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THE EFFECT OF GREEN TEA EXTRACT (CAMELLIA SINENSIS) ON THE NUMBER OF OVARIAN FOLLICLES OF FEMALE WHITE RAT (RATTUS NORVEGICUS) EXPOSED TO MSG (MONOSODIUM GLUTAMATE): A LITERATURE REVIEW

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ABSTRACT

This literature review aims to determine the effect of green tea extract on the number of ovarian follicles of female rats exposed to MSG. The journals studied in this literature review included ten journals published in 2010-2020 and discussed the effect of green tea on the number of ovarian follicles of female rats exposed to MSG. Based on the literature review results, it was found that giving antioxidants (green tea, vitamin C, Dooshivishari, Chlorella Vulgaris, and Spirulina platensis) can increase the number of ovarian follicles. Another opinion states that the administration of MSG can significantly reduce the number of ovarian follicles. If antioxidants (green tea) are given, the reduction in the number of follicles becomes insignificant. Other sources also state that the administration of MSG can reduce the number of ovarian follicles, while sources other than the above state that the administration of MSG can increase the number of ovarian follicles in mice without giving any antioxidants. There are also sources stating that MSG can increase follicle atresia, and after being given an antioxidant (Ceratonia siliqua), there is a decrease in atretic follicles. Overall it can be concluded that the administration of antioxidants (green tea, vitamin C, Ceratonia siliqua, Dooshivishari, Chlorella Vulgaris, and Spirulina platensis) can increase the number of ovarian follicles in rats exposed to MSG because antioxidants can neutralize free radicals and the adverse effects resulting from MSG.

Review Paper

Keywords: MSG (Monosodium Glutamate), MDA (Malondialdehyde), Ovary, Green Tea, Mice.

INTRODUCTION

Whom data for 2010 stated that the highest infertility was shown to women, with a percentage rate of 62% compared to men, only 38% for cases of married couples. A similar case occurs in Indonesia, where 15% of women out of 239 million Indonesians experience infertility (Prawirohardjo, 2011). *Infertility* is a problem that women usually experience because their reproductive system has a high sensitivity to various adverse chemicals, both natural and manufactured, such as food additives (Buriti et al., 2014)). The additive most often encountered in everyday life is monosodium glutamate (MSG) which functions as a food umami flavor enhancer (Rachma & Saptawati, 2021).

MSG is a crystallized sodium salt compound used as an umami flavor enhancer of dishes (Abdulghani et al., 2022). The most frequently encountered MSG is in fast food. Currently, fast food has become everyone's lifestyle. Most people in Indonesia are also fond of this dish. This food contains many flavor enhancers in MSG, which can stimulate one's appetite and cause addiction (Kesherwani et al., 2022). MSG is widely used around the world in varying doses. The average use of MSG in Indonesia is 0.6 g/day (Hamza & Diab, 2020). High MSG in the body will be fatal to the nucleus of the

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hypothalamus aquartha cells, which can activate glutamate receptors to trigger excess oxidative stress reactions (Mason et al., 2020). Oxidative stress or an imbalance in radical oxygen levels in the body is a case where the body's antioxidant levels cannot neutralize free radicals. The level of antioxidants in the body is inversely proportional to the level of its handler in overcoming free radicals. In this case, the higher the level of free radicalization that occurs, the more antioxidants in the body cannot overcome this because the levels are low. The body exposed to excess MSG quickly experiences oxidative stress, especially in the hypothalamic glutamate receptor organ.

Furthermore, this organ then causes infection in the ovaries, causing dysfunction in the follicles (Tondy et al., 2021). Changes in the follicles include a decrease in the number of primordial, primary, secondary, and tertiary follicles, which results in a significant loss of the corpus luteum (Xue et al., 2022). One of the compounds that play an essential role in reducing MSG levels in the body as antioxidants.

Antioxidants function as inhibitors of free radicalization due to high oxidative stress reactions in the human body, especially in the female reproductive organs (Aboubakr et al., 2021). Various studies have been conducted to find solutions that can minimize glutamate levels which cause free radicalization in the female reproductive organs, one of which is using ingredients with high levels of antioxidants, such as green tea. Based on research by Menzel et al., (2021), green tea has excess antioxidants in the form of catechins which can be inhibitors of Reactive Oxygen Species (ROS), hydroxides, peroxides, and negative superoxide ions. The content of catechins in green tea leaves is an essential compound because it has an excess level of antioxidants which help counteract free radicalization. Greening et al., (2015) also states that the catechin content in green tea can neutralize free radicals due to excess oxidative stress with a ratio of 100:1 in vitamin C and 30:1 in vitamin E.

