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FABP4 and Metabolite Profile in Lipopolysaccharide-Induced Mice Model Treated with *Moringa oleifera* Ethanol Leaf Extract

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Abstract

Sepsis, a life-threatening organ dysfunction resulting from a dysregulated host response to infection, induces changes in blood cells and metabolic alterations. Fatty acid binding protein 4 (FABP4), a lipid chaperone predominantly expressed in adipose tissue, is modulated in sepsis and may contribute to metabolic and immunologic changes. *Moringa oleifera* (*M. oleifera)* leaf extract (MOLE) is known to modulate immune system activity, but its potential for treating acute inflammatory conditions like sepsis remains unclear. This study investigates the ability of MOLE to modulate metabolite and hematological profiles in lipopolysaccharide (LPS)-induced sepsis in mice. Thirty-five male Swiss Webster mice (*Mus musculus*) were divided into five groups, including healthy control pre-treated with 0.5% carboxymethyl cellulose (CMC), an LPS-induced negative control, an LPS-induced positive control treated with dexamethasone (DMX) 7mg/KgBW/day and two MOLE treatment groups with doses of 5.6 and 11.2 mg/20 gBW. Mice received MOLE pre-treatment for three days before LPS induction. Three hours post-LPS injection, the LPS-induced group exhibited leukopenia (1.4 [0.9-2.5] x109 cells/L) and a 68.3% increase in triglyceride levels. However, the MOLE-treated group showed improved erythrocyte levels compared to the positive control group; [(9.9(9.3-10.0) x1012 cell/L) vs (7.7(7.0-9.0) x1012 cells/L), $p<0.05$]. The study suggests that MOLE administration may positively impact sepsis conditions, particularly by enhancing RBC levels. Further research with an extended observation period is recommended to address limitations in metabolite level assessment.

Keywords: *Moringa oleifera,* fatty acid binding protein – 4 (FABP-4), metabolite profiles, hematological profiles, lipopolysaccharide

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Introduction

Sepsis, a life-threatening condition,[1] is associated with various cellular changes, including the induction of free radicals, an imbalance in oxidation reactions, and a decline in liver energy metabolism.[2] At the symptomatic level, sepsis triggers inflammatory reactions such as hypothermia or hyperthermia, tachycardia, tachypnea, and changes in white blood cell (WBC) count.[1,3] This imbalance results in a systemic response,[4] causes damage to cells and organs.[5] Sepsis also affects metabolic homeostasis, mainly carbohydrate and lipid metabolism. Cytokine activation, such as TNF-α, IL-6, and IL-1β, plays a significant role in these conditions. Additionally, sepsis affects energy and lipid metabolism.[6,7] TNF-α, similar to cachetin, is believed to increase lipolysis during sepsis. IL-1β and IL-6 work together to induce a hypermetabolic state.[7]

Mitochondrial damage is considered a primary cause of metabolic disorders in sepsis, influencing the metabolism of all macronutrients. Carbohydrate metabolism experiences intensified glycolysis, leading to increased lactate formation due to the failure to introduce pyruvate into the tricarboxylic acid cycle. In lipid metabolism, sepsis induces lipolysis in adipose tissue, resulting in elevated levels of fatty acids and triglycerides in the blood. Simultaneously, disrupted energy substrate utilization may lead to the accumulation of lipids and their toxic metabolites.[6]

Moreover, adipocyte tissue undergoing lipolysis releases fatty acid binding protein 4 (FABP4) acting as an adipokine. FABP4 is expressed by adipocytes and macrophages, playing a crucial role in insulin resistance and atherosclerosis development, especially in metaflammation. During inflammatory states, FABP4 is expressed by macrophages upon LPS administration. In our previous study, there were alterations in pro-inflammatory cytokines, and increased glycogen and triglycerides in the heart of LPS-induced mice. Additionally, a decrease in blood glucose levels and an increase in triglyceride and non-esterified fatty acid (NEFA) levels were observed in mice induced with LPS.[8]

