The Effect Of Giving Cloves Extract (*Syzygium Aromaticum*) On Kidney Function And Kidney Histopathology Of Male Wistar White Rats Infected With Staphylococcus Aureus Bacteria

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ABSTRACT

This research aims to test and analyze the effectiveness of administering clove extract (*Syzygium aromaticum*) on the kidney function of male white rats (Rattus norvegicus) of the Wistar strain infected with Staphylococcus aureus and how the histopathological picture is. The research sample, namely 24 male rats (Rattus norvegicus) of the Wistar strain were divided into 4 groups and received different treatments. The treatment group was given an infection with a suspension of Staphylococcus aureus bacteria and clove extract (*Syzygium aromaticum*) with different doses of 150 mg/KgBW, 350 mg/KgBW, and 450 mg/KgBW. The results of the observation showed that clove extract contains secondary metabolites in the form of saponins, tannins, flavonoids, and steroids that help repair damaged kidney cells and decrease kidney function due to Staphylococcus aureus bacterial infection. The active compound that plays the most role in improving kidney function is flavonoids. Administration of clove extract at a dose of 450 mg/KgBW is effective in improving kidney function in white rats (*Rattus norvegicus*) of the Wistar strain infected with *Staphylococcus aureus*. This improvement can be seen through the levels of urea, creatinine, and the histological structure of the kidneys that have improved. The results of histopathological observations of kidney tissue in treatment group 3, namely clove extract with a dose of 450 mg/KgBW, experienced the most significant improvement and approached the control group compared to the other groups.

Keywords: *Infection, Staphylococcus aureus, Kidney, Clove*

INTRODUCTION

Staphylococcus aureus is a Gram-positive bacterium and the causative agent of various infectious diseases such as skin infections, bacteremia, endocarditis, pneumonia, and food poisoning. This organism was initially a major nosocomial pathogen and then epidemiologically distinct clones emerged in the community environment. This

bacterium exhibits many virulence factors that aid in infection by facilitating tissue attachment, tissue invasion, and evading the host immune response (Gnanamani et al., 2017). *Staphylococcus aureus* bacteria are commensal pathogens that are often found on the skin, nose, and mucous membranes of healthy people. This bacterium is a common global cause of human infections and easily acquires antimicrobial resistance through mutation or horizontal transfer of resistance genes from other bacteria (Linz et al., 2023).

The kidneys are complex organs that are essential for maintaining normal body function. They are essential organs that remove waste products, balance body fluids, release hormones that regulate blood pressure, produce the active form of vitamin D, promote bone health, and control red blood cell production (Ray & Reddy, 2023).

The human kidneys primarily function to eliminate metabolic waste products, such as urea, creatinine, uric acid, and other metabolites, by filtering plasma at different rates (Costanzo, 2021). In addition, these organs perform various important homeostatic functions, regulating fluid and electrolyte balance and concentration, regulating arterial pressure, maintaining acid-base balance, secretion, metabolism, excretion of hormones, and gluconeogenesis (Costanzo, 2021). To maintain homeostasis, the kidneys adjust their excretion rate to match the intake of various substances.

Clove extract has been used as a perfume and food flavoring. Medically, it is widely used to relieve toothache or cavities, asthma, rheumatoid arthritis, acne, scars, various allergic disorders, and as an antiseptic for oral infections (Wongsawan et al., 2019). In addition, the antimicrobial properties of clove oil and its application in any product, such as food and health products, have been tested and shown inhibitory activity against various pathogens, including *Staphylococcus aureus* (Xu et al., 2016).

Previous research by Utami (2019) found that clove extract has a main component in the form of eugenol of 90 – 95%, which functions as an antibacterial that can inhibit bacterial growth. Cloves also contain large amounts of phenolic components and antioxidants. The antioxidant capacity of clove extract is mainly due to the high eugenol content (Harlina et al., 2018). Based on this explanation, researchers are interested in seeing the effect of clove extract on improving kidney function in mice infected with *Staphylococcus aureus*

LITERATURE REVIEW

Syzygium aromaticum, also known as cloves, are dried flower buds belonging to the *Myrtaceae* family that are native to the Maluku Islands in Indonesia but have recently been cultivated in various places around the world (Batiha et al., 2019). The clove tree

consists of leaves and shoots (the commercial parts of the tree) and the production of flowering shoots begins four years after planting. After that, they are collected by hand or using natural phytohormones at the pre-flowering stage (Cortes-Rojas et al., 2014). Interestingly, cloves are used commercially for various medicinal purposes and in the perfume industry, and cloves are considered one of the spices that have the potential to be used as a preservative in many foods, especially in meat processing, to replace chemical preservatives due to their antioxidant and antimicrobial properties (Cortes-Rojas et al., 2014).

