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Research Article

ANTIOXIDANT AND HEPATOPROTECTIVE EFFECTS OF VIRGIN COCONUT OIL AT MAXIMUM PHYSICAL ACTIVITY

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ABSTRACT

Objective: 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 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 ml/200 g BW, 2 ml/200 g BW, and 4 ml/200 g BW, 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 the 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 (MDA), glutathione peroxidase (GPx), and serum glutamic-oxaloacetic transaminase and serum glutamic-pyruvate transaminase level were then measured.

Results: VCO increased swimming time to exhaustion, levels of GPx in the liver, which were accompanied by corresponding decreases in the MDA, alanine transaminase, and aspartate transaminase content.

Conclusion: The results from this study indicate that VCO is effective in the prevention of oxidative stress following maximum physical activity.

Keywords: Virgin coconut oil, Antioxidant, Hepatoprotective, Malondialdehyde, Maximum physical activity.

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INTRODUCTION

Exercise that is carried out routinely, regularly, and according to needs, is beneficial for health such as reducing the risk of degenerative diseases such as cardiovascular disease, some cancers, diabetes, and osteoporosis [1]. Aerobic exercise can provide many benefits to human health by increasing the fitness of the cardiorespiratory system which can improve quality of life, work efficiency, musculoskeletal function, and the strength of the cardiopulmonary system [2]. Besides, giving a positive impact on the body, physical exercise also has a negative impact. Maximum physical activity 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 through a process called oxidation [3,4]. The release of ROS could result in lipid peroxidation in the mitochondrial membrane. Cellular respiration and regeneration of adenosine triphosphate (ATP) can be disrupted if there are damaged mitochondria, they are also a major cause of fatigue [5].

Maximum physical activity or high-intensity resistance can produce malondialdehyde (MDA), one of the oxidized species of membrane lipids. The level of MDA can be used as a general indicator for free radical level and indirectly pointed the oxidant capacity [6,7,8]. The results showed that maximum physical activity can cause an increase in MDA levels [9] and 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) [11-14].

One of the natural sources that contain antioxidants is virgin coconut oil (VCO), oil that comes from fresh old coconut (Cocos nucifera), which is processed at low temperatures [15]. Scientifically, VCO has been reported to exert various pharmacological activities such as antiarthritis and antioxidant [16], anti-thrombogenicity [17], antihyperlipidemia [18], cardioprotective [19], antimicrobial [20-22], antiosteoporosis [23], hepatoprotective [24], and antinociceptive and anti-inflammatory [25]. Interestingly, recent clinical studies demonstrated that VCO possesses at least the antihypercholesterolemic [26] and anti-Alzheimer [27]. The purpose of this study was to determine the antioxidant and hepatoprotective effects of VCO at the maximum physical activity.

MATERIALS AND METHODS

Tools

The tools used in this research were laboratory 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, parchment, spatula, thermometer, air pump, and ruler.

Materials

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

Chemicals

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

Animal

Wistar strain male rats weighing 200–220 g were obtained from the Animal House, Faculty of Pharmacy, University of Sumatera Utara. They are placed in a plastic cage in a room under standard laboratory conditions (temperatures from 20 to 30°C, relative air humidity of 45–55%, and 12/12 h of light/dark cycles). Basal diets and water *ad libitum* are given to meet rat nutrition. Animal trial permits carried out during this study were obtained from the 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. Rats were divided into four groups randomly consisting 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 4 ml/200 g BW, 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 the maximal activity by putting the rats in water with no exit. 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 s. Blood samples were collected immediately after the exhaustive exercise, and then, all rats were killed and liver tissues were collected. The MDA, GPx, and AST and ALT level were then measured.

Biochemical assay

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

Phosphate buffer at pH 7.4 is used for homogenization of fresh liver tissue. The homogenate was used to estimate the levels of GPx and MDA. MDA and GPx were analyzed using an MDA and GPx assay kit according to the manufacturer's instruction.

Statistical analysis

Data of research were tested for homogeneity and normality to determine the type of statistics to be used. One-way ANOVA test was used for data analysis to determine the average difference between treatments using the SPPS 19.0 program. Then, if there is a difference, it will be followed by the Tukey test to determine the difference in value between treatment groups. Based on the significance value, $p<0.05$ is considered statistically significant.

RESULTS

Effect of VCO on swimming time to exhaustion of rats

Endurance exercise is an important parameter to evaluate antifatigue treatments, and the forced swimming test has been widely used for this purpose with high reproducibility [28]. The level of exercise tolerance and fatigue can be seen with a long swim time for fatigue. As shown in Fig. 1, swimming time to exhaustion of the VCO-1, VCO-2, and VCO-4 groups was significantly longer than that of the control (C) group ($p<0.05$) with increased rates of 103.93, 145.29, and 210.59%, respectively. This result indicates that VCO enhanced the exercise endurance and had antifatigue effects.

