Original Article

Extra Virgin Olive Oil Nanoemulsion Attenuated Inflammatory Response in Lipopolysaccharide-Induced Sepsis

Anna Surgean Veterini, Nancy Margarita Rehatta, Subijanto Marto Soedarmo[1], Heni Rachmawati[2], Widjiati[3], Widodo Jatim Pudjirahardjo[4], Annis Catur Adi[5], I Ketut Sudiana[6]

Department of Anesthesiology and Reanimation, Faculty of Medicine, Universitas Airlangga – Dr. Soetomo General Academic Hospital Surabaya, [1]Department of Pediatrics, Faculty of Medicine, Universitas Airlangga - Dr Soetomo General Academic Hospital Surabaya, [2]School of Pharmacy, Bandung Institute of Technology, Bandung, [3]Department of Veterinary Anatomy, Faculty of Veterinary Medicine, Universitas Airlangga, [4]Department of Health Administration and Policy, Faculty of Public Health, Universitas Airlangga, [5]Department of Nutrition, Faculty of Public Health, Universitas Airlangga, [6]Department of Anatomical Pathology, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia

Abstract

The provision of nutritional components in critical illness such as sepsis remains a big issue in clinical application, particularly through oral route due to intestinal integrity damaged-associated absorption problem. The aim of this research was to develop Extra Virgin Olive Oil (EVOO) nanoemulsion as a nutrient carrier to improve its permeability while maintaining the intestinal mucosa integrity in mouse model of lipopolysaccharide-induced sepsis. EVOO nanoemulsion was prepared by using ultrasonication-mild agitation method. EVOO nanoemulsion (1.5 mL) was administered to the mice through orogastric tube. The effect of EVOO nanoemulsion was evaluated by assessing the histopathological alterations in lung, measuring the activation of NFκB-p65 by immunohistochemistry of lung tissue, the levels of circulating Surfactant Protein-D (SP-D), tumor necrosis factor-alpha, interleukin (IL)-8, and IL-10. The main result, EVOO nanoemulsion decreased circulating SP-D level after 24 h. In conclusion, EVOO nanoemulsion is a promising carrier to improve nutrition absorption and decrease circulating SP-D as organ injury biomarker.

Keywords: Circulating surfactant protein-D, extra virgin olive oil, nanoemulsion, sepsis mice, translational nutrition research

Introduction

Administration of enteral nutrition in critically ill patient often encounters absorption problems. Several benefits derived from EN formed the basis for selecting the route of nutrition in this study. One of the advantages is the prevention of bacterial translocation and stress ulcerations. In this study, the potential benefit of extra virgin olive oil (EVOO) nanoemulsion in mouse model of lipopolysaccharide (LPS)-induced sepsis was evaluated. A chronic inflammatory condition was indicated by increased blood level of both pro-and anti-inflammatory mediators. To demonstrate the effect of EVOO nanoemulsion on this disease model, assessment on the levels of inflammatory mediators as well as Surfactant Protein-D (SP-D) was conducted.

Under basal conditions, the intestinal epithelium absorbs nutrients and plays a critical role as the first-line protection against pathogenic microbes and as the central coordinator of mucosal immunity.[1,2] However, there is impaired absorption of nutrients in sepsis and critically ill patient. In addition, gastric emptying is delayed to some extent in almost all critically ill patients, with many having intestinal ileus, particularly after surgery. This may be compounded by opioids used for analgesia or sedation, which further reduce gastric emptying and gut peristalsis. Hypoperfusion, venous congestion, mucosal edema, motility dysfunction, continuous feeding regimens, nasogastric suctioning, and changes in intraluminal pH are all factors that affect GI absorption (alkalinization).

Address for correspondence: Dr. Anna Surgean Veterini, Department of Anesthesiology and Reanimation, Faculty of Medicine, Universitas Airlangga – Dr. Soetomo General Academic Hospital, Surabaya, Indonesia.
E-mail: annasurgean@gmail.com

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Table 1: The division of groups

Group	Induction*	Treatment	Time observed after induction (h)
K1	PBS	-	1
K2	LPS	-	
K3	PBS	EVOO nanoemulsion	8
K4	PBS	-	
K5	LPS	EVOO nanoemulsion	
K6	LPS	-	
K7	PBS	EVOO nanoemulsion	24
K8	PBS	-	
K9	LPS	EVOO nanoemulsion	
K10	LPS	-	

*The noninduced mice received PBS injection instead of LPS. PBS: Phosphate buffer saline, LPS: lipopolysaccharide, EVOO: Extra virgin olive oil

The use of vasoactive agents for circulatory support may also decrease splanchnic blood flow, gut perfusion, and therefore absorption.[3]

To overcome the above-mentioned issue, the nutrient was formulated into nanoemulsion in this study. EVOO is one of the most consumed food ingredients. The bioactive in EVOO that plays a role in multiple positive effects on humans and animals is oleic acid and hydroxytyrosol.[4] Administration of EVOO nanoemulsion was expected to improve pharmacokinetics and pharmacodynamics in critically ill patients.

