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ORIGINAL ARTICLE

Combined high dose vitamin C and E increases oxidative stress and visceral fat mass in rats treated by depot-medroxyprogesterone acetate

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KEYWORDS

Adipose;
Obesity;
Visceral fat;
Tocopherol;
Ascorbic acid

Abstract *Objective:* This study was aimed to investigate the effect of combined vitamin C and E on serum leptin, visceral fat mass, and visceral fat oxidative stress in rats treated by depot-medroxyprogesterone acetate (DMPA).

Method: Twenty-five female Wistar rats, were divided into the following groups ($n = 5$ rats each): control (untreated) group (C); depot-medroxyprogesterone acetate (DMPA) group; DMPA group received vitamin C (at dose 0.2 mg/g; 0.4 mg/g; 0.8 mg/g) and vitamin E (0.04 IU/g). The treatment with combined vitamin C and E was performed for four weeks. Analysis of leptin serum level was done by enzyme linked immunosorbent assay (ELISA) technically. Visceral fat mass was measured by Ohauss scales. Analysis of malondialdehyde (MDA) level in visceral fat was done by spectrophotometer.

Results: The level of serum leptin and visceral fat mass was not significantly different between groups ($P > 0.05$). The lowest and highest dose of combined vitamin C and E in DMPA group significantly increased the visceral fat mass compared with control group ($P < 0.05$).

Conclusion: DMPA treatment induces oxidative stress in visceral fat. Combined high dose vitamin C and E increases oxidative stress and visceral fat mass in rats treated by depot-medroxyprogesterone acetate.

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1. Introduction

Hormonal contraception is most often contraception that was routinely used by women in developed and developing countries, including Indonesia. Depot medroxyprogesterone acetate (DMPA) is one of hormonal contraceptives as an injectable form of progestin. The effectiveness of this substance was approximately 90 days with the pregnancy rate of less than

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1% (1–4). Several studies indicate that DMPA modifies the lipid metabolism and adipose tissue. The DMPA contraceptives are associated with changes of lipid and apolipoprotein levels in Nigerian women (5). Besides, there was an increase in body weight (1.9 kg) in DMPA users at one year of use, resulting from an increase in fat mass of 1.6 kg (6). DMPA was associated with significant weight gain in Navajo women (7). Leptin is an adipokine which was synthesized and secreted by adipose tissue in controlling body weight, energy balance, glucose and lipid metabolisms, reproduction, immunity, inflammation, as well as tissue remodeling. When this level down, severe dysfunction of body homeostasis will happen (8–10). As far as we know there is no study evaluated the association between leptin and body weight in subject received DMPA treatment.

To date, the effect oxidative on adipogenesis is unclear. On the one side, oxidative stress is beneficial for the expression of adipogenesis-related regulators and the maturation of preadipocytes into adipocytes. On the other side, oxidative stress also acts as a harmful agent, disturbing the process of adipocyte differentiation (11). Vitamin C and vitamin E are vitamin antioxidants that have the potential to inhibit the development of atherosclerosis and cardiovascular disease by modulating redox status associated with disease pathogenesis (12). To the best of our knowledge, there were no study has evaluated the protective effects of combined vitamin C and E on serum leptin, visceral fat mass, and visceral fat oxidative stress in rats treated by DMPA.

2. Materials and methods

2.1. Animals

Twenty-five, Wistar rats were divided into the following groups ($n = 5$ rats each), including control (untreated) group (C); depot-medroxyprogesterone acetate (DMPA) group; DMPA group supplemented with a combined vitamin C and E at several doses. The dose of vitamin E was 0.04 IU/g body weight per day. The dose of vitamin C was 0.2, 0.4, and 0.8 mg/g body weight per day. These animals were purchased at weighing 100–125 g, from Physiology Laboratory, Faculty of Medicine, Brawijaya University, and sexed as female rats. These animals were housed in an air-conditioned room at 25 ± 1 °C and 65–70% relative humidity with a 12 h light–dark cycle. The animals had access to food and water *ad libitum* during the experimental period. Diets were made according to American Institute of Nutrition (AIN) standard.

2.2. DMPA treatment

DMPA (Depo Progestin®) was injected at a dose of 2.7 mg/rat/week (in single injection each week) for four weeks. This drug was diluted with 0.2 ml of aquadest, and then injected intramuscularly. The DMPA dose in this study was calculated according to previous toxicity study in rats (13).

2.3. Vitamin C and E

The vitamin C was dissolved with 0.5 cc of aquadest, but vitamin E was dissolved with 0.5 cc sesame oil. All these substances were treated by oral gavage into rats at 10 a.m. every day for four weeks.

2.4. Leptin analysis

The Rat LEP (Leptin) ELISA assay kit (Catalog No: E-EL-RO582) was purchased from Elabscience Biotechnology Co., Ltd (Wuhan, PR China). The analysis was done according to detail procedures in the kit.

2.5. Malondialdehyde analysis

The BIOXYTECH MDA-586™ Spectrophotometric Assay for Malondialdehyde assay kit (Catalog No: 21044) was purchased from Oxis International, Inc. (Foster City, CA 94404 United States). The analysis was done according to detail procedures in the kit.

2.6. Visceral fat mass

Before euthanasia, the body weight was weighed. After euthanasia, the visceral fat mass was obtained and weighted. Visceral fat mass is the total weight of the fat taken from the intraperitoneal fat, including fat in the abdominal cavity around the digestive tract and the fat that surrounds the kidney. This fat was measured with scales (Ohaus®) which has a sensitivity of up to 0.0001.

2.7. Ethics

This research has been approved by the research ethics committee, Faculty of Medicine, University of Brawijaya, Malang, Indonesia.

2.8. Statistical analysis

Data are presented as mean ± SD and differences between groups were analyzed using 1-way ANOVA with SPSS 15.0 statistical package. The post Hoc test was used if the ANOVA was significant. $P < 0.05$ was considered statistically significant.

3. Results

Table 1 presents the serum leptin level of control and experimental group. The level of serum leptin was not significantly different between groups ($P > 0.05$).

Table 2 presents the visceral fat mass, body weight, and body weight/visceral fat mass ratio for each experimental group. The visceral fat mass was insignificantly elevated in the DMPA group compared to the untreated control group ($P > 0.05$). The visceral fat mass was insignificantly increased at first and third doses of combined vitamin C and E compared with a DMPA group ($P > 0.05$), or insignificantly decreases in second dose than that in DMPA group ($P > 0.05$). The lowest and highest dose of combined vitamin C and E in DMPA group significantly increased the visceral fat mass compared with the control group ($P < 0.05$).

Table 2 also presents the body weight, and body weight/visceral fat mass ratio for each experimental group. The body

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