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Empirical Study of Anti-Inflammatory Effects of Kecombrang (*Etlingera elatior*) in *Mus musculus* Sepsis Model

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Abstract

Mice used as experimental animals in the laboratory belong to the genus Mus, subfamily Murinae, family Muridae, superfamily Muroidea, order Rodentia, and class *M. musculus*, these mice are also referred to as home mice. Mice have been used as standard animals in toxicology, teratology, and carcinogenesis tests, even today, mice have also been used for behavioral, neurologic, nutritional, genetic, immunological, infectious, metabolic, and degenerative disease studies. Animal models of sepsis with intraperitoneal or intravenous injection of lipopolysaccharide (LPS) have been widely used for sepsis research. LPS induces systemic inflammation that mimics the early phase of sepsis. LPS injection causes kidney injury, including a decrease in glomerular filtration rate, an increase in blood urea nitrogen, and an increase in neutrophil infiltration in the kidney. The injectable dose of LPS can be titrated to mimic early sepsis without hemodynamic compromise, which has been useful for studying the systemic and renal responses. The response during the early phase of sepsis is that doses of LPS are usually used to induce systemic hypotension and decrease glomerular perfusion, whereas low doses of LPS do not cause systemic hypotension but still decrease glomerular perfusion. There are several advantages of LPS compared to others, namely, the method used is simple and the model is very controlled and standardized. The dose of endotoxin that causes 50% mortality in mice is 1–25 mg/kg. In this study, mice were given intraperitoneal injection of LPS at a dose of 0.3 mg/kg BW. LPS injection was given to the positive control group and treatment group 1, treatment group 2, and treatment group 3 at the start of the study.

Introduction

The problem of infectious diseases with complications of sepsis still becomes a health problem in both developed and developing countries. Sepsis is a disease with the most expensive treatment costs in the United States around 5.2% of total hospital costs [1]. Approximately 15% of patients with sepsis will develop septic shock which contributes to 10% of patients admitted to the intensive care unit [2], [3]. Sepsis causes one-third to half of the deaths of patients [4], [5], [6]. Research conducted at Cipto Mangunkusumo Hospital in 2012 reported that the incidence of sepsis and septic shock in the intensive care unit was 23 cases of sepsis out of 84 cases treated in the intensive care unit with a mortality rate of 47.8% and a mortality rate in the early phase of 34.7% [7]. Research in the internal medicine department of RSUD Dr. Moewardi Surakarta in 1997 found that 130 (97%) out of 135 sepsis patients died [8]. In 2004, the mortality rate due to sepsis in RSUD Dr. Moewardi Surakarta reached 74 (83.1%) out of 89 sepsis patients [9]. Research conducted on January 2006–December 2007 in the PICU/NICU section of RSUD Dr. Moewardi Surakarta reported the incidence of sepsis of 33.5% with a mortality rate of 50.2% [10].

The pathophysiology and pathogenesis of sepsis begin with a reaction to infection. Sepsis occurs as an exaggerated inflammatory response to pro-inflammatory cytokines against infection [11]. Activation of nuclear factor B (NF-B) mediates the transcriptional expression of most pro-inflammatory genes that play an important role in the pathophysiology and pathogenesis of sepsis [12]. One of the pro-inflammatory cytokines activated by NF-B is interleukin-1β (IL-1β). The production of reactive oxygen species and IL-1β is the main etiology of endothelial dysfunction in sepsis [13]. In the case of sepsis, the infection is followed by an increase in caspase 3 mRNA for the implementation of apoptosis [14]. Based on the results of research through biocomputation, some compounds have the potential to inhibit NADPH oxidase through p47-phox, namely, vanillic acid (VA) compounds [4]. The results of the study by Satpute *et al*. reported that VA has antimicrobial, anti-inflammatory, and antioxidant properties [15]. *In vitro* studies showed that VA 100 g/ml can inhibit the growth of *Salmonella enterica* and *Streptococcus mutans* bacteria [16]. Besides, VA acts as an antihypertensive, antihyperglycemic, and antioxidant at a dose of 50 mg/kg body weight of *Mus*

