JMU
Jurnal Medika Udayana

ISSN: 2597-8012 JURNAL MEDIKA UDAYANA, VOL. 15 NO.7, JULI, 2026

Received: 2026-03-13 Revision: 2026-04-13 Accepted: 30-06-2026

GASTROPROTECTIVE ACTIVITY OF CLOVE (*Syzygium Aromaticum*) IN ANTI-TUBERCULOSIS DRUG-INDUCED RATS BY ENHANCING GASTRIC FOVEOLAR CELL GRANULES

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ABSTRACT

Background: The standard regiment of anti-tuberculosis drug (ATD) therapy includes a combination of four drugs, namely isoniazid, rifampicin, ethambutol, and pyrazinamide. However, these combination drug therapies often cause gastrointestinal adverse effects. Clove (*Syzygium aromaticum*) contains eugenol as its main component, which has gastroprotective activity to prevent those adverse effects. The purpose of this study is to prove gastroprotective activity of clove by increasing gastric foveolar granules.
Methods: Experimental rats were treated for 21 days. Treatment groups were treated with ethanolic extract of clove 200, 400, and 800 mg/kg in ATD-induced rats. The positive control group only received ATD 350 mg/kg, and the normal control group received no treatment. After 21 days, the rats were sacrificed and the pylorus section of the stomach stained with hematoxylin-eosin (HE) and were examined under light microscope.
Results: The administration of ATD in positive control group reduced the amount of gastric foveolar granule compared to the normal control group which received no treatment (P<0,05) and clove ethanolic extract in any doses significantly increase the amount of gastric foveolar granules compared to the positive control group (P<0,05).
Conclusion: The standard regiment of ATD decreased granules of foveolar cells in gastric mucosa of rats. The administration of clove ethanolic extract stimulated secretory granules of gastric foveolar cells, which contain mucus as an important gastric protective factor in ATD induced rats.

Keywords : Clove (*Syzygium aromaticum*), Anti-tuberculosis drug, , Gastroprotective.

INTRODUCTION

Tuberculosis (Tb) is an infectious disease caused by *Mycobacterium tuberculosis*. The bacteria can be transmitted through the air and cause primary infection in the lungs, although it can cause extrapulmonary infection.[1–3] Tuberculosis remains a major global public health challenge, requiring prolonged and rigorous multi-drug therapy to ensure eradication and prevent resistance. According to the WHO, estimated 10.7 million people got infected with Tb and 1.23 million died because of the infection in 2024.[4]

The treatment options for drug-susceptible Tb according to the WHO guideline include a 6-month regimen consisting of isoniazid, rifampicin, ethambutol, and pyrazinamide.[4] However, these drugs usage may cause adverse effects in the gastrointestinal system such as stomach ache, nausea, vomiting, and loss of appetite.[5,6] These side effects may impact on compliance with the medication, resulting in discontinuation of therapy.[5]

The pathogenesis of ATD-induced gastric injury is multifactorial, often involving increased oxidative stress, the depletion of protective endogenous antioxidants, and an imbalance between aggressive and defensive factors.[7,8] One critical component of gastric mucosal defence is the layer of mucus and bicarbonate secreted by the foveolar cells.[8,9] These superficial epithelial cells contain specialized granules that release mucins, forming a physical and chemical barrier against luminal aggressors.[9–11]

There is a growing interest in exploring natural product as alternative or adjunct therapy.[12] *Syzygium aromaticum*, also known as clove, is a native Indonesian herb that has long been used as ingredient for culinary spice and traditional medicine.[13,14] The main contents of clove are eugenol, eugenol acetate, and β-caryophyllene. clove has been known to possess antibacterial, antimicrobial, anti-inflammatory, antioxidant, cure respiratory and gastrointestinal disease, and other health benefits.[13–17] Evidence suggests that the gastroprotective mechanism of clove is by

promoting mucus synthesis, which is one of the protective factors in the gastrointestinal system.[15]

Despite the known therapeutic properties of clove, its specific potential to counteract ATD-induced gastric toxicity remains insufficiently explored. Furthermore, the underlying cytoprotective mechanisms, particularly its influence on gastric mucin production and foveolar cell integrity, require detailed investigation. Therefore, the study aims to evaluate the gastroprotective activity of clove extract in ATD-induced rats, which were evaluated by the amounts of secretory granules of gastric foveolar cells.

