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Antioxidant and Hepatoprotective Effects of Virgin Coconut Oil at Maximum Physical Activity

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Abstract: Objectives: The purpose of this study was to determine the protective effects of virgin coconut oil (VCO) treatment on hepatic oxidative stress and antioxidant defenses after maximum physical activity. Methods: This study used 24 healthy male ratss. The rats were divided into four groups randomly consisted of six rats in each group. The control group (P0) was given 2 mL water, the treatment groups (VCO-1, VCO-2, and VCO-4) were given VCO 1 mL/200gBW, 2 mL/200gBW and 4 ml/200gBW, respectively, per day using gavage spuit. The rats were trained to swim for a month, 30 min/day in the 1st week, 35 min/day in the 2nd week, 40 min/day in the 3rd week, and 45 min/day in the 4th week. After 28 days, the rats were forced to perform maximal activity by putting the rats in water with no exit. Blood samples were collected immediately after the maximum physical activity, and then all rats were killed and liver tissues were collected. The malondialdehyde; glutation peroxidase;serum glutamic oxaloacetic transaminaseand serum glutamic pyruvat transaminase level was then measured.Results: Virgin coconut oil increased swimming time to exhaustion, levels of glutathione peroxidase in liver, which were accompanied by corresponding decreases in the malondialdehyde (MDA), alanine transaminase (ALT) and aspartate transaminase (AST) content. Conclusion: The results from this study indicate that virgin coconut oil is effective in the prevention of oxidative stress ollowing maximum physical activity.

Keywords: Antioxidant, hepatoprotective effects, virgin coconut oil

INTRODUCTION

Regular and appropriate exercise benefits health, such as reducing cardiovascular disease risk, some cancers, diabetes, and osteoporosis [1]. Aerobic exercise can provide human health benefits by improving cardiorespiratsory fitness which can increase the quality of life, work efficiency, musculoskeletal function, and cardiopulmonary system strength [2]. Besides giving a positive impact on the body, physical exercise also have a negative impact. Maximum physical activity, as well as exhaustive exercise, can elevate oxidative stress, leading to an imbalance between the body's oxidation system and antioxidant enzymes. Hence, accumulation of free radicals such as reactive oxygen species (ROS) can cause damage to many parts of the cells such as proteins, DNA, and cell membranes by stealing their electrons via a process called oxidation[3,4]. The release of ROS could result in lipid peroxidation in the mitochondrial membrane. Damaged mitochondria were found to reduce cellular respiratsion and adenosine triphosphate (ATP) generatsion; they are also among the primary causes of fatigue [5].

During maximum physical activity, some organs such as liver, kidneys and other organs will experience hypoxia and ischemia because of the higher amount of oxygen consumption in the working muscles. After physical activity is complete, blood flow will return to normal through the reperfusion process. In the reperfusion process free radicals are produced, which will damage the cell membrane through the reaction of lipid peroxidation [6]. Malondialdehyde (MDA) is one of the oxidized species of the membrane lipid that can be produced by maximal physical activities or by high intensity endurance exercises. The level of MDA can be used as a general indicator for free radical level and indirectly pointed the oxidant capacity [7,8].

The results showed maximum physical activity can cause an increase in MDA levels [9], decreased levels of enzymatic antioxidants in liver tissue [9,10] which resulted in liver damage which was characterized by increased levels of alanine transaminase (ALT) and aspartate transaminase (AST) [11,12,13,14].

In human body there is an endogenous mechanism of antioxidant or anti-free radicals. The free radicals formed will be neutralized by the elaboratsion of a defense system between antioxidant enzymes and a number of non-enzyme antioxidants [15,16]. The results of the study report that the administratsion of antioxidants derived from natural or synthetic sources from outside the body is needed to neutralize free radicals formed during physical activity, especially maximum physical activity [17,18,19,20].

One of the natural sources that contain antioxidants is virgin coconut oil (VCO), oil that comes from fresh old coconut (Cocosnucifera), which is processed at low temperatsures [21]. Virgin coconut oil is one of the excellent products that not only takes a lot of attention from the people of Indonesia and the world but also has been consumed as a health product, even the Food and Drug Administratsion (FDA) has included it in the list of safe natural foods [22]. The active compounds contained in the VCO include tocopherols, tocotrienols, phytosterols, phytostanol, flavonoids, polyphenols, phospholipids, and medium chain triglycerides [23].

