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Histopathological Description of Mouse Liver in a Sepsis Model Infected with *Escherichia coli* Treated with *Paederia foetida* L. Leaf Extract for Sepsis Prevention

Lisa Savitri*, Ana Retnowati, Elfred Rinaldo Kasimo, Rochmad Krissanjaya, Syntia Tanu Juwita
Department of Medical Laboratory Technology, Faculty of Health Sciences, Kadiri University, Jalan Selomangleng No. 1, Kediri, East Java, Indonesia.

Corresponding author*
lisasavitri@unik-kediri.ac.id

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Abstract

The leaf of *Paederia foetida* L. is one type of medicinal plant that can be used as a preventive medicine for sepsis. This plant contains secondary metabolites such as alkaloids, flavonoids, triterpenoids, saponins, and other active compounds. The objective of this study was to determine the histopathological description of the liver in a mouse sepsis model infected with *E. coli*, with the administration of *Paederia foetida* L. leaf extract for sepsis prevention, and to ascertain the influence and effective dosage of the leaf extract as a preventive measure against liver histopathology in the sepsis model induced by *E. coli*. The method employed was a Completely Randomized Design (CRD). The study used 24 male white mice divided into 6 (six) groups. Data analysis was conducted using One Way ANOVA. The results of the study revealed the histopathological profile of liver cell degeneration in group PI (100mg/kg BW) at 20.79%±0.03, group PII (200mg/kg BW) at 21.63%±0.02, and group PIII (500mg/kg BW) at 9.08%±0.02. Necrosis rates were observed in group PI (100mg/kgBW) at 22.62%±0.04, group PII (200mg/kg BW) at 17.63%±0.02, and group PIII (500mg/kg BW) at 6.05%±0.02. The presence of polymorphonuclear leukocytes (PMN) was detected in group PI (100mg/kgBW) at 39.56%±0.03, group PII (200mg/kgBW) at 28.05%±0.02, and group PIII (500mg/kg BW) at 18.45%±0.03. The test results showed a significant effect of *P. foetida* L. leaf extract as a preventive measure against liver histopathology in the mouse sepsis model infected with *E. coli*, with significant values for necrosis (p=0.000), cell degeneration (p=0.000), and PMN (p=0.000). The most effective dosage of *P. foetida* L. leaf extract as a preventive measure against liver histopathology in the mouse sepsis model infected with *E. coli* was the dosage used in group PIII (500mg/kgBW).

Keywords: Kentut leaves; liver histopathology; sepsis; *Escherichia coli*.

Abbreviations: ANOVA (Analysis of Variance), Completely Randomized Design (CRD), polymorphonuclear leukocytes (PMNs)

INTRODUCTION

Sepsis is defined as life-threatening organ dysfunction caused by a dysregulated host response to infection (Purwanto & Astrawinata, 2018). Sepsis represents a systemic response to infection within the body that can progress to severe sepsis and septic shock (Singer et al., 2016). It can be a severe and potentially life-threatening medical condition caused by a widespread infection in the body. Sepsis can occur when the body's response to an infection becomes uncontrolled. This unhealthy response can lead to tissue and organ damage, potentially resulting in sepsis (Jarczak et al., 2021). In 2017, WHO estimated there were 48.9 million cases and 11 million deaths related to sepsis worldwide, contributing to nearly 20% of all global deaths. Almost half of all global sepsis cases occur in children, with an estimated 20 million cases and 2.9 million deaths globally in children under 5(five) years old. WHO found significant regional disparities in sepsis incidence and mortality, with approximately 85.0% of sepsis cases and sepsis-related deaths occurring in low- and middle-income countries (WHO, 2021). Severe sepsis and septic shock are leading causes of death in critically ill patients treated in intensive care units (ICUs) in the United States. Meta-analysis research findings indicate that sepsis incidence ranges from 22 to 240 cases per 100,000 people, severe sepsis from 13 to 300 cases per 100,000 people, and septic shock at 11 cases per 100,000 people, with mortality rates reaching 30% for sepsis, 50% for severe sepsis, and 80% for septic shock (Purwanto & Astrawinata, 2018).