MATERIALS AND METHODS

Animal

This study involved 30 male wistar rats weighing between 150-200g and 90-120 days old were obtained from the Histology Department Unit of Udayana University. Animals used in this research were housed and handled according to the animal use of the institution's guideline following ethical clearance approval. Obtained animals were supplemented with food and water ad libitum with proper cage and environment. This study experimental protocol was approved by Udayana University Ethical Committee, Bali, Indonesia (Ethical clearance number: 1750/UN14.2.2.VII.14/LT/2023), in accordance with International Conference on Harmonisation - Good Clinical Practice (ICH-GCP) guidelines.

Extract Preparation

Dried clove flower bud was pulverised into powder using a mechanical grinder. Next, the clove powder was extracted using a soxhlet apparatus set at 60-70℃ for 6 hours in ethanol 50%. The solution was then suspended using distilled water and 1% gum acacia as a suspending agent.

Anti-tuberculosis Drug

This study used a 900 mg fixed dose combination (FDC) anti-tuberculosis drug (ATD) produced by PT Phapros Tbk, Indonesia. The drug consists of 150 mg rifampicin, 75 mg isoniazid, 400 mg pyrazinamide, and 275 mg ethambutol. The drug dose was converted to 350 mg/kg for the experimental rat. The tablets were pulverised into a fine powder using a mechanical grinder. The powder then dissolved in 1 ml of distilled water for every 350 mg of the drug.

Grouping and Dosing

Thirty (30) male wistar rats were randomly grouped into 5 groups. Each group consisted of 6 rats. Group A (normal control) received no treatment. Group B (positive control) was given ATD only. Group C-E (treatment groups) were given ATD each and treated with 200 mg/kg, 400 mg/kg, 800 mg/kg doses of clove ethanol extract. ATD and clove extract were given once a day orally for 21 days.

Histopathological Analysis

After 21 days, the animals were sacrificed by cervical dislocation under anaesthesia. The stomach was excised and placed in a container with 10% phosphate buffer formalin. The stomach then was sliced and stained with hematoxylin-eosin (HE). Pylorus sections of the stomach were examined under light microscope with 400x magnification and then granules of foveolar cells were counted manually using ClickMaster2000.

Statistical Analysis

The data were analysed using IBM-SPSS version 20 and presented as mean ± standard deviation. The data were also compared using one-way analysis of variance (ANOVA) and followed by Fisher's least significant difference (LSD) to determine which groups were different compared to the control group.

RESULT

The HE-stained tissue section showed the granules of gastric foveolar cells in the gastric mucosa can be seen in figure 1. Foveolar cells referred to mucus-producing cells found on the mucosal surface and gastric pits. Under light microscope, the mucin granule of the foveolar cell is identified as a circle-to-oval droplet. The droplet located in the apical cytoplasm and appeared "empty" because stained poorly with HE. This was because mucus was prone to washed out during the processing.

The amounts of granules of gastric foveolar cells in the positive control group B (121.8 ± 11.4) was lower and statistically significant difference ($P<0.05$) compared to the normal control group A (141.2 ± 7.0) granules. In the treatment groups, the values of granules of gastric foveolar cells for group C (150.6 ± 14.6), D (163.7 ± 22.3), and E (196.4 ± 9.8) were significantly ($P<0.05$) higher compared to the normal control group A granules and positive control group B granules. This result also showed that administration of clove increased the amount of the secretory granule in dose-dependent manner. The mean and analysis of granules of gastric foveolar cells in experimental animals is summarized in table 1 and figure 2.

Tabel 1. Effect of *Syzygium aromaticum* and anti-tuberculosis drug on the number of granules of foveolar cells in gastric mucosa of rats. Statistical comparison was performed using ANOVA then followed by Fisher's least significant difference. $^{+}$: P<0.05 compared with the normal control group (A), *: P<0,05 compared with positive control group (B).

