

The Effect of Coconut Water on Decreasing MDA and IL-6 Levels in Male Wistar Rats Induced with High-Fat Diet



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ABSTRACT: Dyslipidemia is a condition in which there are abnormal levels of lipids in the blood, including increased levels of cholesterol, LDL (*Low-Density Lipoprotein*), and triglyceride levels, as well as decreased HDL levels (*High-Density Lipoprotein*) which causes oxidative stress. Oxidative stress results in tissue damage which triggers an inflammatory reaction that releases proinflammatory cytokines such as *Interleukin-6* (IL-6) which increases in blood endothelial cells and triggers *Malondialdehyde* (MDA) which increases as a result presence of lipid oxidation in tissues. This condition can be neutralized by consuming antioxidants from outside the body such as young coconut water. This research aims to know the effect of giving young coconut water on the rate of MDA and IL-6 in male *Wistar* rats who are given a high-fat diet. The research method is a *posttest-only control group design*. The subjects of the study were 24 male *Wistar* rats which were randomly divided into 4 groups. Group K1 was given standard feed and distilled water. Group K2 was given a high-fat diet without coconut water. Groups K3 and K4 were given a highfat diet and young coconut water at doses of 4mL/200gBW/day and 8mL/200gBW/day, respectively. The study was conducted for 29 days by taking blood to check MDA levels using the TBARS and IL-6 methods using the ELISA method. The lowest average of MDA level was in the K3 group (0.071 ng/L) and the lowest average of IL-6 level was in the K4 group (4.377 ppm). *Kruskal Wallis* test result showed the p-value of MDA was 0.004 ppm and at IL-6 levels was 0.020 ng/L. Differences in MDA levels between groups K1-K2 (0.004), K2-K3 (0.004), and -K4 (0.004). Differences in IL-6 levels between groups K2-K3 (0.016), K2-K4 (0.004). The administration of coconut water can significantly reduce the levels of MDA and IL-6 in male *Wistar* rats induced with a high-fat diet.

KEYWORDS: Coconut water, MDA levels, IL-6 levels

I. INTRODUCTION

Dyslipidemia is defined as a characteristic disorder of lipid metabolism and is characterized by elevated total cholesterol levels, low-density lipoprotein (LDL), triglycerides, and decrease high-density lipoprotein (HDL).¹ The increase in LDL due to the consumption of foods that contain fat causes free fatty acid metabolism to occur. Dyslipidemia occurs and affects the high free radicals in the body. The imbalance between free radicals and the body's natural antioxidants causes a condition of oxidative stress which results in tissue damage. This can trigger an inflammatory reaction that releases pro-inflammatory cytokines such as interleukin-6 (IL-6) in blood endothelial cells and triggers Malondialdehyde (MDA) consequences presence of lipid oxidation in tissues.² This condition causes the capacity of antioxidant enzymes to decrease so that natural antioxidant supplies are needed from outside the body such as young coconut water. Young coconut water has the potential to reduce oxidative stress levels, but the effect on levels is unknown Malondialdehyde (from MDA) interleukin-6 (IL-6) in *Wistar* rats fed a high-fat diet.³

Dyslipidemia is a risk factor for cardiovascular disease.⁴ Global Burden of Disease Study reported that nearly 17.6 million deaths were caused by cardiovascular Disease (CVD).⁵ Cardiovascular disease accounts for the highest mortality in the world where around 80% of global deaths come from developing countries.⁶ The prevalence of cardiovascular disease in Indonesia in 2013 was 0.5% or 883,447 people, meanwhile, in 2018, this figure increased to 1.5% or around 1,017,290 people.⁷ Previous studies also proved that effective management of patients with dyslipidemia can reduce the incidence, mortality, and disease burden of CVD.⁸ Coconut water is a natural source of antioxidants and easily available in Indonesia. Natural antioxidants are able to protect the body against cell damage caused by ROS, are able to inhibit the occurrence of degenerative diseases, and are able to inhibit lipid

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peroxidation.⁹ Coconut water contains antioxidants such as Polyphenols, L-Arginine, and Vitamin C.⁹ Several studies have shown that coconut water at a dose of 8 mL/200 gBW has an effect on IL-6 and MDA in Wistar rats with metabolic syndrome.¹⁰ Research IJMRA, Volume 6 Issue (Month) 2023

