The Antidiabetic and Antioxidant Activities of Hydrolyzed Virgin Coconut Oil in Streptozotocin-induced Diabetic Rats

Linda Margata[1*], Jansen Silalahi[1], Urip Harahap[2], Dwi Suryanto[3] and Denny Satria[4]
[1]*Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Universitas Sumatera Utara, Medan, Indonesia*
[2]*Department of Pharmacology, Faculty of Pharmacy, Universitas Sumatera Utara, Medan, Indonesia*
[3]*Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara, Medan, Indonesia*
[4]*Department of Pharmaceutical Biology, Faculty of Pharmacy, Universitas Sumatera Utara, Medan, Indonesia*

Keywords: Virgin coconut oil, Enzymatic hydrolysis, Blood glucose, Haemoglobin A1c, Superoxide dismutase

Abstract: The aim of this study was to examine the antidiabetic and antioxidant effect of enzymatically hydrolyzed virgin coconut oil (HVCO) in streptozotocin (STZ) induced rats. VCO was hydrolyzed enzymatically using lipase from *Rhizomucor miehei* (active on sn-1,3 position). Thirty male rats were induced with 40 mg/kg body weight (BW) STZ. Rats with blood glucose level ≥ 250 mg/dl were divided into six groups which were given with sodium carboxymethylcellulose (CMC Na) 0.5%, metformin 45 mg/kgBW, VCO (4 and 6 ml/kgBW) and HVCO (4 and 6 ml/kgBW). Blood glucose, Haemoglobin A1c (HbA1c), superoxide dismutase (SOD), soluble receptor advanced glycosylation end-product (sRAGE) levels, and immunihistochemistry assay on pancreas were analyzed after 30 days of treatment. It is shown that HVCO 4 ml/kgBW and metformin were not significantly different in lowering blood glucose level. Blood glucose level in groups treated with HVCO 4 ml/kgBW and metformin were 409.2 and 364.40 mg/dl. HVCO also lowered HbA1c and sRAGE levels (55.50 ng/ml and 148.40 pg/ml, respectively), while increased SOD level (76.96 pg/ml). Insulin expression of rats treated with 4 ml/kgBW HVCO and metformin also did not differ significantly which were 11.40% and 11.70%, respectively. HVCO exerted higher antidiabetic and antioxidant effects that VCO did.

1 INTRODUCTION

Diabetes Mellitus (DM) lowers life's quality, productivity and increases mortality rate in either developing or developed countries. DM is metabolic syndrome which is marked by the occurence of hyperglycemia. Hyperglycemia in DM could trigger oxidative stress which then causes microvascular complications (retinopathy, nephropathy and neuropathy) and also macrovascular complications (heart attack, stroke and peripheral blood vessel disease). Risk of heart attack and stroke happen two to four times more in diabetic patients, 50% diabetic patients died because of cardiovascular disease (Vassalotti, 2006; Paliyath, et al., 2011).

Insulin plays an important role in controlling blood glucose. Insulin is a hormon produced by β-cell which is located in Langerhans islet of pancreas. Insulin stimulates the uptake of blood glucose by cell, hence lowers blood glucose level. DM which is caused by the low secretion of insulin from β-cell pancreas is classified as Type I DM, while DM which is caused by insulin resistance is classified as Type II DM (Joshi, et al., 2007; Triplitt, et al., 2005).

Hyperglycemia stimulates protein glycosylation which is a reaction between aldehyde group in glucose and amino group in protein. This reaction produces schiff base via Amadori process. Amadori product then undergoes further autooxidation becomes advanced glycosylation end-product (AGE). Crosslinking protein causes AGE accumulation in extracellular matrix. AGE in diabetic patient causes erythrocyte dysfunction because haemoglobin is glycated. This erythrocyte will bound to receptor of advanced glycosylation end-product (RAGE) in endothelium and causes endothelium dysfunction (Baynes and Domonickzak, 2003; McKee and McKee, 2003; Rayfield and Valentine, 2006; Mechanick, 2006).

Virgin coconut oil (VCO) is one of the sources of medium chain triglyceride (MCT) oil. MCT has

Margata, L., Silalahi, J., Harahap, U., Suryanto, D. and Satria, D.
The Antidiabetic and Antioxidant Activities of Hydrolyzed Virgin Coconut Oil in Streptozotocin-induced Diabetic Rats.
DOI: 10.5220/0009862301520159
In *Proceedings of the 2nd International Conference on Tropical Medicine and Infectious Disease (ICTROMI 2019)*, pages 152-159
ISBN: 978-989-758-469-5

been known to have benefits in glycemic control and insulin secretion. MCT helps in the effectiveness of glucose usage (Vala and Kapadiya, 2014; Bach and Babayan, 1982; Fife, 2004). Studies about the antidiabetic and antioxidant effects of VCO has been reported (Siddalingaswamy, et al., 2011; Mohammed and Luka, 2013; Iranloye, et al., 2013; Akinnuga, et al., 2014; Kolondam, et al., 2008; Elsayed, et al., 2015; Essien, et al., 2014; Dalmacion, et al., 2012; Dewi and Aryadi, 2010). However, study about the effect of hydrolyzed VCO (HVCO) on blood glucose level and its antioxidant effect have never been reported. The objective of this study was to determine the antidiabetic and antioxidant effects of HVCO in STZ-induced diabetic rats.

