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Experimental Article

Protective effect of green tea on tunica adventitia and endothelial changes resulting from depot medroxy progesterone acetate

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الملخص

أهداف البحث: تهدف هذه الدراسة إلى فحص آثار الشاي الأخضر في تثبيط ضمور الرحم وتغيرات الأوعية الدموية الناتجة عن استخدام "ديبو مدروكسي بروجستيرون أسيتَيت".

طرق البحث: تم تقسيم ٢٥ فأرة "وستر" تتراوح أعمارهن بين شهر إلى شهرين عشوائيا إلى خمس مجموعات علاجية: المجموعة الضابطة والمجموعة المعطاة "ديبو مدروكسي بروجستيرون أسيتَيت" والمجموعات المعطاة "ديبو مدروكسي بروجستيرون أسيتَيت" مع مستخلص الشاي الأخضر عن طريق الفم (بجرعة ١٠.٨ملغ/يوم وجرعة ٢١.٦ملغ/يوم وجرعة ٤٣.٢ملغ/يوم). وتم إجراء التحليل النسيجي لأنسجة الرحم والأوعية الدموية باستخدام صبغة هيموتوكسلين-إيوسين.

النتائج: خفضت الـ "ديبو مدروكسي بروجستيرون أسيتَيت" من سماكة بطانة الرحم والطبقة الخارجية للأوعية الدموية بالإضافة إلى انخفاض ذي قيمة في عدد خلايا الطبقة المبطنة. أمكن تثبيط الانخفاض في سماكة الطبقة الخارجية للأوعية الدموية بالإضافة إلى انخفاض عدد خلايا الطبقة المبطنة بشكل كبير باستخدام مستخلص الشاي الأخضر مقارنة بمجموعة الـ "ديبو مدروكسي بروجستيرون أسيتَيت".

الاستنتاجات: خلصت هذه الدراسة إلى أن الـ "ديبو مدروكسي بروجستيرون أسيتَيت" يؤدي إلى استنفاذ بطانة الرحم والطبقة الخارجية للأوعية الدموية ويقلل من عدد الخلايا البطانية. يمكن لتوفير مستخلص الشاي الأخضر في أعلى جرعة إعادة الطبقة الخارجية للأوعية الدموية وبطانة الرحم إلى مستواهما الأساسيين.

الكلمات المفتاحية: منع الحمل؛ ديبو مدروكسي بروجستيرون أسيتَيت؛ شاي أخضر؛ بطانة الرحم؛ الطبقة الخارجية للأوعية الدموية

Abstract

Objective: This study aimed to analyse the effects of green tea in inhibiting uterine atrophy and vascular changes due to the use of depot medroxy progesterone acetate (DMPA).

Methods: Twenty-five female Wistar rats aged one to two months were randomly assigned to five treatment groups: control group, DMPA-induced group, and DMPA-induced group orally treated with green tea extract (at 10.8 mg/day, 21.6 mg/day, or 43.2 mg/day). Histologic analysis of uterine and vascular tissues was performed with haematoxylin-eosin staining.

Results: DMPA decreased the thickness of endometrium and tunica adventitia, as well as significantly decreased endothelial cell count ($p < 0.05$). DMPA-induced decreases in the thickness of tunica adventitia and endothelial cell count could be significantly inhibited by green tea extract ($p < 0.05$).

Conclusion: This study concluded that DMPA triggered the depletion of uterine endometrium and vascular tunica adventitia and decreased endothelial cell count. Green tea extract at the highest dose normalized tunica adventitia and endothelial changes to the basal value.