Microorganisms associated with pathogens causing sepsis and septic shock include Gram-positive, Gram-negative bacteria, and fungi. Microbial entry into the bloodstream is not fundamental to the onset of severe sepsis, as local infections with bacteria producing pathogenic products like exotoxins can trigger systemic inflammatory responses leading to organ dysfunction and hypotension (Purwanto & Astrawinata, 2018). The liver

is the largest gland located in the right upper abdomen. Liver damage often occurs due to ineffective protozoa attacking erythrocytes indirectly, first developing within hepatocyte cells, leading to cell death. This mechanism causes hepatocyte cells to undergo damage in the form of cell degeneration, which if not promptly addressed, can progress to irreversible cell death or necrosis. Acute infections result in the infiltration of inflammatory cells, namely polymorphonuclear leukocytes (PMNs). This cell damage can be observed by directly examining the histopathological appearance of the mouse liver organ under a microscope (Dwiridal et al., 2021).

Severe sepsis occurs due to infections acquired from the community and healthcare settings. Gram-negative bacteria that can cause such infections include *E. coli* (Angus & Poll, 2015). *Escherichia coli* is a facultative anaerobic bacterium that can grow under aerobic and anaerobic conditions. Antibiotic therapy must be administered promptly after a sepsis diagnosis is established using a de-escalation strategy, starting with empirical antibiotic treatment and then adjusting or discontinuing based on clinical response or culture results. Empirical antibiotic therapy involves broad-spectrum antibiotics that can be administered singly or in combination, targeting a range of potential pathogens based on clinical syndromes and previously collected pathogen patterns (antibiogram) (Kemenkes RI, 2017). However, an increasing number of people are becoming aware of the side effects of antibiotic drugs, leading to a growing interest in herbal remedies. Traditional medicine remains popular for treating diseases due to its easy availability of raw materials, affordability, and potential for self-cultivation. A traditional plant historically trusted by communities for anti-inflammatory purposes is kentut leaves (Silaban, 2021). Kentut leaves, have the Latin name *P. foetida* L. and exhibit antibacterial activity against *E. coli*. This is due to the presence of secondary metabolites in kentut leaves such as α and β paederine alkaloids, flavonoid flavanol, friedelin triterpenoid, β-sitosterol, campesterol, triterpenoid saponin, gallotannin, and other active compounds. Ethanol 96% and methanol maceration of kentut leaves result in alkaloid, saponin, tannin, and flavonoid compounds (Salamah & Halim, 2021).

MATERIALS AND METHODS

Research Type

This study falls under experimental research using a Completely Randomized Design (CRD) method with six treatments conducted over 15 days. The test animals used in this research were randomly selected white male mice, ensuring that all mice had an equal chance of being sampled for treatment. This research aimed to determine the antiseptic activity of ethanol extract from kentut leaves in white male mice. In this study, a total of 24 white male mice were used, divided into 6 (six) groups, with each group consisting of 4 mice.

Tools and Materials

Tools

Digital scale, filter paper/cloth, oral gavage, Pyrex beaker glass, stirring rod, spatula, glass funnel, mortar and pestle, measuring flask (100mL, Pyrex), maceration vessel (glass jar), feeding tube syringe (3mL), tweezers, surgical knife, pin needle, urine pot, oven, tissue processor, microtome, water bath, tray, a set of hematoxylin and eosin staining tubes, prepared tissue slides, microscope, tissue cassette, glass slides, coverslips, organ measuring tools, and microtome knife.

Materials

Kentut leaf extract, white male mice, *E. coli* bacterial liquid media, 10% Neutral Buffered Formalin, hematoxylin stain, and eosin stain.