2 MATERIALS AND METHODS

Apparatus used in this study were analytical balance, hot plate, magnetic stirrer, thermometer, oven, water bath, separating funnel, Easytouch test strips, microtube, centrifugator, microplate reader, incubator, micro pipette and laboratory glassware. Chemicals used were lipase from *Rhizomucor miehei* (≥ 20000 U/g, Sigma Aldrich), Tris-HCl (molecular biology grade, Vivantis), distilled water (Bratachem), n-hexane (Macron Chemicals). Calcium chloride and sodium sulfate anhydrous were the products of Merck. All reagents used in this work were of analytical grade unless otherwise stated. VCO used was the product of Palem Mustika, Indonesia. STZ used to induce diabetic in rats was from Nacalai Tesque. Metformin 500 mg tablets were purchased from the local pharmacy. Rat HbA1c, SOD and sRAGE ELISA kits were the products of FineTest.

2.1 Enzymatic Hydrolysis of Virgin Coconut Oil

Thirty (30) g of VCO, 30 ml of distilled water, 12.5 ml of 0.063 M calcium chloride, 25 ml of buffer Tris-HCl 1 M pH 8 and 3 ml of lipase from *R. miehei* were transferred into 250 ml Erlenmeyer flask, respectively. The mixture was incubated for 10 hours at 50°C and stirred at 200 rpm for 10 minutes every 1 hour. After 10 hours, the mixture was transferred into the separating funnel and extracted with 50 ml of n-hexane. The extract was allowed to stand for a while until two layers were formed. The upper layer (n-hexane fraction) was separated (filtrate I), while the bottom layer (water fraction) was extracted again with 50 ml of n-hexane. The second extract was allowed to stand for a while and then the upper layer formed was separated (filtrate II). Filtrate I and II were mixed and then 250 g of sodium sulfate anhydrous was added and allowed to stand for 15 minutes to absorb the water residue. The mixture was filtered, then n-hexane was evaporated using water bath to obtain HVCO (Margata, et al., 2018).

2.2 Preparation of Diabetic Rats

Thirty male rats which had been acclimatized for one week were fasted for 18 hours and water was given *ad libitum* during fasting period. Blood glucose level was measured using Easytouch test strips. Rats were given STZ 40 mg/kgBW i.p. and then 10% sucrose *ad libitium* for one day. After 10 days, blood glucose level was measured and rats with blood glucose level ≥ 250 mg/dl were used as experimental animals (Furman, 2015).

2.3 Experimental Design

Diabetic rats were divided into six groups (five animals each) and were given orally: (1) CMC Na 0.5% (control group), (2) 45 mg/kgBW metformin, (3) 4 ml/kgBW VCO, (4) 6 ml/kgBW VCO, (5) 4 ml/kgBW HVCO, and (6) 6 ml/kgBW HVCO, for 30 days. Blood glucose level was measured every three days during experimental period. After the end of treatment, rats were fasted for 18 h and anesthetized with 70 mg/kgBW ketamine i.p. Blood was collected directly from the heart and centrifuged for 10 min at 3000 rpm to obtain serum. Serum collected was then analyzed for HbA1c, SOD and sRAGE levels using ELISA (Afriadi, 2010; Silalahi, et al., 2016).

2.4 HbA1c, SOD and sRAGE Analysis

HbA1c, SOD and sRAGE were measured enzymatically using ELISA kit. Absorbance of color intensity was read using microplate reader at 450 nm (Ashraf, et al., 2015).

2.5 Immunohistochemical Staining of Pancreas

Insulin expression in pancreas was done using IHC technique. Pancreas tissue which had been sectioned into 3-4 µm was soaked intu xylol, ethanol 100, 90, 80, 70 and 50%, respectively, each was done 2 times for 90 min. Peroxidase blocking was done using

0.3% H_2O_2 in methanol for 20 min, washed with 10% phosphat buffer saline (PBS) 3 times for 5 min. Non-specific blocking was done using 10% normal serum for 30 min and then tissue was incubated with primary antibody at 4°C for 18-22 hrs, washed with 10% PBS 3 times for 5 min. Tissue was given with some drops of secondary (universal) antibody and allowed to stand for 30 min and then washed with 10% PBS 3 times for 5 min. Tissue was given with some drops of chromogen 3,3-diaminobenzidine and allowed to stand for 5-10 sec, then washed with distilled water, counterstained with Hematoxylin Mayer for 5-10 sec and then with running tap water for 10-15 min. Dehydration was done using ethanol 80, 90% and xylol (each for 2 times). Mounting was done using E. Z mount (Lab Vision, Cat#MS-1378-PO)

2.6 Statistical Analysis

All data were statistically analyzed using one-way ANOVA followed by Tukey's test using computerized SPSS package program (SPSS 17.0 software for Windows). Results are expressed as mean±standar deviation and considered significantly different at $p<0.05$.

