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Effects of Extremely Low Frequency Electromagnetic Fields and Simultaneous Treatment with Allium Cepa on Biochemical Parameters and Ultrastructure of Ovarian Tissues of Rats

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ABSTRACT

Introduction: This study investigated the effects of extremely low frequency electromagnetic fields (ELF-EMF) (50 Hz, 3 mT) on biochemical parameters of rats' ovarian tissues and the impact of Allium cepa on the reduction of potential adverse influences of electromagnetic exposure.
Material and Methods: In this study 40 female Wistar rats were divided into four groups, including (1) control group (with 3 cc normal saline), (2) ELF-EMF group (exposed to ELF-EMF, 50 Hz), (3) Allium cepa group (received 3 cc Allium cepa), and (4) ELF-EMF and Allium cepa group (exposed to ELF-EMF and simultaneously received Allium cepa daily for 6 weeks.
Results: The MDA levels significantly increased in the second group, which were exposed to ELF-EMF and decreased in normal rats that received Allium cepa. Although, SOD, GPx, and CAT activities significantly decreased in ELF-EMF group, the combination treatment with Allium Cepa on exposed rats restored their activities to normal levels. The conduction of transmission electron microscopy study on ELF-EMF group revealed the changes regarding cytoplasmic organelles in the ovarian follicles of exposed rats. Moreover, irregular oocyte with damaged heterochromatic nuclei was observed. In degenerative oocyte, mitochondria lost their cristae
Conclusion: The results of the present study suggested that ELF-EMF exposure might cause deleterious effect on ovarian tissues in rats, which may lead to infertility and subfertility. Moreover, using Allium cepa as a nutritional supplement can have beneficial effects in the protection of biological antioxidants and reproductive systems in cases exposed to ELF-EMF.

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Introduction

Electromagnetic waves and fields that are emitted in a wide range of energy and frequency spectrums can have both positive and negative impacts on the life of organisms. The Electromagnetic spectrum is divided into ionizing and non-ionizing segments. Recently, there has been an increasing interest in non-ionizing electromagnetic fields (EMF) from the health and safety point of view. All electric-based devices create extremely low frequency electromagnetic fields (ELF-EMF) and wireless communication equipment emits radio frequency EMF (RF-EMF). Therefore, the non-ionizing section of the electromagnetic spectrum could be categorized into ELF-EMF (up to 100 Hz) and radio frequency EMF (1 KHz to 1000 KHz) [1].

In recent decades, due to the steadily increasing application of the electricity, including electric power systems, electronic appliances, and industrial devices, the exposure to EMF has been one of the major environmental and health concerns [2, 3]. Human and other living creatures are constantly floating in a sea of EMF, especially those of 50-60 Hz frequency, which are emitted from sources, such as power lines, interior and exterior residential wiring, transformers, and domestic appliances that use ELF-EMF at 50-60 Hz [4].

There are several possible mechanisms through which ELF-EMF might influence the body [5]. The exposure of ELF-EMF could result in the production of reactive oxygen species (ROS) in various tissues [6].

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The exhaustion and loss of antioxidant systems are one of the results of oxidative stress that occurs due to increased ROS; therefore, antioxidant defense systems could be damaged by long-term exposure to EMF [7]. Furthermore, a strong body of evidence suggests that oxidative stress can have adverse effects on female fertility [8]. Therefore, dietary and pharmacological interventions can be used as an effective strategy to preserve female fertility [8, 9].

The human body can easily process herbs. Antioxidant compounds are found in various foods and play a vital role in human life [10, 11]. Allium cepa, which has long been used in traditional medicine, is one of many types of onions that is commonly used in the everyday diet [12]. In the present study, it was investigated whether ovarian tissue and serum malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) levels could benefit from Allium cepa treatments in ELF-EMF (50 Hz) exposed rat.

