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Research Article

Effect of Virgin Coconut Oil from Green Coconut on High-Density Lipoprotein (HDL) Levels in Blood Serum of White Mice

Iswendi Iswendi[1*], Iryani Iryani[1], Regi Fadila Putra[2]

[1] Department of Chemistry, Faculty of Mathematics and Natural Science (FMIPA), Universitas Negeri Padang, Padang, Indonesia

[2] Graduate School of Science and Engineering, Kansai University, Osaka, Japan.

[*] *Corresponding author: Iswendy956@gmail.com*

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Abstract: This study aims to determine the effect of High-Density Lipoprotein (VCO) dose on mice's blood serum High-Density Lipoprotein (HDL) levels. This study is an experimental study using 24 mice divided into four groups. Control (-), Control (+) were fed egg yolks 0.025 mL/g body weight, Dose 0.02 and Dose 0.025 were experimental groups, given egg yolks 0.025 mL/g mice body weight and VCO at a dose of 0.020 mL, and 0.025 mL/g body weight of mice. HDL levels were determined on days 7, 14, and 21 by enzymatic method using 20 D* spectrophotometry. The statistical test found that the length of time giving VCO significantly affected blood serum HDL levels in mice with $F_{count} > F_{table}$ (F_{count} = 7.128 and F_{table} = 5.14). In contrast, variations in VCO dose did not significantly affect blood serum HDL levels. mice with $F_{count} < F_{table}$ (F_{count} = 3.33 and F_{table} = 4.76). The highest HDL level was at a dose of 0.020 mL/g body weight of mice with a treatment period of 14 days, namely 164,033 mg/dL of blood.

Keywords: Virgin coconut oil, HDL, White mice, Green Coconut

Introduction

Indonesia is the largest coconut producer after the Philippines. Coconut plants grow a lot in coastal areas. In 2015, coconut plantations in Indonesia reached 3,585,599 hectares [1]. The coconut plant can be called a plant of a thousand uses. All parts of the coconut plant are instrumental, from the roots of the coconut. It is instrumental as a colorant, and the stems can be building materials. Coconut leaves can be used as broomsticks and woven goods. Coconut fiber can be made as craft mats, brooms, and mattresses. Coconut shells can be used as activated carbon and handicrafts. The fruit's flesh can be used as raw material to produce copra, coconut oil, and coconut milk. Coconut water can be used to make vinegar and nata de coco. In addition, coconut is used as a processed product in pure VCO, which is beneficial for humans [2][3].

Figure 1. Green Coconut [4]

VCO, one of the products that come from coconut, has been part of the daily life of people due to its numerous medicinal properties. These include anti-inflammatory, analgesic, antipyretic, antioxidant, anti-stress, and antimicrobial properties[5]. It has also been a significant source of dietary fat and is completely non-toxic to humans[6] VCO also provides health benefits that could reduce some risks of certain diseases, including chronic disease. It has been reported that VCO has similar beneficial effects in mother's milk that can give babies an immunity to disease due to its medium-chain fats from C8 and C12, similar to breast milk[7].

Virgin Coconut Oil (VCO) is oil that comes from the flesh of old coconuts, which is extracted at low temperature (< 60°C), where the Physico-chemical properties of the coconut oil do not change during extraction [3][8]. The term virgin is used to distinguish between virgin coconut oil and conventional coconut oil. Pure coconut oil is processed from fresh coconut raw materials without a refining process, so the temperature used in this process is lower [9][10]**.** Oil processing at low temperatures aims to maintain its chemical structure in order that it does not decompose, especially medium-chain fatty acids (MCFA), such as lauric acid, capric acid, caprylic acid, and myristic acid [4][11][12]. The MCFA contained in VCO is very beneficial for health [12]. VCO contains 90% saturated fatty acids and 10% unsaturated fatty acids. The saturated fatty acids in coconut oil are almost dominated by lauric acid (**Table 3**) [11][13]. The presence of lauric acid in the VCO is responsible for its potential to prevent cardiovascular diseases because it initiates high-density lipoprotein (HDL) and decreases the total/HDL cholesterol ratio[14]. VCO can decrease the total cholesterol, triglycerides, phospholipids, low-density lipoprotein, and very-low-density lipoprotein cholesterol and can increase high-density lipoprotein cholesterol in serum and tissue comparison to copra oil

Table 1. Coconut Oil Fatty Acid Composition (VCO) [11]

Fatty Acid	Amount(%)
Unsaturated Fatty Acids	
Laurate Acid	43,0 – 53,0
Myristate Acid	16,0 – 21,0
Capric Acid	4,5 – 8,0
Palmitic Acid	7,5 – 10,0
Caprylic Acid	5,0 – 10,0
Caproic Acid	0,4 – 0,6
Saturated Fatty Acids	
Oleic acid	1,0 – 2,5
Palmitic acid	2,0 – 4,0
Polyphenols	80 mg/dl