Effect of VCO on 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 [29]. Recent studies have demonstrated a tissue-specific expression of GPx, with their highest activities occurring in the liver [4,12,28].

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 rates of 57.68, 96.33, and 169.62%, respectively.

Effect of VCO on MDA level

Maximum physical exercise increases the production of ROS, which consequently attacks the membrane lipids and results in lipid peroxidation product formation. Significant increases in lipid peroxidation products in the liver after exhaustive exercise have been recorded in several studies [30]. MDA, one of the final products of polyunsaturated fatty acid peroxidation, has been widely investigated in exercise studies as a marker of oxidative stress [31]. As shown in Fig. 3, the MDA content of the liver of the VCO-1, VCO-2, and VCO-4 groups was significantly lower than that of the C group ($p<0.05$). Moreover, the decreased rates in the liver were 58.05, 70.16, and 85.81%, respectively. These results indicate that VCO effectively reduced lipid peroxidation.

Effect of VCO on ALT and AST level

The 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 ALT and AST are the most sensitive markers of liver damage because their location is in cytoplasmic and are released into the circulation after hepatocellular damage.

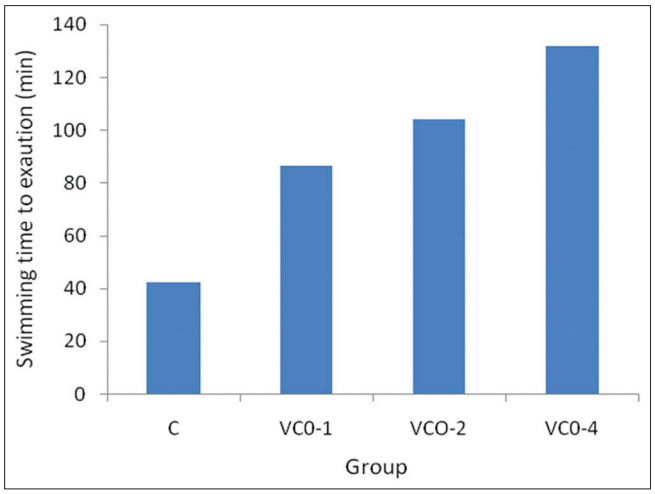

Fig. 1: Effects of virgin coconut oil on swimming time to exhaustion of rats. Data are mean ± standard deviation; n=6, *p<0.05 compared with control (C) group

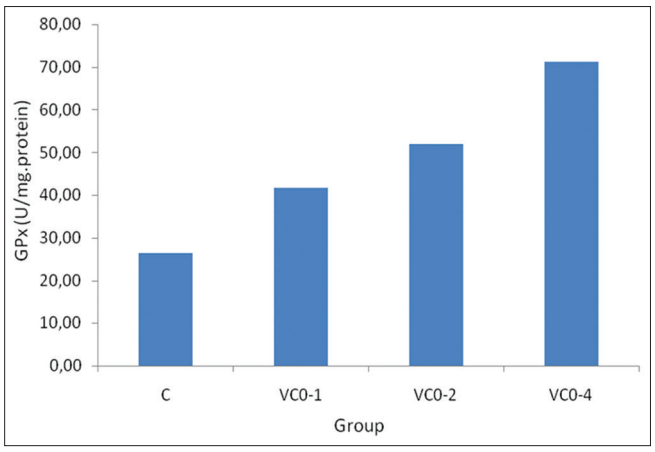

Fig. 2: Effect of virgin coconut oil on the glutathione peroxidase levels in the liver of rats. Data are the mean ± SD. *p<0.05 compared with the control (C) group

As shown in Fig. 4, the AST and ALT level of the 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 was 38.27, 47.87, and 65.10%, respectively, and ALT level decreased 31.51, 47.13, and 58.07%, respectively.

DISCUSSION

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. Cellular respiration and regeneration of ATP can be disrupted if there are damaged mitochondria, they are also a major cause of fatigue.

In this study, the administration 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 polyphenol compounds. The antioxidant content of VCO includes tocopherols, tocotrienols, flavonoids, and some polyphenol compounds [32]. The content of antioxidants and polyphenol compounds in VCO can reduce the occurrence of lipid peroxidation which is characterized by a decrease in MDA concentration (Fig. 3) and increase in the concentration of GPx levels (Fig. 2). Nevin in his study reported that VCO showed a significant antithrombotic effect in animal studies, where animals were fed with VCO, antioxidant vitamin levels also increased [33].

