The Effect of VCO Processing Method on Blood Glucose, Cholesterol and Pancreatic Profile of Diabetic Mellitus Rats (Sprague Dawley)

Pengaruh Metode Pengolahan VCO terhadap Glukosa Darah, Kolesterol dan Profil Pankreas Tikus Diabetes Melitus (Sprague Dawley)

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ABSTRACT: Virgin Coconut Oil (VCO) can be produced by different methods related to health function. The objective was to study the efficacy of VCO processing method in reducing blood glucose level, cholesterol profile and pancreatic tissue profile of diabetic mellitus (DM) Sprague Dawley rats. Four rat groups from five rat groups were injected using alloxan to induce the diabetic condition, while the rest was negative control. VCO-A produced without heat and VCO-B produced with controlled-heat treatment and Coconut Cooking Oil (RBD-CNO) produced with the application of severe heating and chemical (MG) were fed respectively to three rat groups, while the other two rat groups were fed by water and used as negative control (healthy rats) and positive control (DM rats). During 28 days observation, all of the rats blood glucose level were assessed, and at the end of the observation day the rats were terminated to assess the cholesterol and pancreatic tissues profile. VCO-A significantly positive reduced blood glucose level than the others. VCO did not influence DM rats cholesterol profile and all VCO inhibits the rate of pancreatic β cells damage. On 28 days observation all VCO treatments suppressed blood glucose level of DM rats.

Keywords: processing method of virgin coconut oil, diabetes mellitus, blood glucose, pancreatic tissues
1. Introduction

Virgin Coconut Oil (VCO) is one of value added products produced from coconut meat, which has higher economic value than coconut cooking oil. In the domestic and international market the price of VCO has a sale value of 10 to 20 times than the price of coconut cooking oil. This attractive price is due to the health benefits of VCO for human, such as for body health care, cosmetics and spa industries.

Rindengan and Novianto (2005), stated that there are various methods of manufacturing VCO i.e. the changing of the emulsion form of coconut milk, fermentation, enzymatic, centrifugations, direct heating, and the Intermediate Moisture Content (IMC) methods (Supriatna, et al., 2000).

Virgin Fats and Oils means edible vegetable fats and oils obtained by mechanical procedures and the application of heat only, they may have been purified by washing with water settling, filtering and centrifuging (Codex, 2001). Based on these descriptions, VCO made by heat application without any chemicals for purification.

The Lauric acid of VCO, as bioactive compound, will be metabolized into monolaurin in the body which is effective to kill germs of viruses, bacteria, fungi and protozoa (Enig, 1996). The VCO reduce the levels of HIV / AIDS in the blood of patients (Dayrit 2005). VCO was also reported not cause heart problems in Kerala study (Rethinam et al, 2005). Medium chain fatty acids in coconut oil are similar to the fatty acids of breast milk, and VCO can be used to help in degenerative diseases treatment such as atherosclerosis, diabetes, cancer, obesity, stroke and others (Kabara, 2000).

Among various degenerative diseases, diabetes may cause complications, such as renal disease, cardiac disorders, gastrointestinal disorders, blood vessel blockage, decay and leg amputations, blindness, erectile dysfunction, disturbances of touch sensitivity (numbness), pain throughout the body, and death (Wibowo, 2005).

VCO may prevent complications of diabetes mellitus by inducing insulin secretion in the diabetics as VCO is easily absorbed. Inside the body, the lauric acid and capric acid of VCO, stimulates the secretion of insulin by Langerhans cells of the pancreas (Garfinkel et al, 1992).

According to the International Diabetes Federation (IDF) there are approximately 177 million people worldwide suffered from diabetes, and mostly type-2 diabetes (Anon, 2006), and WHO predicts it may increase up to 300 million people in the next 25 years.

This research was conducted to find out the health benefits of VCO, especially for diabetes mellitus, and to find out scientific evidence on efficacy of VCO produced with and no-heat well as low heat treatments, especially on the the efficacy medium chain saturated fatty acids (C6-C12) in VCO in lowering blood glucose levels of diabetes mellitus in rodent model.

