

The Effect of Virgin Coconut Oil (VCO) on Malondialdehyde, Glutathione Peroxidase, and Cortisol Hormone Levels



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ABSTRACT: Vigorous physical activity is a physical movement that produces excessive free radicals due to leakage of electrons from the mitochondrial transport system. The body's antioxidant system is reduced due to the inability of the body's defenses to reduce the production of free radicals resulting in oxidative stress, so it requires a supply of antioxidants from outside the body such as Virgin Coconut Oil (VCO). The purpose of this study is to determine the effect of VCO administration on malondialdehyde levels, glutathione peroxidase levels and cortisol hormone levels in male Wistar rats subjected to strenuous physical activity. The research method uses an experimental with posttest only control group design. The experimental subjects were 24 male Wistar rats which were randomly divided into 4 groups (K1, K2, K3, and K4). The administration of VCO at dose of 25 mL and 50 mL are based of human weight 70 kg and the conversion dose in mice (BB = 200 g) resulted in 0.45 mL/200 g BW/day and 0.9 mL/200 g BW / day which is made into a 1 mL solution and given orally for 28 days. K3 and K4 groups were given strenuous physical activity and given VCO at a dose of 0.45 and 0.9 mL/200 g BW/day. The measurement of malondialdehyde levels using the TBARS method. The glutathione peroxidase (GPx) and the cortisol hormone using ELISA method at PSPG UGM in June 2022. The lowest average levels of MDA and GPx were in the K1 group. One-Way Anova test result showed that the levels of malondialdehyde, glutathione peroxidase, and the cortisol hormone in the K3 and K4 groups had significant differences with K2 group ($p < 0.05$). The administration of VCO at doses of 0.45 mL/200 g BW/day and 0.9 mL/200 g BW/day could affect the levels of malondialdehyde, glutathione peroxidase, and the hormone cortisol in male Wistar rats subjected to strenuous physical activity.

KEYWORDS: Virgin coconut oil, malondialdehyde, glutathione peroxidase, cortisol hormone

I. INTRODUCTION

Physical activity is a physical movement that generates free radicals as a result of electron leakage from the mitochondrial transport system in metabolic processes.(1) Excessive strenuous physical activity triggers an increase in cortisol levels which are regulated by the hypothalamus through Hypothalamus-Pituitary-Adrenal (HPA) axis.(2) Excessive production of free radicals can cause oxidative stress resulting in increased lipid peroxidation which is marked by increased levels malondialdehyde (MDA) in blood endothelial cells. One of Antioxidants produced by the body are glutathione peroxidase (GPx), which will decrease when there is an increase in free radicals, therefore antioxidants are needed from outside the body.(3) Virgin Coconut Oil (VCO) is pure coconut oil made from fresh coconut meat which is processed without heating, which is rich in antioxidants α - *tokoferol* and polyphenols which can reduce oxidative stress. So far research on the effect of VCO administration on MDA levels, GPx and the hormone cortisol in strenuous physical activity are still limited.(4)

Excessive physical activity can cause fatigue that affects immune function, causing respiratory infections in athletes. Psychological and physiological factors also play an important role in the relationship between exercise and respiratory infections.(5) Heavy physical activity causes oxidative stress due to increased free radical production which exceeds the capacity of antioxidant enzymes in the body. The increase in free radicals in strenuous physical activity can be neutralized by consuming antioxidants from outside the body such as Virgin Coconut Oil (VCO).(6) VCO is food that is easily available, affordable and convenient to consume. VCO contains antioxidants (*a-tocopherol*, *beta carotene*, and high phenolic compounds which are effective in counteracting free radicals. Sinaga (2014) proved that administering VCO at doses of 1 mL/day, 2 mL/day, and 3 mL/day can reduce MDA levels in rats with maximum physical activity for 4 weeks.(7) Angrela (2020) proved that giving VCO at a dose of 4 mL/200 g BW for rats/day for 28 days can reduce blood creatinine levels in male rats *Rattus Norvegicus*.(8)

The use of animals as models in biomedical research is common in experiments directly on humans. Apart from being at risk of threatening health, it is also considered unethical and can cause physical and psychological disturbances which can be fatal. According to Fitria (2014) rats of the Wistar strain *Rattus Norvegicus* species are one of the most ideal laboratory animals so they are often used in preclinical research.(9)

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II. MATERIAL AND METHOD

The research type is experimental with posttest only control group design. The research sample consisted of 24 male Wistar rats using simple random sampling techniques according to the inclusion criteria and then divided into four groups, namely the control group and three treatment groups. The rats are given food pellets, the standard Citra feed™ and drinking water in the form of distilled water, the temperature of the maintenance room is around 28^o– 32^o C with sufficient ventilation and room area.

