



Effects of Aerobic Exercise Along with Garlic Supplementation on Oxidative Stress and Sex Hormones in the Ovarian Tissue of Rats Under Wi-Fi Electromagnetic Radiation

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Abstract

Background: Electromagnetic radiation has different effects on body organs, including gonads and fertility. Information about the harmful impacts of Wi-Fi radiation on the oxidant-antioxidant system of ovarian tissue is limited.

Objectives: Therefore, the present study aimed to evaluate the influence of eight weeks of aerobic exercise along with garlic supplements on the levels of superoxide dismutase (SOD), malondialdehyde (MDA), glutathione peroxidase (GPx), and sex hormones in the ovarian tissue of rats under Wi-Fi electromagnetic radiation.

Methods: This experimental study was performed on 40 Sprague Dawley female rats aged about 8 weeks. The animals were randomly divided into 5 groups: control, Wi-Fi radiation, aerobic exercise + Wi-Fi radiation, garlic supplement + Wi-Fi radiation, and aerobic exercise + garlic supplement + Wi-Fi radiation. Wi-Fi radiation was induced 1 h/day at a frequency of 2.45 GHz from a Wi-Fi modem. Endurance exercise was performed as three 60-minute sessions per week for 8 weeks with 50%-60% maximum running speed. Garlic supplement was given to rats for 8 weeks in drinking water at the dose of 800 mg/kg daily.

Results: The results of this study showed that Wi-Fi radiation led to a decrease in the SOD and GPx levels, as well as an increase in the follicle-stimulating hormone (FSH) and MDA levels in rats ($P \leq 0.05$). Moreover, garlic supplements diminished the FSH and MDA concentrations, as well as elevated the SOD levels in rats under Wi-Fi radiation. It was observed that aerobic exercise caused a decline in the FSH and MDA levels and a rise in the luteinizing hormone and SOD of the rats under Wi-Fi radiation. Furthermore, aerobic exercise along with garlic supplements reduced the FSH levels and augmented the SOD and GPx concentrations in rats under Wi-Fi radiation ($P \geq 0.05$).

Conclusions: According to the findings of the present study, aerobic exercise and garlic supplements both have lowering effects on oxidative stress and synergistically increase antioxidants. In addition, aerobic exercise and garlic supplements alone improve the sex hormones of rats exposed to Wi-Fi radiation.

Keywords: Aerobic Exercise, Garlic Supplements, Glutathione Peroxidase, Malondialdehyde, Sex Hormones, Superoxide Dismutase, Wi-Fi Radiation

1. Background

Wi-Fi, as a wireless technology, has led to a dramatic increase in background radiofrequency radiation in the human environment during the last years through smartphones, laptops, and other electronic devices that connect to the Internet (1). It may lead to considerable environmental changes and serious impacts on human biology and health (2). Electromagnetic radiation, one of

the waves emitted by Wi-Fi routers, has been proposed as a new oxidant that affects living cells. Ionizing radiation has direct and indirect biological effects, causing damage to DNA molecules and genetic materials, making it highly dangerous for human and animal health (3). Some studies have shown that Wi-Fi causes neuropsychiatric influences, apoptosis, oxidative stress, sperm/testicular damage, endocrine changes, and cellular DNA damage (4). It has been demonstrated that the exposure of

female rats' glands to Wi-Fi for a long time altered oocyte ultrastructure, changed the endocrine system, and reduced the success rate of mating in female mice (5).

On the other hand, the natural antioxidant systems in humans and animals play an important role in inhibiting and diminishing the reactive oxygen species (ROS) and the negative effects of ionizing radiation (6). The results of some investigations indicated that the function of these antioxidant systems increases with exercise (7). Therefore, moderate- and low-intensity exercises can improve sexual function by reducing oxidative stress levels (8). However, information regarding the effects of exercise on sex hormones and infertility is not complete.

Recently, the usage of herbs has been taken into consideration because of their antioxidant influences. Medicinal plants are suitable treatments in advanced and traditional systems (9). For example, garlic has numerous properties and therapeutic effects due to its antioxidant effects. Garlic, as a member of the *Liliaceae* plant family, contains a variety of compounds, including minerals, carbohydrates, protein, fat, and vitamins (10). Garlic extract stimulates the secretion of gonadotropins and ovarian hormones, as well as enhancing the function of estrogen receptors by activating the pituitary gland (11). Garlic contains organosulfur and phenolic compounds that it makes a potent antioxidant compound. The pharmaceutical effect of garlic is related to allicin, the effect of which is characterized by antimicrobial, antilipidemic, antiviral, and anticancer properties (12, 13).

