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

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ABSTRACT

This study was conducted to test and analyze the differences in histopathological features of liver tissue between the control group and the treatment group given clove extract (*Syzygium aromaticum*) with dose variations of 100mg/KgBW, 300mg/KgBW, and 500mg/KgBW. Clove extracts have many bioactivities, including antioxidant, anti-inflammatory, antimicrobial, anticancer, and neuroprotective effects. The antioxidant activity of clove extract is due to the presence of phenolic compounds that can capture free radicals and reduce oxidative stress. The sample in this study was male white rats (Rattus norvegicus) Wistar strain weighing 160-200gr and aged 2-3 months. The results of phytochemical tests showed that clove extract contains secondary metabolites in the form of saponins, tannins, flavonoids, alkaloids, and steroids that help repair liver cells damaged by Staphylococcus aureus bacterial infection. The results of histopathological observations of liver tissue in treatment group 3, namely clove extract with a dose of 500 mg/kg BW, experienced the most significant improvement and approached the control group compared to the other groups

Keywords: Infection, *Staphylococcus aureus, Liver, AST, ALT, Clove*.

INTRODUCTION

The liver is the largest gland in the body and is ideally located to receive absorbed nutrients and detoxify absorbed drugs and other harmful substances. It functions as both an exocrine

organ and an endocrine organ. The exocrine functions of the liver are primarily in the synthesis and excretion of bile salts into the common hepatic duct and the conjugation of bilirubin and excretion into the intestine. The endocrine functions of the liver include involvement in glycemic control via insulin and glucagon. The liver synthesizes important proteins such as fibrinogen, albumin, prothrombin, and other amino acids and modifies proteins into enzymes and peptide hormones. The liver participates in fatty acid metabolism and synthesizes lipoproteins, cholesterol, and phospholipids. In addition, it is involved in carbohydrate metabolism including glycogen storage and gluconeogenesis. It is also involved in lactic acid metabolism and the conversion of ammonia to urea. The liver stores vitamins, and minerals such as iron. In summary, the liver is an important mediator from the gut to the blood and plays a vital role in the metabolism of macronutrients, hormones, blood plasma components, and exocrine and endocrine substances (Vernon et al., 2022). Staphylococcus aureus is initially cleared by specialized liver macrophages called Kupffer cells. Circulating platelets assist this process by aggregating around the bacteria on the surface of Kupffer cells, encapsulating Staphylococcus aureus until phagocytosis. This clearance process is sufficient in most cases to prevent serious infection, but a small proportion of phagocytosed Staphylococci may survive and multiply within Kupffer cells, ultimately turning the liver into a reservoir for intracellular Staphylococcus aureus (Pollitt et al., 2018). Staphylococcus aureus is released from this intracellular niche into the peritoneal and hepatic circulation, where it encounters peritoneal macrophages and bloodstream neutrophils that act as a second line of phagocytic defense. However, a small proportion of ingested Staphylococcus aureus can survive again within these phagocytes, and carry the intracellular bacteria to other parts of the body, ultimately leading to systemic dissemination (Pollitt et al., 2018). Medicinal plant extracts have been shown to have antibacterial activity, one of which is clove. Clove extract shows strong antioxidant and antibacterial activity compared to eucalyptus and lavender (Vella et al., 2020). Clove essential oil (CEO) has shown strong antioxidant activity with an EC50 of 0.36 μL/mL and is the most potent essential oil compared to eucalyptus, fennel, and lavender (Vella et al., 2020). Phenols and flavonoids are associated with antioxidant effects. Antioxidant activity is mediated through free radical scavenging and increased antioxidant enzyme activity (Xue et al., 2022)..

LITERATURE REVIEW

The liver is a spongy parenchymal mass penetrated by tunnels (lacunae) containing an interdigitating network of afferent and efferent vessels. The adult human liver weighs 1300 to 1700 g, depending on sex and body size. It is relatively small compared to other species (2% of body weight) – in mice and rats, the liver occupies 4–5% of the body (Nagy et al., 2020).