Procedure

Kentut Leaf Determination

Plant determination was performed at the UPT Herbal Materia Medica Laboratory in Batu, Jl. Lahor 87, Batu City, East Java Province. The purpose of plant determination was to confirm the identity of the kentut leaves used in this study. Based on the determination results, it was confirmed that the leaves used in this study were kentut leaves.

A Sampling of Kentut Leaves

The leaf samples used were collected from Mlaten Hamlet, Ngrami-Sukomoro Village, Nganjuk Regency.

Preparation of Extract

The kentut leaf extract was prepared using the maceration method with 96% ethanol. A total of 500 grams of kentut leaf powder was mixed with 1 litre of 96% ethanol (1:10 ratio) and left to stand for 72 hours. The filtrate was then filtered using filter paper to obtain the filtrate, and the resulting macerate was evaporated using an oven at approximately 40°C.

Preparation of Experimental Animals

The experimental animals used were healthy male mice aged 3-4 weeks with a body weight of 30-40 grams. Before use, the male mice were acclimatized for 14 days under experimental conditions.

Preparation of Materials

The materials used included distilled water as a negative control, ciprofloxacin suspension as a positive control, untreated normal mice, ethanol extract suspension of kentut leaves as the test material, and *E. coli* bacteria as the sepsis inducer.

Antiseptic Activity Testing of Kentut Leaf Ethanol Extract

In this study, a total of 24 white male mice were used and divided into 6 (six) groups, with each group consisting of 4 white male mice.

Table 1. Treatment Groups.

No.	Group	Treatment
1.	Group 1	Served as negative control (-) receiving aqua dest
2.	Group 2	Served as positive control (+) receiving Ciprofloxacin suspension
3.	Group 3	Served as standard control without treatment
4.	Group 4, 5, and 6	Treatment groups receiving ethanol extract of parsley leaf at doses of 100 mg/kg BW, 300 mg/kg BW, and 500 mg/kg BW

In conducting this experiment, 24 white male mice were randomly divided into 6 (six) groups. Each group received different treatments: Group 1 received distilled water orally as a negative control, Group 2 received Ciprofloxacin suspension as a positive control, and Group 3 received no treatment. Meanwhile, Groups 4, 5, and 6 were administered kentut leaf ethanol extract orally at doses of 100 mg/kg BW, 300 mg/kg BW, and 500 mg/kg BW, respectively. The researcher repeated these actions daily for 15 consecutive days. After 15 days of treatment in the experimental groups, on day 16, the mice were injected with *E. coli* bacteria into the peritoneal cavity to induce sepsis. The mice were then observed for 24 hours after *E. coli* injection, followed by surgery to extract the liver organ (hepar). The parameters observed in this study included normal cells, cell degeneration, necrosis, and polymorphonuclear leukocytes (PMN).

Data Analysis

Data analysis in this study was performed descriptively using qualitative methods presented in tables and diagrams to understand the histopathological profile of the mouse liver in the sepsis model induced by *E. coli*, with the administration of kentut leaf extract for sepsis prevention. Data analysis was conducted using Microsoft Excel 365 software, evaluating the level of damage or changes in the histopathological profile of the liver in each group of mice using a microscope at 10X and 40X magnifications with 5 (five) fields of view. The assessment was based on hepatocyte cells showing normalcy, degeneration, necrosis, and PMN. The observations were totalled, averaged, and compared across each treatment group.

The percentage of liver organ damage in mice was calculated using the formula by Baldatina (Dwiridal et al., 2021). Further data analysis utilized One-Way ANOVA (Analysis of Variance). Before applying One-Way ANOVA, normality and homogeneity tests were conducted. If the data were normally distributed and homogeneous, One-Way ANOVA was used to determine significant differences among the test groups, with significance indicated by <0.05 (considered significant) or >0.05 (considered not significant). If significant differences were found among the test groups, Post Hoc LSD (Least Significant Difference) tests ($p<0.05$) and Duncan's Post Hoc tests were conducted to identify significant differences among individual groups. If the data were not normally distributed, Kruskal-Wallis tests in SPSS were employed to determine significant differences among two or more test groups, with significance indicated by <0.05 (considered significant) or >0.05 (considered not significant). If <0.05, Mann-Whitney tests were performed to identify specific groups with significantly different values (Ghozali, 2009).

