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 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/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 transaminase and 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 following 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 cardiorespiratory 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 respiratration and adenosine triphosphate (ATP) generation; 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 elaboration of a defense system between antioxidant enzymes and a number of non-enzyme antioxidants [15,16]. The results of the study report that the administration 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 temperatures [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 Administration (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 demonstrated 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 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 were 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 (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 apparatus 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 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, 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 were tested 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 and fatigue. 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 rates 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 glutathione 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 demonstrated 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 of rats. Data are the mean±SD.

*P<0.05 compared with the control (C) group.

As shown in Fig 2, the liver 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 as 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 result 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 polyunsaturated 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 rates 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 alanine transaminase and aspartate transaminase are the most sensitive markers of liver damage because their location is 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
respiration and adenosine triphosphate (ATP) generation; they are also among the primary causes 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 [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 concentration (Fig. 3) and an increase in the concentration 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 administration 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 is 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 administration 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 administration 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. MCT 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 administration 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 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 [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.

REFERENCES
[1] Khan KM, Thompson AM, Blair SN, Sallis JF, Powell KE, Bull FC, Bauman AE. Sport and exercise as contributors to the health of nations. Lancet 2012; 380:59-64.
[2] Imayama I, Alfano C, Mason CE, Wang C, Xiao L, Duggan C, Campbell K, Foster-Schubert K, Wang CY, McTiernan, A. Exercise adherence, cardiopulmonary fitness and anthropometric changes improve exercise self-efficacy and health-related quality of life. J. Phys. Act. Health 2013; 10: 676-689.
[3] Powers SK, Ji LL, Kavazis AN, Jackson MJ. Reactive oxygen species: impact on skeletal muscle. Compr Physiol 2011; 1:941-969.
[4] Lin Y, Liu HL, Fang J, Yu CH, Xiong YK, Yuan K. Anti-fatigue and vasoprotective effects of quercetin-3-O-gentiobiose on oxidative stress and vascular endothelial dysfunction induced by endurance swimming in rats. Food Chem Toxicol 2014;68:290-296.
[5] Echtay KS, Roussel D, St-Pierre J, Jekabsons MB, Cadenas S, Stuart JA, Harper JA, et al. Superoxideactivates mitochondrial uncoupling proteins. Nature 2002;415:96–99.
[6] Daniel, RM, Stelian S, Dragomir C. The effect of acute physical exercise on the antioxidant status of the skeletal and cardiac muscle in the Wistar rat. Romanian Biotechnological Letters 2010; 15:56-61.
[7] Wang L, Zhang HL, Zhou YJ, Ma R, Lv QJ, Li XL, et al. The decapeptide CMS001 enhances swimming endurance in mice. Peptides 2008;29:1176-1182.
[8] Lyle N, Gomes A, Sur T, Munshi S, Paul S, Chatterjee S, et al. The role of antioxidant properties of Nardostachys jatamansi in alleviation of the symptoms of the chronic fatigue syndrome. Behav Brain Res 2009;202:285-90.
[9] Sholikhah AM, Wirjatmadi B, Adriani M. Effects of Purple Sweet Potatoes on Oxidative Stress Biomarkers in Rats Subjected to Exhaustive Exercise. Health Notions 2018;2:174-177.
[10] Yunarsa IPPA, Adiatmika IPG. Levels of antioxidant superoxide dismutase (SOD) rat liver in heavy physical activity. Jurnal Medika Udayana 2018; 7: 143-147.
[11] Droge W. Free Radicals in the Physiological Control of Cell Function. Physiol Rev 2002; 82: 47-95.
[12] Chevion S, Moran, DS, Heled Y, Shani, Y, Regev Y, Abbou B, et al. Plasma Antioxidant Status and Cell Injury After Severe Physical Exercise. Proc.Nati.Acad.Sci.USA 2003; 100: 5119-5123.
[13] Pettersson J, Hindorf U, Persson P, Bengtsson T, Malmqvist U, Werkstrom V, et al. Muscular exercise can cause highly pathological liver function tests in healthy men. Br J Clin Pharmacol 2007; 65:253-259.
[14] Amah UK, Okoli SN, Ogbodo EC, Ezeugwunwe IP, Analike RA, Onuegbu AJ, et al Effect of short term aerobic exercise on liver function of Students College of Health Sciences and Technology, Nnamdi Azikiwe University.Nnewi Campus, Anambra State, Nigeria. Global Scientific Journals 2017;5:67-77.
[15] Christopher PI, Wenke JC, Nofal T, Armstrong RB. Adaptation to lengthening contraction-induced injury in mouse muscle. J Appl Physiol 2004;97:1067-1076.
[16] Urso ML, Clarkson PM. Oxidative stress, exercise, and antioxidant supplementation. Toxicology 2003;189:41-54.
[17] Ostojic SM, Stojanovic MD, Djordjevic B, Jourkesh M, Vasiljevic N. The Effect of a 4-week coffeefubulation on antioxidant status, endurance, and anaerobic performance in college athletes. Res Sports Med 2008;16:281-94.
[18] Gupta C, Gupta PH, Singh B. Effect of vitamin supplementation on exercise induced oxidative stress in trained elite Indian cyclists. Am J Biomed Sci 2009;1:166-70.
[19] Bentley DJ, Dank S, Coupland R, Midgley A, Spence I. Acute antioxidant supplementation improves endurance performance in trained athletes. Res Sports Med 2012;20:1-12.
Kalpana K, Kusuma DL, Lal PR, Khanna GL. Effect of spirulina on antioxidant status and exercise-induced oxidative stress of Indian athletes in comparison to a commercial antioxidant. Asia J Exerc Sports Sci 2012;9:36-48.

