Protective effect of green tea extract on ovary tissue function in rats treated by Malathionsecticide

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Abstract

Introduction:
This study was conducted to investigate the protective effect of green tea extract on ovary tissue function in rats treated by Malathion insecticide.

Materials and Methods:
A total of 72 mature Wistar rats aged 2 to 3 months (approximate weight of 200±15 g) were experimented. Rats were divided into 9 groups of eight. Control group did not receive any medication. Sham group was given 0.2 cc physiological serum and experimental groups 1, 2 and 3 received 100, 200 and 400 mg/KG bw green tea extract respectively, experimental 4 was given 40 mg/KG bw Malathion and experimental groups 5, 6 and 7 received 100, 200 and 400 mg/KG bw green tea extract respectively and 40 mg/KG bw Malathion. After 15 days, the serum levels of sex hormones were measured, and ovaries were removed for counting the ovarian follicles.

Results:
Serum concentration of estrogen, progesterone, FSH and LH were significantly decreased in experimental group 4 compared to control and other groups. The primary and Graafian follicles decreased in experimental group 4 compared to control group. Primary and secondary follicles and corpus luteum significantly decreased in experimental group 4 compared to control group. All examined parameters except Graafian follicles and corpus luteum increased in the experimental groups 5, 6 and 7 compared to experimental group 4. Follicular atresia increased in experimental group 4 compared to other groups and significantly decreased in experimental groups 5, 6 and 7 compared to experimental group 4.

Conclusions:
Malathion had an adverse effect on secretion of sex hormones in female rats, as well as the process of oogenesis, and green tea extract decreased those negative effects.

Keywords: Malathion, Green Tea, Oogenesis, Sex Hormones, Rat

Introduction
Green tea is currently considered a useful source for human health with biological and pharmacological activities. The health benefits of tea extract and its polyphenolic catechins have attracted most scientific research in the prevention and treatment of many diseases. Tea leaves, as the only food product having Epigallocatechin -3-
gallate (EGCG) have active compounds with eight free OH-groups and high antioxidant activity (1, 2). Tea is called khatai tea in traditional medicine and Shay in Arabic, and its shrub or bush is called Theier in French and Teaplant in English. This plant belongs to Temsastromiaceae, Camelliaceae, and Theaceae families, and Camellia sinensis and Camellia Theifer are its scientific names. Tea is a big shrub with a maximum height of one and a half meters and glossy leaves and beautiful white flowers. This plant contains polyphenolic compounds, including Epigallocatechin -3- gallate (EGCG), Epigallocatechin -EGC, Epicatechin -3- gallate-ECG, and Epicatechin –EC (3, 4, 5, 6). The most potent of these antioxidants, Epigallocatechin -3- gallate (EGCG) has been reported to be 100 times as potent as vitamin C and 25 times as potent as vitamin E (7). The protective effect of green tea on body tissues may be due to its antioxidant property (8). The most important ingredient in green tea is catechin (ECGC, the most abundant catechin). This substance is an extremely potent and effective antioxidant and many beneficial effects of green tea are attributed to it (9, 10, 11). Studies have shown that a cup of green tea (2.5 g green tea in 200 ml of water) may contain 90 mg EGC (12). Green tea has been introduced as an anti-inflammatory, anti-cancer, anti-cholesterol, anti-diabetic, anti-mutation, anti-microbial, anti-stroke, and anti-oxidative agent (13). In addition, bioactive components of green tea (catechins and caffeine) stimulate the sympathetic system and increase heat production and fat oxidation in the body and accordingly, exert their anti-obesity effects (14, 15).
Catechin, as a natural antioxidant, affects the reproductive system and stimulates testosterone production by the Leydig cells and decreases the content of total cholesterol, triglycerides and phospholipids in testicular tissue. It also prevents the mutagenicity of mutagenic chemicals on chromosomes (8). The ability of sperm viability, mobility, and fertilization highly depends on its antioxidant capacity expressed in seminal plasma. Being rich in polyphenols, as potent antioxidants, green tea inhibits reactive oxygen and nitrogen species, and thus improves sperm quality (9).
Malathion (S-(1,2-dicarboethoxyethyl) O, O-dimethyl phosphorodithioate) is one of the most widely used insecticides in Iran. Malathion is a yellowish dark brown concentrated and oily liquid that is used as a broad-spectrum pesticide in agriculture (16). This substance is a derivative of D-tonic phosphoric acid and one of the oldest P-group insecticides introduced in 1950 (17). Organophosphates (OPs) are a class of pesticides widely used in agriculture, veterinary medicine, industry, residential areas, and as nerve agents in wars. These compounds cause the most accidental poisonings with acute or chronic complications (18, 19).
Studies have shown that toxic effects of some OPs are not limited to cholinesterase inhibition, but as a result of cholinergic crisis (increased acetylcholine), changes such as cell membrane damage, free radical production, and the antioxidant system malfunction are also observed (20, 21). By conducting a study on the genotoxic effects of Malathion, Giri et al. (2002) found that this toxin affects sperm count and causes cell mutation (22). Weisburger (2002) reported that tea polyphenols have potent antioxidant activity and can reduce LDL oxidation and oxidized DNA metabolism, and subsequently decrease the risk of heart diseases and cancer (13). Crespy and Williamson (2004) reported that green tea extract has antioxidant properties and eliminates free radicals (23). Since green tea is high in catechins, it is considered a potent antioxidant. Azarnia et al. (2013) conducted a study on the impact of hydroalcoholic extract of green tea on rats with polycystic ovary syndrome (PCOS) and reported reduced serum concentrations of LH, CRP, IL-6, insulin, and glucose in polycystic ovary syndrome (PCOS) and reported reduced serum concentrations of LH, CRP, IL-6, insulin, and glucose in...
groups treated with green tea extract. Histomorphometric studies also indicate an increase in the number of follicles and changes in the thickness of the follicular theca cells for improving PCOS. Green tea can be a potential medication for the treatment of PCOS and type 2 diabetes (24).