Research Instruments

The rat cages build in dimensions of L: 40 cm, W: 30 cm, H: 30 cm with food place. Nigushi Scale™ mouse scales, gloves, dropper pipette, Eppendorf tube, spectrophotometer, micropipette, ELISA reader.

Sample Preparation

A 24 of male Wistar rats were acclimatized in the PSPG UGM laboratory and divided into 4 groups there are control group and three treatment group and each group are consisting of 6 rats. The rats were adapted for 7 days before giving a treatment. The experimental animals were given a standard food consisting of 20-25% protein, 45-55% starch, 10-12% fat, and 4% crude fiber and drink the same water every day for 7 days.(10)

Physical Activity

Swimming in a container which is no way out as hard as it can until almost exhausted and drowning. The Swimming duration is approximately 30 minutes for 28 days.

The Administration of Virgin Coconut Oil (VCO)

The Virgin Coconut Oil (VCO) using SIOLA™ is given orally at a dose of 0.45 mL/200 g BW/day and 0.9 mL/200 g BW/day for 28 days.

Malondialdehyde (MDA)

The measurement of MDA levels (mg/dL) in the blood of male Wistar rats using the TBARS method and spectrophotometer at 532 nm. The blood samples were taken on 28th day.

Glutathione Peroxidase (GPx)

The measurement of GPx levels (ng/mL) in the blood of male Wistar rats which using ELISA method and ELISA reader at 450 nm. Blood samples were taken on 28th day.

Cortisol Hormone

The measurement of hormone cortisol levels (ng/L) in the blood of male Wistar rats using the ELISA method and ELISA reader at 450 nm. Blood samples were taken on 28th day.

Data analysis

The average levels of malondialdehyde (MDA), glutathione peroxidase (GPx) and the hormone cortisol are presented descriptively in the form of tables and graphs. Data normality was tested using Shapiro Wilk and data homogeneity was tested using Levene test. The distribution of grade data MDA, GPx and cortisol hormone were found to be normal and homogeneous, so it was continued with parametric One-Way Anova test those who get a value of p <0.05 continue with Tukey test.

III. RESULT

The effect of Virgin Coconut Oil (VCO) on the levels of malondialdehyde (MDA), glutathione peroxidase (GPx) and the hormone cortisol in male rats with heavy physical activity was carried out for 28 days. Until the end of the study, none of the experimental animals were sick or died. The results of examining levels of MDA, GPx and the hormone cortisol are listed in Table 1.

Table 1. Test for Normality, Homogeneity, and Mean Levels of MDA, GPx, and Cortisol Hormone

Variable		Group				Sig.(p)
		K1 N=6	K2 N=6	K3 N=6	K4 N=6	
up to MDA (nmol/mL)	Mean	1.43	8.96	3.63	2.47	
	Std. deviation	0.26	0.36	0.32	0.26	
	Shapiro Wilk	0.985*	0.648*	0.439*	0.583*	
	Levene Test					0.806**
	One Way Anova					0.000***

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up to GPx (u/mL)	Mean	73.70	23.67	54.92	65.47	
	Std. deviation	1.44	1.67	2.04	2.46	
	<i>Shapiro Wilk</i>	0.960*	0.963*	0.863*	0.983*	
	<i>Levene Test</i>					0.496**
	<i>One Way Anova</i>					0.000***
Hormone Cortisol (ng/L)	Mean	21.88	15.13	21.24	18.32	
	Std. deviation	0.33	0.35	0.48	0.48	
	<i>Shapiro Wilk</i>	0.873*	0.242*	0.151*	0.573*	
	<i>Levene Test</i>					0.503**
	<i>One Way Anova</i>					0.000***

Information: * *Shapiro Wilk* $p > 0,05$ ** *Levene Test* $p > 0,05$ *** *One Way Anova* $p < 0,05$

Table 1 shows the average of MDA levels. The lowest average was in the K1 group which only received standard feed without being given heavy physical activity, then followed successively by group K4 who was given VCO at a dose of 0.9 mL/200 g BW/day and group K3 who was given VCO at a dose of 0.45 mL/200 g BW/day and both were given physical activity heavy. In group K2 which received standard feed and given strenuous physical activity to obtain the average of MDA level the highest. Based on Shapiro Wilk test all groups of MDA levels were normally distributed ($p > 0.05$) and homogeneity test using Levene test the results are homogeneous ($p > 0.05$). One-Way Anova test results showed significant differences in all groups ($p = 0.000$).

