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Blood cholesterol levels of hypercholesterolemic rat (*Rattus norvegicus*) after VCO treatment

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Abstract. *Harini M, Astirin OP. 2009. Blood cholesterol levels of hypercholesterolemic rat (*Rattus norvegicus*) after VCO treatment. Nusantara Bioscience 1: 53-58.* This study aims to determine treatment effect of VCO on blood cholesterol levels in hypercholesterolemic white rat (*Rattus norvegicus* L.). This study used 25 male rats of Wistar strain divided into five treatment groups, namely: control, simvastatin (1.3 mL/270 g BW), cholesterol (9:1 lard), VCO 1 (1 mL/270 g BW), and VCO 2 (1.3 mL/270 g BW). Treatment was given orally. Total cholesterol, LDL and HDL cholesterol levels were measured at day 1, day 14 and day 28. Cholesterol data (total cholesterol, LDL and HDL) were analyzed by Ancova and followed by contrast test at significance level of 5%.. The results showed that treatment of VCO at different doses significantly affected the decrease in blood total cholesterol, blood LDL levels, increasing blood HDL in hipercholesterolemic white rat.

Key words: cholesterol, atherosclerosis, VCO.

Abstrak. *Harini M, Astirin OP. 2009. Kadar kolesterol darah tikus putih (*Rattus norvegicus*) hiperkolesterolemik setelah perlakuan* VCO. *Nusantara Bioscience 1: 53-58.* Penelitian ini bertujuan untuk mengetahui pengaruh perlakuan VCO terhadap kadar kolesterol darah tikus putih (*Rattus norvegicus* L.) hiperkolesterolemik. Penelitian ini menggunakan 25 tikus putih jantan galur Wistar yang dikelompokkan menjadi lima kelompok perlakuan, yaitu: kontrol, simvastatin (1,3 mL/270 g BB), kolesterol (lemak babi 9:1), VCO 1 (1 mL/270 g BB), dan VCO 2 (1,3 mL/270 g BB). Perlakuan diberikan secara oral. Kadar kolesterol total, kadar LDL dan kadar HDL diukur pada hari ke-1, ke-14 dan hari ke-28. Data kadar kolesterol (kolesterol total, LDL dan HDL) dianalisis dengan ANCOVA dan dilanjutkan dengan uji *contrast* pada taraf signifikansi 5%. Hasil penelitian menunjukkan bahwa perlakuan VCO pada berbagai dosis berpengaruh nyata terhadap penurunan kadar kolesterol total darah, kadar LDL darah dan peningkatan kadar HDL darah tikus putih (*R. norvegicus*) hiperkolesterolemik.

Kata kunci: kolesterol, aterosklerosis, VCO.

INTRODUCTION

Cardiovascular disease (CVD) is a degenerative disease that most often occur and becomes a major killer in industrialized countries. In Indonesia, the National Household Health Survey 1992 states, CVD became the first rank as the cause of death to people over 40 years of age. A major cardiovascular disease of the productive age is coronary heart disease (CHD), which is closely related to atherosclerosis (Kalim et al. 1996). Atherosclerosis is the hardening of the arteries caused by accumulation of cholesterol in the blood vessels due to the imbalance of influx - reflux of cholesterol (Prabowo et al. 1995). Hypercholesterolemia is a major risk factor for atherosclerosis that underlies the formation of CHD (Marinetti 1990; Wresdiyati 2006). The occurrence of CVD can be reduced by decreasing the formation of atherosclerosis by lowering cholesterol levels in the blood and increase the concentration of high density lipoprotein (High Density Lipoprotein/HDL) (Nogrady 1992).

Early signs of atherosclerosis is the occurrence of injury on the blood vessel wall, especially endothelial followed by the deployment of lymphocytes and monocytes, macrophage formation, lipid deposition, smooth muscle proliferation and extracellular matrix synthesis. Various efforts to reduce levels of cholesterol in the blood can be performed using chemical drugs containing compounds or lipid-lowering agents as well as traditional medicine. Therapies with traditional medicine are perceived to be cheaper and the procedure is easier than synthetic chemical drugs.