The results of the study of Sanaei et al. (2014) also showed that administration of green tea extract for 42 days significantly increased the number of primary, growing, and antral follicles (25). Shariatzadeh and Mohammadi (2014) reported that the use of green tea can significantly compensate damage caused by the use of sodium arsenite, sperm count, motility, viability, and normal morphology, as well as the diameter of the lumen of the seminiferous tubules, germinal epithelial thickness, and the levels of MDA (P<0.001) (26).

The present study aimed to investigate the protective effect of green tea extract on ovary tissue function in rats treated by Malathion insecticide.

Materials and Methods

Animals used: A total of 72 mature female Wistar rats aged 2 to 3 months (approximate weight of 200±15 g) were purchased from Animal House of School of Veterinary Medicine, Shiraz, Iran. Rats were transferred to the Animal House of Jahrom University of Medical Sciences and were housed in special sterilized containers at a temperature of 22-20 °C and moisture content of 60-40 percent for two weeks to be adapted to the environment. During the experiment, they were given foods provided from Fars Livestock and Poultry Company and tap water in special drinkers.

Medications and materials used: Green tea plant (medicinal herb shops in the city), Malathion (Moshkfarfars factory), the FSH hormone kit (Iran Pishtaz Teb, serial number Catnom BYEK1370), the LH hormone kit (Iran Pishtaz Teb, serial number Catnom BYEK1370), the progesterone hormone kit (REF DKO 006, Germany), the estrogen hormone kit (REF DKO 003, Germany).

Green tea extraction method: To prepare the extract, green tea was first milled and soaked in 80% ethanol for 24 hours. The solution was then filtered and the extract was obtained through vacuum concentration method. The extract will be soluble in distilled water (27, 2).

Methods: This experimental study was conducted in Animal House of Islamic Azad University, Jahrom Branch, May 2013. All ethical considerations were observed in relation to animal care and working with laboratory animals during the study and the topic was approved by the Ethic Committee of Islamic Azad University, Jahrom Branch (D/P/2990 dated February 28, 2013). According to previous articles, rats were divided into 9 groups of eight as follows:

Control group did not receive any medication. Sham group was given only physiological serum. Experimental groups 1, 2, and 3 received 100, 200, and 400 mg/kg bw green tea extract respectively, experimental 4 was given 40 mg/kg bw Malathion, and experimental groups 5, 6, and 7 received 100, 200, and 400 mg/kg bw green tea extract respectively and 40 mg/kg bw Malathion. Rats were injected intraperitoneally with green tea extract and Malathion for 14 days.

Determination of serum levels of hormones: On the 15th day, rats were anesthetized with diethyl ether and blood samples were taken from their hearts. Blood samples were placed in a steam bath at 37 °C to be clotted. After centrifugation at 3000 rpm for 10 minutes, the serum was separated. LH, FSH, progesterone, and estrogen levels were measured using the ELISA method. After the sampling, the abdominal region of the rats was incised with a scalpel. Left and right ovaries were removed with a scalpel and forceps from the surrounding fat tissues and the fallopian tube and after washing with physiological saline, they were placed in separate containers of 3% formalin.
solution. Tissue sections were then prepared from the samples. Prepared slides of left and right ovaries of rats were studied separately by a light microscope. In each slide, the number of primary, secondary, Graafian, and atretic follicles, and corpus luteum were counted with 40x and 100x magnification.

