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Efficacy of lycopene in reducing malondialdehyde (MDA) levels due to Lipopolysaccharide (LPS) exposure in Mice (Mus musculus)

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Abstract

Objective: Lipopolysaccharide (LPS) is a major component of the outer membrane of gram-negative bacteria and can trigger inflammatory and oxidative stress responses. This study aims to observe the efficacy of lycopene in reducing malondialdehyde (MDA) levels due to lipopolysaccharide (LPS) exposure in mice (*Mus musculus*).

Method: 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: no LPS or lycopene was administered. K(+): 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 on days 1 and 8 intraperitoneally, and lycopene was administered daily for 14 days. MDA levels were measured using the ELISA method, and data analysis was performed using ANOVA followed by Tukey's Test ($p<0.05$).

Result: LPS exposure significantly increased MDA levels ($p<0.05$), and lycopene administration significantly reduced MDA levels ($p<0.05$) to normal levels similar to the control (-).

Conclusion: LPS exposure significantly increased MDA levels, and lycopene administration was able to reduce MDA levels to normal limits.

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

1. Introduction

Colibacillosis is one of the most common diseases caused by pathogenic *E. coli* in poultry, particularly broilers and layers. This disease often leads to airsacculitis, peritonitis, and perihepatitis, which affect the production and mortality rates of chickens. Avian Pathogenic *E. coli* (APEC) is one of the poultry diseases that causes respiratory tract infections, which can spread into septicemia [1]. The high pathogenicity of Escherichia coli (*E. coli*) bacteria is primarily due to the presence of its cell wall components, especially lipopolysaccharide (LPS), which plays a crucial role in its virulence [2]. The presence of an outer membrane containing lipopolysaccharide (LPS) contributes significantly to the virulence and pathogenicity of gram-negative bacteria such as *E. coli*. The presence of LPS in gram-negative bacteria makes them far more virulent than gram-positive bacteria because LPS triggers a very strong immune response and can cause tissue damage and organ failure through endotoxemia and septic shock.

LPS stimulates immune cells such as macrophages and neutrophils to produce reactive oxygen species (ROS) through the activation of the NADPH oxidase enzyme complex. ROS, such as superoxide (O_2-), are highly reactive molecules that

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serve to destroy pathogens. However, excessive ROS production induced by LPS not only targets pathogens but can also damage the body's own cells and tissues. ROS can oxidize lipids, proteins, and DNA, causing severe cellular damage and contributing to the development of chronic inflammatory diseases. While ROS are necessary for defense against infections, prolonged excessive ROS production can lead to pathological conditions such as atherosclerosis, diabetes, and neurodegenerative diseases [3]. Oxidative stress is a condition of excess free radicals in the body that exceed the antioxidant system's ability to neutralize them. One result of oxidative stress is lipid peroxidation, which produces malondialdehyde (MDA) as an end product. MDA is a key indicator of oxidative damage to cellular membrane lipids and is often used as a biomarker of oxidative stress [4].

Lycopene is one of the most potent and effective antioxidants in combating free radicals. It works by neutralizing free radicals, preventing them from damaging the body's cells and tissues. Lycopene protects cell components, especially lipids in cell membranes, from damage caused by free radicals, thus preventing the onset of lipid peroxidation [5]. Lycopene can prevent the formation of MDA by inhibiting lipid peroxidation. Lycopene supplementation can reduce MDA levels in the blood, indicating reduced oxidative damage to membrane lipids. In this way, lycopene plays a role in protecting cells from damage caused by free radicals and oxidative stress [6].

2. Materials 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), ELISA kit for MDA analysis, 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 research 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, EDTA tubes, 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. P1 was the first treatment group, administered LPS 0.042 mg/kg and lycopene 0.3 mg/kg. P2 was the second treatment group, administered LPS 0.042 mg/kg and lycopene 0.6 mg/kg. P3 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. MDA levels were measured using the ELISA method.

2.4. MDA Measurement

MDA levels were measured using the ELISA method on day 15. Blood samples were collected, and 200µl was transferred to a centrifuge tube. The blood samples were centrifuged at 10.000 rpm for 10 minutes. The formed serum was separated and stored in a freezer (-80°C) until ready for use. The collected serum was pipetted, with 100 µl of the mouse serum placed into the wells of an ELISA plate. Then, 100 µl of MDA antibody reagent was added to the same well, and the plate was incubated for 1 hour at 37°C. The ELISA plate was washed three times using buffer wash. Then, 100 µl of substrate reagent was added to all wells. The plate was incubated for 15 minutes at 37°C in darkness. The reaction was stopped by adding 50 µl of stop solution. Absorbance was measured using an ELISA plate reader. For MDA level calculation, a standard curve provided in the ELISA kit was used to determine the MDA levels in the mouse serum.

2.5. Data Analysis

Data were compiled into tables and analyzed statistically using ANOVA, followed by Tukey's Test ($p<0.05$).

3. Results

Based on the results of MDA level examination using the TBARS method, the obtained MDA levels had a normal and homogeneous distribution. A data comparison was then conducted to observe the effects of the treatments based on the resulting MDA levels. Based on the ANOVA comparison of the MDA levels, the significance value was less than 0.001, indicating a significant difference in MDA levels between the treatments. Therefore, statistical analysis was continued with Tukey's post hoc test, and the results are shown in Table 1.

Table 1 Malondialdehyde (MDA) levels after lipoprotein administration and lipopolysaccharide (LPS) exposure in mice (*Mus musculus*)

	Group	**N**	**Subset for alpha = 0.05**		
			1	**2**	**3**
Tukey HSD	Control (-)	5	2.6176[a]		
	Control (+)	5			5.32460[c]
	P1	5	3.3568[a]	3.3568[ab]	
	P2	5		4.3068[b]	4.3068[bc]
	P3	5		4.3170[b]	4.3170[bc]

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.