Based on the above description, research problems are formulated as follows: Does the processing method of VCO affect the activity bioactive compound of medium chain saturated fatty acid on decreasing blood glucose levels, affecting the cholesterol value and pancreatic tissue profile of diabetes mellitus rats.

2. Materials and Methods

2.1. Materials

The materials consisted of VCO, coconut cooking oil, male Sprague Dawley rats, rats feed, and chemicals. Two types of VCO, VCO-A is the VCO which is processed without heat (VA) and bought from VCO supplier in Bogor, and VCO-B is the VCO which is processed using the controlled heat (VB) and made in CABI. Meanwhile, the commercially branded coconut cooking oil (MG) represents the coconut oil processed using high heat and chemical purification and purchased from a supermarket in Bogor.

Sprague Dawley male rats with body weight ranging from 150 to 250 grams, were purchased from "Langit Biru" a breeder rats at Taman Kencana Bogor. The materials for the rat feed formulation were purchased from a supermarket in Bogor, and the Chemistry Stores in Bogor. Alloxan for inducing rats into diabetes mellitus was ordered from Singapore. The chemical substances for chemical analysis such as monochloride iodine, acetic acid, sico-hexan, 20% potassium iodide, sodium thiosulfate 0.1 N, Wijs solution, 95% neutral alcohol, NaOH, KOH, phenolttalein 0.5%, chloroform, HgO crystals, helium, hydrogen, BF-3 Methanol, petroleum ether p.a. were obtained from CABI. Chemical substances for treatment of rats pancreatic organs such as Bouin solution, a solution of dehydration (alcohol 70%, alcohol 80%, alcohol 90%, alcohol 95% and absolute alcohol), xylo, haematoxylin, eosin, antibody monoclonal insulin (Sigma I2018), PBS, diaminobenzidine, normal serum, H2O2, methanol, distilled water (DW), conjugated antibodies, paraffin, adhesive, neoferand etc. were obtained from the Histological Laboratory Veterinary Faculty of Bogor Agricultural University.

2.2. Methods

Based on field observations, the VCO-A was made by centrifuge technology without any heat application.
VCO-B (VB) was produced by CABI using the Intermediate Moisture Content (IMC) technology without going through coconut milk step. In IMC technology VCO obtained from fresh grated coconut meat which is dried directly using artificial drying method with controlled temperature at 60°C prior to pressing.

The coconut cooking oil used in the study was commercially branded coconut cooking oil in the market. Based on field observations, the coconut cooking oil production is carried out through the process by pressing copra and then the oil obtained is purified in a special purification plants using high heat and chemicals.

2.2.1. Animal experiment

Before starting the research, all Sprague Dawley rats were subjected to an adaptation period for 6 days, and were observed for their health condition and fed with basic feed and water on ad libitum.

After going through a period of adaptation, the rats were fasted for 1 night, and then injected intravenously with alloxan (110 mg / kg of rat body weight). After 2 days, their serum glucose level was measured by using glucometer. The rats with serum glucose levels more than 200 mg/dl categorized as suffered from diabetic mellitus, and then classified to 4 groups (n = 5). The groups consisted of positive control (K+), VA, VB and MG, in addition, one group of healthy rats (n = 5) was not injected with alloxan, as a negative control group (K-).

All 5 groups of rat were fed with basic feed and drinking water every day ad libitum. In addition, VCO and coconut cooking oil were administrated orally to each group of the rats every day, respectively for 28 days. The dose of VCO or coconut cooking oil was 0.81 ml per day per 200g of rat weight. This dosage was obtained from a conversion factor of 0.018 on the basis of human weight of 70 kg (Harmita and Maksum, 2005) supplemented with 1 tablespoon VCO or MG 3 times a day, equivalent to 45 ml per day. Both the positive and negative control groups were not administrated orally neither with VCO nor coconut cooking oil and were administrated orally with 1 ml drinking water (irrespective of the rat weight).

2.3. Observation and analysis

During the research, every 4 days theirs blood glucose levels of the rats in each group were observed from each tail using a Glucometer instrument (Henry, 2002). The blood withdrawn from each rats tail using syringe of Glucometer. The drop of blood from the tail swab to the test strips apparatus of glucometer (Allmedicus, USA). At the end of the research on the 29th observation day all the rats were sacrificed, and their cholesterol, LDL and HDL level of the blood obtained from their heart organ were analyzed. In addition, at the end of treatment, the following activities were performed: the sampling of pancreas organ, fixation, dehydration, clearing, paraffin infiltration, embedding, sectioning, staining mounting and making photomicrograph.