**Statistical analysis method:** One-way analysis of variance (ANOVA) was used for statistical analysis. Since the data were normally distributed according to the Kolmogorov-Smirnov test results, parametric tests were used. Duncan’s test was applied to find the difference between the means when there was a significant difference between different groups. Statistical analysis was performed using SPSS-16 and the significance level was considered P<0.05. The results are presented as Mean±SEM in the results section. Figures were drawn by Excel software.

**Results**

**Changes in plasma concentration of LH:**
The results of changes in plasma concentrations of LH showed that LH levels reduced significantly in experimental group 4 (Malathion group) compared to control group and other experimental groups at P<0.05. A significant increase was observed in experimental groups 5, 6, and 7 (groups receiving minimum, average, and maximum Malathion and tea) compared to Malathion group at P<0.05 (Figure 1).

![Figure 1: Comparison of LH concentrations between the groups studied](attachment:image.png)

- The values are shown as Mean±S.E.
- According to Duncan’s test, if columns have at least one common letter, it indicates there is no significant difference between columns.

**Changes in plasma concentration of FSH:**
The results of changes in plasma concentrations of FSH among the groups studied showed that FSH levels reduced significantly in experimental group 4 (Malathion group) compared to control group and other experimental groups at P<0.05. A significant increase was observed in experimental groups 5, 6, and 7 (groups receiving minimum, average, and maximum Malathion and tea) compared to Malathion group at P<0.05 (Figure 2).
Changes in plasma concentration of estrogen:
The results of changes in plasma concentrations of estrogen among the groups studied showed that estrogen levels significantly increased in experimental group 2 (group receiving average dose of tea) and significantly decreased in experimental group 4 (Malathion group) compared to control group at P<0.05. A significant increase was also observed in experimental groups 5, 6, and 7 (group receiving minimum, average, and maximum Malathion and tea) compared to Malation group at P<0.05 (Figure 3).

Changes in plasma concentrations of progesterone:
The results of changes in plasma concentrations of progesterone among the groups studied showed that progesterone levels significantly decreased in experimental group 4 (Malathion group) and 5 (the group receiving Malathion and the minimum tea) and significantly increased in experimental group 2 (group receiving average dose of tea) compared to control group (P<0.05). A significant
increase was also observed in progesterone levels in experimental groups 5, 6, and 7 (group receiving minimum, average, and maximum Malathion and tea) compared to Malathion group at P<0.05. A significant increase was also observed in plasma concentrations of progesterone in experimental groups 6 and 7 compared to experimental group 5 (Figure 4).

The number of primordial follicles:
The results of the number of primordial follicles among the groups studied showed that the number of primordial follicles increased in experimental groups 2 and 3 (the group receiving average and maximum doses of tea) and significantly decreased in experimental group 4 (Malathion group) compared to control group (P<0.05). A significant increase was also observed in experimental 5, 6, and 7 (the group receiving minimum, average, and maximum Malathion and tea) compared to Malathion group (P<0.05) (Figure 5).
Changes in the number of primary follicles:
The results of the number of primary follicles among the groups studied showed that the number of primary follicles significantly decreased in experimental group 4 (Malathion group) compared to control group and other groups (P<0.05). A significant increase was also observed in experimental 5, 6, and 7 (the group receiving minimum, average, and maximum Malathion and tea) compared to Malathion group (P<0.05) (Figure 6).

Changes in the number of secondary follicles:
The results of the number of secondary follicles among the groups studied showed that the number of secondary follicles decreased in experimental group 4 (Malathion group) and significantly increased in experimental group 2 (average dose of tea) compared to control group (P<0.05). A significant increase was also observed in experimental 5, 6, and 7 (the group receiving minimum, average, and maximum Malathion and tea) compared to Malathion group (P<0.05) (Figure 7).
Changes in the number of Graafian follicles:
The results of the number of Graafian follicles among the groups studied showed that the number of Graafian follicles increased in experimental groups 2 and 3 (the group receiving average and maximum doses of tea) and significantly decreased in experimental group 4 (Malathion group) compared to control group (P<0.05). A significant increase was also observed in experimental 5, 6, and 7 (the group receiving minimum, average, and maximum Malathion and tea) compared to Malathion group (P<0.05) (Figure 8).

