Cukurova Medical Journal
ÇUKUROVA ÜNİVERSİTESİ TIP FAKÜLTESİ DERGİSİ

Cukurova Med J 2016;41(4):675-679
DOI: 10.17826/cutf.254193

ARAŞTIRMA/RESEARCH

Combined effects of infusion of green tea and depot-medroxyprogesterone acetate on the number of granulosa cells and the number and size of ovarian primary follicles: an in vivo study in female rats

Yeşil çay ve depot-medroxyprogesterone asetat infüzyonunun granüloza hücre sayısı ile ovaryan primer folikül sayı ve büyüklüğü üzerine kombine etkisi: dişi ratlarda in vivo çalışma

Maharani Maharani

Midwifery Study Program, Ministry of Health Polytechnic of Meulaboh, Aceh, Special Region of Aceh, Indonesia

Cukurova Medical Journal 2016;41(4):675-679.

Abstract

Purpose: The purpose of the present study was to analyze the effects of green tea infusion on the toxicity of ovaries of female rats treated with the contraceptive depot-medroxyprogesterone acetate (DMPA).

Material and Methods: A total of twenty-four female rats were randomly divided into four groups, incuding the control group (no treatment), the DMPA-treated group and the group treated with DMPA and infusion of green tea at various doses (165 and 330 mg/gram of body weight per day). The number of granulosa cells, the number of ovarian follicles and the size of ovarian follicles were subjected to histopathological analysis.

Results: The number of granulosa cells did not differ significantly among the study groups. The number of ovarian follicles was significantly higher in the DMPA-treated group than that of the control group. Of doses of 165 and 330 mg/g of body weight of green tea administration, only the low dose decreased the number of ovarian follicles significantly relative to the DMPA-treated group, although it has not yet reached the levels comparable to those of the control group. The size of ovarian follicles did not differ significantly among the study groups.

Conclusion: DMPA led to an increased number of ovarian follicles, which was restored by infusion of low doses of green tea. Thus, green tea constitutes an herb that can be combined with administration of DMPA and useful in inhibiting the adverse effects of contraceptives.

Key words: Xenobiotics, contraceptives, ovary, tea.

Öz

Amaç: Bu çalışmanın amacı, yeşil çayın kontraseptif depot-medroxyprogesterone asetat (DMPA) uygulanmış dişi rat yumurtalık toksisitesine etkisinin araştırılmasıdır.

Gereç ve Yöntem: Toplam 24 dişi rat, kontrol grubu (muamele olmayan), DMPA uygulanmış grup, DMPA uygulanmış ve farklı dozlarda yeşil çay infüzyonu (165 ve 330 mg/gram vücud ağırlığı/ gün) yapılmış olmak üzere rastgele 4 gruba ayrılmıştır. Granüloza hücre sayısı, ovaryan folikül sayısı ve ovaryan folikül büyüklüğü histopatolojik analizlerle belirlenmiştir.

Bulgular: Granüloza hücre sayısı çalışılan gruplar arasında anlamlı bir farklılık göstermemiştir. Ovaryan folikül sayısı DMPA-uygulanmış grupta kontrol grubuna göre daha fazladır. 165 ve 330 mg/gram vücud ağırlığı dozlarında yeşil çay uygulanan gruplarda, sadece düşük doz uygulanan grupta ovaryan folikül sayısı DMPA-uygulanmış gruba göre anlamlı olarak azalmış olmasına ragmen kontrol grupla karşılaştırılabilir seviyede değilidir. Ovaryan folikül büyüklüğü çalışılan gruplar arasında anlamlı bir farklılık göstermemiştir.

Sonuç: DMPA ovaryan folikül sayısının artmasına neden olmuş, bu da düşük doz yeşil çay infüzyonu tarafından eski haline getirilmiştir. Bu çalışmaya göre, yeşil çayın, DMPA uygulaması ile kombine olarak kullanılabilen ve kontraseptiflerinlerin yan etkilerini inhibe eden yararlı bir bitki olduğunu göstermektedir.

Anahtar kelimeler: Ksenobiyotikler, kontraseptifler;, yumurtalık, çay

Yazışma Adresi/Address for Correspondence: Dr. Maharani Maharani, Midwifery Study Program, Ministry of Health Polytechnic of Meulaboh, Aceh, Special Region of Aceh, Indonesia, Email: ma.harani@yahoo.com
Geliş tarihi/Received: 31.03.2016 Kabul tarihi/Accepted: 31.05.2016

INTRODUCTION

Overpopulation represents a public health issue in both the developed and developing countries. In order to control population explosion, contraceptives are used to plan the timing of pregnancy. Hormonal contraceptive is the most widely used contraception among women in the developing countries, including Indonesia. Depot medroxyprogesterone acetate (DMPA) is an injectable form of the hormonal contraceptive progestin. Administration of DMPA will provide protection for 90 days with a failure rate of 1%[1-4]. Nevertheless, hormonal contraceptives have adverse effects, such as menstrual disorders, prolonged menstruation, irregular menstruation and amenorrhea. These will lead to discontinuation of hormonal contraception.