The MCFA in CO can provide health benefits, such as functioning as an anti-microbial, antibody, and anti-virus to defend the body from the liver, AIDS, prostate gland enlargement, and cancer. VCO can lose weight or obesity and increase HDL levels and reduce blood levels of Low-Density Lipoprotein (LDL) by dissolving cholesterol; thus, blood circulation becomes smooth [15][16]. Recently, VCO emerged as a health supplement because of its medium-chain fatty acid (MCFA) content which was found to have potential as an anti-obesity treatment and has been shown to cure some minor ailments such as diarrhea, skin inflammation [17][18]. VCO Additionally contains antioxidants. The capacity of flavonoids to act as antioxidants depends on their molecular structure. Flavonoids will activate the Lecithin Cholesterol acyltransferase (LCAT) enzyme in the endogenous pathway, converting free cholesterol into more hydrophobic cholesterol esters, causing an increase in the bonding process between cholesterol esters and lipoprotein cores in order that HDL formation increases [19]. Antioxidants can protect the body from free radical attacks by donating one electron to oxidant compounds to inhibit the activity of these oxidant compounds[20].

The direct effect is shown by reducing free radicals, giving electrons to free radicals, so they are no longer reactive without generating new free radicals. Indirectly, flavonoids inhibit the production of free radicals. Flavonoids (especially genistein) inhibit protein kinase C enzymes required to produce superoxide radicals and hydrogen peroxide, resulting in the downregulation of superoxide and hydrogen peroxide. Besides that, flavonoids also bind excessive free Fe in plasma to prevent the formation of free radicals (Fenton reaction). Flavonoids also increase the production of endogenous AO such as Cu-Zn, Superoxide dismutase.

As antioxidants, flavonoids (polyphenol groups) work as follows: radical scavenging, directly donating electrons to molecules that have unpaired electrons without forming new radicals; reducing the production of free radicals (superoxide) or their precursors (hydrogen peroxide), by inhibiting the

enzyme protein kinase C; chelation of metals such as Fe, thereby inhibiting the Fenton reaction; inhibits the action of the enzyme xanthine oxidase; and increase endogenous antioxidants (superoxide dismutase, glutathione peroxidase, catalase). In the lipid peroxidation process, flavonoids can act in all phases, either in the initiation, propagation, or termination phases. Flavonoids block the initiation phase by scavenging the primary radical, namely superoxide. In the propagation phase, flavonoids react with peroxyl radicals and break the chain reaction. In contrast, in the termination phase, intermediate flavonoid radicals (which are formed when flavonoids react with peroxyl radicals) will react with other radicals (formed during the propagation phase), which will produce non-radical products (NRP)[20].

VCO is a triglyceride. The normal limit for triglycerides is <150 mg/dL and the high limit is 200-499 mg/dL [21]. Medium-chain fatty acids dominate the triglycerides in VCO with the number of carbon atoms from 6 to 12, which are saturated, such as caproic acid ($C_5H_{11}COOH$), caprylic acid ($C_7H_{15}COOH$), capric acid ($C_9H_{19}COOH$), lauric acid ($C_{11}H_{23}COOH$), and some unsaturated fatty acids[22]. Triglycerides containing medium-chain fatty acids are known as Medium Chain Triglycerides (MCT). In contrast, triglycerides composed of long-chain fatty acids are known as Long Chain Triglycerides (LCT), such as tristearin. MCT has a function as an anti-virus and anti-bacterial. Viruses and bacteria are generally protected by lipids that hold the DNA of organisms together with other cellular materials. MCT does not increase cholesterol nor triglyceride levels, nor does it increase the stickiness of platelets (small protein particles involved like plates that cause blood clots). Thus they do not cause excessive blood clots or plaques that lead to coronary artery disease/atherosclerosis..

MCT metabolism in the body is different from LCT. In the body, LCT derived from food will be converted into LCFA in intestinal cells with the help of lipase enzymes. This LCFA will re-form triglycerides with a larger structure, called chylomicrons. Chylomicrons are carried to the spleen system and then to the liver. From the liver, chylomicrons combine with cholesterol to form Very Low-Density Lipoprotein (VLDL), which enters the blood circulation. On the way, VLDL is transformed into IDL and LDL with the help of Lipoprotein lipase; LDL that is not needed will be brought back to the liver. MCTs derived from food in the body will be converted into MCFA in intestinal cells. These MCFA will be directly converted into energy used to conduct blood vessel wall cells, inhibit the entry of LDL into blood vessel cells, and regulate the breakdown of triglyceride-rich lipoproteins in the plasma.

