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Virgin Coconut Oil (VCO) treatment to reduce the level of LOX-1 and Lp-PLA2: experimental study of high-fat diet on wistar rats

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Abstract

This research aimed to investigate whether Virgin Coconut Oil (VCO) can be effective to reduce the level of lectin-like oxidized LDL receptor 1 (LOX-1) and Lipoprotein-Associated phospholipase A2 (Lp-PLA2) as atherosclerotic biomarker. Thirty Wistar rats were categorized into 5 treatment groups; normal diet (ND), high-fat diet (HFD), HFD with 3 different dosages of VCO consecutively 1 ml, 1.2 ml, and 2 ml per day. The average levels of LOX-1 in three groups of HFD with VCO were significantly lower compared to HFD group (p=0.002, p=0.009, and p=0.038 respectively). The average levels of Lp-PLA2 in three groups of HFD with VCO were also shown to be lower than that of HFD group. However, the differences among these groups were not significant (p=0.93). The treatment of VCO with a dosage of 1.2 ml was significantly effective to reduce the level of LOX-1. Eventhough not statistically significant, VCO could also reduce the level of Lp-PLA2.

Introduction

High-fat diet or atherogenic diet is one type of diet that can accelerate atherosclerosis development (Adekunle *et al*., 2013). High-fat diet is also one of many factors, which significantly causes cardiovascular diseases as it can increase the level of oxidative stress, hypercholesteremia, oxidized LDL (OxLDL), LOX-1 expression on adipose tissue, and Lp-PLA2 activity (Mata *et al*., 1996; Ellis, 2006; De Keyzer *et al*., 2009; Wang *et al*., 2013). LOX-1 mediates OxLDL to penetrate endothelial cells. Thus, LOX-1 is known as the key receptor that bridges the initial and final stage of atherogenesis (Tate, 2007). Another biomarker of atherosclerosis is Lp-PLA2 which is also known as plasma platelet-activating factor acetylhydrolase (plasma PAF-AH). It is one type of enzyme which hydrolyses phospholipids and mostly relates to LDL (Cai *et al.*, 2013). One latest experimental evidence shows that Lp-PLA2 is suspected to be the cause of atherosclerosis development and the loose of plaque, but there are still some controversies in regard to the double effect of anti- and pro-atherogenesis of Lp-PLA2 on atherosclerosis (Immanuel *et al*., 2010; Cai *et al.*, 2013).

Some previous studies showed that the polyphenol in olive oil can help to reduce LOX-1 level and the polyphenol contained in cocoa powder can help to increase HDL level (Baba *et al*., 2007; Castañer *et al.*, 2012). The high level of HDL can reduce the expression and activity of Lp-PLA2 (Guan-ping *et al*., 2012). VCO also contains bioactive components such as medium chain triglyserides (MCT) and polyphenol (Carandang, 2008; Harini *et la*., 2009). However the previous study can only show that VCO improve the level of HDL and reduce the level of serum lipid, and OxLDL (Nevin *et al*., 2004). This research aimed to investigate the effectiveness of VCO to reduce the level of LOX-1 and Lp-PLA2.

Materials and Methods

Materials

The sample of the research consisted of 30 male Rattus novergicus strain Wistar rats, 2 months old, fit (health and active), and had 150-200 gram of body weight. The animals were bred in LPPT Laboratory of Gadjah Mada Univerisity, Yogyakarta-Indonesia were used for this research. The rats were excluded from experiments if they were inactive during adaptation, and were dropped out if they were dead during treatment. This study received approval from the ethic committee of medical faculty of Diponegoro University with number of etichal clearance : 123/EC/FK-RSDK/2014

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Diet and VCO

The rats were divided into 5 groups; normal diet (ND); high-fat diet (HFD); Treatment 1 (T1), which was given a set of HFD and VCO 1ml; Treatment 2 (T2) HFD and VCO 1.2 ml; and Treatment 3 (T3) HFD and VCO 2 ml. The composition of ND was Comfeed AD II, wheat flour, and water; and HFD composition was Comfeed AD II, wheat flour, cholesterol (2%), cholic acid (0.2%), and pig oil (10%) (Murwani *et al*., 2006). VCO was bought from herbal medicine shop. To control the quality of VCO especially the level of polyphenol and MCT, researcher analyzed the polyphenol and MCT content of VCO in UPT Laboratory of Muhammadiyah University Semarang-Indonesia and Chemical Laboratory of Muhammadiyah University Malang-Indonesia before it was given to the rats.

