AMJ
ISSN: 0005-2523

ISSN: 0005-2523
Volume 63, Issue 02, February, 2023

Gastroprotective Activity of Nutmeg Flesh Extract (Myristica Fragrans Houtt.) on Acetosal-Induced White Rats

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Keywords:
anti-ulcer, histology, omeprazole, nutmeg, peptic ulcer.

ABSTRACT

Gastric ulcer is a disease disorder caused by damage to the mucous layer, submucosa, to the muscle layer of the gastrointestinal tract due to the activity of pepsin and stomach acid. This study aims to determine the effect of nutmeg flesh as an anti-ulcer. Tests were carried out using Wistar strain white rats. Nutmeg Flesh Extract (NFE) was obtained by maceration method using 70% ethanol, then divided into three dose groups, NFE 400, 900, and 1800mg/kgbw. Acetosal 500mg/kgbw was given as an ulcer inducer on the 8th day. As a positive control, Omeprazole was used at 20 mg/Kgbw. The test parameters observed were the number of ulcers, ulcer index, % protection, gastric pH, total acidity of gastric juice, and histology of gastric tissue. The results showed that the ethanol extract of nutmeg flesh at doses of 450, 900, and 1800 mg/kgbw had significantly different anti-ulcer activity against negative controls ($p<0.05$) in acetocal-induced rats with the highest protective ratio indicated by a dose of 1800 mg/Kgbw.

1. Introduction

A peptic ulcer is a disease caused by obstruction of the upper gastrointestinal tract caused by excess secretion of acid and pepsin by the gastric mucosa [6]. Peptic ulcer disease (PUD) is one of the gastrointestinal disorders common with maintenance dispensing the highest health. Peptic ulcer or stomach ulcer prevalence ranges from 11-14% in men to 8-11% in women. The prevalence of this disease in Indonesia is 6-15%. With an average age of 20-50 years [14]. A peptic ulcer is an acid-induced lesion of the gastrointestinal tract that is usually located in the stomach or proximal duodenum. It is characterized by a thinned mucosa with defects extending into the submucosa or muscularis propria [6]. Peptic ulcers can be treated with antacids useful as symptomatic treatments, sucralfate, H2 blockers, prostaglandin analogues and triple therapy due to Helicobacter pylori infection consisting of Proton Pump Inhibitors (PPI), amoxicillin, and clarithromycin. In a healthy stomach, there is a balance of defensive factors as mucosal protectors with aggressive factors that can damage the integrity of the gastric mucosa [4].

The bioactivity of the nutmeg content has been widely studied, especially in the fruit section. It was reported that the ethanol extract of nutmeg showed anticancer, anti-inflammatory, and nitrous oxide inhibitory activity. Phytochemical tests of the methanol extract of nutmeg leaves have also been reported to contain alkaloids, flavonoids, terpenoids and tannins, while the ethyl acetate extract contains flavonoids and has antifungal activity against Candida albicans [5].

Flavonoids are also reported to have acted as an antiulcer by reducing gastric and peptic secretions to prevent peptic ulcers. It was reported that tannins are also have activity as an antiulcer by protecting the gastric mucosa [7].

Based on this description, it is necessary to carry out tests related to the gastroprotective activity of the ethanol extract of nutmeg flesh so that a safe, effective and efficient dose is obtained. In addition, this research can also be used as a reference for further research. Various unwanted reactions have been reported in the use of the above drugs, so by considering the side effects and disadvantages of these drugs, it is necessary to search for better drugs that have low toxicity but have gastric acid and ulcer inhibition activity. Natural medicine has low-toxicity therapeutic properties, so it is safe to use as a treatment.

Indonesia has several medicinal plants that are used to treat peptic ulcers. The use of plants for peptic ulcers is not only based on hereditary information but also based on the results of scientific research. The nutmeg (Myristica fragrans) has long been known as a spice plant and has high economic value because every part of this plant can be used as an ingredient for the food and beverage industry, medicines, perfumes and cosmetics [9].

2. MATHERIALS AND METHODS

2.1 Plant Material

Nutmeg (Myristica fragrans Houtt.) comes from Ternate City, North Maluku, Indonesia. The samples are washed using running water, cut into smaller pieces, and dried using an oven at 400C until dry and reached a constant weight. The extraction process was carried out by maceration method using 70% ethanol solvent. The liquid extract was then concentrated using a rotary vacuum evaporator [10], [11].

