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THE EFFECT OF *Paederia foetida* L. EXTRACT ON LIVER WEIGHT OF MICE SEPSIS MODEL INFECTED WITH *Escherichia coli*

Pengaruh Ekstrak *Paederia foetida* L. terhadap Bobot Hepar pada Mencit Model Sepsis yang Terinfeksi *Escherichia coli*

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ABSTRACT

Sepsis is a critical medical condition characterized by a systemic immune response to infection, which can lead to severe organ dysfunction and mortality. Bacterial sepsis, particularly caused by *Escherichia coli*, poses a significant risk due to its potential to harm tissues and organs, including the liver. The liver plays a central role in metabolic processes and host defense during sepsis, making it a key organ of interest. This study aimed to investigate the effect of *Paederia foetida* leaf extract on liver weight in a mice sepsis model. Mice were divided into six groups: normal control (N), negative control (K-) receiving distilled water, positive control (K+) receiving ciprofloxacin, treatment 1 (P1) receiving P. foetida extract at 100 mg/kg BW, treatment 2 (P2) receiving 300 mg/kg BW, and treatment 3 (P3) receiving 500 mg/kg BW. After 14 days of treatment, significant differences in liver weight were observed among the groups, with the highest mean and standard deviation recorded in the P1 group (1.3750 ± 0.3932). Liver abnormalities, including swelling, lobular thickening, and weight increase, were identified, indicating the liver's adaptive response to toxic substances during sepsis. Interestingly, the normal control group exhibited higher liver weights compared to the treatment groups, possibly due to fatty substance accumulation within the liver tissues. These findings suggest that *P. foetida* extract may influence liver weight changes in sepsis, potentially modulating metabolic and detoxification processes. Further studies focusing on histopathological and biochemical mechanisms are needed to clarify the therapeutic potential of *P. foetida* in managing liver dysfunction associated with sepsis.

Keywords: *Paederia foetida extract, Liver weight, Mice, Sepsis, Escherichia coli*

ABSTRAK

Sepsis adalah kondisi medis kritis yang ditandai dengan respons imun sistemik terhadap infeksi, yang dapat menyebabkan disfungsi organ berat dan kematian. Sepsis bakteri, khususnya yang disebabkan oleh *Escherichia coli*, menimbulkan risiko signifikan karena potensinya merusak jaringan dan organ, termasuk hati. Hati memainkan peran sentral dalam proses metabolisme dan pertahanan tubuh selama sepsis, sehingga menjadi organ yang penting untuk diteliti. Penelitian ini bertujuan untuk menyelidiki pengaruh ekstrak daun *Paederia foetida* terhadap berat hati pada model sepsis mencit. Mencit dibagi menjadi enam kelompok: kontrol normal (N), kontrol negatif (K-) yang diberikan air suling, kontrol positif (K+) yang diberikan ciprofloxacin, perlakuan 1 (P1) yang diberikan ekstrak *P. foetida* dosis 100 mg/kg BB, perlakuan 2 (P2) dengan dosis 300 mg/kg BB, dan perlakuan 3 (P3) dengan dosis 500 mg/kg BB. Setelah 14 hari perlakuan, terdapat perbedaan signifikan pada berat hati antar kelompok, dengan rata-rata dan standar deviasi tertinggi tercatat pada kelompok P1 (1,3750 ± 0,3932). Kelainan pada hepar seperti pembengkakan, penebalan lobulus, dan

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peningkatan berat hati teridentifikasi, yang menunjukkan respons adaptif hati terhadap zat toksik selama sepsis. Menariknya, kelompok kontrol normal menunjukkan bobot hepar yang lebih tinggi dibandingkan kelompok perlakuan, yang kemungkinan disebabkan oleh akumulasi lemak dalam jaringan hepar. Temuan ini menunjukkan bahwa ekstrak *P. foetida* dapat mempengaruhi perubahan berat hati pada kondisi sepsis, dengan potensi memodulasi proses metabolik dan detoksifikasi. Studi lanjutan yang berfokus pada mekanisme histopatologis dan biokimia diperlukan untuk memperjelas potensi terapeutik *P. foetida* dalam mengatasi disfungsi hati yang terkait dengan sepsis.