DMPA is classified as a xenobiotic capable of leading to a reduced number of ovarian follicles and loss of various ovarian cells (oogonia, oocytes and somatic cells)[5]. Histologically, the changes in the ovary in the subjects treated with DMPA include follicular degeneration and atresia[6]. The basic mechanism of degeneration is apoptosis as evidenced in previous studies[7]. Thus, there is a need for materials capable of preventing the degeneration of the ovaries due to the administration of DMPA. This is to keep the ovaries in physiological conditions, despite the administration of the contraceptive DMPA.

Green tea is a beverage made by brewing the leaves of Camellia sinensis. The plant contains a variety of active substances, such as (-)-epicatechin (EC), (-)-epigallocatechin (EGC), (-)-epicatechin-3-gallate (ECG) and (-)-epigallocatechin-3-gallate (EGCG)[8,9]. Consuming tea may increase the productive life of the ovary[10]. A previous study showed that green tea may improve ovarian morphology in a rat model of polycystic ovarian syndrome[11]. To date, the pharmacological potential of green tea has been known, but its role to prevent degeneration or toxicity from contraceptive DMPA remains unclear. The purpose of the present study was to analyze the effects of green tea infusion on the toxicity of ovaries of female rats treated with the contraceptive depot-medroxyprogesterone acetate (DMPA).

MATERIALS AND METHODS

The present study passed the ethical review of the Faculty of Medicine, Brawijaya University Malang of East Java, Indonesia. Twenty-four female Wistar rats were 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). These rats 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 ± 1oC 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 (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 ml distilled water. This dose was determined on the basis the previous toxicity study[12]. The green tea used in this study was of Kepala Djenggot brand and prepared in infusion. The green tea that has been brewed with distilled water was given by a feeding tube to each rat. The doses of administration were 165 and 330 mg/gram of body weight per day for 10 weeks[13].

The number of granulosa cells and the size of ovarian primary follicles were analyzed from the 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.

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

Table 1 presents the number of granulosa cells of each treatment group. The number of granulosa

cells did not differ significantly among the study groups ($p > 0.05$). Figure 1 and 2 shows the morphology and the number of ovarian primary follicles in various study groups. The number of ovarian follicles was significantly higher in the DMPA-treated group than that of the control group ($p < 0.05$). Of doses of 165 and 330 mg/g of body weight of green tea administration, only the low dose decreased the number of ovarian follicles significantly relative to the DMPA-treated group ($p < 0.05$), although it has not yet reached the levels comparable to that of the control group ($p > 0.05$). Table 2 shows the size of ovarian primary follicles of each treatment group. The size of ovarian follicles did not differ significantly among the study groups ($p > 0.05$).

Table 1. Number of granulosa cells for the control and treatment groups

			DMPA + Green tea (mg/gram body weight)	
Number (cell)	Control	DMPA	165	330
Granulosa cells	75.67 ± 29.53	113.67 ± 47.89	92.00 ± 29.49	121.17 ± 43.09

Values are presented in mean ± standard deviation. DMPA: depot- medroxy progesterone acetate.

Table 2. Size of ovary follicles for the control and treatment groups

		DMPA + Green tea (mg/gram body weight)		
Size (mm)	Control	DMPA	165	330
Ovary follicles	21.20 ± 4.54	25.60 ± 8.70	22.80 ± 5.89	25.24 ± 5.51

Values are presented in mean ± standard deviation. DMPA: depot- medroxy progesterone acetate.

DISCUSSION

At the cellular level, aging and senescence of ovarian follicles are characterized by a reduction in the specific function of the oocytes and overall cellular dysfunction of granulosa cells. Overall cellular dysfunction is caused by a decrease in mitochondrial activity and energy failure, as well as increased expression of genes and senescence of proteins. The reduction in cellular function is sufficient to increase the sensitivity of the ovarian follicle, oocytes and granulosa cells to apoptosis[14,15]. Follicular atresia is a condition caused by apoptosis of granulosa cells[16]. Furthermore, given that granulosa cells are the main source of estradiol and progesterone synthesis a reduction in its number and function will trigger a decline in hormonal levels[15,16]. In the present study, the number of granulosa cells in the DMPA-treated group tended to increase relative to that of the control group, despite the insignificant difference ($p<0.05$). Thus, these findings indicate that the administration of DMPA does not lead to a change in the number of granulosa cells as hormone-producing cells. These findings extend previous data that DMPA induces amenorrhea without a reduction in serum levels of estradiol due to the reversible nature of ovarian function[17]. In the group treated with DMPA and green tea infusion, there was a tendency for a change in the number of granulosa cells approaching that of the control group, although it was not significantly different relative to those groups treated with DMPA ($p<0.05$). Further studies are required to evaluate the function of granulosa cells as hormone-producing cells.