Materials and Methods

Animals

A total of 40 Wistar female rats (thesis code: 93/2-4/9) with an average age of eight weeks, weighing 220±10 g, were quarantined and acclimatized for one week in plastic cages. The animals were kept under laboratory conditions at 20±2 °C with fluorescent light control (12 h light and 12 h dark), and the humidity was maintained at 35% to 50%. The rats were divided randomly into four groups (each group n=10), including (1) control group, which received 3 cc normal saline (0.9%) daily for 6 consecutive weeks, (2) ELF-EMF group, which were exposed to ELF-EMF of 3 mT, 4 h per day, for 6 weeks, (3) Allium cepa group, which received Allium cepa extract daily for 6 weeks, and (4) ELF-EMF and Allium cepa group, which were exposed to ELF-EMF and simultaneously received Allium cepa extract daily for 6 weeks. During the experiment, the rats were fed with compressed food (Dam Pars Tehran Co, Iran) and urban plumbing water.

Allium cepa supplement preparation

The underground yellowish-white bulbs of Allium cepa (onion) were collected from Ilkhchi district (East Azerbaijan, Iran). The skin was removed and onion juice was extracted using a fruit juice-extracting machine. The rats in ELF-EMF and Allium cepa groups received a daily 3 cc dose of Allium cepa for 6 consecutive weeks [13].

Extremely low frequency electromagnetic fields generator

The ELF-EMF was produced by a pair of Helmholtz coils (Tabriz University of Medical Sciences, Iran), which were able to produce an alternating current of 50 Hz frequency and create a magnetic field of 3 mT and average ELF-EMF of 1.01×10^3 A/m measured by EMF/ELF meter (98191 MIC Taiwan). The equipment consisted of two parts. In the first part, the exposure area, two copper coils were placed (one above the other) by a 50 cm distance. In this part, there was a cylindrical wooden vessel with a chamber for holding rat cages among the coils. The second part was the transformer (Tabriz University of Medical Sciences, Iran) which checked the input and output voltage and current.

In order to prevent temperature rise inside the chamber, a fan was operated for air circulation. During the ELF-EMF exposure, four cages were placed within the chamber (rats from Allium cepa group and ELF-EMF and Allium cepa group) with five rats in each cage. In order to avoid ELF-EMF interference, the room lights were located 3m away from the equipment. Voltage and frequency stability in ELF-EMF exposure system used in this study was in line with the voltage and frequency characteristics of line distributions. The output voltage was 25VAC with the stability of 10% max. The ELF-EMF radiation parameters are presented in Table1.

Table 1. Extremely low frequency electromagnetic fields radiation parameters

Electromagnetic field type	Extremely low frequency electric field
	Extremely low frequency magnetic field
Frequency	50 Hz
Wave strength	24×10^2 V/m $1.01\times10^3\pm0.05$ (A/m)
Magnetic field density	3±0.01mT
Duration of exposure (h per day)	4 h per day for 6 weeks

Transmission electron microscopy preparation

At the end of 6 weeks, the blood samples of the rats were collected from the tail vein. Then, pentobarbital (40 mg/kg) was used for anesthesia through intraperitoneal injection. The peritoneum was opened by transverse abdominal incision in order to remove the right ovary tissue. The ovarian samples for transmission electron microscopy study were cut into pieces of about 1 mm^3 with a scalpel. The primary fixation was performed with 2.5% of glutaraldehyde in a 0.1 M phosphate buffer (Thuringowa Central QLD 4817, Australia) and postfixation in 1% aqueous osmium tetroxide (TAAB, UK). A Leo EM 906 transmission electron microscope (Oberkochen, Germany) was used for transmission electron microscopy study [14].

The thiobarbituric acid assay was utilized for serum MDA level measurement. The concentration of plasma Mda (μmol/L) was determined spectrophotometrically via HPLC (SPD - 6AV/Shimadzu/Japan) coupled with UV-VI spectrophotometer. A calibration curve was prepared to express the percentage of the increase in MDA level compared to that control group [14].

Superoxide dismutase activity assay

The serum SOD activity was measured as previously described by Beyer and Fridovich [15].