Kata kunci: *Ekstrak Paederia foetida, Bobot hepar, Mencit, Sepsis, Escherichia coli*

INTRODUCTION

Sepsis is a critical medical emergency characterized by a systemic immune response to infection, often resulting in severe organ dysfunction and death (Gyawali et al., 2019). Globally, diarrheal diseases and lower respiratory tract infections are major contributors to sepsis cases and related fatalities, with an estimated 9.2 to 15 million annual cases attributed to diarrheal diseases in 2017 (Lawn et al., 2017; Stoll et al., 2011). Among the diverse pathogens responsible for sepsis, *Escherichia coli* poses a significant threat due to its increasing prevalence and virulence (Silaban, 2021).

Sepsis caused by bacterial infection occurs when the body's immune response to the pathogen inadvertently causes harm to its own tissues and organs (Singer et al., 2016). *E. coli*, a Gram-negative bacterium, is frequently isolated from the bloodstream and is the most common causative agent of bacteremia in adult patients (Mora-Rillo et al., 2015). Alarmingly, *E. coli* often carries the beta-lactamase gene on its plasmid, conferring resistance to antibiotics such as extended-spectrum cephalosporins and aztreonam (Paterson and Bonomo, 2005).

A key pathogenic feature of *E. coli* is its lipopolysaccharide (LPS) content, a primary component of the outer membrane of Gram-negative bacteria. LPS, specifically lipid A, becomes toxic when released into the bloodstream, triggering an immune response that leads to systemic inflammation (Guntur, 2006). LPS interacts with circulating proteins and macrophage receptors, initiating a cascade of cytokine release, complement activation, and coagulation pathways. Clinically, this sequence manifests as fever, leukopenia, hypoglycemia, hypotension, shock, intravascular coagulation, and ultimately, organ dysfunction or death (Brooks et al., 2003).

In sepsis, bacterial translocation—the movement of microorganisms across the intestinal barrier into mesenteric lymph nodes and other organs such as the liver and spleen—further exacerbates immune responses (Alexander et al., 1990). Delayed neutrophil apoptosis intensifies inflammation, promoting the development of Multiple Organ Dysfunction Syndrome (MODS), a hallmark of severe sepsis.

The liver plays a pivotal role in metabolic regulation and host defense during sepsis. It filters, detoxifies, and eliminates bacteria, endotoxins, and inflammatory mediators from the bloodstream while producing cytokines, acute-phase proteins, and bioactive lipids to modulate inflammation. Early in sepsis, hepatic dysfunction emerges due to reduced blood flow, leading to structural and functional impairments (Sumantri, 2012). As sepsis progresses, the liver's capacity to eliminate toxins diminishes, perpetuating the release of bacteria, endotoxins, and inflammatory molecules, which exacerbates multi-organ damage.

Paederia foetida L., known locally as sembukan leaf in East Java, has been traditionally used to treat digestive disorders such as diarrhea. The therapeutic potential of *P. foetida* is attributed to its secondary metabolites, including alkaloids, saponins, tannins, and flavonoids, which offer diverse pharmacological benefits. These compounds exhibit antioxidant, antimicrobial, anticancer, anti-inflammatory, antitumor, and immunomodulatory properties, making *P. foetida* a promising candidate for managing inflammation and organ dysfunction associated with sepsis (Savitri and Kasimo, 2022).

MATERIALS AND METHODS

Mice that had been adapted were given treatment for 14 days with the following variations: 1) group 1 as normal control (N), namely mice that were not given gastric tube, 2) group 2 as negative control (K-), namely mice given distilled water with volume 0.5 mL, 3) group 3 as positive control (K+), i.e. mice were given ciprofloxacin at a dose of 500 mg/kgBW with a volume of 0.26 mL, 4) group 4 as treatment 1 (P1), i.e. mice were given 100 mg/kgBW *P. foetida* extract with a volume of 0.5 mL, 5) group 5 as treatment 2 (P2), namely mice were given 300 mg/kgBW *P. foetida* extract with a volume of 0.5 mL, 6) group 6 as a treatment 3 (P3), namely mice given 500 mg/kgBW *P. foetida* extract with a volume of 0.5 mL.

