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Research Article

# THE EFFECT OF VIRGIN COCONUT OIL (VCO) AS AN ANTIDIABETIC AND ON LIPID PROFILE IN ALLOXAN –INDUCED WHITE MALE MICE (*MUS MUSCULUS*)

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**ABSTRACT**

*Virgin Coconut Oil* (VCO) is a supplement with contain short and medium chain fatty acids, mostly high Lauric acids. which quickly absorbed by the body and converted to energy. The purpose of this study was to determine the effect of giving VCO toward blood glucose metabolism and to observe the profile lipids in white male mice (*Mus musculus*) after inducing alloxan. This study used an experimental method using 5 groups and 40 white male mice. Those were negative control group, positive control group, diabetes group with glibenclamide, diabetes group with 2% VCO dose and diabetes group with 4% VCO dose. The data was analyzed using Kruskal-Wallis. The results obtained showed that VCO has an effect on glucose metabolism, lipid in diabetic mice which injected 4% dose of VCO was able to reduce the blood glucose level by 151.75 mg/dl or reduce it by 55%. VCO dose of 2% can reduce total cholesterol, triglycerides, LDL with a total cholesterol value of 145.83 mg/dl, triglyceride 143.50 mg/dl, LDL 55.88 mg/dl and increase HDL with value of 61.16 mg/dl.

**Keywords**: VCO, Antidiabetic, Lipid Profile, Glibenclamide, *Mus musculus*

## INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disease characterized by an increase in blood glucose levels accompanied by disturbances in carbohydrate, lipid, and protein metabolism as a result of failure of insulin secretion, insulin activity or both[1]. Deficiency of insulin secretion can result in the inability of blood glucose to enter the cell, so cells do decomposition (gluconeogenesis) from other energy sources such as fat, protein, and glycogen. The consistency of high blood glucose levels can cause serious diseases that affect the heart, blood vessels, eyes, kidneys, and nerves.

Based on Basic Health Research (Riskesdas) there is an increase in the prevalence of diabetes mellitus in Indonesia. In 2013 the prevalence of diabetes was 2.1% while in 2007 it was 1.1%. West Papua and West Nusa Tenggara are provinces that have a tendency of decrease in diabetes mellitus prevalence, while 31 other provinces in Indonesia showed an increase in the diabetes mellitus prevalence. In West Sumatra the prevalence of diabetes mellitus was 1.3% increase almost every month in diabetic clinic with the most prevalence obtained data showed that more than half (65%) patients who visited the polyclinic of internal medicine were patients with diabetes mellitus. The number of patients in a month ranged from 300 to 900 peoples while all patients 95% of them were regular patients while 5% are new patients. These data also showed that most diabetes mellitus patients who visit the polyclinic were patients who have been repeatedly treated[2]. Diabetes mellitus is considered serious and must be treated by using drugs, together can be combine with natural herbal or supplement.

One of the most widely used oral antidiabetic drugs is glibenclamide. Glibenclamide is an oral sulfonylurea group which potent as a hypoglycemic agent that works to reduce blood glucose levels by stimulating beta pancreas Langerhans cells to produce insulin. But this glibenclamide is associated with many side effects such as hypoglycemia, cholestasis jaundice, agranulocytosis, aplastic anemia, hemolytic anemia, blood dyscrasias, liver dysfunction and allergic skin reactions while fatal side effects such as prolonged hypoglycemia are seen in elderly patients or patients with weak hearts or kidney disease[3]. Recently, many herbal medicine and therapy have been recommended for diabetes treatment. Herbal medicines are widely prescribed because of their effectiveness, lack of side effects and relatively low costs[4,5,6]. One of this can be found in coconut oil (VCO).

*Virgin Coconut Oil* (VCO) is made from local coconut, were grown about 50 m from the beach of Padang Pariaman, which has a high in oil. VCO can be produced in several ways such as heating, fermentation, enzymatic, and settling[7]. VCO contains 60-62% medium chain fatty acids (MCFA) such as caproic acid (0.7%), caprylic acid (4,6 - 10%), capric acid (5,0 - 8,0 %), lauric acid (45.1-53.2%) and myristic acid (16.8 - 21%). Medium chain fatty acids are very easily absorbed by the body because they only

need a little energy and enzymes to facilitate digestion, in contrast to long chain fatty acids. These short and medium chain fatty acids in the body will be metabolized and taken directly to the liver they will be quickly converted to energy. Several studies have stated that MCFA can prevent and eliminate many diseases as a result of its anti-microbial properties, it also has the ability to increase the immune system and help the absorption of magnesium, calcium and amino acids by the body[8]. Lauric acid from VCO in the body will be converted to monoglyceride called monolaurin where this compound is very strong against various kinds of viruses, bacteria and protozoa[9,10,11]. VCO also contain Probiotic LAB as strong antimicrobial[12,13].

