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Original Article

Extract of Papaya Leaf to Inhibit Osteomyelitis in Male Wistar Rats Induced by *Staphylococcus aureus*

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Abstract

Osteomyelitis is a bone infection predominantly caused by *Staphylococcus aureus*, accounting for 30–60% of cases. Its incidence remains unclear globally, and data in Indonesia are limited. Alternative treatments using medicinal plants, such as papaya leaves, are being explored due to their antibacterial properties. This study aimed to determine the effect of papaya leaf extract on inhibiting osteomyelitis progression in male Wistar rats (*Rattus norvegicus)* induced by *Staphylococcus aureus*. This experimental study used a Randomized Controlled Trial with a Post-Test Only Control Group Design. A total of 35 male Wistar rats were divided into five groups: control, *S. aureus* induction, *S. aureus* + cephalexin, and two treatment groups receiving papaya leaf extract at doses of 100 mg/kg of body weight and 300 mg/kg of body weight. Data were analyzed using normality, homogeneity, ANOVA, LSD, and effect size tests. Papaya leaf extract at both doses inhibited osteomyelitis progression, with the 300 mg/kg of body weight dose showing the most significant effect. This was indicated by a reduced ratio of mononuclear to histiocyte cells and a large effect size (Cohen's d). Papaya leaf extract demonstrates potential antibacterial activity against osteomyelitis and may serve as an alternative therapeutic approach. Further studies are recommended to explore its clinical application and mechanism of action.

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INTRODUCTION

Osteomyelitis is an infection of the bone caused by various microorganisms, especially around 30% - 60% caused by invasion by *Staphylococcus aureus* (*S. aureus*) bacteria.[1] The overall incidence of osteomyelitis is unknown, but in the United States (US), about 1 in 675 reports are received from US hospitals annually, or about 50,000 cases each year, while in Indonesia, data on osteomyelitis sufferers are still limited. Data from the orthopedic department at Hasan Sadikin Hospital in Bandung indicate that the incidence of chronic osteomyelitis in Indonesia ranges from 0.5 to 2.4 cases per 100,000 population.[2,3] Based on several hospital-based reports in Indonesia, osteomyelitis remains a clinically significant musculoskeletal infection, particularly among post-traumatic and post-operative patients, indicating the need for further local research regarding alternative therapeutic approaches.[4]

Osteomyelitis can be a serious problem in musculoskeletal organs because it can cause inflammation and damage to the progressive bones.[5] Clinical conditions that arise can be in the form of inflammatory signs, such as calor, dolor, tumor, rubor, and functionolesa.[6] In addition, osteomyelitis can also be confirmed by radiologic examination and histopathologic features. In acute osteomyelitis, neutrophil infiltrates can be found, while in chronic conditions, mononuclear cells are usually found, such as histiocytes, plasma cells, and lymphocytes, so that the treatment can be done by administering antibiotics to surgical removal of the infected bone, depending on the severity of the progressive condition.[7,8]

Antibiotic administration is carried out with the aim of preventing further infection of the affected bone. Cephalosporins are a class of β-lactams that can be used as therapy for infections caused by Gram-positive bacteria, including *S. Aureus*.[9] Cephalexin is a first-generation cephalosporin, is the first-line oral therapy for musculoskeletal infections due to *S. aureus*.[10] The use of antibiotics continuously and not as recommended can cause bacteria to become antibiotic resistant, so management using alternative medicine can be an innovation in the development of antibacterial drugs without side effects.[11,12] One of the plants that can be utilized is papaya leaves. Papaya leaf extract (EDP) is known to have a high effect in inhibiting the growth of *S. aureus* because it contains many active compounds such as flavonoids, alkaloids, tannins, and saponins.[13,14] Previous studies have demonstrated that traditional medicinal plants exhibit antibacterial activity, particularly against Gram-positive bacteria, thereby inhibiting their growth.[15]

The purpose of this study was to prove that EDP influences inhibiting the spread of osteomyelitis in male Wistar rats induced by *S. aureus*. This study analyzed the effect of papaya leaf extract (*Carica papaya L.*) at a dose of 100mg/kg and 300mg/kg on the ratio between the number of histiocytes and the number of mononuclear cells in male Wistar rats.