2. Objectives

Many studies evaluated the antioxidant effects of moderate- and low-intensity exercises on the antioxidant system (14, 15), as well as the positive impacts of garlic on sex hormones (12, 13). In the current century, the use of devices that emit electromagnetic waves is increasing. Consequently, it is essential to investigate the interactive effects of exercise and garlic on the antioxidant system and oxidative stress in ovarian tissue due to the possible oxidative impacts of electromagnetic waves. These results could be useful in finding the most desirable prevention and treatment for infertility-related problems in ovarian tissue and can provide a new view in this regard. Therefore, the present study aimed to evaluate the effect of eight weeks of aerobic exercise along with garlic supplements on the levels of superoxide dismutase (SOD), malondialdehyde (MDA), glutathione peroxidase (GPx), and sex hormones in the ovarian tissue of rats under Wi-Fi radiation.

3. Methods

3.1. Animal Experiments

Sprague Dawley rat models were obtained from the Sports Physiology Laboratory of the Marvdasht Branch of Islamic Azad University, Iran. A total of 40 rats with an average weight of 150-200 g and a mean age of 8 weeks were selected. These rats were randomly divided into five groups: (1) control group, (2) intervention I group: Wi-Fi radiation, (3) intervention II group: Wi-Fi radiation and aerobic exercise, (4) intervention III group: Wi-Fi radiation and garlic supplement, and (5) intervention IV group: Wi-Fi radiation along with aerobic exercise and garlic supplement.

3.2. Wi-Fi Radiation Therapy

The rats of intervention groups I, II, III, and IV were exposed to 2.45 GHz radiation emitted from a Wi-Fi modem one hour per day for eight weeks. Moreover, to reduce the interference of other radiations, all other electromagnetic devices (even cell phones) were placed at least 25 m away from the research site during the study.

3.3. Aerobic Exercise

First, rats were kept in polycarbonate cages with autoclave capability at a temperature of 20 - 24°C, 12:12 light-dark cycle, and 55 - 60% humidity for one week to adapt to standard conditions. Next, the rats of intervention II and IV groups were trained in aerobic exercise with a special treadmill for rats at a speed of 8 m/min and zero slope for 10 min three times a week for eight weeks (16).

The rats started running at a speed of 11.6 m/min, and then 1.6 m/min was added to the initial speed every minute to reach the speed of 20 m/min. Afterward, the running speed was increased by 2.3 m/min until the rats were exhausted. Exhaustion was when rats struck the end of the tape at least five times in one minute. The training program included three warm-up stages of 5 min at a speed of 7 m/min and the main training followed by cooling, in which the speed of the treadmill was gradually reduced.

3.4. Garlic Herbal Supplements

The garlic (*Allium sativum* Linn) bulbs were obtained from a local market in Shiraz, Iran, and were cut into small pieces. Next, 250 mL of distilled water was added to 100 g of garlic and crushed in a mixing machine. The product was squeezed and filtered by a fine mesh, and the filtrate was quickly frozen until usage. The rats of intervention groups III and IV received 800 mg/kg body weight garlic supplements daily in drinking water.

3.5. Enzyme Assessments

The rats were fasted for 12 h at the end of the experimental period and underwent cervical decapitation. Anesthesia was performed using a combination of ketamine (70 mg/kg) and xylazine (5 to 3 mg/kg) (Alfasan, Netherlands). Fasting blood samples were collected from the animals in heparin tubes and were centrifuged at 8000 g for 15 min to obtain plasma, which was stored at -20°C until measurements. The levels of sex hormones (follicle-stimulating hormone (FSH) (Pishtaz, Iran), luteinizing hormone (LH) (Pishtaz, Iran), antioxidant enzymes (SOD and GPx) (Denazist, Iran), and MDA (Pishtaz, Iran) were measured using immunohistochemistry, which is the process of selectively identifying antigens by exploiting the antibodies binding specifically to these antigens. Briefly, 10 μ L of serum was added to the wells, and after adding the antibodies, the absorption was read at the wavelength of 475 nm by the photometric device. Finally, the data were calculated in mg/dL.

3.6. Statistical Analysis

The descriptive data were summarized as mean, standard deviation, and/or percentage. The normality of data distribution was checked prior to data analysis using the Shapiro-Wilk test. The one-way ANOVA was utilized to assess the effect of electromagnetic radiation. Moreover, an analysis of variance was applied to evaluate the hypotheses. All the statistical analyses were performed by the SPSS software version 21. A P-value < 0.05 was considered statistically significant.

