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The anti-inflammatory effect of lycopene on neutrophil infiltration in the spleen of mice exposed to Lipopolysaccharide (LPS)

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Abstract

Introduction: Lipopolysaccharide (LPS) is a major component of the outer membrane of gram-negative bacteria that can trigger inflammatory and oxidative stress responses. The purpose of this study is to observe the anti-inflammatory effect of lycopene in reducing neutrophil infiltration caused by LPS exposure in mice (*Mus musculus*).

Objective: This laboratory experimental study used 25 male mice aged 3 months with body weights of ± 25 g – 35 g, divided into five groups with five replicates each. C(-) is the control group: no LPS or lycopene was administered. C(+): received LPS 0.042 mg/kg without lycopene. P1: received LPS 0.042 mg/kg and lycopene 0.3 mg/kg. P2: received LPS 0.042 mg/kg and lycopene 0.6 mg/kg. P3: received LPS 0.042 mg/kg and lycopene 0.9 mg/kg. LPS was given intraperitoneally on days 1 and 8, and lycopene was administered daily for 14 days. The spleen is located near the liver. After the organ was removed, it was placed in 10% formalin buffer for HE staining, and data were analyzed using the Kruskall Wallis test followed by the Mann-Whitney test ($p<0.05$).

Results: LPS exposure significantly increased the number of neutrophils ($p<0.05$), and lycopene administration significantly reduced the number of neutrophils ($p<0.05$) to normal levels similar to the control (C-) at all doses/groups.

Conclusion: LPS exposure was proven to increase the number of neutrophils, and lycopene administration was able to reduce the number of neutrophils to normal levels in all dose groups.

Keywords: Affordable Medicines; Lipopolysaccharide; Neutrophils; Mice; Lycopene

1. Introduction

Escherichia coli is a gram-negative bacterium that naturally resides in the digestive tract of animals, including humans, but some serotypes can cause serious diseases. E. coli infections can lead to a range of disorders, from mild diarrhea to more severe systemic diseases, such as sepsis and enteritis. In livestock, E. coli infections are often associated with enterotoxigenic (ETEC), enteropathogenic (EPEC), and enterohemorrhagic (EHEC) diseases, which cause significant economic losses in the livestock industry [1].

Lipopolysaccharide (LPS), a major component of the E. coli cell wall, acts as an endotoxin that can trigger a strong inflammatory response. When E. coli is infected, LPS is recognized by the immune system through Toll-like receptor 4 (TLR4), leading to the activation of inflammatory pathways and the production of pro-inflammatory cytokines. This process can cause clinical symptoms such as fever, loss of appetite, and in more severe cases, contribute to the development of sepsis [2].

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LPS is a major component of the cell wall of gram-negative bacteria known as a potent endotoxin capable of inducing systemic inflammatory responses in animals and humans. LPS activates Toll-like receptor 4 (TLR4), which triggers the release of pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6, and increases the recruitment of immune cells, including neutrophils, to infected or inflamed tissues. Neutrophil infiltration plays a role in fighting infection but can also cause tissue damage if excessive. In animal models, LPS administration is often used to mimic acute inflammatory conditions and study the mechanisms of tissue damage due to excessive immune responses [3, 4].

Lycopene, a carotenoid found in tomatoes and other red-colored fruits, has strong antioxidant activity and anti-inflammatory properties. Research has shown that lycopene can reduce the production of pro-inflammatory cytokines and inhibit the activation of the NF-κB pathway, one of the important pathways activated by LPS that regulates inflammation-related genes. Lycopene has also been shown to reduce oxidative stress by neutralizing reactive oxygen species (ROS), which can indirectly reduce the recruitment and infiltration of neutrophils into inflamed tissues [5, 6].