3 RESULTS

The effect of VCO and HVCO on blood glucose level during 30 days of treatment can be seen in Table 1 and Figure 1.

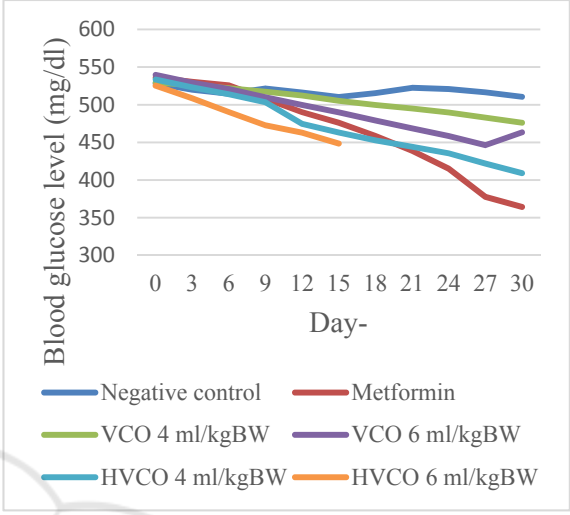

Figure 1: Changes in blood glucose level during 30 days of treatment.

Table 1: Changes in blood glucose level during 30 days of treatment

Treat-ment	Blood glucose level (mg/dl) day-										
	0	3	6	9	12	15	18	21	24	27	30
Negative control	528.40 ± 18.56^{a}	519.80 ± 14.79^{a}	514.60 ± 19.86^{a}	521.60 ± 12.10^{a}	516.20 ± 17.51^{a}	510.40 ± 14.93^{a}	515.40 ± 11.30^{a}	522.40 ± 11.01^{a}	520.80 ± 14.86^{a}	516.40 ± 15.65^{a}	510.60 ± 15.95^{a}
Metfor-min	536.00 ± 47.72^{a}	530.60 ± 46.77^{a}	525.80 ± 45.50^{a}	508.00 ± 32.44^{a}	490.20 ± 29.16^{a}	476.20 ± 23.81^{a,b}	458.60 ± 26.75^{a,b}	439.00 ± 19.34^{b}	415.20 ± 16.39^{b}	377.80 ± 16.90^{b}	364.40 ± 11.50^{b}
VCO 4 ml/kg BW	529.40 ± 31.93^{a}	526.00 ± 31.46^{a}	522.00 ± 32.02^{a}	517.40 ± 30.01^{a}	512.20 ± 30.43^{a}	505.20 ± 31.33^{a,b}	499.80 ± 34.13^{a,b}	495.00 ± 33.59^{a,c}	489.80 ± 33.81^{a,c}	483.00 ± 33.13^{a,c}	476.20 ± 32.84^{a,c}
VCO 6 ml/kg BW	539.60 ± 30.76^{a}	529.20 ± 31.54^{a}	521.00 ± 33.58^{a}	510.00 ± 32.27^{a}	499.80 ± 31.32^{a}	490.00 ± 33.66^{a,b}	479.00 ± 31.02^{a,b}	468.60 ± 32.36^{a,b,c}	458.40 ± 32.12^{b,c}	446.40 ± 27.52^{c,d}	463.40 ± 29.37^{c,d}
HVCO 4 ml/kg BW	533.60 ± 36.58^{a}	523.00 ± 36.82^{a}	514.00 ± 34.16^{a}	503.40 ± 38.04^{a}	474.60 ± 41.60^{a}	463.20 ± 41.79^{a,b}	452.80 ± 40.31^{b}	444.20 ± 39.13^{b,c}	435.60 ± 38.47^{b,c}	422.00 ± 40.85^{b,d}	409.20 ± 46.15^{b,d}
HVCO 6 ml/kg BW	525.20 ± 29.45^{a}	508.40 ± 27.05^{a}	490.40 ± 29.52^{a}	472.80 ± 29.66^{a}	463.00 ± 29.80^{a}	448.40 ± 35.83^{b}	-	-	-	-	-

Means ± SD in each column with different superscript letters differ significantly at $p<0.05$ (n=5).

In Table 1 and Figure 1, it can be seen that at day 0, all rats in each group had blood glucose level ≥ 250 mg/dl. Blood glucose levels at day 0 in all rats were not significantly different. From day 3 to 30, blood glucose levels in all groups, except negative control, were gradually decreasing. In negative control group, blood glucose level was stable until day 30. From day 3 to 12, all all groups had significantly different blood glucose level ($p>0.05$).

At day 15, blood glucose levels in rats given with metformin and HVCO 6 ml/kgBW were 476.20 and 448.40 mg/dl, respectively. Those values were significantly different with blood glucose level in negative control group which was 510.40 mg/dl. At day 18, HVCO 4 ml/kgBW was shown to have a significant decrease in blood glucose level compared to negative control group. At day 30, blood glucose level in rats fed with 4 ml/kgBW VCO did not differ significantly ($p<0.05$) compared to blood glucose level in rats fed with metformin which were 409.20 and 364.40 mg/dl.