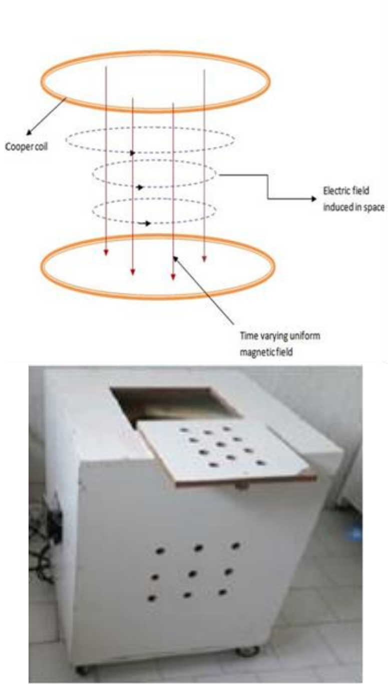

Figure 1. Radiation area of extremely low frequency electromagnetic fields in production device and diagrammatic scheme of radiation area Malondialdehyde assay

Glutathione peroxidase activity assay

The serum activity of GPx was spectrophotometrically determined by measuring the absorbance decrease at 340 nm as Lawrence et al. reported [16].

Catalase activity assay

The Serum activity of CAT was spectrophotometrically determined by measuring the absorbance decrease at 240 nm as Usoh et al. reported [17].**Data analysis**

The statistical analysis was performed using SPSS software (version 16.0). The Oxidative stress was analyzed by one-way analysis of variance followed by Tukey's range test. The results were presented as mean±SEM (i.e., the standard error of the mean). P-value less than 0.05 was statistically considered significant.

Results

Transmission electron microscopy evaluation

As it is shown in the control and Allium cepa groups, micrographs (Figure 2A and 2C), normal healthy granulosa cells, mitochondria, several lipid droplets, oocyte cytoplasmic projection, and microvilli within the zona pellucida were observed. Furthermore, oocytes with oval nuclei and round euchromatin nucleus, which were surrounded by healthy granulosa cells with clear and euchromatin nucleus were noticed.

In ELF-EMF group micrograph (Figure 2B), the morphological changes of oocyte, zona pellucida, and granulosa cells are obvious. Moreover, deformed oocyte nuclei and nucleus (arrows) and cytoplasm's lacking lipid droplets were observed. The number of microvilli that penetrated the zona pellucida was decreased (i.e., microvilli lose), and the appearance of zona pellucida altered and became vague.