2.4. Research place

The research was conducted at Laboratory of Animal Experiment, Department of Food Science and Technology, Faculty of Agricultural Technology and SEAFAST Center (South East Asia Food and Agriculture Science and Technology) Bogor Agricultural University, Laboratory of Histology Faculty of Veterinary Medicine, Bogor Agricultural University, and at the Center for Agro-Based Industries (CABI) Ministry of Industry, Bogor Indonesia.

2.5. Statistical analysis

Experimental Design of the research was Completed Randomize Design. If the treatment effect was significant, continuous test was then conducted using LSD (Least Significant Difference) or Duncan at p value 5% or 1% significant level. Statistical analysis was done using software SPSS 13.

3. Result and Discussion

3.1 Serum blood glucose level

After alloxan treatment, except for the negative group of rats (the healthy rat group), the rest of the rat groups condition of baseline had serum blood glucose level more than 200 mg/dl categorized as suffered from diabetic mellitus. All 5 groups of rat were fed with basic feed and drinking water every day ad libitum. In addition, VCO and coconut cooking oil were administered orally to each group of the rats every day.

Figure 1 shows that the average value of serum blood glucose levels for the K+ (Positive Control) rat groups and MG rat groups (the rats fed by coconut cooking oil) tend to be higher than the other rat groups, since the positive control group were conditioned to suffer from diabetes and no treatment for diabetic. Although statistically no significant different, the blood glucose levels MG rat groups (rats treated with the coconut cooking oil) decreased, with the decreasing value was smaller than that of other groups treated with VCO (VA and VB rat groups), may be caused by the manufacturing process of coconut cooking oil which uses high temperature and chemicals.
There were no significant difference of serum blood glucose level among all group of treatments, but Spearman's rho correlation analysis shows that compared to other rat groups, VA rat group has the most closely correlated in lowering blood glucose of diabetic rats. The data show that the correlation value of VA rats group is -0.929 which means that during the observation time, glucose levels decreased very closely and significantly different at 1% level. Meanwhile VB rat group had a correlation value of -0.762 and significantly different at 5% level. The result shows that the application of heat in the production process of VCO has a significant different effect on the activities of bioactive compound fatty acids in lowering blood glucose levels of diabetic rats.

Fife (2001) stated that the Medium Chain Fatty Acids (MCFA) in coconut oil has small enough molecular weight to enter the cell without the help of the insulin hormone. In other words MCFA can enter the cells regardless the existence of insulin or even if the cell is insulin resistant. Further, Garfinkel et al. (1992) and Han et al (2003) explained that coconut oil can help regulate blood sugar, because the bioactive compound contained is the MCFA which can increase insulin production and sensitivity. In line with the above findings, Opara et al (1994) stated that Lauric acid as a MCFA is fatty acids that increase the highest percentage basal insulin output compared to the short or long chain fatty acids.

3.2 Blood serum cholesterol

Figure 2 shows that total cholesterol value of all rats was below of the danger threshold. The safe threshold for total cholesterol is <200 mg/dl (Henry, 2002 and American Diabetes Association, 2004). This is in accordance with the statement of Rethinam and Bosco (2006), that medium chain saturated fatty acid did not increase blood cholesterol.

Figure 2 indicates that VCO treatments (VA and VB rat groups) relatively did not alter the levels of total cholesterol, HDL, LDL and triglycerides of diabetic mellitus rat blood serum compared to those of the K+ rat group. The European Task Force on Coronary Prevention recommends for decreasing the total cholesterol <190 mg / dl and LDL i.e. <115 mg / dl (Henry, 2002). Research data as showed in Figure 2, that the total cholesterol of K+ rat group is 76.4 while the VA and VB rats groups were 68.0 and 76.0, respectively, means that 28 days consumption did not increase levels of total cholesterol of diabetic rats.

