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Research

Effect of Virgin Coconut Oil Administration to Increase HDL, Decrease LDL and IL-6 in Hypercholesterol Male Wistar Rats

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ABSTRACT

Background: Hypercholesterol is a change in blood lipid profile levels, from rising cholesterol levels that can be triggered by frequent consumption of fatty foods. Hypercholeerol causes adipose tissue and macrophages to release inflammatory cytokines, then adipose cells release IL-6 and spur the formation of CRP. This study in aimed to determine the effect of Virgin coconut oil (VCO) on changes in HDL, LDL, and IL-6 levels in hypercholesterol mice.
Subjects and Method: This was randomized controlled trial was carried out at the PSPG UGM Nutrition Laboratory in August 2021. The sample total number of samples used was 20 tails and 4 spare tails, namely 24 samples male wistar strain rats. Sampling using simple random sampling. The independent variables in this study were the dose of VCO 0.9 ml/200g BW/day, and 0.45 ml/200g BW/day. The dependent variables in this study were HDL, LDL, and IL-6 cholesterol levels in male Wistar rats. The data in the analysis used a normality test with the Shapiro Wilk test and a data homogeneity test with the Levane test. HDL dan LDL levels were measured using a dyasis reagent, while IL-6 was measured using ELISA method.
Results: Average LDL and HDL levels are highest in the P1 group compared to the P2, K0, and K1 groups. The One Way Anova test on HDL levels showed a significant difference between groups with a value of $p<0.001$. One Way Anova test results on LDL and IL-6 levels showed significant differences between the groups ($p<0.001$) and ($p= 0.004$).
Conclusion: Administering VCO at a dose of 0.9 mL/ 200 g BB/day 0.45 mL/200 g BB/day, and 86.4 mg/200 g can increase HDL and IL-6 levels in male rats wistar strain with hypercholesterol and lowered LDL levels by the same dose in male mice with hypercholesterol.

Keywords: hipercholesterol, HDL, LDL, IL-6

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BACKGROUND

Hypercholesterol is a change in blood lipid profile levels, which can be triggered by a diet pattern high in cholesterol (Romadhoni, 2014). A high-cholesterol diet causes an imbalance in the fat metabolism system resulting in LDL and HDL levels in an abnormal range which is a risk factor for cardiovascular disease. Increased levels of IL-6 in such concidients lead to low levels of systemic inflammation. The cytokine IL-6 can inhibit the activity of lipoprotein lipase (LPL), increase the activity of lipolytic endothelial lipase, which is associated with a decrease in HDL levels. Pharmacological therapy efforts can actually cause side effects,

therefore it is necessary to have alternative natural treatments in treating hypercholesterolemia (Susantiningsih and Mustofa, 2018). VCO has anti-inflammatory effects and can improve hypercholesterol, but until now there is still little data on the administration of VCO to lipid levels such as HDL, LDL, and IL-6 in hypercholesterol conditions (Arunima and Rajamohan, 2014). One study to determine the anti-inflammatory activity of VCO by examining gene expression in lipopolysaccaide-induced RAW 264.7 cells in vitro using reverse Transcription-Polymerase Chain Reaction (RT-PCR). Based on research result, VCO administration can reduce the density values of IL-6, TNF-a, Inos, il1b, AND cox-2 (Nasution, 2020).

Cardiovaskular disease is still the leading cause of death in many countries. From 1990 to 2020, mortality from cardiovascular disease increased by 137% for men and 120% foor women in developing countries, while the increase was lower, 48% for men and 29% for women in developed countries. (PERKI, 2013) The high incidence of cardiovascular disease is closely related to lipid profile or hypercholesterolemia at this time affect a person's life habits, such as increased consumption of fatty foods, lack of physical activity, and smoking. Data from the National Health and Nutrition Examination Survei shows 53% of the 105.3 million Americans have abnormal lipid levels (Capewell 2010; Toth et al, 2012). In Indonesia data from the Indonesian Endocrinology Association (PERKENI) population with cholesterol levels >240 mg/dl is estimated at 31.9 million people (13.8%) of the population. Data in Indonesia are taken from the Central Jawa National Basic Health Research (RISKESDAS) showed that 58.41% of the population aged > years had a habit of consuming fatty foods which could affect cholesterol levels, and 1,56% prevalence of heart disease (PERKENI, 2015).