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Keywords: Contraception; DMPA; Green tea; Endometrium; Tunica adventitia

Introduction

Progestin-only injectable contraceptives are safe and effective for women. One example of such contraceptive preparations is Depot Medroxy Progesterone Acetate (DMPA). DMPA is a crystalline suspension administered intramuscularly at a dose of 150 mg/1.0 mL at one or two recommended sites, i.e. the upper arm or buttock. Most recently, DMPA can be administered via subcutaneous injection. The subcutaneous injection sites of DMPA are in the abdomen and quadriceps, as well as the upper arm.[1–4]

The main cause of DMPA inability to use is irregular menstrual bleeding. This side effect is found to occur in 14% women of reproductive age.[5] Abnormal therapies, including the administration of hormone, leuprolide acetate, danazol, NSAIDs, tranexamic acid, and doxycycline, have not been successful.[6,7] The pathophysiological mechanism of this disorder remains unclear. Studies that applied transvaginal Doppler revealed increased uterine perfusion and subendometrial blood vessels.[8] Previous studies have shown that progestin triggers the downregulation of oestrogen and progesterone receptors and triggers endometrial atrophy. Decreased stromal lining is insufficient for the supply of blood vessels, thus triggering vascular fragility and haemorrhage.[9–11] Other studies have also revealed the effect of DMPA as a stimulus of cellular apoptosis.[12] Thus, the improvement of endometrial atrophy in DMPA users is thought to be associated with bleeding prevention.

Contraceptive use is associated with thromboembolic events not only in the endometrium but also in blood vessels. According to Virchow, the risk of thrombosis can be differentiated into three groups of causes, namely decreased blood flow, changes in the blood vessel wall, and changes in blood composition.[13,14] However, few studies have evaluated the effect of DMPA on blood vessels. In DMPA users, there was a decrease in flow-mediated dilatation due to endothelial dysfunction.[15]

Currently, green tea is widely consumed as a beverage, herbal product, capsule, or supplement ready for consumption. Average tea drinkers consume three cups per day. Its effectiveness in the treatment of diseases in humans is unclear, although in experimental animals it has shown positive results.[16–19] The effect of the active ingredients of tea on cell atrophy is controversial. Previous studies have shown EGCG can modulate NF-κβ signals and downstream mediators associated with atrophy in cancer.[20] Other studies have shown that EGCG may suppress transcriptional factors associated with atrophy in diabetes mellitus, such as Foxo1.[21] Other studies have shown that gallic acid inhibits the proliferation of mesenchymal stem cells.[22] In fact, tea can trigger atrophy in the reproductive organs of dogs.[23] Until now, to our knowledge, there has not been a study that evaluates the effect of green tea extracts against DMPA-induced atrophy and changes in the blood vessel walls of the uterus. Therefore, this study aimed to analyse the effects of green tea in inhibiting uterine atrophy and vascular changes due to the use of DMPA.

Materials and Methods

Animals

Twenty-five female Wistar rats aged one to two months were randomly assigned to five treatment groups: control group, DMPA-induced group, and DMPA-induced group treated with green tea extract (at a dose of 10.8, mg/day, 21.6 mg/day, or 43.2 mg/day). These doses were adapted from a previous study.[26] Prior to treatment, the rats were adapted for 7 days under laboratory conditions.

The rats were kept in the laboratory, and they fed on standard feed and drink *ad libitum*. The rats were fed standard feed and drink every day. The drink was placed in a special bottle with a daily requirement of 60 ml/head. The rats were inserted into a plastic cage sized 20 cm × 30 cm × 40 cm, which was padded with rice husk at the base. The enclosure is covered with wire mesh and each cage contains 6 rats. The husk pads were replaced twice a week. Light in the room was controlled in a 12-h light and dark cycle, whereas temperature was controlled between 27 and 28 °C.

DMPA

The DMPA used was Depo-progestin® 150 mg. One bottle of DMPA (3 cc) was diluted with 7 cc of normal saline, stirred until homogeneous, and then injected at 0.2 ml/rat/week. DMPA was injected using a 1 cc spuit by first decontaminating the thigh area of the rat with spray alcohol and then performing aspiration to ensure DMPA did not enter a blood vessel. Next, the rats were put back to their cages. The rats were injected once a week for 4 weeks at a dose of 2.7 mg/rats/week. DMPA injection was performed intramuscularly on the thigh.