Virgin Coconut Oil (VCO) or pure coconut oil is coconut oil produced from fresh coconut milk without heating or the addition of any materials. This virgin coconut oil contains 100% fat consisting of 92% saturated fatty acids, 6% monounsaturated fatty acids, and 2% polyunsaturated fatty acids. Saturated fatty acids in VCO consist of 90% medium-chain fatty acids and 10% long-chain fatty acids. Medium-chain fatty acids in VCO are dominated by lauric acid (C12), namely 45-55%. In the body of saturated fatty acid, this medium chain is broken

and is being used to produce energy and is rarely stored as body fat or accumulates in the blood vessels. These fatty acids can be absorbed easily and quickly burned and used as energy for metabolism thus increasing metabolic activity, so it can help protecting the body from disease and accelerate healing (Enig 2001)

Empirically VCO is known to be beneficial to health. Among others, VCO consumed each day can boost immunity, prevent disease caused by infection of bacteria, fungi and viruses, help to overcome obesity, prevent heart disease, atherosclerosis, and overcome cholesterol, diabetes and cancer.

So far there has been no solid foundation and scientific evidence about the VCO potential as anti cholesterolemic and anti-atherosclerosis agent. Based on this fact, researchers want to know the effect of VCO on blood cholesterol levels of hypercholesterolemia white rats which is an early sign of atherosclerosis.

MATERIALS AND METHODS

Materials

In this study, animals used were 25 of male white rats (*Rattus norvegicus* L.), Wistar strain, at age of 2 months with body weight of 250-290 g and were obtained from LP3HP-LPPT Gadjah Mada University, Yogyakarta. Virgin Coconut Oil was from the Integrated Coconut Processing Center of Yogyakarta. Lard was from LP3HP-LPPT Yogyakarta. Simvastatin was a production of Kimia Farma. The food of test animal was pellets BR-II.

Procedures

Experimental animal. Before being used in the experiment, white rats were being adapted for 7 days in order to get used to the environment. Handling of experimental animals was in accordance with generally accepted protocol (Malole 1989).

Simvastatin dose determination. The dose used for human hyper-cholesterolemia is 10 mg/day. Doses of simvastatin were converted to Rattus norvegicus L based on Laurence and Bacharach conversion table cited by Haznam (1976), and it gave result 10 mg/day x 0.018 = 0.18 mg/day/200 g BW. Simvastatin suspension was obtained by dissolving 0.18 mg of simvastatin in the form of powder into 1 mL of distilled water. For rats weighing 270 g, 1.3 mL suspension of simvastatin was required

Determination of dosage and VCO delivery. VCO given to human therapy is 3 tablespoons or equal to 45 mL/day (Dayrit 2000). When converted to rat, it was 0.018 X 45 = 0.81 mL./200 g BW/day or equal to 1.09 mL/270 g BW/day. In this study, variations of dose was given, namely dose I = 1 mL/day/270 g BW and dose II = 1.3 mL/day/270 g BW.

Treatment of experimental animals. For measurement of blood cholesterol levels, this study used 25 male rats (*Rattus norvegicus* L.), which were grouped into 5 treatment groups, 5 mice for each, as follows:

- Group I: treatment of distilled water and pellets for 28 days (control)
- Group II: treatment of pellets and lard with ratio 9:1 for 14 days, then followed with Simvastatin until day 28th.
- Group III: treatment of a mixture of pellets and lard with ratio 9 : 1 for 14 days, continued with pellets and distilled water until day 28th.
- Group IV: treated with mixture of pellets and lard with ratio 9 : 1 for 14 days, followed by VCO treatment with dose of 1 mL until day 28th.
- Group V: treated with mixture of pellets and lard with ratio 9 : 1 for 14 days, followed by VCO treatment with dose of 1.3 mL up to 28 days

The provision of food and water was in ad libitum way, and VCO and Simvastatin treatment were given orally using a cannula needle. At the beginning of treatment and at the end of their treatment, weight loss of mice was weighed.