VCO (Virgin Coconut Oil) is pure coconut oil derived from fresh coconuts, which is processed naturally without using chemicals or other synthetic ingredients (Liau, 2011). VCO regulates fatty acid oxidation via PPAR-a-dependent patways. The antioxidant content in VCO is dominated by polyphenol groups. Polyphenol components known to prevent LDL oxidation. The polyphenol cmponents found in VCO are ferulic acid, p-kumaric acid, caffeic acid and catechins. Foods containing polyphenols have a beneficial effect on endothelial function (Abujazia, 2012). The fatty acids in VCO can be hydrolyzed more quickly by lipase enzymes to produce monoacylglycerol and free fatty acids, which are then absorbed throught the mucosa and via the portal vein which are transported direcly to the liver (Nurul, 2013). The fatty acids in VCO can be hydrolyzed more quickly by lipase enzymes to produce monoacylglycerol and free fatty acids, then absorbed through the mucosa and through the portal vein which are trasnsported directly to the liver. One Study was conducted to determine the anti-inflammatory activity of VCO by testing gene expression in cells. RAW 264.7 induced by lipopolysaccharide in vitro using Reserve Transcription Polymerase Chain Reaction (RT-OCR).

Based on previous studies, further research needs to be conducted on the effect of VCO administration on LDL, HDL, and IL-6 cholesterol levels in hypercholesterol states at stratified doses (0.45 ml /day and 0.9 ml /day) in a shorter time.

SUBJECTS AND METHOD

1. Study Design

This study uses a True Experimental design with a Post Test Only Control Group Design

research design was carried out at the PSPG UGM Nutrition Laboratory in August 2021.

2. Population and Sample

The population of this study used male wistar rats aged 2-3 months, weighing 150-200 grams, which were obtained from the PSPG Nutrition Laboratory at Gajah Mada University. The research sample was taken using simple random sampling and those who meet the inclusion, exclusion, and drop out criteria.

3. Study Variables

The independent variables in this study were the dose of VCO 0.9Ml/200gr BW/day, and 0,45 Ml/200g BW/day. The dependent variables in this study were HDL, LDL, and IL-6 cholesterol levels in male Wistar rats. Pracondition variable Rats were made hypercholesterolemia by feeding them a high-cholesterol diet, namely using quail eggs given as much as 4ml orally for 7 days.

4. Operational definition of variables

VCO is Virgin Coconut Oil (VCO) is coconut oil that is processed in a way that does not use high heat or chemicals.

HDL is serves to prevent the occurrence of atheroma or narrowing of blood vessels due to fat.

LDL is lipoprotein which carries cholesterol to the blood vessels.

IL-6 is a type of cytokine which is an early marker of a cytokine storm.

5. Study Instruments

IHDL and LDL levels were measured using a spectrophotometer. IL-6 levels were measured using ELISA. The tools used in this study were animal cages, glass objects, oval needles (Gavage), 1 cc syringes, surgical tubes, pipettes, micro plates, yellow type, blue type, spectrophotometer. ELISA Reader, Blood photometer.

6. Data analysis

Data on average levels of HDL, LDL and IL-6 are presented descriptively in the form of tables and graphs. Then the data was tested for normality with the Shapiro Wilk test and the data homogeneity test with the lavene test. The distribution of the data on HDL and LDL cholesterol levels obtained normal and homogeneous results, so it was continued using the One-way Anova test to obtain $p<0,05$ followed by the Post Hoc test with the Tukey Test. The distribution of IL-6 level data showed abnormal and homogeneous result, so it was contued with the Kruskal Wallis test between groups, obtained $p<0.005$ followed by a non-parametric difference test for two groups using the Mann Whitney test.

7. Research Ethics

Ethical Clearance No.253/VIII/2021/Komisi Bioetik Universitas Sultan Agung Semarang.

RESULTS

1. Sample Characteristics

Based on the sample characteristics in table 1, the types of rats used in the study were all 20 wistar strains, male sex, 18 weeks old. Rat body weight 101-120 g which totals 7 with a percentage of 35%, 121-140 g which amounts to 13 with a percentage of 65%.

2. Bivariate Analysis

Table 2 shows that lowest average HDL level was in group K1, then followed successively by the second treatment group (P2) and the control group (K0). The treatment group (P1) had the lowest average HDL level. Considering that all groups of HDL levels based on the Shapiro Wilk test showed that the data were normally distributed ($p>0.005$) and the homogeneity test using the lavene test was homogeneous ($p>0,05$), the data analysis used the One-way Anova test showed significant deferences between groups ($p<0.001$).