According to Henry (2002) low concentrations of HDL <39 mg / dl for men and <43 for women, especially when associated with fasting triglyceride values > 180 mg / dl, estimated to be at high risk for coronary heart disease. Based on Figure 2, the levels of HDL cholesterol and triglycerides of the VCO treated rat groups (VA and VB rat groups) are on safe threshold. This result in line with the study of Rethinam and Bosco (2006) that stated fatty acids of the VCO are rapidly absorbed by the rat’s body do not deteriorate the rat’s body lipid.

Figure 2 shows that VCO consumption in VA and VB compared to control positive group. However, the increased LDL value was still below the threshold. The European Task Force on Coronary Prevention recommends for lowering total cholesterol i.e. <190 mg / dl and LDL i.e. <115 mg / dl. Meanwhile, according to the American Diabetes Association (2004) normal LDL value was <160 mg / dl (Henry, 2002).

Likewise, Figure 2 shows that the K+ rat group or untreated diabetic rats, has higher triglyceride value than VA, VB and MG groups. This shows that the triglyceride value of K+ rats group i.e. 153.6 can be
reduced by the VCO treatment. The triglyceride value of the treated VA and VB rat groups are 86 and 75.6 respectively. In other words VCO did not increase the value of blood triglycerides of diabetic rats. As previously mentioned, this happens because of VCO can be quickly absorbed by the body, converted into energy, and is not stored as body fat, therefore the VCO did not affect the condition of the body lipids (Rethinam and Bosco, 2006).

Based on the results of this research, it was found that during the 28 days observation the VCO orally fed to the diabetic rats did not significantly increased triglyceride values. Similar study conducted by Rajamohan (1997), shows that coconut oil consumption provides the total cholesterol and triglycerides lower than that of peanut oil.

3.3 Profile of pancreatic organ

Haematoxylin-Eosin (HE) tinting was conducted to see in a qualitative way the general morphology of pancreatic tissue of rats. This tinting composed of two color components, haematoxylin and Eosin. Haematoxylin is an alkaline dye colored the acidic cell nucleus whereas Eosin is an acidic dye colored the alkaline cytoplasm (Banks 1993). The observed parameters of HE tinting in the preparations of the pancreas is a general morphology of pancreatic tissue and the number of Langerhans island. The pancreatic organ tissues of K+ and MG rat groups showed the number of degenerating and necrotizing of acinar cells, higher than VA and VB rat groups. The pancreatic organ tissue of VA and VB rat groups have only a very few number of degenerating and necrotizing acinar cells as shown in Figure 3.

The counting results of the pancreatic tissue's Langerhans islands of rat groups per five fields of view are presented in Table 1.

<table>
<thead>
<tr>
<th>Rat Groups</th>
<th>The Number of Langerhans Island (unit)</th>
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</thead>
<tbody>
<tr>
<td>K(-)</td>
<td>34.0 ± 1.18b</td>
</tr>
<tr>
<td>K(+)</td>
<td>140 ± 1.06a</td>
</tr>
<tr>
<td>VA</td>
<td>213 ± 1.46a</td>
</tr>
<tr>
<td>VB</td>
<td>193 ± 2.12a</td>
</tr>
<tr>
<td>MG</td>
<td>153 ± 1.18b</td>
</tr>
</tbody>
</table>

Remark: The Different Superscript on the Same Column Indicates The Significant Difference (p<0.05)

Based on statistical analysis of Variant (ANOVA), the number of Langerhans islands of the K- rat group was significantly higher (p <0.05) compared to other rat groups, since this group is healthy rats. While the K+, VA, VB, and MG groups have no significant difference in Langerhans islands number among the groups, as shown by stable number of Langerhans islands during 28 days. Yet the number of Langerhans islands cannot describe the damage of pancreatic tissues and cells in them. These tissues and cells cannot be detected by haematoxylin-Eosin tinting (HE). Therefore, it needs advanced tinting by using immuno histochemical to detect the β cells within the Langerhans islands. The results of β cell counting of the pancreatic tissues of rats within the Langerhans islands per three fields of view are presented in Table 2.