Excessive neutrophil infiltration is often associated with tissue damage through the release of proteolytic enzymes and ROS by neutrophils. This condition is commonly found in chronic inflammatory diseases such as heart disease, chronic obstructive pulmonary disease (COPD), and even cancer. Therefore, controlling neutrophil infiltration by reducing LPS-induced inflammation is an important target in the therapy of various inflammatory diseases [7].

Lycopene has been reported to suppress neutrophil infiltration in the lung tissue of LPS-treated rats by reducing pro-inflammatory cytokines and ROS. However, the exact mechanism by which lycopene modulates neutrophil infiltration still requires further research. This study aims to evaluate the effect of lycopene administration on LPS-induced neutrophil infiltration and to identify possible mechanisms involved in the process.

2. Material and methods

2.1. Research Materials

The materials used in this study included lipopolysaccharide (LPS from E. coli O111, Singen©) 100 µg/kg, lycopene 10 mg/kg, lycopene 20 mg/kg, lycopene 30 mg/kg, CMC-Na 0.5%, sterile distilled water (Otsuka, DKL991870534341), ketamine, xylazine, povidone iodine, leukoplast, mouse feed in pellet form, drinking water for mice, husk, sterile cotton, sterile gauze, and fornakun buffer 10% (BBC Chemical, 190321-01).

2.2. Research Instruments

The instruments needed in this study included experimental cages, wire mesh as cage covers, feeding and drinking containers, digital scales, gavage needles for lycopene administration, 1 cc syringes, and 3 cc syringes. The equipment used for blood sampling and organ dissection included sterile surgical scissors, sterile scalpels, sterile blades, sterile tweezers, surgical boards, and needles.

2.3. Methods

This laboratory experimental study used 25 male mice aged 3 months with body weights of ± 25 g – 35 g, divided into five groups with five replicates each. K(-) was the control group, consisting of mice not administered LPS or lycopene. K(+) was the positive control group, consisting of mice given LPS 0.042 mg/kg without lycopene. K1 was the first treatment group, administered LPS 0.042 mg/kg and lycopene 0.3 mg/kg. K2 was the second treatment group, administered LPS 0.042 mg/kg and lycopene 0.6 mg/kg. K3 was the third treatment group, administered LPS 0.042 mg/kg and lycopene 0.9 mg/kg. LPS was administered intraperitoneally on days 1 and 8, and lycopene was given daily for 14 days. The spleen was collected on day 15 by euthanizing the mice with an injection of 80 mg/kg ketamine plus 1.4 mg/kg xylazine intraperitoneally. The spleen is located near the liver. After the organ was removed, it was placed in 10% formalin buffer (aquadest 900 mL, CH2O 40% 100 mL, NaH2PO4 4 g, and Na2HPO4 6.5 g, pH 7.0) for HE staining.

2.4. Data Analysis

Data were compiled into tables and analyzed statistically using the Kruskall Wallis test, followed by the Mann-Whitney test ($p<0.05$).

3. Results and discussion

Neutrophil infiltration in spleen tissue is not typically a normal condition and indicates a pathological process. Neutrophils are characterized by their cell nuclei, which consist of three to five lobes connected by thin strands of

chromatin [8]. Neutrophils also contain specific granules in the cytoplasm that cannot be seen with a light microscope, making the cytoplasm appear pale pink [9]. The appearance of neutrophils in the histological preparations of the spleen can be seen in Figure 1.

