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The Effect of Virgin Coconut Oil on TNFα and Cytochrome P450 Aromatase Levels in Obese Female White Rats (*Rattus norvegicus*) Induced with a High-Fat Diet

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ABSTRACT

Background: The high level of various proinflammatory cytokines due to adipocyte hypertrophy such as TNFα in obesity increases cytochrome-P450-aromatase, further inducing increased estrogen production, hypersecretion of Luteinizing Hormone and decreased Follicle Stimulating Hormone, resulting in impaired folliculogenesis, and chronic anovulation. Virgin Coconut Oil is rich in Medium Chain Fatty Acid and phytochemicals as anti-inflammatory.

Aim: The aim of this study was to determine the effect of VCO on reducing TNFα and cytochrome-P450-aromatase levels in obese female white rats.

Methods: Post test only control group, 24 rats were divided into 3 groups, namely: negative control group (K-), positive control group (K+), and treatment group (P). The K+ and P groups were induced using a high-fat diet for 10 weeks, then the P group was given VCO in weeks 7-10. On the first day of week 11, blood was taken from the orbital vein of the eyes of all rats for TNFα levels. Both examinations used ELISA kits. Data analysis used Shapiro Wilks normality test. The value of p>0.05, followed by hypothesis testing using one-way annova and post hoc Bonferroni.

Results: Mean TNFα in the K- group: 141.71±8.87, K+ group: 15.09±19.30, P group: 162,09±13,57. Mean cytochrome-P450-aromatase levels in the K- group: 9.00±0.77, K+ group: 12.22±1.69, P group: 10.58±0.97. The mean levels of one way ANOVA analysis showed that the administration of VCO can significantly reduce TNFα levels (p=0.001) and significantly reduce Cytochrome-P450-aromatase levels (p=0.001).

Conclusion: VCO administration can reduce TNFα levels and cytochorme-P450-aromatase levels in obese female white rats.

Keywords: TNFα, cytochrome-P450-aromatase, infertility, obesity

INTRODUCTION

Obesity is one of the potential causes of infertility. The prevalence of obesity has increased since 1980, reaching about one third of the world's population. Indonesia is the 9th out of 10 countries contributing 50% of the world's obesity (1). Based on the Basic Health Research in 2013 and 2018, the prevalence of obese population in Indonesia and West Sumatra has increased. In 2018 women aged >18 years were more than twice as obese as men, both in Indonesia (29.8%:14.5%) and in West Sumatra (28.1%:12.6%) (2,3). Obese women often suffer from menstrual

dysfunction with ovulation disorders leading to >50% infertility (4,5).

Pro-inflammatory conditions affect reproductive hormone metabolism, increasing the risk of infertility in obese men and women (6). High levels of free fatty acids due to a high fat diet are accommodated by adipocytes by increasing triglyceride storage through hypertrophy (7). High levels of MCP-1 (Monocyte Chemoattractant Protein 1) in obese adipose tissue attract circulating monocytes to adipose tissue, differentiate into macrophages and produce proinflammatory cytokines such as TNFα (8,9).

Adipose tissue also acts as an endocrine organ by secreting biologically active substances called adipokines. Adipokine activity under normal circumstances is essential for maintaining the integrity of the hypothalamic-pituitary-gonadal axis, regulating ovulation, implantation and physiological pregnancy (5). In obesity, chronic inflammation results in an adverse adipokine profile with decreased production of adiponectin (an anti-inflammatory adipokine) and increased production of leptin (a proinflammatory adipokine) (9).

The high level of proinflammatory cytokines and altered adipokine profile in obesity leads to increased estrogen levels by modulating the increase in cytochrome-P450-aromatase in obesity. Cytochrome-P450-aromatase is the most steroidogenesis enzyme in the stromal cells of adipose tissue especially in the inguinal region (9,10). Cytochrome-P450-aromatase mediates the conversion of androstenedione to estrone (9,11).

