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Research Article

The Effect of Epigallocatechin Gallate (EGCG) To Ovary Activity and The Expression of LH Receptors in Female Rat (*Rattus norvegicus*)

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ABSTRACT

Green tea polyphenol, *epigallocatechin gallate* (EGCG) has been proposing as a cancer chemopreventive, antioxidant and has inhibitor effect in steroidogenesis. The aim of the research was to determine the effect of EGCG on the estrous cycle and expression of LH receptors in female rat (*Rattus norvegicus*). This research was using 24 female rats, 2-3 months age divided into four groups rat *(Rattus norvegicus)* were treated with 4.05, 8.1, 12.15 mg EGCG/100 g orally in seven days to study their acute effect on ovarian activity and the expression of luteinizing hormone (LH) receptors. Vagina smear histology has been used as an index of ovarian activity. Daily vagina smears were taken for six days. The expression of LH receptors were examined by immunohistochemistry avidin-biotin complex method. The result showed that EGCG significantly reduced the expression of LH receptors ($p < 0.05$) and extended follicular phase (0.2-19.4%). The decrease in LH receptors were consistent with an increase in the dose of EGCG. The smallest dose of EGCG (4.05 mg) had given the lowest effect in reduced the expression of LH receptors and extended follicular phase.

Keywords: green tea, *Rattus norvegicus,* EGCG, ovary activity, expression LH receptor

INTRODUCTION

Green tea (*Camellia sinensis*) is mainly consumed by some people because it has many benefits to health. Green tea (*Camellia sinensis*) has efficacy as antioxidant, lowering body weight, as cancer chemotherapy subtances which capable of inhibiting the growth of tumor cells and as the anti-inflammatory [1,2]. Besides these benefits, green tea also has an active substance of antisteroidogenic effect that inhibits production the reproductive hormonal both male and female [3]. Green tea contained more than 36 percent of polifenol. Polifenol have seven kinds of the different catechin namely: epigallocatechin-gallate (EGCG), epigallocatechin (EGC), epicatechin-gallate (ECG), epicatechin (EC), gallocatechin (GC)catechin(C) and catechin-gallate (CG) [4].

Epigallocatechin gallate (EGCG) is a catechin from green tea with the largest composition [5] which is known to reduce LH serum levels by up to 40-50% in mice [3,6]. Luteinizing hormone (LH) has an important role in steroidogenesis. Luteinizing hormone (LH) secreted by the anterior pituitary that will bind to its receptors in theca cells. LH signaling increase the transcription of a number of genes that encode the synthesis of enzymes needed to convert cholesterol to androgens (androstenedione and testosterone) [7]. Testosterone and androstenedione will be converted to estradiol-17β by the aromatase enzyme (CYP450arom, CYP19A1) with the help of FSH in granulosa cells. Estradiol-17β or estrogen is an important hormone in determining the sign of estrous. Disturbances in hormonal mechanisms affect ovarian activity, causing disruption of the estrous phase and cycle. Ovarian activity consists of two phases, namely the luteal and follicular phases. The luteal phase of the vaginal swab consists of the metestrous and diestrous phases while the follicular phase consists of the proestrous and estrous phases [8]. The phase can be determined by observing the shape of the vaginal swab cells. Vaginal swab is a fast and inexpensive method for determining the phase [9].

The aim of the research was to determine the effect of EGCG on serum LH levels has been done by [3] but studies to determine the effect of EGCG on the estrous cycle and expression of LH receptors in female rat (*Rattus norvegicus*) ovaries have never been done before. The effect of EGCG

administration affected the hormonal mechanism in the female reproductive organs. Based on the above background, further research is carried out to determine the effect of EGCG administration in the estrous cycle and LH receptor expression of rats *(Rattus norvegicus)*.

MATERIALS AND METHODS

Ethical approval

The study was approved by Faculty of Veterinary Medicine Animal Ethics Committee. All variabels after considerations in accordance to Ethics Committee related to animal handling were observed to ensure no discomfort or pain to animal during sampling (1.KE.107.06.2019).

Materials: Samples were 24 female rats, aged 2-3 months, weighing 100-150 grams. The sample was divided into four groups: control (C) received aquadest, T1 received EGCG 4.05 mg/100 g BW, T2 group 8.1 mg/100 g BW and T3 12.15 mg/100 g BW of rat orally. Vaginal swab is done for seven days. In day 8th ovarian removal and making histology slides of ovarian for the examination of expression of LH receptors with immunohistochemistry.