Determination of blood cholesterol levels. Blood cholesterol levels are determined by *Enzymatic Endpoint Method* with a spectrophotometer (Kayamori et al. 1999). Blood was taken from the orbital sinus by microhematokrit, and then placed in a container, after that it was dripped with heparin as anti-coagulant on day 1st (after acclimation), on day 14th, and on day 28th. The measurement of blood cholesterol contents were to all cholesterol contents, namely HDL and LDL. The contents of HDL and LDL were measured with precipitation and enzymatic method.

Data analysis

Quantitative data (total cholesterol content, LDL cholesterol and HDL cholesterol) were analyzed by T dependent test to determine the effect of 10% of lard. While, data for treatment effect was analyzed by Ancova (Analysis co Variance), and when there were real differences, it continued by a contrast test at significance level of 5% to find out the differences among the treatments.

RESULTS AND DISCUSSION

This study used test animals of Wistar strain male white rat aged 2-3 months. Rats were used because it had similarities with humans in terms of physiology, anatomy, nutrition, pathology, and metabolism and were commonly used in research on cholesterol levels. Male rats are used because they are less affected by hormonal changes (Sitepoe 1992). According to Ganong (2002), estrogen effects blood cholesterol. In male rats, blood lipids are not affected with this estrogen because these animals have less estrogen.

This research went through several stages namely acclimation of animals to adapt to the conditions of surrounding environment, providing high-cholesterol diet and its treatment with VCO. During acclimation, all rats were given pellets food and drinking water ad libitum for 7 days. High-cholesterol diet by mixing the pellets food with lard at a ratio of 90:10 was given for 14 days. In VCO treatment, mice were given VCO for 14 consecutive days and use Simvastatin patented drug as the positive control.

Parameters observed in this study were the content of total blood cholesterol, LDL cholesterol, and HDL cholesterol.

From the statistical tests, giving lard (ratio 9:1) in the food caused a significant increase (P <0.05) on total blood cholesterol level for as high as 10.7%, LDL levels increased by 55.52% and no significant decrease in levels of HDL cholesterol by 2.17%. This situation occurred due to increased accumulation of fat in the liver which resulted in an increasing number of acetil co-A in liver cells to produce cholesterol (Guyton 1991). Lard contains high saturated fatty acid. Triglicerida levels of saturated fat resulted in increased blood cholesterol and were a precursor. Consuming saturated fats may cause an increase of total cholesterol and a decrease of HDL thereby increasing the ratio of total cholesterol and HDL, so the risk of atherosclerosis is greater (Baraas 1993). Eating too much fat may cause hyperlipidemia with the increase of apo B cholesterol and LDL. The increase in apoB cholesterol is associated with the decrease in LDL receptor function (Verde et al. 1999).

Blood total cholesterol level

Cholesterol is present in all tissues and lipoproteins plasma. It exists in the form of free cholesterol or a combination of long chain fatty acids as esters kolestril. This element is synthesized from Acetil-co A and eventually expelled from the body through the bile as cholesterol salt. Free cholesterol is expelled from tissues by HDL and transported to the liver to be converted into bile acids (Murray et al. 1999). The state of Hypercholesterolemia is characterized by increased blood cholesterol levels above normal. In *R. norvegicus* Wistar strain rats, normal blood cholesterol level is 10-54 mg/dl (Smith and Mangkoewidjojo 1998).