Green tea extract

Packaged, dried green tea leaves were obtained from tea plantations in Wonosari, Singosari, Lawang, East Java. Extraction was performed at the Polinema Malang Chemical Laboratory. The process of extraction was conducted by the maceration method, which is an extraction process using a solvent with several times shaking or stirring at room temperature. Extracts were administered orally with oral gavage every day at 9:00 am on a regular basis.

Histopathology analysis

We observed the uterus and vascular organs. Assessment of the uterus included measurement of the thickness of the endometrium, perimetrium, and myometrium. Vascular

assessment included measurement of the thickness of tunica intima, tunica media, and tunica adventitia, as well as the number of endothelial cells. Histological sections were stained with haematoxylin-eosin (HE) and observed using an Olympus BX51 microscope with 400× magnification.

Statistical analysis

Data are presented as mean ± standard deviation and differences between treatment groups were analysed by ANOVA. The analysis was performed with the SPSS 23.0 statistical package program for Windows. Significant difference was determined as the probability value of $p < 0.05$.

Result

Thickness of endometrium in the control and treatment groups is presented in Figure 1. Thickness of endometrium decreased significantly in the DMPA group compared with that in the control group ($p < 0.05$). All doses of green tea extracts increased endometrial thickness, but the increases were not significantly different from that in the DMPA group ($p > 0.05$) (see Table 1).

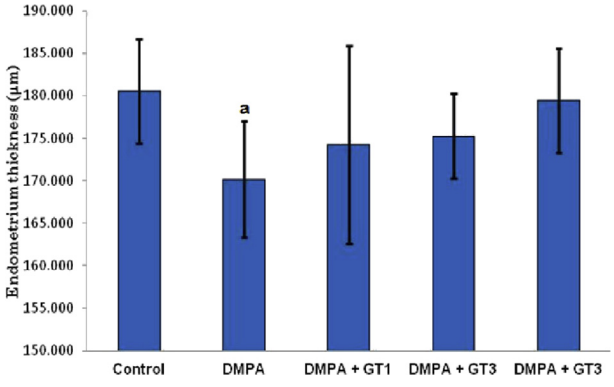

Figure 1: Thickness of endometrium in the control and experimental groups. Note: Data are presented as mean ± standard deviation; [a]$p < 0.05$ compared to the control group; DMPA: depot medroxy progesterone acetate group; DMPA + GT1: DMPA group treated with the first dose of extract; DMPA + GT2: DMPA group treated with the second dose of extract; DMPA + GT3: DMPA group treated with the third dose of extract; µm: micrometre.

Table 2 shows the thickness of perimetrium and myometrium in all groups. There was no significant difference in the thickness of perimetrium and myometrium between all study groups ($p > 0.05$).

Thickness of tunica intima and tunica media in all study groups is presented in Table 2. The thickness of tunica intima and tunica media did not differ significantly between the study groups ($p > 0.05$).

Thickness of tunica adventitia in the control and treatment groups is shown in Figure 2. Thickness of tunica adventitia was significantly lower in the DMPA group than in the control group ($p < 0.05$). The third dose of green tea extract significantly increased the thickness of tunica adventitia compared to DMPA ($p < 0.05$). This increase resulted in the same thickness as that in the control group ($p > 0.05$).

The number of endothelial cells in the control and treatment groups is presented in Figure 3. The number of endothelial cells was significantly lower in the DMPA group than in the control group ($p < 0.05$). The three doses of green tea extract significantly increased endothelial cell count compared to the DMPA group ($p < 0.05$). This increase led to endothelial cell count comparable to that in the control group ($p > 0.05$).

Discussion

Endometrium is a multicellular organ that supports pregnancy and forms the inner lining of the uterus. Endometrial thinning is caused by inhibition of normal endometrial growth. Several causes of endometrial thinning have been revealed, including acute or chronic infections, surgical procedures, administration of clomiphene citrate, and individual structural patterns of the uterus.[24,25,27] To the best of our knowledge, aside from our present study, there is no other study that proves the effect of DMPA on

Table 1: Thickness of tunica intima and tunica media in the control and experimental groups.