The shift from chemical to traditional treatments has led to extensive studies on herbal plants, revealing their immunomodulatory potential in optimizing the immune system during disruptions like infections and inflammation. We previously demonstrated the efficacy of cogon grass (*Imperata cylindrica*) roots extract in ameliorating sepsis conditions, as evidenced by improvements in hematological parameters (lymphocytes, monocytes, and platelets), immunological factors (TNF-a and GPx3), and metabolite levels (FABP4). This observed effect is attributed to the presence of flavonoids in the extract, known for their potential anti-inflammatory properties.[9]

The flavonoid content is also found in other plants, including *M. oleifera*.[10] *M. oleifera* is monogeneric genus *Moringa* within the *Moringaceae* family, gaining widespread recognition since the conference held in 2001 in Tanzania. Subsequent studies have explored the potential of *M. oleifera*, particularly in the field of health, owing to its abundance of bioactive compounds such as vitamins, carotenoids, polyphenols, phenolic acids, flavonoids, alkaloids, glucosinolates, isothiocyanates, tannins, saponins, oxalates, and phytates. Among the numerous compounds found in *M. oleifera*, quercetin is believed to play a role in inhibiting NF-ĸB activation in the inflammatory process.[11]

M. oleifera leaves extracts (MOLE) inhibit the production of human macrophage cytokines

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like tumor necrosis factor alpha (TNF-α), interleukin-6 (IL-6), and IL-8, induced by cigarette smoke and LPS. Furthermore, *M. oleifera* and isothiocyanate concentrates may decrease gene expression and the production of inflammatory markers in RAW macrophages. In particular, they attenuate the expression of inducible nitric oxide synthase (iNOS), IL-1β, and the production of NO and TNFα at 1 and 5μM.1[1,12] Further study indicate MOLE has the potential to reduce triglyceride levels, glucose levels[11,13,14] also increase hemoglobin levels, and reduce granulocyte presentation in mice models of chronic inflammation due to a high-fat diet[15] and diabetes.[11,13,14] However, there have been no studies related to MOLE in sepsis model mice. Thus, the study aimed to determine the effect of MOLE on FABP4 concentration, metabolite profile and hematological profile in sepsis model mice.

Methods

Ethical consideration

Considering the Declaration of Helsinki for the welfare of animals used for research,the Research Ethics Committee Universitas Padjadjaran Bandung has approved the experimental animal protocol (Approval No. 959/UN6.KEP/EC/2022).

Extract and Drug Preparation

M. oleifera leaf powder, approximately 1 kg in quantity, was obtained from PT. Moringa Organik Indonesia. The extraction process involved maceration using a 1:3 ratio of 96% ethanol (Merck, USA) to water for 24 hours, followed by filtration. This procedure was repeated three times. The resulting filtrate was homogenized and subjected to rotary evaporation until it reached one-third of the initial volume.

Subsequently, the filtrate was frozen at −20°C for 24 hours and further desiccated through freeze-drying until it transformed into a greenish concentrate (approximately 1.8 g). The resultant extract was stored in a refrigerator for subsequent applications in treatment as previously described.[16]

The utilization of carboxymethyl cellulose (CMC) as a solvent for MOLE at the optimal concentration of 0.5% involved dissolving it in distilled water.[17] The administration of MOLE, based on previous studies, comprised an initial dose of 5.6 mg/20g body weight (BW) [MOLE1] and a second dose of 11.2 mg/20gBW [MOLE2], each dissolved in CMC 0.5%.16 LPS from Escherichia coli O111 : B4 (Sigma-Aldrich, US), at a dosage of 10 mg/kgBW, diluted in 10 ml phosphate-buffered saline (PBS).[9,18] Dexamethasone, sourced from PT. Harsen, Jakarta, Indonesia, was administered at a dose of 7 mg/kg/BW/day.[19]

Model dan Research Design

The male Swiss Webster mice (*Mus musculus*), belonging to the normal species, which are intended for induction with LPS at a dosage of 10 mg/kg body weight, were procured from D'Wistar Company (Bandung, Indonesia). The selection criteria include an age range of 4-6 weeks and a body weight within the range of 20 – 25 gram. All efforts were made to relieve any pain and distress of the animals by strictly following the procedures. The mice were acclimatized for two weeks in the laboratory. They were then kept in cages at the animal laboratory of Universitas Padjadjaran at a controlled room temperature and on a 12 hours light/12 hours dark cycle. The mice were provided regular food, drinking water ad libitum, and observed daily to confirm lack of behavior.