Results of analysis of blood cholesterol levels are presented in Table 1. Statistical tests, treatment with VCO and Simvastatin can reduce total blood cholesterol levels significantly. Giving VCO 1 mL per day for 14 days can reduce total cholesterol by 19.1% and 1.3 ml of VCO per day for 14 consecutive days can lower total cholesterol 27.83%. Giving Simvastatin can reduce total cholesterol by 28.8%. The most effective dose VCO for lowering total cholesterol levels in this study was 1.3 mL, not so significantly different to the giving of Simvastatin.

In group I (control), rats were not fed with lard from the beginning to the end of treatment, at day 14th, they had decrease in levels of total cholesterol and at the end of treatment an increase of 4.6% occurred. Meanwhile in group III (cholesterol) that were given feed with lard up to day 14th and without being given lard until day 28th, a decline in total cholesterol level with 6.7% which was significantly different from those treated by VCO.

Table 1. The mean of total blood cholesterol levels of white rats (*R. norvegicus*) (mg/dl) on day 1st, 14th and to 28th and the percentage of increase and decrease.

Treatment	1st Day	14th Day	Increase (%)	28th Day	Decrease (%)
Control	52.92±3.08	50.42±4.32	-4.7	52.74±4.17^{a}	-4.6
Simvastatin	52.84±1.65	60.44±2.56	14.38	42.98±4.78^{b}	28.8
Cholesterol	53±5.4	59.94±2.96	13.09	55.9±5.6^{a}	6.74
VCO 1 mL	51.7±0.96	58.84±2.9	13.8	47.6±2.8^{b}	19.1
VCO1,3 mL	52.81±4.55	61.8±6	17.4	44.6±2.76^{b}	27.83

Note: different letters in the same column indicate significant differences (P <0.05)