Sutarmi, Rozaline H. Taklukkan penyakit dengan VCO. Jakarta; Penebar Swadaya: 2006; 5-24.

Alamsyah AN. Perpaduan sang penakluk penyakit VCO+buah merah. Agromedia Pustaka; Jakarta: 2005.

Carandang EV. Health benefits of virgin coconut oil explained. PJCS 2008;31:1-12.

Vysakh A, Ratheesh M, Rajmohan TP, Pramod C, Premlal S, Girish kumar B, et al. Polyphenolics isolated from virgin coconut oil inhibits adjuvant induced arthritis in rats through antioxidant and anti-inflammatory action. Int Immunopharmacol 2014;20:124-130.

Voon PT, Ng TK, Lee VK, Nesaretam K. Virgin olive oil, palm olein and coconut oil diets do not raise cell adhesion molecules and thrombogenicity indices in healthy Malaysian adults. Eur J Clin Nutr 2015;69:712-716.

Nevin KG, Rajamohan T. Beneficial effects of virgin coconut oil on lipid parameters and in vitro LDL oxidation. Clin Biochem 2004;37:830-835.

Babu AS, Veluswamy SK, Arena R, Guazzi M, Lovie CJ. Virgin coconut oil and its potential cardioprotective effects. Postgrad Med 2014;126:76-83.

Shilling M, Matt L, Rubin E, Visitation MP, Haller NA, Grey SF, Woolverton CJ. Antimicrobial effects of virgin coconut oil and its medium-chain fatty acids on Clostridium difficile. J Med Food 2013;16:1079-1085.

Silalahi J, Permata YM, Putra EDP. Antibacterial activity of hydrolyzed virgin coconut oil. Asian J Pharm Clin Res 2014;7:90-94.

Elysa, Harahap U, and Silalahi J. Antibacterial activity of Enzymatic Hydrolysis of Virgin Coconut oil against Salmonella. International Journal of PharmTech Research 2014;6:589-599.

Hayatullina Z, Muhammad N, Mohamed N, Soelaiman IN. Virgin coconut oil supplementation prevents bone loss in osteoporosis rat model. Evid Based Complement Alternat Med 2012;2012:1-8.

Zakaria ZA, Roafie MS, Somchit MN, Zuraini A, Sulaaiman MR, Teh LK, Salleh MZ, Long K. Hepatoprotective activity of dried- and fermented-processed virgin coconut oil. Evid Based Complement Alternat Med 2011;2011:142739.

Zakaria ZA, Somchit MN, Mat Jais AM, Teh LK, Salleh MZ, Long K. In vivo antiinocceptive and anti-inflammatory activities of dried and fermented processed virgin coconut oil. Med Princ Pract 2011;20:231-236.

Cardoso DA, Moreira AS, de Oliveira GM, Raggio Luiz R, Rosa G. A coconut extra virgin oil-rich diet increases HDL cholesterol and decreases waist circumference and body mass in coronary artery disease patients. Nutr Hosp 2015;32:2144-2152.

Hu Yang I, De la Rubia Ortí JE, Selvi Sabater P, Sancho Castillo S, Rochina MJ, Manresa Ramón N, Montoya-Castilla I. Coconut oil: Non-alternative drug treatment against Alzheimer’s disease. Nutr Hosp 2015;32:2822-2827.

Huang WC, Chiu WC, Chuang HL, Tang DW, Lee ZM, Wei L, et al. Effect of Curcumin Supplementation on Physiological Fatigue and Physical Performance in Mice. Nutrients 2015;7:905-921.

