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Gastric Histopathological Image of Winstar Rats Induced by Ethanol After Red Ginger Extract Administration

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Abstract

Background: Alcohol can cause damage to the gastric mucosal barrier and reverse diffusion of HCL, which results in damage to gastric tissue. The flavonoids in red ginger act as exogenous antioxidants, which can ward off free radicals and reduce damage to the gastric mucosa caused by alcohol

Objective: To determine the differences in the histopathology of the rats' gastric induced by 40% ethanol after administration of red ginger (Zingiber officinale var. rubrum) extract at graded doses.

Methods: This research is a post-test-only group design method. Twenty-five rats samples were divided into five groups: K normal control; K(-) negative control treated with ethanol 40% 1.8ml/200g/day; T1 was given red ginger extract 250 mg/KgBW; T2 was given red ginger extract 500 mg/KgBW; and T3 was given red ginger extract 750 mg/KgBW. After 60 minutes, 40% ethanol was given at a 1.8ml/200g/day dose. Treatment was carried out for 30 days, then on day 31, termination was carried out, and gastric histopathology preparations were made with hematoxylin-eosin staining. Calculation of hull damage scores using the Barthel-Manja criteria. Analysis of differences in gastric mucosal damage between groups using the Kruskal Wallis and Mann Whitney Test.

Results: The difference between groups obtained significant results (p=0.003). There were significant differences between the K and K (-) groups (p=0.016), the K and T1 groups (p=0.032), the K and T2 groups (p=0.032), and the K (-) and T3 groups (p =0.032).

Conclusion: Administration of red ginger extract at a dose of 750 mg/KgBW was proven effective in preventing damage to the gastric mucosa induced by 40% ethanol.

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INTRODUCTION

Mucosal damage is the disruption of continuity/integrity of the gastric mucosa under the epithelium or damage to the gastric mucosa, gastric submucosa, and the muscular layer.[1] The most common causes of mucosal damage are Helicobacter pylori infection, non-steroidal anti-inflammatory drug (NSAID) usage, alcohol consumption, and an irregular diet.[2,3]

In Indonesia, the prevalence of alcohol dependence is 1,827,700 people, or 0.7% of total cases. The prevalence of alcohol is 0.8%. According to the Global Status Report on Alcohol and Health 2018, 46% of alcoholics are teenagers.[4,5]

Alcohols are chemical compounds whose molecules are bonded to carbon tetrahedra and contain hydroxyl groups (OH-). 10% of alcohol consumed is absorbed in the gastric system, and the remainder is absorbed in the small intestine.[6] When alcohol enters the gastric, it becomes acetate.[2,7] When the Aldehyde dehydrogenase (ALDH) enzyme does not function properly, it will activate the Reactive Oxygen Species (ROS) and reduce the production of Adenosine Triphosphate (ATP). In the liver, the alcohol is converted into formaldehyde by the aldehydrogenase enzyme; Formaldehyde into gastric cells into phosmic acid by ALDH activating multidrug resistance protein 1 (Mrp1) and releasing glutathione (GSH). Reverse diffusion of hydrochloric acid (HCL) causes gastric mucosal damage because oxidative stress reduces cell membrane permeability.[7,8] The gastric mucosal damage due to alcohol can cause acute and chronic gastritis.[5] Detachment of the superficial mucosal epithelium, or erosion, can also be caused by excessive alcohol consumption. Acute gastrointestinal bleeding is mainly caused by severe mucosal erosion. [9,10]

Indonesian people have long used traditional medicine to treat various diseases, including red ginger (*Zingiber officinale var. Rubrum*).[11] Red ginger is from the *Zingiberaceae* family, which is efficacious for preventing and treating various diseases.[11,12] Red ginger contains flavonoids to ward off exposure to free radicals from alcohol due to the antioxidant content in red ginger.[12,13] A Study by Munadi[14] reported that red ginger rhizome extract contains flavonoids, tannins, saponins, alkaloids, and terpenoids.[14] A 2016 study comparing the benefits of ginger extract with H_2 receptors (ranitidine) found that ginger helped protect the gastric mucosa better than ranitidine by increasing mucus secretion and protecting the gastric mucosa.[15]

In a previous study, Pairul[16] reported that red ginger extract at a dose of 400mg/KgBW was able to reduce gastric rats' mucosal damage.[16] This study aimed to determine the effect of administering red ginger extract on the histopathological image of the Wistar rats' gaster using red ginger extract at a dose of 250mg/KgBW, 500mg/KgBW, and 750mg/KgBW with 40% ethanol induction dose of 1.8 ml/200gramBW/day. Observations were made by observing the rats' gastric mucosal damage.

