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Leaf Extract of Kentut (*Paederia foetida* L.) as a Preventive Measure Against Interleukin-6 Expression in the Liver of Mice in a Sepsis Model Injected with *Escherichia coli*

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Abstract

Kentut leaves (*Paederia foetida* L.) are a medicinal plant that can be used as a preventative medicine against sepsis. This plant contains secondary metabolite compounds such as alkaloids, flavonoids, triterpenoids, saponins, and other active compounds. This research aims to to determine the influence and effective dosage of Kentut leaf extract as a preventive measure against IL-6 expression in the livers of mice in a sepsis model injected with *E. coli*. The method used was a Completely Randomized Design (CRD). The study involved 24 white male mice divided into 6 groups. Data analysis was performed using One way ANOVA. The average values of IL-6 expression in the mouse livers for each group are as follows: KN at 7.09%±0.06; K+ at 26.36%±0.02; K- at 72.60%±0.05; PI (100mg/kgBW) at 71.04%±0.04; PII (300mg/kgBW) at 62.22%±0.02; and PIII (500mg/kgBW) at 40.92%±0.01. The research results indicate an influence of kentut leaf extract as a preventive measure against IL-6 expression in the livers of mice in the sepsis model injected with *E. coli*, with a significance value of 0.000 or p-value < 0.005. The effective dosage of kentut leaf extract as a preventive measure against IL-6 expression is the PIII dosage of 500mg/kg BW. The anti-inflammatory mechanism in sepsis is thought to be caused by the presence of flavonoids, alkaloids, phenolic acids, and terpenoid compounds. The most likely anti-inflammatory mechanism is believed to involve flavonoids inhibiting cyclooxygenase (COX) and lipoxygenase (LOX) enzymes involved in the synthesis of inflammatory mediators such as prostaglandins and leukotrienes, which can trigger IL-6 production.

Keywords: Kentut leaves; IL-6; sepsis; *E. coli*.

Abbreviations: Analysis of Variance (ANOVA); cyclooxygenase (COX); Completely Randomized Design (CRD); Diamono Benzidine (DAB); histone deacetylase (HDAC); Janus kinase (JAK); lipoxygenase (LOX); Least Significant Difference (LSD); mitogen-activated protein kinase (MAPK); prostaglandin H2 (PGH2); phospholipase A2 (PLA2); Strep-Avidin horse radish peroxidase (SA-HRP); systemic inflammatory response syndrome (SIRS); signal transducer and activator of transcription (STAT); Toll-like receptors (TLRs).

INTRODUCTION

Sepsis is a medical emergency condition that describes the body's systemic immune response to an infection process that can lead to end-stage organ dysfunction and death (Turnip et al., 2022). It manifests as a systemic response to an infection within the body, which can progress to severe sepsis and septic shock. Severe sepsis and septic shock pose significant health problems and cause millions of deaths each year. Severe sepsis involves organ dysfunction due to systemic inflammation and a procoagulant response to infection. Septic shock is defined as sepsis accompanied by refractory hypotension (Singer et al., 2016). The complications caused by sepsis can include systemic inflammatory response syndrome (SIRS), which involves phases of inflammatory cytokine release as a response to infection, such as interleukin (IL-6), disseminated intravascular coagulation (DIC), septic shock, and multi-organ failure, including the liver (Kemenkes, 2017).

In 2017, WHO estimated there were 48.9 million cases and 11 million sepsis-related deaths worldwide, accounting for almost 20% of all global deaths. Nearly half of all global sepsis cases occur in children, with an estimated 20 million cases and 2.9 million deaths in children under 5 (five) years old. WHO also found significant regional disparities in sepsis incidence and mortality, with around 85.0% of sepsis cases and associated deaths occurring in low- and middle-income countries (WHO, 2020). The incidence of sepsis in Indonesia remains high, reaching up to 30.29%, with mortality rates ranging from 11.56% to 49%. According to data from the medical records of Abdul Wahab Sjahranie Hospital from 2018 to 2020, there were a total

of 312 sepsis patients, including 69 cases in children. Based on data from the profile of Abdul Wahab Sjahranie Regional Hospital, Samarinda (2017), sepsis ranks among the top 10 causes of death (Verdure et al., 2021).