Methods: Vaginal swab is done with a cotton bud that is rinsed with aquadest and inserted into the vagina of a female rat with an angle of ± 45° and rubbed 2-3 times round. The results of the swab from the cotton bud were made into a slide by applying it to the glass object. Vaginal swab slides are put into 70% fixative alcohol solution for 10 minutes, then rinsed off and dried. The smear is then put into Giemsa dye for 5 minutes, rinsed off with water and dried. Next observed the morphology of epithelial cells in preparations that have been made under a microscope with weak magnification (100x) then strong magnification (400x) [10,11]. Observations were made to determine the phase of ovarian activity before administration of EGCG while observations after administration of EGCG were made every 24 hours for seven days.

Epigallocatechin Gallate (EGCG) doses of 4.05 mg, 8.1 mg and 12.15 mg/100 grams of rat's bodyweight were dissolved in aquadest then given orally with gavage. Rats were given EGCG according to the dose with a volume of 1 ml. administration of EGCG is done for seven days. On the day 8th an ovary collection was performed. Examination of LH receptor expression done by immunohistochemistry techniques in rat ovaries. Immunohistochemistry techniques, using avidin-biotin complex techniques (Thermo Scientific Pierce products) that can identify the expression of LH receptors through the change in color displayed. The presence of brown color indicates the identification of LH receptor expression. LH receptor expression is then compared between control and treatment group.

The parameters observed in this study were ovarian activity, those were the length of the follicular phase and the expression of LH receptors in female ovaries (*Rattus norvegicus*). The length of the follicular phase in the form of a percentage is analyzed descriptively. LH receptor expression was statistically analyzed using the Kruskal Wallis nonparametric test and continued with the Man-Whitney test to find out the differences between the two treatments. They were considered to be significantly different if $P < 0.05$.

RESULTS

The effect of EGCG on ovarian activity can be determined by performing a vaginal swab every 24 hours. The recorded data is used to determine the follicular phase in one estrous cycle (six days). The length of the follicular phase in the form of a percentage is analyzed descriptively as shown in the Table 1.

Table 1. Percentage of follicular phase length and LH receptor expression in white rats (*Rattus norvegicus*)

Group	Average Length of Follicular Phase (%)	LH receptor expression (Mean±SD)	Explanation
Control	38,9	5.10^{a} ±0,62	100%
T1 (4.05mg/100g)	58,3	2.83^{c} ±0,97	Decrease until 55.49%
T2 (8.1mg/100g)	36,1	1.83^{b} ±1,40	Decrease until 64.11%
T3 (12.15mg/100g)	47,3	150^{bc} ±1,14	Decrease until 70.59%

Note: Different superscripts show significant differences ($p < 0.05$).

Data from observations of vaginal cytology are used to determine the number of follicular phases (proestrus and estrus). The number of times the subsequent emergence of the proestrus and estrus phase is compared with the length of one cycle to determine the percentage of follicular phase.

Data displayed in a descriptive analysis showed that administration of EGCG could extend the follicular phase between 11.1 to 44.4% at a dose of 4.05 mg/100 g of mice, 11.1 to 27.8% at a dose of 8.1 mg/100 g and 11, 1 to 27.8% at a dose of 12.15 mg/100 g of rats when compared to the control group. Provision of EGCG affects

ovarian activity by extending the follicular phase (Table 1).

DISCUSSION

Admistration of EGCG is known to reduce LH serum levels [3]. Decreasing in LH serum level would decrease complex hormone binding receptor. The mechanism of LH in steroidogenesis is to increase adenylate cyclase so that cAMP is formed which can cause transcription of a number of enzymes needed in steroidogenesis [12]. A decrease in the LH signaling will inhibit the synthesis of adenylate cyclase so that cAMP will decrease. The role of cAMP is to activate protein kinase-A which increases StAR synthesis. A decrease in cAMP will affect the decrease in StAR protein synthesis so that cholesterol transport into the mitochondria will also be reduced. Reduced cholesterol transport causes a decrease in pregnenolone production as well as progesterone [13].

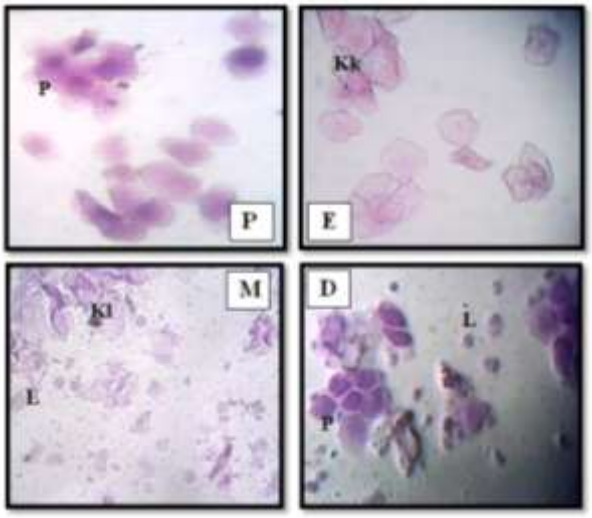

Fig.1: Observation results of cytology with vaginal swabs: Proestrus (P), Estrus (E), Metestrus (M) and Diestrus (D) phases. 400x magnification (P: Intact epithelial cells, Kk: Cells that are cornified with karyorrhexis nuclei, Cl: Cells are cornified with lysis nuclei, L: Leukocyte cells).