Table 2

<table>
<thead>
<tr>
<th>Rat Groups</th>
<th>The Number of β Cells in Langerhans Islands (Unit)</th>
</tr>
</thead>
<tbody>
<tr>
<td>K(-)</td>
<td>63.44 ± 15.29b</td>
</tr>
<tr>
<td>K(+)</td>
<td>10.11 ± 6.95a</td>
</tr>
<tr>
<td>VA</td>
<td>22.78 ± 7.03b</td>
</tr>
<tr>
<td>VB</td>
<td>18.89 ± 8.27b</td>
</tr>
<tr>
<td>MG</td>
<td>8.67 ± 3.12b</td>
</tr>
</tbody>
</table>

Remark: The Different Superscript on the Same Column Indicates The Significant Difference (p<0.05)

Observations of the β cells were quantified by counting the number of β cells in each rat pancreas. β cells detected by immune histochemical staining were indicated by the dark brown color in the Langerhans island cells, while others were blue. The blue color comes from the counter staining by using haematoxylin dye which colors the pancreas cells.

The results of statistical analysis show that the number of β cells of Pancreatic tissues of VA and VB groups is significantly higher (p <0.05) than those of group K (+) and group MG rats although it is significantly lower (p <0.05) than that of the group K (-) rats.

Diabetes mellitus causes a situation in which the body cannot control blood glucose levels due to the disruption of insulin secretion by β cells in the pancreatic tissues of Langerhans islands. The process of glucose metabolism is very important to
generate energy for the survival of cells in the body. However, diabetes mellitus makes the body cells unable to metabolize glucose so the body suffers from energy shortage. Consequently, the body looks for alternative energy through Glycogenolysis and gluconeogenesis. Both processes produce by-products of free radicals that can cause damage to the body's cells including pancreatic cells. Alloxan is one of the substances for inducing experimental diabetes mellitus which selectively destroys pancreatic β cells (Walde et al. 2002). Diabetes mellitus suffered by rats in this study was due to the damaged pancreatic β cells induced by Alloxan which results in the decreased insulin secretion. According to Szkudelski (2001), Alloxan damages pancreatic β cell through the formation of reactive oxygen species preceded by the reduction of Alloxan. Alloxan reacts with reducing agents such as cysteine and enzymes belonging to an SH-group. SH-glucokinase is an enzyme that plays an important role in insulin secretion, therefore, Alloxan has a high affinity for the glucokinase enzyme. Alloxan reacts with two SH-groups of the glucokinase enzyme to form a dimeric bond which inactivates the enzyme and disturbs the insulin secretion. Eventually it damages β cells and results in the state of diabetes.

Alloxan is also the free radicals that can damage biomacromolecules such as lipids, phospholipids, and carbohydrates that are the components of cell walls (Lenzen 2007), and the DNA inside the nucleus. The DNA in the nucleus was also attacked by free radicals. If DNA damage is not too much, it can be improved. However, the recovery process often causes cell mutation which can eventually become cancer (Aruoma 1998). General morphology of pancreatic tissue and cell damage inside acinar cells and the number of Langerhans islands can be seen by haematoxylin-Eosin tinting (HE). The pancreatic tissues of group K (-) has the least amount of acinar cell degeneration and nekrosa but has the highest number of Langerhans islands compared with other treatment groups. This is because pancreatic tissues of treatment group K (-) are not induced by Alloxan, thus diabetic condition does not appear. Meanwhile the pancreatic tissues of the treatment group K (+) has a higher number of degenerated acinar cells to nekrosa compared with the treatment groups of VA and VB. It is because the pancreatic tissues of treatment group K (+) are induced by Alloxan and merely fed with water so the acinar cells deteriorate quickly.