The treated mice were injected into their peritoneum with *E. coli* at a dose of $1x10^5$ CFU/mL. Mice after 24 hours after exposure to septic polymicrobials will show apoptotic events in the liver, so that after 24 hours the mice can be killed. If the mice die before 24 hours, surgery must be carried out immediately to remove the liver so that autolysis does not occur. The organ slices taken were in the middle, left and right edges.

Following the completion of the treatment period, mice were euthanized under appropriate anesthesia to minimize stress and ensure ethical handling. The liver was carefully excised through a midline abdominal incision to avoid tissue damage. Once removed, the liver samples were rinsed gently with cold saline solution to eliminate blood and other contaminants without compromising tissue integrity. Excess moisture was blotted using sterile filter paper to ensure consistent sample preparation.

The liver was then weighed immediately using a calibrated analytical balance to ensure precision, and measurements were recorded in grams. To maintain consistency and reproducibility, all liver samples were processed under the same conditions (e.g., temperature, timing of weighing) and handled by the same researcher. The procedure was performed in triplicate for each sample, and the average weight was recorded to minimize variability.

These liver weight data were subjected to statistical analysis using One Way ANOVA at a confidence level of 95% (α=0.05). If the ANOVA results showed statistical significance, further analysis was conducted using Tukey's HSD post hoc test at a confidence level of 95% (α=0.05). The statistical analysis was performed using SPSS 23.0 software for Windows.

RESULTS AND DISCUSSION

The findings from the conducted research indicate that the treatment group with the highest mean value and standard deviation is P1, with a recorded value of 1.3750 ± 0.3932. On the other hand, the treatment group with the lowest mean value and standard deviation is K+, with a value of 1.1400 ± 0.1639. The mean values and standard deviations for all the treatment groups are presented in Table 1 and Picture 1.

Table 1. Infection The Mean and Standard Deviation of the Treatment Group

Groups	Number of Observations (n)	Mean±SD
N	6	1.2067±0.1695
K-	6	1.3617±0.2049
K+	6	1.1400±0.1639
P1	6	1.3750±0.3932
P2	6	1.1967±0.2068
P3	6	1.1733±0.1687

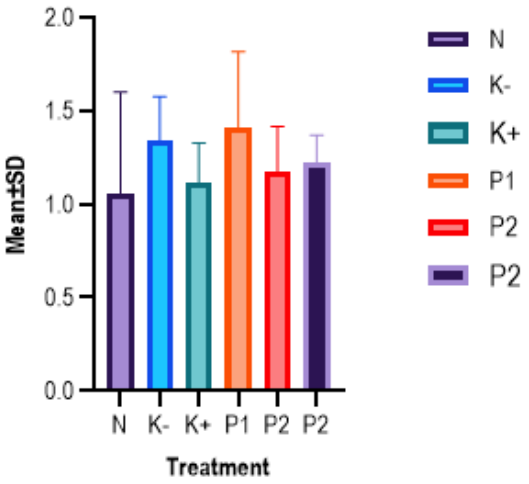

Figure 1. The Graph of Mean and Standard Deviation of the Treatment Group

The gathered data was subjected to statistical analysis employing One Way Single Variant Analysis (One Way ANOVA) at a 95% confidence level (α = 0.05). If the ANOVA calculation demonstrated statistical significance, subsequent analysis was conducted using the Least Significant Difference (LSD) test at a confidence level of 95% (α = 0.05). The statistical analysis was performed using SPSS 23.0 software for Windows, and the outcomes are displayed in Table 2.