The present study showed that the number of ovarian primary follicles was significantly higher in the DMPA-treated group than that of the control group ($p< 0.05$). This indicates that DMPA induces the proliferation of ovarian follicles as part of folliculogenesis. Folliculogenesis is a process that takes place in the ovarian cortex involving antral and pre-antral phases. This study confirms that folliculogenesis can be influenced by xenobiotics, including DMPA[18,19]. Our study expands previous findings that monkeys treated with progestin would undergo apoptosis of ovarian epithelial cells relative to the control group and administration of ethinyl estradiol7 or changes in oxidative stress as the basic mechanisms of cellular apoptosis[20].

Of green tea of doses of 165 and 330 mg/gram of body weight per day, only the lowest dose significantly prevented the increase in the number of ovarian follicles due to DMPA induction. These findings indicate that low doses of green tea may inhibit proliferation or trigger apoptosis of ovarian primary follicular cells.

Green tea contains catechin, epicatechin, epigallocatechin, and epigallocatechin-3-gallate. Previous findings demonstrated that EGCG has anti-proliferative effects[21,22]. Additionally, green tea also has an affinity for the ovaries and protective effects for the ovaries[10,11].

In conclusion, DMPA led to an increased number of ovarian follicles, which was restored by the administration of low doses of green tea. Thus, green tea is an herb that can be combined with the administration of DMPA and useful in inhibiting the adverse effects of contraceptives.

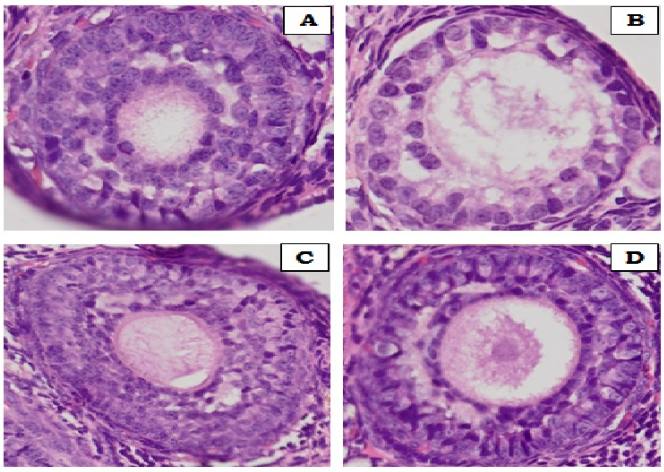

Figure 1. The morphology of ovarian primary follicles for the control and treatment groups.
The control group (no treatment) (A); the DMPA-treated group (B); and the group treated with DMPA + green tea of 165 mg/gram of body weight per day (C); and the group treated with DMPA + green tea 330 mg/gram of body weight per day (D).

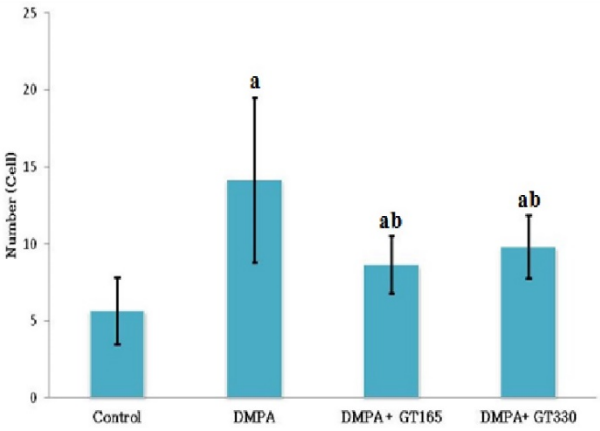

Figure 2. The number of ovarian primary follicles for the control and treatment groups.
Note: Values are shown in mean ± standard deviation; a: $p<0.05$ compared with the control group; b: $p<0.05$ compared with the group treated with DMPA without green tea infusion; DMPA: depot- medroxy progesterone acetate; DMPA + GT165: the group treated with DMPA + green tea of 165 mg/gram of body weight per day; DMPA + GT330: the group treated with DMPA + green tea of 330 mg/gram of body weight per day.