Determination of VCO Dosage

VCO dosage was determined by using VCO human therapy dosage. VCO given to human therapy is 3 tablespoons or equal to 45 ml/day and it was converted to the rat dosage by multiplying with 0.018. First dosage : 45 x 0.018 = 0.81 ≈ 1 ml/day; second dosage : (45 + 90):2 x 0.018 = 1.2 ml/day; third dosage : 90 x 0.018 = 1.6 ≈ 2 ml/day (Pager and Barnes, 1964)

Analysis of LOX-1 and Lp-PLA2

After the 2-month-period of intervention, the blood sample was taken through the rats' eyes to measure the level of LOX-1 and Lp-PLA2 on the serum using the ELISA method with BT-Bioassay Technology Laboratory ELISA KIT. The complete procedure for measurement the level of LOX-1 and Lp-PLA2 were based on BT-Bioassay Technology Laboratory ELISA Catalog with Cat No. E1175Ra for LOX-1 and E0851Ra for Lp-PLA2.

Statistical analysis

The Shapiro Wilk test showed that the data of LOX-1 and Lp-PLA2 did not have normal distribution, the differences among the sample groups on the level of LOX-1 and Lp-PLA2 were analyzed using Kruskal-Wallis test, and followed by Mann Whitney test if the results indicated any significant difference. All data was presented as mean and SD.

Results and Discussion

According to Table 1, there was no differences among groups in body weight before intervention (p=0.287). After 2mo intervention, there was no differences among groups in body weight (p=0.389). There was also no differences intake in rats among groups (p=0.189). The level of cholesterol and LDL in HFD group was higher compared to ND group (p=0.035). This study supports the previous study which showed that HFD is a diet that can cause atherosclerosis development by increasing serum cholesterol level (Murwani *et al*., 2006). However, the level of cholesterol and LDL in treatment groups (T1, T2, and T3) was higher compared to ND dan NFD group.

Effect of high fat diet and VCO to levels of LOX-1

The average levels of LOX-1 of all the sample groups were shown in Table 2. The results of Kruskal-Wallis test showed that there were significant differences among five groups (p=0.01). The average level of LOX-1 in HFD group was significantly higher compared to ND group (p=0.009). The average levels of LOX-1 in T1, T2, and T3 group were all significantly lower than HFD group (p= 0.002, p=0.009, and p=0.038 respectively).

The average level of LOX-1 in HFD group was found to be higher than that of ND group. This is in line with the previous research, which showed that the average level of LOX-1 improves significantly in the rats that were given HFD within 18-week-period of time (Wang *et al.*, 2013). High-fat diet is proven to increase the level of LOX-1 through the increase of pro-inflammatory cytokine especially TNF-α. The increase of TNF-α can restrain the expression of paraoxonase, which covers LDL from oxidation stress so that LDL easily oxidized and become OxLDL (Adekunle *et al*., 2013). Increasing OxLDL resulted in the increase of LOX-1 (Pirillo *et al.*, 2013).

The average levels of LOX-1 in the three dosage of VCO groups were significantly lower than that of HFD group. This may be by the fact that VCO contains an active substance called polyphenol, which functions to capture free radicals and reduces tissue damage caused by free radicals. This research also analyzed the polyphenols compound in VCO. According to the polyphenols test showed that 100 ml VCO contain of 85.76 mg polyphenols. The previous study also suggested that polyphenols compound in VCO was 80 mg/100 oil (Nevin *et al*., 2004).

Polyphenol also restrains LDL oxidation through several mechanisms which include inhibiting lipoxygenase and myeloperoxidase enzyme activity, neutralizing the oxidative stress associated with the damage of endothelial cells in blood vessels due to OxLDL, and acting as an inhibitor of NADPH oxidase in endothel (Tankred, 2011). Through those mechanisms, polyphenols can reduce the level of OxLDL, and the low level of OxLDL can reduce the

Table 1. Mean±SD of Body Weight, Intake, and Profile Lipid of Rats

Groups	Initial Body Weight (g)	2mo Intervention Body Weight (g)	Food Intake (g)	LDL Level (mg/dl)	Cholesterol Level (mg/dl)
ND	183.78±5.74	227.26±13.27	15.69±2.72	20.60±4.03	54.20±7.79
HFD	179.92±7.64	246.06±9.36	13.74±1.89	28.40±8.38	70.00±12.61
T1	173.15±5.11	234.28±15.05	14.08±2.70	31.00±11.46	72.25±6.99
T2	173.40±11.45	234.90±7.37	15.11±1.16	39.50±7.72	83.75±7.85
T3	174.82±11.55	234.28±23.29	17.35±2.34	43.00±17.38	84.50±14.57

Table 2. Mean±SD of LOX-1 and Lp-PLA2 Levels

Groups	Mean ± SD of LOX-1 Levels (ng/ml)	Mean ± SD of Lp-PLA2 Levels (ng/ml)
ND	3,122 ± 1,517*	9,723 ± 7,406
HFD	5,998 ± 1,109*	15,180 ± 8,807
T1	2,774 ± 0,840*	11,793 ± 7,055
T2	2,600 ± 1,741*	12,798 ± 11,717
T3	3,690 ± 1,507*	11,794 ± 10,358

*)Significant different among groups

level of LOX-1 that has also been proven through this research.

In this research, treatment with 1.2 ml dosage can be considered as the optimum dosage in attempt to decrease the level of LOX-1. This dose is equivalent to 67.5 ml or 4.5 tablespoons per day if converted to human doses with the polyphenl contain level of 57.46 mg .