In sepsis, the inflammatory response to attacking pathogens involves both inflammatory and anti-inflammatory processes, humoral and cellular reactions, and circulatory abnormalities (Kaukonen et al., 2015). Several studies have detected nucleotide changes in genes encoding IL-6, resulting in polymorphisms that increase the risk factors or protection against developing sepsis, septic shock, and even death due to sepsis (Tischendorf et al., 2007). Bacteria like *E. coli* can infect hosts, leading to septic shock. The entry of microbes into the bloodstream is not fundamental to the onset of severe sepsis because local infections with bacteria producing pathogenic products like exotoxins can also trigger systemic inflammatory responses, causing organ dysfunction elsewhere and hypotension (Clarias et al., 2018).

Identifying the bacteria causing sepsis is crucial in determining causative therapy. Empirical antibiotic therapy, providing broad-spectrum antibiotics either singly or in combination, can cover various potential causative organisms based on clinical syndromes and previously gathered organism patterns (antibiogram) (Ministry of Health Indonesia, 2017). However, more people are becoming aware of the side effects of antibiotic drugs, leading to a considerable number resorting to herbal treatments due to their easy availability, affordability, and potential for self-cultivation. One traditional plant historically believed to possess anti-inflammatory properties is the kentut (*Paederia foetida* L.) leaf (Silaban, 2021). Another plant that can be used for sepsis prevention is the extract of mangosteen peel (Garcinia mangostana), which possesses antibacterial and anti-inflammatory effects from xanthone compounds (Syahruna et al., 2020). Mangosteen fruit peel also contains compounds from the flavonoid, saponin, alkaloid, triterpenoid, tannin, and polyphenol groups (Dewi et al., 2014).

Kentut leaves are among the indigenous medicinal plants in Indonesia used for sepsis prevention. This plant contains secondary metabolite compounds such as α and β-paederine alkaloids, flavonoid flavanol, triterpenoid friedelin, β-sitosterol, campesterol, triterpenoid saponins, gallo tannin, and other active compounds (Salamah & Halim, 2021). The secondary metabolite contents pharmacologically have benefits such as antioxidants, antibiotics, anticancer, anti-insect, anti-tumor, and immunomodulatory agents (Das et al., 2018). Research on the preventive effects of Kentut leaf extract on interleukin-6 levels revealed anti-inflammatory results in sepsis, presumably due to the presence of saponins, flavonoids, and essential oils in the leaves. It is suspected that saponins can interact with various lipid membranes, such as phospholipids, which are precursors to prostaglandins and other inflammatory mediators (Savitri & Kasimo, 2022).

MATERIALS AND METHODS

Study area

This study falls under experimental research utilizing a Completely Randomized Design (CRD) method. The test subjects used in this study were white male mice selected randomly, ensuring each mouse had an equal chance of being chosen as a sample for treatment. The study aimed to determine the antiseptic activity of ethanol extract from Kentut leaves on white male mice. A total of 24 white male mice were utilized and divided into 6 (six) groups, each comprising 4 mice. Group I comprised untreated mice (normal), Group II received aquadest (negative control), and Group III received ciprofloxacin (positive control). Groups IV to VI (3 groups) were administered kentut leaf extract. Groups II to VI were injected with *E. coli* bacteria, while Group I was not injected with *E. coli*. Materials and equipment: feeding tube, syringe, scale, measuring glass, surgical tools set, urine pot, liver tissue board, Immunohistochemistry kit (IHK), microscope, beaker glass, stirring rod, spatula, glass funnel, white male mice, Kentut leaf extract, *E. coli* bacteria, water (AQUA), 10% formalin, milk pellets A, 96% ethanol, aquadest, ciprofloxacin, and 96% alcohol.

Procedures

Adaptation of Test Animals

During the adaptation process, all groups of mice were fed standard (normal) food consisting of milk pellets A and water, amounting to 90 grams per cage for 14 days.