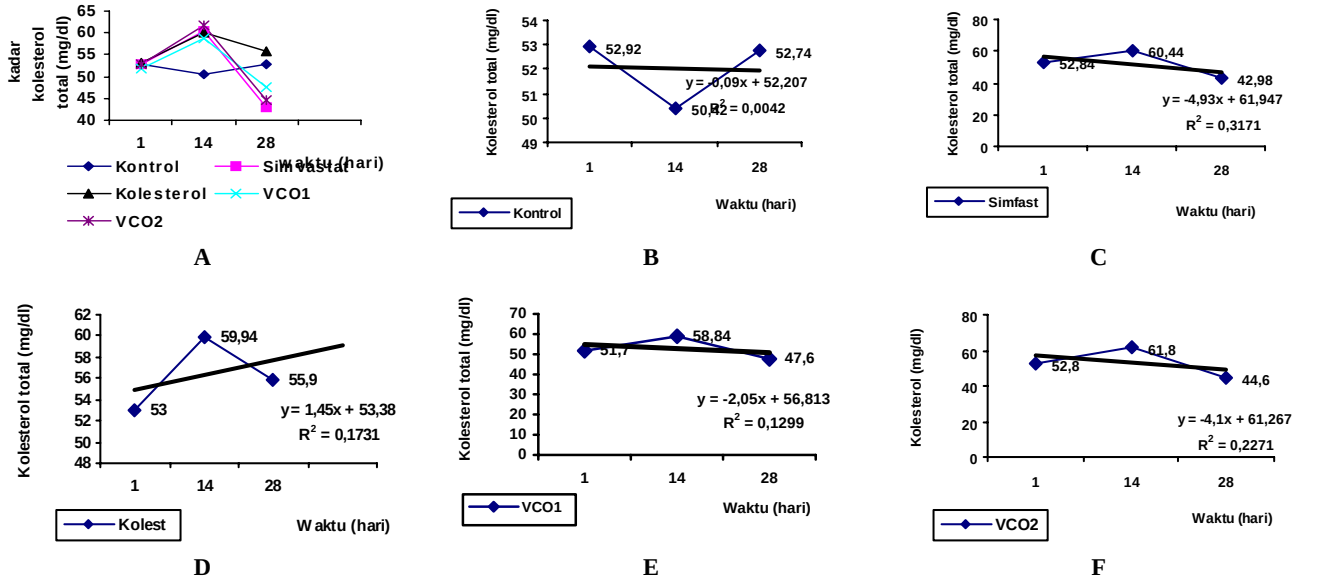

Figure 2. Total blood cholesterol levels of *R. norvegicus*. Note: The level of blood cholesterol (mg/dl) vs time (days): a. The control group, simvastatin, cholesterol, VCO 1 mL, 1.3 mL VCO. b. Control, c. Simvastatin, d. Cholesterol, e. VCO 1 mL, f. VCO 1.3 mL.

According to Shah (2005), the VCO has a saturated fatty acid content which is dominated by medium-chain fatty acids. Chain fatty acids are dominated by lauric acid. Because of the small molecular size, medium-chain fatty acids are easily absorbed through the intestine without enzymatic process. These fatty acids are carried to the liver blood flow to be metabolized and transported to the mitochondria without carnitine to produce energy quickly and efficiently so they are not deposited as fat in the tissue. The results are consistent with research conducted by Nevin (2004) on Sprague-Dawley rats animals fed diets VCO which showed that total cholesterol blood and LDL are decreased and HDL levels are increased. The increase and decrease in total cholesterol levels in blood during the experiment are shown in Figure 2.

LDL blood

Low Density Lipoprotein is a lipoprotein that transports lipids from the liver to the peripheral (extra-hepatic) and is often called "bad" cholesterol. According to Murray et al. (1996), LDL contains a half to two thirds of cholesterol. High levels of LDL are at risk of atherosclerosis.

Results of analysis of blood LDL levels were presented in Table 2. Treatment with a VCO was capable of lowering blood LDL levels significantly. Giving VCO 1 mL per day for 14 consecutive days reduced LDL levels by 12.2% and 1.3 ml of VCO per day for 14 days reduced LDL levels by 28%. Giving VCO 1.3 mL was not significantly different from the provision of Simvastatin, which is 28%.

Giving statins including simvastatin reduces blood LDL levels, inhibits HMG Co A reductase that would inhibit HMG Co A becoming into mevalonat so it inhibits cholesterol synthesis and cause a decrease in the concentration of cholesterol in the liver cells and increase the LDL receptor (E, Apo-B-100).

In this study, giving Simvastatin causes LDL levels decreased from 26.5 at day 14th to 19.08 on day 28th. In group I (Control) which are not fed with lard, until the end of treatment there was a slight decrease at 1.7%. Group III during the first 14 days fed with lard and 14 days later no lard fed, there was an increase in blood LDL levels by 6%, this was possible because the addition of hepatic cholesterol also comes from foods that contain cholesterol, so cholesterol levels in the body will remain high because the body also produces cholesterol. In this study, the giving of VCO lowered blood LDL levels and total cholesterol of blood level. This occurs because 65% of them in the form of LDL cholesterol, so if total cholesterols are decreased the levels of LDL are also decreased. The increase and decrease in LDL levels in blood during the experiment can be shown in Figure 3.

Table 2. The mean levels of LDL blood of white rat (*Rattus norvegicus* L) (mg/dl) on day 1st, to 14th and to 28th and the percentage of increase and decrease.

Treatment	1st Day	14th Day	Increase (%)	28th Day	Decrease (%)
Control	19.1±4.1	25.78±5.12	34.97	25.34±5.44^{a}	1.7
Simvastatin	17.84±3.16	26.5±3.53	48.54	19.08±4.02^{b}	28
Cholesterol	15.3±1.53	32.2±6.83.	110.45	34.14±7.11^{a}	6.02
VCO 1 mL	17.88±4.07	22.58±1.99	26.28	19.82±2.71^{b}	12.22
VCO1,3 mL	15.96±4.66	26.82±4.88	68.04	19.3±1.8^{b}	28

