



A Study of the Protective Effects of Vitamin E and Fennel Extract on Mitochondria Changes in Mice Ovary Due to Electromagnetic Field Exposure

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Abstract

Objective: Everyday use of different types of electrical instruments and appliances has caused a large number of people to constantly be under the influence of electromagnetic fields.

Materials and Methods: For the purpose of this study, 40 female rats were randomly chosen from among 3 months old rats from the animals' laboratory and they weighed 200 ± 20 g. Then, they were randomly divided into 4 groups; control (n = 10), experiment 1 (Ex1) (n = 10), experiment 2 (Ex2) (n = 10), and experiment 3 (Ex3) (n = 10). During the experiment, all 4 groups were maintained in the same conditions and received the same feeding. The experiment groups 1, 2, and 3 were under the influence of a 50 Hz electromagnetic field (EMF) for 8 weeks. Subsequently, the second and third groups were kept away from the EMF effect for another 8 weeks. At the end of the study, after removal of the ovaries by glutaraldehyde, they were prepared for examination using an electron microscope. Group Ex2 rats were not sacrificed and were maintained in the normal laboratory environment for another 8 weeks away from the impacts of EMF. The rats were fed vitamin E (100 mg/kg) and fennel extract (1.5 g per body weight) every day orally and at the end of the second 8 weeks samples were taken. During the second 8 weeks, group Ex3 was kept in normal conditions without the use of vitamin E and fed fennel extract, and then, samples were taken. Samples were taken simultaneously from 10 rats of the control group and Ex1 group.

Results: The results from the mitochondria in the ovary in the groups under the influence of electromagnetic waves indicated that this intracellular organ, compared to samples from the control group, was deformed and the majority of the organs were vacuolated. The mitochondrial vacuolization of the first to fourth groups were 1 ± 0.55, 9 ± 0.55, 6 ± 0.55, and 11 ± 0.55, respectively.

Conclusion: Vitamin E and fennel extract can reduce the damaging effects of non-ionizing radiation with 50 Hz frequency on the ovarian follicles.

Keywords: Electromagnetic Field (EMF), Fennel, Mitochondria, Ovary

Introduction

Everyday use of different types of electrical instruments and appliances has placed a large number of people under the constant influence of electromagnetic fields (EMF). EMFs have many forms and are classified based on their wavelength or frequency (1). The frequency of these fields varies

according to the types of their producers. There is an inverse relationship between the wavelengths and the frequency of EMFs. The production sources of these fields include a variety of printers, vacuum cleaners, mobile phones, televisions, hair dryers, electric razors, microwave ovens, electric mattresses, sewing machines, elevators,

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ferromagnetic material in the industry, high voltage power cables, and etcetera (2). With the dramatic growth in the use of computer technology in the world, more incidences of abnormalities will emerge in the life of human communities. Many studies have been conducted on the ability of EMFs to create a wide range of somatic abnormalities, among which infertility, miscarriage, premature births, retardation, intrauterine growth defects, congenital malformations, genetic disorders, anemia, and depression can be noted. In another study on rats under the influence of EMFs, results showed premature aging in the rats' reproductive organs (3). Ovaries are located in the abdominal cavity. Each ovary contains a large number of sacs or cavities called follicle in which the oocytes are produced. This means that with each follicle enlargement different stages of oogenesis take place. After the sex cells reach the type II oocyte level, follicle opens and ruptures the ovarian surface and the type II oocyte is released. These cells move with the follicular fluid from the ovarian through the egg outflow tract and this phenomenon is called ovulation. The final stage of the production of the oocyte occurs outside the ovary and in the female genital tract, when type II oocyte changes into oocyte (4-7). Encountering EMF with a frequency of less than 200 to 300 Hz can alter the translation and replication of genes followed by changing the rate of cell differentiation and enzymatic activity. It has also been shown that exposure to radiation of EMFs with the strength of 0.1 mT for 24 hours causes an increase in single-stranded and double-stranded DNA fragmentation. Since the effects of EMF with the strength of 3 mT has not been investigated on the ovarian follicles, the aim of the present study was to investigate the effects of long-term exposure to electromagnetic waves on rat follicle and the possible effects of vitamin E and fennel extract on eliminating its destructive effects.