Experimental Treatment of Test Animals

During this period, the six groups of mice received different treatments. Group I (normal) received only standard food. Group II, the mice were administered aquadest (negative control) at 500 mg/kg BW. Group III received ciprofloxacin (positive control) at 500 mg/kg BW. Groups IV to VI were treated (P1, P2, P3) with Kentut leaf extract at 100, 300, and 500mg/kg BW, respectively, via gavage. All six groups of mice were fed 90 grams of food daily for 30 days.

E. coli Bacteria Injection

The mice were injected with 1 mL of *E. coli* intraperitoneally. *E. coli* administration was performed once before surgery, and the mice were monitored for 24 hours.

Surgical Procedure on Test Animals

The examination of IL-6 expression in the mice's liver tissue in this experiment required the liver tissue of the mice. Kentut leaf extract was injected into mice (*Mus musculus*) infected with *E. coli* intraperitoneally,

measuring IL-6 expression. After 15 days of oral Kentut leaf extract administration, twenty-four mice underwent dislocation. The abdomen was then opened, and liver tissue samples were taken and placed in urine pots containing 10% formalin for IL-6 expression examination.

Immunohistochemistry Examination

Immunohistochemistry involves identifying specific proteins in tissues or cells using antibodies, examining IL-6 expression via immunohistochemistry involved rinsing cell cultures with PBS for 30 minutes and fixing them with methanol for 5 minutes. The samples were dried and washed with PBS pH 7.4. Then, 3% H2O2 was applied for 10 minutes, followed by another PBS pH 7.4 wash. Subsequently, a 5% serum PBS solution containing 0.25% Triton X-100 was incubated for 1 hour at room temperature. After washing with PBS pH 7.4, monoclonal anti p50/p65 was applied and incubated overnight. Another wash with PBS pH 7.4 was performed, followed by the application of biotin-labeled secondary antibodies and incubation for 1 hour. A subsequent PBS pH 7.4 wash was done, and Strep-Avidin horse radish peroxidase (SA-HRP) was applied for 40 minutes. The samples were then washed with PBS pH 7.4, and the chromogen for HRP, Diamono Benzidine (DAB), was applied. Counterstaining was done with Mayer hematoxylin for 10 minutes, rinsing with tap water, and washing with H_2O. Finally, the samples were dried and covered with a cover glass (Yolandra, 2013).

Data analysis

Data analysis in this study utilized One-Way Analysis of Variance (ANOVA). Before conducting One-Way ANOVA, tests for Normality and homogeneity were performed. The data were considered normally distributed and homogenous if the p-value was >0.005. If the analysis showed normal distribution and homogeneity, One-Way ANOVA was used to determine if there were significant differences between test groups, signifying significance if the p-value was <0.05 and non-significance if the p-value was >0.05. If there were significant differences between test groups, further analysis was conducted using Post Hoc Tests such as Least Significant Difference (LSD) ($p<0.05$) and Duncan's Test to determine significant differences among the tested individuals (Ghozali, 2009).

RESULTS AND DISCUSSION

Result

The testing of kentut leaf extract (*P. foetida* L) for its antiseptic properties was conducted by observing the expression of IL-6 in the livers of a sepsis model of mice injected with *E. coli*. The observation results are presented in the table below. The research results in the normal group showed an average IL-6 expression value in the livers of mice at 7.09%±0.06, as seen in Table 1.

Table 1. Observation results of IL-6 expression in the liver of normal group mice.

Group	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Average	SD
KN1	17.39%	0.00%	12.00%	9.38%	4.35%	8.62%	0.07
KN2	3.23%	0.00%	12.50%	6.06%	3.23%	5.00%	0.05
KN3	14.29%	11.11%	3.85%	0.00%	10.71%	7.99%	0.06
KN4	7.69%	0.00%	3.13%	13.79%	9.09%	6.74%	0.05
Average						7.09%	0.06

Description:
KN : Normal Group
Rep : Replication

The research findings in the positive control group revealed an average IL-6 expression value in the livers of mice at 26.36%±0.02, as shown in Table 2.