RESULTS AND DISCUSSION

Histopathological Description of Liver

The testing of kentut leaf extract (*P. foetida* L) for its antiseptic properties was conducted by observing the histopathological profile of the liver in a sepsis model induced by *E. coli* injection in mice. In this study, the researchers utilized six (6) groups, with each group consisting of 4 mice. Observations of the histopathological profile of the liver in the sepsis model injected with *E. coli* included cell degeneration, necrosis, and polymorphonuclear leukocytes (PMN) (see Table 2).

Table 2. Histopathological Profile of the Liver in the *E. coli*-induced Sepsis Model.

Group Mean	Degeneration (%)		Necrosis (%)		PMN (%)	
	SD	Mean		Mean	SD	Mean
Normal	2.21%	0.02	2.44%	Normal	2.21%	0.02
Positive (+)	7.72%	0.04	5.18%	Positive (+)	7.72%	0.04
Negative (-)	23.41%	0.04	21.55%	Negative (-)	23.41%	0.04
PI (100mg/kgBW)	20.79%	0.03	22.62%	PI (100mg/kgBW)	20.79%	0.03
PII (300mg/kgBW)	21.63%	0.02	17.20%	PII (300mg/kgBW)	21.63%	0.02
PIII (500mg/kgBW)	9.08%	0.02	6.05%	PIII (500mg/kgBW)	9.08%	0.02

In Table 2, the research results show the histopathological profile of the liver in terms of cell degeneration, necrosis, and polymorphonuclear leukocytes (PMN) in a sepsis model induced by *E. coli* injection.

Cell Degeneration

The study found that the standard group had the smallest average percentage of cell degeneration, at 2.21%±0.02. The positive control group (+) had the second most mirror average at 7.72%±0.04. The group treated with 500 mg/kg BW of kentut leaf extract (Group PIII) had the third smallest average at 9.08%±0.02. Group PIII (500mg/kgBB) also showed the smallest average compared to the other two groups treated with kentut leaf extract.

Necrosis

The normal group had the smallest average percentage of necrosis, at 2.44%±0.02. The positive control group (+) had the second smallest average at 5.18%±0.02. The group treated with 500 mg/kgBW of kentut leaf extract (Group PIII) had the third smallest average at 6.05%±0.02. Similar to cell degeneration, Group PIII (500mg/kgBB) exhibited the smallest average compared to the other groups treated with kentut leaf extract.

Polymorphonuclear leukocytes (PMN)

The normal group had the smallest average percentage of PMN, at 1.62%±0.02. The positive control group (+) had the second smallest average at 16.07%±0.03. The group treated with 500 mg/kgBW of kentut leaf extract (Group PIII) had the third smallest average at 18.45%±0.03. Like the other parameters, Group PIII (500mg/kgBB) showed the smallest average compared to the other groups treated with kentut leaf extract.

Statistical Analysis

Normality tests resulted in p-values of 0.980 for the normal group, 0.807 for the positive control group (+), 0.428 for the negative control group, 0.663 for Group I (100mg/kgBW), 0.662 for Group II (300mg/kgBW), and 0.330 for Group III (500mg/kgBW). All p-values were >0.05, indicating that the data were normally distributed and suitable for further testing. Homogeneity tests across all six groups yielded a significance value of 0.574 (>0.05), indicating homogeneous variance among the treatment groups and meeting the requirements for One-way ANOVA testing. One-Way ANOVA testing resulted in a significance value of 0.000 (<0.005), indicating a significant difference among the groups. Post hoc tests (LSD test and Duncan test) showed significant differences between the treatment groups ($p<0.05$), except for the comparison between Group I (100mg/kgBW) and Group II (300mg/kgBW) with a significance value of 0.205 (>0.05), suggesting similar antiseptic effects in terms of cell degeneration between these two groups.