Layer	Control	DMPA	DMPA + GT1	DMPA + GT2	DMPA + GT3
Intima (µm)	170.095 ± 8.901	139.843 ± 18.935	156.639 ± 14.973	158.111 ± 7.921	157.760 ± 18.823
Media (µm)	179.835 ± 8.509	173.900 ± 5.384	176.395 ± 6.745	176.869 ± 4.946	176.948 ± 7.344

Note: Data are presented as mean ± standard deviation. There were no significant differences between groups. DMPA: depot medroxy progesterone acetate group; DMPA + GT1: DMPA treated with the first dose of extract group; DMPA + GT2: DMPA treated with the second dose of extract group; DMPA + GT3: DMPA treated with the third dose of extract group; µm: micrometre.

Table 2: Thickness of myometrium and perimetrium in the control and experimental groups.

Layer	Control	DMPA	DMPA + GT1	DMPA + GT2	DMPA + GT3
Myometrium (µm)	181.007 ± 4.911	175.122 ± 8.908	176.230 ± 7.557	179.502 ± 8.488	178.191 ± 1.485
Perimetrium (µm)	180.969 ± 6.895	170.870 ± 7.098	175.806 ± 7.330	178.068 ± 3.298	179.710 ± 7.802

Note: Data are presented as mean ± standard deviation. There were no significant differences between groups. DMPA: depot medroxy progesterone acetate group; DMPA + GT1: DMPA treated with the first dose of extract group; DMPA + GT2: DMPA treated with the second dose of extract group; DMPA + GT3: DMPA treated with the third dose of extract group; µm: micrometre.

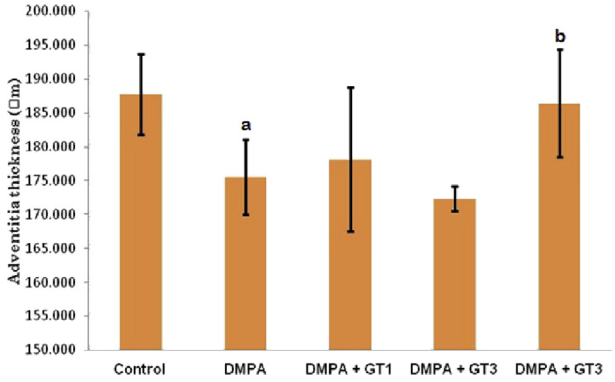

Figure 2: Thickness of tunica adventitia in the control and experimental groups. Note: Data are presented as mean ± standard deviation; $^{a}p < 0.05$ compared to the control group; $^{b}p < 0.05$ compared to the DMPA group; DMPA: depot medroxy progesterone acetate group; DMPA + GT1: DMPA group treated with the first dose of extract; DMPA + GT2: DMPA group treated with the second dose of extract; DMPA + GT3: DMPA group treated with the third dose of extract; µm: micrometre.

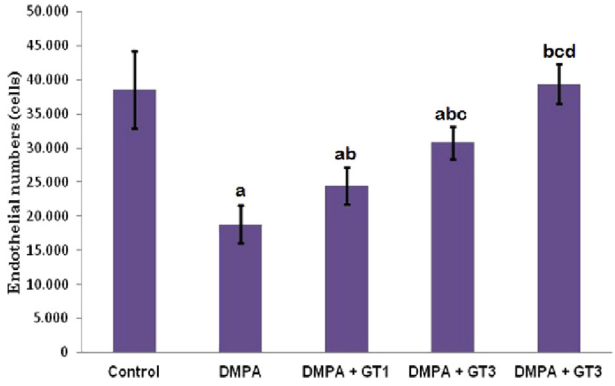

Figure 3: The number of endothelial cells in the control and experimental groups. Note: Data are presented as mean ± standard deviation; $^{a}p < 0.05$ compared to the control group; $^{b}p < 0.05$ compared to the DMPA group; $^{c}p < 0.05$ compared to the DMPA group treated with the first dose of extract; $^{d}p < 0.05$ compared to the DMPA group treated with the second dose of extract; DMPA: depot medroxy progesterone acetate group; DMPA + GT1: DMPA treated with the first dose of extract group; DMPA + GT2: DMPA treated with the second dose of extract group; DMPA + GT3: DMPA treated with the third dose of extract group.