A randomized post-test control group design was used. The number of samples for each treatment group was determined using the Federer formula [(n-1)(t-1) ≥ 15; n ≥ 5]. The

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mice were divided into the following five experimental groups (5-7 mice per group; 35 mice total): group 1 (healthy control) and group 2 (negative control) were treated with (CMC), group 3 (positive control) was treated with CMC and DMX, while group 4 and 5 (treatment group), were treated with MOLE1 and MOLE2, respectively, for three days. On the third day, mice were fasted overnight. Groups 2, 3, 4 and 5 were injected intraperitoneally with LPS. At 3 hours after injection, blood were collected from the abdominal vein, then inserted into the K3-EDTA tube and SST tube.

Measurement of blood parameters

The whole blood in the K3-EDTA tubes was is mixed with the anticoagulant ethylenediaminetetraacetic acid (EDTA). EDTA prevents blood clotting by binding to calcium ions, crucial for the coagulation process, thereby inhibiting the coagulation cascade and maintaining the blood in a liquid, non-clotting state.[15,20–24] The processed blood is then analyzed using the Mindray BC20s hematology analyzer, following the manufacturer's protocol.

The Mindray BC20s utilizes the volumetric impedance method to measure the quantity and characteristics of blood cells. The analyzer draws a small volume of anticoagulated whole blood through an aperture. As cells pass through this aperture, they disrupt an electric current. The resulting impedance changes are proportional to the volume and number of cells passing through, allowing for the quantification of leukocyte (WBC), erythrocyte (RBC), and thrombocytes (PLT), also known as platelets).

Blood parameter tests in this study including WBC, differential count (lymphocyte, granulocyte or neutrophil and mid-range counts or MID encompass cells like monocytes, eosinophils, basophils, blasts, and other precursor white cells falling within a specific size range,[25] PLT, RBC, hematocrit (HCT), hemoglobin (HB), mean corpuscular hemoglobin concentration (MCHC), mean platelet volume (MPV), and red distribution width concentration (RDWC), mean platelet volume (MPV), platelet disribution width concentration (PDWC) dan plateletcrit (PCT).

Measurement of metabolite parameters

The serum in the serum separator tube (SST) tube is separated using centrifugation at 1,500g for 15 minutes at 4°C, and were stored at 80°C for metabolite examination. Metabolites measured include FABP4, NEFA, Triglycerides and Glucose.[8,9,16] FABP4 levels in the samples were measured using commercial enzyme-linked immunosorbent assay (ELISA) kits Mouse FABP4 ELISA Kit (Fine Biotech, Wuhan, China) according to the manufacturer's protocol. NEFA, Glucose and Triglyceride levels in the samples were measured using commercial colorimetric assay kit (Elabscience, United State and DiaSys Diagnostic Systems GmbH, Germany) according to the manufacturer's protocol.

Statistical analysis

Statistical analyses were performed using the GraphPad Prism version 8.0.1 (244) for Windows (GraphPad Software, Inc. CA). All of the data were submitted to the Shapiro-Wilk test of normality. Data are shown as Mean ± SD, on normally distributed, variables using ANOVA One Way and Tukey's test as post hoc. Whereas non-parametric variables are shown as median (min-max) values using Kruskal-Wallis and Dunn's test as post hoc.

Results and Discussion

Numerous methods exist for establishing sepsis models in experimental animals, with among the most frequently employed being cecal ligation and puncture (CLP) and LPS induction.