Table 3. Effectiveness of Kentut Leaf Extract as Prevention Against Hepatic Cell Degeneration in a Sepsis Model of Mice.

Group	a	b	c	d	e
Normal	2.21%				
Positive Control		7.64%			
Treatment III (500mg/kgBW)			9.55%		
Treatment I (100mg/kgBW)				20.79%	
Treatment II (300mg/kgBW)				21.65%	
Negative Control					23.49%

The Duncan test (Table 3) was used by the researchers to assess the effectiveness and determination of the optimal dosage among the groups. The results of the Duncan test in the table indicate that overall comparisons between the treatment groups are in different columns, suggesting that the comparisons between these treatment groups have different effects on preventing cell degeneration in the liver of the sepsis model mice. However, the comparison between Group I (100mg/kgBW) and Group II (300mg/kgBW) falls within the same column. This result aligns with the LSD test earlier, indicating that Group I (100mg/kgBW) and Group II (300mg/kgBW) have similar antiseptic effects in terms of cell degeneration.

The best dosage or dose effectiveness for antiseptic activity in terms of cell degeneration is based on the smallest values or leaning towards the left because antiseptic effectiveness is determined by looking at the average cell degeneration. Based on the Duncan test table, the sequence of the best doses is as follows: (1) normal group; (2) positive control group (+); (3) Group III treatment (500mg/kgBW); (4) Group I treatment (100mg/kgBW); (5) Group II treatment (300mg/kgBW); and (6) negative control group (-).

Necrosis

The normality test results for the normal group yielded a p-value of 0.338, the positive control group (+) had a p-value of 0.975, the negative control group had a p-value of 0.314, Group I treatment (100mg/kgBW) had a p-value of 0.636, Group II treatment (300mg/kgBW) had a p-value of 0.608, and Group III treatment (500mg/kgBW) had a p-value of 0.662. The normality test results for all six groups collectively showed a significance value greater than 0.05 (P>0.05), indicating that the data are normally distributed and suitable for further testing. The homogeneity test results for the six treatment groups yielded a significance value of 0.129

(>0.05), indicating homogenous variance among the treatment groups and meeting the requirements for One-Way ANOVA testing. The One-Way ANOVA test yielded a significance value of 0.000 (<0.005), indicating a significant difference among the groups. Further analysis, including post hoc tests using the LSD test and the Duncan test, is warranted.

Table 4. Effectiveness of Cat's Whiskers Leaf Extract in Preventing Necrosis in a Sepsis Mouse Liver Model.

Group	a	b	c	d
Normal	2.44%			
Positive Control		5.19%		
Treatment III (500mg/kgBW)			6.24%	
Treatment II (300mg/kgBW)				17.16%
Negative Control			21.65%	
Treatment I (100mg/kgBW)				22.62%

The LSD test results indicate that overall comparisons between the treatment groups have a significance value of P < 0.05, suggesting that the comparisons between these treatment groups have different effects, except for the comparison between the positive control group and Group III treatment (500mg/kgBW), as well as the negative control group and Group I treatment (100mg/kgBW). In the comparison between the positive control group and Group III treatment (500mg/kgBW), the significance value obtained is 0.217 (>0.05), indicating that the positive control group and Group III treatment (500mg/kgBW) have similar antiseptic effects on necrosis. Similarly, the comparison between the negative control group and Group I treatment (100mg/kgBW) yielded a significance value of 0.251 (>0.05), indicating that the negative control group and Group I treatment (100mg/kgBW) have similar antiseptic effects on necrosis.

