Anti Inflammatory Effects of Indonesian Kecombrang Fruit (Etlingera Elatior) in The Treatment of Sepsis

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Abstract

Sepsis is a potentially fatal organ dysfunction caused by disruption of the host's response to infection facilitated by inflammation and endothelial stress. In this situation, it is believed that Kecombrang (Etlingera elatior) can suppress inflammation and oxidative stress through the control of NF-kB. Therefore, this study investigates the anti-inflammatory effect of fruit extracts ofKecombrang in the Mus musculus sepsis model. The analysis was conducted using 20 Mus musculus, equally divided into four groups, where the control received lipopolysaccharide (LPS) induction. MP1 group was pretreated with methanol extracts of Kecombrang fruit (4.2 mg/20 gr) for five days before induction of LPS. Meanwhile, MP2 was treated with methanol extracts of Kecombrang fruit (4.2 mg/20 gr) for five days after induction of LPS. MP3 group was simultaneously treated with LPS induction and methanol extracts of Kecombrang fruit (4.2 mg/20 gr). The measured outcome was the serum concentration of NFkB and Caspase 3, and the data were analyzed using ANOVA with $p < 0.05$. Furthermore, pretreated Kecombrang extracts significantly decrease the levels of NFkB and Caspase3 ($p<0.05$). The methanol extract of kecombrang fruits of 4.2 mg/20 gr can reduce levels of NFkB and caspase 3 with the best results in the MP1 group (methanol extract of Kecombrang fruits 4.2 mg/20 gr before LPS). The beneficial effects are evident from the observations since Kecombrang extracts can be exploited to treat sepsis.

Keywords: *Kecombrang fruit; Caspase 3; NF-kB; Sepsis.*
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INTRODUCTION

The problem of infectious diseases with complications of sepsis continues to be a health problem in both developed and developing countries. In the United States, sepsis is a disease with the most expensive treatment, around 5.2% of total hospital costs.[1] Approximately 15% of patients develop septic shock, and 10% are usually admitted to the intensive care unit.[2,3] Furthermore, it causes one-third to half of the deaths of patients.[5,6] A study conducted at CiptoMangunkusumo Hospital in 2012 reported 23 out of the 84 cases treated in the intensive care unit with a mortality rate of 47.8% and 34.7% at the early phase.[7] In 1997,

a study conducted at the internal medicine department of RSUD by Dr. Moewardi Surakarta found that 130 (97%) out of 135 patients died.[8] Meanwhile, the mortality rate due to sepsis in RSUD Dr. Moewardi Surakarta reached 74 (83.1%) out of 89 patients in 2004.[9] A study conducted on January 2006-December 2007 in the PICU/NICU section of RSUD Dr. Moewardi Surakarta also reported the incidence of33.5% with a mortality rate of 50.2%.[10]

The pathophysiology and pathogenesis of sepsis begin with a response to infection. Sepsis occurs as an exaggerated inflammatory response to pro-inflammatory cytokines against disease.[11] The activation of NF-kB mediates the transcriptional expression of most pro-inflammatory genes in the pathophysiology and pathogenesis of sepsis.[12] One of the pro-inflammatory cytokines activated by NF-kB is IL-1β. Primarily, ROS and IL-1β is the cause of endothelial dysfunction.[13] In the siyuation of sepsis, the infection is followed by an increase in Caspase 3 mRNA to initiate apoptosis.[14] The results of biocomputation showed some compounds with the potential to inhibit NADPH oxidase through p47-phox, such as vanillicacid (VA) compounds.[4] The study by Satpute et al. reported that VA has antimicrobial, anti-inflammatory, and antioxidant properties.[15] Meanwhile, an in-vitro study previously conducted showed that VA 100 g/ml can inhibit the growth of S. enterica and S. mutans bacteria.[16] This is because VA acts as an antihypertensive, antihyperglycemic, and antioxidant at a dose of 50 mg/kg body weight of Mus musculus.[17] The compounds were found in Angelica sinensis (Ma, Guo, Jin, 2015) and Etlingera elatior (E. elatior) plants.[16] E. elatior originates from Indonesia, and it is widely spread compared to Angelica Sinensis. The structure of these compounds is determined based on spectroscopic results, specifically 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] In addition, the flower extract with a dose of 200 mg/kg BW can reduce uric acid in Mus musculus induced by hyperuricemia.[18] The ethanol extract of the flowers with an amount of 100, 300, 1000 mg/kg in Mus musculus has an anti-allergic pharmacological activity.[18] The fruit extract at doses of 200, 300, 400 mg/Kg BW of Mus musculus has a neuroprotective effects[19] Meanwhile, E.elatior leaf acetone extract dose of 250 g/mL has antiproliferative and apoptotic activity.[20,21] An in vivo test should be conducted to prove the activity of the methanol extract of the 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 are significant in a cytokine storm.[22,23] This condition is characterized by an inflammatory reaction by pro-inflammatory cytokines, endothelial damage caused by lipid peroxidation, and mitochondrial damage and DNA.[24] The necessities of this present study cannot be overemphasized, considering that the septic patients have a mortality rate above 50%.[1] Early identification and appropriate management can improve the patients' prognosis.[25] Furthermore, the potential of local plants (extract of Kecombrang fruit) for adjuvant therapy for sepsis was explored and developed.