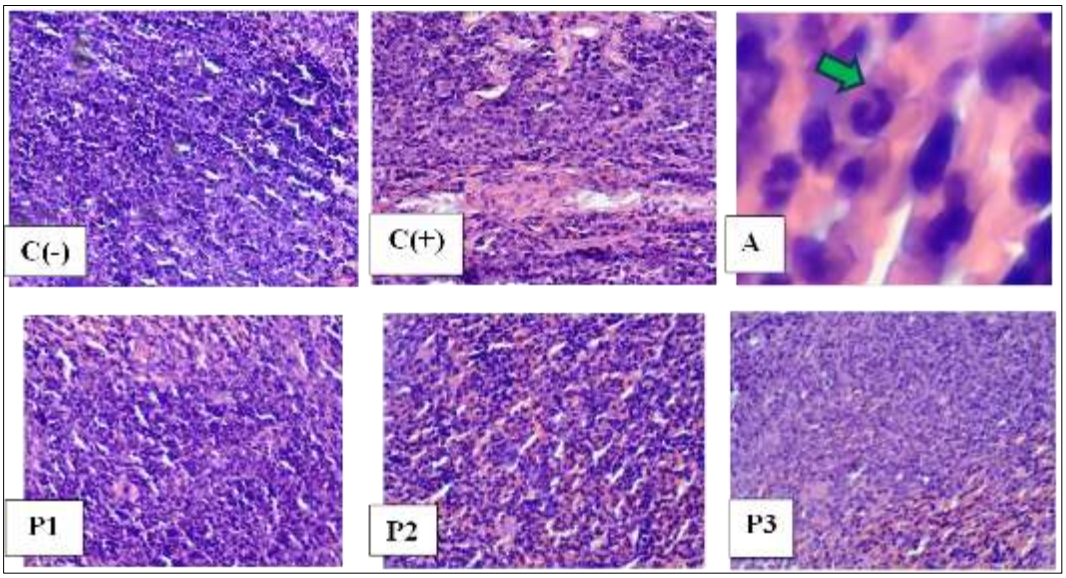

Figure 1 Results of histopathological examination of the spleen organ. Image caption A: Green arrows indicate neutrophil inflammatory cells. He staining. Magnification 400x. C(-): no LPS or lycopene was administered. C(+): received LPS 0.042 mg/kg without lycopene. P1: received LPS 0.042 mg/kg and lycopene 0.3 mg/kg. P2: received LPS 0.042 mg/kg and lycopene 0.6 mg/kg. P3: received LPS 0.042 mg/kg and lycopene 0.9 mg/kg

Table 1 Number of neutrophils after administration of lycopene and exposure to lipopolysaccharide (LPS) in mice *(Mus musculus*)

Group	Mean Rank
C-	10.40[a]
C+	22.80[b]
P1	12.60[a]
P2	9.60[a]
P3	9.60[a]

Note: different superscripts in the same column indicate significant differences ($p<0.05$). C(-): no LPS or lycopene was administered. C(+): received LPS 0.042 mg/kg without lycopene. P1: received LPS 0.042 mg/kg and lycopene 0.3 mg/kg. P2: received LPS 0.042 mg/kg and lycopene 0.6 mg/kg. P3: received LPS 0.042 mg/kg and lycopene 0.9 mg/kg.

The following are the results of observations of spleen microarchitecture characterized by neutrophil infiltration in mice exposed to LPS and lycopene therapy. Based on the calculation of scores from 5 fields of view, the data obtained were tested using the Kruskall Wallis Test. The results obtained from the Kruskall Wallis Test showed significant differences. Because the significance value was ≤ 0.05, it was continued with a post-hoc test using the Mann-Whitney Test. The results of the neutrophil score test for each treatment group can be seen in table 1.

Based on the data in Table 1, it can be concluded that there is a significant difference between the Control group (C+) and the Control (C-), P1, P2, and P3 groups. The Control (C-) group did not show significant differences with P1, P2, and P3. P1 did not significantly differ from P2 and P3, and P2 did not significantly differ from P3. From the results of neutrophil cell score calculations and the Kruskal-Wallis test shown in Table 1. The graph shows that the Control group (C+), which received LPS treatment without lycopene, had the highest neutrophil levels compared to the other groups. The Control (C-), P1, P2, and P3 groups showed similar neutrophil scores.