Table 2. Observation results of IL-6 expression in the liver of positive control group mice.

Group	Rep1	Rep2	Rep3	Rep4	Rep5	Average	SD
K(+)1	25.58%	26.53%	24.32%	28.57%	28.07%	26.62%	0.02
K(+)2	23.40%	24.32%	26.19%	25.93%	25.53%	25.08%	0.01
K(+)3	25.00%	26.83%	29.79%	29.27%	25.00%	27.18%	0.02
K(+)4	27.59%	25.00%	26.47%	26.83%	27.03%	26.58%	0.01
Average						26.36%	0.02

Description:
K(+) : Positive Control Group
Rep : Replication

The research findings in the negative control group showed an average IL-6 expression value in the livers of mice at 72.60%±0.05, as observed in Table 3.

Table 3. Observation results of IL-6 expression in the liver of negative control group mice.

Group	Rep1	Rep2	Rep3	Rep4	Rep5	Average	SD
K(-)1	62.50%	75.00%	69.05%	86.49%	75.86%	73.78%	0.09
K(-)2	72.50%	79.49%	61.54%	71.88%	75.00%	72.08%	0.07
K(-)3	76.60%	76.09%	71.70%	72.50%	73.33%	74.04%	0.02
K(-)4	70.59%	69.23%	69.23%	70.59%	72.92%	70.51%	0.02
Average						72.60%	0.05

Description:
K(+) : Negative Control Group
Rep : Replication

The research results in treatment group I (100mg/kgBW) showed an average IL-6 expression value in the livers of mice at 71.04%±0.04, as shown in Table 4.

Table 4. Observation results of IL-6 expression in the liver of treatment group I (100mg/kgBW).

Group	Rep1	Rep2	Rep3	Rep4	Rep5	Average	SD
(PI)1	72.34%	73.21%	71.43%	70.37%	68.42%	71.15%	0.02
(PI)2	67.35%	66.04%	72.22%	74.00%	73.81%	70.68%	0.04
(PI)3	69.39%	75.00%	74.55%	74.00%	70.37%	72.66%	0.03
(PI)4	77.27%	67.27%	67.31%	75.00%	61.36%	69.64%	0.06
Average						71.04%	0.04

Description:
PI : Treatment Group 1 (100mg/kgBW)
Rep : Replication

The research findings in treatment group II (300mg/kgBW) revealed an average IL-6 expression value in the livers of mice at 62.22%±0.02, as shown in Table 5.

Table 5. Observation results of IL-6 expression in the liver of treatment group II (300mg/kgBW).

Group	Rep1	Rep2	Rep3	Rep4	Rep5	Average	SD
(PII)1	63.41%	60.87%	61.11%	63.04%	62.96%	62.28%	0.01
(PII)2	60.00%	63.83%	61.54%	61.70%	62.22%	61.86%	0.01
(PII)3	62.96%	65.12%	64.58%	65.22%	63.46%	64.27%	0.01
(PII)4	60.34%	61.22%	62.26%	62.22%	56.36%	60.48%	0.02
Average						62.22%	0.02

Description:
PII : Treatment Group II (300mg/kgBW)
Rep : Replication

The research outcomes in treatment group III (500mg/kgBW) displayed an average IL-6 expression value in the livers of mice at 40.92%±0.01, as shown in Table 6.

Table 6. Observation results of IL-6 expression in the liver of treatment group III (500mg/kgBW).

Group	Rep1	Rep2	Rep3	Rep4	Rep5	Average	SD
(PIII)1	39.58%	39.02%	40.74%	40.00%	38.78%	39.62%	0.01
(PIII)2	41.82%	42.86%	42.59%	42.55%	42.00%	42.36%	0.00
(PIII)3	36.59%	37.21%	38.30%	39.22%	39.58%	38.18%	0.01
(PIII)4	43.14%	43.24%	43.75%	42.31%	45.95%	43.68%	0.01
Average						40.96%	0.01

Description:
PIII : Treatment Group III (500mg/kgBW)
Rep : Replication

The research results in the normal group showed an average IL-6 expression in the mice liver of 7.09%±0.06, in the positive control group was 26.36%±0.02, and in the negative control group was 72.60%±0.05. Meanwhile, for treatment group I (100mg/kg BW), it was 71.04%±0.04, for treatment group II (300mg/kg BW) was 62.22%±0.02, and for treatment group III (500mg/kg BW) was 40.92%±0.01. The One-Way ANOVA test resulted in a significance value of 0.000 or a p-value < 0.005, indicating a significant difference among the groups.