An *in vivo* study using mouse model of breast cancer showed the effect of EVOO phenolics nanoemulsion in suppressing tumor growth by 90%.[5] The results in the aforementioned study indicated the potential use of EVOO phenolic formulations for therapeutic and dietary applications.

Methods

Study design and ethical statement

This study is an experimental study with a randomized pre-, posttest only controlled group design different sample. This research was approved and declared ethically feasible by the Animal Care and Use Committee of the Veterinary Medicine Faculty, Airlangga University, with ethical clearance number 2.KE.063.04.2019.

Animals

The animals used in this study were male *Mus musculus* mice, age 10–12 weeks, obtained from the Laboratory of Experimental Animals Universitas Gadjah Mada. The mice underwent acclimatization for a week with adequate food and lighting. Mice were excluded from the experiment if the general condition seemed weak before the experiment or if mice appeared aggressive. Dropout criteria included the death of the mice during the intervention period. The mice were divided into 10 different groups [Table 1].

Extra virgin olive oil nanoemulsion preparation

Materials used to prepare EVOO nanoemulsion are chremofor RH40, PEG 400, and distilled water. Nanoemulsion was produced in School of Pharmacy Institute Technology Bandung. The EVOO brand (Leccino) was chosen for this research because it was proven that this brand has the highest phenol content.[8] The process and manufacture of EVOO nanoemulsion using ultrasonication-mild agitation method has been submitted for publication. The physical characterization of EVOO nanoemulsion consisted of globule size and polydispersity index determination using Particle Size Analyzer.

Table 2: Scoring of changes in lung tissue using the modified Eveillard and Makiuchi method (Makiuchi *et al.*, 2007b) (Eveillard *et al.*, 2010)[6,7]

Grade	Score
Alveolar necrosis (A)	
None (none)	0
Mild (<10%)	1
Moderate (between from 11% to 50%)	2
Severe (between from 51% to 80%)	3
Very severe (over 80%)	4
Vascular congestion (B)	
None (none)	0
Mild (<10%)	1
Moderate (between from 11% to 50%)	2
Severe (between from 51% to 80%)	3
Very severe (over 80%)	4
Inflammatory cell infiltration (C)	
None (none)	0
Mild (<10%)	1
Moderate (between from 11% to 50%)	2
Severe (between from 51% to 80%)	3
Very severe (over 80%)	4
Pulmonary edema (D)	
None (none)	0
Mild (<10%)	1
Moderate (between from 11% to 50%)	2
Severe (between from 51% to 80%)	3
Very severe (over 80%)	4
Alveolar hemorrhage (E)	
None (none)	0
Mild (<10%)	1
Moderate (between from 11% to 50%)	2
Severe (between from 51% to 80%)	3
Very severe (over 80%)	4

Experimental procedure

Sepsis in mice was induced by intraperitoneal injection of LPS (Sigma, 0111:B4) 10 mg/kg body weight. The clinical adjustment of sepsis was adopted from the sepsis score by Gonçalves-de-Albuquerque's clinical score.[9] The clinical parameters examined based on the score (Gonçalves *et al.*, 2017) were the presence of piloerection, Altered respiratory rate, Fecal alteration, Lacrimation/eyelid change, contraction of abdomen, Lack of strength of gasping, change in body temperature, Alert response (scape aftertouch), and Exploration

of the environment and compromized activity. We measured mice abdomen temperature (t_{abd}) using noncontact infrared thermometer (Thermoworks TW2®, Lindon, USA). Each parameter of Goncalves *et al*. 2017 is worth 1 if it occurs in clinical observations. The total sum of these nine parameters, if 0, then there is no sepsis, 1–3 mild sepsis, 4–7 moderate sepsis, and 8–9 severe sepsis.

To support the data for the occurrence of sepsis, the inflammatory responses were evaluated by measuring the circulating levels of tumor necrosis factor-alpha (TNF-α), interleukin (IL)-8, IL-10 circulation using enzyme-linked immunosorbent assay (ELISA), and lung tissue NFκB p65 activity by immunohistochemical staining. SP-D circulation was also measured to evaluate the markers of early organ failure.

EVOO nanoemulsion (1.5 ml) was administered to mice through orogastric tube 1 h after intraperitoneal injection of LPS or phosphate buffer saline (PBS). According to Table 1, there were 4 mice groups receiving EVOO nanoemulsion treatment, i.e., K3, K5, K7, and K9. While foods given to the mice were in the form of pellets consisting of 70% carbohydrates, 20% protein, and 10% fat made in laboratory.