The effect of VCO and HVCO on HbA1c, SOD and sRAGE levels in diabetic rats can be seen on Table 2 and Figure 2.

Table 2: The effect VCO and HVCO on HbA1c, SOD and sRAGE levels in diabetic rats

Treatment	HbA1c (ng/ml)	SOD (pg/ml)	sRAGE (pg/ml)
Negative control	92.45 ± 1.44[a]	43.95 ± 0.58[a]	193.49 ± 2.53[a]
Metformin	45.75 ± 0.77[b]	82.15 ± 0.39[b]	133.82 ± 1.63[b]
VCO 4 ml/kgBW	68.38 ± 0.73[c]	52.82 ± 0.22[c]	167.55 ± 2.53[c]
VCO 6 ml/kgBW	64.88 ± 1.41[d]	61.49 ± 0.50[d]	159.30 ± 1.93[d]
HVCO 4 ml/kgBW	55.50 ± 1.59[e]	76.96 ± 0.32[e]	148.80 ± 1.99[e]

Means ± SD in each column with different superscript letters differ significantly at $p<0.05$ (n=5).

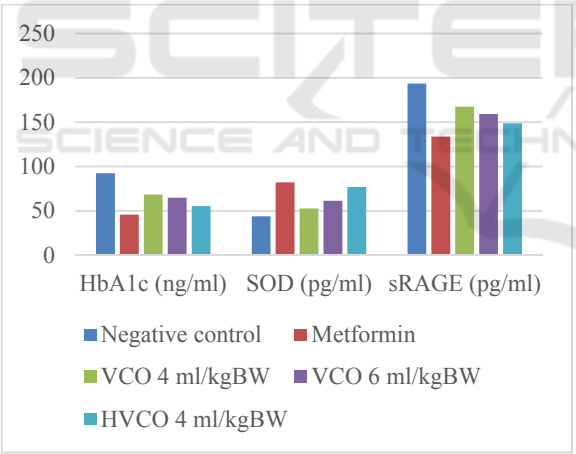

Figure 2: The effect of VCO and HVCO on HbA1c, SOD and sRAGE levels in diabetic rats.

As seen in Table 2 and Figure 2, HbA1c levels in all groups were significantly different ($p<0.05$). The highest to the lowest HbA1c levels started from negative control, 4 ml/kgBW VCO, 6 ml/kgBW VCO, 4 ml/kgBW HVCO to metformin, respectively. SOD levels in all groups were also significantly different ($p<0.05$). The lowest SOD level was in negative control group which was 43.95 pg/ml, while the highest was in metformin group which was 82.15 pg/ml. SOD level in rats fed with 4 ml/kgBW HVCO (52.82 pg/ml) was significantly higher than those in 4 and 6 ml/kgBW VCO (52.82 and 61.49 pg/ml). All groups also had significantly different sRAGE levels ($p<0.05$). The highest sRAGE level was in negative control group, while sRAGE level in groups fed with metformin, 4 ml/kgBW HVCO, 4 and 6 ml/kgBW VCO were shown to decrease significantly compared to negative control group.

The effect of VCO and HVCO on insulin expression in pancreas of diabetic rats can be seen on Figure 3 and Table 3.

Table 3: The effect VCO and HVCO on insulin expression in diabetic rats

Treatment	Mean insulin expression ± SD	Insulin expression (%)
Negative control	6,40 ± 1,82[a]	3.20
Metformin	23,40 ± 1,14[b]	11.70
VCO 4 ml/kgBW	10,60 ± 1,82[c]	5.30
VCO 6 ml/kgBW	15,60 ± 1,67[d]	7.80
HVCO 4 ml/kgBW	22,80 ± 1,48[b]	11.40

Means ± SD in each column with different superscript letters differ significantly at $p<0.05$ (n=5). Percentage of insulin expression was calculated from 200 cells which was counted using Image Raster