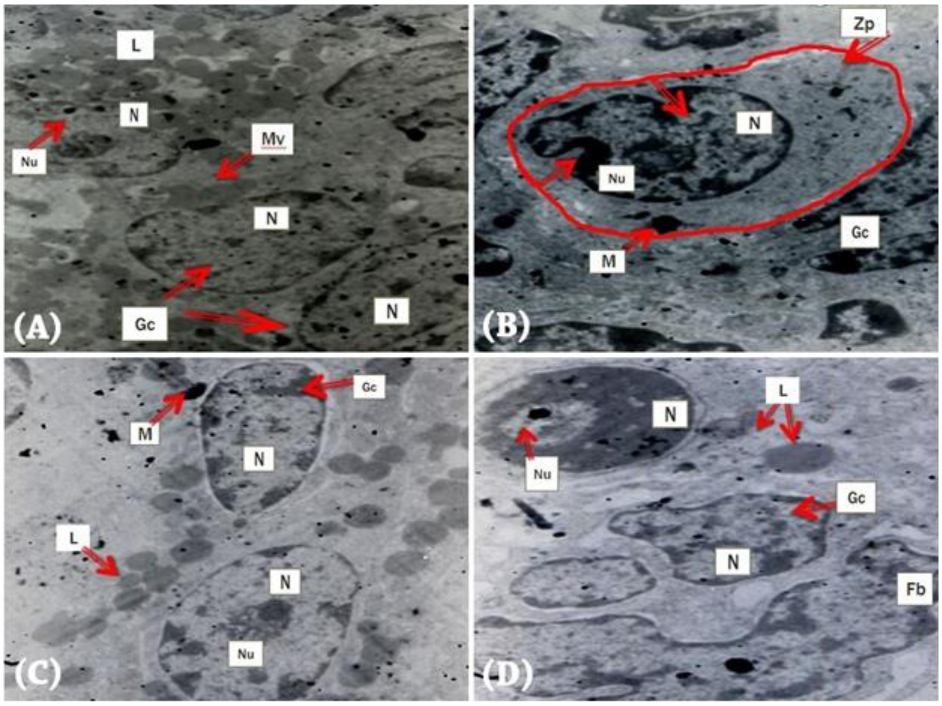

Figure 2. Transmission electron micrographs of rat ovarian tissues in (1) control group (×3000), (2) extremely low frequency electromagnetic fields (ELF-EMF) group (×4000), (3) Allium cepa group (×3000), and (4) ELF-EMF and Allium cepa (×7000) group

Fb: fibroblast; Gc: granulosa cell; L: lipids; Mv: microvilli; M: mitochondria; Nu: nucleoli; N: nucleus; Zp: zona pellucida

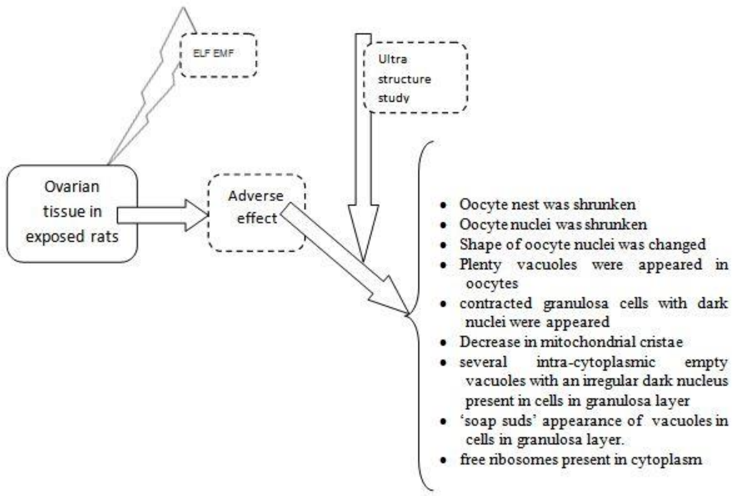

Figure 3. Ultrastructure of ovarian tissue study in exposed rats to 50 Hz and 3 mT extremely low frequency electromagnetic fields

However, the toxic effects of ELF-EMF declined in ELF-EMF and Allium cepa group, exposed to ELF-EMF and received 3 cc Allium cepa. Oocyte with oval nuclei and round euchromatin nucleus were noticed in ELF-EMF and Allium cepa group micrograph (Figure 2D). In addition, cytoplasmic vacuolization was observed. Compared to those in ELF-EMF group, granulosa cells experienced less damage; microvilli presence in zona pellucida increased; cytoplasmic vacuolization reduced, and the number of damaged granulosa cells and damaged cytoplasmic mitochondria decreased. Figure 3 demonstrates the ultrastructure of ovarian tissue in rats exposed to ELF-EMF of 50 Hz at 3 mT.

Serum malondialdehyde levels

The serum MDA levels in control group was 4.99±1.11 nmol/mL, whereas the ELF-EMF group presented the highest level with 6.97±7.11 nmol/mL compared to the control group (P<0.001). Although the treatment with Allium cepa in normal rats significantly decreased MDA levels (2.05±2.11 nmol/mL, P<0.001), the treatment did not significantly attenuate the adverse effects of the ELF-EMF in terms of MDA levels (Figure 4).

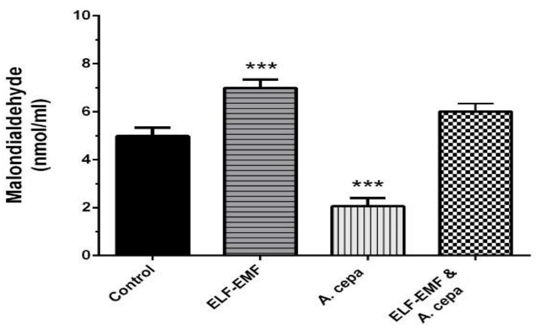

Figure 4. Serum malondialdehyde levels in different experimental groups; each bar representing mean±SEM, (n=10); ***P<0.001 compared with that in control group