The liver is the largest organ in the mammalian body and has very versatile and complex functions. Its special role is demonstrated by the fact that, despite great efforts, the activity of the liver cannot be replaced by artificial devices. The liver participates in the maintenance of the organism's homeostasis as an active bidirectional biofilter. It is classified as bidirectional because it filters the portal blood that transports nutrient compounds and toxins from the environment through the digestive tract and filters the systemic blood (the body's own products, e.g. bilirubin), providing the body's only channel, the bile duct system through which water-insoluble substances can be eliminated. It is classified as an active filter because it rapidly metabolizes most nutrient compounds and neutralizes and is ready to eliminate exogenous (xenobiotics) and endogenous (obsolete) toxic materials (Nagy et al., 2020). Liver organ evaluation can begin with performing liver function tests. Liver function tests, are a group of serum tests that relate to liver tissue injury or function. These biochemical tests represent the liver at a static point in time and do not evaluate actual liver function. However, the term 'liver function test' has been used for decades to represent the following tests: aspartate transferase (AST), alanine transferase (ALT), alkaline phosphatese (ALP), gamma glutamyl transferase (γ-GT), lactic dehydrogenase (LDH), and bilirubin (total and direct). These tests relate to various aspects of liver tissue and are commonly used in the evaluation of liver disease (Sourianarayanane, 2017). Aminotransferases are enzymes involved in the transfer of amino acid groups to keto groups. They are involved in gluconeogenesis. AST is involved in the transfer of the amino acid aspartate to oxaloacetate, while ALT transfers alanine to pyruvic acid. Because these enzymes are present in hepatocytes, hepatocellular injury or disease causes these tests to be elevated. Aspartate transferase AST (formerly called serum glutamic oxalo-acetic transaminase, or SGOT) is present in the cytoplasm and mitochondria of most tissues, but in the liver, AST is predominantly present in the mitochondria of periportal hepatocytes (80%). Therefore, elevated AST reflects mitochondrial injury to the hepatocyte. The half-life of serum AST is 17 hours, with a rapid decline occurring after acute injury, such as ischemia or drug exposure. AST may be falsely elevated in patients with macro-AST, in whom AST is bound to immunoglobulins and is not cleared. AST can be very low in patients undergoing chronic hemodialysis, which is associated with pyridoxine deficiency (Sourianarayanane, 2017). Syzygium aromaticum or commonly called cloves, is a tree belonging to the Myrtaceae family and is a medicinal plant that promises antimicrobial benefits. This plant is native to Indonesia, especially the Maluku Islands, and requires a warm and humid climate. The evergreen tree can reach a height of 12–15 m, and is characterized by oval-lanceolate leaves, flowers with 4 red sepals and 4 white-pink petals, and berries as fruit (Maggini et al., 2024).

Clove is a spice plant with a distinctive taste and various health benefits that are widely used in traditional medicine and culinary activities (Khanal et al., 2021). In recent years, there has been increasing interest in clove valorization, or the process of creating higher-value clove products, beyond its traditional applications. The main bioactive component of cloves is eugenol, which accounts for 85% (El-Saber et al., 2020). Many health benefits have been identified, including anti-inflammatory, analgesic, and antioxidant properties. Eugenol has been shown to have antibacterial and antifungal properties, making it effective against a variety of microorganisms (Marchese et al., 2017). Acetyl eugenol is another important bioactive compound in cloves, accounting for up to 15% of its essential oil. Similar health benefits to eugenol have been found, including anti-inflammatory and analgesic properties. Acetyl eugenol has also been found to have anticancer properties, making it a potential cancer treatment agent. Cloves and other plants, including black pepper and cinnamon, contain the bioactive compound caryophyllene. It has been found to have anti-inflammatory and analgesic properties, making it effective in treating pain and inflammation (Baron, 2018).

METHODS

This study is a True experimental study, with the selection of the type of research design used is Post Test Only Control Group Design, which is a type of research that only observes the control and treatment groups after being given an action. The sample of this study was male rats (Rattus norvegicus) of the Wistar strain weighing 160-200 grams and aged 2-3 months. Researchers chose the Wistar strain of purih rats as the subject of the research test because this animal has characteristics and physiology that are almost the same as humans and is also one of the most widely used animals in biomedical research. Simplasia extraction was carried out using the maceration method. Weighed 100 grams of butterfly pea flower powder (Clitoria ternatea) using 96% ethanol in a ratio of (1:10) for 4 days. The extract was then filtered and the dregs and filtrate were separated. The extract that had been filtered was then evaporated from the solvent using a rotary evaporator to obtain a thick extract. The doses of butterfly pea flower extract given were 200 mg/kgBW, 400 mg/kgBW, and 600 mg/kgBW. Clove samples in the form of flowers, flower stalks were ground to a size of 60-70 mesh, hereinafter referred to as simplicia. The extraction process began by soaking the simplicia in 90% ethanol solvent with a ratio of 1:10, during the soaking process the sample was stirred. Furthermore, the solution was filtered every three days, then the simplicia was soaked again using ethanol, and so on until the filtering process was carried out three times. The resulting filtrate was evaporated using a rotary evaporator until all the solvent had evaporated and finally formed a paste. The paste is then called ethanol extract which is ready to be tested. Acclimatization is