![Figure 8: Comparison of the number of Graafian follicles between the groups studied](image)

Changes in the number of corpus luteum:
The results of the number of corpus luteum among the groups studied showed that the number of corpus luteum increased in experimental group 2 (average dose of tea) and significantly decreased in experimental groups 3 (maximum dose of tea) and 4 (Malathion group) compared to control group and other groups (P<0.05). A significant increase was also observed in experimental 5, 6, and 7 (the group receiving minimum, average, and maximum Malathion and tea) compared to Malathion group (P<0.05) (Figure 9).

![Figure 9: Comparison of the number of corpus luteum between the groups studied](image)
Changes in the number of atretic follicles:
The results of the number of atretic follicles among the groups studied showed that the number of atretic follicles significantly increased in experimental group 4 (Malathion group) compared to control group and other groups (P<0.05). A significant decrease was also observed in experimental 5, 6, and 7 (the group receiving minimum, average, and maximum Malathion and tea) compared to Malathion group (P<0.05) (Figure 10).

![Figure 10: Comparison of the number of atretic follicles between the groups studied](image)

Discussion
The results showed that progesterone, estrogen, FSH, and LH levels significantly reduced in experimental group 4 compared to control group and other groups, and significantly increased in experimental groups 5, 6, and 7 (the group receiving minimum, average, and maximum Malathion and tea) compared to Malathion group (P<0.05). Increased serum levels of sex hormones have been reported useful for the evaluation of human and animal fertility (28). In general, a significant reduction in concentration of sex hormones in fertility activities leads to fertility disorders in people who are exposed to chemicals (29).

Exposure to chemicals led to ovarian tissue destruction and significant changes in progesterone and estrogen in rats which it was also confirmed in previous studies. Yarbe et al. (2009) reported that reduced serum levels of progesterone and estrogen occur due to ovarian damage which is consistent with the results of this study. At the time of fertility and ovulation, estrogen and progesterone are normally affected by the pituitary gland producing LH and FSH (30). Thus, it can be said that ovarian tissue damage and impaired secretion of estrogen and progesterone which can occur increasingly or decreasingly justify a significant reduction in estrogen and progesterone in experimental group 4 compared to control group. LH and FSH changes may also be due to reduced GnRH which decreased the serum levels of LH and FSH. The hypothalamic-pituitary-gonadal axis regulates reproductive activities, a multifactorial process, by genetic and hormonal control. In this study, the results of histological evaluation showed that treatment with Malathion leads to ovarian cell damages and induces extensive histopathological changes in ovarian follicles. Malathion can cause toxic effects on the reproductive system through oxidative stress (31), which can be reduced with concurrent administration of antioxidants. This result is consistent with our results. By conducting a study on the
protective effect of green tea extract on ovary function in mice treated by Paclitaxel drug. Mohseni Kouchesfahani and Asoudeh (2014) reported that green tea extract improves ovarian parameters treated by Paclitaxel. Green tea extract with its antioxidant properties has protective effects on ovarian tissue parameters in mice and LH and FSH levels after treatment with Paclitaxel (32). This protective effect of green tea on the parameters of ovarian tissue is consistent with our results. According to a study conducted by Ghaafariyan et al. on the effect of green tea extract on the histology of the ovary in polycystic ovary syndrome, it was found that green tea affects oocyte maturation and follicular development in polycystic women. Green tea extract reduces the theca layer thickness in rats and may increase lipolysis and reduce the hypertrophy of this layer. Accordingly, the production of androgens and steroids also decreases by the mentioned layer (33). It seems that the difference in the type of animal, developmental stage, treatment duration, and the type of field is the main reason for these differences. The results of the study of Allahdadian et al. showed that green tea consumption has a significant effect on reducing free testosterone in patients with polycystic ovarian disease (34). According to a laboratory study conducted by Figueiroa et al. on the effect of green tea compounds on testosterone production in rabbit Leydig cells, it was found that green tea compounds inhibit basic and stimulated production of testosterone (35). According to a study conducted by Wu et al. on the effect of two months of green tea consumption on testosterone level in healthy postmenopausal women, it was reported that green tea supplements caused no constant changes in the testosterone levels (36). According to a study conducted by Shayeghi et al. on the effect of Malathion insecticides on the inhibition of cholinesterase enzyme among the agricultural sprayers, it was found that Malathion, as a phosphate insecticide, has gastrointestinal toxicity and fumigant property. They also found that decreased cholinesterase activity will be less if health regulations are observed when using Malathion (37). Fortunato et al. (2006) reported that Malathion induces the production of free radicals and oxidative stress in the brain and increases the activity of antioxidant enzymes (31). Oxidative stress is caused by free radicals and mitochondria are identified the main region of the production of free radicals (38). By evaluating the effect of Malathion-induced stress on fat metabolism, scientists found that the lethal and sub-lethal doses of this toxin increase fatty acids, glycerol and lipase activity in tissues studied and can have a negative impact on reproductive activity. Malathion also has a negative impact on reproductive activity and decreases the amphibian population which is consistent with the present study (39). Studies conducted in recent years emphasize the role of reactive oxygen species as a contributing factor in some tissue damage. An imbalance in the production of reactive oxygen species and antioxidant enzyme activities leads to tissue damage and it can occur through either increased production of reactive oxygen species or decreased antioxidant enzyme activities, or both, suggesting the existence of oxidative stress conditions (40). Reactive oxygen species can severely endanger cell life by weakening the structure and function of plasma membrane and intracellular membranes (41). By examining ovarian tissues and counting the follicles using a light microscope, a decrease was observed in the number of primordial and Graafian follicles in experimental group 4 (Malathion group) and a significant increase was found in experimental groups 2 and 3 (the average and maximum doses of green tea) compared to control group (P<0.05). The number of Graafian follicles also increased in experimental group 6.
compared to Malathion group. The number of primordial follicles increased in experimental groups 5, 6, and 7 (the group receiving minimum, average, and maximum Malathion and tea) compared to Malathion group (P<0.05). The number of atretic follicles significantly increased in experimental group 4 (Malathion group) compared to other groups (P<0.05). Also, the number of atretic follicles in experimental groups 5, 6, and 7 (the group receiving minimum, average, and maximum Malathion and tea) significantly reduced compared to Malathion group (P<0.05).