SEM: standard error of mean; ELF: extremely low frequency; EMF: electromagnetic fields; A. cepa: Allium cepa

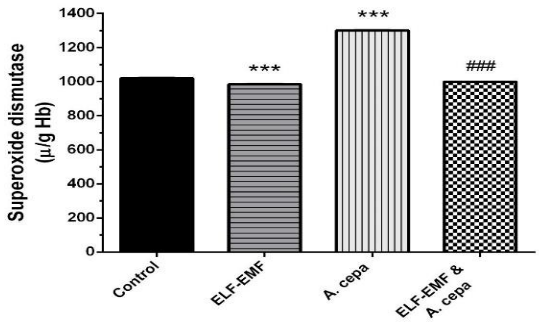

Figure 5. Serum superoxide dismutase activity in different experimental groups; each bar representing mean±SEM, (n=10); ***P<0.001 compared with that in control group; ###P<0.001 compared with that in ELF-EMF group

SEM: standard error of mean; ELF: extremely low frequency; EMF: electromagnetic fields; A. cepa: Allium cepa

Serum superoxide dismutase activity

The exposure to 50 Hz ELF-EMF significantly reduced SOD activity from 1020±5.05 µ/gHb in control group to 985±3.11 in ELF-EMF group (P<0.001). In the ELF-EMF and Allium cepa group, the SOD activity was significantly higher than that in ELF-EMF group (1000±5.05, P<0.001). In addition, the combination treatment with Allium cepa in normal rats remarkably increased SOD activity in comparison to that in control group (1300±4.82 ng/ml, P<0.001) and significantly increased and modified, compared to that in the ELF-EMF group (P<0.001) (Figure 5).

Serum glutathione peroxidase activity

The serum GPx activity in control group was 123±7.55 µ/mgHb, while the ELF-EMF group showed lower activity with 103±5.66 µ/mgHb, compared to the control group (P<0.001). The data from ELF-EMF and Allium cepa group indicated that six-week treatment with herbal agents significantly increased GPx activity in comparison to that in ELF-EMF group (117±4.66

μ/mgHb, P<0.001). Furthermore, the Allium cepa group displayed higher GPx activity, compared to those in control group (145±6.41 μ/mgHb, P<0.001) and ELF-EMF group (P<0.001) (Figure 6).

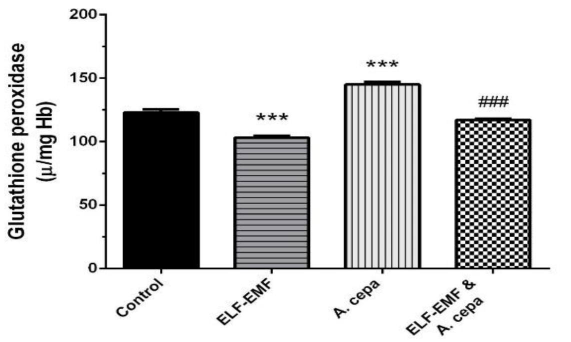

Figure 6. Serum glutathione peroxidase activity in different experimental groups; each bar representing mean±SEM, (n=10); ***P<0.001 compared with that in control group; ###P<0.001 compared with that the ELF-EMF group

SEM: standard error of mean; ELF: extremely low frequency; EMF: electromagnetic fields; A. cepa: Allium cepa

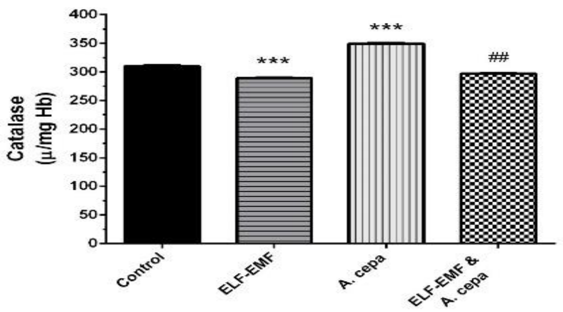

Figure 7. Serum catalase activity in different experimental groups; each bar representing mean±SEM, (n=10), ***P<0.001 compared with that in control group; ##P<0.01 compared with that in ELF-EMF group