Based on the data in Table 1, it can be concluded that the control (-) group did not differ significantly from P1, but differed significantly from groups P2, P3, and control (+). The P1 group did not show a significant difference from the control (-), P2, or P3 groups, but did differ significantly from the control (+). P2 did not differ significantly from P1 or P3, but was significantly different from both control (+) and control (-). The control (+) group showed significant differences from all other treatment groups. From the results of MDA level analysis using ANOVA and Tukey HSD tests, as presented in Table 1, the graph shows that the control (+) group, which was treated with LPS without lycopene, had the highest MDA levels compared to the other groups. Lycopene administration in the P1 group resulted in the lowest MDA levels compared to P2 and P3. This indicates that LPS exposure had a significant effect on the MDA levels in the serum of treated mice, and lycopene administration reduced MDA levels to normal levels, similar to the control (-).

4. Discussion

Based on the average MDA levels obtained using the Thiobarbituric Acid Reactive Substances (TBARS) test, treatment with LPS significantly increased MDA levels compared to the negative control group. This result is consistent with previous studies that reported LPS induces oxidative stress, characterized by increased lipid peroxidation. The positive correlation between MDA levels and pro-inflammatory cytokines suggests that inflammation triggered by LPS contributes to increased oxidative stress [7]. Based on the results of the comparison test, significant differences were found between the negative control group and the P1 group, and between the P2, P3, and positive control groups. The P1, P2, and P3 groups showed different average MDA levels. This was influenced by the administration of lycopene in different doses to the treated mice groups. The lycopene dose in P1 (0.3 mg) showed the potential to reduce MDA levels more effectively than P2 (0.6 mg) and P3 (0.9 mg). This aligns with the study by Veeramachaneni et al [8], which reported that lycopene supplementation induced hepatic CYP2E1 protein, TNF mRNA, and inflammatory foci in alcohol-fed rats.

Lipopolysaccharide (LPS) is a component of the outer membrane of gram-negative bacteria and a potent pathogen-associated molecular pattern (PAMP), capable of inducing systemic inflammatory responses and oxidative stress in various animal models. Oxidative stress induced by LPS is often associated with increased malondialdehyde (MDA) levels, an end product of lipid peroxidation widely recognized as a marker of oxidative damage [9]. When LPS triggers macrophage activation and the immune system in the spleen, an increase in the production of free radicals such as Reactive Oxygen Species (ROS) occurs. These free radicals cause lipid peroxidation in cell membranes, producing MDA as the end product of lipid damage. LPS exposure triggers a strong inflammatory response in various organs, including the spleen. MDA is involved in the pathogenesis of various diseases, such as cardiovascular disease, neurodegeneration, and neoplasms [4].

The mechanism of MDA formation induced by LPS involves a complex cascade of reactions. LPS activates NADPH oxidase, an enzyme that produces superoxide radicals, significantly increasing ROS levels [10]. Additionally, LPS stimulates the expression of COX-2 and 5-LOX, enzymes involved in arachidonic acid metabolism, contributing to the production of inflammatory mediators and increased oxidative stress [11]. Furthermore, LPS induces the expression of iNOS, which increases NO production [12]. NO interacts with superoxide to form peroxynitrite, a highly reactive oxygen species that exacerbates lipid peroxidation and MDA formation [13].

Lycopene is a tetraterpene with a unique structure that contributes to its strong antioxidant properties [14]. Lycopene is a carotenoid antioxidant that is effective in neutralizing free radicals. When administered to individuals or animals exposed to LPS, lycopene can reduce ROS in the spleen and inhibit lipid peroxidation, thereby lowering MDA levels. This is because lycopene acts as a scavenger, eliminating free radicals before they damage cell membrane lipids [15]. Lycopene inhibits the activation of the NF-κB pathway, which is important in inflammatory processes, thereby reducing inflammation and oxidative stress, contributing to the decrease in MDA levels [16].

One of the mechanisms by which lycopene inhibits MDA is through the modulation of inflammatory mediators. Lycopene has been shown to inhibit the expression of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6), which are increased in response to LPS stimulation [17]. By reducing these cytokine levels, lycopene indirectly lowers ROS production and subsequent MDA formation. Additionally, lycopene is known to enhance the expression of Nrf2 and its downstream antioxidant genes, such as superoxide dismutase (SOD), NAD(P)H quinone dehydrogenase 1 (NQO1), and heme oxygenase 1 (HO-1), in various models, including broiler chickens and human cells [18].

The spleen exposed to LPS experiences severe oxidative stress, leading to tissue damage and increased MDA levels as a marker of cell damage. Lycopene administration can help repair damaged tissues and prevent further degradation, as evidenced by the reduction in MDA levels in spleen tissue. This decrease in MDA reflects lycopene's protective effects against oxidative damage induced by LPS. In vivo studies on animals exposed to LPS have shown that lycopene administration significantly reduces MDA levels in various organs, including the spleen. This confirms that lycopene can reduce lipid peroxidation and oxidative stress caused by LPS, thus reducing measurable cellular damage through MDA. Lycopene, as a potent antioxidant, can protect the spleen from the harmful effects of free radicals generated by LPS exposure. Lycopene supplementation reduces free radical production and prevents lipid peroxidation, thereby reducing MDA formation in spleen tissue.

5. Conclusion

LPS exposure significantly increased MDA levels, and lycopene administration was able to reduce MDA levels to normal limits.

Compliance with ethical standards

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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