Pharmacogn J. 2023; 15(3): 378-384
A Multifaceted Journal in the field of Natural Products and Pharmacognosy
www.phcogj.com

Research Article

Anti-Gastritis Activity of Cloves (*Eugenia caryophyllata* Thunberg) and Lime (*Citrus aurantifolia*) Leaf Extracts Combination in Absolute Alcohol Induced-Gastric Injury Mice

Herra Studiawan, Sukardiman*, Indana Lazulfa, Rosita Handayani

Herra Studiawan, Sukardiman*, Indana Lazulfa, Rosita Handayani

Pharmaceutical Science Department, Faculty of Pharmacy, Airlangga University, Surabaya, East Java, INDONESIA.

Correspondence

Sukardiman

Pharmaceutical Science Department, Faculty of Pharmacy, Airlangga University, Surabaya, East Java, INDONESIA.

E-mail: sukardiman@ff.unair.ac.id

History

- Submission Date: 11-03-2023;
- Review completed: 22-04-2023;
- Accepted Date: 27-04-2023.

DOI : 10.5530/pj.2023.15.88

Article Available online

http://www.phcogj.com/v15/i6

ABSTRACT

Background: Clove *(Eugenia caryophyllata* Thunberg) and lime (*Citrus aurantifolia*) are medicinal plants traditionally used to treat various diseases such as gastritis. Some studies reported that cloves leaf and lime leaf extract showed a gastroprotective effect by decreasing the gastric acid secretion and increasing the gastric mucus. **Aim:** This study aims to investigate the anti-gastritis activity of the combination of ethanolic extract of cloves leaf and lime leaf extract in alcohol absolute-induced mice. **Methods:** Mice were treated with the combination extract 0.7 g/kg BW, 1.4 g/kg BW, and 2.8 g/kg BW. Famotidine was used as a positive control, and Na CMC suspension was used as a negative control. After 45 minutes of oral administration, 0.14 ml/20g BW alcohol absolute was given to all mice. All groups were sacrificed one hour later. The evaluation showed that extract combination in all doses significantly decreased ulcer index (UI) compared to a negative control group ($p<0.005$) in macroscopic evaluation. **Results:** In histopathologic evaluation, all doses significantly decreased mucosal edema and epithelial cell loss ($p<0.005$), but in gastric bleeding evaluation, only 0.7 and 1.4 g/kg BW doses showed a significant decrease. **Conclusion:** This study showed that a combination of clove and lime leaf extracts has anti-gastritis activity and could be a possible therapeutic of anti-gastritis. The most effective dose is 1.4 g/kg BW in mice which showed the lowest ulcer index and gastric mucosal edema, bleeding and epithelial cells loss reduction on histopathological observation.

Key words: Clove, *Eugenia caryophyllata*, *Citrus aurantifolia,* Lime, Gastritis, Ulcer.

INTRODUCTION

Acid reflux disease includes gastritis and peptic ulcers. All these conditions are characterized by inflammation of the gastric mucosa with a histological appearance of the reddish gastric mucosa. Symptoms include pain and dyspepsia, which include nausea, vomiting, or upper abdominal discomfort.[1] Gastritis is caused by damage to the gastric mucosal barrier, which usually prevent mucosal irritation due to gastric acid secretion. Damage to the gastric mucosal barrier can occur due to *Helicobacter pylori* infection or NSAIDs, which can increase gastric acid secretion.[2] In addition, gastritis can also be caused by alcohol consumption.[3]

Drugs used to treat gastritis include antacids, H2 receptor antagonists, proton pump inhibitors, sucralfate, prostaglandin analogues, and bismuth compounds which can reduce gastric acid secretion and increase mucosal defense.[4] None of these drugs is free of side effects, especially with long-term use of large doses. These side effects include osteomalacia, osteoporosis, neurotoxicity, nausea, abdominal pain, constipation, flatulence, and diarrhea. Due to these side effects, it is necessary to develop natural product therapy, which generally has fewer side effects.[5] Some potential plants that can be developed as natural products for gastritis are clove (*Eugenia caryophyllata* Thunberg) leaf and lime (*Citrus aurantifolia*) leaf.

Eugenia caryophyllata Thunberg syn *Syzygium aromaticum* or clove belongs to the Myrtaceae family. It is usually used as a flavouring agent, a spice for perfumes, and as an ingredient to control nausea, vomiting, coughing, diarrhea, dyspepsia, flatulence, and gastrointestinal pain.[6] The main content of clove essential oil is eugenol, which gives the strong aroma.[7] In the extract obtained from extraction using 70% ethanol, clove extract also showed the same dominant compound content as the essential oil resulting from the distillation of clove leaf, namely eugenol.[8]

The eugenol content contained in cloves has a gastroprotective effect. The mechanism of action is by increasing mucus secretion on the gastric mucosal barrier.[9] In addition, research by Morsy and Fouad (2008) regarding the gastroprotective effects of eugenol in experimental rats showed that eugenol could reduce gastric ulcers by reducing gastric acid secretion and increasing mucus secretion to protect the gastric mucosal barrier.[10] In our unpublished preliminary *in vitro* study, 70% ethanolic extract of cloves leaf at a dose of 3% could neutralize gastric acid for the most prolonged duration. It also provided an anti-flatulent effect.