Based on the description above, a literature review is needed regarding the Effect of Green Tea (Camellia sinensis) Extraction on the Number of Ovarian Follicles of Female White Rats (Rattus norvegicus) exposed to MSG (Monosodium Glutamate) in order to see the link regarding consuming green tea to increasing the number of ovarian follicles of female rats exposed MSG.

METHOD

The research design uses a library research approach or literature review (literature review). This literature review was conducted from August 2020 to May 2021. The dependent variable in the study was the number of ovarian follicles. The independent variables are green tea and MSG. The inclusion criteria in this study were 1) Journals published in the last ten years, namely 2012-2022. 2) The type of journal is original research articles. 3) Using Indonesian and English. 4) Reputable national and international journals. 5) The journals obtained come from databases such as science direct, research gate, google scholar, and PubMed. 6) Journals that use rats or mice as subjects. 7) Journals that discuss the content of green tea or other plants containing catechins, polyphenols, flavonoids, and other antioxidants on ovarian follicles exposed to MSG.

The literature collection procedure is carried out by determining the theme of the literature content to be used per the inclusion criteria. Researchers search for original research literature in databases such as Google Scholar, Research Gate, Science Direct, and PubMed by typing in keywords: green tea, antioxidants, catechins, MSG, number of follicles, and ovaries. Then select literature based on predetermined inclusion criteria and download it and identify national journal accreditation on the Sinta website and international journals on the scimago website. Ten pieces of literature can be obtained, while 30 pieces of literature need to be more suitable and used. The literature used in this literature review was obtained from Google Scholar with four pieces of literature, Research Gate with three pieces of literature, Science Direct with one piece of literature, and PubMed with two pieces of literature. Data quality analysis in this study used questions of quality assessment criteria (Quality Assessment).

RESULT AND DISCUSSION

Study Characteristics

Based on the ten journals used by researchers in this literature review, six journals discuss the effect of antioxidants (green tea, vitamin C, Dooshivishari, Chlorella Vulgaris, and Spirulina platensis) on the number of follicles in experimental animals exposed to MSG, there is one journal that discusses the effect of antioxidants (Ceratonia siliqua) on follicle atresia, and there are three journals that discuss the effect of MSG on the number of ovarian follicles in experimental animals without giving antioxidants. On average, these studies are conducted in Asian countries, namely three studies in Egypt, two studies in India, one in Iraq, three in Indonesia, and one in Islamabad. The research design in the journals used in this literature review uses experimental studies.

Characteristics of Study Population/Sample

The samples used in the ten journals used female rats with an average age of 2-3 months with an average body weight of 100-150 grams. The average length of research is 14-40 days.

Intervention Method

Administration of MSG and antioxidants (green tea, vitamin C, Dooshivishari, Ceratonia siliqua, Chlorella Vulgaris, and Spirulina platensis) in experimental animals was usually carried out orally. The dosage of MSG and antioxidants given in each study is different.

Data Synthesis Results

Five of the ten journals studied stated that giving antioxidants (green tea, vitamin C, Dooshivishari, Chlorella Vulgaris, and Spirulina platensis) can increase the number of ovarian follicles. There is one journal stating that MSG administration can significantly reduce the number of ovarian follicles. If antioxidants (green tea) are given, the reduction in the number of follicles becomes insignificant. As many as two journals stated that MSG administration could reduce the number of ovarian follicles. In contrast, one stated that MSG administration could increase the number of ovarian follicles in rats without any antioxidant administration. There is also one journal stating that MSG can increase follicle atresia, and after being given an antioxidant (Ceratonia siliqua), there is a decrease in follicle atresia.

The Effect of MSG on the Number of Ovarian Follicles

Ten journals discuss the effect of MSG on ovarian follicles, 3 of which are not given any antioxidants. In comparison, the other seven journals are given antioxidants such as green tea, vitamin C, carob extract, Chlorella Vulgaris, Spirulina platensis, and Dooshivishari agada.

The results of the data analysis showed that the administration of MSG to female rats could reduce the number of ovarian follicles. This is because consuming excess MSG can lead to higher levels of glutamate in blood plasma and can trigger free radicals, which can cause cell death, then can attack the rest of the organs with glutamate receptors, such as the hypothalamus. A dysfunctional hypothalamus can cause the anterior pituitary to secrete FSH and LH hormones to be disrupted. Of course, this can affect the development of ovarian follicles because FSH and LH have an essential role in ovarian follicles, where FSH stimulates the growth and development of follicles in the ovaries (Laili et al., 2015).