The Duncan test (Table 4) was used by the researchers to assess the effectiveness and determination of the best dosage among the groups. The results of the Duncan test in the table indicate that overall comparisons between the treatment groups are in different columns, suggesting that the comparisons between these treatment groups have different effects on preventing necrosis in the liver of the sepsis model mice. However, the comparison between the positive control group and Group III treatment (500mg/kgBW), and the negative control group and Group I treatment (100mg/kgBW), falls within the same column. This result aligns with the previous LSD test, indicating that the positive control group with Group III treatment (500mg/kgBW) and the negative control group with Group I treatment (100mg/kgBW) have similar antiseptic effects on necrosis.

The best dosage or dose effectiveness for antiseptic activity in terms of necrosis is based on the smallest values or leaning towards the left because antiseptic effectiveness is determined by looking at the average necrosis. Based on the Duncan test table, the sequence of the best doses is as follows: (1) normal group; (2) positive control group (+); (3) Group III treatment (500mg/kgBW); (4) Group II treatment (300mg/kgBW); (5) negative control group (-); and (6) Group I treatment (100mg/kgBW).

Polymorphonuclear Leukocytes

The normality test results for the normal group yielded a p-value of 0.370, the positive control group had a p-value of 0.868, the negative control group had a p-value of 0.613, Group I treatment (100mg/kgBW) had a p-value of 0.318, Group II treatment (300mg/kgBW) had a p-value of 0.863, and Group III treatment (500mg/kgBW) had a p-value of 0.617. The normality test results for all six groups collectively showed a significance value greater than 0.05 (P>0.05), indicating that the data are normally distributed and suitable for further testing. The homogeneity test results for the six treatment groups yielded a significance value of 0.398 (>0.05), indicating homogenous variance among the treatment groups and meeting the requirements for One-Way ANOVA testing. The One-Way ANOVA test yielded a significance value of 0.000 (<0.005), indicating a significant difference among the groups.

Further analysis, including post hoc tests using the LSD test and the Duncan test, shows that overall comparisons between the treatment groups have a significance value of P < 0.05, suggesting that the comparisons between these treatment groups have different effects. The Duncan test was used by the researchers to assess the effectiveness and determination of the best dosage among the groups. The results of the Duncan test in the table indicate that overall comparisons between the treatment groups are in different columns, suggesting that the comparisons between these treatment groups have different effects on preventing PMN in the liver of the sepsis model mice. However, the comparison between the positive control group and Group III treatment (500mg/kgBW), and the negative control group and Group I treatment (100mg/kgBW), falls within the

same column. This result aligns with the previous LSD test, indicating that the positive control group with Group III treatment (500mg/kgBW) and the negative control group with Group I treatment (100mg/kgBW) have similar antiseptic effects on PMN.

Table 5. Effectiveness of Cat's Whiskers Leaf Extract in Preventing PMN in a Sepsis Mouse Liver Model.

Group	a	b	c	d	e	f
Normal	1.62%					
Positive Control		16.07%				
Treatment III (500mg/kgBW)			18.45%			
Treatment I (100mg/kgBW)				28.05%		
Treatment II (300mg/kgBW)					39.56%	
Negative Control						48.63%

The optimal dose or dose effectiveness for PMN antisepsis is based on the smallest value or leftward direction because antisepsis is determined by looking at the average PMN. Based on the Duncan test table, the best dose sequence is as follows: (1) normal group; (2) control group (+); (3) treatment group III (500mg/kgBW); (4) treatment group II (300mg/kgBW); (5) negative control group (-); and (6) treatment group I (100mg/kgBW).