Increased estrogen in obesity leads to hypersecretion of LH and decreased FSH, along with the overall endocrine changes causing hypothalamic-pituitary-ovarian-axis disruption, resulting in impaired folliculogenesis, follicular atresia and impaired ovulation. Several drugs have been FDA (Food and Drug Administration) approved for weight management, but were discontinued due to adverse effects. The high MCFA in VCO has been shown to have potential for weight management in obesity and reducing adipocyte size (12). VCO also contains a large amount of phytochemicals (vitamins, polyphenols and phytosterols) that act as anti-inflammatory. It has previously been shown that the administration of VCO to obese rats can reduce leptin concentrations, increase adiponectin levels, and reduce TNF-α, IL-6, PGE2 and COX-2 (12,13,14). There has been no research on the effect of VCO administration on cytochrome-P450-aromatase levels. The objective of this study was to determine the effect of VCO on reducing TNFα and cytochrome-P450-aromatase levels in obese female white rats.

MATERIALS & METHODS

This study is experimental with a Post Test Only Control Group Design. The independent variables in this study were omega-3 doses (EPA/DHA: 180mg/120mg), vitamin E doses of 300 mg tocopherols that were converted to rat doses and administered orally, while the dependent variables were CRP levels and NO levels. The preeclampsia induction substance used is L-NAME dose 50 mg/kg/B.BB. B. The samples in this study were divided into five groups, namely the negative control group (K-) without treatment, the positive control group (K+) was given the induction substance L-NAME, the group (P1) was assigned L-NAME +omega-3, the group (P2) was given L-NAME + vitamin E, the P3 group was given L-NAME + omega-3 + vitamin E given at the gestational age of days 10-19. The sample criteria in this study were female mice with an aging period of 10 weeks, an average body weight of 200 gr, and mice in good health and did not experience abortus or die during the study. This research was carried out at the Animal House and Biomedical Laboratory of the Faculty of Medicine, Andalas University, Padang, from August 2020-May, to 2021. NO Inspection with Colorimetric Method Assay kit brand Elabscience no catalog: E-BC-K035-M while the CRP examination by Elisa method no catalog E0053Ra. Both

measuring instruments use a spectrophotometer. This research was conducted after obtaining a research implementation permit and has been tested through an ethical test process by the ethics committee of the Medical Faculty, Universitas Andalas with Certificate No: 241 / UN.16.2 / KEP-FK / 2021. The data were analyzed using the Shapiro Wilks normality test. After the parametric test is met, the hypothesis test is continued using *One Way ANOVA.*

Ethical approval

This study has obtained ethical clearance from the Research Ethics Committee of the Faculty of Medicine, Universitas Andalas Padang with certificate No. 606/un.16.2: 606/UN.16.2/KEP-FK/2022.

Settings and Design

This type of research was experimental with Post test only control group design. The samples in this study were 24 female Wistar strain white rats which were divided into 3 groups, namely the negative control group (given standard feed for 10 weeks), the positive control group and the treatment group (given a high-fat diet for 10 weeks, on the first day of week 7, obesity was determined with the criteria of an increase in body weight >40% compared to the control group and Lee index >310). The high-fat diet consisted of mixing 60% standard feed with 30% beef tallow and 10% duck eggs given ad libitum. In the treatment group, after being confirmed obese, 0.54 ml of VCO per 200 grams of body weight was administered orally every day from week 7 to week 10. The researcher used Siti Nurbaya brand VCO which contains 51.1% lauric acid.

Collection of orbital venous blood and adipose tissue

On the first day of the 11th week of the study, all rats were weighed and 3 ml of orbital venous blood was taken. Then the blood was centrifuged at 5000 xg for 30 minutes, the serum was separated for immediate examination or the sample could be stored in a refrigerator at -200C for further examination procedures. After that, all rats were euthanized with diethyl ether. Rat adipose tissue was taken, then washed with Phospate Buffer Saline (PBS) solution (pH 7.4). Then, the adipose tissue was weighed and crushed into small pieces, then homogenized in PBS (pH 7.4) using a tissue grinder [tissue weight (g): PBS (mL) volume = 1:9]. Cell preparations were centrifuged at 5000 xg within 5 minutes. The supernatant is separated for immediate examination or the sample can be stored in a refrigerator at -200C for further examination procedures.

