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EFFECTS OF *ANDROGRAPHIS PANICULATA* LEAF EXTRACT ON C-REACTIVE PROTEIN AND SERUM FERRITIN IN LIPOPOLYSACCHARIDE-INDUCED SEPSIS MODEL RAT

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ABSTRACT

Background: C-reactive protein (CRP) and ferritin are acute-phase reactants that have demonstrated association with the severity of a variety of inflammation-mediated illnesses, including infection and sepsis. Sambiloto (Andrographis paniculata) leaf is traditionally used as an anti-inflammation and anti-bacterial herb. Sambiloto leaf extract possesses anti-inflammatory, antiallergenic, immuno-stimulatory, antiviral, and antioxidant activities. **Objective:** To evaluate the effect of Sambiloto leaf extract (Andrographis paniculata) on inflammation biomarker (CRP and serum ferritin) in a rat model of sepsis-induced by lipopolysaccharide (LPS). **Methods:** This research was a true experimental study with a post-test-only control group design. Twenty five male Wistar rats were randomly divided into 5 groups consisting of a healthy control (HC), Negative control (NC), Treatment 1 (T1) Sambiloto 200mg/kgBW, T2 Sambiloto 400mg/kgBW, T3 Sambiloto 500mg/kgBW). Sambiloto leaf extract was given on days 8-21 and LPS 5mg/kgBW was injected intraperitoneal on day 22. On the 25th day, blood was drawn and CRP and serum ferritin levels were analyzed using ELISA method. The data were analyzed using One-Way ANOVA and Kruskal Wallis test. **Results:** The mean CRP levels of HC, NC, T1, T2,T3 were 7.72 ± 2.53; 8.12 ± 1.08; 5.22 ± 1.71; 6.40 ± 1.19; 5.14 ± 1.37 ng/dl respectively. The mean serum ferritin level of HC, NC, T1, T2, T3 were 293.60 ± 66.53; 601.20 ± 100.17; 433.40 ± 194.65; 331.60 ± 75.97; 318.00 ± 69.64 ng/dl respectively. There were differences in CRP levels in groups T1(p=0.012) and T3(p=0.010), serum ferritin levels in groups T2(p=0.014) and T3(p=0.008) to the control group. **Conclusion:** Sambiloto (Andrographis paniculata) leaf extract has anti-inflammatory effects in the rat model of sepsis.

Keywords: *Andrographis paniculata, CRP, serum ferritin, lipopolysaccharide, sepsis*

INTRODUCTION

Lipopolysaccharide (LPS) endotoxin can induce systemic inflammatory response, called sepsis. LPS is well-known bacterial components that derives from the cell wall of Gram-negative bacteria.[1] Increased stimulatory response on the immune system, and LPS-mediated signalling, in severe cases, can lead to sepsis shock.[2,3] A 10-years meta-analysis study noted that estimated mortality rates in North America, Europe, and Australia ranging from 26.4 – 33.7%.[4] Referring to high-mortality rate, increasing awareness of sepsis-related action was receiving attention from World Health Organization (WHO).[5]

Unfortunately, there is no gold standard for sepsis diagnosis. Despite the commonly used sequential organ failure assessment (SOFA) score, recently found higher risk of premature of the sign of a potentially fatal infection.[6] Development of sepsis biomarker definitely help in prognosis prediction and diagnosis of sepsis cases.[7] C-Reactive Protein (CRP) and Ferritin are the most common biomarker used for diagnosis and severity of sepsis.[8] A prospective observational cohort study in America revealed combination value of CRP and ferritin simultaneously affected mortality risk up to 80% in children with sepsis.[8] With cut-off point of CRP and ferritin value are 7.1 mg/dL and 373 ng/mL respectively.[9]

In the light of discovery sepsis-related drug, *Andrographis paniculate* stands out because provides various benefits, including antioxidant, antiviral, anti-pyretic, and anti-inflammatory properties. *Andrographis panilculata* is, well-known as *Sambiloto*, very popular among traditional population in Indonesia. Several clinical studies regarding *A. paniculata* are showing certain benefits in adults with acute respiratory tract infections, cold, fever, and sore throat.[10] Sumiyati et al (2022) showed significant role of *A. paniculata* in liver necrosis prevention by preventing lipid accumulation in sepsis model rat.[11] Thus, encourage the author to further

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investigate *A. paniculata* effect on CRP and ferritin serum level in LPS-induced sepsis model rat.

METHODS

Study Design

This research is an experimental study with a post-test-only control group design. The subjects of this study used experimental male Wistar rats (*Rattus norvegicus*) aged 8-12 weeks. The study was conducted from June to August 2021, at Center for Food and Nutrition Studies, Gadjah Mada University.

Animal Treatment

A. paniculata extract were obtained from PT. Industri Jamu dan Farmasi Sido Muncul (Semarang, Indonesia).

To induced sepsis in wistar rats. The authors are using LPS 5 mg/kgBW dissolved in NaCl 0.9% 3 ml on the day 25 of study.

This study were using 25 healthy male wistar rats randomly allocated into 5 groups; healthy control (hC), negative control (Cneg), treatment one group treated with 200 mg/kgBW *A. paniculata* extract (T1); treatment two group treated with 400 mg/kgBW *A. paniculata* extract (T2); and treatment three group treated with 500 mg/kgBW *A. paniculata* extract (T3). Adaptation was carried out for 7 days, followed by treatment for 14 days, and injection LPS injection on day 22. Throughout the study, rats which experienced weight loss after adaptation period and death during study period were excluded from study. On day 25, all rats were terminated using excessive chloroform gas, and blood sample was collected to further measure CRP and serum ferritin level by ELISA.

Statistical Analysis

Statistical analysis was done using SPSS Software for Mac version 26.0. CRP and serum ferritin data normality analysed by Saphiro-Wilk test, followed by ANOVA and Kruskal Wallis, for CRP level and serum ferritin data respectively. All analysis were considered significant with $p < 0.05$.

This study has been approved by Ethical Commission of Health Study, Diponegoro University (No. 50/EC/FK-UNDIP/V/2021).

RESULTS

Table 1. Bivariate analysis of CRP and serum ferritin levels

Group	Variable	
	CRP (ng/mL)	Ferritin Serum (ng/mL)
hC	7.72 ± 2.53	293.6 ± 66.53
Cneg	8.12 ± 1.08	601.2 ± 100.17
T1	5.22 ± 1.71	433.4 ± 194.65
T2	6.4 ± 1.19	331.6 ± 75.97
T3	5.14 ± 1.37	293.6 ± 66.53
p	0.027*†	0.022*‡

Note: †One-Way ANOVA; ‡Kruskal-Wallis

Table 1 showing the descriptive and bivariate analysis of CRP and serum ferritin level of research subject. Normality test by Saphiro-Wilk revealed that data of CRP level was normally distributed ($p > 0.05$), while data of serum ferritin was not normally distributed ($p < 0.05$). Hence, bivariate analysis was done differently per variable mentioned, ANOVA and Kruska-Wallis were used for CRP and serum ferritin data respectively. It was found both CRP and ferritin serum levels were statistically significant compared to every group in this study.

Table 2. Post Hoc Analysis of CRP levels

Groups	Compared Group	*p*
hC	Cneg	0.708*
	T1	0.027*
	T2	0.224
	T3	0.023*
Cneg	T1	0.012*
	T2	0.117
	T3	0.010*
T1	T2	0.275
	T3	0.940
T2	T3	0.245

Note: *Significant

Further analysis done by post hoc LSD test to investigate difference value of CRP in every groups. This analysis resulted that hC-Cneg, hC-T1, hC-T3, Cneg-T1, and Cneg-T3 group comparison were statistically significant. These results are indicating *A. paniculata* effectiveness in decreasing CRP level in sepsis-model rats.

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Table 3. Mann-Whitney Analysis of serum ferritin level

Groups	Compared Group	*p*
hC	Cneg	0.003*
	T1	0.169
	T2	0.606
	T3	0.764
Cneg	T1	0.112
	T2	0.014*
	T3	0.008*
T1	T2	0.390
	T3	0.283
T2	T3	0.830

Note: *Significant

Further analysis done by Mann-Whitney test to investigate difference value of serum ferritin in every groups. This analysis resulted that hC-Cneg, Cneg-T2, and Cneg-T3 group comparison were statistically significant. These results are indicating *A. paniculata* effectiveness in decreasing serum ferritin level in sepsis-model rats. This results also indicating overall anti-inflammatory activities of *A.paniculata* in reducing inflammatory response caused by LPS-induced sepsis in vivo.

DISCUSSION

In LPS-injected rats, they will develop condition where pro-inflammatory cytokines such as TNF-α, IL-6, and IL-1 are very high, leading to septic conditions.[12] Sepsis occurs because the body responds excessively to infection.[13] The role of the body against infection is the introduction of microorganisms through pattern recognition receptors (PRR) such as toll-like receptors (TLRs) to pathogen-associated molecular pattern (PAMPs).14 First-line responds are monocytes and macrophages which will then induce pro-inflammatory cytokines such as TNF-α, IL-6 and chemokines to strengthen the inflammatory response.[13] Followed by activation of lymphocyte cells as an immune response and nitric oxide (NO) to induce vasodilation and an increase in leukocytes to the body's immune activity.[15] This type of increased immune response-related to sepsis can be reflected by several inflammatory biomarkers, such as CRP and serum ferritin level.