2.2 Animals

A total of 30 male Wistar rats (age, 3-4 months; weight, 200-250 g) were purchased from Hasanuddin University. All procedures carried out in this study were approved by the animal research ethics committee at Khairun University (05/KEPH/PH/2022). Animals were housed with controlled conditions, light cycle (12-h light/dark cycle), relative humidity (46-50%) and temperature (22℃). The animals had free access to rat chow and water. All were maintained individually in polyethylene cages. The rats were fasted for 18h prior to the start of the experiment but had free access to water.

2.3 Animal grouping and dosing

Rats were randomly divided into six groups containing five animals each. Group I (Normal Control) was treated with Sodium CMC 0.5%, Group II was negative control, Group III (Positive Control) was treated with Omeprazole 20mg/kgbw, Group IV-VI were treated with different doses of Nutmeg Flesh Extract (NFE) 400mg/kgbw, 900mg/kgbw and 1800mg/kgbw. The standard drugs and plant extracts were administered orally.

2.4 Acetosal induced gastric ulcer

AMJ
ISSN: 0005-2523
ISSN: 0005-2523
Volume 63, Issue 02, February, 2023

All groups of rats were given treatment every day for seven consecutive days orally except for group II. On the 6th day, all groups of rats fasted for 16 hours, then administered an acetosal dose of 500 mg/kgbw orally while the rats remained fast. The animals were sacrificed on the 8th day, 10 hours after being induced. Then the animals were dissected, and the stomach organs were taken for macroscopic and microscopic examination. Ulcers were scored according to severity (Table 1). The average gastric number and severity score was then calculated to determine the ulcer index and percent protection [1]. The sum of length (mm) of all lesions for each stomach was used as the ulcer index (UI), and the inhibition percentage was calculated by the following formula.

Table 1. Gastric ulcer severity score [2]

Severity	Score
Loss of normal morphology	1 point
Discoloration of mucosa	1 point
Mucosal edema	1 point
Hemorrhages	1 point
Petechial points (until 9)	2 point
Petechial points (>10)	3 point
Ulcers up to 1 mm	*n x 2 points
Ulcers >1mm	*n x 3 points
Perforated ulcers	*n x 4 points

*number of ulcers found

Ulcer index (UI")=UN+US+(UP/10)"

Were UI = ulcer index, UN = average number of ulcer per animal, US = average of severity score, And UP = percentage of animals with ulcer [1]. And percentage protective ratio was calculated as follow

$$\% \text{ of protection} = 100 - \frac{(UI\ Pretreated)}{(UI\ Control)}\ x\ 100$$

2.5 Determination of Gastric pH.

The gastric fluid that has been obtained is collected in a tube and then measured using a pH meter, centrifuged at 3000 rpm for 10 minutes. 1 mL gastric juices was diluted with 1 mL of distilled water and and pH of the solution was measured using pH meter [1].

2.6 Determination of total acidity.

The solution obtained is added with 2-3 drops of Toppfer's reagent, titrated with 0.01 N NaOH. The endpoint of the titration is determined if the colour changes from orange to yellow, and the final volume of the titration is recorded as the free acid content. The titration is then continued with the addition of 2-3 drops of phenolphthalein indicator. The end point of the titration is determined if the colour changes from yellow to reddish-orange, and the final volume of the titration is recorded as the combined acid content. The total acidity was expressed as mEq/L and calculated by the following formula [1], [12].

$$\text{Acidity} = \frac{V\ x\ NaOH\ x\ N\ x\ 100mEq/L}{0{,}1}$$

where V is volume and N is normality.

2.7 Gastric Histological Examination.

The stomach corpus was put in Bouin's solution (a mixture of 70 parts of saturated picric acid, 25 parts of

formalin, and five parts of glacial acetic acid), and soaked for 24 hours in a tightly closed container. Then observe the ulcers that form under the microscope [8].