METHODS

The study used a laboratory experiment and a post-test-only control group design. It was conducted at the Biology Laboratory, Faculty

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of Mathematics and Natural Sciences, Semarang National University, and the Satmoko Clinical Anatomy Pathology Laboratory, Semarang, for reading preparations. Approval for this study was granted by the Health Research Ethics Committee Faculty of Medicine, Muhammadiyah University Semarang No. 052/EC/FK/202.

The sample consisted of 25 Wistar rats divided into five groups, which were calculated using the Federer formula, which met the inclusion criteria of being male, weighing 150-200 grams, 6-8 weeks old, healthy, and having no anatomical abnormalities. The dropout criteria were Wistar rats that died during the study period. The study began with an adaptation of Wistar rats for seven days, then divided into five treatment groups consisting of group K given standard feed, group K(-) given standard feed and 40% ethanol at a dose of 1.8 ml/200 grams, group T1 given red ginger extract at a dose of 250 mg/KgBW after 60 minutes given 40% ethanol, group T2 was given red ginger extract at a dose of 500mg/KgBW after 60 minutes given 40% ethanol, group T3 was given red ginger extract at a dose of 750mg/kgBW after 60 minutes given 40% ethanol. Treatment was carried out for 30 days. On the 31st day, the rats were terminated with ketamine anesthesia, and the cervical vertebrae were dislocated. Then, surgery was performed to remove the gastric organ. Next, preparations were made with HE staining to examine the histopathological image of the gastric rat using a microscope.

Data processing using the SPSS software was carried out after obtaining primary data in mucosal damage mode from the histopa-thology of gastric tissue between groups, and the results were entered into the *Manja-Bathel* score. Then, the *Krustal-Wallis* test was carried out, and the Mann-Whitney analysis was continued.

RESULTS

A total of 25 rats were divided into five treatment groups, each consisting of 5 rats. Damage data for each group is shown in Table 1 and Figure 1. The data at Tabel 1 shows the damage to gastric tissue in each treatment group; the normal control group was the group with the lowest damage, in the negative control group, the most severe damage was dominated by epithelial erosion and ulceration of the gastric mucosa, and in the T3 group is the group with the lowest damage compared to the other groups, T1 and T2.

Table 1. The result of the damage mode for each group

Group	Score				Mode score	Description
	0	1	2	3		
K	4	1	0	0	0	No damage
K (-)	0	2	3	0	2	Epithelial erosion
T1	0	5	0	0	1	Epithelial desquamation
T2	0	5	0	0	1	Epithelial desquamation
T3	3	2	0	0	0	No damage

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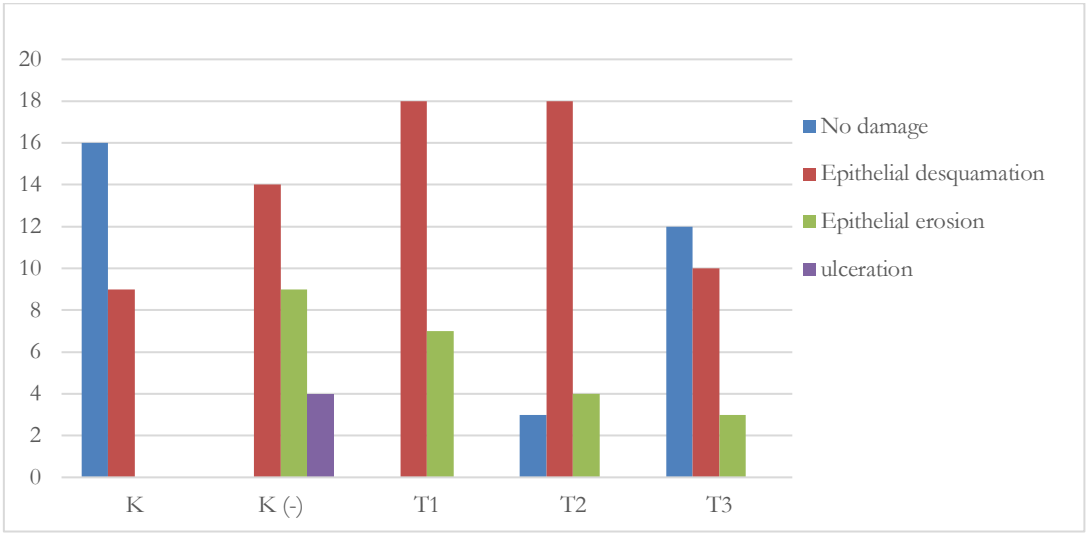

Figure 1. Histogram of Gastric Tissue Damage

Table 2. Mann-Whitney Test

Group	K (-)	T1	T2	T3	***p-value***
K	0,016*	0,032*	0,032*	0,690	0.005*
K (-)		0,151	0,151	0,032*	
T1			1,000	0,151	
T2				0,151	

Note: (*) Significant (p<0.05)

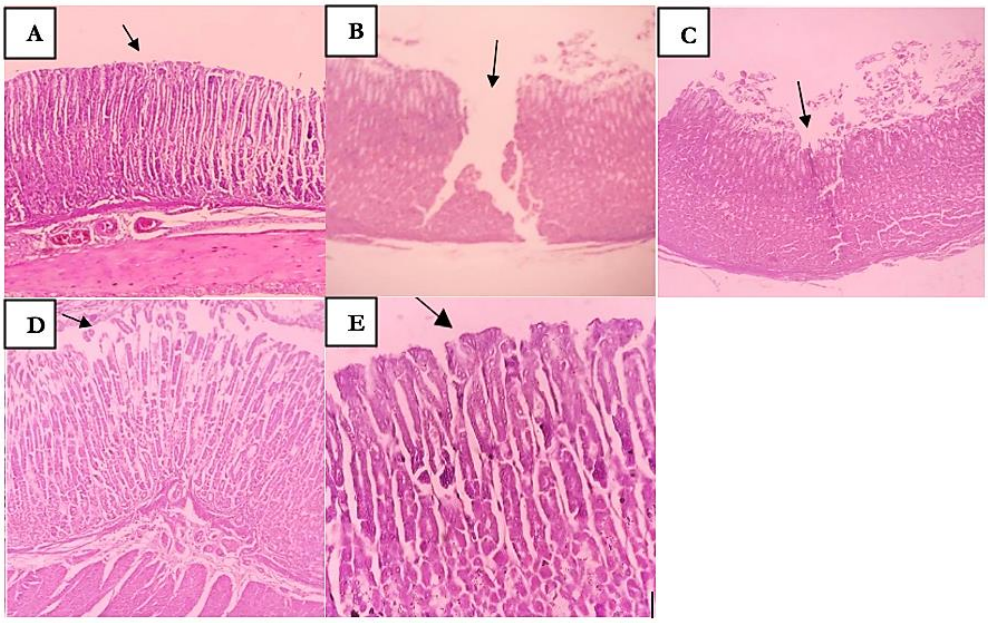

Figure 2. Histopathological features of gastric epithelium between groups (100x magnification); (a) Group K no morphological changes, (b) K (-) group K (-) ulceration, (c) Group T1 epithelial desquamation, (d) Group T2 epithelial desquamation, (e) Group T3 no damage

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The Figure 1 showed damage to the gastric tissue of each group. In the normal control group, the score was dominated by 0 (no damage or intact to the gastric mucosal tissue). The negative control group was dominated by scores 2 (epithelial erosion) and 3 (epithelial ulceration). Treatment group 1 (T1) was dominated by score 1 (epithelial desquama-tion). Treatment group 2 (T2) was dominated by a score of 1 (epithelial desquamation). Treatment group 3 (T3) was dominated by a score of 0 (no damage).

From the mode data at Table 1, a Kruskal-Wallis test was carried out to obtain a p-value of 0.003, meaning there was a significant difference in all groups because P<0.05. Then, a different test was carried out for each treatment group using the Mann-Whitney test.

In Figure 2, The gastric mucosa layer in the normal control group (image A) showed no damage, as shown in image E (group T3). The histological image of the negative control (image B) showed that the gastric mucosal lining appeared ulcerated. Image C was a histological image of the T1 group that shows the gastric mucosal layer showed epithelial desquamation as in image D. From this image, it can be concluded that the group with the worst damage (score 3) had ulceration in the K- group, and in P3 the Damage was less than in P1 and P2.

DISCUSSION

The study aimed to determine the gastroprotective effect of red ginger extract on the gastric mucosa of Wistar rats induced with 40% ethanol at a dose of 1.8 ml/200g daily. The histopathological image was a parameter in this study to identify the damage to the gastric mucosa. The results showed that the normal control group, namely the group given standard feed without red ginger extract and 40% alcohol, had slight damage to gastric mucosal cells (score 0 = no damage), but there was still minimal epithelial desquamation. Desquamation releases epithelial elements, a tissue defense against an irritant, as a normal physiology. The normal appearance of the gastric mucosa was due to no irritative substance damaging the gastric mucosa, namely 40% alcohol. This result was relevant to the study conducted by Setiawan in 2019, which found that the group without alcohol did not experience damage to the gastric epithelial cells.

Comparison between the damage severity to the gastric mucosa in the normal control and negative control groups showed a significant difference (p = 0.016). In the control normal group, there was mostly no damage (score 0), whereas in the negative control group, there was dominated by epithelial erosion (score 2) and ulceration (score 3).

Alcohol can harm stomach health because it damages the gastric mucosal barrier and allows reverse diffusion of HCL, which damages gastric tissue.[17] As a result, the inflammatory mediator histamine will increase HCL secre-tion, which causes edema, capillary damage, and bleeding in the gaster. The more alcohol consumed is related to the more gastric damage, including erosion, increased inflammatory cells, or necrosis.[8,15] Setiawan's study in 2019 stated that the administration of 40% ethanol can cause damage to the integrity of the gastric mucosa, which causes gastric ulcers.[1]

The outcome of the treatment group by administering red ginger extract at doses of

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250mg/KgBW, 500mg/KgBW, and 750mg/KgBW were comparable to the effect of preventing damage to the gastric mucosa. In the group treated with red ginger extract at a dose of 750mg/KgBW (group T3), there was a significant difference in gastric mucosal damage compared to the negative control group (K-) (p=0.032). This group had predominantly no damage due to intact and clean mucosal areas. The results were insignificant in the group of 250mg/KgBW red ginger extract (group T1) (p=0.151). In this group, epithelial desquamation and epithelial erosion were seen. The results were insignificant in the group of 500mg/KgBW red ginger extract (group T2) (p= 0.151). Epithelial desquamation and epithelial erosion were seen. A study from Pairul[16] on mice given red ginger extract at a dose of 400mg/KgBW and induced by piroxicam at a dose of 40mg/200gBW, it showed an effect of preventing damage to the gastric mucosa as indicated by the presence of mild gastric damage to the mucosal lining.[16] This study showed an effect of preventing stomach damage at a dose of 750mg/KgBW with 40% ethanol induction at a dose of 1.8ml/200 gBW. There was a difference in the effectiveness of each study due to different exposures.

The effects and side effects of increasing the dose of red ginger extract are not always positive.[18] Figure 2 shows that epithelial desquamation was highest in groups T1 and T2. Meanwhile, P3 was lower. In Figure 4.1, the T3 group also had normal cells, which increased compared to the P2 and P1 treatment groups. It can be concluded that a dose of 750mg/KgBW of red ginger extract had a more optimal effect than a dose of 250mg/KgBW and a dose of 500mg/KgBW. This study was relevant to Pairul[16] regarding the administration of increasing red ginger extract doses at 400mg/KgBW and 600mg/KgBW to a rat induced with piroxicam 40mg/200 gBW.[16] This study reported that a dose of 750mg/KgBW showed an increased effect in preventing damage to the gastric mucosa, which was indicated by mild gastric damage to the submucosa layer. Red ginger has biological activity that can be used as an antiulcer, antioxidant, and anti-inflammatory.[12,17] The pharmacological effects of red ginger are caused by its constituents, namely zingerone, zingiberene, gingerols, and shogaol, which are phenolic bioactive components that can inhibit inflammation.[16] Gingerol has anti-inflammatory, antipyretic, gastroprotective, cardiotonic, hepatotoxic, antioxidant, anti-cancer, anti-angiogenesis, and anti-atherosclerotic properties.[19] This can reduce damage to the gastric mucosa caused by exposure to free radicals, as shown in this study. Apart from that, the flavonoid content in red ginger also has antiulcerogenic activity, which means it can prevent mucosal damage as the primary defense against endogenous and exogenous ulcerogenic agents.[20]

There were several limitations of this study. We did not observe the stomach macroscopically; it was only observed microscopically, so we could not see the damage to the stomach as a whole. This study was only a post-test control group design study because there was no pre-test control group as an outcome of the treatment and comparison. In addition, this study used tissue histopathological parameters, which were 2-dimensional microscopic images of an organ, so it could not explain the 3-dimensional structure as an actual image of the organ. Future studies are needed to focus on the pure active substances, standardize the red

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ginger extract dosage, and carry out the acute and chronic toxicity tests on red ginger extract.

CONCLUSION

Administration of red ginger extract at a dose of 750mg/KgBW prevented damage to the gastric mucosa induced by 40% ethanol. Red ginger extract has the potential to be an antioxidant; therefore, further research on a higher dose variation is needed.

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