The LSD test showed that overall comparisons between treatment groups had a significance value of P<0.05, suggesting that the comparison between treatment groups had different effects, except for the comparison between the negative control group and treatment group I (100mg/kg BW), which yielded a significance value of 0.196 or 0.196>0.05. This suggests that the negative control group and treatment group I (100mg/kg BW) have the same antiseptic effect.

The researcher employed the Duncan test to determine the effectiveness and the best dosage among the groups. The Duncan test results in the table indicated that overall comparisons between treatment groups were in different columns, suggesting that the comparison between treatment groups had different antiseptic effects, except for the comparison between the negative control group and treatment group I (100 mg), which appeared in the same column. This aligns with the LSD test results, showing that the negative control group and treatment group I (100 mg) have the same antiseptic effect.

The determination of the best dosage or the dosage effectiveness for antiseptics is based on the smallest values or leaning towards the left because antiseptic efficacy is determined by examining the average IL-6. According to the Duncan test table, the sequence of the best dosages is as follows: (1) normal group; (2) positive control group; (3) treatment group III (500mg/kgBW); (4) treatment group II (300mg/kgBW); (5) treatment group I (100mg/kgBW); and (6) negative control group.

Table 7. Effectiveness of kentut leaf extract as a preventive measure against IL-6 expression in the liver of a sepsis model in mice.

Group	a	B	C	d	e
Normal	7.09%				
Positive Control		26.37%			
Treatment III			40.96%		
Treatment II				62.22%	
Treatment I					71.03%
Negative Control					72.60%

Description:

1. Differences in column positions of subset values indicate the level of difference among treatment groups.
2. Values located in the same subset column indicate no significant difference between treatment groups.

Discussion

In this study, the average IL-6 expression values in the livers of each group of mice were as follows: the normal group was 7.09%±0.06; the positive control group was 26.36%±0.02; the negative control group was 72.60%±0.05; treatment group I (100mg/kgBW) was 71.04%±0.04; treatment group II (300mg/kgBW) was 62.22%±0.02, and treatment group III (500mg/kgBW) was 40.92%±0.01. Statistical analysis using One-Way ANOVA showed a significance value of 0.000 or p-value < 0.005, indicating a significant difference between the groups. According to the Duncan test table, the best dosing order was as follows: (1) normal group; (2) positive control group; (3) treatment group III (500mg/kgBW); (4) treatment group II (300mg/kgBW), (5) treatment group I (100mg/kgBW), and (3) negative control group.

The normal group had the smallest average IL-6 expression compared to the other groups, at 7.09%±0.06, as this group was not injected with *E. coli*, thus not undergoing sepsis. Among the sepsis-experiencing mice groups were groups II to VI. Group II was the most effective among the five other groups experiencing sepsis. Group II served as the positive control and contained ciprofloxacin.

Ciprofloxacin is an antibiotic agent in the fluoroquinolone class that to combat gram-negative bacterial infections such as *E. coli* (Thai et al., 2023). In sepsis, ciprofloxacin works by inhibiting the enzymes DNA gyrase and topoisomerase IV. Ciprofloxacin inhibits the activity of these enzymes, which play a crucial role in bacterial DNA replication and maintenance. The inhibition of these enzymes by ciprofloxacin disrupts the bacterial division and replication process, halting the growth and spread of infection (Roberts et al., 2019).