2.8 Data Analysis.

Results were expressed as mean ±S.E.M. The statistical difference between the mean ulcer index of the treated group and that of the control was calculated by using one-way ANOVA and Tukey-/Kramer multiple comparison tests

3. FINDINGS AND DISCUSSION

Gastric ulcer is a disease that occurs in the stomach wound, infection and inflammation so that the stomach wall becomes eroded. which factor causes of gastric ulcer recurrence are HP (helicobacter pylori) and NSAIDs (non-steroidal anti-inflammatory). The sample used in this study was the ethanol extract of nutmeg flesh (Myristica fragrans Houtt.). Several studies have shown that nutmeg contains alkaloids, flavonoids, terpenoids and tannins, in which flavonoid compounds are reported to have antiulcer activity by reducing gastric and peptic secretions to prevent gastric ulcers. In addition, tannin compounds can act as an antiulcer by protecting the gastric mucosa.

Acetosal suspension of 500mg/kgbw was given after the rat group was treated. Then surgery and gastric histopathological examination were carried out to see the gastroprotective effect of the extract on the gastric epithelial cell damage score in white male rats. Acetosal is a non-steroidal anti-inflammatory drug widely used to treat several diseases. A common side effect suffered by acetosal users is gastric ulcers. Aspirin works by non-selectively inhibiting cyclooxygenase (COX) 1 and 2 enzymes. In contrast, the COX-1 enzyme functions to convert arachidonic acid into prostaglandins (PGI2 and PGE2). The secretion of mucus and bicarbonate in the stomach also decreases by inhibiting the synthesis of prostaglandins. This causes the protection of the stomach to be reduced. In addition, aspirin induces ROS formation, making the stomach more susceptible to ulcers or wounds [3].

The results of the study (see table 2) showed that the three doses of the ethanol extract of nutmeg flesh had comparable effectiveness with the standard drug, omeprazole, in preventing the formation of gastric ulcers due to the use of acetosal. Nutmeg Flesh Extract (NFE), at a dose of 900mg and 1800mg body weight, showed a significant increase in pH value compared to the negative control ($p<0.01$). The omeprazole group, as the positive control, also showed a significant increase in gastric pH values ($P<0.01$) compared to the negative control. Omeprazole is a proton pump inhibitor. It works by inhibiting acid secretion by binding to irreversible and can inhibit the hydrogen potassium ATPase pump (an enzyme known as a proton pump) present on the surface luminal parietal cell membrane [13].

Table 2. Effect of nutmeg flesh extract on acetosal induced gastric ulcers in rats

Group / Treatment	Dose (mg/kgbw)	pH (mean± S.E.M)	Total Acidity (mEq/l/100 g)	Ulcer index (mean± S.E.M)	% protection
Normal Control	-	2.85±0.29y	76.17±8.92y	0	-
Negative Control	500	1.78±0.15y	89.92±7.43y	63.16 ± 8.08y	-
Omeprazole	20	4.11±0.39ab	51.15±6.01ab	18.83 ± 0.57ab	70.18
NFE	450	2.77±0.22y	71.66±7.9y	41.66 ± 8.96acy	34.04
	900	3.01±0.14ab	68.63±6.93a	28.33 ± 3.60ab	55.15
	1800	3.89±0.31ab	48.32±4.33ab	21.66 ± 3.05ab	65.70

NFE: Nutmeg Flesh Extract, analysis was performed with One-Way ANOVA followed by Tukey test: a =

ISSN: 0005-2523
Volume 63, Issue 02, February, 2023

compared to negative control p <0.05, b = compared to negative control p<0.01, y = compared to Omeprazole p<0.05.

The ulcer index in NFE at doses of 450 mg/kgbw, 900mg/kgbw and 1800mg/kgbb showed a significant difference compared to the negative control (p <0.05) with a per cent protection of 34.04%, 55.15% and 65.70%, respectively.