Cholesterol bound by HDL is catabolized as cholesterol reserves in the liver. HDL is high-density lipoprotein, especially one that contains protein. These lipoproteins transport lipids from the periphery to the liver. This reserve is used to synthesize VLDL and the biosynthesis of other compounds, thereby reversing cholesterol transport from peripheral tissues to the liver. HDL Additionally inhibits the binding of LDL by receptor molecules in peripheral tissues. The presence of high levels of HDL will prevent the accumulation of LDL in the walls of blood vessels which is the cause of atherosclerosis[23]. HDL has an anti-inflammatory effect to help stabilize atherosclerotic plaques in blood capillaries, making them less prone to rupture and reducing blood clot formation. Therefore, high HDL levels can reduce cardiovascular risk. The most important complications of atherosclerosis are coronary heart disease, cerebral vascular disorders, and peripheral vascular disorders[14].

This research aims to determine the effect of giving VCO and the length of treatment on blood serum HDL levels of mice. This research is expected to benefit the development of science and technology in biochemistry.

Materials and Methods

Research Design

This study used a completely randomized design using two indented factors with two repetitions. The first factor is the length of treatment time with three variations of 7, 14, and 21 days and the second factor is the dose of VCO with two variations, namely the VCO dose of 0.020 mL and 0.025 mL/g body weight of mice (**Table 2**).

Table 2. Research Design

Time (B)	Treatment (A)			
	Control (+) (A_1)	Control (-) (A_2)	Dose 0.020 (A_3)	Dose 0.025 (A_4)
7 Days (B_1)	B_1A_1	B_1A_2	B_1A_3	B_1A_4
14 Days (B_2)	B_2A_1	B_2A_2	B_2A_3	B_2A_4
21 Days (B_3)	B_3A_1	B_3A_2	B_3A_3	B_3A_4

Description :
A = Treatment (A)
B = Time (B)
AB = Amount (HDL) White Mice Serum
Control (-) = VCO 0 mL, Egg yolk 0 mL
Control (+) = VCO 0 mL, Egg yolk 0.025 mL/g Mice Weight
Dose 0.02 = VCO 0.020 mL, Egg yolk 0.025 mL/g Mice Weight
Dose 0.025 = VCO 0.025 mL, Egg yolk 0.025 mL/g Mice Weight

Test Animals

The experimental animals were female white Swiss mice, 2.5 months old, as many as 24 (average weight 20-25 g) were divided into four groups. Control (-), Control (+) were fed egg yolks 0.025 mL/g body weight, Dose 0.020 and Dose 0.025 were experimental groups, given egg yolks 0.025 mL/g mice body weight and VCO at a dose of 0.020 mL, and 0.025 mL/g body weight of mice.

HDL Level Measurement

HDL levels were measured on (7, 14, and 21) days. Blood was taken from the neck of the mice, then allowed to stand for 15 minutes, and then centrifuged for 20 minutes at 3000 rpm. The transparent liquid portion of the blood (serum) is used to measure HDL levels. Absorbance readings were carried out by reacting with 100 µL of supernatant added with 1000 µL cholesterol reagent, mixed until homogeneous, then incubated at 20-25 °C for 20 minutes. The absorbance was measured at a wavelength of 500 nm against the blank. 1 mL of HDL reagent was used as a blank. Then a standard HDL solution with a concentration of 50 mg/dL is used 1 mL. The standard measurement is the same as measuring the absorption of HDL levels. Furthermore, the determination of HDL levels, each determination of levels and absorption using a 20 D* spectrophotometer.

Analysis Data

Data analysis was carried out statistically using the ANOVA (one-way ANOVA) method. There is a significant difference if the P-value <0.05.

Results and Discussions

The results of the study obtained HDL levels in the blood serum of experimental mice with a dose of VCO 0.020 mL/g and 0.025 mL/g body weight of mice with a treatment time of 7,14,21 days as in (**Table 3**)

Table 3. Average HDL Level (mg/dL)

Treatment(A)	**Time (B)** Amount HDL (mg/dL)		
	7 Days	**14 Days**	**21 Days**
Control (-)	42.415	98.819	55.689
Control (+)	39.984	64.873	29.283
Trial C	44.49	164.033	71.283
Trial B	70.608	115.714	108.981

The VCO dose of 0.020 mL/g bodyweights of mice on day 7, the average HDL level of mouse blood serum was 44.490 mg/dL, on day 14, it increased to 164.033 mg/dL, while on day 21, it decreased to 71.283 mg/dL. For a dose of VCO 0.025 mL/g body weight of mice, on day 7 obtained HDL levels of 70.608 mg/dL, day 14 of 115.714 mg/dL, on day 21 of 108.981 mg/dL (**Table 3**). In the control group [control (+) and control (-)], the increase in HDL levels with treatment time was the same as the group given VCO. The following is a graph of mice's average HDL blood serum levels (

Figure 2).