4. Results

The effects of aerobic exercise and garlic supplements on plasma FSH and LH are presented in Table 1. Statistical analysis showed that the LH level was not significantly different between the control group and the Wi-Fi radiation groups ($P = 0.15$, $t = 1.5$). Aerobic exercise did not have a significant effect on increasing LH levels in rats under Wi-Fi radiation ($P = 0.76$, $t = 0.091$, $F_2 = 0.003$), while garlic supplementation significantly elevated LH levels in rats under Wi-Fi radiation ($P = 0.03$, $t = 4.79$, $F_2 = 0.14$). Furthermore, aerobic training along with garlic supplementation did not augment LH levels in rats under Wi-Fi radiation ($P = 0.09$, $t = 2.91$, $F_2 = 0.094$).

The FSH levels in the Wi-Fi radiation groups were significantly lower than in the control group ($P = 0.008$, $t = 3.07$). Aerobic exercise ($P = 0.03$, $t = 4.96$, $F_2 = 0.15$) and garlic supplementation ($P = 0.03$, $t = 5.18$, $F_2 = 0.15$) significantly increased FSH levels in rats under Wi-Fi radiation. Aerobic exercise, along with garlic supplements, changed FSH

levels in rats under Wi-Fi radiation. However, the latter result was not significant ($P = 0.46$, $t = 0.54$, $F_2 = 0.01$).

The results of SOD, GPx, and MDA measurements are shown in Table 2. Statistical analysis revealed that SOD levels were significantly lower in the Wi-Fi radiation groups than in the control group ($P = 0.001$). Aerobic exercise ($P = 0.14$, $t = 2.21$, $F_2 = 0.085$) and garlic supplementation ($P = 0.26$, $t = 1.28$, $F_2 = 0.051$) significantly affected SOD activity. However, aerobic exercise along with garlic supplements significantly diminished SOD activity ($P = 0.004$, $t = 10.35$, $F_2 = 0.301$). In addition, aerobic exercise in combination with garlic supplements had agonistic effects on reducing SOD levels in the ovarian tissue of rats under Wi-Fi radiation.

The MDA levels were significantly higher in the Wi-Fi radiation groups than in the control group ($P = 0.001$). Aerobic exercise ($P = 0.001$, $t = 25.23$, $F_2 = 0.51$) and garlic supplement ($P = 0.007$, $t = 8.35$, $F_2 = 0.260$) significantly reduced MDA activity in the ovarian tissue of the rats under Wi-Fi radiation. However, aerobic exercise along with garlic supplements did not have a significant impact on MDA activity ($P = 0.03$, $t = 0.73$, $F_2 = 0.03$).

The GPx levels were significantly lower in the Wi-Fi radiation groups compared to the control group ($P = 0.001$). The effect of aerobic exercise ($P = 0.103$, $t = 2.74$, $F_2 = 0.111$) and garlic supplement ($P = 0.67$, $t = 0.17$, $F_2 = 0.007$) on the changes in GPX activity were not significant. However, aerobic exercise along with garlic supplements significantly increased GPX activity ($P = 0.001$, $t = 16.92$, $F_2 = 0.414$). Therefore, aerobic exercise and garlic supplements have interactive effects on rising GPX levels in the ovarian tissue. However, aerobic exercise has an agonistic influence on garlic supplement intervention.

5. Discussion

Electromagnetic radiation has different effects on the organs of the body, including the gonads. Information about the harmful effects of Wi-Fi radiation on the oxidant-antioxidant system of ovarian tissue is limited. Therefore, we evaluated the effect of eight weeks of aerobic exercise along with garlic supplements on SOD, MDA, GPx, and sex hormones (LH and FSH) levels in the ovarian tissue of rats under Wi-Fi radiation. The results of this study showed that Wi-Fi radiation led to decreased SOD and GPx levels, as well as increased FSH and MDA levels in rats. However, the reduction in LH was not significant. The garlic supplement reduced FSH and MDA, as well as elevated SOD levels in rats under Wi-Fi radiation. Moreover, aerobic exercise caused a decline in FSH and MDA levels, along with a rise in the LH and SOD levels of rats under Wi-Fi radiation. Aerobic exercise along with

Table 1. Serum Levels of FSH and LH in Study Groups

Experimental Groups	LH, mL/min		FSH, mL/min	
	Mean ± SE	P-Value	Mean ± SE	P-Value
Control (1)	2.34 ± 0.69	0.88	4.86 ± 0.3	0.008
Wi-Fi (2)	1.74 ± 0.55	0.15	4.15 ± 0.57	0.008
Wi-Fi + aerobic exercise (3)	2.04 ± 0.69	0.003	3.83 ± 0.48	0.15
Wi-Fi + garlic supplement (4)	2.11 ± 0.5	0.14	3.20 ± 0.79	0.15
Wi-Fi + aerobic exercise + garlic supplement (5)	2.32 ± 0.12	0.094	3.82 ± 0.48	0.01

