**Effect of VCO Administration on Increased HDL Cholesterol Levels, Decreased LDL Levels, And IL-6 in Male Wistar Rats With Hypercholesterol**

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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.

Objective: To determine the effect of virgin *coconut oil* (VCO) on changes in HDL, LDL, and IL-6 levels in hypercholeleerol mice.

**Method:** Research uses *True experimental design* with Post Test Only Control Group Design research design. The study subjects numbered 24 male wistar strain rats that were randomly divided into 4 groups, namely K0, K1, P1 and P2. The K0 group was fed standard without being given a high-cholesterol diet and K1 was fed standard with a high-cholesterol diet. P1 and P2 are given VCO at doses of 0.9 mL/200 g BB/ day and 0.45 mL/200 g BB/ day, on the 22nd day blood intake is carried out for examination of LDL, HDL, and IL-6 levels. The data in the analysis used a normality test with the Shapiro Wilk test and a data homogeneity test with the Levane test.

**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=0.000) 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 hypercholeleerol. And lowered LDL levels by the same dose in male mice *with* hypercholeleerol.

*Keyword : Hiperkorestrol, HDL, LDL, IL-6*

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**1. 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 & 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 & Rajamohan, 2014)

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.

**2. Subjects and Methods**

 This study used a True experimental design with a Post Test Only Control Group Design research design. 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 of Gajah Mada University. Research sampling using simple random sampling method. The sample size according to WHO is at least 5 heads per group with a reserve of 10% (1 head). So that the total number of samples used is 20 heads and 4 reserve tails, namely 24 samples.

**Inclusion criteria**

Male Wistar Rats Rats in an active, healthy, behavioral, and normal activity Age 2-3 months Weight 150-200 grams

**Exclusion criteria**

Rats have anatomical abnormalities

**Research Instruments**

The tools used in this study were experimental animal cages, glass objects, oval needles (Gavage), 1 cc size syringes, surgical tubs, dissecting sets, test tubes, drip pipettes, micro plates, micro pipettes, yellow type, blue type, ELISA Reader Spectrophotometer, Blood photometer.

**Research Materials**

Male Wistar rats age 2-3 months weighing 150-200 grams, 24 heads. Virgin Coconut Oil (VCO) Hypercholesterol feed using Serum quail egg yolk, rat blood Reagent dyasis ELISA kit Rat IL-6 EDTA 10% Solution Turk Aquades.

**VCO Dosage**

The recommended dose of VCO is 2.5-3 tablespoons 3 times a day or equivalent to 37.5 - 45 mL /day for adults with a body weight of 45-67 kg and 50 mL for adults with a body weight of 70 kg. The optimal dose if converted provided that : human 70 kg equivalent to rat 200 gr is 0.018. The doses of VCO given in this study were 50 mL x 0.018 = 0.9 mL /200 g BB (optimal dose) and 25 mL x 0.018 = 0.45 mL /200 gr BB (moderate dose) for 14 days (Ogedengbe et al., 2016).

**High Cholesterol Diet Feeding**

Feeding experimental animals hypercholesterolmya using quail egg yolk is given as much as 4 mL perorally for 7 days. quail egg yolks contain cholesterol as much as 2,139.17mg / 100g, the cholesterol content is higher than other foodstuffs.

**Place and Time of Research**

Research using male Wistar rats was conducted at the PSPG UGM Nutrition Laboratory, the examination of HDL, LDL, and IL-6 levels was carried out at the PSPG UGM Nutrition Laboratory.

**Data Analysis**

The average data on HDL, LDL, and IL-6 levels are presented descriptively in the form of tables and graphs. Then the data in the normality test with the Shapiro Wilk test and the data homogeneity test with the Levene test. The distribution of HDL, LDL, and IL-6 cholesterol cholesterol levels data obtained normal and homogeneous results, so continued using the One Way Anova test, p<0.05 values were obtained followed by the Post Hoc test with the Tukey test.

**3. Result**

Research on the administration of Virgin Coconut Oil to HDL, LDL, and interleukin-6 (IL-6) cholesterol levels in male wistar strain rats with hypercholesterol has been carried out for 21 days. The results of the study are listed in table 1. The results of the analysis of the average HDL, LDL, and IL-6 cholesterol levels after treatment are listed in table 1.