musculus [17]. VA compounds were found in *Angelica sinensis* (Ma, Guo, Jin, 2015) and *Etlingera elatior* (*E. elatior*) plants [16]. *E. elatior* is a plant originating from Indonesia and widely spread in Indonesia compared to *Angelica sinensis*. The structure of these compounds is determined based on the study of spectroscopic data, especially spectral data of 1H- and 13C-NMR [16]. The methanol extract of *E. elatior* fruit contains bioactive compounds, such as flavonoids, tannins, saponins, steroids, and triterpenoids [5]. *E. elatior* flower extract with a dose of 200 mg/kg BW can reduce uric acid in *Mus musculus* induced by hyperuricemia [18]. Besides, the ethanol extract of *E. elatior* flowers with a dose of 100, 300, and 1000 mg/kg in *Mus musculus* has pharmacological activity as an anti-allergic [18]. *E. elatior* leaf acetone extract dose of 250 g/mL has antiproliferative and apoptotic activity [14], [17]. An *in vivo* test is needed to prove the activity of the methanol extract of *E. elatior* fruit as an anti-inflammatory and antioxidant in the rat model of sepsis.

The cellular activation complex (neutrophils, monocytes, and microvascular endothelial cells), the neuroendocrine system, complement activation, coagulation activation, and the fibrinolytic system play a role in the sepsis state leading to a cytokine storm [12], [13]. This condition is characterized by an inflammatory reaction by pro-inflammatory cytokines, endothelial damage caused by lipid peroxidation, mitochondrial damage, and DNA [17]. This present study is urgent and important, considering the septic shock patients have a mortality rate of higher than 50% and, as mentioned earlier, sepsis is a disease with the most expensive treatment costs in the United States with 5.2% of total hospital costs [1]. Early identification of sepsis and prompt and appropriate management can improve the patients' prognosis [18]. Further, this study tries to explore and develop the potential of local plants (extract of Kecombrang fruit) for adjuvant therapy for sepsis in accordance with the UNS research roadmap 2011–2025 in the field of Health, Tropical Diseases, Nutrition, and Medicine as well as suitable with the research topics of the Ministry of Higher Education in the medicine-health field, namely, the development of local resource-based phytopharmaceuticals.

Materials and Methods

The research was conducted in the laboratory of the Center for Food and Nutrition Studies, Gadjah Mada University.

The research focused on finding the dose of methanol extract of *E. elatior*. The treatment sample for each group was eight male BALB C *Mus musculus*. Group I received lipopolysaccharide (LPS) induction as the control group. Group II received LPS induction and pretreated with methanol extracts of Kecombrang fruit (4.2 mg/20 g) for 5 days before induction of LPS. Group III received LPS induction and treated with methanol extracts of Kecombrang fruit (4.2 mg/20 g) for 5 days after induction of LPS. Group IV received LPS induction and treated with methanol extracts of Kecombrang fruit (4.2 mg/20 g) at the same time induction of LPS. The sepsis model was performed by induced intraperitoneal LPS of 0.3 mg/kgBW. Treatment of the methanol extract of Kecombrang fruit was performed every day and after 7 days, *Mus musculus* were sacrificed. The expression of NF-B and caspase 3, and IL-1β was tested using ELISA. The fruit of *E. elatior* was obtained from the area of Langkalir, Pangandaran, West Java. The validation of the determination of plant species was carried out by the Faculty of Biology, Muhammadiyah University, Surakarta, with the Latin name *E. elatior* (Jack) R.m. Sm., from the Zingiberaceae tribe. The chemicals used are chemicals with pro-analytical specifications, namely: 70% ethanol (Merck).