The number of primary and secondary follicles and corpus luteum significantly reduced in experimental group 4 compared to control group (P<0.05). The number of primary and secondary follicles significantly increased in experimental groups 5, 6, and 7 (the group receiving minimum, average, and maximum Malathion and tea) compared to Malathion group (P<0.05).

Organophosphate insecticides have alkylating properties and thus can affect the cell nuclear DNA (42). These chemicals have also electrophilic property. Therefore, they can affect cellular proteins and lead oocytes and follicular cells to lose their efficiency. In this study, the destructive impact of Malathion was also confirmed (43). Malathion increases malondialdehyde in ovarian tissue that may be caused by released radicals or lipid and body metabolism. With increasing doses, Malathion reduces the number of healthy follicles, increases the number of atretic follicles, and causes changes in the corpus luteum which is consistent with the results of the present study (44). Salvadori et al. (1988) found that Malathion induces chromosomal aberrations in somatic cells (bone marrow) and primordial germ cells (primary spermatocytes) (45). Studies have also indicated that oxidative stress plays an important role in pathogenesis of various diseases, including cancer, diabetes, cardiovascular diseases, Parkinson's disease, schizophrenia, atherosclerosis, pulmonary diseases, and cataracts (46). Oxidative stress is caused by free radicals and mitochondria are identified in the main region of the production of free radicals (38). When cell properties are changed, oocytes and follicular cells lose their normal efficiency. According to what was mentioned and the fact that the production of primordial follicles in mammals in embryonic period is a local phenomenon caused by ovarian factors and germ cells, and considering the fact that the continued growth of the follicles is influenced by the hypothalamic and pituitary hormones, a significant decrease in primordial, primary, and secondary follicles can thus be justified. In this study, significant changes in the pituitary hormones were also observed. As mentioned, cellular damage caused by pesticides can be caused by various reasons, but the impact of Malathion on the structure of DNA and cellular proteins can change cell function. Considering the possible effect of Malathion on changes in natural concentrations of gonadotropins at the time of injection, it can be concluded that with abnormal development of follicular cells, reduced thickness of granulosa layer and follicular sheath, estrogen production decreases which is consistent with our results. Development of follicular sheath is directly affected by factors secreted from the granulosa layer and reduced granulosa layer can thus affect the growth of follicular sheath (38). Studies have shown that green tea has antioxidant properties and eliminates free radicals (23) through the inhibition of cytochrome P450 expression (47, 48). It has also been reported that green tea extract can reduce oxidative damage caused by cyclosporine A (27). Studies have shown that green tea catechins prevent lipid peroxidation caused by chemicals in the liver and kidneys of animals (49).
Conclusion

According to the results of histological studies and ovarian hormones, it can be concluded that Malathion insecticide destroys ovarian tissue and reduces the activity of the pituitary-gonadal hormones. These effects can be generalized to humans to a less extent. However, the manner of use and extent of exposure to this insecticide is very important. Therefore, care should be taken in generalizing these results to humans. Due to its antioxidant property, green tea extract can reduce the negative effects of Malathion insecticide, so the use of green tea extract is recommended to reduce the harmful effects of Malathion.

References: