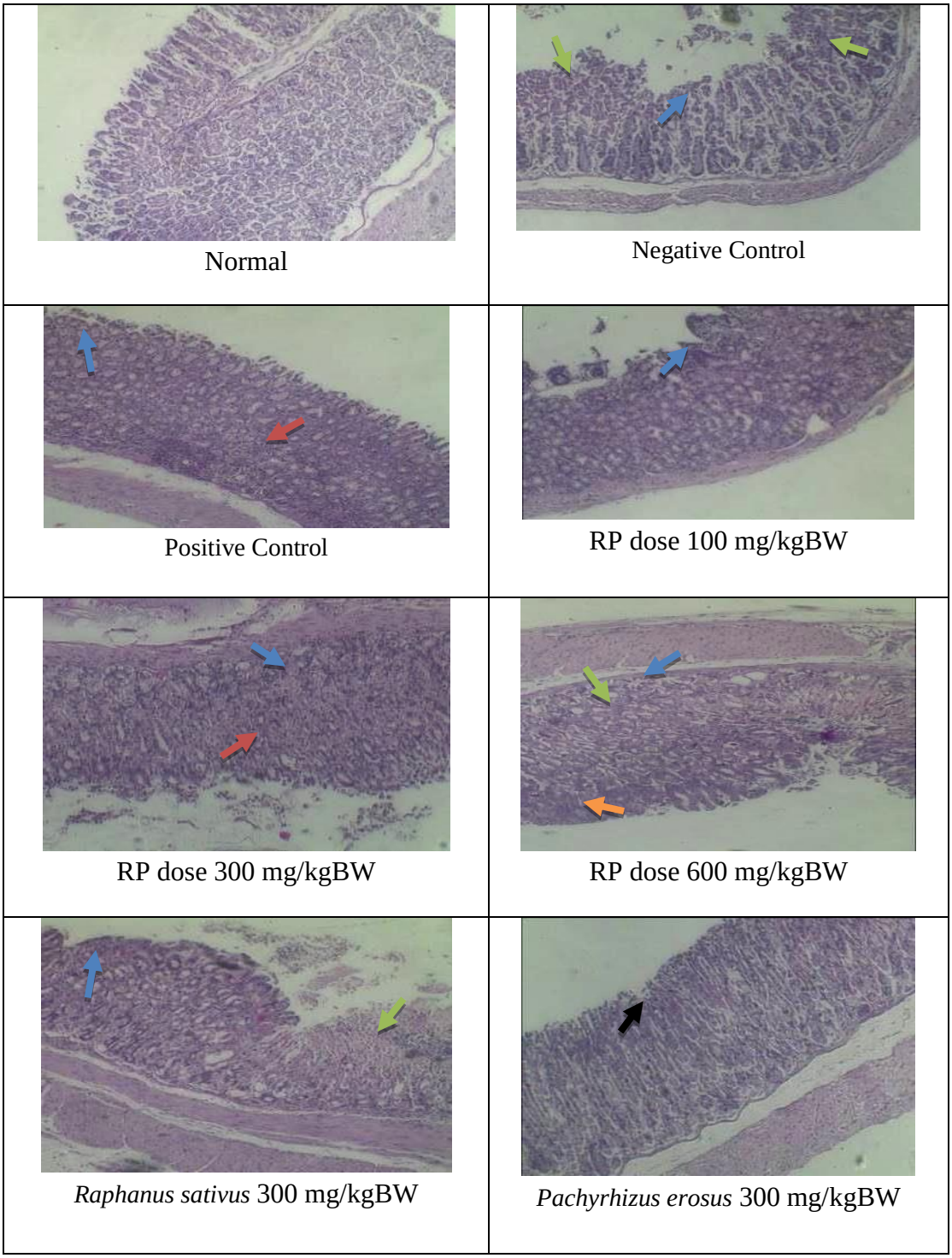

Figure 2. Histological preparation of mice's gastric after *Raphanus sativus and Pachyrhizus erosus* uber Juices were given (RP: *Raphanus sativus* and *Pachyrhizus erosus*; : ulcer; : lesions on the mucosa; : bleeding; : hemorrhage; : gastric cell repair and no ulcer)

Based on the mice's gastric anatomy observation, there were no characteristics that indicated gastric ulcers, such as hyperemia, hemorrhage petechiae, hemorrhage ecchymoses, hemorrhage purpura, or erosion (loss of gastric wall tissue). There were no bleeding and injuries seen from the macroscopic anatomy in the gastric of mice after being treated with *Pachyrhizus erosus* at a dose of 300 mg/kgBB. *Pachyrhizus erosus* tubers are identified to contain adenine, choline, saponins and flavonoid compounds (Catteau et al., 2013).

Flavonoids can become cytoprotective agents with various mechanisms, namely stabilizing membranes and influencing several intermediate metabolic processes, peroxiding lipid by increasing the activity of the Superoxide Dismutase (SOD) enzyme, and increasing the prostaglandin content of the gastric mucosa by stimulating cyclooxygenase (COX-1) and having anti-inflammatory activity. In addition, *Helicobacter pylori* can prevent recurrence (Islamiah and Sukohar, 2017). According to Robiyanto and Marsela, (2018), alkaloids can increase the secretion of alkali and mucus, reduce gastric acid secretion, and help cure and prevent gastric ulcers against irritant agents/factors by improving blood flow to the gastric mucosa. Alkaloids are responsible for proton pump of H+ inhibition, K+ ATPase and can increase mucus secretion (Do Nascimento et al., 2015). Antimicrobial effects in tannins can help the defense against *Helicobacter pylori* to prevent gastric ulcer recurrence (Indraswari, 2011). Saponins also have an inhibitory effect on the formation of lesions on the gastric mucosa (Islamiah and Sukohar, 2017).

From the results of histopathological observations, it can be seen that there is an improvement in the gastric tissue of mice when the *Pachyrhizus erosus* juice was given. The histopathological picture of the gastric shows a picture that is almost the same as the histopathology of the normal gastric.

CONCLUSION

The *Pachyrhizus erosus* juice can prevent gastric damages in mice caused by ethanol induction. However, in this study, *Pachyrhizus erosus* is proven to be better at preventing gastric damage compared to *Raphanus sativus* juice and the combination of *Raphanus sativus* and *Pachyrhizus erosus* juices.

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