Table 2. Serum Level of SOD, GPx, and MDA in Study Groups

Experimental Groups	SOD, pg/mL		GPx, pg/mL		MDA, pg/mL	
	Mean ± SE	P-Value	Mean ± SE	P-Value	Mean ± SE	P-Value
Control (1)	939.85 ± 60.21	0.001	0.135 ± 0.024	0.001	9.76 ± 1.90	0.001
Wi-Fi (2)	610.82 ± 58.4	0.001	0.022 ± 0.01	0.001	23.42 ± 2.92	0.001
Wi-Fi + aerobic exercise (3)	733.08 ± 71.55	0.14	0.0844 ± 0.04	0.111	16.18 ± 3.45	0.001
Wi-Fi + garlic supplement (4)	620.03 ± 86.58	0.26	0.036 ± 0.011	0.67	9.51 ± 3.22	0.007
Wi-Fi + aerobic exercise + garlic supplement (5)	664.90 ± 53.5	0.004	0.062 ± 0.031	0.0001	19.86 ± 7.41	0.38

garlic supplement diminished FSH levels and augmented SOD and GPx levels in rats under Wi-Fi radiation. However, the latter combination did not affect LH and MDA levels.

Studies on animals have shown that electromagnetic radiation can affect the body in diverse aspects, including the reproductive system, enzymes, sex hormones, bone marrow, hematological and mutagenic changes, heart rate, and neurobehavioral disorders (1). The results of Marci et al. revealed that Wi-Fi electromagnetic radiation can affect the secretion of gonadotropins, such as FSH and LH. Electromagnetic radiation reduces fertility and the chance of fertilization (17). Moreover, electromagnetic radiation might cause physiopathological or morphological changes in the ovarian tissues of rats (5).

The Wi-Fi radiation increases the production of ROS and MDA, as well as a reduction in antioxidant enzymes, such as SOD and GPx (18). The elevated ROS generation leads to the accumulation of these species in cells, oxidative stress, and irreversible tissue damage. Some experiments also indicated that Wi-Fi radiation changes the structure of the gonads and endocrine system, augments the number of ovarian follicles, and reduces fertility in females (19).

Baharara et al. demonstrated that cell phone waves significantly changed the levels of FSH and LH in female Balb/C mice (20). The Wi-Fi radiation results in a significant rise in MDA and a significant reduction in the GSH and SOD levels in rats (21). Another study reported that Wi-Fi

radiation could increase MDA levels and decrease GPx activity (22). Jonwal et al. demonstrated that exposure to Wi-Fi radiation reduces the activity of SOD and GPx while increasing the MDA concentration (23). The results of the current study, similar to other investigations, showed that the LH, SOD, and GPx levels declined following Wi-Fi radiation. Recently, most electronic devices operate with electromagnetic radiation, and the dangers of exposure to electromagnetic fields are inevitable. Therefore, it is necessary to find out the factors that reduce the destructive effects of this radiation on the body's systems.

Physical activity and exercise have been physiologically proven to influence the endocrine glands (24). Al-Mahdawi et al. reported that physical activity elevates the levels of hormones, such as LH, prolactin, and testosterone, in women with polycystic ovary syndrome (25). Prior studies have shown that regular exercise leads to higher metabolic and hormonal function in women (26). Heidarianpour and Zamiri Dalir demonstrated that the FSH levels increased and LH levels decreased after two weeks of aerobic exercise (27). However, another investigation revealed that gonadotropin hormones were not correlated with physical activity (28). Our results showed that aerobic exercise reduced FSH and increased LH in rats under Wi-Fi radiation. Different studies have reported a decrease, increase, or no change in these hormones (29-31). It should be noted that hormonal responses to exercise are dependent on diverse factors, such as the duration and type of exercise, the intensity of

muscle activity, genetics, gender, nutrition, age, circadian cycle, and the amount of exercise.