MATERIALS AND METHODS

Materials

This study used 35 male Wistar rats aged 2 - 3 months with a body weight of 200 - 250 grams obtained from the Animal Laboratory of the Faculty of Medicine, University of Surabaya. Wistar rats are in a healthy condition and have never been used as experimental animals in research. The materials used in this study are: papaya leaves, male Wistar white rats, 96% ethanol (Brataco, Indonesia), Neutral Buffered

Formaline (NBF) 10% (local laboratory supply), 1% Na CMC Suspension (Brataco, Indonesia), Cephalexin (Kimia Farma, Indonesia), Phosphate Buffered Saline (PBS) pH 7.4 (Brataco, Indonesia), ether (local laboratory supply), and Aquadest (local laboratory supply). The bacterial strain used was *Staphylococcus aureus ATCC* from the microbiology laboratory of the Faculty of Medicine, University of Surabaya.

The instruments used in this study are: an animal drum with a drum cover, food, standard laboratory equipment, and specialized devices for extraction and microbiological analysis. Extraction was performed using a rotary evaporator and maceration apparatus. Microbiological procedures utilized an incubator, McFarland densitometer, centrifuge, and sterile culture tools. Animal handling was supported by standard surgical instruments and monitoring devices such as a digital scale and thermometer.

Methods

Determination of *Staphylococcus aureus* Dosage

Determination of the dose of *S. aureus* in this study induced *S. aureus* intratibia with a dose of $4x10^6$ CFU/ml. One colony of bacteria will be inoculated on nutrient agar and incubated at 37˚C for 24 hours. The bacteria are then subcultured and incubated for 12 hours, after which the bacterial colonies are transferred into 15 ml tubes using loops, loops containing 10 ml of PBS pH 7.4, and homogenized using a McFarland Densitometer.[16]

Determination of Cephalosporin Dosage

In this study, one of the treatment groups will be given the drug cephalexin which has been proven to overcome infections caused by *S. aureus* by inhibiting the peptidoglycan of the bacterial cell wall, thus causing cell damage.[17] So that it can cause cell damage, which is used as a comparison of papaya leaf extract. Researchers used other research references with a 500 mg dose. The dose of cephalexin administration in rats is converted based on dose conversion calculations. Dose of cephalexin:

$= theoretical\ dose \times conversion\ factor\ (rat)$
$= 500\ mg \times 0.018$
$= 9$ mg/200 grams of body weight

Preparation of cephalexin suspension begins with grinding until smooth, mixing with 1% Na CMC and distilled water, and then given to the experimental animals orally through a gastric tube for 14 days.

Research Implementation Procedure

This study used the experimental method of Randomized Controlled Trial (RCT) with the research design, namely Post-test Only Controlled Group Design, using male Wistar strain white rats. The samples used in this study were 35 male Wistar white rats aged 2-3 months with a body weight of 200-250 grams, which had never been used as a research sample, in a healthy condition, with active movement, and no anatomical abnormalities. The samples were divided into 5 groups, with 7 rats in each group. The groups consisted of a normal control group, a negative control group that was induced by *S. aureus*, a positive control group induced by *S. aureus* and given antibiotics, and 2 treatment groups that were induced by *S. aureus* and given EDP at doses of 100 mg/kg of body weight and 300 mg/kg of body weight. The number of experimental animals used in this study was determined based on previous similar studies on the antibacterial effects of plant extract in Wistar rats.[16]

Before treatment, the animals will be adapted for fourteen days to increase body weight to fit the inclusion criteria. On the sixteenth day, groups II, III, IV, and V were

induced with *S.aureus* to make the animals develop osteomyelitis, then will be waited for ten days to wait for the infection to occur in the animals. During the ten days, the animals will be monitored to see their clinical development of the animals. On the twenty-sixth day, the animals were given EDP for fourteen days in groups IV and V. The administration of papaya leaf extract in this study used an extract obtained from papaya leaves. Papaya leaves were washed, air-dried, and ground into powder. The powdered leaves were extracted using the maceration method with 96% ethanol as the solvent. The extraction process was carried out for 72 hours at room temperature with periodic stirring. The extract was then filtered and concentrated using a rotary evaporator to obtain a thick papaya leaf extract. Papaya leaf extract acts as an antibacterial, so it can inhibit the spread of osteomyelitis, which is characterized by reduced MN cells, namely histiocyte cells, plasma cells, and lymphocyte cells. EDP was given until the fortieth day. At the end of the study (forty-first day), termination or euthanasia with ether inhalation was performed and followed by surgery to take the rat tibia bone tissue and make histopathology preparations. The tibia bone was then submitted to the Anatomical Pathology Laboratory, Faculty of Medicine, University of Surabaya, to make histopathology preparations, and then the number of mononuclear cells was observed using a 400x magnification light microscope.