Discussion

The research results (Table 3) indicate decreased cell degeneration damage in hepatocytes after mice were infected with *E. coli* bacteria. The normal group had the smallest average at 2.21% ± 0.02, because the normal control group was not injected with *E. coli*, thus not experiencing sepsis that causes cell degeneration damage. In the sepsis-experiencing mouse group (Groups II to VI), Group II had the most effective dose among the sepsis-experiencing groups compared to the other 4 (four) groups infected with *E. coli*. Group II served as the positive control containing ciprofloxacin. In the treatment groups with parsley leaf extract, namely Treatment Group I (100mg/kg BW), Treatment Group II (300mg/kg BW), and Treatment Group III (500mg/kg BW), Treatment Group III (500mg/kg BW) was the most effective in preventing cell degeneration damage in the sepsis model mouse liver. Although Treatment Group I (100mg/kg BW) and Treatment Group II (300mg/kg BW) had different average values of cell degeneration damage, based on statistical test results, Treatment Group I (100mg/kg BW) and Treatment Group II (300mg/kg BW) had the same antisepsis effect on cell degeneration.

The effective dose of parsley leaf extract can be determined in the statistical analysis results using the ANOVA method at a 95% confidence level. The analysis results show a significant difference in the parsley leaf extract dose as a preventive measure for cell degeneration damage in the sepsis model mouse liver. The reduction in hepatocyte cell degeneration damage is suspected to be due to various active compound contents found in parsley leaves such as flavonoids and saponins. Cell degeneration damage in mice is caused by *E. coli* bacteria induced in the mice. The inflammation occurs due to the release of cytokines that can disrupt normal cellular signalling pathways in hepatocytes, including cell degeneration. Flavonoids in parsley leaves are believed to potentially prevent inflammation and protect normal hepatocytes from cell degeneration damage in the liver (Maleki et al., 2019). Flavonoids have strong antioxidant properties that help protect normal hepatocytes from oxidative stress. Oxidative stress is a condition where there is an imbalance between damaging free radical production and the body's antioxidant defences. Flavonoids help neutralize free radicals and reduce cell degeneration damage caused by oxidative stress (Panche et al., 2016). Flavonoids can inhibit the production of pro-inflammatory mediators such as cytokines and enzymes involved in the inflammatory process. By reducing inflammation, flavonoids help protect normal hepatocytes from cell degeneration damage caused by excessive inflammatory responses (Maleki et al., 2019).

Saponins in kentut leaves not only act as anti-inflammatory agents that reduce cell degeneration damage in the liver but also enhance liver cellular defence, thus helping maintain balance in the immune system and reducing inflammation that damages hepatocyte degeneration. The saponin content in kentut leaves can enhance liver detoxification systems, aiding in eliminating harmful compounds such as *E. coli* bacteria in mice. Improved liver detoxification can help protect normal hepatocytes from damage caused by toxic substances and inflammation (Wang et al., 2019).

Research results (Table 4) indicate a decrease in cell degeneration damage in hepatocytes after mice were infected with *E. coli* bacteria. The normal group had the smallest average at 2.21% ± 0.02 because the normal control group was not injected with *E. coli*, thus not experiencing sepsis that leads to necrosis. In the sepsis-experiencing mouse group (Groups II to VI), the positive control (+) group and Treatment Group III (500mg/kgBW) showed similar effects on liver necrosis. It was the most effective doses among sepsis-

experiencing mice compared to other groups infected with *E. coli*. In the treatment groups with parsley leaf extract, namely Treatment Group I (100mg/kgBW), Treatment Group II (300mg/kgBW), and Treatment Group III (500mg/kgBW), Treatment Group III (500mg/kgBW) was the most effective in preventing cell degeneration damage in the sepsis model mouse liver. In Treatment Group I (100mg/kgBW), based on statistical test results, it was found to be equally effective as the negative control (-) group in preventing necrosis.