The highest average levels of GPx was in the K1 group which was giving standard feed without being given heavy physical activity, then successively followed by group K3 who was given VCO at a dose of 0.45 mL/200 g BW/day and group K4 who was given VCO at a dose of 0.9 mL/200 g BW/day and both were given physical activity heavy. Group K2 which was giving standard feed and strenuous physical activity have the lowest average levels of glutathione peroxidase. Based on Shapiro wilk test result, all group of glutathione peroxidase were normally distributed ($p > 0.05$) and homogeneity test using Levene test the result is $p = 0.496$ ($p > 0.05$) means that the data is homogeneous so that the data analysis uses One-Way Anova test fulfilled. One-Way Anova test result shows a value of $p = 0.000$, which means that there is a significant difference in all groups.

The lowest average of cortisol hormone levels was in the K2 group which was giving the standard feed and were given strenuous physical activity, followed successively by group K4 who were given VCO at a dose of 0.9 mL/200 g BW/day and group K3 who were given VCO at a dose of 0.45 mL/200 g BW/day and both were given physical activity heavy. In the K1 group that received standard feed without being given strenuous physical activity obtained the highest average cortisol hormone. Based on the Shapiro Wilk test result, all groups of cortisol hormone levels were normally distributed ($p > 0.05$) and the homogeneity test results using Levene test get a value of $p = 0.503$ ($p > 0.05$). One-Way Anova test results get a value of $p = 0.000$ which means there are significant differences in all groups.

Malondialdehyde (MDA) Levels

The differences in MDA levels between the two groups is known by the text after this using the Tukey test as presented in Table 2.

Table 2. Results of analysis of MDA levels with the Tukey test

Group	<i>p-Value</i>
K1 vs K2	0.000*
K1 vs K3	0.000*
K1 vs K4	0.000*
K2 vs K3	0.000*
K2 vs K4	0.000*
K3 vs K4	0.000*

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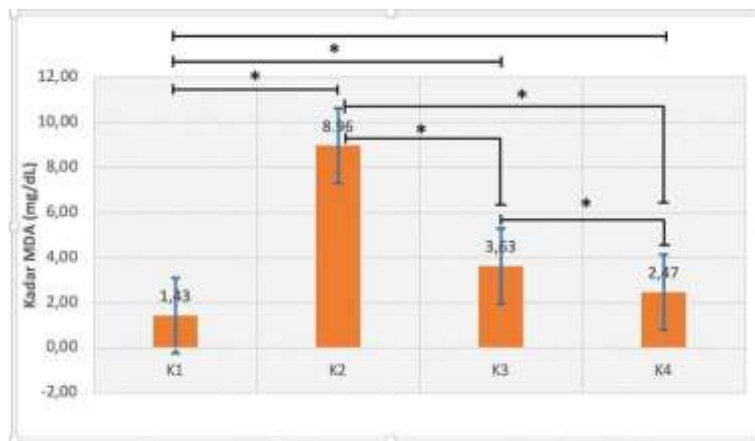


Figure 1. Average graph of Malondialdehyde levels

(Remarks: Posthoc test and Tukey test * $p < 0.05$)

Tukey test results in Table 2 and Figure 1 show that MDA levels in the K1 group were significantly different from those in the K2, K3, and K4 groups with a value of $p = 0.000$ ($p < 0.05$). There is a significant difference between K2 and K3 ($p = 0.000$), and K4 ($p = 0.000$). The K3 group was significantly different from K4 ($p = 0.000$). It can be concluded that administration of VCO at a dose of 0.45 mL/200 g BW/day and a dose of 0.9 mL/200 g BW/day had a significant effect on MDA levels in male Wistar rats subjected to strenuous physical activity.

Glutathione Peroxidase (GPx) Levels

The difference levels of glutathione peroxidase between the 2 groups known by test after this using Tukey test which is presented in Table 3.

Table 3. Analysis of GPx Levels with the Tukey Test

Group	<i>p-Value</i>
K1 vs K2	0.000*
K1 vs K3	0.000*
K1 vs K4	0.000*
K2 vs K3	0.000*
K2 vs K4	0.000*
K3 vs K4	0.000*