SEM: standard error of mean; ELF: extremely low frequency; EMF: electromagnetic fields; A. cepa: Allium cepa

Serum catalase activity

The six-week exposure to 50 Hz ELF-EMF significantly decreased CAT activity from 310±5.55 μ/mgHb in control group to 289±4.11 μ/mgHb in ELF-EMF group (P<0.001). On the other hand, the simultaneous treatment with Allium cepa significantly increased CAT activity to normal levels (297±5.11 μ/mgHb, P<0.01). Moreover, the Allium cepa group exhibited higher CAT activity compared to control group (350±4.11 μ/mgHb, P<0.001) and ELF-EMF group (P<0.001) (Figure 7).

Discussion

As the use of electricity has become an integral part of everyday life, concerns about potential effects of EMF on both human and animal health have increased. The present study assayed the effects of extremely low frequency electromagnetic field of 3 mT on antioxidant activity and ultrastructure of ovarian tissue.

Mature egg production in mammals is a result of coordinated sequence of events in ovarian follicle. The healthy ovarian follicle consists of an oocyte surrounded by a layer of granulosa cells. In addition, the ultrastructural morphology of healthy oocyte shows round or oval shapes of mitochondria with small numbers of cristae Normal and healthy oocyts were observed surrounded by healthy granulosa cells with clear and euchromatin nucleus in control group micrographs. The oval nuclei and round and euchromatin nucleus of occyt, multiple lipid droplets in cytoplasm, and microvilli in zona pelucida were visible in control group micrographs.

The present ultrastructural study on ovarian tissues using transmission electron microscopy on ovarian tissues of exposed rats demonstrated vast changes in follicular cells. The shrunken nuclei of oocyte were observed in ELF-EMF-exposed (50 Hz, 3 mT) rats.

Furthermore, the irregular contracted granulosa cells with dark nuclei and increased cytoplasmic vacuolization were exhibited in exposed rats. Moreover, the nuclear congestion of granulosa cells, loss of microvilli, and abnormal mitochondrial cristae, as well as alternation in the appearance of the zona pellucida were observed by ultrastructural transmission electron microscopy study. Cheronff et al. reported the morphological changes of oocyte and congestion of nuclear granulosa cells congestion [18].

In a similar study on the effects of 50 Hz electromagnetic field on ovarian follicles, Bacacak et al. showed t ELF-EMF could change nuclei oocyte (shrunken nuclei) [19]. Roshangar et al. indicated that ELF-EMF could increase cytoplasmic vacuolization and cause the morphologic changes of oocyte and granulosa cells nuclei [20]. Nonetheless, more clinical studies are required to detect the effects of increasing EMF exposure in daily life, on the reduction of ovary reserve, which can result in infertility [18-21].

Oxidative stress is a condition caused by an imbalance between ROS systematic production and the detoxification ability of biological systems [22]. In addition, the role of ROS in developing various diseases in women has been reported [23, 24]. The oxidative stress has been implicated in pathology of female infertility, including abortion, recurrent pregnancy loss [25], defective embryogenesis, endometriosis, ovarian cancer, and polycystic ovary disease [26]; furthermore, it can affect assisted fertility treatments [23]. in addition, the high levels of ROS could cause pathological changes in female reproductive tracts [27]. According to the literature, the reason behind almost 40% to 50% of infertility is female fertility factors [7].

The ELF-EMF could induce the production of ROS in various tissues. The physical interactions on atomic surfaces are the basis of interaction between biological molecules exposed to ELF-EMF [28]. Magnetic fields could affect the chemical bonds of adjacent atoms and change energy levels and the rotation direction of electrons. This could increase activity, concentration, and the longevity of free radicals [29].

