The effects of green tea on spiral artery density and endometrial thickness in rats treated with the contraceptive depot-medroxyprogesterone acetate

Abstract: The purpose of the present study was to investigate the effects of green tea on the spiral artery density and endometrial thickness in female rats treated with the contraceptive depot-medroxyprogesterone acetate (DMPA). A total of twenty-four female rats were randomly divided into four groups: the control group (no treatment), the DMPA-treated group and the group treated with DMPA and green tea of various doses (165 and 330 mg/gram of body weight per day). Spiral artery density and endometrial thickness were subjected to histopathological analysis. Spiral artery density decreased in the DMPA-treated group, despite the insignificant difference (p > 0.05). With regard to the administration of green tea at doses of 165 and 330 mg/gram of body weight per day, only green tea at 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, administration of DMPA and/or DMPA with green tea did not cause significant changes in endometrial thickness relative to the control group (p > 0.05). In conclusion, 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 green tea. Therefore, green tea could be a candidate herb to prevent the adverse effects of the contraceptive DMPA.

Keywords: xenobiotics; progestin; contraceptive; endometrium; uterus; histology.
ABSTRACT

Background: The purpose of the present study was to investigate the effects of green tea on the spiral artery density and endometrial thickness in female rats treated with the contraceptive depot-medroxyprogesterone acetate (DMPA). Methods: A total of twenty-four female rats were randomly divided into four groups: the control group (no treatment), the DMPA-treated group and the group treated with DMPA and green tea of various doses (165 and 330 mg/gram of body weight per 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 green tea at doses of 165 and 330 mg/gram of body weight per day, only green tea at 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, administration of DMPA and/or DMPA with green tea 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 green tea. Therefore, green tea could be a candidate herb to prevent the adverse effects of the contraceptive DMPA.

KEYWORDS: xenobiotics; progestin; contraceptive; endometrium; uterus; histology.
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 three months) for long-term contraception effect. Once injected, a serum peak level of 1.0 ng/ml will be reached for 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 changes local in 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 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 intrauterine administration of levonorgestre relative to that of DMPA [10].

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

Animals
Twenty-four female Wistar rats were divided into two groups, the control group (no treatment), the DMPA-treated group and the group treated with DMPA green tea of various doses (165 and 330 mg/gram of body weight per day). These mice were purchased with a body weight of 150–200 grams from the Molecular Physiology Laboratory, Medicine Faculty of Brawijaya University Malang, East Java, Indonesia. They were maintained in the laboratory conditions at 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 hours. 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 (AIN).

DMPA treatment
DMPA (Depo Progestin®) was administrated by intramuscular injection at a dose of 2.7 mg/rat/week for 10 weeks. Prior to 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 green tea (Kepala Djenggot brand) that has been brewed with distilled water 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 400x magnification.
Ethics
The present 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 by means of one-way ANOVA tests using the SPSS 15.0 statistical software package. Further tests were performed by means of the post-hoc tests when ANOVA found significant differences. A $p$-value of <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 green tea at doses of 165 and 330 mg/gram of body weight per day, only green tea 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 ($p>0.05$).
DISCUSSION

The present 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 [19]. In this study, high doses of green tea 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 green tea 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 green tea 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 VEGF-A [20].

In the present 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 depot-medroxyprogesterone acetate (Depo-Provera) [10].

In 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 green tea. Thus, green tea could be a candidate herb to prevent the adverse effects of the contraceptive DMPA.

**Conflict of interest statement**

The author declares that there is no conflict of interest in the publication of this article.
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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 DMPA without green tea; \(^c\): \(P < 0.05\) is compared with the group administered with DMPA plus green tea at dose of 330 mg/gr body weight per day; DMPA: depot-medroxy progesterone 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.