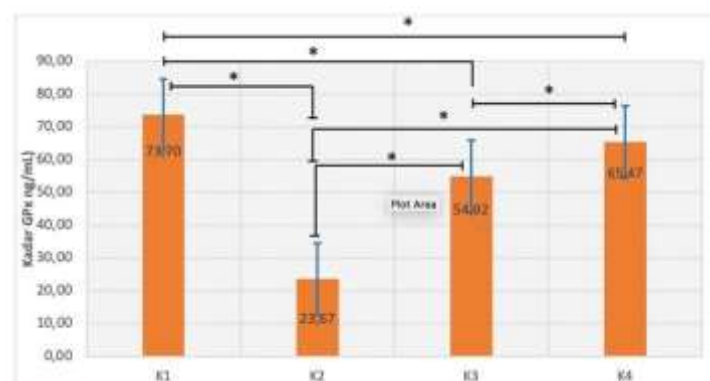


Figure 2. Graph of Mean Levels of Glutathione Peroxidase

(Remarks: Posthoc test and Tukey test * $p < 0.05$)

Tukey test result in Table 3 and Figure 2 shows the levels of glutathione peroxidase K1 is significantly different from K2, K3, and K4 with $p = 0.000$ ($p < 0.05$). There was a significant difference in GPx levels between K2 and K3 ($p = 0.000$), K4 ($p = 0.000$). GPx K3 levels were significantly different from K4 ($p = 0.000$). It can be concluded that administration of VCO at a dose of 0.45 mL/200 g BW/day and a dose of 0.9 mL/200 g BW/day has an effect on the levels of glutathione peroxidase in male Wistar rats subjected to strenuous physical activity.

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Cortisol Hormone Levels

The difference in cortisol hormone levels between the 2 groups using Tukey test as presented in Table 4.

Table 4. Results of Analysis of Cortisol Hormone Levels with the Tukey Test

Group	p-Value
K1 vs K2	0.000*
K1 vs K3	0.067
K1 vs K4	0.000*
K2 vs K3	0.000*
K2 vs K4	0.000*
K3 vs K4	0.000*

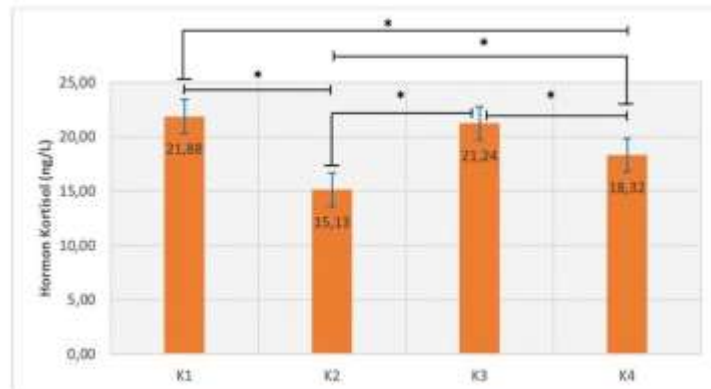


Figure 3. Graph of Average Cortisol Hormone Levels

(Remarks: Posthoc test and Tukey test * $p < 0.05$)

Tukey test result in Table 4 and Figure 3 show that cortisol levels in the K1 group were significantly different from those in the K2 and K4 groups with a value of $p = 0.000$ ($p < 0.05$). Cortisol hormone levels in the K1 group were not significantly different from those in the K3 group ($p = 0.067$). The results of cortisol levels in K2 were significantly different from those in K3 ($p = 0.000$) and K4 ($p = 0.000$). The K3 group was significantly different from K4 ($p = 0.000$). Based on the data above, it can be interpreted that administration of VCO at a dose of 0.45 mL/200 g BW/day and a dose of 0.9 mL/200 g BW/day has a significant effect on cortisol hormone levels in male Wistar rats given strenuous physical activity so that the hypothesis is accepted.

IV. DISCUSSION

Heavy physical activity is excessive physical activity, caused by too much activity volume, too high activity intensity, too long activity duration, and too frequent activity frequency. Strenuous physical activity can increase the formation of oxidants which form free radicals resulting in oxidative stress.(11) The group of rats that were treated with strenuous physical activity were K2, K3 and K4. The results of examining MDA levels in the K2 group that were given strenuous physical activity without VCO administration experienced a significant increase as shown in table 1. This means that the rats were already in a state of oxidative stress. MDA levels are a marker of lipid peroxidation as a result of oxidative stress. The increase in plasma MDA levels is due to the high activity carried out every day for 28 days, resulting in an imbalance between the amounts of antioxidants and free radicals in the body. The amount of antioxidants will be greatly influenced by the amount of free radicals that are formed in the body during high physical activity.(12) The results of this study are in line with research conducted by Arsana (2014) that the group with excessive physical activity has the highest average of MDA levels.(13) Research by Rosidi (2013) also states that there is an increase in MDA levels after physical activity.(14)

The decrease of MDA levels in the K3 group which was given strenuous physical activity and VCO administration at a dose of 0.45 ml/200 g BW/day experienced a significant difference with the control group (K1) and the treatment group with a dose of 0.9 mL/200 g BW/day (K4) as in table 1. This is because the antioxidant content in VCO seems to be able to bind the free radicals that are formed. This study is in accordance with that conducted by Sinaga (2014)(7) stating that VCO can prevent increased production of free radicals due to maximum physical activity.(7) The results of this study are in line with research conducted by Dosumu et al.,(2010)(15) reported that VCO at a dose of 6.7mL/KgBW could reduce testicular MDA levels of rats induced by giving alcohol at a dose of 7mL/KgBW.