Scientifically, VCO has been reported to exert various pharmacological activities such as anti-arthritis and antioxidant [24], anti-thrombogenicity [25], antihyperlipidemia [26], cardioprotective [27], antimicrobial [28-30], anti-osteoporosis [31], hepatoprotective [32], and antinociceptive and anti-inflammatory [33]. Interestingly, recent clinical studies demonstratsed that VCO possesses at least the antihypercholesterolemia [34] and anti-Alzheimer [35].

The purpose of this study was to determine the antioxidant and hepatoprotective effects of virgin coconut oil at maximum physical activity.

METHOD

Tools

The tools used in this research were laboratsory glassware, vortex (Thermo), test tube (Iwaki), Beckman coulter (Beckman), link Dako epitope retrieval (Dako), tissue processor (Leica), spectrophotometer (Shimadzu), analytical balance (Boeco), syringe for oral feeding, flask 10 ml, stopwatch, hairdryer, animal box, syringe 1 ml, funnel, pipette, parchament, spatula, thermometer, air pump and ruler.

Materials

Materials used in this study were virgin coconut oil (VICO®) is the production of PT. Patria Wiyata VICO Indonesia that has been registered with the Food and Drug Supervisory Agency with the registratsion number POM TR.052 652 611.

Chemicals

Commercial assay kits for the detection of MDA and GPx were purchased from PT. Biozatix Indonesia. All other chemicals used were of analytical grade and purchased from local suppliers.

Animal

Male rats of Wistar strain weighing 200-220 g were obtained from the Animal House Faculty of Pharmacy, University of Sumatera, Utara. They were placed in plastic cages in a room under standard laboratsory conditions (temperatsure 20 to 300C, relative air humidity 45 to 55%, and 12/12 h light/dark cycle). The rats were fed with a basal diet and water ad libitum. All animal experiments conducted during the present study got prior permission from Institutional Animal Ethics Committee, Department of Biology, Faculty of Mathematics and Science, University of Sumatera Utara.

Experimental design

This study used 24 healthy male rats. The rats were divided into four groups randomly consisted of six rats in each group. The control group (P0) was given 2 mL water, the treatment groups (VCO-1, VCO-2, and VCO-4) were given VCO 1, 2, and 4ml/200gBW, respectively, per day using gavage spuit, for 28 days. The rats were trained to swim for a month, 30 min/day in the 1st week, 35 min/day in the 2nd week, 40 min/day in the 3rd week, and 45 min/day in the 4th week. After 28 days, the rats were forced to perform maximal activity by putting the rats in water with no exit. The apparatsus used was an acrylic plastic pool (60, 50, and 50 cm in length, width, and height, respectively) filled with fresh water, which was maintained at 25 ± 0.5 °C at a depth of 40 cm. Exhaustion was determined by observing the loss of coordinated movements and failure to return to the surface within 10 seconds. Blood samples were collected immediately after the exhaustive

exercise, and then all rats were killed and liver tissues were collected. The MDA; GPx; AST and ALT level was then measured.

2.6 Biochemical assay

Blood sample (3ml) was collected into plain tube and allowed to clot for 45 min at room temperatsure. Serum was separatsed by centrifugation at 2500 rpm at 30°C for 15 min and utilized for the estimation of various biochemical parameters, namely, alanine transaminase (ALT) and aspartate transaminase (AST). The level of ALT and AST was measured by spectrophotometer.

Fresh liver tissue was homogenized in phosphate buffer at pH 7.4. The homogenate was used to estimate the levels of glutathione peroxidase (GPx) and malondialdehyde (MDA). MDA and GPx were analysed by using a malondialdehyde and glutathione peroxidase assay kit according to the manufacturer's instruction.

2.7 Statistical analysis

Data of research weretested for homogeneity and normality to determine the type of statistics to be used. Data were analyzed using one-way ANOVA test to determine the mean difference between treatments using SPPS 19.0 program. If there is a significant difference, further proceed with the Tukey test to determine the differences value between treatment groups. Based on the significance value, $p<0.05$ is considered statistically significant.

