ORIGINAL ARTICLE

EFFECTS OF GREEN TEA EXTRACT ON GRAAFIAN FOLLICLES AND SERUM 17β-ESTRADIOL IN MONOSODIUM GLUTAMATE-EXPOSED RATS

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Received: August 2, 2018 Accepted: October 10, 2018

ABSTRACT

Monosodium glutamate (MSG) is widely consumed as food preservative and flavor enhancer. MSG administration in rats has been reported to induce oxidative stress causing damage to ovarian histological structures by affecting number of follicles and production of reproductive hormones. Polyphenols in green tea are suggested as powerful antioxidants that inhibit free radicals responsible for causing oxidative stress. This study aimed to investigate the effects of various doses of green tea on the Graafian follicles and serum 17β-estradiol levels in rats exposed to MSG. The design of this study was the post test only control group experiment. A total of twenty-five white female rats (*Rattus norvegicus*) were divided into 5 treatment groups. MSG in a dose of 0.7 mg/g body weight (BW) and green tea extract (*Camellia sinensis*) in doses of 0.7, 1.4, and 2.8 mg/day were administered orally over 30 day period starting after the rats' proestrus phase. Graafian follicles were counted using light microscope while serum 17β-estradiol level was measured using ELISA kit and the data was statistically analyzed using one-way ANOVA. A significant difference of Graafian follicles number ($p<0.04$) and serum 17β-estradiol level ($p<0.006$) was observed across the treatment groups. The results showed that green tea extract at a dose of 1.4 mg/day significantly increased the number of Graafian follicles and serum 17β-estradiol level in female white rats exposed to MSG.

Keywords: 17β-estradiol, Graafian follicles, green tea extract, monosodium glutamate.

1. INTRODUCTION

Infertility in females is a real medical problem. The female reproductive system is very sensitive to different hazardous environmental factors[1]. Chemicals, environmental and industrial pollution, and food additives have been linked to cause harmful effects in humans[2].

Most food additives act either as preservatives or as flavor enhancers. One of the flavor enhancers widely known and used to public under various trademarks is the salt of glutamic acid, i.e. monosodium glutamate (MSG)[3]. Since 1963, Japan along with Korea has pioneered mass production of MSG and its use is growing all over the world. Before the political crisis in 1997, Indonesia's MSG production reached 254,900 tons/year with a concomitant increase in the amount of consumption of around 24.1% per year[3]. Based on Indonesia's Basic Health Research Data in 2013, MSG is consumed by 77.8% of Indonesian population[4]. The consumption of MSG in adults exceed the safe limit when consumed above 3 g/day[5]. Moreover, packaged food sometimes does not properly indicate MSG contents because many other additives also contain MSG, such as flavor enhancers, hydrolyzed protein, yeast, natural flavoring agents, modified starch, textured protein, seasoned salt, soy protein, and similar additives. As a result, the actual MSG levels are often not properly listed[3,6].

MSG causes damage to the arcuate and

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ventromedial nuclei in the hypothalamus when an impaired hypothalamic-pituitary-gonad-axis is present. The results of one study showed that MSG lesions could cause hyperplasia in gonadotropin luteinizing hormone (LH). Although this is not the case in gonadotropin follicle stimulating hormone (FSH) and showed a significant difference in each case[7]. Another study has suggested that MSG administration to white rats cause damage to ovarian histological structures[8]. Ovaries in rats exposed to MSG for 14 days showed structural changes including some follicle degeneration accompanied by oocyte degeneration. There was a significant decrease in the number of primordial, primary, secondary and tertiary follicles in the absence of the corpus luteum even though the number of follicular atresia significantly increased[9].

Estrogen is one of the female reproductive hormones formed by granulosa cells in the ovarian follicle through a series of enzymatic reaction conversions. The synthesis of estrogen increases along with the development of follicles in the ovaries. Fluctuations of the 17β-estradiol hormone during one estrus cycle are consistent with the development of follicles in the ovary[10]. The administration of about 4 mg/g BW of MSG can cause oxidative stress that can lead to the production of reactive oxygen species (ROS)[11]. The ROS can be scavenged by antioxidants while some extracellular antioxidants such as vitamin C, vitamin E, and polyphenols can be obtained from various foods[12].