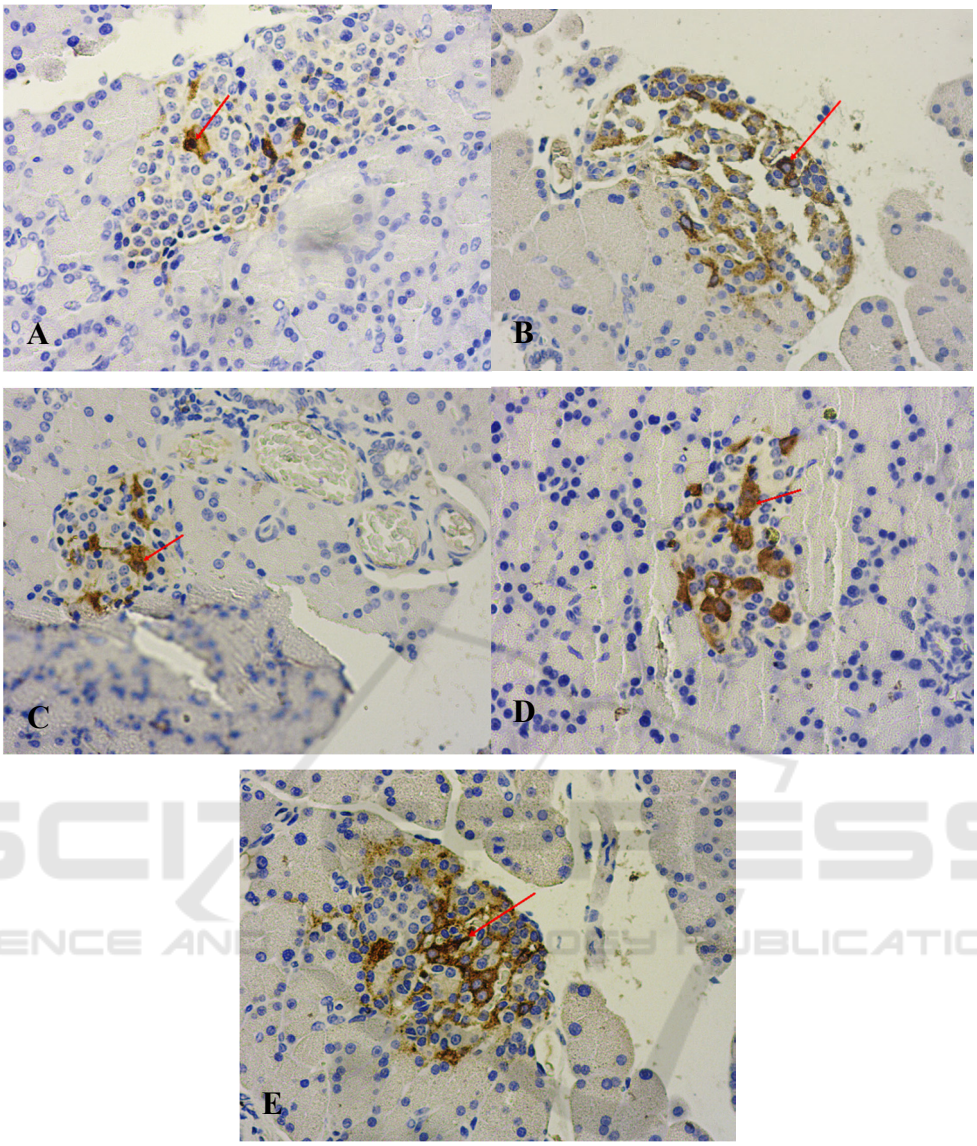

Figure 3: The effect of VCO and HVCO on insulin expression in diabetic rats. A = negative control, B = metformin 45 mg/kgBW, C = VCO 4 ml/kgBW, D = VCO 6 ml/kgBW, E = HVCO 4 ml/kgBW, (→) = show positive reaction between antigen and insulin antibody on β-cell which is marked by brown color; magnificent 10 x 40.

Based on Figure 3, it can be seen that rats from negative control had the least brown color expressed compared to other groups. In addition, it is also shown that rats treated with metformin and 4 ml/kgBW HVCO showed highest insulin expression. From Table 3, it can be seen that insulin expression in negative control group was the lowest insulin expression with score 6.40 (3.20%). Insulin expression in each group differs significantly at $p<0.05$, except groups treated with metformin and HVCO 4 ml/kgBW with insulin expression of 27,60 (13,80%) and 22.80 (11.40%), respectively.

4 DISCUSSION

In this study, 40 mg/kgBW STZ ~~was shown to~~ give uniform response of blood glucose levels in all groups. STZ is a broad spectrum antibiotic which is toxic to insulin producing β-cell in pancreas Langerhans islet. STZ is uptaken via glucose transporter GLUT2 and causes DNA alkylation, and eventually causes β-cell death (Szkudelski, 2011; Deeds, et al., 2011). β-cell damage was ~~shown to be~~ stable until day 30 in this study. Blood glucose level

was not return to normal during experiment period if intervention was not done. Structural changes in β-cell pancreas (total granulation) occur 48 hours after STZ administration and continue for 4 months. The low rate of β-cell regeneration in diabetic individual causes the importance of finding a way to increase its regeneration rate (Eleazu, et al., 2013; Yin, et al., 2006).

Blood glucose level ~~was~~ decrease after 15 day of treatment in groups fed with metformin and 6 ml/kgBW. It shows that the bioavailability of metformin and 6 ml/kgBW HVCO were enough to decrease blood glucose level in diabetic rats after 15 days. After day 15, blood glucose level in group fed with 6 ml/kgBW was not recorded because all rats in those group did not survive until day 18. Therefore, HbA1c, SOD and sRAGE levels in that group were not available.