Hematoxylin and eosin (H and E) staining was carried out to evaluate the evidence of acute lung injury through histology examination. The obtained staining was observed under Nikon Eclipse Ci microscope (×400). Scoring the occurrence of acute lung injury on histopathological examination with H and E staining using the criteria of the modified scoring method [Table 2].[6,7]

After histopathological examination with H and E staining, we examined the activity of NFκB p65 by immunohistochemistry immunofluorescent method. The obtained staining was observed under Confocal Laser Scanning Microscopy (Olympus FV1000). Protein expression of TNF-α, IL-8, IL-10, SP-D in mice serum was evaluated by using ELISA method. One-way analysis of variance was used to analyze the difference between groups. A two-sided statistical significance test is used to get all *P* values.

Results

LPS-induced sepsis in mice was used as a model in this study to represent the condition of sepsis in human. The results of the clinical examination in the 1-hour LPS injection group were worth 9 so that the clinical score of the mice in this study experienced severe sepsis. In Figure 1, we show the change in body temperature of mice after 1 h of LPS injection as one of the supporting data for the occurrence of the sepsis model. After 1 h, the abdomen temperature of LPS-induced mice increased significantly [Figure 1; $P < 0.05$].

To support the occurrence of organ damage in addition to circulating SP-D examination, we also examined histopathology with H and E staining. We performed histology examination with the H and E staining method to see changes in lung injury features at various time points. At 1 h after LPS injection, there were no significant changes in lung injury. However, changes in the reduction in acute lung injury scoring occurred in the LPS group given nanoemulsion [Figure 2]. An example of histological examination using the H and E staining method is shown in Figure 3 which also showed pathological alterations that are characteristics of acute lung injury at 1 h after LPS induction.

The activity of NFκB-p65 was measured by determining the ratio between NFκB-p65 in the nucleus and cytoplasm.[10,11] The staining results were presented in Figure 4, and the ratio quantification was presented in Figure 5. It shows that at the early time of endotoxemia, there was a significant increase in NFκB-p65 activity and a significant decrease in NFκB p65 activity occurred at 24th-h groups of endotoxemia mice given EVOO nanoemulsion [Figure 5].

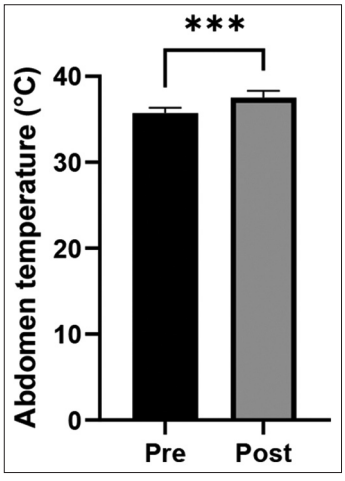

Figure 1: A significant increase in abdomen temperature of lipopolysaccharide-induced mice was observed 1h after receiving intraperitoneal injection of lipopolysaccharide 10 mg/kg

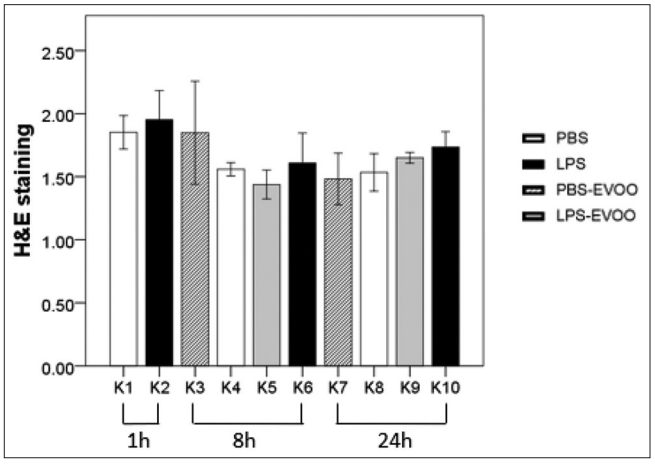

Figure 2: Histopathological examination of the lung using the modified Eveillard (Eveillard et al., 2010) and Makiuchi (Makiuchi *et al*., 2007a) scoring method. The data for each sample is the value observed in ten different fields of view at ×200 and ×400 magnifications.