The spleen is an important organ in the immune system, particularly in eliminating pathogenic microorganisms and regulating immune responses to infections. Neutrophils are the first immune cells to respond to bacterial infections and

other pathogens, including endotoxins like lipopolysaccharide (LPS). LPS is known to trigger inflammation in several diseases and can cause both acute and chronic inflammatory reactions [10]. This is indicated by the neutrophil infiltration observed in the histopathological preparations examined. Several studies have explained that inflammation induced by LPS triggers neutrophil migration [11], as LPS activates TLR4 in the spleen, macrophage cell surfaces, dendritic cells, and various other immune cells, leading to the release of pro-inflammatory cytokines (IL-1, IL-6, TNF-α, and interferon-gamma), which in turn induces neutrophil migration to target organs, increasing neutrophil infiltration in the spleen [12].

Neutrophil activation induced by LPS is usually accompanied by early regulation of L-selectin and upregulation of CD11b/CD18 on the cell surface. L-selectin and CD11b/CD18 are important molecules that mediate neutrophil adhesion to the vascular endothelium. Other studies have mentioned that LPS increases the expression of adhesion molecules such as ICAM-1 (intercellular adhesion molecule-1) on the endothelial cells of the spleen, facilitating the attachment and migration of neutrophils from the bloodstream into spleen tissue. This adhesion response forms the basis of neutrophil movement from circulation into tissue, accompanying the innate immune response during acute inflammation [13]. This is consistent with the findings of this study, which shows that the inflammation observed is still in the acute or subacute phase, where neutrophil migration can still be detected. The subacute phase occurs between the acute and chronic inflammation phases and can last for 2 to 6 weeks [14].

During the acute inflammatory phase triggered by LPS, neutrophil infiltration into the spleen can cause structural changes in the organ. A large number of neutrophils infiltrating the spleen causes splenomegaly, accompanied by an increase in macrophages and other immune cells. If the inflammatory response persists, the accumulation of activated neutrophils can damage spleen tissue through the release of proteolytic enzymes and ROS, leading to tissue necrosis and further damage [15].

In cases of sepsis caused by gram-negative bacterial infections, including E. coli, neutrophil infiltration into organs like the spleen increases significantly. Activated neutrophils in large numbers release proteolytic enzymes such as elastase and collagenase, which not only destroy pathogenic microorganisms but also damage surrounding healthy cells and tissues. This leads to organ dysfunction, including spleen damage, which contributes to the development of multiple organ failure [16]. Some recent studies suggest that enzyme inhibitors, such as neutrophil elastase inhibitors, can reduce tissue damage caused by neutrophil infiltration in the spleen. Treatment with elastase inhibitors or other anti-inflammatory agents can reduce neutrophil infiltration and slow tissue damage caused by excessive inflammatory responses to LPS [17].

Recent studies show that neutrophil infiltration into the spleen of animal models after LPS administration from E. coli occurs within a few hours and is accompanied by structural changes in spleen tissue. In mouse models, LPS is known to increase neutrophil numbers in spleen tissue by upregulating chemokines such as CXCL1 and CXCL2. This process demonstrates that LPS triggers an intense inflammatory reaction, including neutrophil accumulation in lymphoid organs [18]. Experimental studies show that neutrophil infiltration caused by LPS can disrupt spleen architecture, particularly in the context of systemic inflammatory responses. The large accumulation of neutrophils can cause tissue damage through the release of proteolytic enzymes and reactive oxygen species (ROS). This can lead to spleen dysfunction in filtering microorganisms and regulating immune responses. Research on mouse models shows that after LPS administration, the spleen experiences increased production of pro-inflammatory cytokines and tissue damage due to neutrophil infiltration. This emphasizes the importance of controlling the inflammatory response to prevent excessive tissue damage [19].