Group	Dose (mg/kg)		Number of granules (Mean ± SD)
	Syzygium aromaticum	ATD	
A	-	-	141.2 ± 7.0*
B	-	350	121.8 ± 11.4^{+}
C	200	350	150.6 ± 14.6*
D	400	350	163.7 ± 22.3*$^{+}$
E	800	350	196.4 ± 9.8*$^{+}$

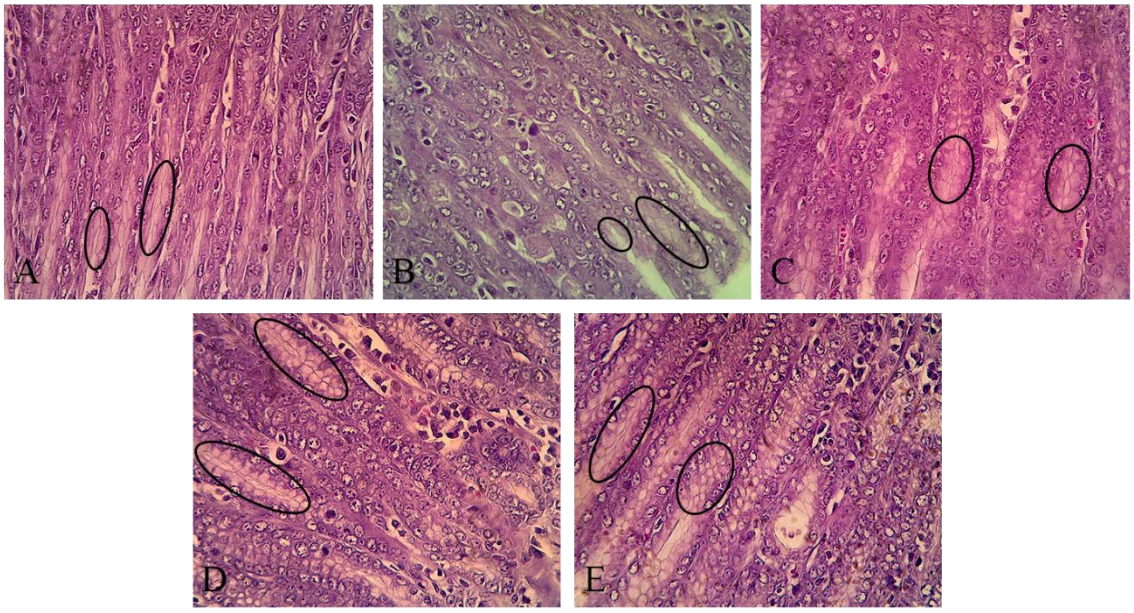
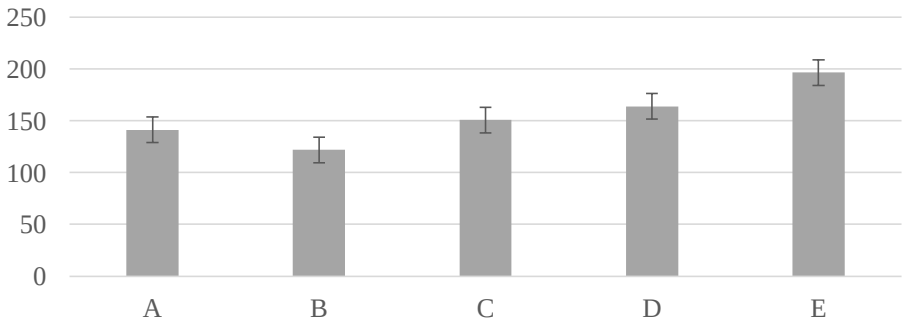


Figure 1. Histology slide of the pylorus section of the stomach with hematoxylin-eosin staining at 400x magnification by light microscope. Secretory granule of gastric foveolar cell is indicated by circle. A: Normal control group, B: positive control group, and C-E: treatment groups received 200, 400, 800 mg/kg of *Syzygium aromaticum* respectively.

Tabel 1. Chart showing the mean and standard deviation of the number of granules of foveolar cells in gastric mucosa of experimental animals. A: normal control group; B: negative control group; C-E: treatment group.