Conducted stated that coconut water at a dose of 4 mL/100 gBW/day can reduce IL-6 levels in male white rats induced by STZ-Na.¹¹

Dyslipidemia causes an increase in the accumulation of lipids in the liver, thereby reducing the body's ability to lower blood fats.¹² Accumulation of cholesterol in endothelial cells, hepatocytes, leukocytes, erythrocytes, and platelets triggers its production of reactive oxygen species (ROS) and reduces antioxidant defense mechanisms.¹³ This condition causes oxidative stress and affects body changes.¹⁴ Consumption of unbalanced foods such as foods high in cholesterol and high in carbohydrates causes changes in lipid profiles, oxidative stress, and inflammation.¹⁵ A high-fat diet causes an increase in Lipopolysaccharides (LPS) plasma and activates toll-like receptor 4 (TLR4) resulting in increased levels of inflammatory cytokines, such as interleukins (IL-6, IL-17, and TNF- α) which can lead to increased ROS.¹⁶ Increased ROS can be neutralized by consuming antioxidants from outside the body such as coconut water. Coconut water has anti-inflammatory and antioxidant properties that can work to neutralize free radicals in the body, which is expected to prevent an increase in MDA and IL-6 levels. Further research is needed on the effect of giving coconut water on MDA and IL-6 levels. In mice fed a high-fat diet.

II. MATERIAL AND METHOD

Experimental Subjects

The research subject was male Wistar rats which were divided into four groups. The control group (K1) and the treatment group (K2, K3, and K4).

Administration of Coconut Water

The control group was not given a high-fat diet (K1), the control group was given a high-fat diet (K2), the treatment group was given young coconut water therapy at a dose of 4mL/200gBW/day (K3), and the control group treatment given young coconut water therapy at a dose of 8 mL/200 gBW/day (K4)

Data Analysis

The normality and homogeneity data test using Shapiro Wilk and Levene test. The data of MDA and IL-6 levels were analyzed using the One-Way ANOVA, the Tamhane, and the LSD test.

III. RESULT

The Effect of Coconut Water on MDA Levels

Table 1 shows that the highest average level of MDA was in the second treatment group (K2) which was given a high-fat diet and distilled water (0.096 ppm). The third treatment group (K3) obtained the lowest MDA level (0.071 ppm) by giving the coconut water dose of 4mL/200gBW/day and a high-fat diet, then followed by the control group (K1) by feeding standard and distilled water, then the fourth treatment group (K4) by giving young coconut water a dose of 8mL/200gBW/day. Test results KruskalWallis showed significant differences in all groups with a p-value of 0.004 ($p < 0.05$).

Table 1. Results of Average Analysis, Normality Test, Homogeneity Test on MDA Levels and IL-6 Levels

Variable	Group				Sig.(p)
	K1 N=6	K2 N=6	K3 N=6	K4 N=6	
MDA (ppm)					
Average	0.072	0.096	0.065	0.058	
Std. deviation	0.011	0.003	0.033	0.020	
Shapiro Wilk	0.073*	0.050*	0.644*	0.627*	
Levene Test					0.001
					One Way Anova
					<u>0.021***</u>
IL-6 (ng/L)					
Average	5.043	5.760	4.513	4.812	
Std. deviation	0.985	0.543	0.966	0.861	

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Shapiro Wilk	0.154*	0.993*	0.612*	0.118*
Levene Test				0.334**
One Way Anova				0.108

Information: *Normal $p > 0.05$ **Homogeneous $p > 0.05$ ***Significant $p < 0.05$

Table 2. Differences in MDA levels between the 2 groups using LSD test

Group	p-Value
K1 vs K2	0.004*
K1 vs K3	0.935
K1 vs K4	0.808
K2 vs K3	0.004*
K2 vs K4	0.004*
K3 vs K4	0.623



Figure 1. The average of MDA levels between groups

Mann Whitney test result shows the MDA levels in the K1 group had a significant difference to the K2 group with a p-value of 0.004 ($p < 0.05$) while there was no significant difference to the K3 group with a p-value of 0.935 and the K4 group with a p-value of 0.808 ($p > 0.05$) (Table 2). The K2 group had a significant difference from the K3 and K4 groups with a p-value of $p = 0.04$ ($p < 0.05$). There was no significant difference between the K3 and K4 groups with a p-value of $p = 0.623$ ($p > 0.05$). Based on the data above, it can be concluded that giving coconut water at 4 mL/200gBW/day and 8 mL/200gBW/day has a significant effect on reducing MDA levels in male Wistar rats induced with a high-fat diet.