Regarding the treatment groups with *P. foetida* L. 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 IL-6 expression in the livers of the sepsis model mice. Determination of the effective dose of *P. foetida* L. leaf extract can be observed in the statistical analysis results with ANOVA at a confidence level of 95%. The analysis showed a significant difference in the *P. foetida* L. leaf extract doses in preventing IL-6 expression in the livers of the sepsis model mice. The post hoc test results from LSD and Duncan showed that the negative control group had the same antisepsis effect as treatment group I (100 mg/kgBW). This indicates that the dose of treatment group I (100 mg/kgBW) was less effective as an antiseptic due to having the same effect as the negative control group. Researchers suspect this is due to the low dose, which counteract the inflammation caused by the *E. coli* injection.

P. foetida L. is one of the plants long used in traditional medicine for various conditions, including as an anti-inflammatory agent. This study's anti-inflammatory mechanism in sepsis is thought to be caused by various secondary metabolite compounds found in *P. foetida* L. leaves, such as flavonoids, alkaloids, phenolic acids, and terpenoid compounds (Rosanti, 2016). IL-6 is a proinflammatory cytokine involved in the inflammation process. When inflammation occurs in the body, whether due to infection, injury, or chronic disease, immune system cells release IL-6 into their surrounding environment. IL-6 then interacts with IL-6 receptors on various types of cells, including immune cells, endothelial cells, and other tissue cells. IL-6 plays a role in stimulating and enhancing the inflammatory response. This cytokine can increase the production and release of other proinflammatory cytokines, such as IL-1β and TNF-α, triggering further inflammatory responses. IL-6 can also enhance the activity of immune cells, such as macrophages and neutrophils, involved in the inflammatory response (Tanaka et al., 2014).

Flavonoids in *P. foetida* L. leaves are believed to possess anti-inflammatory properties that can inhibit the release of proinflammatory cytokines, including IL-6. Flavonoids can inhibit the activation of the NF-κB signalling pathway, the main pathway involved in regulating the release of proinflammatory cytokines. Flavonoids inhibit NF-κB activation by blocking NF-κB transcription factors and preventing the nuclear migration of proteins involved in the transcription of proinflammatory cytokine genes, including IL-6 (Ginwala et al., 2019). Flavonoids can inhibit the activation of mitogen-activated protein kinase (MAPK) pathways such as ERK, JNK, and p38 MAPK, involved in of proinflammatory cytokine gene expression. By inhibiting MAPK pathway activation, flavonoids can reduce IL-6 production. They also inhibit the activity of COX and LOX enzymes. Flavonoids also can inhibit cyclooxygenase (COX) and lipoxygenase (LOX) enzymes. COX and LOX are involved in synthesising inflammatory mediators such as prostaglandins and leukotrienes, which can trigger IL-6 production (Maleki et al., 2019).

The inhibition of COX-1 and COX-2 enzymes, responsible for converting arachidonic acid into prostaglandin H2 (PGH2). PGH2 becomes a precursor for various inflammatory mediators such as prostaglandins and thromboxanes. Flavonoids can interact with the active site of COX enzymes, inhibiting the access of arachidonic acid substrate to the enzyme reaction site. They can also inhibit lipoxygenase (LOX) enzyme activity, which is responsible for converting arachidonic acid into leukotrienes. Leukotrienes are inflammatory mediators that trigger the inflammatory response. Flavonoids can inhibit LOX enzyme activity by directly binding to the enzyme and inhibiting the process of converting arachidonic acid into leukotrienes (Maleki et al., 2019).

In addition to flavonoids, *P. foetida* L. leaves also contain phenolic acids, which have strong antioxidant properties that help protect cells from oxidative damage and oxidative stress associated with inflammation. Phenolic acids can reduce IL-6 production and relieve inflammation by inhibiting oxidative stress. Phenolic acids can directly interact with free radicals involved in oxidative stress by donating electrons or hydrogen to free radicals, neutralizing their activity, and preventing cell damage by free radicals. Phenolic acids stimulate the production of endogenous antioxidant enzymes in cells, such as superoxide dismutase (SOD), catalase, and glutathione peroxidase. These enzymes play a role in eliminating free radicals and preventing oxidative damage (Maleki et al., 2019).