The reduction in necrosis damage in hepatocytes is suspected to be due to various active compound contents found in parsley leaves such as flavonoids, saponins, and tannins. Necrosis damage in the sepsis model mouse liver is caused by *E. coli* bacteria induced in the mice. The reduction in necrosis damage is likely due to the flavonoid content in parsley leaves, which can influence gene expression in the inflammatory pathway, thereby reducing the production of molecules that cause excessive inflammation in sepsis-induced mouse livers. Flavonoids can protect hepatocytes from direct damage and necrosis by stabilizing cell membranes and reducing oxidative damage caused by *E. coli* infection leading to liver injury (Tan et al., 2022).

The strong reduction in necrosis damage is also suspected to be due to the saponin content in parsley leaves. Saponins in parsley leaves have detoxifying effects on the liver. Saponins are believed to help eliminate necrosis in *E. coli*-infected mouse livers and reduce liver workload, thereby reducing the risk of necrosis in sepsis-induced mouse livers. Saponins can affect the immune response, including the immune system in the liver. By regulating immune responses, saponins can help control excessive inflammatory reactions that can cause necrosis in sepsis-induced mouse livers (Waheed et al., 2012).

Kentut leaves containing saponins can function as inhibitors of inflammatory enzyme activity and modulate cellular signalling pathways involved in inflammation and necrosis in the liver. Saponins inhibit the production of inflammatory mediators that can reduce inflammation in the liver caused by *E. coli* infection and protect liver cells from damage leading to necrosis. Tannins inhibit the activity of pro-inflammatory enzymes such as pro-inflammatory cytokines and other inflammatory mediators, thereby reducing the production of inflammatory mediators and helping prevent excessive inflammatory responses that can cause necrosis in sepsis-induced mouse livers (Fikru et al., 2012).

Research results (Table 5) indicate a decrease in damage to PMN in hepatocytes after mice were infected with *E. coli* bacteria. The normal group had the smallest average at 1.62% ± 0.02, because the normal control group was not injected with *E. coli*, thus not experiencing sepsis that leading to PMN increase. In the sepsis-experiencing mouse group (Groups II to VI), Group II was the most effective dose among sepsis-experiencing mice compared to the other 4 groups infected with *E. coli*. Group II served as the positive control containing ciprofloxacin. Among the treatment groups with parsley leaf extract, Treatment Group III (500mg/kgBW) was the most effective in preventing PMN increase in the sepsis model mouse liver.

PMN refers to granulocyte leukocytes or polymorphonuclear white blood cells, a type of white blood cell that is part of the immune system. Tannins in parsley leaves are believed to modulate the immune system by affecting immune responses and regulating the activity of immune cells in PMN to reduce excessive inflammation and prevent cell damage caused by *E. coli* infection in sepsis-induced mouse livers (Chen et al., 2023). Alkaloids in parsley leaves are also believed to play a role in inhibiting the release of inflammatory mediators such as pro-inflammatory cytokines by PMN in inflammatory responses, thereby helping reduce excessive inflammation and prevent tissue damage. Alkaloids can affect PMN activity, including migration and the release of proteolytic enzymes. By regulating PMN activity, alkaloids can control inflammatory responses involving PMN cells (Bai et al., 2021).

CONCLUSIONS

The research results show the histopathological picture of liver cell degeneration in Group PI (100mg/kgBW) at 20.79%±0.03, Group PII (200mg/kgBW) at 21.63%±0.02, and Group PIII (500mg/kgBW) at 9.08%±0.02. For necrosis, Group PI (100mg/kgBW) exhibited 22.62%±0.04, Group PII (200mg/kgBW) showed 17.63%±0.02, and Group PIII (500mg/kgBW) had 6.05%±0.02. Regarding PMN, Group PI (100mg/kgBW) recorded 39.56%±0.03, Group PII (200mg/kgBW) registered 28.05%±0.02, and Group PIII (500mg/kgBW) showed 18.45%±0.03. Statistical tests indicate the effect of parsley leaf extract as a preventative measure against liver histopathology in a sepsis model of *E. coli*-infected mice, with significant values for necrosis (p=0.000), cell degeneration (p=0.000), and PMN (p=0.000).

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