VCO was shown to have antidiabetic effect and the effect increasing as the dosage increasing. This study corresponds to the studies reported before which showed that antidiabetic effect of VCO is dose dependant (Iranloye, et al., 2013; Afriadi, 2010; Handajani and Dharmawan, 2009). At day 30, group fed with 4 ml/kgBW and metformin were shown to have a decrease in blood glucose level. It might be because those groups undergo β-cell recovery at day 30. MCFA contained in HVCO is suspected to have important role in the recovery of β-cell pancreas. In addition, lauric acid also has the ability to stimulate insulin secretion (Vala and Kapadiya, 2014).

Metformin is an antihyperglycemic commonly used in the treatment of Type II DM. Metformin increases hepatic and peripheral insulin sensitivity by inhibiting hepatic glucose production and increasing the uptake of glucose in skeletal muscle and adipose. Although metformin was not commonly recommended as adjunct therapy in Type I DM (Beysel, et al., 2018), this study shows that metformin was able to lower blood glucose level in single dose STZ induced diabetic and this finding corresponds to the works reported before (Silalahi, et al., 2016; Erehywa, et al., 2011; Han, et al., 2017). This might be because diabetic type induced by STZ has similarity either with Type I or Type II DM (Eleazu, et al., 2013).

VCO enzymatic hydrolysis using lipase from *Rhizomucor miehei* which is active on sn-1 and sn-3 position in triglyceride molecule produces two MFAs (especially lauric acid) and one molecule 2-monoglyceride (especially 2-monolaurin) (Aehle, 2004). MCFA is known to help blood glucose control, increase insulin secretion and help in glucose usage, hence MCFA can be used in diabetic prevention and treatment (8,10). In β-cell, MCFA activates fatty acid 1 receptor (FFAE1/GPR40) and induces mitochondira ketogenesis, hence increases β-cell function (Pujol, et al., 2018).

VCO and HVCO ~~was shown to~~ decrease HbA1c levels although the decrease were not more effective than that of metformin. HVCO lowered HbA1c levels better than VCO. HbA1c is an indicator used to monitor diabetic condition and hyperglycemia. Glycemic control helps in decreasing the risks of heart failure, myocardial infarction, etc. Recent study shows that high HbA1c involved in the increase risk of cardiovascular disease mortality in diabetic patients (Wong, et al., 2018).

HVCO ~~was shown to~~ helps in decreasing excessive oxidative stress condition in diabetic rats, hence, it prevented the occurence of DM complications. This result corresponds to the studies reported before that VCO increases SOD level in diabetic rats (Siddalingaswamy, et al, 2011; Iranloye, et al., 2013). Oxidative stress is ~~known to be~~ the key role of diabetic complications pathogenesis. Human body is constantly protected from excessive oxidative stress by a complex system of enzymatic and non-enzymatic antioxidant. Enzymatic antioxidant SOD involves in reactive oxygen species (ROS) metabolsim. Superoxide anion is highly reactive ROS which is converted by SOD into hydrogen peroxide which is then reduced to water by catalase and gluthation peroxidase (Wong, et al., 2018; Ng, et al., 2013).

HVCO and VCO decreased sRAGE in diabetic rats, although it was not more effective than metformin. RAGE is a cell surface type receptor from immunoglobulin superfamily which binds to various ligands, including AGE. Soluble RAGE (sRAGE) is a RAGE isoform found in blood circulation. The binding of ligand and sRAGE prevents the condition of oxidative stress, inflammation and apoptosis which occur from the interaction between RAGE and ligand. In hyperglycemia condition, ROS and AGE induces metalloproteinase-9 matrix which cleaves the cell surface receptor which produces sRAGE, hence increasing sRAGE levels in DM patients (Wong, et al., 2018).

Antidiabetic drug metformin is known to ameliorates oxidative stress status in DM. Metformin prevents SOD inhibition caused by aldehyde modification and increase its antioxidant

activity in diabetic patient. In addition, metformin also prevents oxidative stress by decreasing ROS and increasing enzymatic antioxidant. DM condition causes proteins to undergo non-enzymatic glycation with reducing sugar and produces AGE. Production of AGE is followed by oxidative reaction which produces radical compounds. The interaction between AGE and its receptor (RAGE) involves in the development of microvascular and macrovascular complication (Ng, et al., 2013).

In this study, it was found that rats given with VCO showed increased insulin expression compared to rats in control group, although the score was not higher than in metformin group. However, HVCO showed insulin expression which was not significantly different from metformin. This finding corresponds to the work reported before which VCO was able to increase β-cell and serum insulin in diabetic rats (Iranloye, et al., 2013).

VCO and HVCO had the ability to inhibit the continuous damage of β-cell in Langerhans islet. This might be caused by their abilities to decrease oxidative stress which happens in diabetic condition. MCFAs like lauric, palmitic and capric acid which contained in VCO and HVCO are able to increase insulin secretion by releasing intracellular calcium from calcium channel in β-cell membrane plasma. Increase in insulin secretion causes lower production of free radicals, hence decrease β-cell damage. In addition, VCO also contains vitamin E and antioxidants which are able to neutralize free radicals accumulatted in diabetic condition (Supriatna, et al., 2018).