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In our study, the LPS induction method was chosen based on several advantages, including (1) a simpler technique,[26] (2) the formation of an inflammatory response resembling the response to a direct infection,[27] and (3) higher reproducibility with a faster recovery time.[26] The LPS dose used in this study aligns with previous studies, specifically 10 mg/kgBW.[8,9] As an initial observation, mice induced with LPS exhibited conditions such as lethargy, curling up, and clustering, indicative of behaviors aimed at maintaining body heat.[28] The post-LPS induction observation period in our study was conducted for 3 hours. This choice was made to spesifically target early onset of sepsis and focus on the inflammatory response. However, a few parameters exhibited different results when compared to previous studies that utilized longer durations for post-LPS induction observation.

WBC parameter, typically used as a reference for sepsis conditions, manifests conditions such as leukocytosis or leukopenia.1 In our study, leukopenia occurred, wherein the leukocyte levels were significantly lower in LPS treatment-only group compared to healthy group [1,4 (0,9-2,5) x109 cells/L vs 9,2 (5,7-11,8) x109 cells/L, $p<0,05$]. Furthermore, the results of the WBC differential count indicated a significant decrease in absolute counts of lymphocytes and granulocytes, with a simultaneous significant increase in mid-range relative count.

The reduction in lymphocyte absolute count was observed between the healthy group and the LPS treatment-only group and MOLE2 treatment group [6.9(4.7-9.7) x109 cells/L vs. 0.95(0.6-2.2) x109 cells/L and 0.9(0.3-2.0) x109 cells/L, $p<0.05$]. A decrease in granulocyte absolute count was also noted between the healthy group and the LPS treatment-only group and positive control group [1.3(0.9-2.9) x109 cells/L vs. 0.2(0.2-0.5) x109 cells/L and 0.2(0.1-0.7) x109 cells/L, $p<0.05$]. Meanwhile, an increase in mid-range relative count occurred between the healthy group and the LPS treatment-only group and MOLE^2 treatment group [1.3(1.2-1.5) x109 cells/L vs. 19.5(14.5-23.3) x109 cells/L and 18.6(14.5-25.6) x109 cells/L, $p<0.05$].

Our research reveals a decrease in WBC counts, consistent with findings reported in numerous prior studies related to the ability of endotoxins to induce inhibition of hematopoiesis processes through the administration of bacterial endotoxin[29] or LPS induction.[30] In other experimental animals, apart from mice, this commenced within the first hour following LPS induction and escalated after 8 hours post-induction.[36,37] The reduction in WBC count (leukopenia) correlates with a decrease in the number of circulating lymphocytes and monocytes[30] due to the migration of bloodstream into tissues. This is evidenced by absolute lymphocyte count data, whereas there were no differences in mid-range absolute count, and a significant increase in mid-range relative count was observed. This difference is presumed to be a result of the overall decrease in WBC count that occurred across all treatment groups due to LPS induction.

Therapeutic administration of MOLE in immunomodulated mice trials increased WBC count.[32] Meanwhile, in the administration of MOLE with sub-acute toxicity, here was an increase in WBC count.[33] The administration of MOLE without specific conditions in mice showed a significant increase in WBC count.[34] This condition aligns with the improvement trend observed after mice were provided with MOLE therapy in this study.

The RBC parameter showed a significant increase in treated with MOLE1 compared to positive control [(9,9 (9,3-10,0) x1012

cells/L vs (7,7 (7,0-9,0) x1012 cells/L), p<0,05]. RBC count in mice tends to increase after endotoxin induction,[35] providing significant results with MOLE therapy. This is consistent with previous research conducted on healthy mice[34,36] and mice induced with cyclophosphamide as an immunomodulation condition, which experienced an increase in RBC count.[32] However, there were no significant differences observed in platelet levels among the treatment groups (Table 1).

In our previous study, it was observed that there is a propensity for a reduction in WBC parameters, alongside an elevation in RBC and PLT parameters following *M. oleifera* therapy administered to a mouse model with a high-fat diet (HFD).[15] In the other hand, *M. oleifera* can increase WBC and decreased RBC and PLT in parasite infection.[37] These indicate that the difference of hematological parameter might be influenced by varying inflammatory conditions and disease background.