In this study, VCO was made by extracting coconut milk and fermentation with probiotic *Lactobacillus plantarum* for 15 hours then filtered with cotton wrapped in Whatman filter paper and vacuumed[14]. VCO can be used to reduce cholesterol levels in the blood due to high levels of lauric acid and antioxidant activity, it also able to accelerate wound healing accompanied by an increase in the number of fibroblast cells appearing in wounds and fewer complaints of pain[15]. VCO can also be used to reduce blood glucose levels and increase glucose tolerance due to antioxidant effects which result in increased insulin secretion[16]. The aim of this study was to see the effect of VCO on blood glucose metabolism and observe the profile lipids in white male mice induced by alloxan. The use of alloxan in this study was to induce diabetes in animals with hyperglycemic models. The giving of alloxan is a quick way to produce experimental diabetic conditions (hyperglycemic) in experimental animals. Giving of alloxan can have a very significant effect on blood glucose levels of hyperglycemic mice and can increase blood glucose levels in white mice and is suitable for use in animals with hyperglycemic models[17].

## MATERIALS AND METHOD

This research has been carried out ethical feasibility test by the Research Ethics commission of the Faculty of Medicine of Andalas University with an ethical certificate No.170/KEP/FK/ 2019.

### Animal Experiment Mice (*Mus musculus*)

The animals used were 40 white male mice of DDY Japan strain with a body weight of 20-30 grams, aged 2-3 months, healthy and had normal activities obtained from the Andalas University Pharmacy Faculty Laboratory. Before the treatment, all experimental animals were acclimatized for 1 week. Weight weighing was carried out during acclimatization and during treatment. 40 of these mice were kept in 5 cages made of plastic tubs measuring 30 cm long, 20 cm wide, 15 cm high and covered with wire at the top and bottom of the cage coated with rice husks as thick as 0.5-1 cm and replaced every day to prevent infections that occur due to dirt. Each cage contains 8 mice. Experimental animals are kept in the Andalas University Pharmacy Laboratory. The conditions during acclimatization and treatment are controlled at a fixed range of environments with the aim that the test animals adapt to the conditions that will be placed during the experiment. During the experiment the room temperature ranged from 23$^{o}$C-27$^{o}$C. The food given is in the form of DT2 as much as 10 grams / mice and 10 cc drink in the form of boiled water by *ad libitum*. Lighting is carried out for 12 hours of dark / light cycles[18].

### Experimental Design

The experimental design carried out has been modified by giving VCO concentration[16]. Furthermore, the mice used were 40 consisting of 5 groups, which were:

a) Group I : negative control, given standard food and drinks during the study
b) Group II : positive control, given standard food and drinks, alloxan
c) Group III : experimental, given standard food and drinks, alloxan and glibenclamide
d) Group IV : experimental, given standard food and drinks, alloxan and VCO 2%
e) Group V : experimental, given standard food and drinks, alloxan and VCO 4%

For group 3 glibenclamide was given for 4 weeks every day orally and for groups 4 and 5 VCO was given for 4 weeks every day orally.

### Induction of Diabetes

After the acclimatization period, alloxan monohydrate was injected intraperitoneally at a dose of 175 mg / kg body weight which was used to induce diabetes in groups 2, 3, 4 and 5. Mice were fasted from food for 8-12 hours (only water was provided). Blood glucose levels of mice were observed on day 5 and mice with blood glucose levels > 200 mg/dl were considered diabetes which would be used for research [16].

### Blood Glucose Measurement

Blood glucose level measurements were carried out at the beginning of the experiment and after 4 weeks of treatment. Mice are fasted for 12 hours before measuring their blood glucose level. Measuring blood glucose levels is measured using the one touch ultra easy glucometer. Blood is obtained from the blood vessels of the mice tail, then blood is dropped on the test strip which has been included in one touch of the ultra easy glucometer. The blood glucose level of the mice is displayed for about 5 seconds[16].