Citrus aurantifolia, or in English known as lime, belongs to the Rutaceae family, used in traditional medicine to treat stomach aches and to increase appetite.[11] Lime leaf ethanolic extract has the main component, namely limonene.[12] The limonene content in lime can increase the secretion of gastric barrier mucus to provide a gastroprotective effect. Mucus on the gastric barrier will protect gastric from lesions caused by alcohol or NSAID drugs; therefore, it can prevent gastritis.[9] A study by Moraes

Cite this article: Studiawan H, Sukardiman, Lazulfa I, Handayani R. Anti-Gastritis Activity of Cloves (*Eugenia caryophyllata* Thunberg) and Lime (*Citrus aurantifolia*) Leaf Extracts Combination in Absolute Alcohol Induced-Gastric Injury Mice. Pharmacogn J. 2023;15(3): 378-384.

(2009) showed that lime essential oil at 250 mg/kg BW p.o. had a gastroprotective effect in ethanol and NSAID drug-induced mice.[3]

Based on empirical utilization, reports on the beneficial compounds, and preliminary studies that have been done, an *in vivo* anti-gastritis activity assay was carried out on the combination of 70% ethanolic extract from clove and lime leaf in the gastric mice that have been induced by absolute alcohol thus the combination of the two is expected to have stronger anti-gastritis effect. To induce gastritis, several inducers, including NSAID drugs and alcohol can be used. Based on the study by Saputri (2008), which compared the induction of gastritis in experimental white rats using indomethacin, aspirin-HCl, and absolute alcohol orally, showed that the alcohol-induced group provided the highest gastric ulcer index.[13] In another study conducted by Moraes *et al*., (2009) which compared the induction of gastritis using alcohol and indomethacin, stated that after administration, ethanol-induced experimental animals could be sacrificed after 1 hour because it produced the highest ulcer index, while those induced by indomethacin could be sacrificed after 5 hours. These results indicate that using inducer ethanol can accelerate the observation time.[3]

MATERIALS AND METHODS

Clove (*Eugenia caryophyllata* Thunberg) leaf and lime (*Citrus aurantifolia*) leaf collected fom Batu City, East Java. The plant samples were authenticated by Purwodadi Botanical Garden. Absolute alcohol, Sodium carboxymethyl cellulose (Na CMC), distilled water, formalin 10%, hematoxylin and eosin stain (HE).

Extract preparation

Clove and lime leaf extracts were prepared separately using the exhaustively extraction by overnight maceration (24 hours) for three consecutive days with 10 volumes of 70% ethanol as solvent. The extract was then filtered to separate the filtrate and residue. The filtrate obtained was then concentrated with a rotary evaporator and vacuum oven for 24 hours until a thick, concentrated extract was formed.

Preparation of clove and lime leaf extracts suspension

The suspension was prepared by mixing the extract and Na CMC. Each mice received 0.2 ml of suspension with a combination dose of 0.7 g/kg BW, 1.4 g/kg BW, and 2.8 g/kg BW.

Preparation of experimental animals

In this study, BALB/c white mice (*Mus musculus* L) was used with an average body weight of 20-40 grams. These experimental animals were obtained from animal laboratories of the Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Airlangga University. Experimental animals were adapted to the environment where the study was conducted for approximately 14 days. Mice were placed in a cage with a temperature of 22 ± 2°C (adjusted with an air conditioner). All mice were reared in the same way and received the same diet.[14] The mice to be used were previously weighed to calculate the dose arrangement.

Anti-gastritis activity assay

Experimental animals were randomly grouped into five groups. Each group consisted of 6 mice. The groups include negative control, positive control and treatment groups with the following details:

Negative control group, treated with 1 ml Na-CMC 0,5%.

Positive control group, treated with standard drug (antacids). Famotidine 2.8 g/kg BW.

Treatment group, showed in table 1

Dose 1, treated with the combination of clove and lime leaf extract at 0.7 g/kg BW

Dose 2, treated with the combination of clove and lime leaf extract at 1.4 g/kg BW

Dose 3, treated with the combination of clove and lime leaf extract at 2.8 g/kg BW

After receiving treatment orally for 45 minutes, all mice were given 0.14 ml/20 g BW of absolute alcohol orally. One hour after the administration of absolute alcohol, the mice were sacrificed. The gastric tissue was taken, stored in 10% formalin buffer, and then attached to paraffin. The preparations were then cut using a microtome with a thickness of 6 µm and stained with hematoxylin-eosin (HE). Histopathological changes were evaluated using the scoring method with the criteria shown in Table 2, 3, and 4. The ulcer index was then calculated. The total gastric and ulcerated areas were observed using a computer imaging program and calculated using the following formula:

UI=(Ulcerated area of the gastric mucosal barrier)/(Total area of the gastric mucosal barrier)

Statistical analysis

Anti-gastritis activity was evaluated with the ulcer index value of the combination of clove and lime leaf extract compared to the positive control and negative control. All results are presented in mean ± SD form. Differences in the results of each test group against the control group were statistically analyzed using one-way analysis of variant (ANOVA) followed by the LSD Post Hoc Test method. In the histopathological observation of the gastric mice, a non-parametric statistical test was performed to assess gastric mucosal edema, bleeding, and epithelial cell loss. The non-parametric Kruskal-Wallis statistical test ($\alpha = 0.05$) was carried out to determine the statistical significance anti-gastritis effect of treatment with a combination of clove and lime leaf extract in various doses compared to positive and negative controls.