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The GPx test results in the K2 group that was given strenuous physical activity without administration of VCO experienced a significant decrease as shown in Table 1. This is due to high levels of free radicals in the body during strenuous physical activity resulting in decreased activity of endogenous antioxidants such as glutathione peroxidase so that the body is unable to neutralize free radicals. Naturally the body has the ability to ward off free radicals by forming endogenous antioxidants produced by the body whose levels can be measured through GPx (Glutathione Peroxidase), catalase and Superoxide Dismutase (SOD) activity.(16)

The increase of glutathione peroxidase levels in the K3 group that was given heavy physical activity and given VCO at a dose of 0.45 mL/200 g BW/day experienced a significant difference with the control group (K1) and the treatment group at a dose of 0.9 mL/200 g BW/day (K4) as in table 1. This is because Virgin Coconut Oil (VCO) contains antioxidants such as polyphenols *α-tokoferol*. The high polyphenol content in VCO is able to maintain normal levels of parameters in tissues and serum, and increases the antioxidant enzyme glutathione peroxidase (GPx) so that it can bind reactive oxygen in plasma. Similar research conducted by Hayatulina, *et al* (2018) stated that the polyphenols in virgin coconut oil prevented lipid peroxidation in MC3T3-E1 cells that had been treated with H₂O₂ by increasing the activity of antioxidant enzymes.(17)

The results of hormone cortisol levels in the K2 group that were given strenuous physical activity without VCO administration experienced a significant increase as shown in Table 1. Changes in the internal environment of the body during strenuous physical activity, namely physical and psychological stress. The stress reaction to stressors directly increases ACTH secretion (adrenocorticotropin) and consequently cortisol secretion also increases. This research is in line with that conducted by Huldani (2021) that twelve minutes of medium-intensity aerobic exercise causes a higher increase in cortisol levels in students who are trained in basketball than students who are not trained.(18)

The decrease of cortisol hormone in the K3 group which was given heavy physical activity and VCO administration at a dose of 0.45 mL/200 g BW/day experienced a significant difference from the control group (K1) and the treatment group at a dose of 0.9 mL/200 g BW/day. days (K4) as in table 1. This is due to the presence of the content *α-tokoferol* in VCO which acts not only as an antioxidant but also has an effect on stress hormones. Content *α-tokoferol* may reduce the response to stress by reducing stress-induced radical formation of adrenal catecholamines thereby reducing corticosterone release. Novalia's research (2019) concluded that vitamin E supplements (*α-tokoferol*) can reduce stress conditions as seen from the levels of the hormone cortisol in male Wistar rats. (19) A similar study was conducted by Hidayatik, *et al* (2021) showed that giving vitamin E had a significant effect on decreasing cortisol in albino rats with chronic variable stress (CVS) for 4 weeks.(20)

The results showed that the heavier the physical activity, the more oxidants were released and the decrease in antioxidants resulted in an increase in oxidative stress and an increase in MDA. The presence of MDA can cause tissue damage so that high MDA levels also play a role in aggravating tissue damage. Nevertheless, the results of our study were successful in showing that MDA levels in strenuous physical activity were enforced which were quite sensitive but not specific for detecting tissue damage due to strenuous physical activity.

The research limitation is that the TBARS examination using the spectrophotometric method has several weaknesses, namely some aldehyde compounds other than MDA can also react with TBARS and in the spectrophotometric examination it is also measured at the same absorbance as MDA. In addition, MDA is not a specific product in the lipid peroxidation process so that it can cause false positives which results in a low positive predictive value. Another method, namely High Performance Liquid Chromatography (HPLC), is reported to increase the specificity of examining MDA levels. This study used a posttest only control design which only measured laboratory parameters after treatment. So that it cannot know the description of MDA, GPx and cortisol levels before and after treatment.

V. CONCLUSION

The administration of Virgin Coconut Oil (VCO) can reduce the Malondialdehyde and cortisol hormone levels but can increase the levels of Glutathione Peroxidase (GPx) in male Wistar rats that given strenuous physical activity.

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