The data obtained will be analyzed using a prerequisite test in the form of a normality test. Data is declared significant if ($p > 0.05$). The data from the prerequisite test results obtained were normally distributed, and then we continued the homogeneity test. The data from the homogeneity test results showed homogeneous data. Normally distributed and homogeneous data, then parametric tests were carried out between the control group and the treatment group to see the difference in means using the ANOVA test, and continued with the LSD (Least Significant Difference) post hoc test to analyze the difference between the 4 treatment groups. Effect size was also calculated using Cohen's D test to determine the magnitude of the treatment effect of papaya leaf extract on the inhibition of osteomyelitis progression among experimental groups.

RESULTS AND DISCUSSION

Results

Figure 1 shows that administration of EDP at a dose of 100 mg/kg of body weight and a dose of 300 mg/kg of body weight in groups IV and V is proven to help reduce the number of MN cells in the tibia of male Wistar rats that experience osteomyelitis. The reduction of MN cells in the tibia of rats receiving EDP occurs due to the presence of active compounds in EDP that act as antibacterials, such as flavonoids, alkaloids, tannins, and saponins.

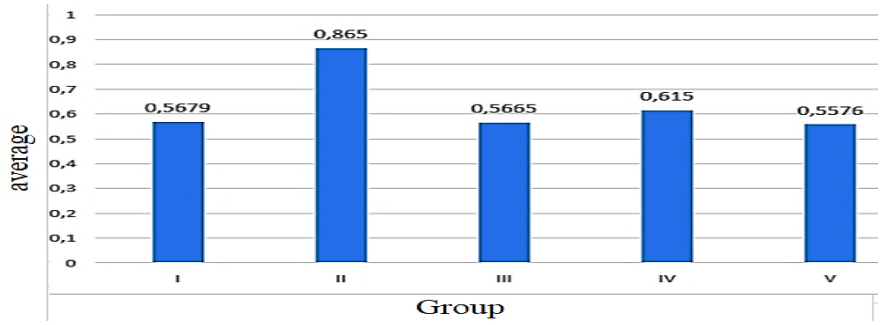


Figure 1. Mean histiocyte to mononuclear cell ratio diagram

Table 1. Normality Test Using Shapiro-Wilk

Group	Normality Test
I	0.274*
II	0.956*
III	0.150*
IV	0.273*
V	0.695*

*Significant if $p>0.05$

Based on the normality test using the Shapiro-Wilk, Table 1 shows a comparison between histiocyte cells and mononuclear cells, where each group is normally distributed ($p>0.05$).

Table 2. Homogeneity Test Using Levene

Group	Homogeneity Test
I	
II	
III	0.761*
IV	
V	

*Significant if $p>0.05$

Based on the homogeneity test using Levene, it is concluded that Table 2 shows that the data are homogeneous ($p>0.05$) and can be continued for parametric tests using the ANOVA test.

Table 3. Parametric Test Using One-Way ANOVA

Group	ANOVA Test
I	
II	
III	0.000*
IV	
V	

*Significant if $p>0.05$

Based on Table 3, it was found that the comparison of data using the ANOVA test obtained the result of p=0.000 (-<0.05), so it was concluded that there was a significant effect on all treatment groups. Data presentation can be continued with post-hoc tests using the Least Significant Difference (LSD) test.