Materials and Methods

For the purpose of this study, 40 female rats were randomly selected from the animals' laboratory, and their age and weight were first determined. They were 3 months old and weighed 20 ± 200 g. Then, they were randomly divided into 4 groups; control ($n = 10$), experiment 1 (Ex1) ($n = 10$), experiment 2 (Ex2) ($n = 10$), and experiment 3 (Ex3) ($n = 10$). During the experiment, the 4 groups were maintained in the same conditions and were fed similarly. Test groups 1, 2, and 3 were under the influence of a 50 Hz EMF for 8 weeks. Then, the second and third groups were kept for another 8 weeks without the effects of EMF. After these periods, the rats were sacrificed with chloroform and their ovaries were removed and placed in fixative. Then, the required samples were prepared for electron microscopy study. Group Ex2 were not sacrificed and were maintained in the normal laboratory environment for another 8 weeks without

EMF effects. The rats were fed vitamin E (100 mg/kg) and fennel extract (1.5 g per body weight) every day orally and in combination with their food. The fennel extract used was the product of Barij Essence Pharmaceutical Company (Tehran, Iran) with the brand name Fennelin. This extract is available in the form of a drop in pharmacies; every milliliter of this product contains 4.11 mg fennel extract. Samples were taken at the end of the second 8 weeks. During the second 8 weeks, group Ex3 rats were kept in normal conditions without the use of vitamin E and were fed fennel extract, and then, samples were taken. Samples were taken simultaneously from 10 rats of the control group and Ex1 group. To avoid the influence of environmental variables such as temperature, a fan was placed in the upper part of the magnetic field generating device, so when the animals were placed in the field, with its constant functioning, it prevented the device temperature from rising. A temperature gauge was also installed to constantly control the inside temperature of the device and it was consistently controlled by a technician and the project manager. In this study, there were no other important confounding variables except the heat from the device, which, as previously mentioned, was controlled. By measuring the outside and inside temperature of the device, the effect of the heat was found to be insignificant. Even if there were other confounding factors, their possible impact would be the same on both groups, since the rats in the control group and the rats in the EX groups were maintained in similar conditions, and thus, would have no effect on the results.

In the present study, an EMF generator was used that produced a 50 Hz magnetic field. The EMF generating device was made based on the theory of Helmholtz. The Helmholtz coils had a diameter of 20 cm and contained 200 windings of 0.8 mm copper wire located at a distance of 10 cm from each other. The devices used in this study were: an AC power supply with a voltage of 0 to 250 volts, a two-channel oscilloscope to check the parameters and waveforms of the windings input and output, a multimeter to measure the current voltage, AC and DC teslameter with an accuracy of 0.001 mT, a thermometer with an accuracy of 0.1 °C, and an incubator. In the selection of these devices, parameters such as the need to achieve a uniform field with certain intensity were carefully considered.

Fennel seeds were purchased from local markets and authenticated by a botanist (School of Pharmacy, Tabriz University of Medical Sciences, Iran). The extract was prepared according to the World Health Organization (WHO) protocol for preparation of an alcoholic extract (7). Briefly, 100 g of fruit was shed-dried, powdered, and added to 1000 ml of 70% ethanol (v/v) and left to macerate at room temperature for 20 hours. The basin was slowly rotated during this time. After filtration, ethanol evaporated at low pressure at 30 °C.

Ovarian tissue samples with a clean surface were

transferred on a plate containing washing solution (PH Phosphate buffer = 7.4) and washed several times to remove clots and tissue debris, so the blood stains, clot adhesion, and debris were cleaned. Then, the samples were divided into 5 mm sections. The samples, after being placed in a solution of glutaraldehyde 2.5%, were washed with phosphate buffer solution 0.1 M (pH = 7.4) for 6 hours. Subsequently, they were kept for 2 hours in osmium tetroxide solution 1%. Later, they were washed 3 times with phosphate-buffered saline 0.1 M, (pH = 7.4). For hydration, alcohol (ethanol) was used with increasing concentration gradient. Replacement was performed using propylene oxide. Samples' molding procedure was performed by Epon 812 resin. Trimmed samples were installed on the ultramicrotome (Reichert-Jung, Germany). Semi-thin sections with 500-700 nanometers thickness and a speed of 2.5 mm per second were prepared. They were stained with toluidine blue solution. After preparing the ultra-thin sections, in order to stain the grids, uranyl acetate solution 3% and lead citrate were used for 1-2 hours. This stage of the research was conducted at the research center of Tabriz University of Medical Sciences. To evaluate the changes, 100 microscopic sections were evaluated.

Statistical analysis

Data analysis was performed using ANOVA and SPSS software (version 16, SPSS Inc., Chicago, IL, USA).

Results

The results of mitochondria in the ovarian tissue in the groups affected by EMF indicated that this intercellular organ, compared to samples from the control group, was deformed and the majority of the organs were vacuolated. The mitochondrial vacuolization of the first to fourth groups were 1 ± 0.55 , 9 ± 0.55 , 6 ± 0.55 , and 11 ± 0.55 , respectively.