Metabolically, a significant 68.3% increase in blood triglyceride levels was observed in the LPS treatment-only group compared to healthy group (Figure 1.D). Elevated triglyceride levels have also been documented in various condition such as cholera, polymicrobial infections, and sepsis.[38–40] Previous research has identified triglyceride levels as predictive of sepsis-related mortality.[39,40] Sepsis triggers the release of catecholamines, which contribute to the liberation of free fatty acids through adipose tissue lipolysis. The liver metabolizes these free fatty acids, releasing them as triglycerides in lipoproteins.[2]

In gram-negative bacteria-induced sepsis (LPS), high doses (50ug/100gBW), lead to decrease Lipoprotein Lipase (LPL) activity in adipose tissue, diminishing catabolism and clearance of triglyceride-rich lipoproteins in the bloodstream.[2,38,41] Conversely, at low doses (100ng/100gBW), there is stimulation of de novo liver fatty acid synthesis and lipolysis.[38] This stimulation results in elevated blood triglyceride levels (hypertriglyceridemia).[42,43] Lipoproteins, particularly those containing large triglycerides (chylomicrons and VLDL), may function as an innate immune response to lipopolysaccharide-induced sepsis.[7,11] These lipoproteins can bind to lipopolysaccharides (LPS) to form lipoprotein-LPS or chylomicron-LPS complexes.[2,38] This binding modulates the immune response and inhibits LPS-induced toxicity. The chylomicron-LPS complex can inhibit nitric oxide (NO) production[45,46] y hepatocytes, suggesting that the chylomicron-LPS bond can inhibit the nuclear factor kappa-B (NF-ƘB) pathway and prevent liver damage.[2]

Additionally, glucose levels exhibited a significantly reduction of 71.8% in the positive control group, 74.9% in MOLE1-treated group and 66.2% in MOLE2-treated group compared to the healthy group (Figure 1.C). Previous studies have reported a substantial decrease in glucose levels[26] within the first 3 hours following endotoxin (LPS) administration,[47] persisting from 6 to 24 hours.[48] Dexamethasone therapy in other groups showed a decreasing trend within the 6 to 24 hours timeframe, with a slight increase at 12 hours post-induction.[48]

The decline in blood glucose levels is linked with the release of inflammatory cytokines. IL-1 is a prerequisite for hypoglycemia in LPS-induced mice, with IL-1α, β and TNF-α inducing hypoglycemia due to LPS induction.[49] This observation is consistent with research by Schmidt et al which revealing that TNF-α, IL-1β and INF-γ decrease the regulation of sodium-glucose co-transporter (SGLT)-2, SGLT-3, glucose transporter (GLUT)-2 and Na-K-ATPase, while SGLT-1 and GLUT-1 activity increased.

Table 1. Results of Hematological Parameter Examination Conducted using Hematology Analyzer (Mindray BC20s)

Parameter	Group				
	Healthy	LPS-only	LPS-DMX	LPS-MOLE1	LPS-MOLE2
Leukocyte					
WBC (10^9/L)	9.2(5.7-11.8)[ab]	1.4(0.9-2.5)[a]	1.5(0.9-3.8)	2.2(1.3-3.8)	1.3(0.5-3.9)[b]
Lymph (10^9/L)	6.9(4.7-9.7)[ab]	0.95(0.6-2.2)[a]	1.0(0.6-2.4)	1.5(0.8-2.1)	0.9(0.3-2.0)[b]
Mid (10^9/L)	0.1(0.1-1.4)	0.2(0.0-0.3)	0.3(0.0-0.7)	0.2(0.0-0.4)	0.2(0.1-0.7)
Gran (10^9/L)	1.3(0.9-2.9)[ab]	0.2(0.2-0.5)[a]	0.2(0.1-0.7)[b]	0.4(0.2-0.4)	0.3(0.1-0.7)
Lymph%	83.4(49.8-88.1)	68.5(58.2-91.7)	75.5(57-91.6)	75.6(60.5-88.7)	56.5(45.4-67.8)
Mid%	1.3(1.2-1.5)[ab]	19.5(14.5-23.3)[a]	16.7(4.8-20.9)	11.8(8.8-17.5)	18.6(14.5-25.6)[b]
Gran%	15.2(10.7-28.8)	12.4(9.2-23.2)	13.1(6.6-22.1)	12.2(6.2-23)	25.7(6.6-34.2)
Erytrocyte					
RBC (10^{12}/L)	7.9(7.4-8.3)[a]	8.3(7.5-9.1)	7.7(7.0-9.0)[b]	9.9(9.3-10.0)[ab]	8.5(8.0-8.9)
HGB (g/dL)	12.1(11.6-14.2)[a]	13.4(11.9-13.9)	14.0(11.7-15.7)	14.9(13-15.8)[a]	14.6(13.6-14.8)
HCT (%)	38.3(34.9-41.1)[a]	44(37.6-49.5)	46.4(38.4-53.0)	48.8(44.0-52.7)[a]	46.7(43.7-50.1)
MCV (fL)	48.2(47.0-50.9)[a]	52.8(49.7-59.6)	53.6(50.2-56.2)	44.15(43-50.7)[b]	54.9(53.2-56.2)[ab]
MCH (pg)	15.9(14.6-17.6)	16.0(15.2-16.8)	16.2(14.9-17.4)	14.6(13.2-16.8)	16.6(16.2-18.3)
MCHC (g/L)	328.5(304-346)	294(281-337)	303.5(287-310)	304.5(295-313)	308(288-335)
RDW-CV (%)	15.4(13.4-18.5)	16.2(13.2-17.7)	14.9(14.0-17.5)	16.5(13.6-21.6)	15.9(12.6-16.7)
RDW-SD (fL)	26.1(23.1-31.4)	30.5(23.2-33.9)	29.2(24.9-33.8)	28.6(24.4-33.5)	31.4(24.3-32.6)
Thrombocyte					
PLT (10^9/L)	480(162-781)	482(311-805)	516.5(364-835)	454(205-737)	614(182-921)
MPV (fL)	6.6(5.5-6.8)	6.0(5.9-6.6)	6.4(5.7-7.0)	6.6(5.7-7.0)	6.2(5.8-6.9)
PDW	15.0(14.7-15.0)	15.2(14.8-16.5)	15.1(14.8-15.6)	15.3(14.7-15.9)	15.0(14.8-15.3)
PCT (mL/L)	3.7(2.8-6.8)	2.9(2.1-5.0)	3.4(2.5-4.9)	1.7(0.4-4.5)	3.8(1.1-5.3)

LPS decreased leukocyte parameter in a sepsis mouse model
LPS increased mid-range relative count in a sepsis mouse model
Treatment of MOLE, increased erythrocyte parameter in sepsis mouse model
Data are represented as the median(min-max), n = 5 per group.
Kruskal-Wallis and post-hoc Dunn's test were performed for difference between median
a-b: Similar superscript in the same line indicate significant statistical differences ($p<0.05$)

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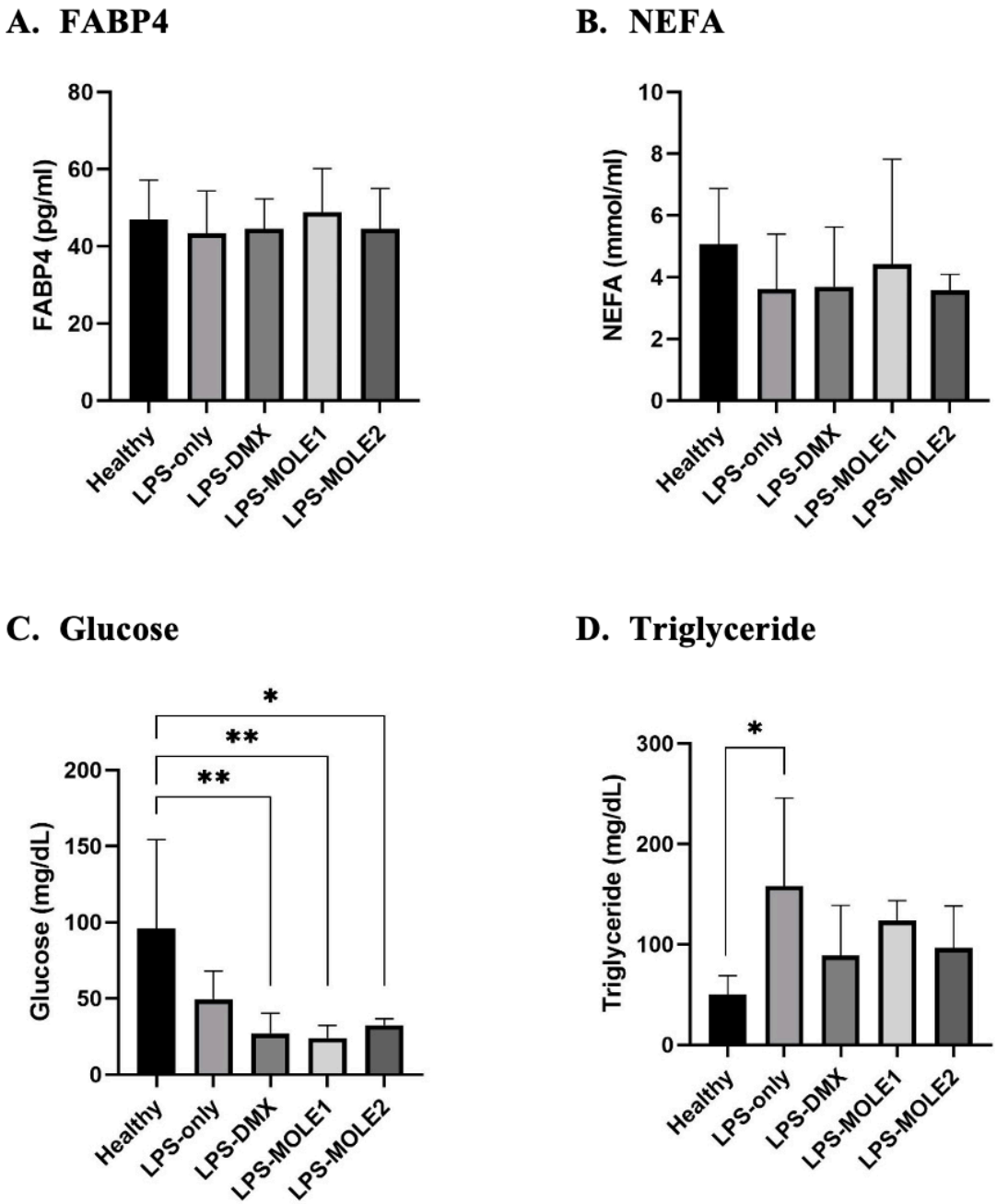

Table 1. Results of Hematological Parameter Examination Conducted using Hematology Analyzer (Mindray BC20s)

IL-6 inhibits of SGLT-2 and GLUT-2 activity and IL-1β reduces Na-K-ATPase activity in in vitro studies.[48] The use of glucocorticoids like dexamethasone as anti-inflammatories reverses changes in glucose transport activity by suppressing the spread of inflammatory cytokines in the NF-ƘB pathway.[48,50–52]