5 CONCLUSION

HVCO is more effective than VCO in lowering blood glucose, HbA1c and sRAGE levels, while increasing SOD level and insulin expression in diabetic rats. Blood glucose, HbA1c, SOD, sRAGE levels and insulin expression in metformin and 4 ml/kgBW HVCO groups were not significantly different after 30 days of treatment, hence, HVCO is effective as antidiabetic and antioxidant in STZ-induced diabetic rats.

ACKNOWLEDGEMENTS

If any, should be placed before the references section without numbering.

REFERENCES

Aehle, W., 2004. *Enzyme in Industry,* Wiley-VCH. Weinheim.

Afriadi, A., 2010. *Uji Efek Virgin Coconut Oil (VCO) terhaap Berat Badan dan Penurunan Kadar Gula Darah (KGD) Tikus Putih Diabetes yang diinduksi Streptozotocin (STZ).* Tesis. Program Studi Magister dan Doktor Ilmu Farmasi. Fakultas Farmasi. Universitas Sumatera Utara.

Akinnuga, A.M., Jeje, S.O., Bamidele, O. and Sunday, V.E., 2014. Dietary consumption of virgin coconut oil ameliorates lipid profiles in diabetic rats. *Physiology Journal, 2014.*

Ashraf, J.M., Ahmad, S., Choi, I., Ahmad, N., Farhan, M., Tatyana, G. And Shahab, U., 2015. Recent advances in detection of AGEs: Immunochemical, bioanalytical and biochemical approaches. *IUBMB Life, 67*(12), pp.897-913.

Bach, A.C. and Babayan, V.K., 1982. Medium-chain triglycerides: an update. *The American Journal of Clinical Nutrition*, *36*(5), pp.950-965.

Baynes, J. and Domonickzak, M.H. 2003. *Medical Biochemistry*, Elsevier Science Mosby. London.

Beysel, S., Unsal, I.O., Kizilgul, M., Caliskan, M., Ucan, B. and Cakal, E., 2018. The effects of metformin in type 1 diabetes mellitus. *BMC Endocrine Disorders, 18*(1), p.1.

Dalmacion, G.V., Ortega, A.R., Pena, I.G. and Ang, C.F., 2012. Preliminary study on the in-vitro susceptibility of *Mycobacterium tuberculosis* isolates to virgin coconut oil. *Functional Foods in Health and Disease, 2*(8), pp.290-299.

Deeds, M.C., Anderson, J.M., Armstrong, A.S., Gastineau, D.A., Hiddinga, H.J., Jahangir, A., Eberhardt, N.L. and Kudva, Y.C., 2011. Single dose streptozotocin-induced diabetes: considerations for study design in islet transplantation models. *Laboratory Animals, 45*(3), pp.131-140.

Dewi, S.S. and Aryadi,T., 2010. Efektifitas virgin coconut oil (VCO) terhadap kandidiasis secara in vitro. In *Prosiding Seminar Nasional & Internasional*.

Eleazu, C.O., Eleazu, K.C., Chukwuma, S. and Essien, U.N., 2013. Review of the mechanism of cell death resulting from streptozotocin challenge in experimental animals, its practical use and potential risk to humans. *Journal of Diabetes and Metabolic Disorders, 12*(1), p.60.

Elsayed, H.H., Elrahman, M.K.A., Emara, A.H. and El-Hafez, A., 2015. Compare effect of fatty acid composition (olive, coconut oil and butter) on adipose liver tissue, and serum lipid profile in albino rats. *IOSR-JBB, 1,* pp.28-38.

Erejuwa, O.O., Sulaiman, S.A., Ab Wahab, M.S., Sirajudeen, K.N.S., Salleh, M.S.M. and Gurtu, S., 2011. Glibenclamide or metformin combined with honey improves glycemic control in streptozotocin-induced diabetic rats. *International Journal of Biological Sciences, 7*(2), pp.244.

Essien, N.M., Bassey, S.C., Nna, V.U. and Ofem, O.E., 2014. Comparative effect of chronic consumption of some edible vegetable oils on lipid profile and some haematological parameters in rats. *Annals of Biological Research, 5*(7)*,* pp.16-21.

Fife, B., 2004. *The Coconut Oil Miracle*, Penguin Group. USA.

Furman, B.L., 2015. Streptozotocin-induced diabetic models in mice and rats*. Current Protocols in Pharmacology, 70*(1), pp.5-47.

Han, X., Tao, Y.L., Deng, Y.P., Yu, J.W., Cai, J., Ren, G.F., Sun, Y.N. and Jiang, G.J., 2017. Metformin ameliorates insulitis in STZ-induced diabetic mice. *PeerJ, 5,* p.e3155.

Handajani, N.S. and Dharmawan, R., 2009. Effect of VCO to leucocyte differential count, glucose levels and blood creatinine of hyperglycemic and ovalbumin sensitized *Mus musculus* Balb/c. *Bioscience, 1*(1), pp.1-8.