METHODS

The study was conducted in the laboratory of the Center for Food and Nutrition Studies, Gadjah Mada University. The treatment sample for each group was eight male BALB C Mus musculus. Group I as the control received lipopolysaccharide (LPS) induction. MP1 group was pretreated with methanol extracts of Kecombrang fruit (4.2 mg/20 gr) for five days before induction of LPS. MP2 group was treated with methanol extracts of Kecombrang fruit (4.2 mg/20 gr) for five days after induction of LPS. Meanwhile, the MP3 group was simultaneously treated with the induction of

LPS and methanol extracts of Kecombrang fruit (4.2 mg/20 gr). The sepsis model was performed by induced intraperitoneal LPS of 0.3 mg/KgBW. Furthermore, treatment of the methanol extract ofKecombrang fruit was performed daily. After seven days, the Mus musculus were sacrificed, and the expression of NF- B and Caspase 3 were tested using ELISA. The ethical clearance was issued by the Health Research Ethics Committee of Dr. Moewardi General Hospital number: 477/IV/HREC/2021

RESULTS AND DISCUSSION

Effect of the methanol extracts ofkecombrang fruit on nfkb levels in various treatment preparations

Differences in levels of NFkB in the group MP1, MP2, MP3, and the control group were identified using ANOVA because the data were normally distributed (appendix). The results of the differences in NFkB levels 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
MP 1	99.71 ±2.15
MP 2	123.43 ±3.02
MP 3	111.09 ±2.50
Control	671.94 ±8.42
p-value	<0.001*

Note:
MP1 = methanol extract of kecombrang fruits of 4.2 mg/20 gr before LPS
MP2 = methanol extract of kecombrang fruits of 4.2 mg/20 gr 5 days after LPS
MP3 = methanol extract of kecombrang fruits of 4.2 mg/20 gr simultaneously LPS
Control = only LPS
*Significant at α=5%

Table 1 showed that the average value of NFkBlevels in the MP1, MP2, MP3, and control group is 99.71 +2.15, 123.43 +3.02, 111.09 +2.50, and 671.94 +8.42, respectively. Therefore, the methanol extractof Kecombrang fruit of 4.2 mg/20 gr can reduce levels of NFkB with the best results in the MP1 (the methanol extract of Kecombrang fruit of 4.2 mg/20 gr before LPS).

The statistical test obtained the p-value of 0.001 ($p < 0.05$), means that there is a significant difference in NFkB levels for all the groups as presented in Figure 1.