Table 3 shows that the lowest average HDL levels were in the K1 group, followed by the second treatment group (P2) and the first treatment group (P1). The control group (K0) got the lowest average HDL levels. In

all groups of HDL levels based on the shapiro wilk test, the data were normally distributed (p>0.005) and the homogeneity test using the levene test the results were homogeneous (p=0.969) then data analysis using the One-way Anova parametric test. One-way Anova test results showed significant differences between groups (p<0.001). To find out which groups differ meaningfully performed a post Hoc test with the Tukey test as presented in table 3. Table 2 shows that the lowest average HDL levels were in the K1 group, followed by the second treatment group (P2) and the first treatment group (P1). The control group (K0) got the lowest average HDL levels. In all groups of HDL levels based on the shapiro wilk test, the data were normally distributed (p>0.005) and the homogeneity test using the levene test the results were homogeneous (p=0.969) then data analysis using the One-way Anova parametric test. One Way-anova test results showed significant differences between groups (p<0.001). To find out which groups differ meaningfully performed a post Hoc test with the Tukey test as presented in Table 3.

The result of the Tukey test in table 4 show that there is a significant difference in LDL levels in the control group (K0) with the negative control group (K1) with a value (p<0.001), the control group (K0) has a significant difference with the P1 treatment group (p<0.001), K0 there is a significant difference with group P2 (p<0.001). K1 there is a significant difference with the treatment group P1 (p<0.001), K1 there is a significant difference with the P2 group (p<0.001). Group P1 had a significant difference with group P1 (p<0.001) based on the dataabove it can be concluded that administration of VCO at a dose of 0.9 Ml/200g BW/day of VCO had a significant effect followed by a dose of 0,45mL/200g BW/day on decreasing LDL levels in male wistar rats with hypercholesterol so that the hypothesis statement can be accepted.

The Result of the Mann Whitney test in table 5 show that there is no significant difference in total cholesterol levels in the control group (K0) with the negative control group (K1) with the treatment group P1 (p=0.107), K0 there is No. significant difference with the P2 treatment group (p=0.004), P1 there was a significant difference with group P2 (p=0.521). Based on the data above, it can be concluded that administration of VCO at a dose of 0.9 ml/200 g BW/day had a significant effect, followed by a dose of 0.45 ml/200g BW/day on decreasing total cholesterol levels in male Wistar rats with hypercholesterolemia so that the hypotesis statement can be accepted.

Table 1. Sample characteristics

Characteristics	Category	Frequency	Percentage
Type of Rat	Wistar Strain	20	100%
Gender	Male	20	100%
Age	18 weeks	20	100%
Weight	101-120 g	7	35%
	121-140 g	13	65%

Table 2. Results of the analysis of average HDL, LDL, and IL-6 cholesterol levels

Variable	Group				
	K0 (N = 6)	K1 (N = 6)	P1 (N = 6)	P2 (N = 6)	p
HDL	0.80	0.72	0.98	0.96	0.001
LDL	0.80	0.83	0.79	0.77	0.001
IL-6	0.82	0.28	0.94	0.74	0.001

Table 3. Correlation of cardiorespiratory fitness on systolic blood pressure in the elderly

Group	Mean	SD	p
K0 vs K1	12.31	6.92	0.312
K0 vs P1	-18.17	6.92	0.071
K0 vs P2	-14.28	6.92	0.199
K1 vs P1	-30.48	6.92	0.001
K1 vs P2	-26.60	6.92	0.005
P1 vs P2	3.88	6.92	0.942

Table 4. Table.4 Differences in LDL levels between 2 groups using the Tukey test

Group	Mean	SD	p
K0 vs K1	-19.52	1.84	0.001
K0 vs P1	38.03	1.84	0.001
K0 vs P2	29.88	1.84	0.001
K1 vs P1	57.55	1.84	0.001
K1 vs P2	49.40	1.84	0.001
P1 vs P2	-8.15	1.84	0.001

Table 5. Differences in IL-6 levels between the 2 groups using Mann Whitney test

Group	Mean	SD	p
K0 vs K1	28.00	-1.68	0.920
K0 vs P1	29.00	-1.60	0.100
K0 vs P2	29.00	-1.61	0.100
K1 vs P1	24.00	-2.40	0.010
K1 vs P2	21.00	-2.88	0.040
P1 vs P2	35.00	-0.64	0.500

DISCUSSION

This Study used a sample of 24 male Wistar rats which were divided into 4 groups of 6 rats each, namely the control group (K0) with standard feed without being given a high cholesterol diet, the negative control group (K1) with standard feed with high cholesterol diet, the treatment group (P1) was given VCO at a dose of 0,9 ml/200g BW/day which was given a high cholesterol diet, and the treatment group (P2) was given VCO at a dose of 0,45 ml/200g BW/day which was fed a high cholesterol diet. On the 21st day, HDL, LDL and IL-6 levels were examined. This study used wistar rats because they are similiar to humans in terms of physiology, anatomy, and many human symptoms and conditions that can be replicated in rats. Hypercholesterol is a condition where there is an increase in cholesterol levels in the blood and the trigger is the High fat diet lifestyle. Hypercholesterol contributes to oxidative stress that can cause tissue damage or inflammation. This is because a high-cholesterol diet can increase cholesterol synthesis in the liver through increasing the activity of the HMG-CoA enzyme, which is the catalyst of the first step in cholesterol biosynthesis, causing an increase in LDL cholesterol levels in the blood (Dean, 2013). This study is in line with research conducted by Venty (2017) where the result of his research stated that administration of 0.8 Ml OF vco/day to male white Wistar rats fed a high-cholesterol diet was able to reduce lipid profile.