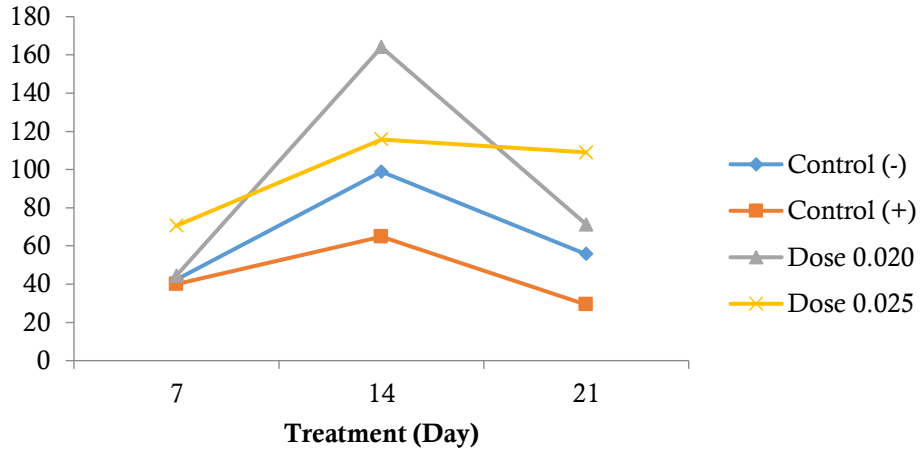

Figure 2. Graph of Average HDL Serum Blood Levels of Mice

At a dose of VCO 0.020 mL/g body weight of mice, HDL levels increased on day 14, while on day 21, it decreased again. At a dose of 0.025 mL/g VCO of mice body weight. HDL levels increased on day 14, while on day 21 decreased again. The decrease in HDL levels on day 21 was caused by a dose of VCO that was too large; therefore, the metabolic system of mice had not metabolized the triglycerides from VCO. Besides, it is caused by the active estrogen hormone in female mice. Cholesterol in the body is partly used to form estrogen or other steroid compounds such as thyroid hormone [24]. To see the effect given by the treatment variables were analyzed using analysis of variance (ANOVA). The variance of ANOVA is presented in (**Table 4**).

Table 4. Print Variety of Average HDL Levels

SK	Db	JK	KT	FB	F_{table} 0.05
Treatment(A)	3	5651.2	1993.73	3.33**	4.76
Treatment(B)	2	8074.25	4037.125	7.128*	5.14
Random	6	3397.78	566.295		
Total	11	17223.2			

The ability of VCO to increase blood serum HDL levels is thought to be related to the lipoprotein lipase enzyme, where VCO can increase the activity of the lipoprotein lipase enzyme, thereby increasing VLDL catabolism. Free cholesterol and phospholipids resulting from VLDL breakdown are transferred to HDL, so HDL levels increase. Giving VCO will increase lipoprotein lipase enzyme activity according to the number of doses given[24]. VCO Additionally contains antioxidants, where antioxidants can protect the body from free radical attacks by donating one electron to oxidant compounds to inhibit the activity of these oxidant compounds[25]. The direct effect is shown by reducing free radicals, giving electrons to free radicals, so they are no longer reactive without generating new free radicals. Indirectly, flavonoids inhibit the production of free radicals. Flavonoids (especially genistein) inhibit the enzyme protein kinase C, which is needed to produce superoxide radicals and hydrogen peroxide, resulting in the downregulation of superoxide and hydrogen peroxide. Besides that, flavonoids also bind excessive free Fe in plasma to prevent the formation of free radicals (Fenton reaction). Flavonoids also increase the production of endogenous

AO such as Cu-Zn, Superoxide dismutase [26]. Furthermore, the capacity of flavonoids to act as antioxidants depends on their molecular structure. Flavonoids will activate the Lecithin Cholesterol Acyl Transferase (LCAT) enzyme in the endogenous pathway, converting free cholesterol into more hydrophobic cholesterol esters, causing an increase in the bonding process between cholesterol esters and lipoprotein cores in order that HDL formation increases [19].

Conclusion

The results showed that to begin with, The statistical test that the length of time giving VCO significantly affected blood serum HDL levels in mice with $F_{count} > F_{table}$ (F_{count} = 7.128 and F_{table} = 5.14). In addition, variations in VCO dose did not significantly affect blood serum HDL levels. mice with $F_{count} < F_{table}$ (F_{count} = 3.33 and F_{table} = 4.76). Furthermore, the highest HDL level was at a dose of 0.020 mL/g body weight of mice with a treatment period of 14 days, namely 164,033 mg/dL of blood.

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