Exercise stimulates endogenous antioxidant defenses. However, sports activities can increase oxidative stress to some extent in a balanced and beneficial way for the cell (31). In this regard, researchers indicated that aerobic exercise caused a significant reduction in oxidative stress by reducing the amount of MDA (32). Li et al. showed that high-intensity exercise leads to increased MDA and decreased antioxidant enzymes, such as SOD and GPx (33). It was found in another study that SOD and CAT significantly increased, while MDA declined after 8 weeks of combined exercise in postmenopausal women with type 2 diabetes (34). We observed that the MDA levels diminished and SOD augmented following aerobic exercise in rats under Wi-Fi radiation. Increased SOD activity in exercise might result from biochemical and histological changes evoked by free radicals in muscle tissue (35). Exercise can stimulate SOD production depending on the type, intensity, and length of training. After short and intense anaerobic training, MDA in inactive people was higher than in semi-professional and professional athletes (36, 37). Therefore, it seems that the intensity, duration, and type of exercise have different effects on the occurrence of oxidative damage and the activity of the antioxidant system.

Garlic, as an antioxidant, increases the expression of intracellular antioxidant enzymes, such as SOD, catalase, and GPx, leading to elevated total serum antioxidant capacity (38). Okada et al. reported that the neutralization of free radicals does not cause a remarkable change in their tissue and serum levels in oxidative stress conditions. Consequently, the changes in MDA, SOD, and GPx are very small (39). Another study showed that garlic can be used to suppress chronic inflammation in obese individuals (40). Our results showed that garlic supplements decreased MDA levels and augmented SOD in rats under Wi-Fi radiation. Garlic supplements might remove oxidative agents rather than increase non-enzymatic antioxidants. Therefore, the results of the current study can be related to the antioxidant mechanism of garlic to some extent and the rise in serum antioxidants.

Garlic supplementation stimulates the secretion of gonadotropins and ovarian hormones by activating the pituitary gland and enhancing the ability to unbound estrogen receptors (41). Modaresi and Mohajer demonstrated that garlic supplement consumption leads to increased LH and FSH levels in heat-stressed female mice (42). The findings of the current research showed that garlic supplements could reduce the FSH level in rats under Wi-Fi radiation. Generally, garlic supplement affects sex hormones via stimulating gonadotropins. However,

our study and another investigation revealed controversial results, which could be attributed to garlic concentration, duration of use, and the condition of the body when consuming garlic supplements. However, garlic interferes with the production of some sex hormones by disrupting free cholesterol transfer to the mitochondria of gamete cells.

Our findings indicated that aerobic exercise in combination with garlic supplement diminished the FSH concentrations, as well as raised SOD and GPx levels in rats under Wi-Fi radiation. However, these interventions did not influence LH and MDA levels. There is limited information in the literature about the interactive effect of aerobic exercise and garlic supplements on the oxidant-antioxidant system of ovarian tissue. During exercise, activated gonadotropin receptors increase secretion, as well as the growth and proliferation of sex cells, resulting in augmented gonadotropic hormones, LH, and FSH (27). Furthermore, reduced oxidative stress combined with antioxidant-stimulating exercise can synergistically lead to an increase in antioxidant enzymes. The consumption of garlic supplements along with exercise may have different outcomes because the secretion of hormones depends on the dose of garlic and the distinct mechanisms of aerobic exercise.

There is still limited information about the impact of garlic supplements and exercise on sex hormones. Therefore, further studies on this subject are recommended. Diverse exercises and the dose of garlic may have variable effects on sex hormones, which should be taken into consideration in future investigations. Moreover, it is suggested to investigate the influence of garlic supplements and exercise on male sex hormones.

5.1. Conclusions

The results of the present study showed that Wi-Fi radiation leads to decreased SOD and GPx, as well as increased FSH and MDA levels in rats. The concentrations of FSH and MDA diminished after using garlic supplements and aerobic exercise. However, aerobic exercise along with garlic supplements only reduces the FSH levels. The SOD levels augmented after aerobic exercise combined with a garlic supplement. The LH concentration exclusively increased following aerobic exercise. The GPx level only elevated after the combination of aerobic exercise and garlic supplement. Aerobic exercise and garlic supplements alone seem to have antioxidant effects and improve sex hormones in rats exposed to Wi-Fi radiation. In addition, these two antioxidant approaches interact to increase antioxidant capacity.

Footnotes

Authors' Contribution: MJ designed the study and drafted the manuscript. FD participated in designing the evaluation, performed parts of the statistical analysis, and helped to draft the manuscript. AS revised the manuscript and performed the statistical analysis. SOG collected the data, interpreted them, and revised the manuscript. MGA drafted the manuscript, revised the manuscript, and performed parts of the statistical analysis. All authors read and approved the final manuscript.

Conflict of Interests: The authors have no conflict of interest.

Ethical Approval: The Ethical approval was received from Veterinary Department, Behbahan Branch, Islamic Azad University, Behbahan, Iran (IR. Beh. E. no. 5394). This article contains studies with animals that the care was performed according to the instruction of animal care codes approved in IAU (IR. IAU. 119-2010).

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