RESULT

Effect of VCO on swimming time to exhaustion of rats

Endurance exercise is an important parameter to evaluate anti-fatigue treatments, and the forced swimming test has been widely used for this purpose with high reproducibility [36]. The lengths of the swimming time to exhaustion indicate the degree of exercise tolerance andfatigue. As shown in Fig. 1, swimming time to exhaustion of the VCO-1, VCO-2, and VCO-4 groups were significantly longer than that of the control (C) group ($p < 0.05$) with increased ratses of 103.93, 145.29, and 210.59%, respectively. This result indicates that VCO enhanced the exercise endurance and had anti-fatigue effects.

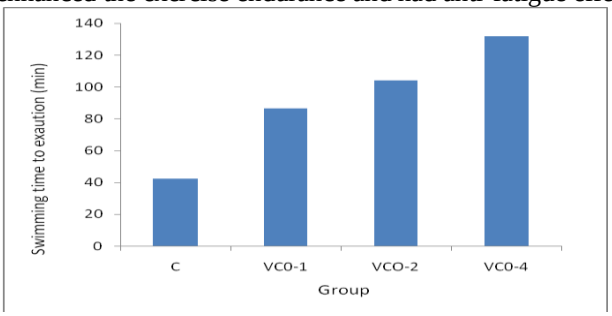

Figure. 1: Effects of virgin coconut oil on swimming time to exhaustion of rats. Data are mean ± standard deviation (SD); n=6, *$p < 0.05$ compared with control (C) group.

Effect of VCO on glutation peroxidase (GPx) level

The liver is a critical physiological metabolic organ in organisms, involved in almost all metabolism substance, and contains higher levels of antioxidant enzymes than other tissues, which in turn release more ROS with increased lipid peroxidation products [37]. Recent studies have demonstratsed a tissue-specific expression of GPx, with their highest activities occurring in the liver [4,12,36].

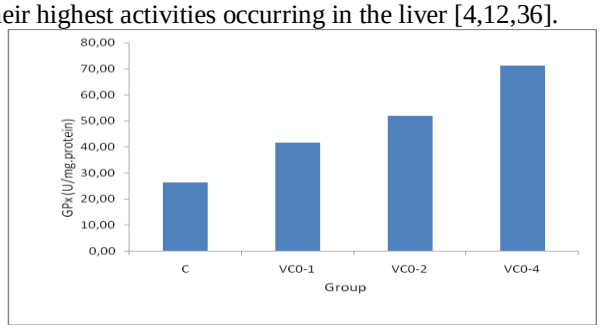

Figure. 2: Effect VCO on the glutathione peroxidase levels in the liver o f rats. Data are the mean±SD. *P<0.05 compared with the control (C) group.

As shown in Fig 2, the lever GPx levels of the VCO-1, VCO-2, and VCO-4 groups were significantly higher than that of the C group ($p < 0.05$), with increased ratses of 57.68, 96.33, and 169.62 %, respectively.

Effect of VCO on malondialdehyde (MDA) level

Maximum physical exercise increases the production of ROS, which consequently attack the membrane lipids and results in lipid peroxidation product formation. Significant increases in lipid peroxidation products in liver after exhaustive exercise have been recorded in several studies [38]. Malondialdehyde (MDA), one of the final products of polyunsaturatsed fatty acid peroxidation, has been widely investigated in exercise studies as a marker of oxidative stress [39]. As shown in Fig. 3, the MDA content of liver of the VCO-1, VCO-2, and VCO-4 groups were significantly lower than that of the C group ($p < 0.05$). Moreover, the decreased ratses in the liver were 58.05, 70.16, and 85.81 %, respectively. These results indicate that VCO effectively reduced lipid peroxidation.

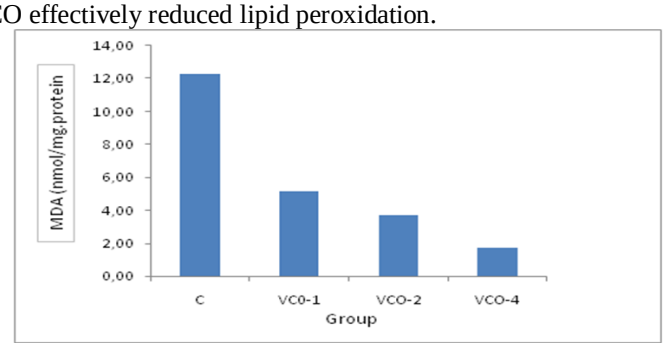

Figure.3: Effect VCO on the malondialdehyde (MDA) levels in the liver tissues of rats. Data are the mean±SD. *P<0.05 compared with the control (C) group.