Discussion

Free radicals, active oxygen species, and their effects on biological systems is one of the important issues raised in medicine today. These factors can cause irreversible damages on biological molecules such as nucleic acids, proteins, lipids, and lipoproteins. Antioxidants can protect biological systems against these agents. Some medicinal plants contain large amounts of antioxidants, and thus, their use can be effective on human health (8-11). Much of the human health crises results from the effects of a type of hostile and destructive oxygen for the body. This means the risk of the most vital element of life, which is oxygen, and that can be fatal. Human cells are constantly exposed to incompatible and traumatic oxygen. If the cells are unable to resist the damage caused by oxygen free radicals, they will be damaged and destroyed one by one. The dangerous effects of this oxygen on the cells can cause clogs in the arteries, produce cancer cells, and serious damage to the cells in joints and the human nervous system. There are a variety of oxidants, the most famous and dangerous of which are the free radicals. The molecules of the free

radicals have an unstable form due to losing one electron and being in search of electrons to complete their molecular structure. These free radicals combine with every healthy cell in the body that they come in contact with, and thus, more damaging free radicals are produced. This new finding about oxygen generates new discussions and researches. Preventive methods for the damage caused by the destructive oxygen have been introduced in the world. Free radicals, meaning oxygen without an electron, can attack the DNA of the cell nucleus that is the essential genetic matter of the cell and cause genetic mutations in the cell. This is the first step in the process of cancer cells. It can also attack the fatty part of the cell membrane, and destroy and cause decay in the cells that lack the needed antioxidizing materials and are defenseless. This corruption causes complete disruption of the cell membrane. The fat molecules and the molecules around them will be damaged. This process may continue until the indication of an acute form. Oxidizers often intentionally or unintentionally enter the body from the outside environment in forms such as air pollution, toxic chemicals, pesticides, cigarette smoke, some chemical medications, and etcetera. One can quit smoking and be cautious about the use of some substances, but in general, consumption of foods rich in antioxidants in the daily diet should be increased. Vitamin E is a fat-soluble vitamin. This vitamin, like vitamin C, has antioxidant properties and prevents the damaging effects of chemicals that can harm body tissues. Vitamin E isolated from wheat germ is called alpha-tocopherol. This name is derived from the Greek word tokos meaning "child birth", and pherein meaning giving birth, and ol is to show the alcoholic structure. This vitamin is placed in the fat layer of the cell wall and within the cell and prevents the destruction of the cell wall. Vitamin E is also a name for a group of molecules which have similar effects as that of alpha-tocopherol (12). Increasing use of home equipment and appliances which generate EMF (refrigerator, computer, television, and etcetera) has drawn the attention of many research centers such as the World Health Organization to their harmful effects on human health. Increasing use of modern equipment and the key role of the health management in the society in controlling diseases such as allergies and cancers are indicators of the determinant role of these factors in health promotion and health management in the community. Such researches and studies can guide health managers and medical sciences researchers in finding more effective ways to promote community health (6,9). Multiple surgical and pharmaceutical procedures have been used to treat infertility, but unfortunately they all have side effects. Over the past years, there have been attempts to find the appropriate treatment methods for infertility with minimal side effects around the world. Therefore, plant derivatives have increasingly been considered in this regard. Regarding the use of fennel as an antioxidant in this study, it should be noted that in human history plants have been used as a great source for medicines, and fennel has been used since ancient times in the preparation of food and drugs. The antioxidant effects of

fennel in terms of tissue protection and reduction of carcinogenic effects of EMF have been illustrated. It is also conjectured that this plant can have protective effects on the ovarian tissue against reactive oxygen species (ROS) (11). Moreover, its protective effects on spermatogenesis are also considered (12,13). Several studies have shown that antioxidants and medicinal plants play an important role in the maintenance of healthy cells and their protection against damaging elements (10,14). Low vitamin E intake increases the risk of cancer, cardiovascular problems, and reproductive disorders. Supply of this vitamin in the body will be sufficient for 10 to 15 years. However, after this period and usually in those who have trouble digesting fats, visual disturbances, muscle weakness, lack of response to environmental stimuli, inability to maintain balance, and skin's inability to heal rapidly are observed. This vitamin is also very effective in preventing miscarriages (14). The results of this study confirm that combined use of these two antioxidants has significant effects on the vacuolization of the mitochondria in ovarian cells ($P < 0.05$).

Conclusion

Antioxidant consumption (fennel and vitamin E) reduces the damaging effects of electromagnetic waves and its inclusion in the daily diet is recommended.

Ethical issues

This research was approved by the Ethics Committee of Tabriz University of Medical Sciences.

Conflict of interests

We declare that we have no conflict of interests.

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