Table 1: Dose combination of clove and lime leaf extracts.

Treatment group	Cloves leaf extract	Lime leaf extract	Total dose combination
Dose 1	0.175 g/kg BW	0.525 g/kg BW	0.7 g/kg BW
Dose 2	0.35 g/kg BW	1.05 g/kg BW	1.4 g/kg BW
Dose 3	0.7 g/kg BW	2.1 g/kg BW	2.8 g/kg BW

Table 2: Scoring and interpretation of mucosal edema.

Score	Interpretation
0	No lesions
1	Mild edema
2	Mild edema of the mucosal
3	Mild edema of the mucosal and submucosal
4	Moderate-severe edema of the mucosal and submucosal

Table 3: Scoring and interpretation of gastric bleeding.

Score	Interpretation
0	No bleeding
1	Hyperemia (bleeding spots)
2	Minor area bleeding
3	Bleeding in large areas
4	Bleeding is evenly distributed almost in all parts

Table 4: Scoring and interpretation of epithelial cell loss.

Score	Interpretation
0	No ulcers
1	Small ulcer
2	Large ulcer
3	The gastric surface is covered with ulcers

If there is a significant difference, the statistical test was continued with the Mann-Whitney method with a value of $\alpha = 0.05$.

RESULTS AND DISCUSSION

Anti-gastritis activity assay was performed on absolute alcohol-induced mice. Absolute alcohol was used as an inducer of gastritis because it can give higher gastric ulcers than NSAIDs and has a faster induction time.[3,13] In addition, alcohol is also known as the leading cause of gastritis in humans.[15] Absolute alcohol could cause severe peptic ulcers by stimulating extreme instability in the gastric mucosa, such as decreased bicarbonate secretion, mucus production, and gastric blood flow.[16] The ulcerogenic activity of ethanol is driven by the capacity to dissolve gastric mucus and reduce transmucosal action potentials, thus increasing the flow of Na^+ and H^+ ions in the lumen and stimulating the secretion of pepsin enzymes and gastric acid. Furthermore, ethanol also reduces the protein, DNA, and RNA levels, leading to *Sanguineous* drainage resulting in tissue lesions.[3]

Induction was carried out after negative control, positive control, and extracts combination were orally administered to the experimental animals under the gastroprotective mechanism of clove and lime leaf extracts, which increased mucus secretion to protect the gastric barrier.[9,10] Famotidine was selected as the positive control because of the similarity of the mechanism of action with eugenol and limonene, which were found abundant in the combination of the extracts.[17]

Macroscopic observations were carried out on the stretched mice gastric by calculating the ulcer area indicated by bleeding lesions on the gastric mucosal barrier (Figure 1). The ulcer index was calculated by comparing the area of the ulcer and the total surface area of the gastric (Figure 2). Smaller ulcer index value indicates greater anti-gastritis activity. The smaller the ulcer index, the greater the anti-gastritis activity. Microscopic observation was carried out through histopathological observation of gastric. Indications of gastritis were assessed from changes in mucosal edema, gastric bleeding, and epithelial cells loss.[17]

The average ulcer index after treatment was as follows: negative control (0.09±0.05), positive control (0.02±0.00), dose 1 (0.04±0.01), dose 2 (0 .03±0.01), dose 3 (0.05±0.01). Ulcer index data showed that the dose 2 group had a lower ulcer index than doses 1 and 3 but not lower than the famotidine as the positive control (Figure 2).

The normality test results showed that in each group, the p-value>0.05 was obtained, which indicated that the observed data were normally distributed and met the variance analysis requirements. The data were then analyzed using one-way ANOVA to determine the significance value of the difference between the control and treatment groups. From the one-way ANOVA statistical analysis results, the calculated F value was 9.42 with a significance value of 0.000 ($p < 0.05$). It can be concluded that there are at least a pair of treatment groups that are significantly different. To find out which group pairs had significant