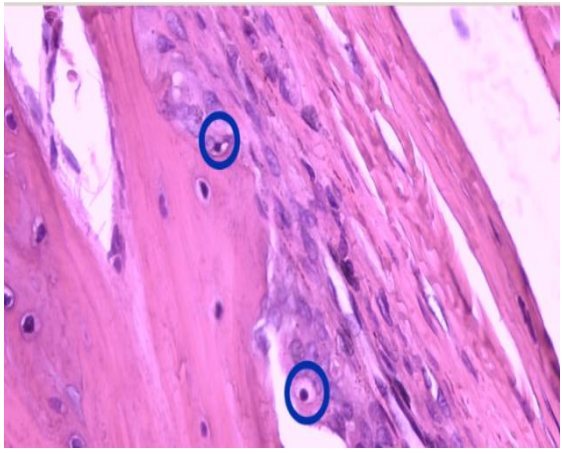
Table 4. Comparison of Histiocyte Cells with Tibia Mononuclear Cells in Each Group Using the LSD Test

Group	I	II	III	IV	V
I	-	0.000*	0.884	0.000*	0.293
II	-	-	0.000*	0.000*	0.000*
III	-		-	0.000*	0.363
IV	-			-	0.000*
V	-				-

*Significant if $p>0.05$

Table 4 shows that there is a significant difference ($p<0.05$) between group I and group II ($p=0.000$) and group IV ($p=0.000$). Comparison between group II and group III ($p=0.000$), group IV ($p=0.000$), and group V (p=0.000) showed significant differences ($p<0.05$). Comparison between group III and group IV ($p=0.000$) and comparison between group IV and group V ($p=0.000$) found a significant difference ($p<0.05$). There was no significant difference ($p>0.05$) in the comparison between group I and group III ($p=0.884$) and group V ($p=0.293$), and group III with group V ($p=0.363$). Based on Cohen's D calculation, papaya leaf extract administration at doses of 100 mg/kg of body weight and 300 mg/kg of body weight demonstrated large effect sizes, indicating strong treatment effectiveness in reducing mononuclear inflammatory cell infiltration.

Group I



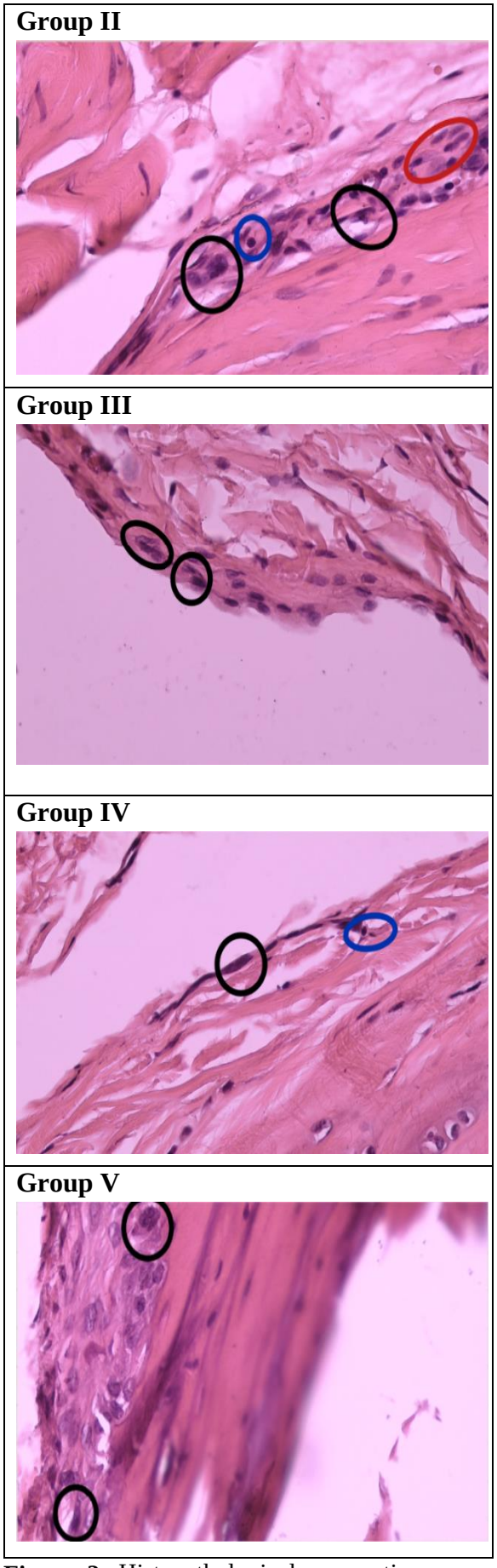


Figure 2. Histopathological preparations were stained using Hematoxylin-Eosin (HE). Group I showed normal tibial histology with minimal inflammatory infiltration. Group II showed mononuclear inflammatory cells, indicating osteomyelitis. Group III showed reduced inflammatory infiltration after antibiotic treatment. Groups IV and V showed a gradual reduction of inflammatory cells following papaya leaf extract administration. (**O**) The image shows the presence of histiocyte cells in the mouse tibia, (**O**) in the image shows the presence of lymphocyte cells in the mouse tibia, and (**O**) in the image shows the presence of plasma cells in the mouse tibia.