### Measurement of Total Cholesterol, Triglycerides, HDL and LDL[15]

At the end of 4 weeks administration of glibenclamide and VCO, measurements of total cholesterol and triglycerides were carried out in the blood plasma, blood was taken by cutting blood vessels in the neck, blood was collected in a test tube, and then left for 15 minutes. The blood was then centrifuged for 10 minutes at 3000 rpm, so that plasma was separated from hemoglobin and platelets. For measurement of total cholesterol and triglycerides, each serum was piped for 10 µl. Then it was put in a test tube and added with 1000 µl of cholesterol and triglyceride reagent solution. Next, the solution was mixed using vortex and left for 20 minutes at room temperature and absorbance measured at 500 nm compared to blank.

The standard measurement of absorption was done in the same way for measuring absorption of total cholesterol. For the determination of HDL cholesterol levels, as much as 0.02 ml of the total squeezed serum was put into a centrifugation tube, then a 0.5 ml precipitator solution was added. Then it was mixed with vortex, left for 10 minutes at room temperature and centrifuged for 10 minutes at 4500 rpm. After that, 0.1 ml of the supernatant was piped and put in a test tube. Then a cholesterol reagent solution was added, the solution was mixed using vortex and left for 10 minutes at room temperature and the absorbance was

measured at 500 nm. The LDL level was determined by the formula:

$$LDL = \text{Total Cholesterol} - \frac{\text{Trigliserida}}{5} - \text{HDL}$$

**Data Analysis**

The data obtained was processed statistically using SPSS. The analysis used was one way ANOVA test by paying attention to the homogeneity and normality of the data. If it fulfilling the requirements then it was continued with one way ANOVA test, if it does not meet the requirements, an alternative test was used with the Kruskal-Wallis test.

**RESULT AND DISCUSSION**

**Provision of Alloxan Induction on Blood Glucose Levels**

In this study the mice were conditioned to suffer from diabetes mellitus by induction of alloxan with a single dose of 175 mg / kg BW intraperitoneally.

Alloxan injections in experimental animals are thought to cause diabetes mellitus, this is evident from an increase in blood glucose levels above the normal limit of >200 mg/dl. After the alloxan induction, the mice was allegedly to experiencing type 1 diabetes mellitus. Induction of intraperitoneal alloxan with a dose of more than 40 mg/kg was able to make mice suffering from diabetes mellitus type 1[19]. Type 1 diabetes mellitus is characterized by pancreatic beta cell damage and increased blood glucose levels exceed the normal limit[20].

Alloxan is a hydrophilic and unstable substance. Its half-life at neutral pH and 37 ° C is about 1.5 minutes and longer at lower temperatures[21]. On the other hand, when a diabetogenic dose is used, the time of decomposition of alloxan is sufficient to allow it to reach the pancreas in a destructive amount.

**VCO Treated to Mice Blood Glucose Levels**

From the research, it was shown that all tested VCO doses can reduce blood glucose levels. The effect of VCO on reducing blood glucose levels in mice can be seen in Figure 1.

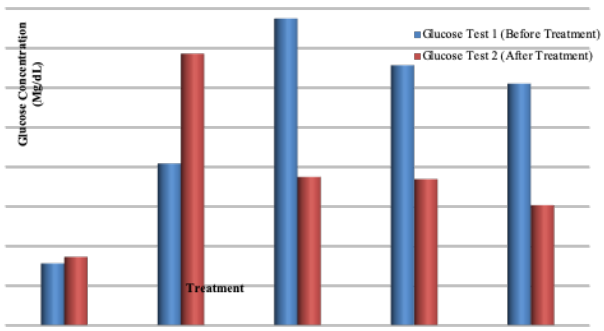


**Figure 1. Glucose Test Results, A = Control (-); B = Control (+); C = Alloxan and Glibenclamide; D = Alloxan and VCO 2% ; E= Alloxan and VCO 4%**

After treated by VCO to diabetic mice shows a decrease glucose level. This results can be seen after treated VCO 2% and 4%. At 2% decrease in blood glucose levels was 184,62 mg/dl or 46% while 4% dose caused the decrease of blood glucose levels by 151.75 mg/dl or decrease more than 55%. When compared with the control and treated by glibenclamide drugs, the treated of VCO can reduce blood glucose levels. These data showed that 4% VCO caused the optimum results in reducing blood glucose levels in mice.