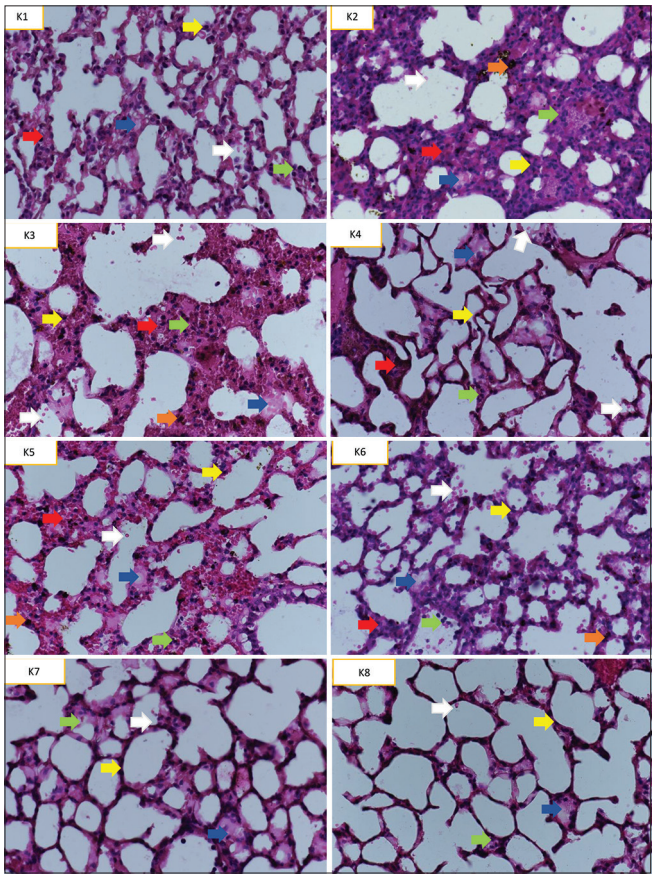

Figure 3: Histopathological features of the lung for various conditions. The red arrow indicated congestion. The blue arrow indicated pulmonary edema. White arrow indicated bleeding erythrocyte infiltration (hemorrhage) into the alveoli. Green arrows indicated the presence of inflammatory cell infiltration. Yellow arrows indicated alveolar necrosis. The orange arrow indicated the presence of alveolar macrophages (Nikon Eclipse Ci. DS-Ri2. 16 Megapixel).

The activation of NFκB signaling pathway was evaluated by determining the expression of p65-NFκB using immunohistochemistry technique. The activity of NFκB-p65 was determined by measuring the expression ratio between NFκB-p65 in the nucleus and cytoplasm.

The results showed that LPS significantly increased the NFκB-p65 activity in lung tissue after 1 h. The control group showed increased activity of NFκB-p65 after 8 h but much lower compared to LPS-induced mice which was not treated with EVOO. After 24 h, the NFκB-p65 activity was decreased in LPS-induced mice and significantly decreased in control mice. EVOO treatment affected the NFκB-p65 activity and decreased it in control mice after 24 h.

The effect of EVOO nanoemulsion on circulating TNF-α, IL-8, IL-10, SP-D, and NFκB-p65 activity was evaluated after 8th h and 24th h [Figure 6].

Before EVOO nanoemulsion treatment, the expression of circulating TNF-α was significantly higher in mice at 1 h after LPS-induction [Figure 6; $P < 0.05$]. However, the expression of SP-D was lower in LPS-induced mice, and there was no difference in the expression of circulating IL-8 and IL-10 between LPS-induced mice and control (PBS) after 1 h [Figure 6]. This result was in line with the proposal that TNF-α could orchestrate the production of other cytokines.[12]

The results in Figure 6 shows that there was no significant difference in IL-8 expression of LPS-induced and control mice after 1 h and 8 h but a decrease in IL-8 expression was seen in all groups given EVOO nanoemulsion and the peak of the decrease occurred at 24 h. Interestingly, there was an increase of IL-8 levels in PBS groups after 24 h. This might be due to the effect of molecular clock or circadian clock.[13]

In this study, IL-10 expression was significantly higher in LPS-induced mice compared to control mice after 8 h. This result was in line with several other studies showing that IL-10 production was enhanced several hours after TNF-α synthesis. A significant decreased of IL-10 expression was observed in LPS-induced mice after 24th h. These low circulating IL-10 level supports the theory that IL-10 plays an important role in downregulating the potentially detrimental mediators, one of them is TNF-α.[14] Since TNF-α expression was already low after 24th h, IL-10 production was also declined [Figure 6]. On the contrary, the control mice showed increased IL-10 expression after 24 h. Interestingly, the contrast effect was also observed in EVOO-treated mice after 24 h. EVOO increased the IL-10 expression in LPS-induced mice, but the IL-10 expression was decreased in control mice. Nevertheless, this result might be explained by other study showing that blockade of endogenous TNF-α may lead to increased production of IL-10. It was clear in this study that EVOO nanoemulsion decreased TNF-α expression after 8 h, nonetheless the effect on IL-10 expression was observed later after 24 h. Other studies also showed the ability of certain drugs, i.e., cyclosporin[15] and chlorpromazine,[16] to potentiate the release of LPS-induced IL10 which correlated to its suppressive effect on TNF-α.

Circulating SP-D expression was remained similar after 8 h, but it was significantly lower after 24 h in LPS-induced mice. Treatment with EVOO nanoemulsion decreased the SP-D expression significantly after 24 h in LPS-induced mice. SP-D plays a pivotal role in the innate immunity and the regulation of inflammatory responses in various infectious diseases.[17,18] A study on the role of SP-D in acute kidney injury (AKI) showed that SP-D attenuates AKI in the sepsis by modulating renal apoptosis, inflammation, and NF-κB signaling.[18] The exact mechanism of EVOO nanoemulsion that triggered a decrease in serum SP-D levels was unclear. Nonetheless, this result supports the positive outcome from EVOO nanoemulsion in reducing excessive inflammatory response in LPS-induced mice.