Pancreatic tissues of group VA and VB have fewer acinar cells degenerating to nekrosa compared with the K (+) and MG, because the pancreas of group VA and VB induced by Alloxan receive VCO intake from the body, so the rate of damaged cells can be inhibited. VCO contains Lauric Acids, a medium chain saturated fatty acids, which have been reported to increase insulin secretion (Garfinkel et al 1992). Increased secretion of insulin causes the search for glucose alternatives to be reduced which results in fewer by products of free radicals. The rate of cell damages in pancreatic tissue group VA and VB can be inhibited so that the amount of cell damage becomes fewer than the group K (+) and MG. With HE tinting the number of Langerhans islands is not significantly different among group K (+), VA, VB, and MG. To be able to detect β cells contained in the Langerhans islands, the advanced colouring is done with immunohistochemical techniques. β Cell detection in immunohistochemical may indicate a β cell damage in diabetes mellitus condition. Pancreatic tissues of group K (+) has the least amount of β Cell compared with other groups, this is because the pancreatic tissues of K (+) were induced by Alloxan and treated with drinking water only resulting in the high rates of β cellular damages while the insulin secretion becomes very low. Reduction of insulin secretion that occurs in β cells of group K (+) was caused by Alloxan which inactivated the glucokinase enzyme. Glucokinase is an enzyme-with SH group which has an important role in the secretion of insulin in the pancreas. Alloxan has a high affinity with proteins having SH groups, therefore, Alloxan reacts with the two SH-groups of glucokinase which results in the dimer bond causing the inactivation of enzymes and being responsible for the β cell decay (Szkudelski 2001). In addition, Alloxan also reduces ATP production, causing the opening of K + channels and the plasma membrane hyperpolarization. Then, it was followed by the closing of the gate voltage that causes Ca2 + concentration decrease resulting in decreased secretion of insulin and cell decay (Drews et al. 2000).

In the MG treatment group, the number of β cells produced was not much different from that of the K (+). In a diabetic condition, the search for glucose alternatives produces by products of free radicals. The high amount of free radicals due to the diabetic condition will increase further with the consumption of cooking oil containing a few unsaturated fatty acids that will increase lipid oxidation, which will also produce by products of free radicals. As a result, the free radicals present in the pancreas of MG treatment group will be even higher. This will eventually make the function of β cells of the pancreas decrease causing the reduced insulin secretion which will make the faster rate of β cells decay in pancreatic tissues of MG treatment group faster. Consequently, the number of β cells becomes fewer. Pancreatic tissues of treatment group VA and VB have higher amount of β cells than that of the treatment group K (+) and MG. It indicates that there is an inhibition of the rate of
damage to the Langerhans island of β cells in the treatment group VA and VB. The secretion of insulin in the group VA and VB is triggered by the increased concentration of intracellular Ca2 + released by the gate voltage of Ca2 + channels within the β cell plasma membrane. The medium chain saturated fatty acids play an important role in the activation of the Ca2 + channel resulting in mobilization of intracellular Ca2 + that triggers the release of insulin from β cells (Gravena et al. 2002). The saturated fatty acids that play an important role in stimulating insulin secretion is Lauric acid, palmitic acid, and capric acid contained in the VCO (Garfinkel et al. 1992). Increased secretion of insulin causes the search for glucose alternatives to be reduced, and the by product of free radicals produced is less. Thus β cells damage can be inhibited and the number of β cells present in the pancreas of treatment group VA and VB is higher than that of K (+) and MG. However, the number of β cells in the pancreas of the treatment group VA and VB is still less than that of the treatment group K (-). It is because pancreatic tissues of treatment group K (-) are not induced by Alloxan.

As diabetic condition did not arise in this group, the number of β cells remains intact. In addition to medium chain saturated fatty acids, VCO also contains vitamin E and antioxidants. Antioxidants contained in VCO can neutralize free radicals that accumulate in cells due to diabetes mellitus so β cell damage caused by free radicals can be inhibited.

4. Conclusion

Non heated and controlled heated VCO showed no significant effect on lowering blood glucose level, but on lowering blood glucose, VCO A (without heat) has closer correlation and very significant difference (-0.929 correlation value at p < 1%), compared to VCO B (controlled heat) (correlation value of -0.762, at p <5%) and coconut cooking oil ( -0.524 correlation value at p > 5%) 

Non heated VCO (VA) showed significant positive correlation with blood glucose level (-0.929 correlation value at p <1%), while controlled heated VCO (VB) significantly different at p 5% level but coconut cooking oil could not lowering the blood glucose level.

VCO and coconut cooking oil showed no significant effects on raising the level of total cholesterol, HDL value, LDL value and serum triglyceride of diabetic mellitus rats at p 5% level.

The virgin coconut oil significantly inhabited cell damage of pancreatic tissue on the Langerhans island of diabetic mellitus rats.

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Reference