VCO can reduce blood glucose levels in mice by giving 7.5 ml/kg dose and 10 ml/kg[16]. This is because VCO contains 48-50% lauric acid, which is a medium chain fatty acid with C-12 atoms that has the ability to increase metabolic rate and absorption of nutrients in the body. VCO also contains antioxidants that can increase metabolism and monolaurin compounds from lauric acid which can damage the fatty sheath of the virus so that it can increase the endurance of test animals against attacks caused by viruses[22]. VCO contain probiotic life microorganism and benefit total metabolism[14]. VCO contains fat which is composed mostly by medium chain fatty acids (MCFA) that are not harmful to the body, these fatty acids are also useful to increase the body's metabolism and increase energy[23].

**VCO Treated to Total Cholesterol Levels, Triglycerides, HDL and LDL**

The results of total cholesterol examination in diabetic mice after treated by *Virgin Coconut Oil* (VCO) are shown in Figure 2.

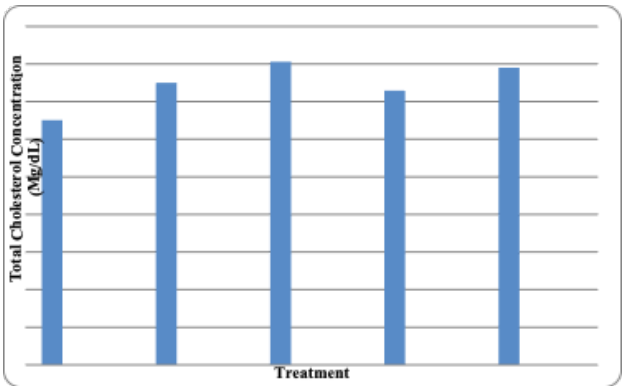


**Figure 2. Total Cholesterol Determination Results, A = Control (-); B = Control (+); C = Alloxan and Glibenclamide; D = Alloxan and VCO 2%; E= Alloxan and VCO 4%**

The results of triglycerides examination in diabetic mice after treated by *Virgin Coconut Oil* (VCO) are shown in Figure 3.

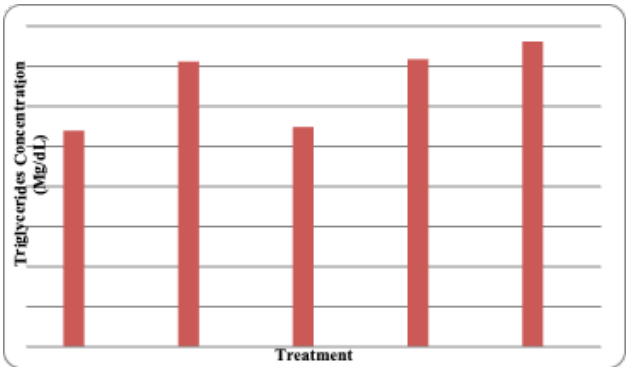


**Figure 3. Triglycerides Determination Results, A = Control (-); B = Control (+); C = Alloxan and Glibenclamide; D = Alloxan and VCO 2%; E= Alloxan and VCO 4%**

The results of high density lipoprotein (HDL) examination in diabetic mice after treated by *Virgin Coconut Oil* (VCO) are shown in Figure 4.

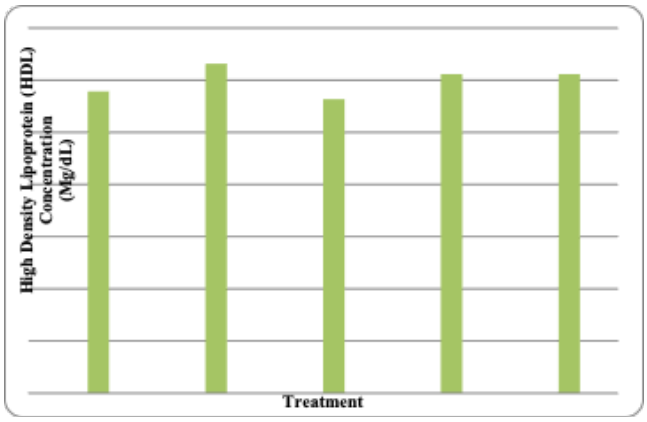


**Figure 4. High Density Lipoprotein (HDL) Determination Results, A = Control (-); B = Control (+); C = Alloxan and Glibenclamide; D = Alloxan and VCO 2%; E= Alloxan and VCO 4%**

The results of low density lipoprotein examination in diabetic mice after treated by *Virgin Coconut Oil* (VCO) are shown in Figure 5.