Extraction is done by maceration method. Extraction was started by preparing *E. elatior* fruit. *E. elatior* fruit peeled, washed, and drained. Then, the shelled fruit is cut into small pieces and dried. After drying, the samples were crushed using a blender until a dry powder was produced. Maceration was carried out for 24 h followed by maceration for 2 h at room temperature. The mixture was separated using a centrifuge at low temperature (4°C) at 4200 rpm for 20 min. The mixture was then filtered using filter paper using a vacuum filter. The filtrate obtained was put into an extraction flask and evaporated with a rotary evaporator at a temperature of 50°C. The fruit extract of *E. elatior* was then tested for high-performance liquid chromatography (Farida and Maruzy, 2016). Based on the results of qualitative identification, it was found that the fruit extract of *E. elatior* with methanol as a solvent contained bioactive compounds, namely, flavonoids, tannins, saponins, steroids, and triterpenoids. The purple methanol extract of *E. elatior* fruit contained more total phenolic, namely, 1.51 ± 0.04 mg GAE/g extract, red fruit (1.34 ± 0.02 mg GAE/g extract), and pink (1.21 ± 0.04 mg GAE/g extract). The highest total flavonoids in the purple-colored *E. elatior* fruit methanol extract were 0.38 ± 0.03 mg QE/g extract followed by pink fruit extract (0.20 ± 0.02 mg QE/g extract) and red (0.15 ± 0.02 mg QE/g extract) (Isyanti *et al*., 2019). Based on qualitative tests conducted in other studies, it is known that *E. elatior* plants contain phytochemical tannins, terpenoids, flavonoids, phenolics, and saponins (Samarang *et al*., 2016).

The ethical clearance of the research is issued by the Health Research Ethics Committee of Dr. Moewardi General Hospital.

Results

Effect of the methanol extracts of Kecombrang fruit on nuclear factor KB levels in various treatment preparations

Differences in levels of NFkB in the group MP1 (the methanol extract of Kecombrang fruit of 4.2 mg/20 g before LPS), MP2 group (5 days of methanol extract of Kecombrang fruit 4.2 mg/20 g after LPS), MP3 group (the methanol extract of Kecombrang fruit of 4.2 mg/20 g with LPS), and the control group (LPS only) were identified using ANOVA because the data were normally distributed (appendix). The results of the differences in NFKB levels in the MP1 group, MP2 group, MP3 group, and control group are presented in Table 1.

Table 1: Differences in NFKB levels in the MP1 group, MP2 group, MP3 group, and control group

Treatment	NFKB Levels
MP1	99.71 ± 2.15
MP2	123.43 ± 3.02
MP3	111.09 ± 2.50
Control	671.94 ± 8.42
p	< 0.001*

*Significant at α = 5%. MP1: Methanol extract of Kecombrang fruits of 4.2 mg/20 g before LPS, MP2: Methanol extract of Kecombrang fruits of 4.2 mg/20 g 5 days after LPS, MP3: Methanol extract of Kecombrang fruits of 4.2 mg/20 g simultaneously LPS, control: Only LPS. LPS: Lipopolysaccharide.

Based on Table 1, the average value of NFKB levels in the MP1 group is 99.71 + 2.15. The average value of NFKB levels in the MP2 group is 123.43 + 3.02. Meanwhile, the average value of NKB levels for the MP3 group and the control group is 111.09 + 2.50 and 671.94 + 8.42, respectively. Thus, the methanol extract of Kecombrang fruit of 4.2 mg/20 g can reduce levels of NFkB with the best results in the MP1 (the methanol extract of Kecombrang fruit of 4.2 mg/20 g before LPS).

The results of the statistical test obtained the p-value of 0.001 ($p < 0.05$) meaning that there is a significant difference in NFKB levels in the MP1 group, MP2 group, MP3 group, and control group as presented in Figure 1.

Based on the ANOVA test, it was found that there was a significant difference in the NFKB levels in the MP1 group, MP2 group, MP3 group, and control group with $p < 0.05$. Then, a further test of *post hoc* LSD test was carried out and the results can be seen below.

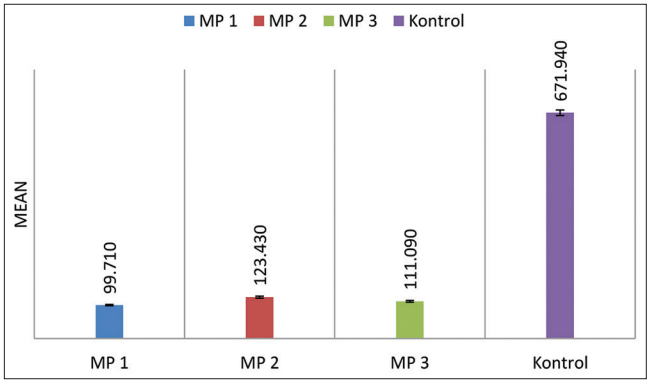

Figure 1: Bar chart description of NFKB levels in each treatment preparation

Table 2 shows that the value of NFKB levels is significantly different in the MP1 group, MP2 group, MP3 group, and the control group partially with $p < 0.05$. Based on the description above, it can be seen that the use of the methanol extract of Kecombrang fruit lowers NFkB with the best results in the methanol extract of Kecombrang fruit of 4.2 mg/20 g before LPS (MP1).