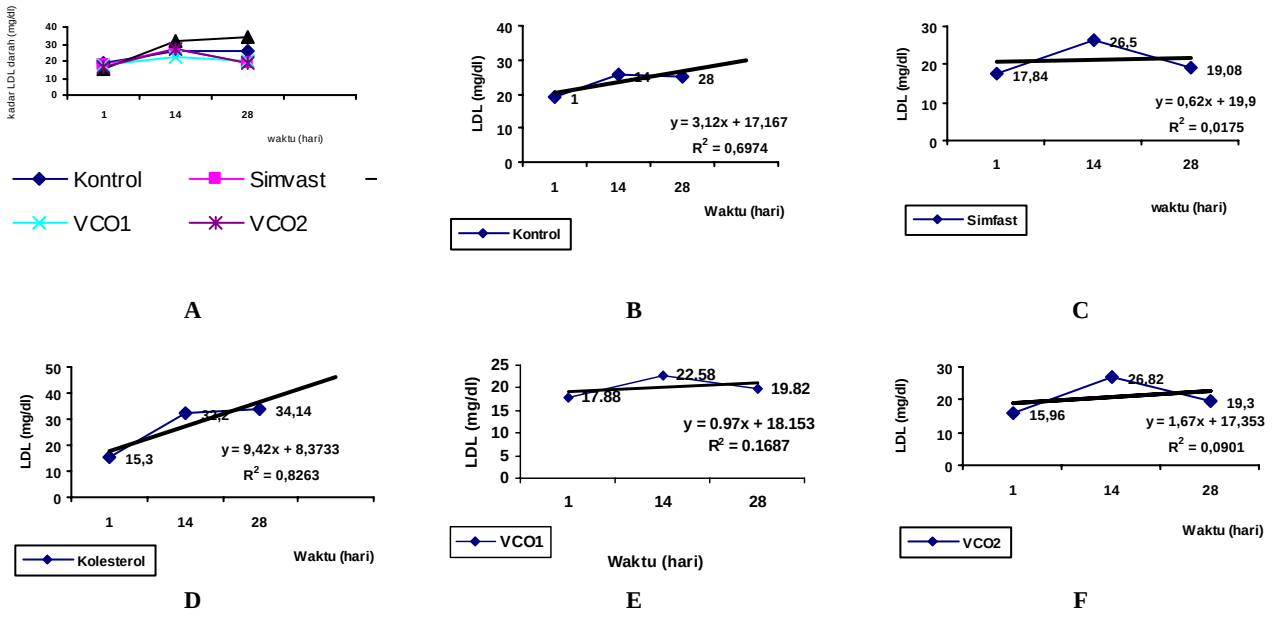

Figure 3. Blood levels of LDL *R. norvegicus*. Note: The level of blood LDL (mg/dl) vs time (days): a. The control group, simvastatin, cholesterol, VCO 1 mL, VCO 1.3 mL. b. Control, C. Simvastatin, d. Cholesterol, e. VCO 1 mL, f. VCO 1.3 mL.

HDL blood

High Density Lipoprotein (HDL) cholesterol is often called "*good*" because it is a lipoprotein that transports lipids from the periphery to the liver. Because the molecules are relatively small compared to other lipoproteins, HDL can pass through the vascular endothelial cells and into the intima to bring back the accumulated cholesterol in macrophages, besides, the HDL also has antioxidant properties that can prevent the oxidation of LDL. The low levels of HDL in the blood will increase the risk of atherosclerosis and coronary heart disease (Moeliandari and Wijaya 2002).

