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Protective Effect of Green Tea Extract on Leptin and Lipid Profile Levels as Result of Induction of Depot Medroxyprogesterone Acetate

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ABSTRACT
Objective: The purpose of this study was to investigate the effects of administration of green tea extract in reducing visceral fat, leptin level, and improvement of lipid profile in female rats injected with depot medroxyprogesterone acetate (DMPA). Material and methods: A total of thirty Rattus norvegicus rats were divided into five groups (n=6), consisting of control group (without treatment), group receiving DMPA injection, and group receiving DMPA injection and administration of various doses of green tea extract. Fat tissues were weighed by Ohaus scale. Leptin analysis was done by ELISA technique. Analysis of HDL and LDL levels was performed by spectrophotometry. Results: DMPA induced the increase in leptin level significantly compared with control group ($p<0.05$). This increase can be reduced by all doses of green tea extract, even reaching a value equal to control group for both highest doses ($p>0.05$). DMPA significantly increased LDL level compared with control group ($p<0.05$), and can be restored by control group level by administration of the highest green tea extract dose. DMPA triggered a decrease in HDL level significantly compare to the control group ($p<0.05$). Management of first green tea extract dose can achieve HDL level equal to control group ($p>0.05$). Conclusions: It was concluded that green tea extract can protect metabolic status through decreased leptin and improvement of lipid profile induced by DMPA.

Keywords: *Catechin, Contraception, Lipid metabolism, Tea, Weight.*

1. INTRODUCTION

Depot medroxyprogesterone acetate (DMPA) is a synthetic progesterone derivative. In the reproductive system, this compound can suppress ovulation, induce endometrial atrophy, and even inhibit the hypothalamic-pituitary-gonadal axis. Other effects include inhibition of reproductive cycle, binding to androgenic receptor and glucocorticoids and associated with insulin resistance and worsening of cardiometabolic parameters [1-3]. In DMPA users, the main problem for discontinuation of the use of contraception is weight gain [4].

Body fat can be divided into adipose visceral tissue and subcutaneous adipose tissue. The adipose visceral tissue has close relationship with metabolic disease, as risk factor for cardiovascular disease [5]. In previous research it has been proven that DMPA users experienced weight gain and have body fat. However, the weight gain mechanism is unclear, it is allegedly due

to appetite effect, glucocorticoid-like effect, or an increase in resting metabolic rate [1,6,7].

Leptin is 16kDa protein, one of adipokines mainly produced by white adipose tissue. Leptin is also produced by breast epithelial tissue and placenta [8]. Leptin functions pleiotropically in physiology or pathophysiology of energy homeostasis, endocrinology, metabolism, reproduction, immunity, and fat oxidation [8, 9]. Physiological functions of leptin include decreased appetite, increased energy storage, regulation of glucose utilization, and improved insulin sensitivity [10]. Under normal eating cycle condition, leptin concentration reflects the adipose tissue quality, increased leptin concentration will be in line with increased adiposity. Leptin levels in women are higher than men. Increased leptin level in women occurs at birth [11]. Previous study showed that leptin level did not increase in DMPA-induced rats [12]. In women who received DMPA for 6 months, basal leptin level did not correlate with weight gain [13]. Interestingly, one case of DMPA injected woman was reported to have lipodystrophy, as a leptin deficiency state and should be treated with leptin [14].

Green tea is a very popular drink in Asian countries and has been widely consumed throughout the world. This drink attracts the attention of scientific community for its health benefits. Unlike black tea and oolong tea, green tea is not fermented to ensure the availability of polyphenols as active ingredients that are beneficial to health. Various polyphenols from green tea are known as catechins, including epigallocatechin gallate, epicatechin gallate, and gallocatechin gallate. Many studies have proven the mechanism of green tea as antiobesity, reducing body weight and fats. Such mechanisms include fat oxidation stimulation, adipogenesis modulation, decreased fat synthesis, and inhibition of digestive enzymes and nutrient absorption [15-16]. Until now, no study has been done to evaluate the benefits of green tea in reducing visceral fat, leptin level, and lipid profile improvement as result of DMPA administration. Therefore, this study aimed at investigating the effects of administration of green tea extract in reducing visceral fat, leptin level, and lipid profile improvement as result of DMPA administration.