In this study, there were no significant difference in the levels of FABP4 and NEFA; only a trend towards a decrease was observed among the treatment groups (Figure 1.A. and 1.B.). This finding contrasts with prior research that reported an increase in FABP4 levels after 8 hours[9] and an increase in FABP4 mRNA after 12 hours[53], as well as an increase in NEFA after 12 hours post-LPS induction.[8] However, there is research indicating a significant decrease in FABP4 gene expression after 12 hours post-LPS induction.[8] These differences are presumed to be associated with distinct inflammatory conditions between the early and later stagesThe unbalanced secretion of cytokines such as IL-10 and TNF-α as seen in sepsis, increases insulin resistance, affecting adipocyte cells that become insensitive to insulin. Such conditions can induce lipotoxicity and hinder insulin's ability to stimulate the absorptivity of NEFA cells, generated by the lipolysis cycle of triglycerides, leading to the inhibition of lipolysis of endogenous triglycerides into NEFA.[54]

M. oleifera identified as having major components isoquercetin and quercetin-3-O-malonylglucoside inhibits adipogenesis by suppressing marker CCAAT / enhancer-binding protein beta (C/EBPβ), adiponectin, FABP4, peroxisome proliferator-activated receptor (PPAR)-γ,[13,55] fatty acid synthase (FAS), acetyl-CoA carboxylase (ACC),[56] resulting in reduced cellular lipid level (triglycerides, LDL and VLDL).[57] The extract enchances lipolysis by inhibits α-glucosidase, and lipase[56] increasing phosphorylation of AMP-activated protein kinase α (AMPKα) and ACC,55 and activating uncoupling protein 1 (UCP1), sirtuin 1 (SIRT1), PPARα, and Peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC1α). It also induces the antioxidant enzyme heme oxygenase-1 (HO-1).[58] *M. oleifera* exhibits potential in improving glucose tolerance, reducing blood glucose levels, and demonstrating antidiabetic effects,[59–61] possibly due to quercetin,[62] myricetin[11] and isothiocyanate.[57] which have anti-diabetic effects.[61]

The compound (4-[2-o-Acetyl-alpha-l-rahamnoslyloxy)benzyl]thiocynate isolated from *M. oleifera* effectively inhibits nitric oxide in Raw264.[7] cells.[20,44] Active compounds such as tannins, phenols, alkaloids, flavanoids, carotenoids β-sitosterol, vanillin, and moringin have anti-inflammatory properties.[61] MOLE treat atopic dermatitis[20,45] protection against oxidative stress from methotrexate[20,46] and exhibits antioxidant potential against diclofenac sodium-induction liver toxicity.[20.47] Bioactive components contribute to reactive oxygen spices (ROS).[12,61,66–68]

The aqueous extract capturis free radicals,[59,61] while kaempferol, isoquercetin, astragalin, and crypto-chlorogenic acid act as an antioxidant.[61,69,70] Additionally, *M. oleifera* acts as an oxidative and inflammatory marker, inhibiting IKBα phosphorylation, preventing nuclear translocation and suppressing inflammatory proteins such as TNF-α, cyclooxygenase-2 (COX-2), IL-6, and inducible nitric oxide synthase (iNos) offering therapeutic potential for as obesity, arthritis, cancer, diabetes, and ulcers.[61]

Although previous research supports the elevation of certain metabolites in sepsis, our study yields conflicting results with some of those findings. The limitation of this study lies

in the brief duration of observing the effect of LPS-induced sepsis in mice, compared to prior studies that conducted observed for 8 to 12 hours.[8,9] Additionally, the duration of therapy with *M. oleifera* is believed to influence anti-inflammatory, antioxidant, and immunomodulatory cabilities. Therefore, further understanding of these mechanisms could aid in the development of therapeutic strategies to address complications induced by sepsis.

Conclusion

In this study, we established that MOLE has the potential to change metabolite and hematological parameters in a sepsis mouse model. This suggests that *M. oleifera* may ameliorate sepsis by reducing glucose levels and downward trend triglyceride levels, as well as by increasing erythrocyte parameters, and upward trend leukocyte parameters, FABP4 levels, and NEFA levels.

Further understanding of the mechanisms involved in the metabolic and inflammatory effects of *M. oleifera* is needed. This requires continued exploration of the NF-ĸB pathway and other metabolic markers associated with sepsis by period. Our findings have the potential of *M. oleifera* as an herbal medicine for sepsis.

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Conflict of Interest

None declared

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