The K1, P1, and P2 treatment groups showed an increase in cholesterol levels (>200 mg/dL) after being given each high-cholesterol diet by 4 mL perorally for 7 days. The increase in HDL cholesterol levels was found in the P1 treatment group who were given a high cholesterol diet and given VCO at a dose of 0.9 mL / 200 g BB / day experienced insignificant differences with the control group and the P2 group who were given a high cholesterol diet and given VCO at a dose of 0.45 mL / 200 g. Meanwhile, the decrease in LDL cholesterol levels was found in the P1 treatment group who were given a high-cholesterol diet and given VCO at a dose of 0.9 mL / 200 g BB / day experienced significant differences with the control group and the P2 group who were given a high-cholesterol diet and given VCO at a dose of 0.45 mL / 200 g. This is because VCO contains MCTs that can function as ligands that can activate PPAR-α receptors which play an important role in lipid metabolism. Activation of PPAR-α will improve the regulation of genes related to the oxidation of fatty acids such as carnitine palmitoyl transferase (CPT 1) and acyl CoA oxidase so that it can suppress postprandial lipidemia and lipid accumulation, and will increase the activity of reserve cholesterol transport. Previous research results also said that giving 10% VCO in diabetic mice for 3 weeks showed that there was a decrease in triglyceride concentration, total cholesterol, LDL, VLDL, and enhancement on HDL parameters (Dosumu et al., 2010). a high-cholesterol diet can increase cholesterol synthesis in the liver through increased activity of the enzyme HMG-CoA, which is a catalyst from the first stage in cholesterol biosynthesis causing an increase in LDL cholesterol levels in the blood.58 This study is in line with research conducted by Venti where the results of his study stated that the administration of VCO 0.8 mL /day in male white rats of the wistar strain who were given a high cholesterol diet, able to lower the lipid profile (Agustina and Murwani R, 2013).

The administration of VCO has been shown to suppress IL-6 as a pro-inflammatory cytokine that can cause inflammatory inization or inflammation, which is caused by oxidative stress conditions due to the administration of a diet high in cholesterol. The content of VCO in the form of lauric acid plays an important role in the process. The acid that enters the body will be converted into monolaurin. The monolaurine can modulate the proliferation of immune cells, which are able to suppress inflammatory processes in the body.56 Research conducted by Nasution invitro on Raw 264.7 cells using VCO with a concentration of 62.5 micrograms /mL showed results that VCO was able to inhibit IL-6, and other cytokines. Final conclusion VCO effectively has anti-inflammatory activation (Grassi et al., 2010)

Based on the results of research and discussion on the effect of VCO administration on HDL, LDL, and IL-6 cholesterol levels for 21 days, it can be concluded that: Giving VCO at a dose of 0.9 mL/200 g BB/ day 0.45 mL/200 gr BB/day can increase blood HDL cholesterol levels in male wistar rats with hypercholesterol VCO administration at a dose of 0.9 mL/ 200 g BB/day 0.45 mL/200 gr BB/day can reduce blood LDL cholesterol levels in male wistar rats with hypercholesterol Administration of VCO at a dose of 0.9 mL/200 g BB/day 0.45 mL/ 200 gr BB/day may increase blood IL-6 cholesterol levels in male wistar rats with hypercholesterol.

Administration of VCO has been shown to suppress IL-6 as a pro-Inflammatory cytokine capable of causing inflammation, wgich is caused by oxidative stress due to high cholesterol diet. The content of VCO in the form of lauric acid plays an important role in this process. Acid that enters the

body will be converted into monolaurin (Hamsi, 2015). Monolaurin can modulate immune cell proliferation which is able to suppress inflammatory processes in the body. A study conducted by Nasution (2020) in vitro on raw 264.7 cells using VCO at a concentration of 62.5 micrograms /ml showed that VCO was able to inhibit IL-6, and other cytokines. The final conclusion is that VCO is effective in having anti-inflammatory activity.

AUTHOR CONTRIBUTIONS

Titiek Sumarawati as a background designer and provider of input regarding research methods. Joko Wahyu Wibowo as a giver of input in the literature review and writing a bobliography. Meli Maulida as a writer and data analysis and result discussion thiker.

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CONFLICT OF INTEREST

There is no conflict of Interest in this study.

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