Green tea contains polyphenols which are powerful antioxidants, useful in capturing free radicals that do not oxidize fats, proteins and DNA in the cells as well as inhibits the proliferation of malignant cells. The flavonoids in polyphenols are responsible for the antioxidant activity and are scavengers for anion radicals, oxygen, lipid peroxyl radicals and can also remove the reactive power of metal ions by attachment to them[12]. The oral administration of green tea at a dose of 14 mg/L has shown some beneficial effects on the toxicity of cadmium chloride on the gonadotropin hormone. Therefore, consumption of green tea is recommended to reduce the toxicity of cadmium chloride[13].

The main objective of this study is to investigate the effect of green tea extract (*Camellia sinensis*) administration on the number of Graafian ovarian follicles and serum 17β-estradiol level in female rats exposed to MSG.

2. MATERIALS AND METHODS

2.1. Study Design

This was an experimental study of 30 day period based on the post test only control groups design approach with in vivo laboratory work. A total of 25 female white rats (*Rattus norvegicus*) of eight weeks of age and 120–150 g in weight were selected. Rats were given standard normal diet and ad libitum access to water. The rats were caged in a 12 hourly light–dark cycle at optimum room temperature with 50–60% humidity. The rats were grouped into five treatment groups:

i. K– (negative control group): No MSG and green tea extract administered.
ii. K+ (positive control group): Only 0.7 mg/g BW MSG administered orally.
iii. PI: Administered with 0.7 mg/g BW MSG and 0.7 mg/day green tea extract orally.
iv. PII: Administered with 0.7 mg/g BW MSG and 1.4 mg/day green tea extract orally.
v. PIII: Administered with 0.7 mg/g BW and 2.8 mg/day green tea extract orally.

A vaginal smear was used to determine the estrus cycle in the rats. Vaginal smears were prepared before dissection in order to determine the proestrus phase of the rats.

2.2. Administration of MSG

MSG (L-glutamic acid monosodium salt hydrate, 99%, Sigma-Aldrich, USA) powder was dissolved in 1 ml of distilled water and the solution was administered orally over a 30 day period using a dose of 0.7 mg/g BW[8].

2.3. Administration of Green Tea Extract

Dry tea leaves were obtained from Wonosobo Tambi Tea Plantation, Central Java, Indonesia. Leaves were

processed by maceration method using 96% ethanol as solvent. Green tea extract was diluted using 1 ml of distilled water. The solution was taken up into a 1 ml syringe and administered orally during the study period of 30 days. The green tea extract was given after 2 h of the MSG administration.

2.4. Measurement of the Number of Graafian Follicles and 17β-Estradiol Serum Level

The number of Graafian follicles was counted using an Olympus XC10 Dot slide light microscope (Tokyo, Japan). Serum 17β-estradiol levels were examined using the ELISA rat kit (CSB E05110r, Cusabio, Houston, USA).

2.5. Statistical Analysis

All data were statistically analyzed using SPSS software (version 23.0, IBM, New York, USA). The data was tested for normality using Shapiro-Wilk and homogeneity test using the Levene's test. One-way analysis of variance (ANOVA) test followed by least significant difference (LSD), were performed to compare the negative and positive control groups over various treatment doses. A two-tailed *p*-value less than 0.05 ($p<0.05$) was considered statistically significant.

3. RESULTS AND DISCUSSION

3.1. Effect of Green Tea Extract on the Number of Graafian Follicles

The effects of three different doses of green tea extract (0.7, 1.4, and 2.8 mg/day) on the number of Graafian follicles are shown in Table 1. The lowest number of Graafian follicles was found in positive control (K+) group (3.20±1.48) while the highest were found in PII group (7.00±1.58). PII treatment group with 1.4 mg/day of green tea extract dose demonstrated the highest mean number of Graafian follicles and lowest mean number of atresia follicles as compared to other doses. On the other hand, the positive control group exposed to MSG at a dosage of 0.7 mg/g BW showed that MSG in white female rats can decrease the number of Graafian follicles in the ovaries. The consumption of MSG can decrease the number of primordial, primary, secondary, tertiary follicles, and increase the number of follicular atresia, decrease the fertility rate and can cause abnormality of ovarian function that can lead to infertility[1,9,14]. Infertility is reported to result from oxidative stress conditions caused by MSG, which is characterized by the formation of free radicals[15].