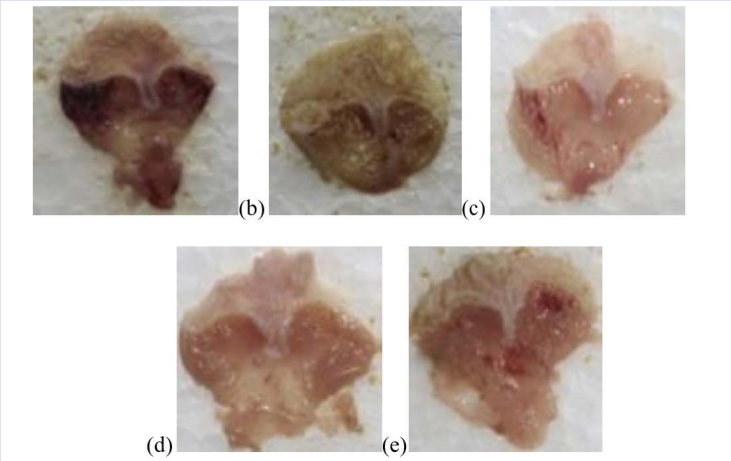


Figure 1: Macroscopic observation of mouse gastric slices; (a) negative control; (b) positive control; (c) dose 1 (0.7 g/kg BW); (d) dose 2 (1.4 g/kg BW); (e) dose 3 (2.8 g/kg BW)

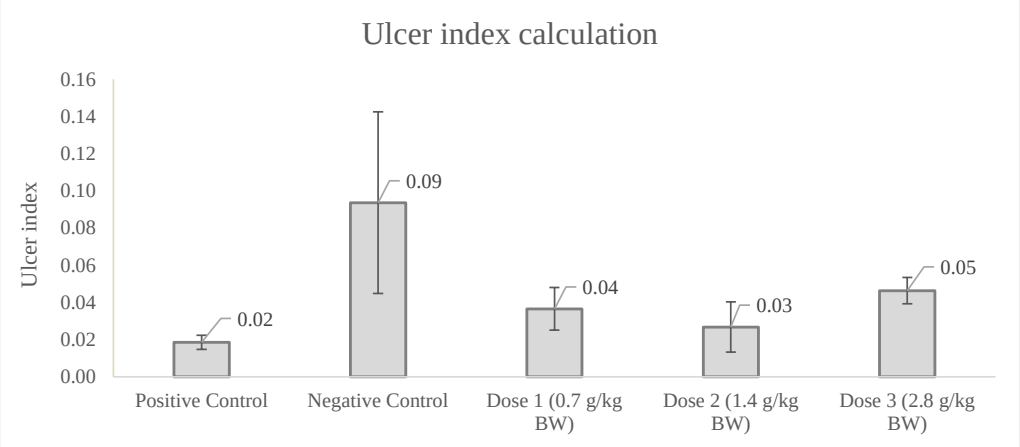


Figure 2: Ulcer index calculation

differences, Post Hoc LSD was performed (α=0.05). The results of the LSD Post Hoc Test (table 5) showed that there was a significant difference between the negative control group and all treatment groups (dose 1, dose 2, dose 3), indicated by a significance value ($p < 0.05$). It can be interpreted that the combination of clove and lime leaf extracts can significantly reduce the ulcer index in absolute alcohol induced-mice. The negative control and positive control groups also showed a significant difference in the significance value ($p < 0.05$).

In a microscopic histopathological observation of the gastric mice (figure 3), non-parametric statistical tests were performed to score mucosal edema, bleeding, and loss of epithelial cells. From histological observations, scoring was carried out to assess changes in the mucosa, edema, bleeding, and loss of epithelial cells. Obtained data as shown in table 6.

Further non-parametric Kruskal-Wallis statistical test was performed (α = 0.05) to evaluate the effect of clove and lime leaf extract combination as an anti-gastritis compared to the negative control. Based on the results of the non-parametric statistical analysis of Kruskal-Wallis (Table 6) there was a significant difference ($p<0.05$) in

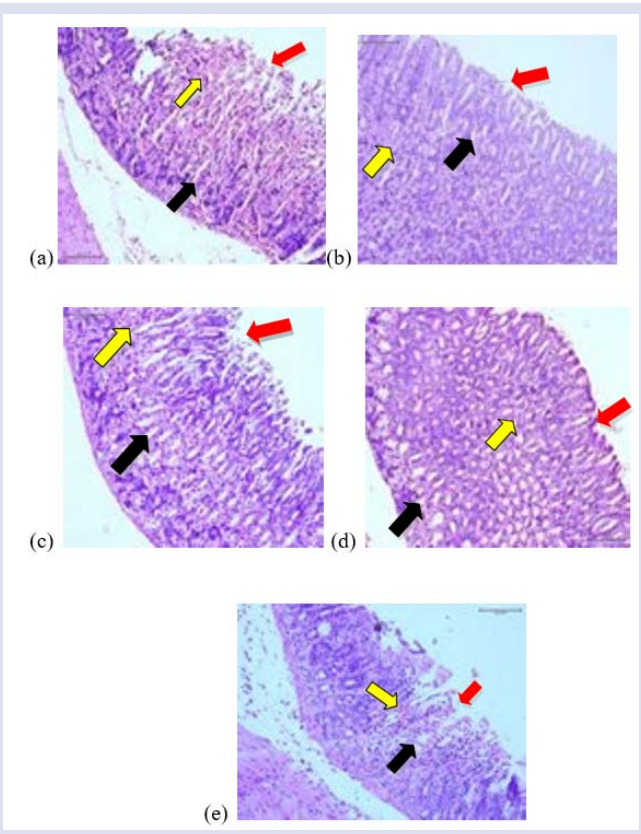


Figure 3: Gastric histopathology; (a) negative control; (b) positive control; (c) dose 1 (0.7 g/kg BW); (d) dose 2 (1.4 g/kg BW); (e) dose 3 (2.8 g/kg BW) (description: black arrow: edema; yellow: bleeding; red: loss of epithelial cells, magnification 100x)

Table 5: Significance value (p-value) on the post hoc LSD test.