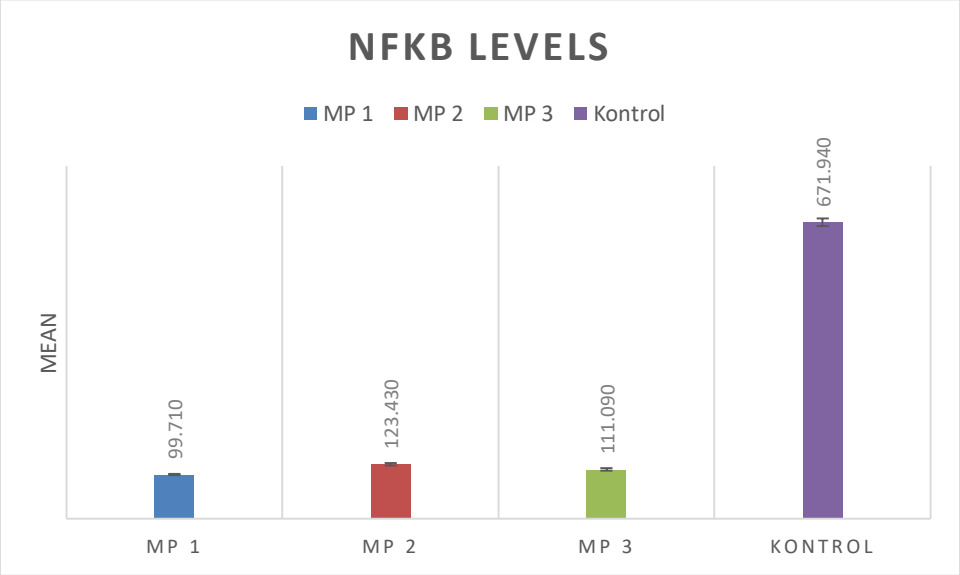

Figure 1. Description of NFkB levels in each treatment preparation

The ANOVA results showed that there was a significant difference in the NFkB levels of all the groups with $p < 0.05$. Then a test of posh hoc LSD was carried out, and the results can be seen in Table 2.

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Table 2. Posh Hoc Test of NFkB levels in the MP1 group, the MP2 group, the MP3 group, and the control group

Tratment	NFkB p-value		
	MP1	MP2	MP3
MP2	<0.001*		
MP3	0.002*	0.001*	<0.001*
Control	<0.001*	<0.001*	<0.001*

Note:
MP1 = methanol extract of kecombrang fruits of 4.2 mg/20 gr before LPS
MP2 = methanol extract of kecombrang fruits of 4.2 mg/20 gr 5 days after LPS
MP3 = methanol extract of kecombrang fruits of 4.2 mg/20 gr simultaneously LPS
Control = only LPS
*Significant at α=5%

Table 2 showed that the value of NFkB levels is significantly different across the groups with a p-value of < 0.05. Based on the description above, it can be seen that the use of the methanol extract of Kecombrang fruit lowered NFkB, and the best result was in MP1.

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 across the groups were identified using the ANOVA test because the data were normally distributed, and the result is presented in Table 3.

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**
MP 1	3.61 ±0 .28
MP 2	5.64 ±0 .18
MP 3	4.82 ±0 .23
Control	7.83 ±0 .29
p-value	<0.001*

Note:
MP1 = methanol extract of kecombrang fruits of 4.2 mg/20 gr before LPS
MP2 = methanol extract of kecombrang fruits of 4.2 mg/20 gr 5 days after LPS
MP3 = methanol extract of kecombrang fruits of 4.2 mg/20 gr simultaneously LPS
Control = only LPS
*Significant at α=5%

Based on Table 3, the average value of the level of Caspase 3 in the MP1,MP2, MP3 and control group is3.61 +0.28, 5.64 +0.18, 4.82 +0.23 and 7.83 +0.29, respectively. Therefore, the methanol extract of Kecombrangfruit of4.2 mg/20 gr can reduce the levels of CASPASE 3 with the best results in the MP1 Group (methanol extract of Kecombrangfruit of4.2 mg/20 gr before LPS).

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

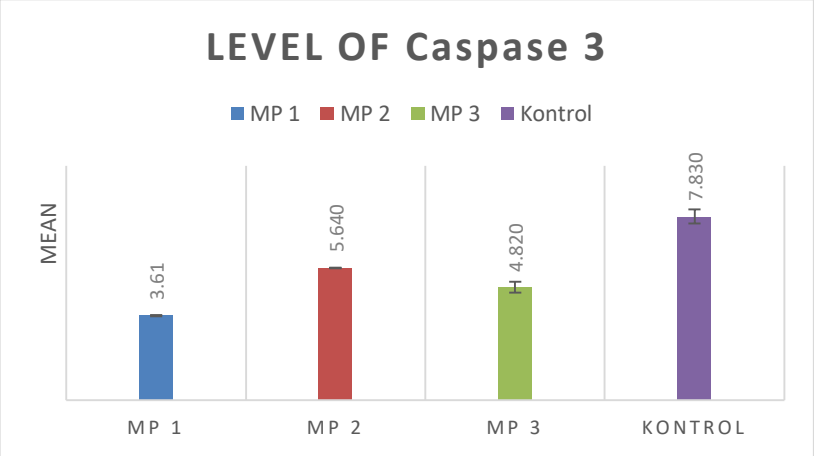

Figure 2. Description of levels of Caspase 3 in each treatment preparation

The ANOVA test showed a significant difference in the level of Caspase 3 across the groups with p< 0.05. Then, an LSD posh hoc test was carried out to obtain the following results in Table 4.