Discussion

In our study, we divided the sacrifice at 8th-h and 24th-h groups considering that the sampling at 8th h represents the acute condition after intraperitoneal injection of LPS. Meanwhile,

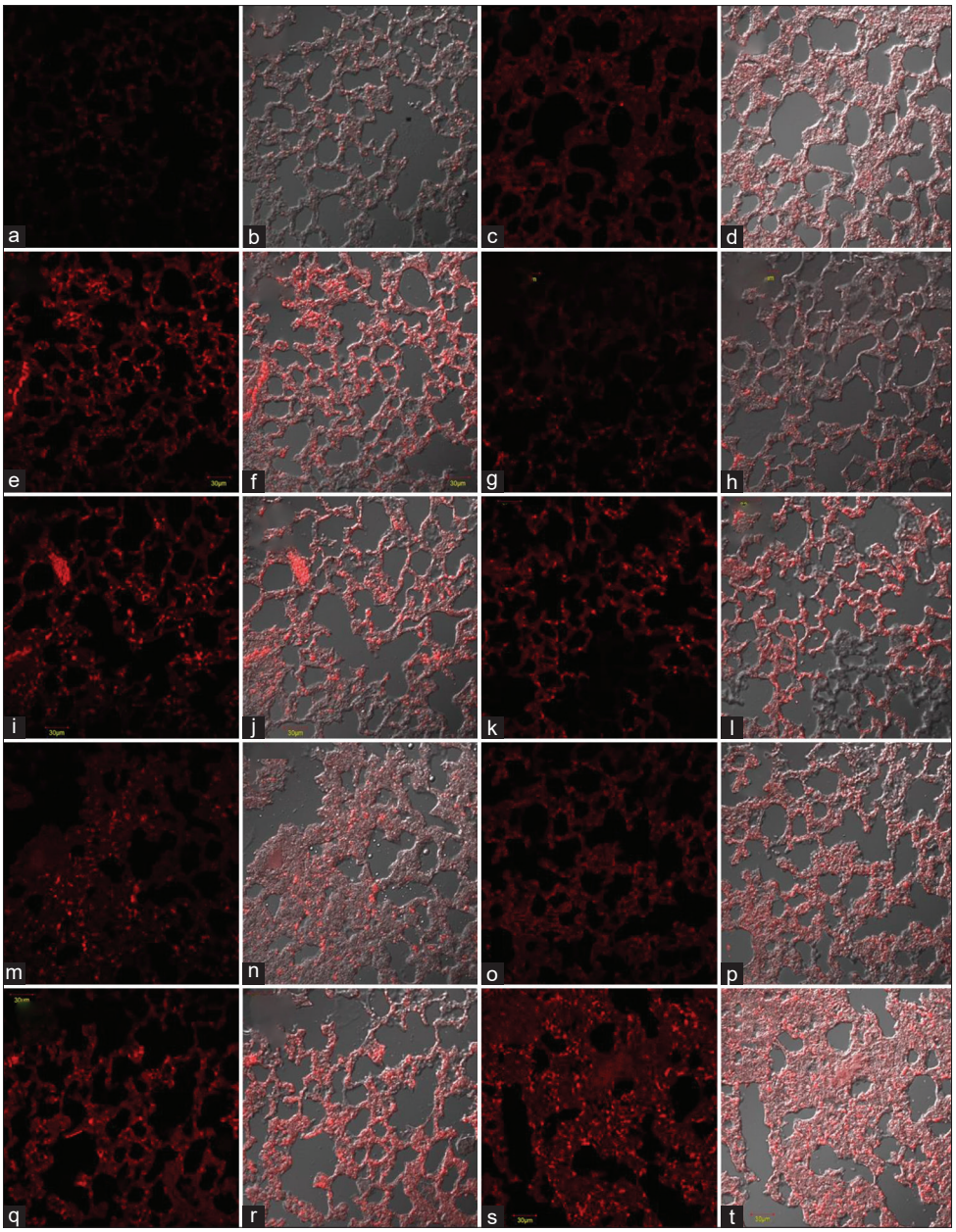

Figure 4: Examination of NFκB intensity p65 Lung Tissue with a Confocal Microscope. (a and b) Mice Group K1, (c and d) Mice Group K2, (e and f) Mice Group K3, (g and h) Mice Group K4, (i and j) Mice Group K5, (k and l) Mice Group K6, (m and n) Mice Group K7, (o and p) Mice Group K8, (q and r) Mice Group K9, and (s and t) Mice Group K10

the 24th h sampling represents the end of the acute phase toward chronic conditions.