The potential production of wet ripe flowers is 80 kg/tree/year, and dry flowers are 25 kg/tree/year (Alfian et al., 2019). Clove extract is traditionally used in the treatment of burns and wounds, as a pain reliever in dental care and to treat dental infections and toothaches. In addition, its use has been documented in various industrial applications and is widely used in perfumes, soaps, and as a cleaning agent in histological work (Sarrani et al., 2002). Traditionally, cloves have been used for centuries in the treatment of vomiting; flatulence; nausea; liver, intestinal, and gastric disorders; and as a nerve stimulant. In tropical Asia, cloves have been shown to relieve various microorganisms such as scabies, cholera, malaria, and tuberculosis. In addition, in America, cloves are traditionally used in inhibiting foodborne pathogens to treat viruses, worms, candida, and various bacterial and protozoal infections. In addition, eugenol has been widely used in dentistry because it can penetrate dental pulp tissue and enter the bloodstream (Martinez-Herrera et al., 2016).

Previous studies have found that clove extract has substantial antimicrobial activity against a broad spectrum of microorganisms, including bacteria, fungi, and viruses (Swamy et al., 2016). This activity is thought to be due to eugenol, the main bioactive compound in clove extract. Eugenol is known to disrupt the cell membrane of microorganisms and inhibit their growth and reproduction (Devi et al., 2010). Oulheir et al. (2017) showed that clove extract has strong antibacterial activity against a variety of bacteria, including Gram-positive and Gram-negative bacteria. Clove extract was also found to inhibit the growth of methicillin-resistant Staphylococcus aureus (MRSA), a bacterial strain that is resistant to antibiotics (Alanazi et al., 2022).

METHODS

This study is a True experimental study, with the selection of the type of research design used Test Only Control Group Design, which is a type of research that only observes the

control and treatment groups after being given treatment. The preparation of histopathology preparations is carried out by fixing the kidney organs using 10% Neutral Buffer Formalin (NBF) for 18-24 hours, after fixation is complete, the dehydration process is carried out with one solution session consisting of: 70% alcohol, 80% alcohol, 90% alcohol, 96% alcohol, and absolute alcohol each for two hours. The clearing process is carried out using toluene and impregnation or paraffin infiltration using paraffin. Organ samples are printed into blocks using an embedding set that is poured with liquid paraffin and cooled. All of these processes are carried out in stages within one day. The cold blocks are cut using a microtome with a slice thickness of about 4-5 microns. The last process is staining with the Harris Hematoxylin-Eosin method. The stained specimen block is placed on an object glass then given mounting media as an adhesive and covered with a cover glass. Data from histopathological observations through microscopic examination were collected and then scored. The research data were tabulated, and then the changes found were analyzed and presented descriptively.

Data were analyzed using SPSS (Statistic of Package for Social Science) 25.0. for windows. The data normality test was analyzed using the Kolmogorov-Smirnov test approach ($p > 0.05$). To test the significance between trial groups, one-way analysis of variance or One-way ANOVA was used at a 95% confidence level ($p < 0.05$). Further analysis or testing was carried out using the Post Hoc Test with the LSD technique.

The first stage of the research procedure is to carry out acclimatization. Acclimatization of test animals is the process of adjusting to a new environment, climate, conditions, or atmosphere. Before giving treatment, all male Wistar strains went through an acclimatization process for seven days at the Department of Pharmacology and Therapeutics, Faculty of Medicine, University of North Sumatra. The mice were given time to adapt to the new environment, as well as their food and drink (ad libitum). The acclimatization treatment of test animals in this study was based on the research of Gusbakti et al., (2022) which has been adapted by researchers. Test animals in the form of mice were kept in groups in experimental animal cages in the laboratory. The mouse cages measuring (30 cm x 20 cm x 10 cm) were made of plastic and covered with fine wire mesh. The base of the cage was covered with 0.5–1 cm thick rice husks which were replaced every day during the study. The room lights were controlled to produce a 12-hour light/12-hour dark cycle, the temperature was set to 25–27 °C, and the room humidity was adjusted to the normal range of 35–50%. Mice were fed standard mouse pellets and given distilled water ad libitum (Gusbakti et al., 2022).