The results of Blood HDL analysis are presented in Table 3. From the statistical tests, the treatment with lard feeding for 14 days in different groups decreased HDL levels which were not significantly different. While at the end of treatment, increased HDL levels were significantly different. In group I (control), an increase levels of HDL cholesterol by 3.58% occured at the end of treatment. In group II (Simvastatin), an increase in HDL levels by 16%. This group had HDL levels that were not significantly different with the group given with 1.3 mL VCO. Group III (cholesterol) that are not fed with lard after day 14th, until the end of treatment on day 28th there was an increase in HDL cholesterol by 8% only. Groups of mice that have the highest HDL levels are group which was fed with 1 mL of VCO by 29.68 mg/dl, and they are not significantly different with the group given by 1.3 mL VCO for 29.6 mg/dl. Giving VCO with two kinds of doses can raise levels of HDL better than giving Simvastatin. The decrease and increase of HDL levels in the blood are shown in Figure 4.

Virgin coconut oil is coconut oil composed by medium-chain fatty acids (C12), which are dominated by lauric acid. Medium-chain fatty acids (MCT) are more polar (faster in releasing H ions) than long-chain fatty acids (LCT). The nature of MCT solubility in water which is higher than LCT makes easier its entry into the liver directly via the portal vein after absorbed by the intestine without pancreatic lipase and rapidly metabolized into energy. Medium-chain fatty acids are not included in the cycle of cholesterol and do not accumulate as fat in body tissues. (Dayrit 2003). According to Enig (2001), lauric acid contained in the VCO is able to burn fat from other sources, and quickly made the energy and increase metabolism. In this study, the effect of VCO with its lauric acid content causes an increase in HDL levels. The function of HDL is to transport cholesterol from peripheral tissues to the liver, removing excess cholesterol and inhibits the development of atheroma plaque, so the increase in HDL levels in the blood will prevent the risk of atherosclerosis.

Table 3. The mean levels of blood HDL (mg/dl) of white rat (*R. norvegicus*) on day 1, to 14 and to 28 and percentage changes.

Treatment	1st Day	14th Day	Increase (%)	28th Day	Decrease (%)
Control	21.38±1.94	23.98±4.74	-0.12	24.84±2.8^{b}	3.58
Simvastatin	25.26±	23.44±2.58	7.2	27.18±3.68^{a}	16
Cholesterol	21.32±1.95	19.94±3.19	6.47	21.58±2.28^{b}	15.95
VCO 1 mL	23.98±4.74	22.26±2.19	7.17	29.68±4.75^{a}	33.3
VCO1,3 mL	23.2±2.93	22.68±	2.24	27.6±3.43^{a}	21.69