Table 2: *Post hoc* test of NFKB levels in the MP1 group, the MP2 group, the MP3 group, and the control group

Treatment	NFKB p		
	MP1	MP2	MP3
MP2	< 0.001*		
MP3	0.002*	0.001*	< 0.001*
Control	< 0.001*	< 0.001*	< 0.001*

*Significant at α = 5%. MP1: Methanol extract of Kecombrang fruits of 4.2 mg/20 g before LPS, MP2: Methanol extract of Kecombrang fruits of 4.2 mg/20 g 5 days after LPS, MP3: Methanol extract of Kecombrang fruits of 4.2 mg/20 g simultaneously LPS, control: Only LPS. LPS: Lipopolysaccharide.

Effect of the methanol extract of Kecombrang fruit on the level of CASPASE 3 in various treatment preparations

Differences in the levels of caspase 3 in the MP1 group (methanol extract of Kecombrang fruit of 4.2 mg/20 g before LPS), MP2 group (5 days of methanol extract of Kecombrang fruit of 4.2 mg/20 g after LPS), MP3 group (methanol extract of Kecombrang fruit of 4.2 mg/20 g with LPS), and the control group (LPS only) were identified using the ANOVA because the data were normally distributed (appendix). The results of the differences in the level of CASPASE 3 in the MP1 group, MP2 group, MP3 group, and control group are shown in Table 3 as follows:

Table 3: Effect of the methanol extract of Kecombrang fruit on the level of caspase 3 in various treatment preparations

Treatment	Levels of caspase 3
MP1	3.61 ± 0.28
MP2	5.64 ± 0.18
MP3	4.82 ± 0.23
Control	7.83 ± 0.29
p	<0.001*

*Significant at α = 5%. MP1: Methanol extract of Kecombrang fruits of 4.2 mg/20 g before LPS, MP2: Methanol extract of Kecombrang fruits of 4.2 mg/20 g 5 days after LPS, MP3: Methanol extract of Kecombrang fruits of 4.2 mg/20 g simultaneously LPS, control: Only LPS. LPS: Lipopolysaccharide.

Based on Table 3, the average value of the level of CASPASE 3 in the MP1 group and MP2 group is 3.61 + 0.28 and 5.64 + 0.18, respectively. Then, the average value of the level of CASPASE 3 in the MP3 group and control group is 4.82 + 0.23 and 7.83 + 0.29. Thus, the methanol extract of Kecombrang fruit of 4.2 mg/20 g can reduce levels of CASPASE 3 with the best results in the MP1 group (methanol extract of Kecombrang fruit of 4.2 mg/20 g before LPS).

The results of the statistical test showed a p-value of 0.001 ($p < 0.05$) meaning that there is a significant difference in the level of CASPASE 3 in the MP1 group, MP2 group, MP3 group, and the control group as presented in Figure 2.

Based on the ANOVA test, there was a significant difference in the level of caspase 3 in

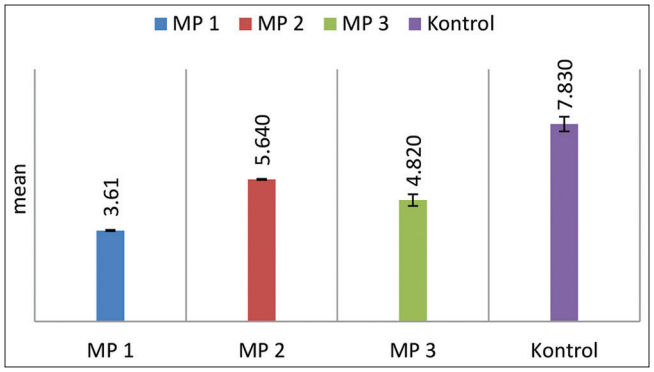

Figure 2: Bar chart description of levels of caspase 3 in each treatment preparation

the MP1 group, MP2 group, MP3 group, and control group with $p < 0.05$. Then, a further test, the LSD *post hoc* test, was carried out and obtained the following results.