Kan NW, Huang WC, Lin WT, Huang CY, Wen KC, et al. Hepatoprotective effects of ixora parviflora extract against exhaustive exercise-induced oxidative stress in mice. Molecules 2013;18:10721-10732.

Narkhede AN, Jagtap SD, Nirmal PS, Giramkar SA, Nagarkar BE, OP, Kulkarni, et al. Anti-fatigue effect of amarkand on endurance exercise capacity in rats. Complementary and alternative medicine 2016;16:1-7.

Roohi BN, Moradlou AN, Booboli L. Influence of curcumin supplementation on exercise-induced oxidative stress. Asian J Sports Med 2017;8:e35776.

Carandang EV. Health Benefits Of Virgin Coconut Oil Explained. PJCS 2008;31:1-12.

Srivastava, Y., Semwal, A.D., Majumdar, A., Yildiz, F., 2016. Quantitative and qualitative analysis of bioactive components present in virgin coconut oil. Cogent Food Agric 2(1): 1164929.

Dosumu OO, Duru FIO, Osinubi AA, Oremosu AA, Noronha CC. Influence of virgin coconut oil (VCO) on oxidative stress, serum testosterone and gonadotropic hormones (FSH, LH) in chronic ethanol ingestion. Agric. Biol. J. N. Am 2010;1:1126-1132.

Yeap SK, Beh BK, Ali NM, Yusof HM, Ho WY, Koh SP, et al. Antistress and antioxidant effects of virgin coconut oil in vivo. Experimental And Therapeutic Medicine 2015;9:39-42.
Nevin KG, Rajamohan T. Virgin coconut oil supplemented diet increased the antioxidant status in rats. Food Chem 2006;99:260-266.

Nandakumar M, Al-sarraf H, Al-fadhli R, Al-shammari M, Al-harmi J, Al-saleh E. Effect of oral administration of coconut oil on hematological and metabolic parameters in female adult rats. Nutritional Therapy & Metabolism 2009;27:183-188.

Iranloye B, Oludare G, Olubiyi M. Anti-diabetic and antioxidant effects of virgin coconut oil in alloxan induced diabetic male Sprague Dawley rats. Journal of Diabetes Mellitus 2013; 3:221-226.

Bach AC and Babayan VK. Medium-chain triglycerides: an update. Am J Clin Nutr 1982;36:950-962.

Fushiki T, Matsumoto K., Inoue K, Kawada T, and Sugimoto E. Swimming Endurance Capacity of Mice Is Increased by Chronic Consumption of Medium Chain Triglycerides. Jurnal of Japan Institute of Nutrition 1994;22:531-539.

Che Man Y and Manaf MA. Medium-Chain Triacylglycerols. In: (editor fereidoon) nutraceutical and specialty lipids and their co-product. United State of America: Taylor and Francis Group 2006; 27-28, 33.

Silalahi J, Pertiwi D, Dalimunthe A, and Silalahi Y.C.E. Effect of acute consumption of coconut and palm oil on swimming capacity endurance of mice (Mus musculus). International Journal of PharmTech Research. 2015;8:55-59.

Rowell LB, Blackmon JR, and Bruce RA. Indocyanine green clearance and stimulated hepatic blood flow during mild to maximal exercise in upright man. Journal of Clinical Investigation 1964; 43: 1677-1690.

K. Ohnishi, M. Saito, T. Nakayama et al., “Portal venous hemodynamics in chronic liver disease: effects of posture change and exercise,” Radiology, vol. 155, no. 3, pp. 757–761, 1985.

Duan FF, Guo Y, Li JW, Yuan K. Antifatigue effect of luteolin-6-C-neohesperidoside on oxidative stress injury induced by forced swimming of Rats through modulation of Nrf2/ARE signaling pathways. Oxidative Medicine and Cellular Longevity 2017; 1:13.

Huang WC, Chiu WC, Chuang HL, Tang DW, Lee ZM, Wei L, et al. Effect of curcumin supplementation on physiological fatigue and physical performance in mice. Nutrients 2015;7: 905-921.

Zakaria ZA, Rofiee MS, Somchit MN, Zuraini A, Sulaiman MR, Teh LK, et al. Hepatoprotective Activity of Dried- and Fermented-Processed Virgin Coconut Oil. Evidence-based complementary and alternative medicine 2011;2011:1-8.

Rofiee MS, Zakaria ZA, et al: Involvement of CYP450 system in hepatoprotective activity of Malaysian Agricultural Research and Development Institute (MARDI)-produced virgin coconut oils. AJPP 5: 2526-2536, 2011.