Iranloye, B., Oludare, G and, Olubiyi, M., 2013. Anti-diabetic and antioxidant effects of virgin coconut oil in alloxan induced diabetic male sprague dawley rats. *Journal of Diabetes Mellitus*, *3*(4), p.221-226.

Joshi, S.R., Parikh, R.M. and Das, A.K., 2007. Insulin – history, biochemistry, physiology and pharmacology. *Supplement of JAPI, 55,* p.19-25.

Kolondam, B.J., Pokatong, W. And Tallei, T., 2008. Kadar trigliserida dan kolesterol tikus wistar (*Rattus novergicus*) setelah konsumsi virgin coconut oil. *Biosaintifika, 1,* pp.35-44.

Margata, L., Silalahi, J., Harahap, U. and Satria, D., 2018. The effect of dietary oils and hydrolyzed coconut oil on minerals absorption in rats. *Asian Journal of Pharmaceutical and Clinical Research, 11*(1), p.185-190.

McKee, T. and McKee, J.R., 2003. *Biochemistry: The Molecular Basis of Life*, McGraw-Hill. New York, 3rd edition.

Mechanick, J.I., 2006. The rational use of dietary supplements, nutraceuticals, and functional foods for the diabetic and prediabetic patients. In: Mechanick and Brett, editors. *Nutritional Strategies for the Diabetic and Prediabetic Patient*, Taylor & Francis, CRC. New York, p.265-296.

Mohammed, A. and Luka, C.D., 2013. Effect of coconut oil, coconut water and palm kernel oil on some biochemical parameters in albino rats. *Journal of Pharmacy and Biological Sciences*, *6*(3), pp.56-59.

Ng, Z., Chua, K., Iqbal, T. And Kuppusamy, U., 2013. Soluble receptor for advanced glycation end-product (sRAGE)/pentosidine ratio: a potential risk factor determinant for type 2 diabetic retinopathy. *International Journal of Molecular Sciences, 14*(4), pp.7480-7491.

Paliyath, G., Bakovic, M. and Shetty, K., 2011. *Functional Foods, Nutraceuticals and Degenerative Disease Prevention*, Wiley-Blackwell. UK.

Pujol, J., Christinat, N., Ratinaud, Y., Savoia, C., Mitchel, S. and Dioum, E., 2018. Coordination of gpr40 and ketogenesis signaling by medium chain fatty acids regulates beta cell function. *Nutrients, 10*(4), p.473.

Rayfield, E.J. and Valentine, M.V., 2006. Pathophysiology and clinical management of diabetes and prediabetes. In: Mechanick and Brett, editors. *Nutritional Strategies for the Diabetic and Prediabetic Patient*, Taylor & Francis, CRC. New York, p.16-44.

Siddalingaswamy, M., Rayaorth, A. and Khanum, F., 2011. Anti-diabetic effects of cold and hot extracted virgin coconut oil. *Journal of Diabetes Mellitus, 1*(4), pp.118-123.

Silalahi, J., Rosida, R., Putra, E.D.L. and Satria, D., 2016. Hypoglycemic effect of hydrolyzed palm kernel oil in rats. *Der Pharma Chemica, 8*(20), p.182-186.

Supriatna, D., Uray, A.D., Astawan, M., Muchtadi, D. And Wresdiyati, T., 2018. The effect of VCO processing method on blood glucose, cholesterol and pancreatic profile of diabetic mellitus rats (Sprague Dawley). *Warta IHP, 35*(2), p.91-98.

Szkudelski, T., 2001. The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas. *Physiological Research, 50*(6), pp.537-546.

Triplitt, C.L., Reasner, C.A. and Isley, W.L., 2005. Diabetes mellitus. In: DiPiro, J.T., Talbert, R.L., Yee, G.C., Wells, B.G. and Posey, L.M. *Pharmacotherapy: A Patophysiologic Approach,* The McGraw-Hill Companies, Inc. USA, 6th edition.

Vala, G.S. and Kapadiya, P.K., 2014. Medicinal benefits of coconut oil. *International Journal of Life Sciences Research*, *2*(4), pp.124-126.

Vassalotti, J.A., 2006. Nutritional strategies for the patient with diabetic nephropathy. In: Mechanick and Brett, editors. *Nutritional Strategies for the Diabetic and Prediabetic Patient*, Taylor & Francis, CRC. New York, p.149-169.

Yin, D., Tao, J., Lee, D.D., Shen, J., Hara, M., Lopez, J., Kuznetsov, A., Philipson, L.H. and Chong, A.S., 2006. Recovery of islet β-cell function in streptozotocin-induced diabetic mice: an indirect role for the spleen. *Diabetes, 55*(12), p.3256-3263.

Wong, F.N., Chua, K.H., Tan, J.A.M.A., Wong, C.M. and Kuppusamy, U.R., 2018. Glycaemic control in type 2 diabetic patients with chronic kidney disease: the impacts on enzymatic antioxidants and soluble RAGE. *PeerJ, 6*, p.e4421.