Table 4 shows that the level of CASPASE 3 values has significant differences in the MP1 group, MP2 group, MP3 group, and the control group partially with $p < 0.05$. Based on the description above, it can be seen that the methanol extract of Kecombrang fruit can reduce caspase 3 with the best results of 4.2 mg/20 g of the methanol extract of Kecombrang fruit before LPS (MP1).

Table 4: *Post hoc* test for the level of caspase 3 in the MP1 group, the MP2 group, the MP3 group, and the control group

Treatment	Caspase 3 p		
	MP1	MP2	MP3
MP2	< 0.001*		
MP3	< 0.001*	< 0.001*	< 0.001*
Control	< 0.001*	< 0.001*	< 0.001*

*Significant at α = 5%. MP1: Methanol extract of Kecombrang fruits of 4.2 mg/20 g before LPS, MP2: Methanol extract of Kecombrang fruits of 4.2 mg/20 g 5 days after LPS, MP3: Methanol extract of Kecombrang fruits of 4.2 mg/20 g simultaneously LPS, control: Only LPS. LPS: Lipopolysaccharide.

Effect of the methanol extract of Kecombrang fruit on the level of interleukin-1 in various treatment preparations

Differences in levels of IL-1 in the MP1 group (methanol extract of Kecombrang fruit of 4.2 mg/20 g before LPS), MP2 group (5 days of methanol extract of Kecombrang fruit of 4.2 mg/20 g after LPS), MP3 group (methanol extract of Kecombrang fruit of 4.2 mg/20 g with LPS), and the control group (LPS only) were identified using the ANOVA because the data were normally distributed (appendix). The results of differences in the level of IL-1 in the MP1 group, MP2 group, MP3 group, and control group are shown in Table 5.

Table 5: Effect of the methanol extract of Kecombrang fruit on IL-1 levels in various treatment preparations

Treatment	Level of IL-1
MP 1	0.28 ± 0.02
MP 2	0.76 ± 0.03
MP 3	0.60 ± 0.06
Control	0.99 ± 0.07
p	< 0.001*

*Significant at α = 5%. MP1: Methanol extract of Kecombrang fruits of 4.2 mg/20 g before LPS, MP2: Methanol extract of Kecombrang fruits of 4.2 mg/20 g 5 days after LPS, MP3: Methanol extraction Kecombrang fruits 4.2 mg/20 g simultaneously LPS, control: Only LPS. LPS: Lipopolysaccharide, IL-1: Interleukin 1.

Based on Table 5, the average value of the level of IL-1 in the MP1 group and MP2 group is 0.28 + 0.02 and 0.76 + 0.03. Then, the average value of the level of IL-1 in the MP3 group and the control group is 0.60 + 0.06 and 0.99 + 0.07. Thus, the methanol extract of Kecombrang fruit of 4.2 mg/20 g can reduce levels of IL-1 with the best results in the MP1 group (methanol extract of Kecombrang fruit of 4.2 mg/20 g before LPS).

The results of the statistical test showed a p-value of 0.001 ($p < 0.05$) meaning that there is a significant difference in the level of IL-1 in the MP1 group, MP2 group, MP3 group, and control group as presented in Figure 3.

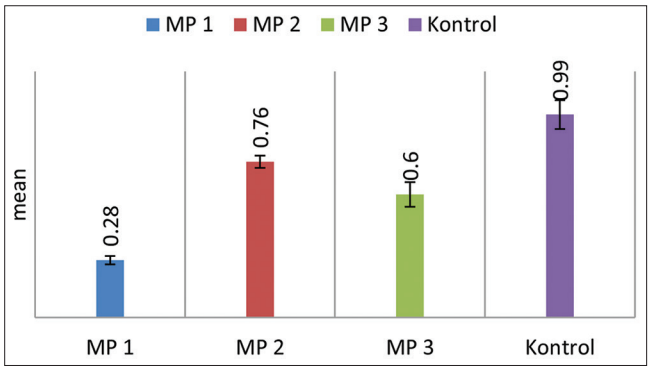

Figure 3: Bar chart description of the level of IL-1 in each treatment preparation

Based on the ANOVA test, there was a significant difference in the level of IL-1 in the MP1 group, MP2 group, MP3 group, and control group simultaneously with $p < 0.05$. A further test was carried out using the *post hoc* LSD test with the following results.