Table 1. Effects of green tea extract on the number of Graafian follicles and serum 17β-estradiol levels in MSG exposed rats

Treatment	Mean ± SD	*p* value	Mean ± SD (pg/mL)	*p* value
K–	4.80±1.48^{abc}	0.040	2.49±0.14^{ad}	0.006
K+	3.20±1.48^{ac}		2.19±0.07^{b}	
PI	5.40±1.14^{abc}		2.40±0.13^{cd}	
PII	7.00±1.58^{b}		2.51±0.24^{d}	
PIII	4.20±2.86^{c}		2.20±0.15^{bc}	

K– (no treatment); K+ (MSG 0.7 mg/g BW); PI (MSG 0.7 mg/g BW + green tea 0.7 mg/day); PII (MSG 0.7 mg/g BW + green tea 1.4 mg/day); PIII (MSG 0.7 mg/g BW + green tea 2.8 mg/day).

$^{a–d}$ Means with different superscript differ significantly ($p<0.05$).

Physiologically, ROS plays an important role through the regulation of various signals and transduction pathways in folliculogenesis, oocyte maturation, corpus luteum, uterine function, embryogenesis, embryonic implantation and fetoplacental formation[16]. Glutamate is the major amino acid neurotransmitter in the human brain and is important in synaptic communication. If it is produced in excess, it will be re-pumped into the glial cells around the neuron and will cause the cells to die. Glutamate opens the calcium neuron channel and allows calcium to enter the cell[17]. Excessive accumulation of intracellular calcium can cause death or nerve injury due to excessive stimulation of the glutamate receptor. High levels of intracellular calcium accumulation can trigger a cascade of membrane, cytoplasm and neurotoxicity events[18]. There are several places in the brain that are not protected by the blood brain barrier including the arcuate and ventromedial nucleus in the hypothalamus. As the center of homeostasis regulation, the hypothalamus regulates hormonal expenditure that acts on the gonads. Excess amount of MSG can cause damage to the arcuate and ventromedial nucleus in the hypothalamus resulting in decreased gonadotrophin releasing hormone (GnRH) secretion thus affecting the anterior pituitary in decreasing the secretions of follicle stimulating hormone (FSH) and luteinizing hormone (LH)[19].

It has also been observed in the study that MSG exposure had a significant effect on the mean number of Graafian follicles (p<0.040). This happened because MSG can cause hormonal disorders. Granulosa cells that have FSH receptors in the follicles are affected by MSG thus causing disturbance of antrum enlargement. The even formation of a granulosa zone around the edge of the follicles also becomes non-optimal. Granulosa cells not only produce hormones but also supply nutrients to the ova. The destruction of these cells will result in the death of the egg cell causing many atretic follicles[8].

Based on the result of post hoc test with 5% LSD, it can be concluded that the highest number of Graafian follicles are produced by green tea extract with a dosage of 1.4 mg/day. However, this number was not significantly different to the mean number from green tea extract with dose of 0.7 mg/day. Previously, it has been reported that green tea extract can significantly increase the GnRH levels (LH and FSH) in female rats when exposed to cadmium chloride[13]. Furthermore, green tea contains flavonoids that play an important role in scavenging free radicals. The scavenging activity of flavonoids begins with the allocation of hydrogen or electron groups in free radicals (R•). The allocation of hydrogen groups to free radicals will produce flavonoid radical molecules (FlO•) and stable molecules (RH). Radical flavonoids (FlO•) have lower reactivity than free radicals (R•). The flavonoid radicals (FlO•) will bind to other radicals to form non-reactive compounds[20]. Green tea acts as an excellent antioxidant for biological activity such as inhibition of oxidative enzymes, inhibition of transcription factors in cancer, cleansing reactive oxygen and reduction of active metals chelation[21]. Tea leaves contain polyphenols as their main component, comprising about 30–42% of dry weight, and most are catechins that play a direct role as a radical inhibitor for reactive oxygen and nitrogen species. Chemically, catechins have some hydroxyl substituent's that are responsible for antioxidant activity[22]. Catechins give an indirect effect through the activation of antioxidant enzymes[23]. Thus the ROS trapped by green tea provides protection from the effects of MSG consumption on ovarian structures[9].