Table 2. ANOVA One Way

Source of Variation	Sum of Squares	Mean Square	Variance	F
Between Groups	1.6886	0.3377	0.0185	8.5350
Within Groups (Er-ror)	0.9337	0.1556		
Total	2.6223			

The analysis using One Way ANOVA indicated a significant difference among the treatment groups, with an F-ratio value and p-value of 0.0005. The standard deviation was calculated for each group to assess the variability within them. Post hoc analysis using Tukey's HSD test identified several significant differences between the groups. Specifically, a significant difference was observed between group N and group P1 (p = 0.0369), indicating that the average liver value in group N differed significantly from that in group P1.

Furthermore, significant differences were found between the K- group and the K+, K-P2, K-P3, and K+-K+ groups, with p-values below 0.05. This suggests significant variations in mean liver values among these groups. Similarly, the K+ group showed significant differences in mean liver values compared to the P2, P3, and K+-P2 groups, with p-values below 0.05. No significant differences were detected between the N group and the K- and K+ groups. Likewise, there were no significant differences between the K- group and the P1 and P2 groups, as well as between the K+ group and the P1 and P3 groups. In conclusion, there are significant differences in liver data 1-6 among the groups. Further analysis could be conducted to gain a more comprehensive understanding of these differences within each group.

The liver is one of the organs in the body that functions as a detoxification tool so that the liver is very susceptible to toxic substances. Previous research stated that red calliandra leaf extract contains compounds that can damage liver cells including alkaloids, saponins, and caffeic acid (Onyeama et al., 2012; Moharram et al., 2006). This can be related to *P. foetida* leaf extract which contains the same compound, so it can be seen that *P. foetida* leaf extract has a risk that can damage liver cells.

A similar study using neem leaf extract conducted by Kupradinun et al. (2012) stated that the side effects of neem are thought to cause structural damage to the liver and kidneys. Sitaswi et al. (2018) has proven that the ethanol extract of neem leaves causes an increase in liver weight. Ghimeray et al. (2009) stated that neem leaf extract at 200 g/kg body weight can cause weight loss in animals accompanied by symptoms such as weakness, anorexia and histopathological defects. Omotayo et al. (2012) has proven that the ethanol extract of neem bark causes an increase in the ratio of body weight to liver weight of rats.

The toxicology of alkaloids, saponins, and caffeic acid to organs is still being debated because several studies conducted on alkaloids, saponins, and caffeic acid have shown benefits for the body. However, other studies have shown that these three compounds can damage cells and tissues and even cause death in experimental animals. Pyrrolizidine alkaloids can cause liver enlargement (hepatomegaly) (Hanafi, 2023). According to Irfai (2013), abnormalities in the liver are characterized by an increase in the size and weight of the liver where swelling and thickening occurs in one of the liver lobules. In addition, the liver will work harder so that these toxic substances do not damage the body so that the weight of the liver will increase. According to Anggraini (2008), if fat degeneration occurs in the liver, it will result in weight gain of the liver. In this study, the liver in the treatment group was heavier than the control, besides that fatty degeneration also occurred. The increase in weight that occurs is caused by fatty substances found in the tissues so that it can affect the total weight of the liver.

While this study demonstrates the effect of *Paederia foetida* L. extract on liver weight in a murine sepsis model infected with *Escherichia coli*, its limitations include the lack of detailed histopathological and biochemical analyses to identify underlying mechanisms of liver weight changes. Further research involving histological evaluations, inflammatory marker profiling, and dose-response studies is essential to better understand the therapeutic potential and safety of *P. foetida* in managing sepsis-induced liver dysfunction.

CONCLUSION

Based on the research it is known that there are significant differences between the treatment groups. This can be explained further by conducting a more in-depth analysis of each group. The highest mean and standard deviation values were 100 mg/kg BW with a volume of 0.5 mL for 14 days, namely 1.3750 ± 0.3932. Abnormalities in the liver are characterized by an increase in the size and weight of the liver where there is swelling and thickening of one of the liver lobules. In addition, the liver will work harder so that these toxic substances do not damage the body so that the weight of the liver will increase. In addition, if there is degeneration of fat in the liver, it will result in an increase in the weight of the liver. In this study, the liver in the normal group was heavier than the treatment group. The increase in weight that occurs is caused by fatty substances found in the tissues so that it can affect the total weight of the liver.

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