Table 6 explains that the value of the level of IL-1 showed a significant difference in the MP1 group, MP2 group, MP3 group, and the control group partially with $p < 0.05$. Based on the description above, the use of the methanol extract of Kecombrang fruit can lower the level of IL-1 with the best results in the methanol extract of Kecombrang fruit of 4.2 mg/20 g before LPS (MP1).

Table 6: *Post hoc* test for the level of IL-1 in the MP1 group, the MP2 group, the MP3 group, and the control group

Treatment	IL-1 p		
	MP1	MP2	MP3
MP2	< 0.001*		
MP3	< 0.001*	< 0.001*	< 0.001*
Control	< 0.001*	< 0.001*	< 0.001*

*Significant at α = 5%. MP1: Methanol extract of Kecombrang fruits of 4.2 mg/20 g before LPS, MP2: Methanol extract of Kecombrang fruits of 4.2 mg/20 g 5 days after LPS, MP3: Methanol extract of Kecombrang fruits of 4.2 mg/20 g simultaneously LPS, control: Only LPS. LPS: Lipopolysaccharide, IL-1: Interleukin 1.

Discussion

Sepsis is a disease with the most expensive treatment costs in the United States around 5.2% of total hospital costs [1]. In the case of sepsis, after the infection, the caspase 3 mRNA for the implementation

of apoptosis increases [14]. Based on the results of research through biocomputation, some compounds have the potential to inhibit NADPH oxidase through p47-phox, including VA compounds [4]. The results of a study by Satpute *et al*. reported that VA has antimicrobial, anti-inflammatory, and antioxidant properties [15]. *In vitro* studies showed that VA 100 g/ml can inhibit the growth of *S. enterica* and *S. mutans* bacteria [16]. Besides, VA acts as an antihypertensive, antihyperglycemic, and antioxidant at a dose of 50 mg/kg body weight of *Mus musculus* [17]. VA compounds were found in Angelica sinensis (Ma, Guo, Jin, 2015) and *E. elatior* plants [16]. *E. elatior* is a plant originating from Indonesia and widely spread compared to *Angelica sinensis*.

This study is urgent and important considering that patients with septic shock have a mortality rate of more than 50% and sepsis is a disease with the most expensive treatment costs in the United States, around 5.2% of total hospital costs [1].

This study was conducted on a total of 20 *Mus musculus*, equally divided into four groups. Group I received LPS induction as control group. Group II received LPS induction and pretreated with methanol extract of Kecombrang fruit (4.2 mg/20 g) for 5 days before induction of LPS. Group III received LPS induction and treated with methanol extract of Kecombrang fruit (4.2 mg/20 g) for 5 days after induction of LPS. Group IV received LPS induction and treated with methanol extraction Kecombrang fruit (4.2 mg/20 g) at the same time induction of LPS. The measured outcome is the serum concentration of IL-1ß. Data were analyzed using ANOVA with $p < 0.05$. Pretreated Kecombrang extracts significantly decrease the serum levels of IL-1ß ($p < 0.05$).

The effect of the methanol extract of Kecombrang fruits on NFkB levels in various treatments showed that methanol extract of Kecombrang fruits of 4.2 mg/20 g can reduce levels of NFKB with the best results in the MP1 group (methanol extract od Kecombrang fruits of 4.2 mg/20 g before LPS). Based on the ANOVA test results, there are significant differences in NFKB levels in the MP1 group, MP2 group, MP3 group, and control group simultaneously with $p < 0.05$. Further, the *post hoc* test shows that the use of methanol extract of Kecombrang fruits can reduce NFB with the best results in MP1.

The effect of the methanol extract of Kecombrang fruits on levels of caspase 3 in various treatments showed that the methanol extract of Kecombrang fruits of 4.2 mg/20 g can reduce levels of caspase 3 with the best results in the MP1 group (methanol extract of Kecombrang fruits 4.2 mg/20 g before LPS). Based on the ANOVA test results, there are significant differences in the level of caspase 3 in the MP1 group, MP2 group, MP3 group, and control group simultaneously with $p < 0.05$. It indicates that the use of methanol extract of Kecombrang fruits can reduce caspase 3 with the best results in MP1.