Effect of VCO on alanine transaminase and aspartate transaminase level

Liver is the largest organ in the human body and key organ of metabolism, including glycogen storage, decomposition of red blood cells, plasma protein synthesis, and detoxification. Serum alanintransamninase and aspartattrnasaminase are the most sensitive markers of liver damage because theirlocation are in cytoplasmic and are released into the circulation after hepatocellular damage.

As shown in Fig. 4, the AST and ALT level of liver of the VCO-1, VCO-2, and VCO-4 groups were significantly lower than that of the C group ($p < 0.05$). AST level decreased in the liver were 38.27, 47.87, and 65.10 % respectively, and ALT level decreased 31.51, 47.13, and 58.07 % respectively.

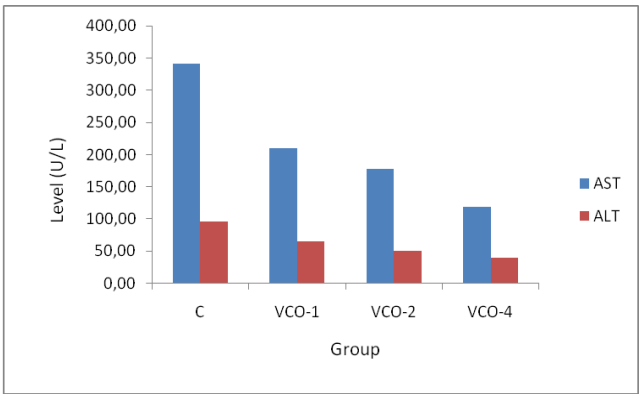

Figure.4: Effect VCO on the AST and ALT levels in serum of rats. Data are the mean±SD. *P<0.05 compared with the control (C) group.

It is known, one of the causes of the decline in performance during physical activity, especially heavy physical activity is the increase in ROS. ROS are highly reactive molecules that cause lipid peroxidation in the membrane structure and damage the cellular structure. The release of ROS could result in lipid peroxidation in the mitochondrial membrane. Damaged mitochondria were found to reduce cellular

respiratsion and adenosine triphosphate (ATP) generatsion; they are also among the primary causes of fatigue.

In this study, the administratsion of VCO during an exercise program can increase rats swimming time (Fig. 1). One of the supporting theories is that VCO can increase the durability of rats when doing maximum physical activity is because the content of VCO is rich in antioxidants and polyfenol compounds. The antioxidant content of VCO includes tocopherols, tocotrienols, flavonoids and some polyphenol compounds [23,41]. The content of antioxidants and polyphenol compounds in VCO can reduce the occurrence of lipid peroxidation which is characterized by a decrease in MDA concentratsion (Fig. 3) and an increase in the concentratsion of glutathione peroxidase levels (Fig. 2). Yeap et al. examine the anti-stress and antioxidant effects of virgin coconut oil in vivo. In his study, the rats that were soaked in water caused lipid peroxidation which was characterized by an increase in MDA levels from 3.51 ± 0.88 to 16.82 ± 1.76 nmol / g protein and a decrease in SOD levels of 12.57 ± 1.3 to 5, 97 ± 1.77 U / mg protein. In the group of rats given VCO at a dose of 10ml / KgBW reduced the lipid peroxidation process which was characterized by a decrease in MDA levels to 5.38 ± 1.59 nmol / g protein accompanied by an increase in SOD levels of 9.85 ± 1.26 U / mg protein . This study shows a decrease in MDA levels, increased levels of endogenous antioxidants due to VCO administratsion were also reported by many researchers [42-46]. Dosumu et al. reported that VCO with a dose of 6.7ml / KgBW could reduce testicular MDA levels of rats induced with alcohol at a dose of 7ml / KgBW with a significance level of $p < 0.001$. The study was conducted on five treatment groups, namely group I (control group), II (alcohol), III (alcohol-VCO), IV (alcohol / VCO), V (VCO / alcohol) and MDA levels obtained for each group of (I = 10.68 ± 1.04; II = 29.24 ± 2.51; III = 8.45 ± 1.07; IV = 6.62 ± 0.70; V = 18.01 ± 2.45 nmol / min) [42]. The anti-stress activity and antioxidants in VCO are associated with the presence of polyphenol compounds and medium chain fatty acids [43]. Nevin and Rajamohan also reported the antioxidant effectiveness of VCO compared to copra (CO) oil and peanut oil (GO) with Vitamin E as a control. Vitamin E, 8% VCO, 8% COC, and 8% GO were administered for 45 days in rats. The results showed that VCO increased the activity of enzyme catalase (CAT), superoxide dismutase (SOD), glutathione reductase (GR), glutathione peroxidase (GPx) and decreased levels of MDA and conjugate dienes (CD) in liver, heart and kidney organs compared to CO and GO [44]. Nandakumaran et al. also reported daily administratsion of VCO to rats for 30 days at a dose of 1ml (group I), 2ml (group II) and 4 ml (group III) can increase SOD levels. It is known that the enzymes SOD, CAT, GPx and GR are endogenous antioxidants that function to neutralize free radicals formed in the body [45]. Increased levels of endogenous antioxidant activity (GSH, CAT, SOD) and decreased MDA levels in diabetic-induced rats due to VCO administratsion have also been reported [46].