REFERENCES

1. Erkkola R, Landgren BM. Role of progestins in contraception. Acta Obstet Gynecol Scand. 2005;84:207–16.
2. Kaunitz AM. Injectable contraception. New and existing options. Obstet Gynecol Clin North Am. 2000;27:741–80.
3. Jain J, Dutton C, Nicosia A, Wajszczuk C, Bode FR, Mishell DR Jr. Pharmacokinetics, ovulation suppression and return to ovulation following a

lower dose subcutaneous formulation of Depo-Provera. Contraception. 2004;70:11–8.
4. Toh YC, Jain J, Rahnny MH, Bode FR, Ross D. Suppression of ovulation by a new subcutaneous depot medroxyprogesterone acetate (104mg/0.65ml) contraceptive formulation in asian women. Clin Ther. 2004;26:1845–54.
5. Regan KS, Cline JM, Creasy D, Davis B, Foley GL, Lanning L et al. Ovarian follicular counting in the assessment of rodent reproductive toxicity. Toxicol Pathol. 2005;33:409–12.
6. Bhowmik T, Mukherjea M. Histological changes in the ovary and uterus of rat after injectable contraceptive therapy. Contraception. 1988;37:529-38.
7. Rodriguez GC, Walmer DK, Cline M, Krigman H, Lessey BA, Whitaker RS et al. Effect of progestin on the ovarian epithelium of macaques: cancer prevention through apoptosis? J Soc Gynecol Investig. 1998;5:271-6.
8. Koo SI, Noh SK. Green tea as inhibitor of the intestinal absorption of lipids: potential mechanism for its lipid-lowering effect. J Nutr Biochem. 2007;18:179–83.
9. Pastore RL, Fratellone P. Potential health benefits of green tea (Camellia sinensis): a narrative review. Explore (NY). 2006;2:531–9.
10. Luo LL, Huang J, Fu YC, Xu JJ, Qian YS. Effects of tea polyphenols on ovarian development in rats. J Endocrinol Invest. 2008;31:1110-8.
11. Ghafurniyan H, Azarnia M, Nabiuni M, Karimzadeh L. The effect of green tea extract on reproductive improvement in estradiol valerate-induced polycystic ovarian syndrome in rat. Iran J Pharm Res. 2015l;14:1215-33.
12. Bakry S, Aseem N, Montaser N. Cytotoxicity and genotoxicity of DMPA on female rats. Toxicol Lett. 2010;96:156-7.
13. Boehm K, Borrelli F, Ernst E, Habacher G, Hung SK, Milazzo S et al.. Green te (Camellia sinensis) for the prevention of cancer. Cochrane Database Syst Rev. 2009;8:CD005004.
14. Zhu M, Lei Z, Yang D. Evaluation of safety in Chinese women with amenorrhea following injection of depot-medroxyprogesterone for contraception. Zhonghua Fu Chan Ke Za Zhi. 1999;34:621-3.
15. Johnson AL. Intracellular mechanisms regulating cell survival in ovarian follicles. Animal Reprod Sci. 2003;78:185–20.
16. Tatone T, Amicarelli F, Carbone MC, Monteleone P, Caserta D, Marci R, et al. Cellular and molecular aspects of ovarian follicle ageing. Hum Rep Update. 2008;14:131–41.
17. Matsuda F, Inoue N, Manabe N, Ohkura S. Follicular growth and atresia in mammalian ovaries: regulation by survival and death of granulosa cells. J Reprod and Dev. 2012;58:44–50.
18. Eppig JJ. Oocyte control of ovarian follicular development and function in mammals. Reproduction. 2001;122:829-38.
19. Regan KS, Cline JM, Creasy D, Davis B, Foley GL, Lanning L et al.. Ovarian follicular counting in the assessment of rodent reproductive toxicity. Toxicol Pathol. 2005;33:409–12.
20. Ismiyati A, Wiyasa IWA, Hidayati DYN. Protective effect of vitamins C and E on depot-medroxyprogesterone acetate-induced ovarian oxidative stress in vivo. J Toxicol. 2016 Article ID 614963.
21. Baker KM, Bauer AC. Green tea catechin, EGCG, suppresses pcb 102-induced proliferation in estrogen-sensitive breast cancer cells. International Journal of Breast Cancer 2005;2005:163591.
22. Farhan M, Khan HY, Oves M, Al-Harrasi A, Rehmani N, Arif H et al.. Cancer therapy by catechins involves redox cycling of copper ions and generation of reactive oxygen species. Toxins. 2016;8 doi:10.3390/toxins8020037.