Table 4. Posh 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-value		
	MP1	MP2	MP3
MP2	<0.001*		
MP3	<0.001*	<0.001*	<0.001*
Control	<0.001*	<0.001*	<0.001*

Note:
MP1 = methanol extract of kecombrang fruits of 4.2 mg / 20 gr before LPS
MP2 = methanol extract of kecombrang fruits of 4.2 mg / 20 gr 5 days after LPS
MP3 = methanol extract of kecombrang fruits of 4.2 mg / 20 gr simultaneously LPS
Control = only LPS
*Significant at α=5%

Table 4 showed that the level of Caspase 3 values have significant differences across the groups with p< 0.05. Based on the description above, it can be seen that the methanol extract ofKecombrang fruit can reduce Caspase 3 with the best results in MP1.

Sepsis is a disease with the most expensive treatment in the United States, around 5.2% of total hospital costs.[1] In the manifestation of this infection, Caspase 3 mRNA is activated to initiate the process of apoptosis.[14] The results of a study by Satpute et al. reported that VA has antimicrobial, anti-inflammatory, and antioxidant properties.[15] Furthermore, in vitro studies showed that VA 100 g/ml can inhibit the growth of S. enterica and S. mutans bacteria.[16] VA acts as an antihypertensive, antihyperglycemic, and antioxidant at a dose of 50 mg/kg body weight of Mus musculus.[17] The compounds were found in Angelica sinensis (Ma, Guo, Jin, 2015) and Etlingeraelatior (E. elatior) plants.[16] This plant originates from Indonesia, and it is widely spread compared to Angelica Sinensis.

The methanol extract of kecombrang fruits of 4.2 mg/20 gr can reduce levels of NFkB with the best results in the MP1 Group (methanol extract od kecombrang fruits of 4.2 mg/20 gr before LPS). The ANOVA results showed that there are significant differences in NFkB levels across the groups with p <0.05. Furthermore, the posh hoc test reported that the use of methanol extract fruits can reduce NFkBwith the best results in MP1.

The methanol extract of kecombrang fruits of 4.2 mg/20 gr can reduce levels of caspase 3 with the best results in the MP1group (methanol extract of Kecombrang fruits 4.2 mg/20 gr before LPS). The ANOVA test showed that there are simultaneous significant differences in the level of Caspase

3 across the groups with p<0.05. Therefore, the use of methanol extract of Kecombrang fruits can reduce Caspase 3 with the best results in MP1. In addition, this study has several limitations, including the number of subjects, variable parameters, and confounding factors.

CONCLUSION

The methanol extract of kecombrang fruits of 4.2 mg/20 gr can reduce levels of NFKB and Caspase 3 with the best results in the MP1 group (methanol extract of kecombrang fruits of 4.2 mg/20 gr before LPS). In sepsis, the beneficial effects of the pretreatment are evident from the observations. Therefore, the extract of Kecombrang can be exploited in the treatment of sepsis.

REFERENCES

Gyawali B., Ramakrishna K., and Dhamoon A.S. 2019. Sepsis: The evolution in definition, pathophysiology, andmanagement. SAGE Open Medicine Vol. 7, p. 1 –13.

Siddharth D., Chirag C., and Abhijit D. 2020. Sepsis andsepticshock: Guideline-based management. *Cleveland Clinic Journal Of Medicine,* Vol. 87, no 1.

Singer, M., et al.(2016) 'The third international concesus definitions for Sepsis and Septic shock', *Jama*, 315(8), p. 801–810. doi: 10.1001/jama.2016.0287.

Laksono A.B. 2020. *IdentifikasiFitokimiaSebagaiPenghambatNadphOksidaseMelalui P47-Phox UntukPengembanganTerapiHipertensiSecaraIn Silico*. Surakarta. Skripsi.

Samarang, Isnawati R., and Murni. PotensiKandunganKarondo (EtlingeraElatior) SebagaiObatCacingTradisional Masyarakat Kulawidi Sulawesi Tengah. *JurnalPenyakitBersumberBinatang*, Vol. 2, No.2, p. 1- 8.

Michael D. Howell, and Andrew M. 2017. Davis Management of Sepsis and Septic Shoc*JAMA Clinical Guidelines Synopsis*,Vol. 317. Issue 8, p. 847-8.

Pedomannasionalpelayanankedokteran sepsis. Kementrian Kesehatan Republik Indonesia. 2017.

Guntur H.A., 1999. Sepsis, imunopatogenesis dan penatalaksanaannya. Simposiumsehari FK-UNS. Surakarta, p. 17-27.