In the PIII treatment group with 0.7 mg/g BW of MSG and 2.8 mg/day of green tea extract, it has been noted that the mean number of Graafian follicles are lower than PII treatment group (Table 1). Although green tea has some health benefits, this effect only works at a certain dosage level and higher doses can cause some side effects. The effect of catechins in green tea differs for each individual. Epigallocatechin gallate (EGCG) from green tea extract is cytotoxic and the consumption of green tea in higher doses may cause acute cytotoxicity. It has been reported that high intake of green tea can lead to oxidative damage of DNA[24]. EGCG can act as a pro-oxidant, depending on its concentration. Nanomolar concentrations of EGCG are generally

accepted to have antioxidant actions whereas pro-oxidant action occurs at micromolar concentrations[25].

3.2. Effect of Green Tea Extract on Serum 17β-Estradiol Levels

The lowest mean serum 17β-estradiol level has been found in K+ group (Table 1), which indicates that MSG can decrease its serum level. K+ group (2.19±0.07 pg/mL) had lower concentrations of serum 17β-estradiol level than K– group (2.49±0.13 pg/mL). The synthesis of estrogen will increase with the development of follicles in the ovaries. Fluctuations of the 17β-estradiol hormone during an estrus cycle are consistent with the development of follicles in the ovaries[10]. Since the development of normal follicles will produce normal hormone levels therefore if the follicle development process is disrupted, many follicular atresia will adversely affect the formation of estrogen hormone. The rats exposed to MSG have an average reduction of FSH and LH levels compared with rats not exposed to it and thus the 17β-estradiol levels would also decrease[8]. Estrogen is formed by granulosa cells in the ovary follicle through a series of enzymatic reaction conversions. The main substrate of estrogen-formation is cholesterol. Cholesterol sequentially changes into pregnenolone, progesterone, 17β-hydroxy progesterone, androstenedione and testosterone. Testosterone is converted to 17β-estradiol, both in theca and granulosa cells in the ovarian follicles. The synthesis of estrogen hormones will increase with the development of follicles in the ovaries[26]. Theca cells have many LH receptors that work through cAMP to increase cholesterol conversion to androstenedione. Some androstenedione is converted to 17β-estradiol, which then enters the blood circulation. Theca cells also distribute androstenedione to granulosa cells. Granulosa cells have many FSH receptors, and FSH facilitates the secretion of 17β-estradiol by activation of cAMP to increase aromatization activation. Mature granulosa cells also have LH receptors, which also stimulates the production of 17β-estradiol[26].

On the basis of ANOVA test results, it can be concluded that green tea extract at various doses can significantly increase the levels of serum 17β-estradiol in female rats. From the post hoc test using LSD 5%, it can be concluded that the highest mean serum 17β-estradiol level was produced by green tea extract treatment with dose of 1.4 mg/day. However, this was not significantly different from the treatment of green tea extract with dose of 0.7 mg/day. Similarly, green tea extract decreased the estrogen hormone levels significantly (Table 1) which has been found consistent with the findings that the consumption of green tea can regulate hormone secretion. It can also affect hormonal receptors and reduce the adverse effects of hormonal imbalance[27]. Moreover, in the PIII group, the MSG exposure of 0.7 mg/g BW and administration of 2.8 mg/day green tea extract, the mean level of serum 17β-estradiol was lower than the PII treatment group (Table 1). Prior study had shown that regular consumption of green tea in large number by the majority of Japanese contains a large number of polyphenols that affected the estrogen metabolism resulting in decreased estrogen levels[28].

4. CONCLUSION

In this study, it has been observed that green tea extract (*Camellia sinensis*) in various doses (with 1.4 mg/day as an optimum dose) has an influence in increasing Graafian follicles numbers and serum 17β-estradiol levels in white rats exposed to MSG.

ACKNOWLEDGEMENT

The authors are thankful to the laboratory of Pharmacology, Faculty of Medicine, Universitas Brawijaya Malang, Indonesia for helping in extraction from green tea leaves.

FUNDING

None mentioned.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ETHICAL APPROVAL

The study was approved by the Animal Ethics Committee of the Faculty of Medicine of Universitas Brawijaya Malang, East Java, Indonesia.

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