DISCUSSION

Administration of first line ATD therapy (isoniazid, rifampicin, pyrazinamide, ethambutol) decreased the amounts of mucous granules of gastric foveolar cells. The most responsible drug of these four combinations therapy is believed to be the rifampicin. A case reported by Zargar *et al.* (1990)[18], administration of rifampicin induced upper gastrointestinal bleeding in patient. The author found that there was a correlation between rifampicin and erosive gastritis as a source of the upper gastrointestinal bleeding by endoscopic finding. This evidence was supported by a research conducted by Paramita *et al.* (2019)[19] showed that administration of rifampicin caused erosion to the surface epithelial cells of gastric mucosa tissue. The acidic characteristic of rifampicin is believed to be the cause of the local erosion to the surface epithelial cells.[20,21]

Physiologically, gastric mucosa integrity is maintained by equilibrium between the aggressive and the protective factor of the gastrointestinal system.[8] A pre-epithelial component known as the mucus-bicarbonate barrier makes up the first line of gastric mucosa defense. The second line of defense is represented by the epithelial component cell along with its continuous regeneration by the stem cells. At last, the third line of defense is recognized by gastric blood flow, mucosa neurovascularization, and neurohormonal regulation.[8,10] The imbalance between aggressive and protective factor toward aggressive factor may lead to mucosal epithelial damage, edema, inflammation, even gastric ulcer.[10]

Mucus is made of 95% of water and 5% of polymeric glycosylated mucin. Mucin is responsible mainly for the viscous and stable framework of mucus.[8] Mucin is secreted by foveolar cells and stored inside the intracellular secretory granules.[8,22] Under physiological condition, granules of foveolar cells will undergo exocytosis when they reach the surface of the lumen and release the mucin within the granules into the lumen, setting up the mucus barrier.[8] The stomach mucus layer is composed of two layers, the inner layer has dense mucus, firmly adhered to the epithelial cells and impermeable to bacteria, while the outer layer has loose mucus layer and can be easily detached from the inner layer. The mucus in the both layers are manufactured by the basic mucin gen such as MUC2, MUC5AC, MUC5B, and MUC6.[9,10]

The efficiency of gastric mucus is based on the thickness and structure of the gel that forms the mucus layer, the thicker the mucus layer, the better the gastroprotection.[8] This mucus layer protects the gastric mucosa physically against both endogenous and exogenous aggressive factors, such as stomach acid, drugs, alcohol, etc.[8,9] Mucus also act as a filter to harmful molecules and pathogens.[10] In addition, mucus also has antioxidant properties which protect the mucosa from oxidative stress which may be caused by the usage of ATD. The intracellular mucus is able to neutralize free radicals, thereby preventing oxidative stress.[15,23]

Several studies earlier had reported the use of clove as a treatment for gastrointestinal disorder.[15,24,25] Mucus secretion can be increased by stimulation of prostaglandin E_2 (PGE_2) and NO.[24] Eugenol, which is the primary component of clove, contributes to the upregulation of PGE_2 synthesis. In the stomach, PGE_2 induces mucus and bicarbonate production via EP1/EP4 receptors.[23] Increase production of mucus and bicarbonate indicating an increased gastric protective factor.[15,23] A study by Santin *et al.*[15] administration of clove oil and eugenol protect gastric mucosa from ethanol-induced ulcer by stimulation of mucus production. Another study by Issac *et al.*[24] reported that clove attenuated ethanol-induced gastric lesion by improving mucous antioxidant activity. From the histological analysis in this study, the secretory granules of gastric foveolar cells increased in amount suggesting better gastroprotection by administration of clove extract. These mucin-contain secretory granules constitute a major part of gastric defensive factor.

CONCLUSION AND RECOMENDATION

In this study, clove (*Syzygium aromaticum*) ethanolic extract exhibited gastroprotective activity based on its ability to stimulate secretory granules of gastric foveolar cells which contain mucus as an important gastric protective factor in ATD induced rats. Exposure of the first line ATD (isoniazid, rifampicin, pyrazinamide, ethambutol) has shown to decrease secretory granules of gastric foveolar cells.

This research was conducted in a short time compared to the time period of ATD therapy in humans. Further research needs to be done regarding the side effects of clove flower ethanol extract and long-term dosage before it can be applied to humans.

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