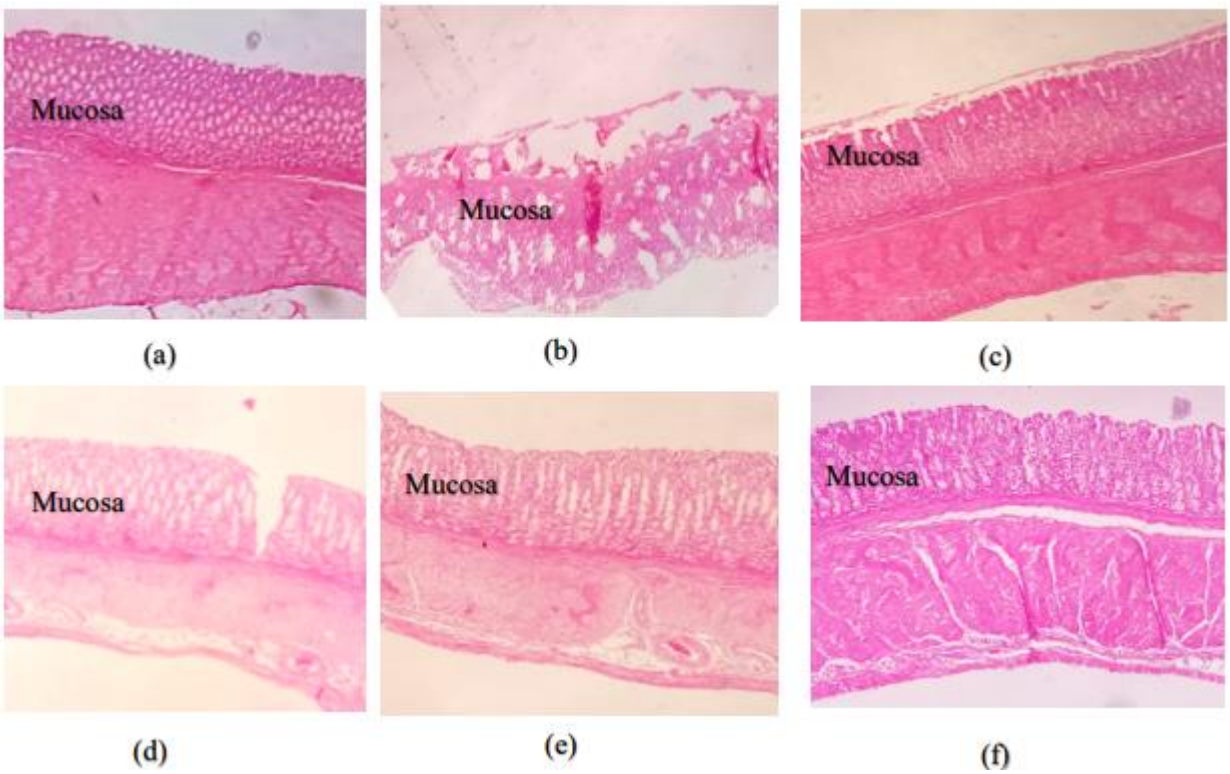

Fig. 1 Microscope appearance of gastric tissue after hematoxylin-eosin (H&E) staining. Magnification 10x. Normal group (a); negative control group (b); group receiving omeprazole 20mg/kgbw (c); group receiving nutmeg flesh extract 450mg/kgbw (d); group receiving nutmeg flesh extract 900mg/kgbw (e); group receiving nutmeg flesh extract 1800mg/kgbw.

Testing continued with histological observations of gastric ulcers (see figure 1). The test results showed damage to epithelial cells in the mucosa to the submucosa of the stomach after administration of acetosal 500mg/kgbw (figure 1b) compared to the normal group (figure 1a). The group that received omeprazole 20mg/kgbw (figure 1c) showed normal repair of gastric mucosal epithelial cells and submucosa. Administration of NFE 450 mg/kgbw (figure 1d) showed erosion of the gastric mucosa and damage to parietal cells. NFE 900 mg/kgbw (figure 1d) showed better results. However, it still damaged the parietal cells (figure 1e). The NFE group at a dose of 1800 mg/kgbw (figure 1f) showed improvement in parietal cells and no erosion of the gastric mucosa.

The macroscopic and microscopic observations showed that the ethanol extract of nutmeg flesh 1800 mg/kg had the best preventive effect against gastric ulcers. According to Patil et al. (2014), flavonoids can prevent the formation of free radicals and act as an anti-inflammatory. In addition, the content of tannins can repair wounds. The presence of these two secondary metabolites is likely to prevent gastric ulcers.

4. Conclusion

The nutmeg flesh extract (Myristica fragrans Houtt.) has a gastroprotective property against experimentally induced ulcers in rats and hence can be used to treat ulcers.

5. Acknowledgments
The authors are thankful to Khairun University for financial support

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