The normal function of ovarian tissue plays a critical role in maintaining fertility and general health and depends on ovarian follicle development. There are solid evidences that the involvement of ROS in the initiation of ovarian follicles apoptosis is linked to environmental, chemical, and physical factors [30]. According to a study conducted on the reproductive toxicity of ELF-EMF, the exposure can have harmful effects on mammalian female fertility [31].

The oxidative damage of lipids induced by ELF-EMF may impair the function of ovarian tissue [30]. Moreover, the adverse effects of ELF-EMF (50 Hz, 1.2 mT) on pregnancy and infant growth in mice have been observed [32]. Currently, regarding the harmful effects of ELF-EMF, many morphological and biochemical parameters, such as follicular growth, antrum formation, granulosa cells apoptosis, and oocyte meiotic maturation as follicular development indicators have been explored [33].

In the present study, biochemical analysis revealed that serum MDA significantly was increased in exposed groups ($P<0.001$). However, SOD, GPx, and CAT significantly decreased in exposed group ($P<0.001$). The ELF-EMF might alter free radical metabolism and lead to antioxidant capacity reduction [34, 35]. The MDA is one of the most common genotoxic lipid peroxidation products that is considered as an indicator of oxidative stress [6]. The exposure to 50 Hz ELF-EMF could decrease blood serum antioxidants by increasing the level of MDA and ultimately lead to cell damage by ROS [14]. Limited data obtained from studies conducted on rats suggest that many antioxidant enzymes protecting cells against ROS may be able to play an effective role in follicular development and fertility [8, 9].

The antioxidant agents, such as vitamins (e.g., A, E, and C), and endogenous antioxidant enzymes, such as SOD, CAT, and GPx protect cells against lipid oxidation [36]. The importance of SOD in the eradication of free radicals has been established. The effects of EMF exposure on SOD activity as a fundamental antioxidant has been widely investigated [37]. Zwirska-Korczala et al. indicated the harmful effects of electromagnetic fields on the reduction of antioxidant enzymes activity, such as catalase and superoxide dismutase [38].

Kesari et al. reported significant decrease in the levels of SOD, GPx, and CAT in a study conducted on the effects of 50 Hz electromagnetic fields on the reproduction system of Wistar rats [39]. Moreover, a study conducted by Aydin et al. showed significant decrease in catalase levels; however, any significant changes were not reported in MDA or GPx levels in rats exposed to ELF-EMF [40]. In another study carried out by Aksen et al., it was indicated that 1 mT ELF-EMF could increase the MDA concentration in the ovaries and uterus of Wistar rats [41].

Recently, natural antioxidants presented in herbs and spices have attracted widespread interest. It seems that polyphenol compounds are able to enhance body immune system function against oxidative stress due to their antioxidant activity [42]. Additionally, Allium cepa has high antioxidant activity and contains antioxidants, such as glutathione, vitamin E, and C and is a rich source of polyphenolic compounds [12, 43]. Allium cepa has free-radical scavenging properties and could reduce lipid peroxidation index MDA and increase SOD, which indicates the antioxidant effects of Allium cepa [44, 45].

Based on the results of the present study, it was revealed that the treatment with Allium cepa significantly increased serum SOD, GPx, and CAT levels in ELF-EMF exposed (50 Hz, 3 mT) rats in comparison with those in ELF-EMF group ($P<0.001$). However, it should be noted that the treatment with herbal agents could not significantly reduce the serum MDA levels in exposed rats.

In addition, in this ultrastructural study conducted on ovarian tissues using transmission electron microscopy, it was reported that oxidative damages were remarkably reduced in ELF-EMF exposed rats treated with Allium cepa The results of this study clearly indicate that Allium cepa has beneficial effects on ovarian tissue quality enhancement and the antioxidant index of ovarian tissue.

Conclusion

According to the results of this study, it was indicated that ELF-EMF exposure had a deleterious effect on ovarian tissue, which may lead to infertility and subfertility. Furthermore, it seemed that using Allium cepa as a nutritional supplement could have beneficial effects on the protection of biological antioxidants and reproductive systems in populations exposed to ELF-EMF. However, more studies are required to evaluate the effects of antioxidants on the ultrastructure of ovarian tissues and other female reproductive tracts.

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