Table.1 Results of the analysis of average HDL, LDL, and IL-6 cholesterol levels

|  |
| --- |
| **Group** |
| **Variable** | **K0 N=6** | **K1 N=6** | **P1 N=6** | **P2 N=6** | **Sig.(p)** |
|  | **Mean** | **Mean** | **Mean** | **Mean** |  |
| **Kadar HDL** | 73.16 | 24.82 | 64.30 | 42.07 |  |
| Std.deviasi | 1.644 | 1.676 | 1.940 | 1.530 |
| *Shapiro Wilk* | 0.801\* | 0.723\* | 0.985\* | 0.965\* |  |
| *Levene Test* |  |  |  |  | 0.969\*\* |
| *One Way Anova* |  |  |  |  | 0.000\*\*\* |
| **Kadar LDL** | 19,85 | 68.83 | 30.44 | 34.17 |  |
| Std.deviasi | 1.976 | 2.911 | 1.672 | 2.314 |
| *Shapiro Wilk* | 0.800\* | 0.834\* | 0.799\* | 0.770\* |  |
| *Levene Test* |  |  |  |  | 0.711\*\* |
| *One Way Anova* |  |  |  |  | 0.000\*\*\* |
| **Kadar IL-6** | 75.07 | 131.80 | 86.52 | 100.12 |  |
| Std.deviasi | 4.64 | 6.24 | 2.57 | 3.61 |
| *Shapiro Wilk* | 0.826\* | 0.287\* | 0.946\* | 0.746\* |
| *Levene Test* |  |  |  |  | 0.098\*\* |
| *One Way Anova* | 0,000\*\*\* |
| **Keterangan: \***Normal p>0,05 \*\*Homogen p>0,05 \*\*\*Signifikan p<0,05 |

**HDL Levels**

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>0.05) 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=0.000). 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>0.05) 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=0.000). To find out which groups differ meaningfully performed a post Hoc test with the Tukey test as presented in table 3.

**LDL Levels**

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

|  |  |
| --- | --- |
| **Group** | ***p-Value*** |
| K0 vs K1 | 0.000\* |
| K0 vs P1 | 0.000\* |
| K0 vs P2 | 0.000\* |
| K1 vs P1 | 0.000\* |
| K1 vs P2 | 0.000\* |
| P1 vs P2 | 0.000\* |

Table 2 shows that the lowest mean LDL levels were in the control group (K0), followed by the first treatment group (P1) and the second treatment group (P2). The negative control group (K1) got the highest average LDL levels. The group was tested using the shapiro wilk test with the results of existing data that were normally distributed (p>0.05) and using the levene test for homogeneity showed homogeneous results (p=0.711) then data analysis using One Way Anova and showed meaningful differences between groups (p=0.000) to find out which group was meaningful to do post Hoc test with Tukey test as presented in table 3.

**IL-6 Levels**

Table.3 Differences in LDL levels between 2 groups using the Tukey test

|  |  |
| --- | --- |
| **Kelompok** | ***p-Value*** |
| K0 vs K1 | 0.000\* |
| K0 vs P1 | 0.000\* |
| K0 vs P2 | 0.000\* |
| K1 vs P1 | 0.000\* |
| K1 vs P2 | 0.000\* |
| P1 vs P2 | 0.045\* |

Table 2 shows that the lowest mean IL-6 levels were in the control group (K0), followed by the treatment group (P1) and the second treatment group (P2). The negative control group (K1) got the highest average IL-6 levels. The group was tested using the shapiro wilk test with normal distributed data results (p>0.05) and using the levene test for homogeneity showed homogeneous results (p=0.098), then data analysis using the One Way Anova parametric test. One Way Anova test results showed significant differences between groups (p=0.000). To find out which groups are different, post Hoc test is carried out with Tukey test as presented in table 4.

**4. Discussion:**

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. 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.9 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 & 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).

**5. Conclusion:**

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 grBB / 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 grBB / 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 grBB / day may increase blood IL-6 cholesterol levels in male wistar rats with hypercholesterol.

**6. Compliance with Ethical Standards:**

This work is approved by institutional ethical committee.

**7. Conflict of interest:**

There is no conflict of Interest in this study.

 **8. Acknowledgement:**

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