endometrial depletion. This is a novelty of this study. In this study, the thickness of endometrium in the group receiving DMPA was significantly lower than that in the control group, indicating that DMPA induced the depletion of uterine endometrium layer. The results of this study are consistent with previous findings that progestin triggers endometrial atrophy.[9–11,28] Our findings also support previous studies that DMPA triggers apoptosis by increasing the expression of pro-apoptotic proteins and decreasing the expression of anti-apoptotic proteins.[12] Thus, the results of this study are consistent with previous findings that DMPA interferes with the homeostasis of endometrial regeneration.[7]

The three doses of green tea increased endometrial thickness, although not significantly different from that in the DMPA-group. This finding did not indicate the ability of green tea extract in improving DMPA-induced endometrial atrophy. It is known that endometrial proliferation depends on oestrogen level, which causes the progressive growth of functional endometrium in the proliferative phase.[29] Flavonoids and triterpenes are components of green tea that move like oestrogen,[30–33] but this study showed no significant result.

In this study, it was proved that DMPA significantly decreased the thickness of vascular tunica adventitia compared with that in the control group. This indicated that the dominant tunica adventitia contained connective tissue that is sensitive to DMPA. We suspected that this depletion was caused by the activity of metalloproteinase matrix. This supports the previous finding that there is an increase in the activity of MMP-1 and MMP-9 in the uterus of DMPA users.[34] The third dose of green tea extract increased the thickness of tunica adventitia to a thickness comparable to that of the control group. We suspected that the active ingredient of green tea can inhibit the activity of metalloproteinase matrix. Previous studies have shown that green tea may inhibit MMP-9 activity.[34,35]

In this study, the number of endothelial cells was significantly lower in the DMPA group than in the control group. This suggested that DMPA disrupted endothelial cell homeostasis. We suspected that its mechanism was through endothelial cell apoptosis and/or apoptosis of endothelial progenitor cells. This result of this study is consistent with previous findings that DMPA triggers endothelial cell apoptosis.[12] This apoptotic effect of DMPA can be inhibited by the three doses of green tea. This finding is consistent with earlier findings that green tea may inhibit apoptosis of endothelial cells.[36,37] Green tea contains various catechins, including epicatechin gallate (ECG), epigallocatechin (EGC), epicatechin (EC), gallocatechin gallate (GCG), catechin gallate (CG), and cathecin (CT). EGCG is the most abundant catechin (50–80%) in green tea.[38] All of these active components work synergistically to repair DMPA exposure-induced damage to the endothelial layer. This effect extends the pharmacological function of green tea by improving endothelial dysfunction.[39]

It was concluded that DMPA triggered the depletion of uterine endometrium and vascular tunica adventitia, and suppressed endothelial cell count. Provision of green tea extract at the highest dose can normalize changes in tunica adventitia and endothelium to the basal value.

Conflict of interest

The authors have no conflict of interest to declare.

Ethical approval

This research was approved for ethics by the Health Research Committee of the Health Polytechnic, Ministry of Health, Langsa, Aceh, Indonesia.

Authors' contributions

EAS, NV, CM, IS, and RM conceived and designed the study, provided research materials, collected and organized the data, analysed and interpreted the data, wrote the initial and final drafts of the article, critically reviewed the final draft, and are responsible for the content and originality of the manuscript. All authors have critically reviewed and approved the final draft and are responsible for the content and similarity index of the manuscript.

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