The main source of ferritin is macrophages, when stimulated with non-canonical inflammatory agonists, will produce the highest amount of ferritin.[16] In addition to macrophages, the function of hepatocytes is to encourage caspase-11 activation in macrophages and facilitate the absorption of lipopolysaccharide into myeloid cell lysosomes.[17] Ferritin functions to fight cytokine inflammatory cells such as interleukin 1 (IL-1), IL-6, TNF-α.[18] Extracellular ferritin can also act as a pro-inflammatory cytokine and induce NF-kB signalling.[18] Most of the body's ferritin stores are in the reticuloendothelial system macrophages and hepatocytes.[19] Yunita E, et al stated that andrographolide is a bioactive component consisting of many pharmacological aspects, including anti-inflammatory as an antioxidant by stopping the chain reaction of a free radical molecul.[20] In a previous study, Fu S, et al revealed that increasing ferritin levels is capable of causing macrophage accumulation, increasing ROS formation, decreasing transferrin levels and increasing hemoglobin levels during inflammation.[21] This is supported by the study of Mussard E, et al, which indicated that *A. paniculata* was able to reduce ROS production in serum ferritin-mediated oxidative stress and decrease IL-6 secretion and LPS-induced TNF- expression.[22]

CRP is used as an acute phase marker in tissue injury, infection and inflammation.[23] This protein is synthesized by macrophages and smooth muscle cells in hepatocytes and secreted in blood plasma which is regulated by the cytokine IL-6.[24] Factors that influence the increase in CRP levels during inflammation are increases in proinflammatory cytokines such as IL-6, TNF-a, IL-1ß and NF-kB.[25] This endotoxin will increase pro-inflammatory mediators such as TNF-a, IL-6, IL-1, and IL-17 to activate macrophages, then macrophages will damage tissue resulting in inflammation.[25] Acute inflammation can cause sepsis precipitated by an infectious agent.[26] Burgos et. al (2020) noted that *A. paniculata* exhibit inhibition of NF-kB to DNA in neutrophils thereby reducing the expression of pro-inflammatory proteins.[27] Zhu N, et al showed that *A. paniculata* has 23 bioactive anti-inflammatory compounds which able to reduce CRP stimulated by proinflammatory cytokines such as IL-6 and TNF-a.[28,29] Recent study also supported by study results of Fu K, et al which examine the inflammatory response in *A. paniculata* which was induced by LPS determined using CRP as biomarker. The results of the analysis showed that *andrographolide* was able to

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reduce CRP expression compared to the group induced only by LPS.[30]

CONCLUSION

The results of this study indicate that rat model of sepsis which receive pre-treatment of *Andrographis paniculata* for fourteen days has lower CRP and serum ferritin level than control groups. These results show that pretreatment *Andrographis paniculata* has inhibition ability of CRP and serum ferritin level in LPS-induced model rats.

ETHICAL APPROVAL

This research has received ethical clearance from the Research Ethics Commission of the Faculty of Medicine, Diponegoro University (No. 50/EC/FK-UNDIP/V/2021).

CONFLICTS OF INTEREST

The author declare, there is no conflict of interest.

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AUTHOR CONTRIBUTIONS

Conceptualization, Nyoman Suci Widyastiti, Rezya Salsabela and Neni Susilaningsih; methodology, Rezya Salsabella and Nyoman Suci Widyastiti; software, Rezya Salsabela, Dwi Retnoningrum and Ariosta; validation, Nyoman Suci Widyastiti, Neni Susilaningsih, and Dwi Retnoningrum; formal analysis, Rezya Salsabela and Nyoman Suci Widyastiti; investigation, Rezya Salsabela, Nyoman Suci Widyastiti; resources, Rezya Salsabela; data curation, Rezya Salsabela, Nyoman Suci Widyastiti; writing—original draft preparation, Rezya Salsabela and Nyoman Suci Widyastiti; writing—review and editing, Rezya Salsabela, Nyoman Suci Widyastiti, Neni Susilaningsih, Dwi Retnoningrum; visualization, Rezya Salsabela and Ariosta; supervision, Nyoman Suci Widyastiti; project administration, Dwi Retnoningrum and Ariosta; funding acquisition, Nyoman Suci Widyastiti.

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