Administration EGCG before ovulation (follicular phase) will reduce LH serum levels, causing ovulation failure. During ovulation, LH secretion should increase by up to 10 times. LH has a special effect on granulosa cells and theca cells, which change the two cells to be more progesterone-secreting and less estrogen. But due to a decrease in LH, progesterone production will also inhibit. A decrease in progesterone causes a decrease in the follicular wall space. Progesterone together with PGE and PGF will trigger the formation of plasminogen activators that convert plasminogen to plasmin. Plasmin has a function to activate cholegenase which weakens the collagen in tunica albugenia and theca layer which also stimulates granulosa cells to actively produce follicular fluid. However, the decrease in progesterone causes inhibition of plasmin formation which causes the production of cholegenase also be reduced so that the layers of theca and tunica albugenia are still strong. Inhibition of plasmin also causes decreased follicular fluid secretion. Decreased follicular fluid secretion, decreased follicular wall space resistance and decreased amount of active collagenase which causes obstruction or failure of ovulation [14].

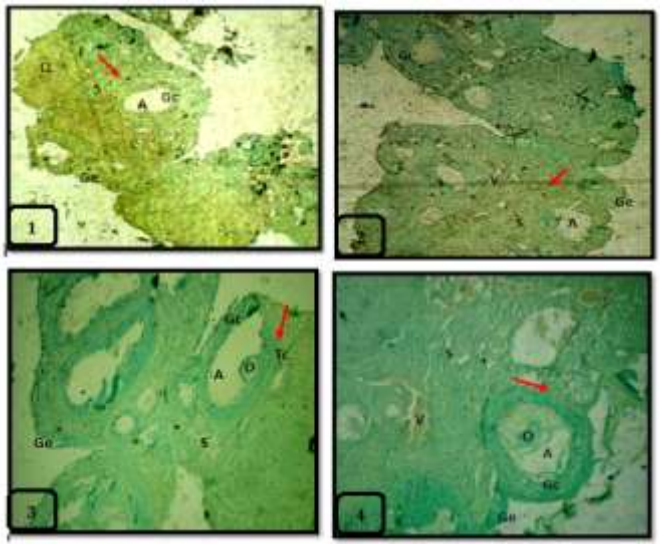

Fig.2: Administration of EGCG in the control group (1), doses of 4.05 mg (2), 8.1 mg (3) and 12.15 mg/100 g (4) of LH receptor expression in rat ovaries.

Failure of ovulation causes accumulation of Estradiol17β in the follicles in sufficient amount so the rat will experience long or continuous estrous stage (nimfomani/high libido). High libido is due to reduced LH levels but FSH is produced in sufficient quantities [15]. LH receptor expression in each sample was assessed semi quantitatively according to the modified Remmele method [16]. Remmele scale index (IRS) is the result of multiplying the percentage score of immunoreactive cells (A) with the color intensity score produced on cells (B). All treatments were tested with the Kruskal Wallis Test and the results were significantly different ($p < 0.05$) and then followed by the Mann Whitney Test between two treatments each (Table 1).

Epigallocatechin gallate (EGCG) is known to be able to exert a significant influence on the expression of LH receptors in the ovaries. Giving EGCG is known to reduce LH levels up to 40-50% in both male and female rats [3]. Decreasing of LH serum levels will reduce the complex of hormone-receptor binding on the cell surface. LH receptors are G-protein pairs that modulate cell function by activating the second messenger signaling pathway and binding to peptide hormone ligands [17,18].

Luteinizing hormone (LH) which is secreted from the anterior pituitary will bind to its receptors in ovarian theca cells. LH signals cause transcription of a number of genes that encode the synthesis of enzymes that function to convert cholesterol into androgens (androstenedione and testosterone) [19]. A portion of androstenedione will diffuse into follicular fluid in granulosa cells. In response to the induction of CYP450arom in granulosa by FSH stimulation, androstenedione will undergo aromatization to estrone which will later be converted to estrogen. Decreased LH secretion due to EGCG administration will reduce the hormone-receptor binding complex so that it will affect the synthesis of enzymes needed for the production of a number of steroid hormones, follicular development and inhibit ovulation [20].

CONCLUSION

EGCG significantly reduced the expression of LH receptors and extended percentage follicular phase. The decrease in LH receptors were consistent with an increase in the dose of EGCG. The effective dose of EGCG administration in reducing LH receptor expression is 4.05mg/100 g rat.

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Conflict of interests: The authors declare that there is no conflict of interests regarding the publication of this article.

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