Group	Negative control	Positive control	Dose 1	Dose 2	Dose 3
Negative control		0,000*	0,000*	0,000*	0,002*
Positive control	0,000*		0,196	0,551	0,051
Dose 1	0,000*	0,196		0,475	0,479
Dose 2	0,000*	0,551	0,475		0,161
Dose 3	0,002*	0,051	0,479	0,161	

* significance value (p-value) <0.05

Table 6: Scoring results of histopathological changes in mice gastric preparations.

Group	Sample number	Gastric mucosal edema	Gastric bleeding	Epithelial cell loss
Negative control	1	4	3	3
	2	4	3	3
	3	3	3	2
	4	4	4	3
	5	4	2	2
	6	4	4	3
Positive control	1	3	0	1
	2	2	2	1
	3	3	0	1
	4	3	0	1
	5	0	2	1
	6	1	2	1
Dose 1	1	4	3	2
	2	2	2	1
	3	2	2	2
	4	1	2	2
	5	2	2	2
	6	1	2	1
Dose 2	1	3	2	1
	2	4	2	2
	3	3	0	1
	4	2	2	1
	5	3	3	2
	6	3	2	1
Dose 3	1	2	2	1
	2	3	3	1
	3	2	3	2
	4	1	2	1
	5	1	2	1
	6	1	2	1

indicators of mucosal edema and epithelial cells loss. Further analysis using the Mann-Whitney Post Hoc Test also confirmed that there was a significant difference ($p < 0.05$) between the negative control group and each treatment group (dose 1, dose 2, dose 3). These results statistically indicate that combining cloves leaf and lime leaf extracts can reduce gastritis indicators, in term of mucosal edema and loss of epithelial cells. Meanwhile, the Kruskal-Walls non-parametric statistical analysis on the indicator of gastric bleeding showed a significant value ($p < 0.05$), which meant that there were at least a pair of test groups that had significant differences. However, the results of further analysis using the Mann-Whitney Post Hoc Test showed that the significant difference ($p < 0.05$) is only valid between the negative control group and the treatment groups at doses 1 and 2, whereas for dose 3 there was no significant difference ($p>0.05$). The results conformed with the macroscopic observations, which showed that the group in dose 3 has higher ulcer index value compare to other treatment groups.

In previous studies, it was known that the highest content in the ethanol extract of clove leaf was eugenol and in the ethanol extract of lime leaf was limonene.[8,12] Both of these compounds have a gastroprotective effect. The mechanism of action is to increase mucus secretion, which will protect the gastric lesions caused by alcohol or NSAID drugs to prevent gastritis.[9] A previous study by Isaac *et al.* reported that pretreatment with standardized polyphenol-rich clove extracts (Clovinol) containing 38.6% w/w polyphenols and 5.8% volatile oil significantly ($p<0.01$) in Wistar rat inhibited the formation of ethanol-induced gastric ulcers in a dose-dependent manner. The highest

antiulcerogenic activity was shown at a dose of 100 mg/kg BW which showed a reduction of ulcer index (1.82 ± 0.40) and the average ulcer inhibition of 78.9% compared to ranitidine (50 mg/kg BW) as a standard drug which showed ulcer index of 3.60 ± 0.80 ($p < 0.01$) and 54.31% ulcer inhibition. The antiulcerogenic effect of clove extract is related to the ability of the phenolic compounds in clove to act as antioxidants through upregulation of superoxide dismutase (SOD), glutathione (GSH), and catalase (CAT) enzymes activity.[14] The antioxidant activity of clove and eugenol extracts has also been investigated using the ABTS *(2,2′-Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid)* and DPPH *(1,1-diphenyl-2-picrylhydrazyl)* methods. Both showed similar activities, with IC_{50} against DPPH and ABTS, respectively, IC_{50} = 0.33 and 0.16 mg/mL for the clove and IC_{50} = 0.19 and 0.15 mg/mL for eugenol. Eugenol (150 mg/kg) was able to inhibit the release of inflammatory cytokines (TNF-α, IL-1β and IL-6) in lipopolysaccharide-induced mice.[18] Studies have shown that inflammation and oxidative stress are interrelated phenomena. Compounds that can modulate oxidative stress can reduce essential mediators in inflammatory events; thus, they act as anti-inflammatory agents, even indirectly. In cases of gastritis, reducing inflammation can improve the gastric mucosa from ulcer formation.[19] The effect of eugenol on gastric mucosal production was assessed by nitric oxide (NO) formation, PGE2 synthesis, and measurement of lipid peroxidation. Pre-treatment with eugenol on ethanol-induced ulcers reduced plasma NO levels and increased PGE2 concomitantly with decreased TNF-α and IL-6 concentrations. In addition, significant transcriptional and translational upregulation of HSP70 and downregulation of iNOS were detected in the gastric tissues of eugenol-treated mice.[20]