Phenolic acid affects the expression of transcription factors involved in regulating pro-inflammatory cytokine gene expression. Inhibiting the activity of transcription factors NF-κB and AP-1, which play a role in proinflammatory cytokine production, can reduce the production of proinflammatory cytokines and alleviate inflammation (Lopez et al., 2022). Phenolic acid inhibits the activation of proinflammatory immune cells, such as macrophages and T cells, involved in the inflammatory response. This reduces the production of proinflammatory cytokines like IL-6 and suppresses the inflammatory response. Phenolic acid can also inhibit the activation of the NF-κB signaling pathway, which regulates the expression of proinflammatory cytokine genes. Activated macrophages and T cells will produce proinflammatory cytokines, including IL-6 (Lopez et al., 2022).

Alkaloid compounds present in the kentut leaves can inhibit the production of inflammatory mediators, such as prostaglandins and leukotrienes, which can trigger IL-6 production by inhibiting arachidonic acid synthesis. One mechanism involved in alkaloid-mediated arachidonic acid synthesis inhibition is the enzyme phospholipase A2 (PLA2). Alkaloids can inhibit the activity of

phospholipase A2, which is arachidonic acid from cell membrane phospholipids. Inhibition of PLA2 by alkaloids will reduce the availability of arachidonic acid required for prostaglandin and leukotriene synthesis (Lopez et al., 2022).

Cellular receptor and transcription factor inhibition by alkaloids are mechanisms involved in reducing the inflammatory response. Alkaloids can inhibit the expression or activity of cellular surface receptors involved in activating proinflammatory immune cells. Inhibiting the expression of Toll-like receptors (TLRs) that stimulate the inflammatory response. Inhibition of TLR activity by alkaloids can hinder the activation of proinflammatory immune cells and the production of proinflammatory cytokines such as IL-6. Alkaloids can also inhibit the Janus kinase (JAK)-signal transducer and activator of transcription (STAT) signaling pathway, which regulates proinflammatory cytokine gene expression. Additionally, alkaloids can affect histone deacetylase (HDAC) activity gene transcription regulation (Lopez et al., 2022).

Kentut leaves also contain terpenoid compounds that can affect the inflammatory response through various mechanisms. One of these mechanisms is inhibiting the activity of proteolytic enzymes such as elastase and collagenase in degrading the extracellular matrix and damaging tissues in the inflammatory response. Terpenoid compounds can directly interact with proteolytic enzymes and inhibit their catalytic activity. They can bind reversibly or irreversibly to the enzyme's active site, change the enzyme's conformation, or interfere with cofactors required for enzyme activity. Furthermore, terpenoid compounds can affect the expression of protease inhibitors that act as negative regulators of proteolytic enzymes. They can increase protease inhibitor expression or inhibit the production of factors that suppress protease inhibitor expression. An increase in protease inhibitor levels by terpenoid compounds can inhibit proteolytic enzyme activity and prevent tissue damage (Gallily et al., 2018).

Savitri & Kasimo's (2022) study on the effect of kentut leaf extract (*P. foetida* L.) on reducing interleukin-6 levels found anti-inflammatory results in sepsis, possibly due to the presence of saponins, flavonoids, and essential oils in kentut leaves. It is suspected that saponins can interact with many lipid membranes, such as phospholipids, which are precursors of prostaglandins and other inflammatory mediators. According to the researchers, the anti-inflammatory mechanism in sepsis is likely due to the content of flavonoids, alkaloids, phenolic acids, and terpenoid compounds. The most likely anti-inflammatory mechanism is presumed to be flavonoids inhibiting cyclooxygenase (COX) and lipoxygenase (LOX) enzymes involved in synthesizing inflammatory mediators such as prostaglandins and leukotrienes, which can trigger IL-6 production.

CONCLUSIONS

The research results indicate an influence of the kentut leaf extract in preventing the expression of IL-6 in the livers of sepsis-modeled mice injected with *E. coli*, with a significance value of 0.000 or p-value < 0.005. The most effective dose of the kentut leaf extract in preventing the expression of IL-6 in the livers of sepsis-modeled mice injected with *E. coli* is the dosage in treatment group III (500mg/kg BW).

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