The effect of the methanol extract of Kecombrang fruits on levels of IL-1 in various treatments showed that the methanol extract of Kecombrang fruits of 4.2 mg/20 g can reduce levels of IL-1 with the best results in the MP1 group (methanol extract of Kecombrang fruits of 4.2 mg/20 g before LPS). Based on the ANOVA test results, there are significant differences in the level of IL-1 in the MP1 group, MP2 group, MP3 group, and control group simultaneously with $p < 0.05$. It indicates that the use of methanol extract of Kecombrang fruits can reduce IL-1 with the best results in MP1.

This study has several limitations, including the number of research subjects, variable parameters, and confounding factors. Hence, further studies are required.

Several studies have used *in vitro* and *in vivo* tests with regard to *E. elatior* extract, including:

1. Mai *et al*./2009/antiproliferative and apoptotic studies of the standardized extracts of *E. elatior* on human colorectal carcinoma cells. *E. elatior* leaf acetone extract at a dose of 250 g/mL had antiproliferative and apoptotic activity against colorectal carcinoma (HT-29) cells and did not interfere with normal Chinese hamster ovary cell proliferation. Apoptotic-induced cell death was demonstrated by detection of phosphatidylserine translocation and activation of caspase 3. This study used the extract of the leaves instead of the fruit of *E. elatior*. The solvent used is acetone. Apoptotic parameters were seen by examining phosphatidylserine translocation and activation of caspase 3 with staining indicators. Cell death was assessed quantitatively by ELISA. In this study, we did not observe the effect of *E. elatior* leaf extract on mice with sepsis models.
2. Dewi *et al*. /2016/antihyperuricemic activity of ginger flower (*E. elatior* Jack.) extract in beef broth-induced hyperuricemic rats (*Rattus norvegicus*). *E. elatior* flower extract with a dose of 200 mg/kg BW can reduce uric acid in rats (*Rattus norvegicus*) induced by hyperuricemia. There was a decrease in blood uric acid levels up to 31.78% in the discontinuous hyperuricemia group (the treatment group up to 16 days) given *E. elatior* extract. Antihyperuricemic activity is associated with high levels of polyphenols, flavonoids, and saponins. The structure of flavonoids plays an important role in inhibiting xanthine oxidase. The sample used was flower extract of *E. elatior* with distilled water as a solvent, the infundation method. In this study, what was observed was a decrease in uric acid levels, not sepsis.
3. Vinothiya and Ashokkumar/2017/modulatory effect of VA on antioxidant status in high fat

diet-induced changes in diabetic hypertensive rats. Administration of VA 50 mg/kg BW for 8 weeks in diabetic and hypertensive rat models with a high-fat diet significantly had antihyperglycemic, antihypertensive and antioxidant activity effects. The results showed a significant decrease in blood glucose levels, aspartate transaminase (AST) and alanine transaminase (ALT), urea, and creatinine. There was an increase in antioxidant activity with the assessed parameters, namely, levels of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (Gpx), glutathione (GSH), Vitamin C, and Vitamin E. Antioxidant activity can also protect tissues from lipid peroxidation which is assessed to be delayed. The thiobarbituric acid reactive substances VA parameters used in this study were not derived from the ethanol extract of the fruit of *E. elatior*. This study observed antioxidant activity in diabetic and hypertensive rat models, not in sepsis model mice.