If the LH and FSH hormones decrease, several changes can occur. Namely, if the LH decreases, ovulation becomes disrupted. The development of the corpus luteum can also decrease so that the production of the hormone progesterone is disrupted. In contrast, if the hormone FSH is disturbed, it can inhibit the development of ovarian follicles. Ovarian atresia occurs, which can disrupt the production of the hormone estrogen (Hanum et al., 2020).

Consuming excess MSG causes glutamate levels to accumulate in the intestines, then flows slowly in the blood so that it spreads throughout the body until it penetrates the blood-brain barrier and binds to receptors. Glutamic acid levels in the body have a high level of toxicity, so it can damage nerves if it exceeds the brain's capacity to maintain it at low levels. Overstimulation of glutamate receptors drives a variant cascade that can potentially damage and kill cells. Glutamate-activated NMDA receptors significantly affect high calcium ions (Ca2+) levels and increased sodium ions via the AMPA receptors. MGlu receptors (especially mGluR1) that have been activated can also increase the release of calcium ions through the endoplasmic reticula (Khaled et al., 2022; Pereira et al., 2022; Saygin et al.,

2018). The unusual behavior of increased calcium ions in nerves can encourage the activation of various pathways that can damage synapses to cause cell death. The behavior of increasing calcium ions also harms mitochondrial function resulting in free radicalization (Sun et al., 2022).

Free radicals are molecules with an excess of free electrons, also called oxidative stress. These free electrons then look for partners with other compounds, such as carbohydrates, proteins, and fats, to cause organ dysfunction in various systems (Jakubczyk et al., 2020). Oxidative stress can cause changes in a person's lifestyle to become addicted to unhealthy food, known as Chinese restaurant syndrome. When someone is addicted to MSG, they experience symptoms such as numbness in the neck and hands, weakness, and damage to other organs that can trigger organ dysfunction (Xue et al., 2022).

In Septadina's study (2011), which examined the effect of MSG on the number of ovarian follicles, it was shown that MSG at a dose of 30 mg/kg/day caused the levels of primary, secondary, and tertiary follicles to decrease. Likewise, the study of Kesherwani et al., (2022) showed that the highest reduction in the number of follicles was in the administration of MSG at a dose of 0.018 mg/grBB. This happens because the toxic substance MSG causes the secretion of the FSH hormone to be not optimal, so the follicle's development is disrupted. These results follow seven other studies that the administration of MSG to female rats can reduce the number of ovarian follicles.

Furthermore, Mondal et al. (2017) stated that MSG administration could significantly increase the number of follicles. It is known that MSG can impair the function of the reproductive organs by increasing the function of the hypothalamus-pituitary-godanal (ovary) axis. MSG acts as a neurotransmitter because of its action on glutamate neurons in the brain. Glutamate neurons found in the brain probably add to the neurons responsible for synthesizing and releasing LHRH and FSHRH, increasing LH and FSH secretion. FSH secreted from the anterior pituitary with ovarian estradiol aids in ovarian follicle maturation, and LH helps release oocytes from mature graft follicles. The difference in results with other studies used in this literature review is probably due to the different doses used.

The Effect of Green Tea on the Number of Ovarian Follicles Exposed to MSG

Based on the ten journals used in this study, 7 used green tea and other sources of antioxidants to reduce the adverse effects on the ovarian follicles of female rats exposed to MSG. The results showed that green tea and other antioxidants increased the number of ovarian follicles in female white rats.

Two journals Abdulghani et al., (2022); de Oliveira et al., (2019); Intra & Kuo, (2007) observed the effect of vitamin C as an antioxidant on the number of ovarian follicles exposed to MSG. The two journals showed that there was an improvement or an increase in the number of ovarian follicles given MSG. The study by Tondy et al., (2021) stated that vitamin C is an effective antioxidant for reducing the effects of oxidative stress from MSG. Joshi et al., (2021) showed that the lowest number of ovarian follicles was found in the MSG group, and then there was an improvement in the MSG + vitamin C group. The improvement in the follicles was due to vitamin C increasing the integrity of the follicular membrane and synthesizing collagen needed for follicle growth (Weanbiao, 2013). Vitamin C can also counteract the effects of free radicals, pollution, and toxic compounds and is the primary antioxidant that dissolves in water, plasma, and tissues (Kitchenham et al., 2011).

Vitamin C has one molecule of ascorbate anion (AscH-), which is the leading antioxidant donor that reacts with free radicals. Vitamin C can also increase progesterone levels in infertile women, reduce MDA levels in the testes, and increase FSH and LH (Amiri-Rigi & Abbasi, 2016). Beltagy et al. (2016) obtained research results in the form of MSG administration that can reduce ovarian follicles' development. With the administration of antioxidants (Ceratonia Siliqua), it is known that there are improvements in the histological structure of the ovaries. Because Ceratonia Siliqua is rich in flavonoids and polyphenols, Ceratonia Siliqua exhibits vigorous antioxidant activity.