Besides containing antioxidants and polyphenol compounds, VCO also contains MCT. When viewed from the energy system in sports, the potential use of VCO which is rich in MCT is very potential to be used as a fast energy source available especially for endurance sports. MCT is fast hydrolyzed, more complete than LCT, and absorbed faster. The higher nature of MCT solubility in water so that MCT can enter the circulatory system, enter the liver directly through the veins (veins) and quickly burn it into energy, which means that MCT is not stored (buried) in body tissues [47]. MCT is delivered in the form of free fatty acids into the blood faster than LCT. LCT is re-esterified in the mucosa of the small intestine into chylomicron, which is a combination of LCT and albumin that enters the lymph channels and requires the enzyme carnitine to enter the mitochondria. MCT does not bind to albumin because MCT in the form of fatty acids is easier to interact with water (polar), rapidly absorbed into the portal vein directly into the liver and into the mitochondrial membrane to be oxidized to energy so that MCT is not accumulated in adipose tissue [48 , 49]. The nature of MCT that is not metabolized like conventional fat can be a good source of energy so it can increase endurance in rats that do maximum physical activity. The results of this study are supported by Silalahi et al who examined the effect of acute VCO administratsion on rats with a dose of 0.1ml, 0.2ml, and 0.4ml / 20grBW compared to palm oil. The results of his study concluded that the administratsion of VCO and palm oil in an acute manner could increase stamina, where the higher the concentratsion of fatty oil given the stronger the stamina produced. When compared to the effect of VCO with palm oil, VCO results are stronger to increase stamina compared to palm oil which is measured by the ability of swimming rats [50].

Exercise has various effects on skeletal muscle and hepatic function, enhancing both nutrient metabolism and antioxidant capacity. Accumulating evidence indicates that exhaustive exercise could injure liver cells by decreasing blood flow in the liver [51] and the portal vein [50], which often causes hypoxia of hepatocytes, eventually inducing their necrosis. Alanine transaminase (ALT) and AST is a liver specific enzyme. High levels of ALT and AST are indicative of liver injury [53,54].

As shown in Fig. 4, the AST and ALT levels of liver of the VCO-1, VCO-2, and VCO-4 groups were significantly lower than the C group ($p < 0.05$). The decrease in AST and ALT levels in this study due to

antioxidant activity and the content of polyphenol compounds found in VCO can reduce MDA levels (Fig. 3) and increase the antioxidant levels of GPx (Fig. 3). The results of this study are supported by research that reports the VCO dose of 10ml / kgBW for 7 days can reduce liver damage induced by giving paracetamol dose of 3g/kgBW in rats. Reduced liver damage is known from histopathological examination, decreased levels of AST, ALT, alkaline phosphat (ALP), liver weight, and increased viability of rats liver cells [55,56].

CONCLUSIONS

The results of the study show that giving virgin coconut oil during exercise can increase levels of endogenous antioxidant, reduce lipid peroxidation, alanine transaminase and aspartate transaminase. These results indicate that VCO has an antioxidant and hepatoprotective effect on maximum physical activity

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