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Original Research

High dose of green tea infusion normalized spiral artery density in rats treated with the depot-medroxyprogesterone acetate

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ABSTRACT

Aim: The purpose of this study was to investigate the effects of green tea (GT) on the spiral artery density and endometrial thickness in female rats treated with the depot-medroxyprogesterone acetate (DMPA). **Material and Methods:** A total of 24 female rats were randomly divided into four groups (n = 6 each): The control group (no treatment), the DMPA-treated group, treated with DMPA and GT doses of 165 mg/kg of body weight/day, and treated with DMPA and GT doses of 330 mg/kg of body weight/day. Spiral artery density and endometrial thickness were subjected to histopathological analysis. **Results:** Spiral artery density decreased in the DMPA-treated group, despite the insignificant difference ($P > 0.05$). With regard to the administration of GT at doses of 165 and 330 mg/g of body weight/day, only GT at the high dose was capable of significantly preventing a decrease in spiral artery density ($P < 0.05$). At this dose, the spiral arteries achieved a density comparable to that of the control group ($P > 0.05$). Meanwhile, the administration of DMPA and/or DMPA with GT did not cause significant changes in endometrial thickness relative to the control group ($P > 0.05$). **Conclusions:** DMPA induced a decrease in spiral artery density, despite the insignificant differences, and these changes could be normalized by the administration of high doses of GT. Therefore, GT could be a candidate herb to prevent the adverse effects of the contraceptive DMPA.

KEY WORDS: Contraceptive, endometrium, histology, progestin, uterus, xenobiotics

INTRODUCTION

Depot-medroxyprogesterone acetate (DMPA) is a suspended solution of pregn-4-ene-3,20-dione,17-(acetyloxy)-6-methyl-(6α), which injected intramuscularly (150 mg once every 3 months) for long-term contraception effect. Once injected, a serum peak level of 1.0 ng/ml will be reached in 3 months and will be followed by a gradual decline. Unpredictable changes in menstrual patterns are often found among women who use DMPA. This constitutes the reason for discontinuation of DMPA as a contraceptive [1-3].

Changes in menstrual patterns of women using DMPA are associated with the local changes in the endometrial microvasculature. To date, the mechanism for the bleeding remains unknown. The underlying mechanism is not directly related to changes in the levels of endogenous and exogenous steroid hormones [4-7]. In addition to changes in the density of the spiral arteries, changes in endometrial thickness are also thought to be involved in the changes in the menstrual pattern of women using DMPA. A decrease in spiral artery density and an increase in endometrial thickness are found among women who use DMPA [8,9]. Increased thickness of the endometrium is proportional to the intrauterine administration of levonorgestrel relative to that of DMPA [10].

Green tea is a beverage derived from the plant *Camellia sinensis* (L). O. Kuntze. It contains a variety of catechins, such as -epicatechin (EC), (-)-epigallocatechin (EGC), (-)-EC-3-gallate (ECG), and (-)-EGC-3-gallate, are found in 30% of the dry weight of plant [11,12]. Previous studies showed that green tea (GT) was capable of affecting angiogenesis and inducing proliferation [13-17]. Thus, the researchers speculated that GT could be an adjunct to the administration of DMPA, as an herb capable of normalizing changes in spiral artery density and endometrial thickness. Thus, this study was to demonstrate the effects of the administration of GT on the number of the spiral arteries and endometrial thickness in DMPA-treated female rats.

MATERIALS AND METHODS

Animals

A total of 24 female Wistar rats were divided into four groups: The control group (no treatment), the DMPA-treated group and the group treated with DMPA and GT of various doses (165 and

330 mg/g of body weight/day, respectively). These mice were purchased with a body weight of 150 (200 g), from the Molecular Physiology Laboratory, Medicine Faculty of Brawijaya University, Malang, East Java, Indonesia. They were maintained in the laboratory conditions in an air-conditioned room at a temperature of 25 ± 1°C with a relative humidity of 65 (70%) and a cycle of dark and light per 12 h. Those rats were given drinking water and feed *ad libitum*. The feed given was in accordance with the standard recommendation from the American Institute of Nutrition.

DMPA Treatment

DMPA (Depo Progestin®) was administrated by intramuscular injection at a dose of 2.7 mg/rat/week for 10 weeks. Before injection, the drug was dissolved in 0.2 distilled water. This dose was determined on the basis the previous toxicity study [18].

Green Tea

The GT (Kepala Djenggot brand) that has been brewed with distilled water (15 min, 90°C) was administered by a feeding tube to each rat.

Histopathology

Spiral artery density and endometrial thickness were calculated from transverse sections of endometrial tissue. The tissue was then subjected to hematoxylin-eosin staining and photographed using a Dotslide Olympus Camera XC 10. Overall, an analysis was carried out on five fields at ×400 magnification.

Ethics

This study passed the ethical review of the Faculty of Medicine, Brawijaya University, Malang of East Java, Indonesia.

Statistical Analysis

All data were presented in mean ± standard deviation. Differences among groups were analyzed using ANOVA tests using the SPSS 15.0 statistical software package. Further tests were performed using the *post-hoc* tests when ANOVA found significant differences. A $P < 0.05$ was considered significantly different.

RESULTS

The density of spiral arteries is presented in Figure 1. The density of spiral arteries in the DMPA-treated group was lower than that of the control group, despite the insignificant difference ($P > 0.05$). With regard to the administration of GT at doses of 165 and 330 mg/g of body weight/day, only GT at the high dose was capable of significantly preventing a decrease in spiral artery density, reaching a density comparable to that of the control group ($P > 0.05$).