2. MATERIAL AND METHODS

2.1. Animal

Five study groups (n per group) consisting of 30 female rats (Rattus norvegicus) 12 months old and weighing 130,200 grams, consisting of a control group (without treatment), a group receiving DMPA injection, and a group receiving DMPA. = 6). Injections received different doses of green tea extract (10.8 mg / day, 21.6 mg / day and 43.2 mg / day).

2.2. Administration of DMPA

DMPA dose administered was determined by human dose conversion, that is 150 miligram. Of this dose, it was obtained the dose for this research of 2.7 milligram/week administered in four weeks [34]. DMPA was administered intramuscularly to the quadriceps muscle.

2.3. Green Tea Extract Preparation

Dry tea leaf extraction was obtained from Wonosari tea plantation, Lawang, East Java, and processed by maceration method with 96% ethanol solvent in Biochemistry Laboratory of Polinema Malang, East Java, Indonesia.

2.4. Measurement of Leptin

Measurement of leptin hormone levels using the Elisa test technique, using Elisa's Kit from Elabscience. Samples were obtained from blood serum taken from mouse heart.

2.5. Measurement of Visceral Fat Weight

Visceral fat weight was known after the animals were executed at the end of treatment. Abdominal part was incised to open the abdominal skin. The intraperitoneal fat in the abdominal cavity around the digestive tract and fats surrounds the ren were removed. Fats were weighed by using Ohaus scale with sensitivity up to 0.0001 and the results were recorded.

2.6. Analysis of HDL and LDL Levels

Analysis of HDL and LDL levels was made in serum using EnzyChrom HDL and LDL/VLDL Assay Kit. The analysis was done according to the detailed instructions available in the kit.

2.7. Ethics

This research has passed the research ethical review from institutional ethics committee of the Faculty of Medicine of Brawijaya University, East Java, Indonesia.

2.8. Statistical Analysis

The data were presented in mean ± standard deviation and analyzed by the ANOVA test. For statistical analysis, we used the statistical package SPSS for Windows version 14.0. The p-value & lt; 0.05 was statistically significant.

3. RESULTS

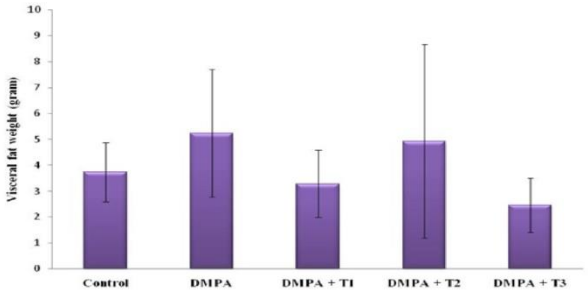

Figure 1 Visceral fat weight in various groups treated with DMPA

Figure 1shows visceral fat weight in various groups. There was no significant difference in visceral fat in various groups ($p>0.05$).

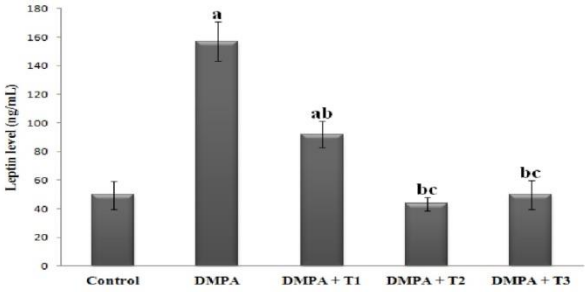

Figure 2 The leptin level in various groups treated with DMPA

The leptin level in all groups showed in Figure 2. Leptin level increased significantly in the group treated with DMPA compared with control group ($p<0.05$). Leptin level decreased significantly in all DMPA groups treated with green tea extract compared with DMPA-induced group ($p<0.05$), even the extracts with second and third doses could significantly decrease the leptin level, achieving value equal to control group ($p>0.05$).

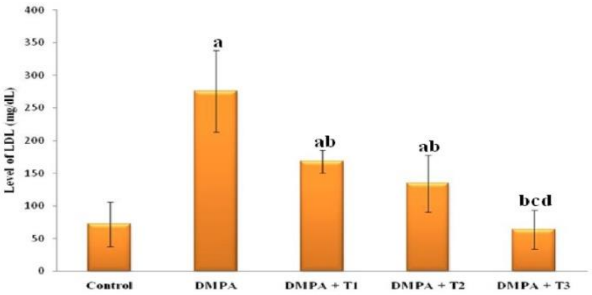

Figure 3 The LDL levels in various groups treated with DMPA

Figure 3 shows the LDL levels of all groups. There was significant increase in LDL level in the group receiving DMPA ($p<0.05$). All groups receiving DMPA and administration of green tea extract showed significant decrease in LDL levels than DMPA group without administration of extract ($p<0.05$). This decrease can achieve level equal to control group in the administration of highest dose extract ($p > 0.05$).