Essential oil (EO) of lime and limonene compound showed effective gastroprotection against absolute ethanol and NSAID-induced lesions in rats. OEC and LIM did not interfere with gastric H^+ secretion, gastrin serum, or glutathione levels (GSH) in the gastric mucosa. The gastroprotective effects of lime EO and limonene are supposedly originated from stimulating gastric mucus production by maintaining basal PGE_2 levels.[21] Further study reported that limonene 50 mg/kg reduced up to 93% of the formation of gastric ulcers and showed higher mucus production to maintain gastric mucosal integrity. Mucus plays an essential role in gastroprotection, maintaining the integrity of the mucous membranes and neutralizing acids.[22] Lime and limonene essential oils were tested in an ethanol-induced gastric injury model. The results demonstrated an increase in mucus production as their gastroprotective action. Oxidative stress induces gastric mucosal ischemia, which causes increased ROS production and lipid peroxidation. In this context, increased SOD levels play an essential role in protection from oxidative stress.[23] In addition, neither Lime nor limonene oil changed GSH or SOD levels in the ethanol injury model, indicating that the protective effect exhibited by these products was not related to antioxidant activity but to increased mucus production. However, different study results were revealed by Souza (2019) and Perico (2020). They mentioned that limonene reduced MPO activity, a biomarker of neutrophil infiltration, and increased GPx activity (as confirmation of its antioxidant effect). Aside from being an antioxidant, limonene also exhibited anti-inflammatory activity by reducing TNF-a, IL-6, and IL-1β levels and increasing levels of IL-10, downregulate the expression of Nf-κb, Il-1β, and Mpo and increase the expression of GPx which is needed to trigger inflammation.[24,25]

This study discovered an anomaly in the effect of dose 3. The administration of a combination of cloves leaf extract and lime leaf extract with the highest combined dose of 2.8 g/kg BW (0.7 g/kg BW cloves leaf extract and 2.1 g/kg BW lime leaf extract) instead showed the highest ulcer index compared to the other two lower doses. This non-linear result is probably caused by the eugenol content, which is too high in the combined dose, causing an acute toxic effect. Signs of acute toxicity induced by high doses of eugenol include sloughing of the gastric mucosa, capillary bleeding, and gastritis. The LD_{50} value of eugenol and relative toxicity for mice was 3 g/kg BW; thus, the LD_{50} value of eugenol is relatively high. Although eugenol is safe for human use, various studies have indicated that it has cytotoxic, genotoxic and immunotoxic capacities.[26] A recent study by Longo *et al*. (2021) showed that eugenol plays a dual role in healing ulcers efficiently at lower doses and worsening gastric lesions at high doses. Eugenol at a dose of 100 mg/kg given for 7 days increased the area of ulceration by 49%.[27] The histopathological findings of the gastric specimen at dose 3 agreed with the previously observed macroscopic findings. Therefore, dose regulation is necessary to regulate the level of eugenol in clove extract, avoiding gastric ulcer worsening. Hobani uses eugenol at a dose of 5-10 mg/kg BW. Both doses (5 and 10 mg/kg) showed mild or moderate gastric mucosal irritation, and the effect was dose-dependent. Pre-treatment up to a dose of 10 mg/kg BW still showed a gastroprotective effect by protecting the tissue bleeding and maintaining morphological integrity by increasing the amount of total gastric mucus production.[20] The limitation of this study is that there was no standardization and determination of marker compound levels in the extract. By knowing the levels of marker compounds (eugenol in clove and limonene in lime leaf extracts), the study could explain more comprehensively how non-linear effects appear at large dose combinations.

CONCLUSION

The combination of 70% ethanol extract of clove leaf (*Eugenia caryophyllata* Thunberg) and lime leaf (*Citrus aurantifolia*) exhibited anti-gastritis activity with the most effective dose is 1.4 g/kg BW in mice which showed the lowest ulcer index and gastric mucosal edema, bleeding and epithelial cells loss reduction on histopathological observation.