4. Meng and Zhang/2019/modulating effects of VA on sepsis-induced oxidative liver injury in rat model. Administration of VA significantly protects against oxidative stress and inflammatory cytokines and inhibits liver damage in septic rat models. Administration of VA 100 mg/kg BW on oxidative liver injury in sepsis model mice significantly increased levels of GSH, GPx, SOD, and CAT compared to the control group and placebo. The levels of liver damage marker enzymes (alkaline phosphatase [ALP], AST, and ALT) in the VA treatment group significantly decreased ($p < 0.05$). In addition, based on the results of the ELISA test, the VA also decreased the levels of the pro-inflammatory cytokines TNF-α and IL6. In this study, the VA used was not from the ethanolic extract of *E. elatior* fruit. Septic model mice were induced by the cecal ligation and puncture method instead of using LPS. The parameters of sepsis improvement in this study were assessed by increasing levels of GSH, GPx, SOD, CAT, as well as decreasing ALP, AST, and ALT pro-inflammatory cytokines TNF-α and IL6.
5. Sumandjar *et al*., 2019/the ethyl acetate fraction of *Moringa oleifera* leaves effects on endothelial stress in rat sepsis model. The concentrations of HPA, MDA, and CRP were assessed using the ELISA method, while the expression of e-selectin, Nf-kB, renal, and neuropathological histopathology was assessed using the immunohistochemical method. Mice were injected with LPS 0.25 mg/kg BW at the beginning of the study. *Moringa oleifera* ethyl acetate fraction administration with doses of 10, 20, and 40 mg/kgBW was administered 5 days before LPS injection to 7 days after LPS injection. It was found that the ethyl acetate fraction of *Moringa oleifera* significantly decreased the serum HPA and MDA levels on days 3 and 7, and decreased CRP levels on day 7 ($p < 0.05$). In other variables, the ethyl acetate fraction of *Moringa oleifera* did not show significant results. In this study, mice were used as a sepsis model instead of mice. The sample used was the ethyl acetate fraction of *Moringa oleifera* leaves. Sepsis improved parameters were assessed by decreasing concentrations of HPA, MDA, CRP, e-selectin, Nf-kB, renal, and ovarian histopathology.
6. Fristiohady *et al*./2020/nephroprotective effect of extract *E. elatior* (Jack) R.M. Smith on CCl4-induced nephrotoxicity in rats. The ethanolic extract of *E. elatior* fruit had a nephroprotective effect as evidenced by the normal examination of albumin, total protein, urea, and creatinine in CCl4-induced rats. Urea levels decreased at doses of ethanol extract of *E. elatior* 200, 300, and 400 mg/kg BW, while creatinine decreased at doses of *E. elatior* fruit ethanol extract 400 mg/kg BW. Based on histopathological examination, doses of 200 and 300 mg/kg BW of the ethanolic extract of *E. elatior* fruit had activity to protect kidney cell tubules, while at a dose of 400 mg/kg BW, there was necrosis in the tubules of kidney cells. Nephrotoxic model mice were induced by CCL4 administration not because of the septic process. Parameters used to determine the effect of *E. elatior* fruit ethanol extract as a nephroprotective agent, namely, albumin, total protein, urea, creatinine, and histopathological examination. In this study, NGAL examination was not performed. The doses of the ethanolic extract of *E. elatior* fruit in this study were 200 and 300 mg/kgBW, while the proposed dose would be 75, 100, and 150 mg/kgBW.
7. Aldi, Husni, and Yesika/2020/activity of Kincung flowers (*E. elatior* (Jack) R.M.Sm.) on total leukocytes and percentage of leukocytes in allergic male white mice. Administration of *E. elatior* flower ethanol extract to allergic model mice at doses of 100, 300, and 1000 mg/kg BW was proven to be significant in increasing leukocytes. The increase in total leukocytes makes the ethanol extract of *E. elatior* can be used as an immunomodulator. The decrease in basophil and eosinophil cells makes the ethanol extract of *E. elatior* used as an allergy medicine. The ethanol extract was taken from the flower parts of *E. elatior*. Observations on immunomodulatory and antiallergic effects were observed in allergic model mice, not observed in sepsis model mice.

Based on the information above, there is no research that explains the effect of methanolic extract of *E. elatior* fruit as an anti-inflammatory against sepsis model mice. The results of this study showed an anti-inflammatory effect of the methanolic extract of *E. elatior* fruit, which was indicated by a decrease in the levels of caspase 3, IL1B, and NfKB in septic model mice.

Conclusion

The effect of giving methanol extract of Kecombrang fruits with the levels of NFkB, caspase 3, and IL-1ß in various treatment preparations shows that the methanol extract of Kecombrang fruits of 4.2 mg/20 g can reduce levels of NFKB, caspase 3, and IL-1ß with the best results in the MP1 group (methanol extract of Kecombrang fruits of 4.2 mg/20 g before LPS). The beneficial effects of Kecombrang extract pretreatment in sepsis are evident from the observations. It can be said that the extract of Kecombrang can be exploited in the treatment of sepsis.

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