Discussion

Figure 2 shows the presence of MN cells in the tibia of rats. Administration of EDP to rats with osteomyelitis due to *Staphylococcus aureus* showed a decrease in the number of MN cells in the tibia of male Wistar rats. This is evidenced by the results of parametric tests, namely the One-Way ANOVA test and LSD test that have been carried out on the whole group. Treatment of experimental animals by inducing *S. aureus* in the tibia of rats causes infection, so that an inflammatory process appears, which MN cells to migrate more to the infected area to fight the infection, resulting in an increase in MN cells compared to the normal group. The number of different MN cells in groups I and II is evidenced by using the LSD test which has a result of p = 0.000, which indicates there is a significant difference. The test results between the normal group and the EDP treatment group with a dose of 300 mg / kg of body weight were declared to have no significant difference based on the calculation of the LSD test; the comparison between group I and group V obtained the result of p = 0.293. The absence of significant group differences I and group V proves that EDP at a dose of 300 mg/kg of body weight can help reduce the number of MN cells in rats that experience osteomyelitis.

Papaya leaves have many health benefits because they have active compounds that function as antibacterials, such as flavonoids, alkaloids, tannins, and saponins.[15] Flavonoids and alkaloids in papaya leaves suppress protein and nucleic acid synthesis, damage bacterial cell membranes and walls, and inhibit energy metabolism in *S. aureus*, making it difficult for bacteria to spread.[18,19] Alkaloids specifically work by disrupting the function of the peptidoglycan wall in Gram-positive bacterial cells.[20,21] Tannins, which are active compounds in EDP, also have an antibacterial effect, namely as bacteriostatic, so that tannins can pass through the bacterial cell wall to the internal membrane.[22] Quinoa saponins can cause degradation of bacterial cell walls, disrupt cytoplasmic membranes and membrane proteins.[23] In previous studies, it was stated that alkaloids can interfere with peptidoglycan constituents in bacterial cells, so that no bacterial cell wall layer is formed, besides that, it is also said that saponins can cause bacterial cell wall lysis.[24,25]

STRENGTH AND LIMITATION

The limitations of this study include technical difficulties during bacterial induction, particularly variability in bacterial inoculation success and differences in immune response among experimental animals, which may affect the consistency of osteomyelitis development. To address this issue, bacterial suspension concentration was standardized and induction procedures were performed uniformly in all treatment groups.

Despite these limitations, this study has several strengths. It used a controlled experimental design with standardized procedures, allowing for comparison between groups. Furthermore, the use of an established osteomyelitis model and objective outcome measures enhances the validity of the findings.

CONCLUSIONS

The final results of this study showed that the administration of EDP at a dose of 100 mg/kgBB and 300 mg/kg of body weight can inhibit the spread of osteomyelitis. The most effective dose was 300 mg/kgBW, as indicated by a reduced number of MN cells in this treatment group. EDP can be one of the alternative treatments that can be done as an effort to reduce MN cells in conditions of infection caused by *S.aureus*, thus inhibiting the spread of osteomyelitis. Based on the calculation of effect size using Cohen's D, it is known that the administration of EDP at a dose of 100 mg/kg of body weight and 300 mg/kgBB has a large effect, namely 13.58 and 15.204, so it can be concluded that EDP has an antibacterial effect in inhibiting the spread of osteomyelitis, which is characterized by a reduced number of MN cells.

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ETHICAL CLEARANCE

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CONFLICT OF INTEREST

The authors emphasize that they have no conflict of interest.

AUTHOR CONTRIBUTION

Experimental design: YPMC. Materials preparation: YPMC, IGNDMR. Implementation: YPMC. Research supervision: YPMC, SW, IGNDMR. Manuscript writing: YPMC. Manuscript editing: YPMC.

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