RESULTS

This study used test animals in the form of male white rats (Rattus norvegicus) Wistar strain weighing 160-200gr aged 2-3 months. The test animals were divided into 4 groups, the control group was only given regular feed and distilled water, and the treatment group was given infection and clove extract with different doses, namely 150mg/KgBB, 350mg/KgBB, and 450mg/KgBB.

Reporting Research Results

The number of samples was calculated based on the Ferderer formula for 4 groups and the results were 6 per group, so the total sample in this study was 24 mice. The following characteristics of the test animals can be seen in Table 2 and Table 3 shows the results of phytochemical screening of the test samples in this study.

Table 2. Characteristics of test animals

Component	Group			
	Control	**P1**	**P2**	**P3**
Types of Rats	Wistar strain white rats			
Gender	Male			
General Condition	White fur color, healthy and active			
Average Initial Body Weight	188gr	189gr	192gr	190gr
Average Final Weight	187gr	189gr	191gr	189gr

Table 3. Phytochemical Test

Secondary Metabolites	Testing	Color	Results
Flavonoid	Wilstater	Yellow	+
Saponins	Forth	Yellow and foamy	+
Tannin	FeCl3	Blackish blue	+
Alkaloid	Wagner	Yellow	+
Steroid	Lieberman – Burchard	Green	+

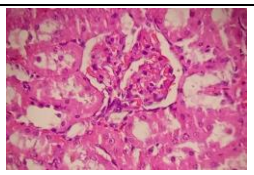
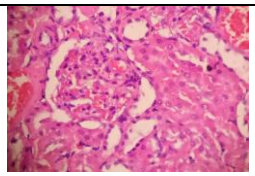
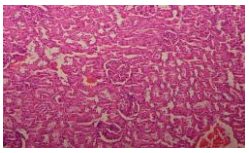
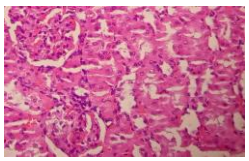
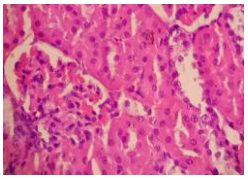
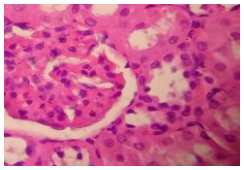
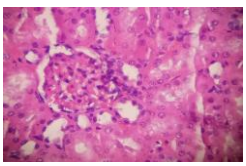
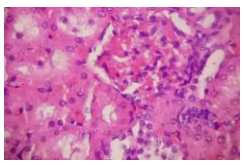
Description: (+) = Contains the compound group being tested

(-) = Does not contain the tested compound

Phytochemical testing is carried out to examine the content of secondary metabolite compounds contained in clove extract (*Syzygium aromaticum*). Phytochemical tests include tests for flavonoids, saponins, tannins, alkaloids, and steroids/triterpenoids. First, a flavonoid test is carried out. 1 gram of clove extract (*Syzygium aromaticum*) is put into a test tube, then concentrated HCl is added and heated for 15 minutes in a water bath. If a red or yellow color is formed, it means that flavonoids (flavones, chalcones, and aurones) are positive. In testing flavonoids, a yellow liquid is formed, which means that it is positive for flavonoids.

The results of the histology testing of animal kidneys from each test group can be seen in Table 4.

Table 4. Histopathology of Kidney Tissue

No	Group	Histopathological Image of Kidney Tissue	
1	Control (Aquades)		
2	Treatment 1 (150mg/KgBW)		
3	Treatment 2 (350mg/KgBW)		
4	Treatment 3 (450mg/KgBW)		

The results of histopathological observations showed different cell appearances. The control group that was not infected with Staphylococcus aureus bacterial suspension and was given distilled water had normal kidney histology and was included in the score

category 0, which means there was no histopathological damage to the kidney tissue. The kidney histopathology in the control group was in normal form because it was not given a bacterial infection diet, so it was used as a reference to describe other groups and as a comparison with the treatment group that was infected with Staphylococcus aureus bacterial suspension and given clove extract. Urea normality test results can be seen in Table 5.