Examination of TNF-α and Cytochrome P450 Aromatase Levels

Samples in the form of serum from the orbital vein of rats were examined by ELISA method using Rat TNF-α ELISA Kit for the examination of TNF-α levels. Samples in the form of supernatant derived from rat inguinal adipose tissue were examined by ELISA method using Rat Cytochrome P450 19A1 ELISA Kit for examination of cytochrome P450 aromatase enzyme levels. Each sample was made in duplicate to ensure better results.

STATISTICAL ANALYSIS USED

Data analysis used Sapiro-Wilk normality test (p value≥0.05). Hypothesis testing using One Way ANOVA test with 95% confidence level (p<0.05). Then the analysis was continued with the Bonferroni Post Hoc Test to see differences between groups.

RESULT

Rat Body Weight

Table 1. Mean body weight, weight gain and Lee index of control and treated rats

Group	Weight Gain (gram) mean±SD	Weight Gain compared to K- (%)	Lee Index
K-	28,75±2,05	-	301
K+	43,25±6,49	50,434	315
P	41,62±6,39	44,782	313

Based on Table 1, the average body weight gain of rats in the positive control group and treatment group compared to the negative control group were >50.434% and >44.782%, respectively. The Lee index of the negative control, positive control and treatment groups were 301; 315 and 313, respectively. These data indicate that the high-fat diet-induced group were obese. Rats induced by diet can be declared obese if there is an increase in body weight >40% compared to the control. In addition, it can also use the Lee index [square root of body weight (g) divided by naso-anal length (cm) and multiplied by 1000] where rats that are declared obese have a Lee index value >310 (15).

The high-fat diet consisting of a combination of beef fat and duck eggs can induce obesity in this study because of the high content of saturated fatty acids in beef by 68% (16) and saturated fatty acids in duck eggs by 31.85% (17). High consumption of saturated fatty acids can lead to greater accumulation of body fat through the resynthesis of new triglycerides, thereby increasing the body adiposity index and increasing the production of proinflammatory cytokines which is a classic change in human obesity (18).

TNF-α Level

Table 2. Mean TNF-α levels of female white rats (Rattus norvegicus) in control and treatment groups

Group	TNF-α Level (ng/ml) mean±SD	p value
K-	141,71 ± 8,87	0,001
K+	185,09 ± 19,30	
P	162,09 ± 13,57	

Table 2 shows that the mean TNF-α levels were highest in K+ compared to K- and P. Obese rats that had been given VCO (P) had lower mean TNF-α levels than the obese group that was not given VCO (K+) and higher than the normal weight group (K-). The One Way ANOVA test showed that there was a significant difference in the mean TNF-α levels of obese rats between the control and treatment groups (p=0.001).

Tumor Necrosis Factor Alpha is one of the proinflammatory cytokines that increases in obesity. The results of this study prove that feeding a high-fat diet to the positive control group can increase TNF-α levels compared to the negative control group which was given standard feed. Previously it has been proven that by giving a high-fat diet to rats, it can cause an increase in body weight, adipocyte hypertrophy and high levels of proinflammatory cytokines such as TNF-α. This increase stems from the occurrence of adipocyte hypertrophy in obesity. Hypertrophied adipocytes secrete MCP-1, attracting circulating monocytes to adipose tissue. Infiltrated monocytes differentiate into macrophages and produce proinflammatory cytokines, namely TNF-α (8,9).

High levels of TNF-α in obesity lead to increased levels of estrogen by modulating the increase in the enzyme cytochrome-P450-aromatase (9). Tumor Necrosis Factor Alpha is the main driver of 1.4 promoter-mediated aromatase expression in adipose tissue (19). The administration of VCO to obese rats can reduce TNF-α levels compared to obese rats that were not given VCO although not lower than TNF-α levels of non-obese rats (negative control group). Coconut and its derivatives have been proven safe and effective as potential anti-obesity and anti-inflammatory treatments. The anti-inflammatory potential where the consumption of VCO according to the dose has been shown to be able to reduce various pro-inflammatory cytokines. Supplementation of VCO polyphenol fraction to rats induced rheumatoid arthritis

has immunological protective effects by downregulating TNF-α expression (20).