ACKNOWLEDGEMENT

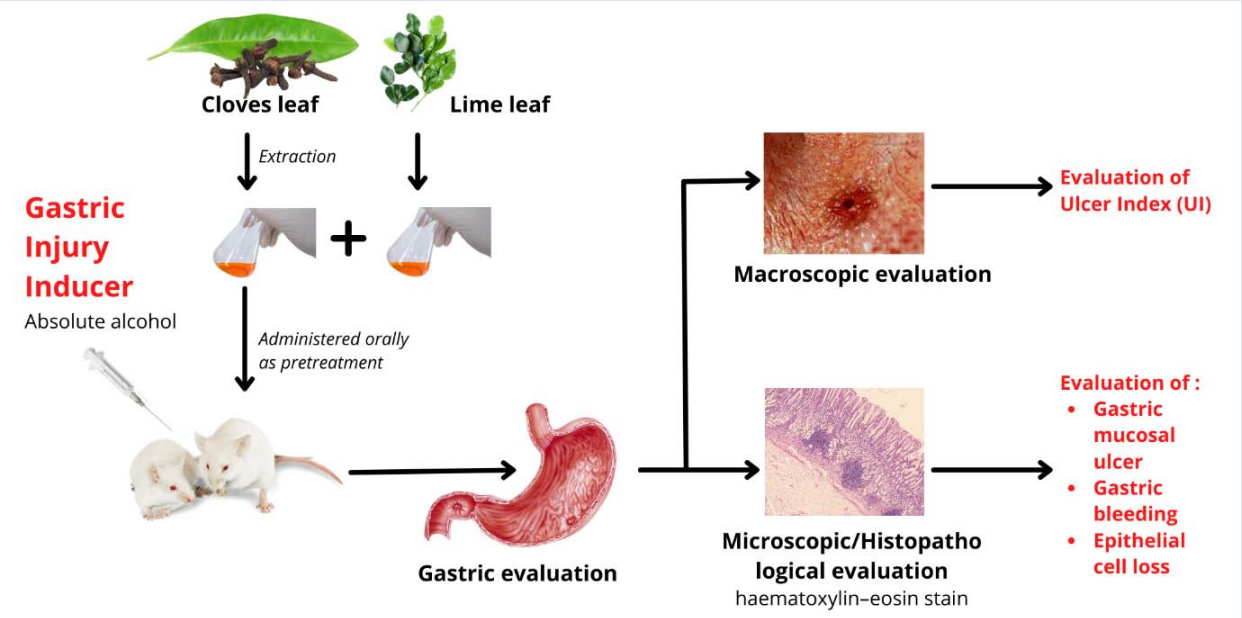
This work was supported by PDUPT Research Grant Year 2020.

REFERENCES

1. Hinkle J. Brunner & Suddarth's Textbook of Medical-Surgical Nursing. 15th edition. Wolters Kluwer Health; 2021.
2. Yuan J, Barrett KE, Barman SM, Brooks HL. Ganong's Review of Medical Physiology, Twenty sixth Edition. McGraw-Hill Education; 2019.
3. Moraes TM, Kushima H, Moleiro FC, Santos RC, Machado Rocha LR, Marques MO, *et al*. Effects of limonene and essential oil from Citrus aurantium on gastric mucosa: Role of prostaglandins and gastric mucus secretion. Chem Biol Interact. 2009;180(3):499-505.
4. Katzung BG, Trevor AJ. Basic and Clinical Pharmacology 15e. McGraw-Hill Education; 2020.
5. Wu T-H, Chen I-C, Chen L-C. Antacid effects of Chinese herbal prescriptions assessed by a modified artificial stomach model. World J Gastroenterol. 2010;16(35):4455-9.
6. Srinivas TL, Lakshmi SM, Shama SN, Reddy GK, Prasanna KR. Medicinal Plants as Anti-Ulcer Agents. J Pharmacogn Phytochem. 2013;2(4):91-7.
7. Jorjani O, Raeisi M, Hezarjaribi HZ, Soltani M, Soosaraei M. Studying the chemical composition in vitro activity of Cinnamomum zeylanicum and Eugenia caryophyllata essential oils on Leishmania major. J Pharm Sci Res. 2017;9(8):1300-4.
8. Myint S, Wan Daud WR, Mohamad AB, Kadhum AA. Gas chromatographic determination of eugenol in ethanol extract of cloves. J Chromatogr B Biomed Sci Appl. 1996;679(1):193-5.
9. Oliveira FDA, Andrade LN, De Sousa ÉBV, De Sousa DP. Anti-Ulcer Activity of Essential Oil Constituents. Molecules. 2014;19(5):5717-47.