Table 5. Urea Normality Test Results

Group	df	Sig
Control	6	.200
P1	6	.200
P2	6	.200
P3	6	.200

Based on the results of the normality test that has been carried out using the Kolmogorov-Smirnov Test. The significance results were obtained at 0.200 in all groups. Data is said to be normally distributed if the p value> 0.05. Creatinine normality test results can be seen in Table 6.

Table 6. Creatinine Normality Test Results

Group	df	Sig
Control	6	.200
P1	6	.200
P2	6	.200
P3	6	.200

Based on the results of the normality test that has been carried out using the Kolmogorov-Smirnov Test. The significance results were obtained at 0.200 in all groups. Data is said to be normally distributed if the p value> 0.05.

DISCUSSION

The urea and creatinine levels data were then tested for homogeneity using the Levene test to determine whether the data came from a population with the same variance. The results obtained showed a significance value of 0.682 for urea levels and 0.329 for

creatinine levels. The probability value of significance obtained was greater than 0.05, so it can be concluded that the results of observations of urea and creatinine levels in the control group, treatment group 1, treatment group 2, and treatment group 3 were homogeneous or came from the same population. The normally distributed and homogeneous data were then tested for effectiveness and significance using the One-Way ANOVA test.

The results of the One-way ANOVA test on the results of observations of serum urea and creatinine levels showed a significance value of 0.000 or greater than 0.05. Based on these data, it can be concluded that there is a significant difference between the control group, treatment group 1, treatment group 2, and treatment group 3 so a further post-hoc LSD test is needed. The post-hoc LSD test was conducted to analyze the differences in average urea and creatinine levels between groups.

The results of the Post Hoc LSD test analysis on the observation of urea levels showed that there was a significant difference between the control group and treatment groups 1 ($p = 0.000$) and 2 ($p = 0.000$) and there was no significant difference with treatment group 3 ($p = 0.909$). Based on the results of this analysis, it can be concluded that treatment group 3 which was given clove extract at a dose of 450 mg/KgBW had urea levels that were not different from the control group. While treatment groups 1 and 2 had different levels from the control group.

The results of the analysis of creatinine levels using the Post Hoc LSD test showed that there was a significant difference between the control group and treatment groups 1 ($p = 0.000$) and 2 ($p = 0.000$). While the control group and treatment group 3 did not have a significant difference with a significance value of 0.058. This means that the control group and treatment group 3 did not differ significantly. The creatinine levels of the control group were not much different from those of treatment group 3.

The histological condition of the rat kidneys that had gone through the trial process was then analyzed. The control group that was not infected with *Staphylococcus aureus* bacterial suspension and given distilled water had normal kidney histology and was included in the score category 0, namely, there was no histopathological damage to the kidney tissue. The kidney histopathology in the control group was in normal form because it was not given a bacterial infection diet so it was used as a reference to describe other groups and as a comparison with the treatment group that was infected with *Staphylococcus aureus* bacterial suspension and given clove extract.

In treatment group 1 which was given *Staphylococcus aureus* bacterial infection and clove extract at a dose of 150 mg/KgBB, there was a difference in the shape of the kidney structure, because the organ had been exposed to the Staphylococcus aureus bacterial suspension. In the histological picture of treatment group 1 which was given clove extract at a dose of 150 mg/KgBB, there was damage to the kidney cells, so it was included in the score category 4 (there was diffuse/severe damage). Treatment group 2 which was given clove extract at a dose of 350 mg/KgBB showed improvement in the histological structure of the kidneys but there was still multifocal/moderate damage, so it was included in the score category 2. Treatment group 3 which was given clove extract at a dose of 450 mg/KgBB showed a histological structure of the kidneys that was close to the control group, so it was included in the score category 0.

CONCLUSION

1. Clove extract contains secondary metabolites in the form of saponins, tannins, flavonoids, and steroids that help repair damaged kidney cells and decrease kidney function due to Staphylococcus aureus bacterial infection. The active compound that plays the most important role in improving kidney function is flavonoids.
2. Administration of clove extract at a dose of 450 mg/KgBW is effective in improving kidney function in white rats (Rattus norvegicus) Wistar strain infected with Staphylococcus aureus. This improvement can be seen through the levels of urea, creatinine, and histological structure of the kidneys which have improved.
3. The results of histopathological observations of kidney tissue in treatment group 3, namely clove extract with a dose of 40 mg/KgBW, experienced the most significant improvement and approached the control group compared to the other groups.

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