Figure 2 shows the values of endometrial thickness for the various treatment groups. There were no significant differences in endometrial thickness among the treatment groups ($P > 0.05$).

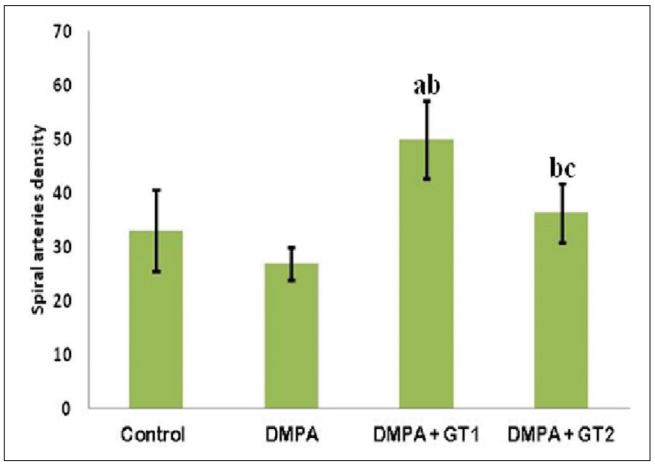

Figure 1: The density of spiral arteries in the control group and the treatment group. Note: Values are presented in mean ± standard deviation; [a]$P < 0.05$ is compared with the control group; [b]$P < 0.05$ is compared with the group administered with depot-medroxyprogesterone acetate (DMPA) without green tea (GT); [c]$P < 0.05$ is compared with the group administered with DMPA plus GT at dose of 330 mg/g body weight/day; DMPA: Depot-medroxyprogesterone acetate; GT: Green tea

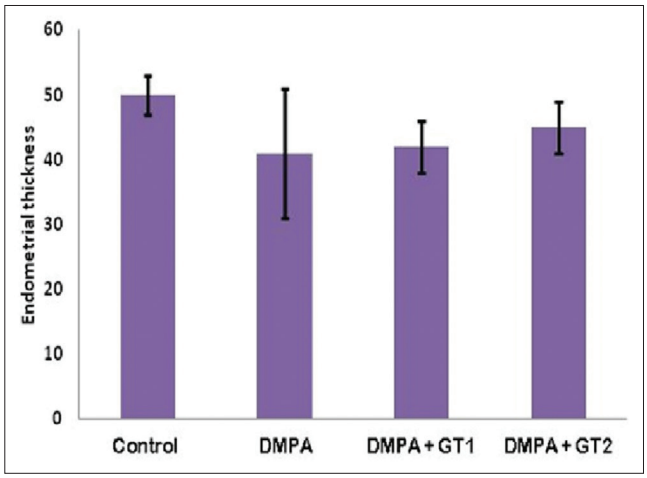

Figure 2: The thickness of the endometrium in the control group and the treatment group. Note: Values are presented in mean ± standard deviation; DMPA: depot-medroxyprogesterone acetate; GT: Green tea

DISCUSSION

The limitation in this study was not conducted analysis of active ingredients of GT infusion. Various literature states that GT contains polyphenolic compounds, minerals, and trace elements [19,20]. In detail, the chemical constituents of GT include polyphenols, caffeine (3.5%), theobromine (0.15-0.2%), theophylline (0.02-0.04%), lignin (6.5%), organic acids (1.5%), chlorophyll (0.5%), theanine (4%), and free amino acid (1-5.5%). Tea also contains flavonoids and flavanols [21-25].

This study showed that the density of spiral arteries tended to decrease in the DMPA-treated group relative to the control group ($P < 0.05$), despite the insignificant difference. This tendency was in accordance with previous studies, showing

that administration of high doses of DMPA was associated with decreased vascular density. Changes in endometrial vascular density are influenced by various factors including the types of hormone, dosage, and methods of administration [8]. Progestin-containing contraceptives will lead to atrophy as seen in vascular changes and characterized by the impaired development of the spiral arteries, dilation, and thin-walled blood vessels near the surface of the epithelium [26]. In this study, high doses of GT were capable of restoring the density of spiral arteries, reaching a value comparable to that of the control group ($P > 0.05$). On the contrary, low doses of GT significantly increased the density of spiral arteries relative to the control group and the DMPA-treated group ($P > 0.05$). This indicates that high doses of GT were capable of normalizing the changes in the spiral arteries as a result of DMPA administration. This is supported by previous findings that the catechins had fluctuating effects on angiogenesis based on the levels of vascular endothelial growth factor (VEGF-A) [27]. However, only ECG that inhibits binding of VEGF to its receptors. Meanwhile, EC, ECG, and EGC cannot inhibit binding of VEGF to its receptor [28].

In this study, endometrial thickness did not differ significantly among the treatment groups ($P < 0.05$). This study differed in the findings for women treated with DMPA, in which there was an increase in endometrial thickness, causing the endometrium to be fluffier and edematous [9]. Our study also extended the previous finding that there was no difference in endometrial thickness between patients treated with levonorgestrel-releasing intrauterine system (Mirena) and those treated with DMPA (Depo-Provera) [10].

CONCLUSION

DMPA induced a decrease in the density of the spiral arteries, despite the insignificant difference, and these changes could be normalized by the administration of high doses of GT. Thus, GT could be a candidate herb to prevent the adverse effects of the contraceptive DMPA.

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