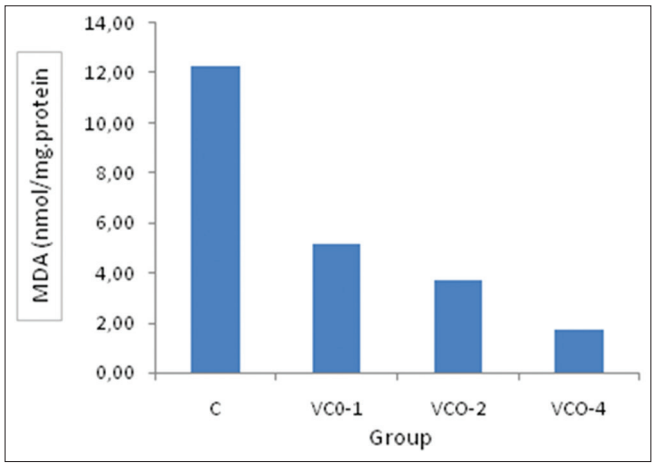

Fig. 3: Effect of virgin coconut oil on the malondialdehyde levels in the liver tissues of rats. Data are the mean ± SD. *p<0.05 compared with the control (C) group

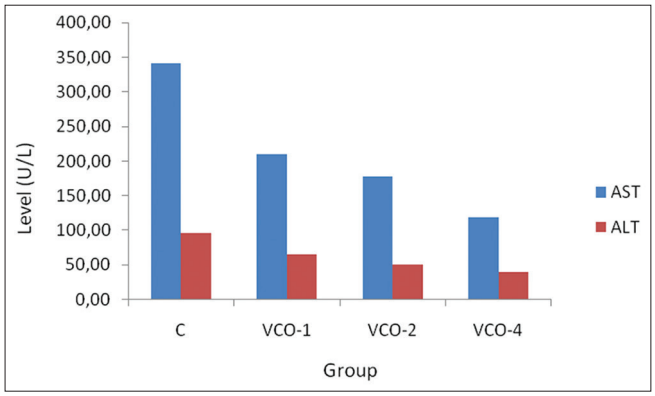

Fig. 4: Effect of virgin coconut oil on the aspartate transaminase and alanine transaminase levels in serum of rats. Data are the mean ± SD. *p<0.05 compared with the control (C) group

Yeap *et al*. examined the antistress and antioxidant effects of VCO *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 superoxide dismutase (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 10 ml/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 administration were also reported by many researchers [28,34-37]. Dosumu *et al*. reported that VCO with a dose of 6.7 ml/kg BW could reduce testicular MDA levels of rats induced with alcohol at a dose of 7 ml/kg BW with a significance level of $p<0.001$. The study was conducted on five treatment groups, namely Groups I (control group), II (alcohol), III (alcohol-VCO), IV (alcohol/VCO), and 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, and V=18.01±2.45 nmol/min [38].

The antistress activity and antioxidants in VCO are associated with the presence of polyphenol compounds and medium chain fatty acids [39]. Antioxidant activities and phenolic compounds such as tocopherol and tocotrienol are found in VCO and are useful in the prevention of various chronic diseases including cancer and cardiovascular disease [40]. Determination of antioxidant status shows that defense against ROS increases with the administration of VCO and prevents lipid peroxidation [40].

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), SOD, glutathione reductase (GR), GPx, and decreased levels of MDA and conjugated dienes in liver, heart, and kidney organs compared to CO and GO [41]. Nandakumaran *et al*. also reported daily administration of VCO to rats for 30 days at a dose of 1 ml (Group I), 2 ml (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 [42]. Increased levels of endogenous antioxidant activity (GSH, CAT, and SOD) and decreased MDA levels in diabetic-induced rats due to VCO administration have also been reported [43].

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. One of the unique natures of MCT is its solubility in water. This nature allows MCT to enter the blood circulatory system and then enter the liver through veins to be turned quickly into energy without being stored (buried) in the body tissues [44].

MCT is delivered in the form of free fatty acids into the blood faster than LCT. LCT is reesterified 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 [34,45]. 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 the maximum physical activity. The results of this study are supported by Silalahi *et al*. who examined the effect of acute VCO administration on rats with a dose of 0.1 ml, 0.2 ml, and 0.4 ml/20 g BW compared to palm oil.

The results of his study concluded that the administration of VCO and palm oil in an acute manner could increase stamina, where the higher the concentration 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 [35].

Exercise has various effects such as increasing nutritional metabolism and antioxidant capacity, also has an effect on skeletal muscle and liver function. Accumulating evidence indicates that exhaustive exercise could injure liver cells by decreasing blood flow in the liver [36] and the portal vein [35], which often causes hypoxia of hepatocytes, eventually inducing their necrosis. ALT and AST are a liver-specific enzyme. High levels of ALT and AST are indicative of liver injury [37,38].

As shown in Fig. 4, the AST and ALT levels of the 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 10 ml/kg BW for 7 days can reduce liver damage induced by giving paracetamol dose of 3 g/kg BW in rats. Reduced liver damage is known from histopathological examination, decreased levels of AST, ALT, alkaline phosphate (ALP), and liver weight, and increased viability of rats liver cells [24,46].

CONCLUSION

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

AUTHORS' CONTRIBUTIONS

All the authors have the same contribution in this research (carried out the research, collected the data, analyzed the data, and formatted the manuscript).

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest in this research and this article.

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