the process of adjusting to a new environment, climate, condition, or atmosphere. Before giving treatment, all male Wistar strains went through a seven-day acclimatization process at the Department of Pharmacology and Therapeutics, Faculty of Medicine, Universitas Sumatera Utara. The rats were given time to adapt to the new environment, as well as their food and drink (ad libitum). In vivo studies of this research were supervised under Universitas Prima Indonesia ethical committee, number: 060/KEPK/UNPRI/IX/2024. Based on the difference in the average ALT levels, the researchers concluded that treatment group 3, namely mice infected with bacteria and given clove extract at a dose of 500 mg/kgBW, had the greatest decrease in ALT levels and was close to the control group. While treatment group 1, namely mice given a dose of 100 mg/kgBW, experienced the lowest decrease or improvement in ALT levels compared to treatment groups 2 and 3. Based on the difference in the average value of AST levels, the researchers concluded that treatment group 3, namely mice infected with bacteria and given clove extract at a dose of 500 mg/kgBW had the greatest decrease in serum AST levels and was close to the control group. While treatment group 1, namely mice given clove extract at a dose of 100 mg/kgBW experienced the least decrease or improvement in AST levels.

RESULTS

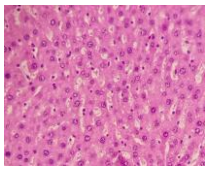
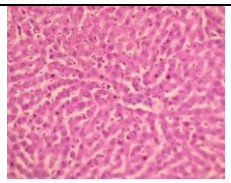
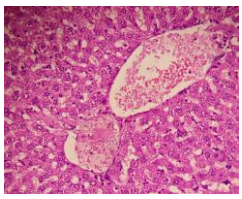
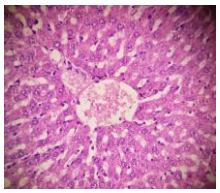
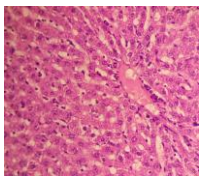
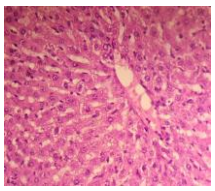
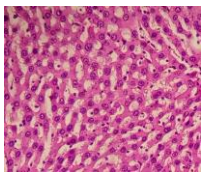
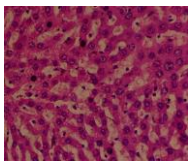
This study used test animals in the form of male white rats (Rattus norvegicus) Wistar strain weighing 160-200gr aged 2-3 months. The test animals were divided into 4 groups, the control group was only given regular feed and distilled water, the treatment group was given an infection with a suspension of Staphylococcus areus bacteria. and clove extract with different doses, namely 100mg/KgBB, 300mg/KgBB, and 500mg/KgBB. The number of samples was calculated based on the ferderer formula for 4 groups and the results obtained were 6 per group, so that the total sample in this study was 24 rats. The following are the characteristics of the test animals: In **Table.1** consist of phytochemical screening of secondary metabolite groups on clove extract (*Syzygium aromaticum*) was carried out to determine the content, which can be used to accelerate the healing of wounds from dermapen and stimulate collagen growth on the backs of white rats (Rattus norvegicus) Wistar strain. The following are the screening results obtained:

Table 1. Screening Phytochemical Tests On Clove Flower Extract

Secondary Metabolites	Color	Results
Flavonoid	Yellow	+
Saponins	Yellow and foamy	+

Tannin	Blackish blue	+
Alkaloid	Yellow	+
Steroid	Green	+

Table 2. Histopathological Image of Liver Tissue

No	Group	Histopathological Image of Liver Tissue
1	Control (Aquades)	 
2	Treatment 1 (200mg/KgBW)	 
3	Treatment 2 (400mg/KgBW)	 
4	Treatment 3 (600mg/KgBW)	 

The results of histopathological observations can be seen in **Table.2** different cell appearances. The control group was not given bacterial infection and clove extract. The control group had a normal histological picture of the liver and was included in the score category 1, which means there was no change in the histological structure of the liver. The histopathology of the liver in the control group was in normal form because it was not given a high-fat diet, so it was used as a reference to describe other groups and as a comparison with the treatment group that was given a high-fat diet andclove extract.

DISCUSSION

This study was conducted to test and analyze the effectiveness of clove extract administration on the liver function of white rats (Rattus norvegicus) Wistar strain exposed to Staphylococcus aureus bacteria. Observation of liver function is based on AST and ALT levels, and how the histopathology is. The sample in this study was male white rats (Rattus norvegicus) Wistar