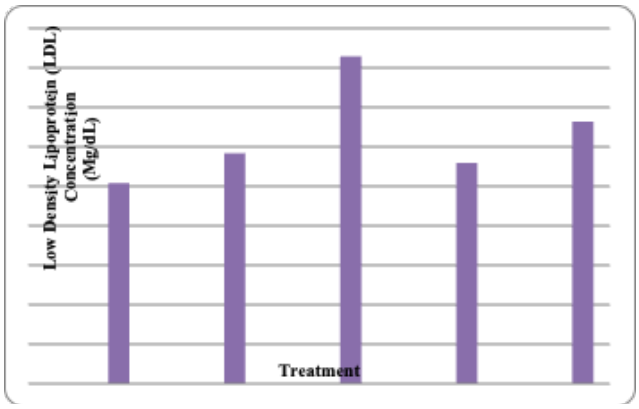


**Figure 5. Low Density Lipoprotein (LDL) Determination Results, A = Control (-); B = Control (+); C = Alloxan and Glibenclamide; D = Alloxan and VCO 2%; E= Alloxan and VCO 4%**

Figure 2, 3, 4 dan 5. The results of lipid profile after administration of VCO in diabetic mice showed that can reduce lipid profile when compared to the control and treated of glibenclamide drugs. After being treated by VCO at 2% showed the optimum of reducing the lipid profile when compared to the administration of VCO at 4%. Giving VCO at 2% can reduce total cholesterol, triglycerides, LDL with total cholesterol of 145.83 mg/dl, triglyceride 143.50 mg/dl, LDL 55.88 mg/dl and increase HDL to 61.16 mg/dl.

VCO can reduce total cholesterol level, triglycerides, LDL, and increase HDL in mice by giving 2% and 4% VCO dose[15]. VCO can reduce cholesterol, phospholipids, triglycerides, LDL cholesterol in serum and rat tissue. Besides that, it can also increase HDL cholesterol levels[25]. VCO for two weeks can increase HDL levels[25]. Wistar rats after atherogenesis was induced, where there was an increase in serum HDL cholesterol in the treatment group by giving VCO for 28 days. Surfa concluded that VCO had an effect on increasing serum cholesterol levels[26].

Furthermore, the data for decreasing blood glucose and profilipid levels in 5 treatment groups were analyzed by the SPSS version 15, using the Shapiro-Wilk test to determine the normality of the data. The Shapiro-Wilk test was used in small-scale group data, which is less than 50 data samples, in this study th data was 30. From the results of the Shapiro-Wilk test all treatments showed a significance value of 0.000, p <0.05, which means blood glucose levels were abnormally distributed.The second statistic test was test of homogeneity of variance. This test uses the level of variance test data and showed that the significance was 0.158 which > 0.05, this means that blood glucose levels and profilipids are homogeneous in mice. Due to the result of the abnormal distribution of blood glucose levels and profilipids, it can be continued with a non-parametric test, which is Kruskal-wallis test to find out whether there is a significant difference in blood glucose and profilipid for each dose difference of 2% and 4%. Results of the Kruskal-wallis test showed that from all treatments a significance value of 0.005 was obtained (p <0.05). Each dose of 2% and 4% had an influence on blood glucose levels and profilipids.

## CONCLUSION

VCO has an good effect on glucose and lipids concentration levels in diabetic mice. Treated VCO at 4% dose was able to reduce blood glucose levels in mice by 151.75 mg/dl or 55%. VCO at 2% dose able to reduce total cholesterol to 145.83 mg/dl, Triglyceride 143.50 mg/dl, LDL 55.88 mg/dl and can increase HDL to 61.16 mg/dl.

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