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Antioxidant Effect of Virgin Coconut Oil on Urea and Creatinine Levels on Maximum Physical Activity

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Keywords: Virgin coconut oil; Antioxidant; Urea; Creatinine; Malondialdehyde; Maximum physical activity

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Abstract

BACKGROUND: Maximal physical activity can produce an imbalance between reactive oxygen species (ROS) and antioxidants which are possibly related to fatigue and tissue injury. One of the natural sources that contain antioxidants is virgin coconut oil (VCO).

AIM: This study aimed to determine the protective effects antioxidant of virgin coconut oil (VCO) treatment on urea and creatine level on 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 gBW, 2 mL/200 gBW and 4 ml/200 gBW, respectively, per day using gavage spuit. 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. The urea, creatinine, malondialdehyde and glutation peroxidase level was then measured.

RESULTS: 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 gBW, 2 mL/200 gBW and 4 ml/200 gBW, respectively, per day using gavage spuit. 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. The urea, creatinine, malondialdehyde and glutathione peroxidase level was then measured.

CONCLUSION: The results of this study indicate that virgin coconut oil is effective in the prevention of oxidative stress following maximum physical activity.

Introduction

It is known that physical activity can increase the production of various types of free radicals which result in cell damage [1]. When the production of free radicals exceeds the antioxidant cellular defence, oxidative stress will occur [2]. High levels of oxidative stress lead to excessive generation of reactive oxygen species (ROS). Reactive oxygen species 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 respiration and adenosine triphosphate (ATP) generation; they are also among the primary causes of fatigue [3]. Malondialdehyde (MDA) is one of the results of lipid peroxidation induced by free radicals during maximum physical exercise or high-intensity endurance training [4], [5], so MDA is a general indicator used to determine the number of free radicals and indirectly assess the body's oxidant capacity [6].

The results showed that maximum physical activity could lead to lower levels of antioxidant enzyme superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione-S-transferase [7] and increased levels of MDA [8], [9]. During physical activity, several organs like the heart, kidneys, and other organ are in hypoxia and ischemia as oxygen is higher at the contracted muscles. This disturbs the metabolism, and cell homeostasis also causes damage in the tissue [10]. After the exercise, the blood quickly flows back into the kidneys. Along with that, a big oxidant is released, which can damage kidney cells and activate leucocyte. Therefore, the kidneys will severely damage [2]. Progressive damage in the kidneys can be measured clinically by measuring serum creatinine and urea level [11].

Naturally, body has a defense mechanism against ROS by an endogenous antioxidant system which consists of superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT) [12]. This enzyme plays an important role as first-line protection against the harmful effects of ROS generated by various sources. However, when the production of ROS is excessive, the function of endogenous antioxidant will be limited. Therefore, the supplementation of exogenous antioxidant from diet becomes important to protect cells against the deleterious effect of ROS [13].

One of the natural sources that contain antioxidants is VCO, is an oil that comes from the fruit of old coconut (Cocos nucifera) which is processed at low temperatures [14]. VCO is one of the prima donna products that not only takes a lot of attention from the people of Indonesia and the world but also has been widely consumed as health products, even the Food and Drug Administration (FDA) has included it in the list of safe, natural foods [15]. The active compounds contained in the VCO include tocopherols, tocotrienols, phytosterols, phytostanol, flavonoids, polyphenol, phospholipid, and medium-chain triglyceride [16]. This study aims to determine the effect of supplementation VCO on MDA, GPx, urea and creatinine level when performing maximum physical activity.

Material 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 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 registration 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 was obtained from the Animal House Faculty of Pharmacy, University of Sumatera, Utara. They were placed in plastic cages in a room under standard laboratory conditions (temperature 20 to 30°C, 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 (C) was given 2 ml water, the treatment groups (VCO-1, VCO-2, and VCO-4) were given VCO 1, 2, and 4ml/200g bw, respectively, per day, using gavage spuit, for 28 days. After 28 days, the rats were forced to perform the maximal activity by putting the rats in water with no exit. The apparatus used was an acrylic plastic pool (60, 50, and 50 cm in length, width, and height, respectively) filled with freshwater, 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. The MDA, GPx, urea and creatinine level were then measured.

Biochemical assay

Blood (3 ml) was collected into a plain tube and were allowed to clot for 45 min at room temperature. Serum was separated by centrifugation at 2500 rpm at 30°C for 15 min and utilised for the estimation of various biochemical parameters, namely, MDA, GPx, urea, and creatinine. MDA, GPx were analysed by using a malondialdehyde and glutathione peroxidase assay kit according to the manufacturer's instruction. Serum levels of urea and creatinine were measured using a blood chemistry analyser.

Statistical analysis

Data of research result determined homogeneity and normality to determine statistical analysis used. Data were analysed 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 value of the difference between treatment groups. Based on the significance value, $p < 0.05$ is considered statistically significant.