Lycopene is a carotenoid compound found in tomatoes and some other fruits and vegetables. Lycopene has strong antioxidant properties, which help reduce oxidative stress and inflammation. Lycopene is associated with a reduction in neutrophil infiltration in organs such as the spleen exposed to lipopolysaccharide (LPS), linked to several mechanisms, including reducing pro-inflammatory cytokine production, increasing antioxidant capacity, and modulating inflammatory signaling pathways. Lycopene can inhibit the production of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin-1β (IL-1β), and interleukin-6 (IL-6), which are stimulated by LPS exposure. These cytokines play a key role in triggering the inflammatory response, including the recruitment and infiltration of neutrophils into tissues. By reducing these cytokine levels, lycopene helps decrease neutrophil infiltration into the spleen [20].

Lycopene, as a strong antioxidant, protects cells from damage caused by free radicals triggered by LPS. Oxidative stress can exacerbate inflammation and promote neutrophil recruitment. By reducing oxidative stress, lycopene can lessen the overall inflammatory response, including neutrophil infiltration in the spleen. Lycopene can affect inflammatory signaling pathways activated by LPS, such as the NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells)

and MAPK (mitogen-activated protein kinase) pathways. Activation of these pathways is crucial in regulating the inflammatory response and immune cell recruitment, including neutrophils. Inhibiting these pathways through lycopene contributes to reduced neutrophil infiltration [21].

Lycopene can inhibit neutrophil chemotaxis, which is the ability of neutrophils to migrate to the site of inflammation. Neutrophil chemotaxis is induced by various inflammatory mediators, including chemokines produced as a result of LPS exposure. By reducing the activity of these mediators, lycopene helps reduce the accumulation of neutrophils in LPS-exposed tissues [22]. Lycopene can decrease the production of chemokines such as interleukin-8 (IL-8) and other pro-inflammatory cytokines that are important for neutrophil chemotaxis. These chemokines direct neutrophil migration to the site of inflammation. Reducing chemokine production by lycopene inhibits neutrophil recruitment, thereby reducing infiltration into damaged or infected tissues [20]. Neutrophil chemotaxis also depends on the activation of certain receptors, such as chemokine receptors (e.g., CXCR1 and CXCR2) on the surface of neutrophils. Activation of these receptors triggers neutrophil movement toward the site of inflammation. Lycopene can disrupt the signals received by these receptors, thereby reducing neutrophil chemotaxis [23]. The signaling pathways activated by inflammatory factors, such as NF-κB and MAPK, are critical in regulating neutrophil chemotaxis. Lycopene is known to suppress the activation of NF-κB, which is a major regulator of pro-inflammatory gene expression. By blocking this pathway, lycopene reduces the chemotactic signals necessary for neutrophil recruitment to the site of inflammation [24].

Excessive neutrophil infiltration into inflamed tissues can cause further tissue damage through the release of proteolytic enzymes and reactive oxygen species (ROS). Normally, neutrophils are essential for clearing microorganisms and debris from damaged tissues, but prolonged inflammation can disrupt tissue repair processes. Lycopene, by reducing neutrophil chemotaxis and infiltration, helps control excessive inflammation and allows the body to focus on the healing phase. Reducing neutrophil infiltration decreases oxidative and inflammatory tissue damage, ultimately speeding up tissue regeneration and repair. After acute inflammation is controlled, the body enters the healing and repair phase. During this phase, cells like type 2 macrophages (M2) take over the role of neutrophils in repairing tissues by producing growth factors that stimulate tissue regeneration. Lycopene supports the transition from the inflammatory phase to the healing phase by reducing excessive inflammatory responses and minimizing tissue damage [25].

4. Conclusion

LPS exposure significantly increased neutrophil numbers, and lycopene administration was able to reduce neutrophil numbers to normal levels across all doses/groups.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest to be disclosed.

Statement of ethical approval

The study was approved by the Faculty of Veterinary Medicine Animal Ethics Committee of Universitas Airlangga. All variables were considered in accordance with the Ethics Committee related to animal handling to ensure that no discomfort or pain was caused to the animals during sampling (certificate registration number: 2024/110-KE).

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