strain weighing 160-200gr and aged 2-3 months. Determination of the sample used the Ferderer formula for 4 groups and the overall results were 24 rats which would be divided into 4 different groups. The first group as the control group, in this group the rats were only given regular pellet feed and distilled water. The treatment group was exposed to Staphylococcus aureus bacteria and clove extract with different doses, namely 100mg/KgBB, 300mg/KgBB, and 500mg/KgBB. The liver performs more than 500 functions in the body. The main function of the liver is filtration, which removes harmful substances from the blood (Mirzaali et al., 2022). These main functions make the liver constantly exposed to intense microbiological and antigenic stimuli that require the function of the innate and adaptive immune systems (Nagy et al., 2020). The liver organ is ultimately the target of many infectious agents (Masia & Misdraji, 2018). One of them is *Staphylococcus aureus* bacterial infection. *Staphylococcus aureus* initially cleared by specialized liver macrophages called Kupffer cells. Circulating platelets assist this process by aggregating around the bacteria on the surface of Kupffer cells, enveloping Staphylococcus aureus until phagocytosis. This clearance process is sufficient in most cases to prevent serious infection, but a small proportion of phagocytosed staphylococci may survive and multiply within Kupffer cells, ultimately turning the liver into a reservoir for Staphylococcus aureus (Pollitt et al., 2018Medicinal plant extracts have been shown to have antibacterial activity, one of which is clove. Clove extract shows strong antioxidant and antibacterial activity compared to eucalyptus and lavender (Vella et al., 2020). Clove essential oil (CEO) has shown strong antioxidant activity with an EC50 of 0.36 μL/mL and is the most potent essential oil compared to eucalyptus, fennel, and lavender (Vella et al., 2020). Phenols and flavonoids are associated with antioxidant effects. Antioxidant activity is mediated through free radical scavenging and increased antioxidant enzyme activity (Xue et al., 2022). Based on the explanation, the researcher suspects that there is an effect of giving clove extract on improving liver function in male white rats (Rattus norvegicus) of the Wistar strain infected with Staphylococcus aureus bacteria. The researcher conducted a trial on male white rats (Rattus norvegicus) of the Wistar strain to prove this suspicion. Researchers first conducted phytochemical tests on clove extract to determine the compounds contained in the extract. The results of the phytochemical tests that have been carried out indicate that clove extract contains secondary metabolites in the form of flavonoids, saponins, tannins, and steroids. The test animals were then given treatment in the form of giving clove extract with their respective group doses. This research procedure was conducted for 14 days and produced data that needed to be processed and tested first, so it was necessary to do some data analysis in the form of normality, homogeneity, and significance tests. The normality test data was obtained with the help of SPSS using the Kolmogorov-smirnov test. The results showed that the data for each group was normally distributed with a significance value of 0.200 in all test groups

for testing ALT and AST levels. So it can be concluded that the data is normally distributed, or can represent the population. The ALT and AST level 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 0.137 for ALT levels and 0.069 for AST levels. The probability value of significance obtained was greater than 0.05, so it can be concluded that the results of observations of ALT and AST 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 ALT and AST 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 that a further post-hoc LSD test is needed. The Post-hoc LSD test was conducted to analyze the differences in average ALT and AST levels between groups. The results of the LSD Post Hoc Test analysis were used to determine whether the group had a significant difference from the other groups. The results of the analysis showed that there was a significant difference between the control group and treatment group 1 ($p = 0.000$) and treatment group 2 ($p = 0.000$). While the control group and treatment group 3 did not have a significant difference ($p = 0.227$). The LSD Post Hoc Test was used to determine whether the group had a significant difference in AST from the other groups. The results of the analysis 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 ($p = 0.376$). The histological condition of the liver of mice that had gone through the trial process was then analyzed. The control group had a normal histological picture of the liver. The results of histopathological observations of the liver in the control group were used as a reference to describe other groups and as a comparison. In treatment group 1 which was given a Staphylococcus aureus bacterial infection and clove extract at a dose of 100mg/KgBB, changes in the shape of the histological structure of the liver were seen from the normal group, because the organ had been exposed to bacteria. In the histological picture of treatment group 1 which was given clove extract at a dose of 100mg/KgBB, there was necrosis in the liver cells, so it was included in the score category 4. Treatment group 2 which was given clove extract at a dose of 300mg/KgBB showed improvement in the histological structure of the liver but there was still damage, so it was included in the score category 3. Treatment group 3 which was given a bacterial infection and clove extract at a dose of 500mg/KgBB showed a histological structure of the liver that was close to the control group, so it was included in the score category 1 (no change in shape or normal).

CONCLUSION

The results of phytochemical tests show that clove extract contains secondary metabolites in the form of saponins, tannins, flavonoids, alkaloids, and steroids which help repair liver cells damaged by Staphylococcus aureus bacterial infection. With the administration of clove extract at a dose of 500mg/KgBW is effective in improving liver function in white rats (Rattus norvegicus) Wistar strain infected with Staphylococcus aureus. This improvement can be seen through the levels of ALT, AST, and histological structure of the liver which has improved. The results of histopathological observations of liver tissue in treatment group 3, namely clove extract with a dose of 500 mg/kgBW, experienced the most significant improvement and approached the control group compared to the other groups.

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