10. Morsy MA, Fouad AA. Mechanisms of gastroprotective effect of eugenol in indomethacin-induced ulcer in rats. Phytother Res. 2008;22(10):1361-6.
11. Apraj V, Thakur N, Bhagwat AM, Mallya RR, Sawant LP, Pandita N. Pharmacognostic and Phytochemical Evaluation of Citrus aurantifolia (Christm) Swingle PEEL. Polym J. 2011;3(1):70-6.
12. Loizzo MR, Tundis R, Bonesi M, Menichini F, De Luca D, Colica C, *et al.* Evaluation of Citrus aurantifolia peel and leaves extracts for their chemical composition, antioxidant and anti-cholinesterase activities. J Sci Food Agric. 2012;92(15):2960-7.
13. Saputri FC, Munim A. Pengembangan Metode Induksi Tukak Lambung. Pharm Sci Res. 2008;5(2).
14. Issac A, Gopakumar G, Kuttan R, Maliakel B, Krishnakumar IM. Safety and anti-ulcerogenic activity of a novel polyphenol-rich extract of clove buds (Syzygium aromaticum L). Food Funct. 2015;6(3):842-52.
15. Salga MS, Ali HM, Abdulla MA, Abdelwahab SI. Gastroprotective activity and mechanism of novel dichlorido-zinc(II)-4-(2-(5-methoxybenzylideneamino)ethyl)piperazin-1-iumphenolate complex on ethanol-induced gastric ulceration. Chem Biol Interact. 2012;195(2):144-53.
16. Boutemine I-M, Amri M, Amir Z-C, Fitting C, Mecherara-Idjeri S, Layaida K, *et al.* Gastro-protective, therapeutic and anti-inflammatory activities of Pistacia lentiscus L. fatty oil against ethanol-induced gastric ulcers in rats. J Ethnopharmacol. 2018;224:273-82.
17. Ortaç D, Cemek M, Karaca T, Büyükokuroğlu ME, Özdemir ZÖ, Kocaman AT, *et al.* In vivo anti-ulcerogenic effect of okra (Abelmoschus esculentus) on ethanol-induced acute gastric mucosal lesions. Pharm Biol. 2018;56(1):165-75.
18. Magalhães CB, Casquilho NV, Machado MN, Riva DR, Travassos LH, Leal-Cardoso JH, *et al.* The anti-inflammatory and anti-oxidative actions of eugenol improve lipopolysaccharide-induced lung injury. Respir Physiol Neurobiol. 2019;259:30-6.
19. Barboza JN, da Silva Maia Bezerra Filho C, Silva RO, Medeiros JVR, de Sousa DP. An Overview on the Anti-inflammatory Potential and Antioxidant Profile of Eugenol. Oxid Med Cell Longev. 2018;2018:3957262.
20. Hobani YH, Mohan S, Shaheen E, Abdelhaleem A, Faruque Ahmad M, Bhatia S, *et al.* Gastroprotective effect of low dose Eugenol in experimental rats against ethanol induced toxicity: Involvement of antiinflammatory and antioxidant mechanism. J Ethnopharmacol. 2022;289:115055.
21. Moraes TM, Kushima H, Moleiro FC, Santos RC, Machado Rocha LR, Marques MO, *et al.* Effects of limonene and essential oil from Citrus aurantium on gastric mucosa: Role of prostaglandins and gastric mucus secretion. Chem Biol Interact. 2009;180(3):499-505.
22. Flemström G, Isenberg JI. Gastroduodenal mucosal alkaline secretion and mucosal protection. News Physiol Sci. 2001;16:23-8.
23. Tariq M, Khan HA, Elfaki I, Arshaduddin M, Al Moutaery M, Al Rayes H, *et al.* Gastric antisecretory and antiulcer effects of simvastatin in rats. J Gastroenterol Hepatol. 2007;22(12):2316-23.
24. de Souza MC, Vieira AJ, Beserra FP, Pellizzon CH, Nóbrega RH, Rozza AL. Gastroprotective effect of limonene in rats: Influence on oxidative stress, inflammation and gene expression. Phytomedicine. 2019;53:37-42.
25. Périco LL, Emílio-Silva MT, Ohara R, Rodrigues VP, Bueno G, Barbosa-Filho JM, *et al.* Systematic analysis of monoterpenes: Advances and challenges in the treatment of peptic ulcer diseases. Biomolecules. 2020;10(2):265.
26. Nejad SM, Özgüneş H, Başaran N. Pharmacological and Toxicological Properties of Eugenol. Turkish J Pharm Sci. 2017;14(2):201-6.
27. Longo B, Sommerfeld EP, da Silva R de CMV de AF, Somensi LB, Mariano LNB, Boeing T, *et al.* Dual role of eugenol on chronic gastric ulcer in rats: Low-dose healing efficacy and the worsening gastric lesion in high doses. Chem Biol Interact. 2021;333:109335.

GRAPHICAL ABSTRACT



ABOUT AUTHORS

Herra Studiawan is a senior lecturer from the Faculty of Pharmacy, Airlangga University. His research interest is about investigation of chemical compounds in several Indonesia Medicinal plants and the exploration of antidiabetic activity from natural products.

Sukardiman is a professor from the Faculty of Pharmacy, Airlangga University. He has a lot of research experiences in the field of herbal plant activities as anti-cancer and anti-diabetic. He also conducted some biochemical, toxicity, and herbal standardization studies. He is a member of the national commission for herbal medicine in Indonesia and has published many scientific articles in reputable journals.

Indana Lazulfa is a fresh graduate and novice researcher from the Faculty of Pharmacy, Airlangga University. Currently she works as a pharmacy practitioner. Indana is a member of the Indonesian Pharmacist Association.

Rosita Handayani is a young lecturer and researcher from the Faculty of Pharmacy, Airlangga University. Her research interest is natural product discovery and development in cosmetics, anti-inflammatory, and anti-cancer agents. Her research includes *in vitro* testing using enzymatic assays and cell culture models, as well as standardization of the quality of raw materials from plants.

Cite this article: Studiawan H, Sukardiman, Lazulfa I, Handayani R. Anti-Gastritis Activity of Cloves (*Eugenia caryophyllata* Thunberg) and Lime (*Citrus aurantifolia*) Leaf Extracts Combination in Absolute Alcohol Induced-Gastric Injury Mice. Pharmacogn J. 2023;15(3): 378-384.