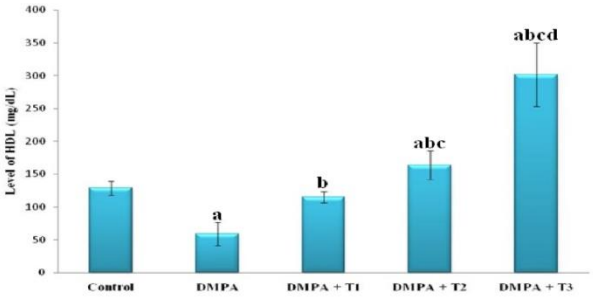

Figure 4 The HDL levels in various groups treated with DMPA

The HDL levels of control and experimental groups were shown in Figure 4. HDL level decreased significantly in group receiving DMPA compared with control group ($p<0.05$). The three doses of green tea extract significantly increased HDL levels compared with group receiving DMPA ($p<0.05$). Administration of first green tea extract dose can achieve HDL level equal to control group ($p>0.05$).

4. DISCUSSION

In this study, there was no significant difference in visceral fat of various groups ($p > 0.05$). This indicates that the administration of DMPA with or without green tea extract does not affect the formation of visceral fat. This study is consistent with previous study on rats, stating that DMPA administration did not induce significant changes in visceral fat level compared with control group [12]. The study on human subjects with DMPA contraceptive acceptor indicated weight gain [18], the weight gain was also found in post-partum women who were considered obese before the pregnancy [19]. This weight gain was caused by glucocorticoid-like effect [20]. We expect the non-significant visceral fat formation in this research is due to progestin and glucocorticoid receptors in adipose cells of female rats. The progestin bond to glucocorticoid receptor will be antagonistic to glucocorticoid [21].

There was significant increase in leptin level in the DMPA group compared with control group ($p < 0.05$). This suggests that administration of DMPA upregulated leptin production by various leptin-producing cells, including white adipose [8]. This study is consistent with previous findings in which the leptin levels increased significantly in women using DMPA for 1 year [21]. We hypothesized that such increase was due to increased synthesis even though the number of cells does not increase as a result of hypertrophy of adipose

cells [1, 22], although in this study there was no significant increase in the quantity of visceral fat ($p > 0.05$).

In this study, administration of second and third green tea extract doses can lower leptin level back to normal level. This indicates that green tea can inhibit leptin increase as result of DMPA administration. The results of meta-analysis research stated that green tea cannot reduce leptin level in the circulation in research that administered green tea less than 12 weeks [23]. This study provides new perspective in reduction of leptin level as result of drug side effects, such as contraception. Other study suggested that quercetin can interact with leptin genes thereby inhibiting leptin production [24].

Regarding the lipid profile, this study demonstrated that administration of DMPA increases the lipid profile, this study demonstrated that DMPA increased LDL level and significantly decreased HDL level compared with control group ($p < 0.05$). These findings are consistent with previous study that found the decreased HDL and increased LDL as result of the use of DMPA [25], but are inconsistent with other researcher. Increased level of LDL was caused by elevated leptin level that triggered decreased expression of LDL receptor and LDL uptake [26]. Administration of green tea extract can significantly lower LDL level and increase HDL level as result of DMPA administration. This suggests that green tea extract can improve lipid profile changes due to DMPA administration. This study extends previous findings that catechin and epigallocatechin gallate can lower LDL level [27] and increase HDL level [28]. Mechanism of this improvement is at least through decreased fat absorption, antioxidant effects, anti-inflammatory effects, decreased fatty acid synthesis, and increased fatty acid oxidation [1].

It is concluded that green tea extract can protect metabolic status through decreased leptin and improvement of lipid profile induced by DMPA. Thus, green tea can regulate leptin and improve lipid profile and become an alternative ingredient that is able to inhibit cardiovascular side effects for women who use contraception.

STATEMENT OF CONFLICT OF INTEREST

The author declares that there is no conflict of interest in the research or publication of this article.

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