The Effect of Coconut Water on IL-6 Levels

The highest average levels of IL-6 (Table 1) is the second treatment group (K2) which was given a high-fat diet and distilled water (5,760 ng/L). The fourth treatment group (K4) was given young coconut water at a dose of 8 mL/200gBW/day with a high-fat diet and had the lowest average of IL-6 level (4,377 ng/L), followed successively by the third treatment group (K3) which was given coconut water dose of 4 mL/200gBW/day with a high-fat diet and the control group (K1) with standard feeding and distilled water. Kruskal-Wallis test results showed significant differences in all groups with a p-value of 0.020 ($p < 0.05$).

Table 3. Differences in IL-6 levels between the 2 groups using LSD test

Group	p-Value
K1 vs K2	0.109
K1 vs K3	0.749
K1 vs K4	0.261

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K2 vs K3	0.016*
K2 vs K4	0.004*
K3 vs K4	0.261

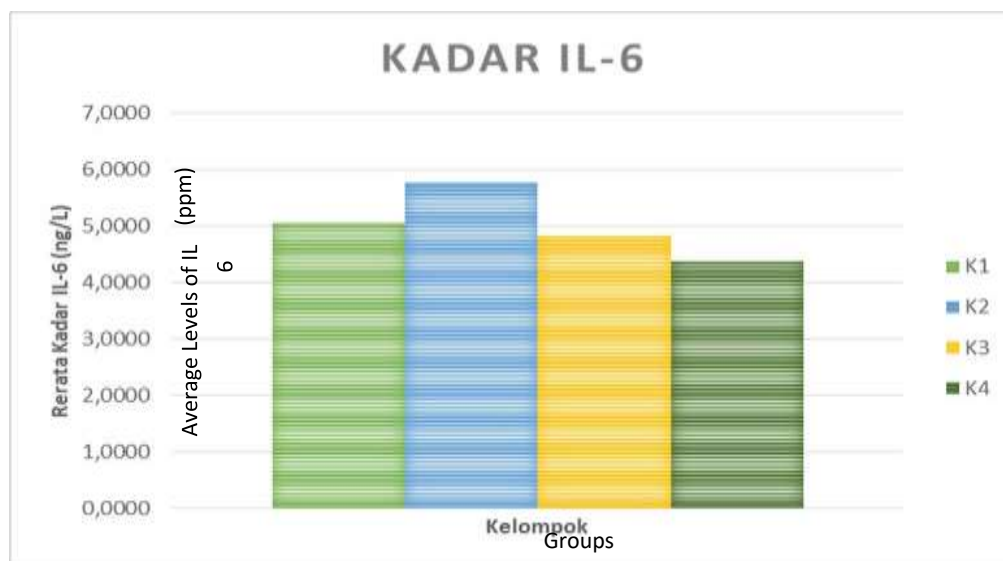


Figure 2. The average of IL-6 levels between groups

Mann Whitney test result (Table 3) shows that IL-6 levels in group K1 did not differ significantly from group K2 with a p-value of 0.109, group K3 with a p-value of 0.749, and group K4 with a p-value of 0.261 ($p > 0.05$). The K2 group had significant differences from the K3 and K4 groups with a $p < 0.05$. There was no significant difference between the K3 and K4 groups with a p-value of $p = 0.261$ ($p < 0.05$). Based on the data above, it can be concluded that giving young coconut water at 4 mL/200gBW/day and 8mL/200gBW/day has a significant effect on decreasing IL-6 levels in male Wistar rats given a high-fat diet.