Results

Effect of VCO on urea and creatinine level

Based on the results of the analysis it was found that the mean level of urea in the control group (C); VCO-1, VCO-2, and VCO-4 is 60.48 ± 0.60, 41.04 ± 1.51, 35.80 ± 0.82; 28.00 ± 1.10 mg/dl, respectively. Meaning analysis using One Way Anova test showed that the mean urea levels in the four groups were significantly different ($p < 0.05$).

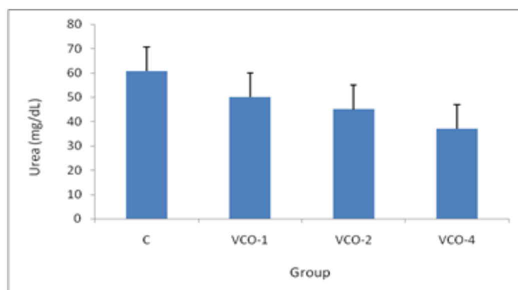

*Figure 1: Effect VCO on urea levels in the serum of rats. Data are the mean ± SD; *P < 0.05 compared with the control (C) group*

As shown in Fig. 1, the urea level of the VCO-1, VCO-2, and VCO-4 groups were significantly lower than that of the C group ($p < 0.05$). Urea level decreased were 17.54, 25.86, and 39.08% respectively.

Based on the results of the analysis it was found that the mean creatinine levels of the control group (C); VCO-1, VCO-2, and VCO-4 is 0.94 ± 0.17, 0.78 ± 0.17, 0.68 ± 0.01, 0.58 ± 0.17 mg/dl, respectively. Meaning analysis with One Way Anova test showed that the mean creatinine levels in all four groups were significantly different ($p < 0.05$). As shown in Fig. 2, the creatinine level of the VCO-1, VCO-2, and VCO-4 groups were significantly lower than that of the C group ($p < 0.05$). Creatinine level decreased were 17.02, 27.65, and 38.29% respectively.

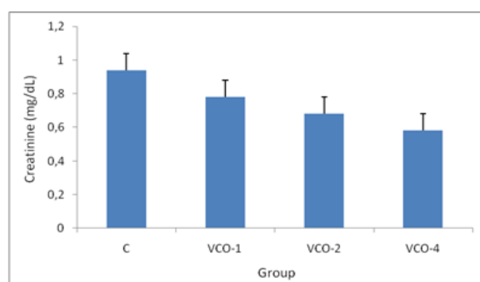

*Figure 2: Effect VCO on creatinine levels in the serum of rats. Data are the mean ± SD; *P < 0.05 compared with the control (C) group*

Effect of VCO on malondialdehyde (MDA) level

Based on the results of the analysis it was found that the mean level of malondialdehyde (MDA) control group (C); VCO-1, VCO-2, and VCO-4 is 12.42 ± 0.61, 6.60 ± 0.44, 4.89 ± 0.51, 2.66 ± 0.42 nmol/L, respectively. Meaning analysis using the One Way ANOVA test showed that the mean malondialdehyde (MDA) levels in the four groups of results were significantly different ($p < 0.05$).

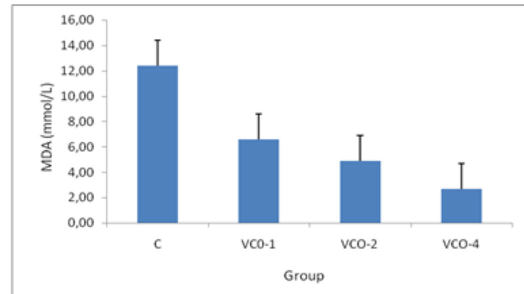

*Figure 3: Effect VCO on the malondialdehyde (MDA) levels in the liver tissues of rats. Data are the mean ± SD. *P < 0.05 compared with the control (C) group*

As shown in Fig. 3, the MDA content of serum 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 serum were 46.85, 60.62, and 78.52 %, respectively. These results indicate that VCO effectively reduced lipid peroxidation.

Effect of VCO on glutathione peroxidase (GPx) level

Based on the results of data analysis, the mean levels of the control group (C), VCO-1, VCO-2, and VCO-4 Glutathione Peroxidase (GPx) were 34.97 ± 1.18, 56.43 ± 1.75, 67.51 ± 1.41, 87.51 ± 1.41, respectively. Meaning analysis using the One Way ANOVA test showed that the mean Glutathione Peroxidase (GPx) levels in the four groups were significantly different ($p < 0.05$).

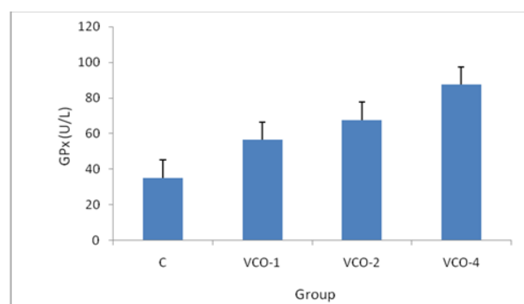

*Figure 4: Effect VCO on the glutathione peroxidase levels in the serum of rats. Data are the mean ± SD; *P < 0.05 compared with the control (C) group*

As shown in Fig 4, the 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 61.36, 93.05, and 150.24 %, respectively.