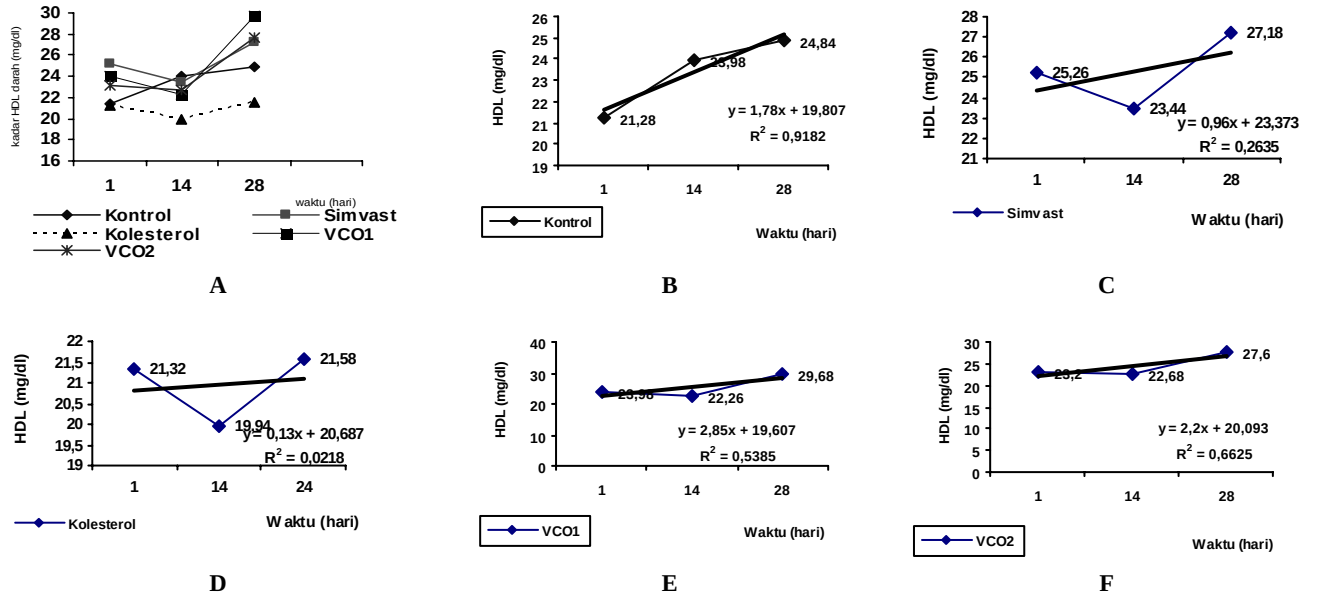

Figure 4. Blood levels of HDL *R. norvegicus*. Note: The level of blood LDL (mg/dl) vs time (days): a. The control group, simvastatin, cholesterol, VCO 1 mL, 1.3 mL VCO. b. Control, c. Simvastatin, d. Cholesterol, e. VCO 1 mL, f. VCO 1.3 mL.

Virgin Coconut Oil (VCO) is coconut oil which contains 92% saturated fatty acids that are dominated by medium-chain fatty acids (MCT), which is 44% -55% are lauric acid. MCT metabolism is different from long chain fatty acids (LCT), MCT can be absorbed rapidly in the intestinum without the need for pancreatic lipase, and carried by the portal vein into the liver and rapidly oxidized into energy. This energy is used to increase metabolism, which can help protect the body from disease and accelerate healing.

According to Carandang (2005), VCO also contains an active ingredient, although in small amounts that can prevent and provide protection against disease and is beneficial to health. The active ingredient is known as tocopherol, an antioxidant that has a phyti saturated side chain, and tocotrienols, a better antioxidant than tocopherol with unsaturated isoprenoid side chain with three double bonds. Both of these active ingredients have a hypocholesterolemic effect, anti-atherogenic and anti cancer. Its anti-atherogenic activity is by inhibiting the oxidation of LDL, suppresses the activity of HMG-Co A reductase and inhibits platelet aggregation (Theriault et al. 1999 in Carandang 2005). This is similar to the mechanism of simvastatin in lowering cholesterol levels and reduces levels of LDL. Other active ingredients in the VCO are flafonoid and polyphenols. Flavonoids as antioxidants have good effects on endothelial function namely reducing the oxidation of LDL and increasing the production of Nitric Oxide (NO) (Vita 2005). The oxidation of LDL would induce inflammatory responses by producing leukocyte and cytokine on endothelial. Flavonoids reduce LDL oxidation and prevent inflammation in indotel. Nitric Oxide is an endogenous vasodilator that has the ability of anti-atherosclerosis. Polyphenols will prevent the oxidation of LDL. Oxidation of LDL would generate Reactive Oxygen Species (ROS) that are toxic, and if it binds with NO, it forms peroxynitrite oxidant. Oxidation of cholesterol is to spur the process of atherosclerosis.

In this study, measurement of total cholesterol and LDL levels in both treatment doses showed a decrease, but not significantly different from simvastatin treatment. Decrease in cholesterol levels will reduce the occurrence of atherosclerosis.

CONCLUSION

Giving VCO (Virgin Coconut Oil) on hypercholesterolemic white rats (*Rattus norvegicus* L.) leads to lower cholesterol levels (total cholesterol, LDL) and HDL levels at a significance level of 5%, not so significantly different from the provision of patented drug Simvastatin as cholesterol-lowering drugs.

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