Cytochrome-P450-aromatase Levels

Table 3. Mean cytochrome-P450-aromatase levels of female white rats (Rattus norvegicus) in control and treatment groups

Group	Cytochrome-P450-aromatase levels (ng/ml)	p value
	mean±SD	
K-	9,00 ± 0,77	0,001
K+	12,22 ± 1,69	
P	10,58 ± 0,97	

Table 3 shows that the mean cytochrome-P450-aromatase levels were highest in K+ compared to K- and P. Obese rats that had been given VCO (P) had lower mean cytochrome-P450-aromatase levels than the obese group that was not given VCO (K+) and higher than the normal weight group (K-). One Way ANOVA test showed that there was a significant difference in the mean cytochrome-P450-aromatase levels of obese rats between the control and treatment groups (p=0.001).

DISCUSSION

The enzyme cytochrome-P450-aromatase mediates the conversion of androgens to estrogen (androstenedione to estrone and testosterone to estradiol) (21). The results of this study prove that feeding a high-fat diet to the positive control group can increase the levels of cytochrome-P450-aromatase compared to the negative control group fed a standard diet. Previously, it has been reported an increase in aromatase in the adipose tissue of the mammary glands of obese rats (22) and in the breasts of obese women compared to thin women (23). This situation is explained by the appearance of Crown-Like Structure (CLS) and increased levels of pro-inflammatory mediators such as TNF-a, IL-1ß, Cox-2, hsCRP, IL-6, and leptin, and decreased levels of adiponectin in obesity (22,23).

Cyclooxygenase-2 catalyzes the conversion of arachidonic acid to PGE2. Increased levels of PGE2 in obesity can inhibit p53 which is a negative regulator of aromatase expression resulting in increased aromatase. Furthermore, IL-6 in the serum of obese individuals was found to induce PGE2 secretion, which in turn induces aromatase expression in adipose stromal cells. PGE2 can also increase aromatase expression by inhibiting the LKB1/AMPK pathway, removing its inhibitory effect on cAMP-responsive element-binding protein-regulated transcriptional coactivators, resulting in increased aromatase. Adipokines also play a role in the regulation of aromatase expression. The LKB1/AMPK pathway is normally activated by adiponectin, leading to transcriptional suppression of aromatase. However, adiponectin secretion is greatly reduced in obesity and may therefore lead to increased aromatase expression. Furthermore, leptin levels increase in obesity and result in inhibition of p53 and further lead to an increase in aromatase (9,24).

Administration of VCO to obese rats can reduce cytochrome-P450-aromatase levels compared to obese rats not given VCO although not lower than the cytochrome P450 aromatase levels of non-obese rats (negative control group). Coconut and its derivatives have been shown to be safe and effective immunomodulatory agents with potential as anti-obesity and anti-inflammatory treatments. The anti-inflammatory potential where the consumption of VCO according to the dose has been shown to be able to reduce various pro-inflammatory cytokines thus inducing a decrease in cytochrome-P450-aromatase levels in obesity.

VCO production comes from the endosperm (pulp) of fresh coconuts, processed without a refining stage so as to maintain a higher content of bioactive compounds such as tocopherols, sterols, and vitamins (28). Previously, it has been shown that VCO can reduce the concentration of TNF-α, IL-6, leptin and increase the concentration of adiponectin in obese rats (12,26). Research on rats given ethyl phenylpropiolate to induce oedema in the legs of rats, VCO has an inhibitory effect on the synthesis and/or

release of mediators released earlier such as histamine, 5-HT, and kinin. Furthermore, in the second phase of edema formation, VCO had an inhibitory effect on the synthesis and/or release of prostaglandins (27). Another study with cadmium administration to induce nephrotoxicity caused by oxidative stress and inflammatory response with VCO polyphenols inhibited the increase of CRP, IL-6 and NO (28,29).

CONCLUSION

VCO administration was shown to reduce inflammation in alveolar macrophages by regulating TLR4/MAPK pathway. The MAPK signaling pathway is one of the important intracellular signal transduction systems activated by various extracellular and intracellular stimuli. TLR4 is an important upstream factor in the MAPK pathway of immune response.

Declaration by Authors

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