Furthermore, Ibrahim et al., (2021) stated in their research that the administration of Dooshivishari Agada (DVA) as an antioxidant showed that the level of ovarian follicles exposed to MSG increased. Most DVA content has antioxidant properties that fight the oxidative

stress caused by MSG. DVA ingredients such as Lodhra, Gokshura, Yashtimadhu, and Jatamansi have antioxidant properties whereby oxidative stress decreases and egg cell growth is normalized. Gokshur stimulates the GDF9 and BMP15 genes, which have essential roles in the regulation of folliculogenesis. Likewise, the study of Abdel et al. (2018) observed the effect of Chlorella Vulgaris (Cv) and Spirulina platensis (Sp) extracts as antioxidants on the number of ovarian follicles exposed to MSG. The study's results also stated an increase in ovarian follicles. *Chlorella Vulgaris* is a single-celled freshwater green microalga belonging to the Phylum Chlorophyta, which is rich in protein, lipids, carotenoids, vitamins, and minerals. At the same time, Spirulina platensis is a blue-green alga used as a human food supplement. Sp has instrumental characteristics as an antibacterial, antifungal, antiviral, anticancer, anti-inflammatory, and antioxidant.

Two journals Greening et al., (2015); Weanbiao, (2013) use green tea as an antioxidant. In Ali et al.'s research. (2014) showed that giving MSG at a dose of 4 mg/gBW caused a decrease in the number of primordial, primary, secondary, and Graafian follicles. The administration of green tea at a dose of 300 mg/kgBW could make the number of follicles insignificant (there was an improvement because it previously significantly decreased). Ali et al. (2014) stated that green tea extraction was able to minimize ovarian dysfunction caused by MSG due to its role as an antioxidant. Likewise, the study of Kesherwani et al., (2022) found that MSG at a dose of 0.8 mg/gBB could reduce the number of gray follicles in the ovaries, and green tea at a dose of 1.5 mg/day significantly increased the number of gray follicles.

The results of all the journals used stated that there was an improvement and was able to increase the ovarian follicle levels of female rats exposed to MSG because the antioxidants contained in green tea function as inhibitors of electron-free radicalization, which causes oxidative stress. Antioxidants inhibiting free radicalization usually propagate to cause lipid peroxidation, which can neutralize various tissue dysfunctions in the human body. Antioxidants with high free electrons give one of their ionic charges to free radicals so that they become neutral from the previous one with an excess of free charges to be balanced (Zheng & Wang, 2009).

Green tea contains catechins, which are included in the flavonoid group and are often referred to as polyphenolic compounds, potent antioxidants, and helpful in capturing the free radicals caused by MSG. Flavonoids are responsible for antioxidant activity and are scavengers of anion radicals, oxygen, and lipid peroxyl radicals, and they can also reduce the strength of metal ions by capturing them (Mason et al., 2020; Menzel et al., 2021). Flavonoid antioxidants function as free radical binding ions and free radical ion scavengers. The antidote function of flanoid is to prevent the formation of free radicals that occur due to the attraction of free radical electrons. In contrast, the critical function is to provide electrons to free radical reactions so that they cannot interfere with other essential compounds in the body.

Excessive amounts of MSG can cause damage to the arcuate and ventromedial nuclei in the hypothalamus, which cause a decrease in the level of GnRH hormone secretion, thereby affecting the anterior pituitary in reducing FSH and LH secretion. The decrease in FSH and LH secretion levels certainly significantly affects the ovarian follicles, where FSH is needed to stimulate the development of follicles in the ovary. If there are obstacles in carrying out FSH secretion, FSH levels may decrease, resulting in the follicles not developing correctly (Abdulghani et al., 2022). Green tea provision can minimize free radicalization formation, causing LH and FSH to increase (Adinugroho et al., 2022). This, of course, can improve the development of ovarian follicles so that the number of ovarian follicles increases because the secretion of the hormones FSH and LH are not inhibited.

CONCLUSSION

From the results of this literature review, it can be concluded that the administration of MSG to female rats can reduce the number of ovarian follicles, and the presence of green tea extract or other antioxidants can improve the level of ovarian follicles increasing. This happens because green tea with high antioxidants can minimize free radicalization and the destructive effects caused by MSG. However, one journal states that giving MSG can increase the number of ovarian follicles

because glutamate neurons in the brain may increase LH and FSH secretion.

To add to the literature and clarify the mechanisms that occur, it is recommended to carry out further research on the effect of green tea extraction on ovarian follicles exposed to MSG.

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