IV. DISCUSSION

Dyslipidemia causes an increase in the accumulation of lipids in the liver, thereby reducing the body's ability to lower blood fats.¹² Accumulation of cholesterol in endothelial cells, hepatocytes, leukocytes, erythrocytes, and platelets triggers its production of reactive oxygen species (ROS) and reduces antioxidant defense mechanisms.¹³ This condition causes oxidative stress and affects body changes.¹⁴ Consumption of unbalanced foods such as foods high in cholesterol and high in carbohydrates causes changes in lipid profiles, oxidative stress, and inflammation.¹⁵ The treatment group showed increased cholesterol levels (> 54 mg/dL), LDL (> 27.2 mg/dL), and HDL (< 35 mg/dL) as a result of being given a high-fat diet using egg yolks of 2 ml/head/day by sonde for 14 days on K2, K3, and K4.

A high-fat diet causes an increase of Lipopolysaccharides (LPS) plasma and activates toll-like receptor 4 (TLR4) resulting in increased levels of inflammatory cytokines, such as interleukins (IL-6, IL-17, and TNF- α) which can lead to increased ROS.¹⁶ Increased ROS can be neutralized by consuming antioxidants from outside the body such as young coconut water. Young coconut water has anti-inflammatory and antioxidant properties that can work to neutralize free radicals in the body, which is expected to prevent an increase in MDA and IL-6 levels. Further research is needed on the effect of giving young coconut water on MDA and IL-6 levels in mice fed a high-fat diet.

The results of examining MDA levels in the K2 group that was given a high-fat diet without giving young coconut water experienced a significant increase compared to the control group (K1), the group that was given coconut water at a dose of 4 mL/200 gBW/day (K3) and 8 mL/day. 200 gBW/day (K4) as shown in table 5.1. This suggests that hypercholesterolemia will trigger lipid peroxidation. Lipid peroxidation is a reaction that occurs between free radicals and polyunsaturated fatty acids (*polyunsaturated fatty acid*, PUFA) which are present in cell membranes and LDL. As a result, the blood vessels can get thicker by this. Polyunsaturated fatty acids that undergo peroxidation form products that are toxic to the body, Malondialdehyde (MDA).¹⁷

MDA levels in the group that was given a high-fat diet and given coconut water at doses of 4 mL/200 gBW/day and 8 mL/200 gBW/day decreased as shown in Table 1. The administration of coconut water is proven to inhibit damage caused by free radicals and can reduce MDA levels. Young coconut water besides containing vitamins and minerals, also contains DPPH free radical scavenging which can reduce the increase in free radicals. Coconut water also contains ascorbic acid. Ascorbic acid plays a role in the synthesis of collagen, carnitine, and neurotransmitters, it also plays a role in microsomal metabolism and has antioxidant

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activity. Ascorbic acid is a reducing agent or an electron donor in the antioxidant system. Ascorbic acid gives ionic groups hydrogen on intracellular ROS/NOS.¹⁸ Another similar study conducted by Nova stated that young coconut water at a dose of 4mL/100gBW was able to increase plasma insulin levels and reduce glucose and MDA levels in type 2 DM pregnant rats.¹⁹

The results of examining IL-6 levels in the K2 group that were given a high-fat diet without giving young coconut water experienced a significant increase compared to the control group (K1), the group that was given coconut water at a dose of 4 mL/200 gBW/day (K3) and 8 mL/200 gBW/day (K4) as in table 5.1. Feeding fat can induce ROS which will have an impact on lipid metabolism. When there is a disturbance in lipid metabolism, there will be an increase in adipose tissue mass caused by the incoming energy being higher than the energy expended, resulting in an increase in the size and number of adipose tissues. An increase in the size and number of adipose tissues leads to the production of pro-inflammatory cytokines, one of which is IL-6. This is caused by oxidative stress which will activate Bax in mitochondria so that release occurs cytochrome-c.²⁰ This release has an impact on inhibiting the flow of electrons in the respiratory chain. Levels β -oxidation and FFA also increased so that there was an increase in the levels of NADH, FADH, and sending electrons to the respiratory chain. This imbalance in sending electrons and removing electrons causes an accumulation of electrons in the respiratory chain which will form an excess nitric oxide (NO) by nitric oxide synthase (iNOS) which then activates NF- κ B expression to induce IL-6.²¹

V. CONCLUSION

The administration of coconut water at a dose of 8 mL/200gBW/day and 4 mL/200gBW/day can decrease the MDA and IL-6 levels in male Wistar rats induced with high-fat diet.

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