Arifin, Guntur A, 2005. Prevalensi sepsis di rumahsakit dr. Moewardi Surakarta tahun 2004. Dalam Guntur A, Pramana TY, Prasetyo DH, (ed). Kumpulan MakalahLengkapKonas PETRI XI, PERPARI VII, PKWI VIII dan PIT PAPDI Cab. Surakarta. Surakarta, p. 105- 10

Pudjiastuti, 2008. Imunoglobulinintravena pada anak dan bayidengan sepsis. Dalam Guntur A, Subagyo Y, Sumandjar T, Reviono (ed). Kumpulan Makalah National Symposium : The 2nd Indonesian Sepsis Forum. Surakarta, hlm 100-5

Siner, J.M. 2009. Sepsis: definitions, epidemiology, etiology and pathogenesis. *PCCSU Article*l, p. 09-15.

Liu S.F, and Malik A.B. 2020. NF-κB activation as a pathological mechanism of septic shock and inflammation. *American Journal of Physiology-Lung Cellul rand Molecular,* p. 662-645

Wu F., Schuster D.P., Tyml K., and Wilson J.X. 2007. Ascorbate inhibits NADPH oxidase subunit p47 phox expression in microvascular endothelial cells. *Free Radical Biology & Medicine*, Vol. 42, p. 124–31.

Chousterman B.G, Swirski F.K., and Weber G.F. 2017 Cytokinestormand sepsis diseasepathogenesis. Seminars in Immunopathology. doi: 10.1007/s00281-017-0639-8.

Satpute MS, Gangan VD, hastri, I. 2019. Synthesis and antibacterial activity of novel vanillic acid hybrid derivatives. *Rasayan Journal of Chemistry*, Vol. 12. No 1, p. 383–8.

Sahidin, Salsabila S, Wahyuni, Fristiohady A, and Imran. 2019. PotensiAntibakteriEkstrakMetanol dan SenyawaAromatikdariBuahWualae (*Etlingeraelatior*). *Jurnal Kimia Valensi*, Vol 5, No. 1, p. 1-7.

Vinothiya K. and Ashokkumar N. 2017. Modulatory effectof vanillic acid on antioxidant status in high fat diet-induced changes in diabetic hypertensiverats.

Biomedicine&Pharmacotherapy, Vol. 87, p. 640–52.

Dewi A.R.I., Nur'Aini I.S, Bahri H.N., Afifah A, Fattah, and Tunjung W.A.S. 2016. Antihyperuricemic activity of gingerflower (Etlingeraelatior Jack.) extract in beef broth-induced hyperuricemic rats (Rattusnorvegicus). *AIP Conf. Proc*., Vol. 1755. 10.1063/1.4958573

Fristiohadya A, Wahyunia W, Ilyas M, Bafadala M, Purnamaa L.O.M.J., Sangadjia , Muhammad Malakaa M.H., Malika F, Hongc J , Yodhaa A.W.M., and Sahidin I. 2020. Nephroprotective effect of extract Etlingeraelatior (Jack) R.M. Smith on CCl4-induced nephrotoxicity in rats. *Current Research on Biosciences and Biotechnology*, Vol 1, No. 2, p. 62-5.

Mai C.W., Wong S.Y., Tan E.L., Balijepalli M.K., and Pichika M.R. 2009. Antiproliferative and apoptotic studies of the standardized extracts of Etlingeraelatioron human colorectal carcinomacells. Malays. *J. Chem,* Vol.11, p. 136-42.

Gotts J. and Matthay M. 2016. Sepsis: pathophysiology and clinical management. *BMJ.* p. 1353-585.

Mera S., Tatulescu D., Cismaru C., Bondor C., Slavcovici A., Zanc V., Carstina D., and Oltean M. 2010. Multiplex Cytokine Profiling In Patients With Sepsis. *APMIS.* No. 119, p. 155–163.

Bernard G.R., Vincent J.L., Laterre P.F., LaRosa S., Dhainaut J.P., Rodriguez A.L., et al.2001. Efficacy and safety of recombinant human activated protein c forsevere sepsis. *N Eng J Med.* Vol. 344. Issue 10, p. 699-709.

Meng J. and Zhang C. 2019. Modulating Effects of Vanillic Acid on Sepsis-induced Oxidative Liver Injury in Rat Model. *International Journal Pharmacology,*Vol. 15, No. 6, p. 752-8.

Rhodes A., Evans L.E., Alhazzani W., Levy M.M., Antonelli M., Ferrer R., et.al. 2017. Surviving sepsis campaign: International guidelines for management of sepsis and septic shock: 2016. *Intensive Care Med*, Vol. 43 Issue 3, p. 304-77.