Interestingly the average level of LOX-1 in HFD and 2 ml dosage of VCO group was higher, although not significant, than that HFD and two other doses of VCO group. This can be explained through the following theory: polyphenols could function as both antioxidant and pro-oxidant, and high doses of polyphenols may promote the pro-oxidant effect (Martin *et al.*, 2010). Oxidant is one of many factors that can cause the increasing level of LOX-1 in endothelial cells, smooth muscle cells, and fibroblast (Pirillo *et al.*, 2013). Another component that may also need to be considered is the MCT in VCO.

MCT is a fatty acid with a carbon chain 6-12, which consists of capronic acid (C6: 0), caprylic acid (C8: 0), capric acid (C10: 0) and lauric acid (C12: 0) (Marten *et al*., 2006). Previous studies showed that lauric acid has more probability to increase the total of cholesterol, both LDL and HDL cholesterol than palmitic acid, and MCT can significantly increase the cholesterol serum in hypercholesterolemic patients (mild hypercholesterolemia) (Hornstra *et al.*, 1996; Cater *et al.,* 1997). This finding was in line with this research, where the HFD and 2 ml dosage of VCO group had the highest LDL and cholesterol levels. Other study showed that MCT was also proven to increase adhesion molecules and cause changes that were related to the increasing inflammation response (Marten *et al*., 2006). The increase of oxidative stress due to the high dosage of VCO, the increase of lipids serum, and inflammation are also suspected to be the cause of the high level of LOX-1 in Wistar rats were given HFD and 2 ml dosage of VCO. This dose is equivalent to 90 ml or 6 tablespoons of VCO if converted into the human dosage with the polyphenols content level of 77.18 mg/dl, 42.59 g of lauric acid, 6.7 g caprylic acid, and capric acid 6.64 g. That was according the result of MCT analysis showed that 100 ml VCO contain of 47.32 g lauric acid, 7.45 g caprylic acid, and 7.39 g capric acid.

The amount of MCT that can be tolerated is only 25 - 30g (Marten *et al*., 2006). The consumption of MCT in a large amount may cause nausea, vomiting, gastrointestinal discomfort, abdominal cramps, and osmotic diarrhea (Jeukendrup *et al.*, 2004). During data collection, 3 rats from the treatment group (especially T2 and T3 groups) experienced diarrhea and died. This phenomenon might be explained with those theory.

Effect of high fat diet and VCO to levels of Lp-PLA2

Another biomarker of atherosclerosis discussed in this research is Lp-PLA2. Table 2 showed that the average level of Lp-PLA2 in HFD group was higher compared to ND group. The average levels of Lp-PLA2 of group T1, T2, and T3 were all lower compared to HFD group. Nevertheless, the results of statistical tests showed that there was not any significant difference among those groups (p=0.932). Although not significant, this research showed that the average level of Lp-PLA2 in HFD group was higher compared to ND group. The high level of Lp-PLA2 may be caused by the high level of OxLDL caused by HFD. The high level of OxLDL may increase the level of Lp-PLA2 through P13k

pathway and p38MAPK (Wang *et al*., 2010). This research supports previous research that showed a significant increase of Lp-PLA2 genes in pigs, which suffered severe atherosclerotic lesions for 6 months. Other researches also used pigs, which suffering atherosclerosis as a result of hypercholesterolemia for 36 weeks, also showed that there was an increase of Lp-PLA2 activity and was associated with the increasing level of lisophospatidilcholine, OxLDL, and inflammation rates (De Keyzer *et al.*, 2009; Karakas *et al*., 2010).

The average levels of Lp-PLA2 in the groups of HFD and three dosage of VCO were lower compared to HFD group only, although not significant. This may be by the fact that polyphenols in VCO could increase the level of HDL (Nevin *et al.,* 2004). The high level of HDL can inhibit the expressions and activities of Lp-PLA2 through PPARγ (Guan-ping *et al*., 2012). Lp-PLA2 is produced by macrophages and foam cells in unstable atherosclerotic plague, and later penetrates the circulation system and becomes Lp-PLA2 secreted (Reddy *et al.*, 2009; Cai *et al.*, 2013). Lp-PLA2 is also strongly expressed by cell nuclei and macrophages that are located around the vulnerable plaque rupture, with relatively weak staining in less advanced lesions (Reddy *et al*., 2009; Guan-ping *et al.,* 2012). Therefore, the increased or decreased level of Lp-PLA2 seem significant in atherosclerosis with unstable plaques. According to this research, the levels of LOX-1 and Lp-PLA2 among VCO groups with different dosage did not have clinical significant differences

Conclusion

VCO treatment can significantly reduce the level of LOX-1 in the Wistar rats, which were given the set of high-fat diet, and the effective dosage is 1.2 ml. VCO treatment can also reduce the level of Lp-PLA2 in the Wistar rats, which were given the set of high-fat diet, although statistically not significant.

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