Providing nutrition in the form of nano size is one of the scientific breakthroughs to answer the challenge of impaired absorption of enteral nutrition in critical illness cases. The result from computational study predicted that that oleic acid, the highest content of EVOO, has the most significant role on macrophages colony-stimulating factor agonist.[19] The protective effect of EVOO is due to the high oleic acid content and the antioxidant component of the polyphenolic compounds including hydroxytyrosol.[20]

Modeling of septic mice was done by intraperitoneal injection of LPS 10 mg/kg body weight. Sepsis in these experimental animals was formed after 1 h from the time of LPS administration. The assessment of the occurrence of sepsis was carried out based on clinical scores: the appearance of piloerection, increased respiratory rate, changes in stool, changes in lacrimation/eyelids, abdominal contractions, gasping and weak breaths, changes in body temperature, ability to respond to warnings (run aftertouch), and ability to explore the environment and ability to perform composing activities.[21] The use of the MSS, M-CASS, and/or MGS clinical scoring system to diagnose sepsis is recommended

for several reasons. The first reason, this parameter provides a general noninvasive assessment of disease progression and can be performed with high frequency, unlike repeated blood draws which risk reducing the red blood cell count and hemoglobin concentration after 5 days of sampling, even though only 35 µl is taken daily. The second reason, evaluation of the symptom pool can be done easily by assessing the clinical status of experimental animals, evaluating physiological responses when testing new therapies, or the effects of comorbidities.[22]

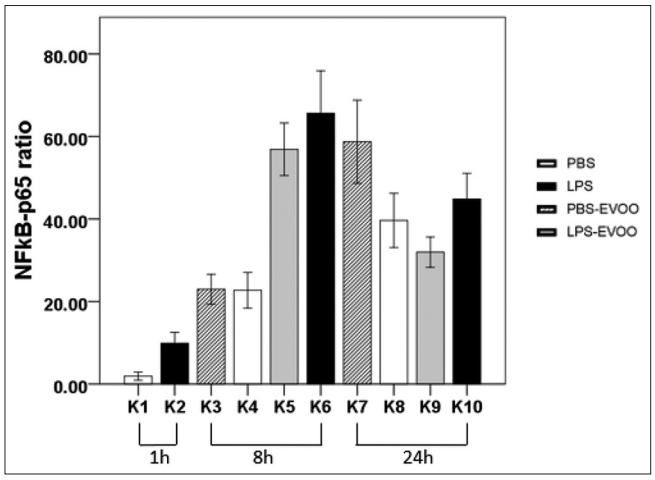

Figure 5: Nucleus/cytoplasm NFκB p65

In addition, we also measured abdominal body temperature in septic mice. The temperature measurement that we did in this study is not the main parameter for establishing the diagnosis of sepsis. Measurement of body temperature of mice in sepsis model mice was used as a tool to predict the progression of sepsis to death. Measurement of body temperature of mice in this study was using the noncontact infrared method. Body temperature measurements using noncontact infrared thermometer were relatively easy, without the need of anesthesia and did not produce any injury to the animal. Infrared from the thermometer was fired at abdomen and anus regions.[23] We did not choose to measure the body temperature of mice using a colon or rectal thermometer