Discussion

It is known, physical activity causes blood flow and metabolism to decrease significantly in the liver and kidneys [17]. In moderate exercise, renal blood flow may fall to 25% of the resting value, although glomerular filtration rate (GFR) is preserved through an increase in the filtration fraction, which may double, limiting the transfer of metabolites or substances through the glomeruli and reducing the extent of exercise proteinuria [18], [19]. Extreme exercise, however, may decrease GFR by up to 50% [20], [21]. This decrease in blood flow causes hypoxia in the kidneys. After the physical activity is complete, the blood will quickly return to the kidney and at the same time will release a large number of oxidants [2]. Increasing amounts of oxidants will damage kidney cells and will activate leukocytes so that damage to the kidneys gets worse. The process of ischemia-reperfusion and leukocyte activation can also cause oxidative stress during and after exercise in the kidneys. Both of these mechanisms are very responsible for the occurrence of oxidative stress in the organs and extra muscular tissues after physical exercise [17]. Several studies have been conducted to see the effect of physical activity on kidney function. Foran et al. found that the short-term effects of marathon training increased creatinine and blood urea nitrogen (BUN) [22]. According to Warburton et al., concentrations of urea and creatinine increased after prolonged strenuous exercise in which this increase was associated with decreased renal blood flow and glomerular filtration rate, increased protein catabolism and creatinine release due to muscle work [23].

The results of this study indicate VCO administration can reduce levels of urea and creatine when rats perform maximum physical activity. It is known, VCO contains antioxidants including tocopherol, tocotrienol, flavonoids and some polyphenol compounds [16], [17], [18], [19], [20], [21], [22], [23], [24]. With the presence of these antioxidants, VCO supplementation for 28 days will increase glutathione peroxidase levels (Figure 4) and reduce the occurrence of lipid peroxidation which is characterised by a decrease in MDA levels (Figure 3).

The results of this study were supported by several researchers who reported the effect of VCO on oxidative stress [25], [26], [27], [28], [29], [30]. Famurewa et al. reported that administration of Cadmium Chloride (CDCl2) 5 mg/kg orally to rats for 5 weeks could increase urea levels (29.81%) and creatinine 36.25% compared to the control group given aqua destilata. Meanwhile, administration of VCO polyphenol (PF) 10 mg/kg + CDCl2 5 mg/kg; PF dose of 20 mg/kg + CDCl2 dose of 5 mg/kg; PF 50 mg/kg + CDCl2 dose of 5 mg/kg can reduce urea levels by 20.57%, 40.66%, 52.63% and decrease in creatinine levels by 30.67%, 39.32% and 50% compared to the group given CdCl2. In his research, the sub-chronic administration of Cd (5 mg/kg) significantly decreased renal activities of SOD, CAT, and GPx, as well as the renal level of non-enzymatic antioxidant, GSH, compared to control. Marker of lipid peroxidation, MDA, significantly increased in renal tissue of rats treated with Cd. Meanwhile, the administration of VCO polyphenol together with Cd, can increase of SOD, CAT, GPx, GSH level and reduce MDA levels when compared with the control group given Cd. [25].

Dosumu et al. reported that VCO with a dose of 6.7 ml/kg BW could reduce testicular MDA levels of mice induced with alcohol at a dose of 7 ml/kg BW [26]. Yeap et al. reported untreated mice undergoing the forced swim test and chronic cold restraint stress were found to exhibit higher lipid peroxidation (MDA) and lower antioxidant enzyme SOD levels. VCO was able to reduce lipid peroxidation and increase the activity of SOD in the serum of mice undergoing the forced swim test and the brains of mice subjected to chronic cold restraint. The results of this study are associated with the presence of polyphenol compounds and medium-chain fatty acids [27]. Nevin and Rajamohan also reported that giving VCO to mice for 45 days in vivo and in vitro can increased the activity of catalase (CAT), superoxide dismutase (SOD), glutathione reductase (GR) enzymes, glutathione peroxidase (GPx) and reduce MDA levels and conjugated dienes (CD) in the heart, heart and kidney organs. Nandakumaran et al. also reported the administration of VCO in rats for 30 days with a dose of 1 ml, 2 ml and 4 ml, respectively, to increase SOD levels [28]. Nandakumaran et al. also reported daily administration of VCO to rats for 30 days at a dose of 1 ml, 2 ml and 4 ml can increase SOD levels. It is known that the enzymes SOD, CAT, GPx and GR are endogenous antioxidants that function to neutralise free radicals formed in the body [29]. Increased levels of endogenous antioxidant activity (GSH, CAT, SOD) and decreased MDA levels in diabetic mice induced by alloxan due to VCO administration have also been reported [30].

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