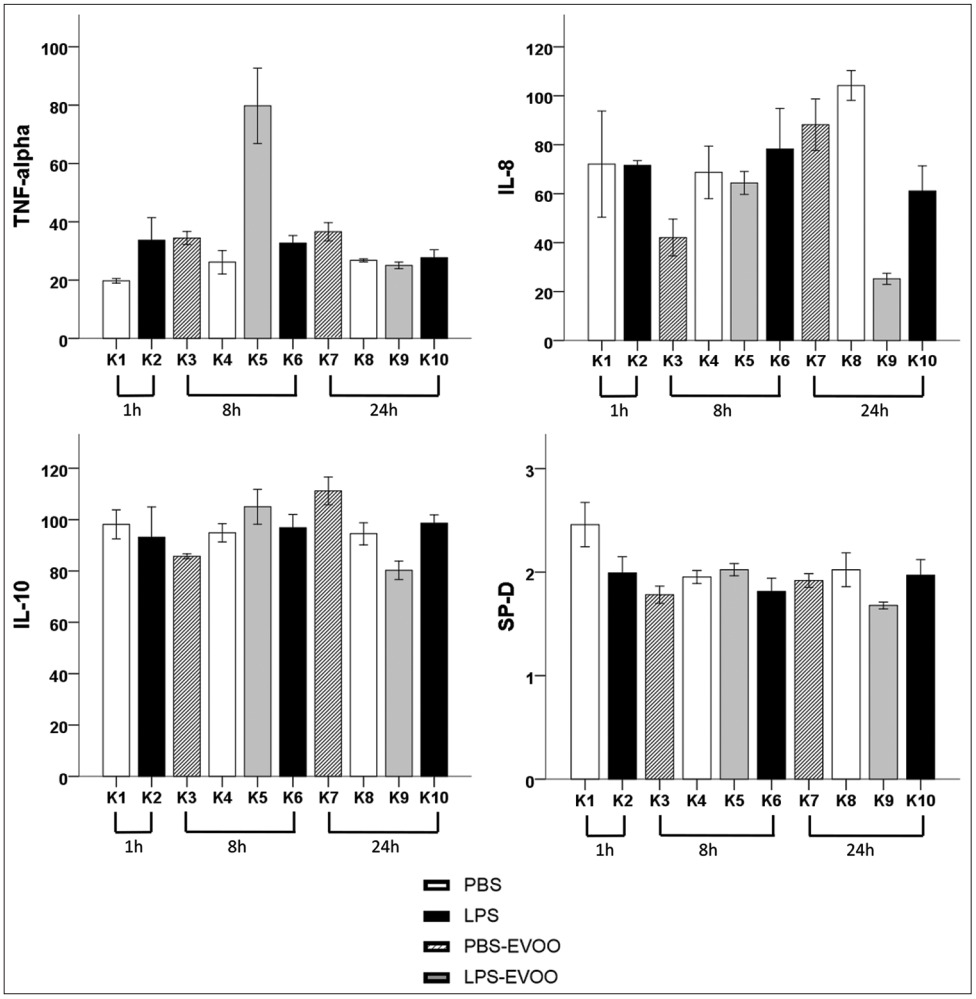

Figure 6: Circulating tumor necrosis factor-alpha, interleukin-8 levels, interleukin-10, and Surfactant Protein-D levels were measured at 1, 8, and 24h after lipopolysaccharide induction. Control mice received phosphate buffer saline instead of lipopolysaccharide. Each of control and lipopolysaccharide-induced mice was divided into two groups, i.e., the nontreated and extra virgin olive oil-nanoemulsion-treated mice. The effect of extra virgin olive oil nanoemulsion on control and lipopolysaccharide-induced mice was evaluated at 8 and 24 h after treatment

because this method is difficult to do. The depth of entry of the thermometer through the anus is recommended to be >2 cm. Measurement with this rectal/colon thermometer takes time, if it is too fast, it will result in a lower value than the value it should have. Measurements that are too long will cause a rapid increase in temperature as a stress response to the treatment.[24] Temperature measurement using the noncontact infrared method also has several advantages and disadvantages. Some of the advantages are that the technique is easy, mice are not stressed, in the tail area, can describe vasoconstriction or vasodilation.[24] The disadvantage of this noncontact infrared temperature measurement technique is that the presence of hair in animal models will give inappropriate measurement values.[24] Some researchers suggest shaving the hair first before measuring the body temperature of animal models using the noncontact infrared method. In this study, we chose to measure in the abdominal area and the tail area close to the anus.

The results of the analysis of temperature measurements in this study gave a significant increase after 1 h of intraperitoneal LPS injection. All the results of our research, the average temperature of the mice in the pretest group after being injected with LPS 10 mg/kg between intraperitoneally in a bright atmosphere was 37.52°C, the average body temperature of mice before being injected with LPS was 35.7°C also in this condition. This confirms that the significant increase in abdominal temperature in our study was actually caused by LPS administration, not due to the influence of environmental conditions. However, the body temperature of mice cannot be used as a single parameter in diagnosing sepsis because the body temperature of mice is strongly influenced by gender, activity, environmental conditions, hormone cycles that mentioned by Sanchez-Alavez *et al*., 2011. To anticipate the influence of hormones and gender, all mice used in this study were male mice.

Sepsis conditions will cause changes in the body cytokines. In this study, we measured the levels of pro-inflammatory cytokines (TNF-α and circulating IL-8) and anti-inflammatory (circulating IL-10) at 1, 8, and 24 h after intraperitoneal LPS injection. As proinflammatory cytokines used to evaluate sepsis patient, TNF-α is released from macrophages within 30 min after the inciting event, following gene transcription and RNA translation. Thus, this mediator is considered to be an early regulator of the immune response.[25] An elevated serum level of TNF-α has been found in up to 90% of sepsis patients.[26] In LPS-induced mice, the expression of TNF-α increased two times after 8th h but decreased again after 24th h. This result was in agreement with other studies showing the rapid and transient induction of TNF-α by LPS.[27-29] This phenomenon also occurred in the circulating IL-10 levels of the LPS group given the EVOO nanoemulsion. As we know, there are currently 2 TNF receptors; there are TNFR1 and TNFR2. The process of releasing pro-inflammatory (TNF-α) into the circulation can be through 2 pathways, first, LPS binds to TLR which will then trigger the transcription process by NFB to produce pro-inflammatory mediators (TNF-α, IL-8, IL-6). Second, also TNF will bind to TNFR1 or TNFR2 triggering the release of pro-inflammatory or anti-inflammatory. When it binds to TNFR1, it is an inflammatory mediator that increases, whereas when it binds to TNFR2, it increases the immune suppression/anti-inflammatory process. The increase in circulating levels of TNF-in this study may be advantageous because it occurs in the acute phase and is accompanied by elevated circulating levels of IL-10. Administration of the EVOO nanoemulsion is thought to increase the modulation of circulating TNF-α and circulating IL-10 in the acute phase, which is the body's defense mechanism against pathogens in the acute phase. Decreased circulating levels of TNF-α and circulating IL-10 in the subacute/early chronic phase so that the prevention of immune suppression that occurs in the chronic phase can be suppressed. The level of pro-inflammatory (TNF-α) at 24 h was close to the level of the control group (pretest). In addition, it also supports the prognostic biomarker role to this cytokine. TNF-α production was also observed in control mice after 8 h but not as high as LPS-induced mice. An elevated TNF-α production was also observed in control mice after 8 h but not as high as LPS-induced mice and it remained similar after 24 h.

The effect of EVOO nanoemulsion treatment was very profound in LPS-induced mice after 8 h. EVOO attenuated TNF-α expression in LPS-induced mice. However, there was no difference in TNF-α expression of EVOO-treated and nontreated LPS-induced mice after 24 h. This can be explained by the decreased TNF-α expression in LPS-induced mice after 24 h, which showed that the inflammatory response may have been declined. EVOO nanoemusion treatment did not affect the TNF-α expression in control mice at any time. Thus, EVOO nanoemulsion has specific effect in reducing TNF-α expression only in inflamed mice. In agreement to our result, a study showed that mice receiving olive oil-enriched diet were resistant to endotoxic shock and showed the lowest TNF-α expression compared to other mice.[30,31]

In addition to the biomarkers mentioned above, in this study, we also examined the effect of EVOO nanoemulsion on decreasing serum SP-D levels as an indicator of target organ damage. The results of our study show that at 24 h, there was a significant decrease in serum SP-D levels in the sepsis model group given the EVOO nanoemulsion. Our hypothesis is that the EVOO nanoemulsion inhibits the activity of NFκB-p65 so that the formation of inhibition of the release of pro-inflammatory cytokines occurs, also triggering the release of anti-inflammatory as a mechanism behind the high pro-inflammatory increase. Furthermore, a decrease in these pro-inflammatory cytokines also led to a decrease in serum SP-D levels.

Studies in various diseases have shown that circulating SP-D is a marker of leakage from the lungs into the circulation. So that if there is alveolar destruction, it will cause an increase in the level of this lung protein in the blood.[32] The exact mechanism that triggers the increase in circulating SP-D levels is still unclear. Of the many hypotheses that have been

widely accepted, the increase in levels is due to changes in alveolar-capillary permeability. The exact function of circulating SP-D is also unknown.[33] In this study, an increased circulating SP-D level after LPS injection was a marker of lung damage or due to changes in alveolar-capillary permeability or the onset of acute lung injury due to sepsis.

The process of absorption of nanoparticles when they reach the gastrointestinal tract goes directly to the intestinal barrier with gradual and multiple processes that supports the process of diffusion through the mucus layer. These nanoparticles will meet with enterocytes and/or M cells, then the uptake process through a cellular entrance or para-cellular transport. The mechanism for uptake of nanoparticles that commonly occurs is through the process of endocytosis with various techniques, such as clathrin-mediated, caveolae-mediated, clathrin-and clavicle-independent as well macropinocytosis. The uptake mechanism is influenced by the physicochemical properties of the nanoparticles, some of which are aggregation ability and size. Several studies have demonstrated that nonlymphoid areas can also be involved in the absorption of nanoparticles, especially conjugation between nutrients or nutrient-like compounds to increase uptake. However, the main absorption through M cells and the lymphoid system cannot be controlled so that the combination of the two can cause side effects, namely increasing the activation of pro-inflammatory pathways. The results of the analysis of the pro-inflammatory pathway (circulating TNF-) in the study showed an improvement in EVOO nanoemulsion group. This strengthens the report written by Kilic *et al*. 2009, which describes that the pathophysiology of sepsis is very complex and requires therapy at the cellular and molecular levels. Therapy that targets systemic levels produces a less safe and effective outcome. The application of nanotechnology has the possibility of providing promising therapeutic designs targeting the molecular or cellular level.[34-37]

Conclusion

The effect of giving EVOO nanoemulsion through the enteral route of sepsis mice occurred 24th h after administration. This phenomenon probably indicates that enteral absorption of EVOO nanoemulsion can occur through sepsis mice. The mechanism that causes this decrease in circulating SP-D levels is probably via pro-inflammatory and anti-inflammatory cytokine pathways. Further research needs to be continued to explore other paths, for example, genetics, the nature of the infecting pathogen, or environmental factors that may also affect SP-D levels.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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