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Araştırma Makalesi / Research Article

Evaluation of The Effects of ELF-EMF on Oxidative Parameters in Brain, Liver and Heart Tissues

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Abstract

The aim of the present study was to investigate the prospective effects of continuous and intermittent extremely low-frequency magnetic fields (ELF-EMFs) on the levels of malondialdehyde (MDA), nitric oxide (NO) and glutathione (GSH) in brain, heart, and liver tissues. The experiments performed on 18 guinea pigs were divided into three groups (n= 6 for each group). 50 Hz of 1.5 mT a magnetic field was used for exposure. Experimental groups were exposed for 4 h/day either continuously or intermittently (2h on/ 2h off/ 2h on) EMF for a period of 7 days. MDA, NO and GSH levels were determined by spectrophotometric methods. The intermittent exposure was increased GSH levels whereas that was reduced MDA levels in the brain when compared to control and continuous exposure groups. GSH concentrations of both experimental groups were found to be elevated in the liver. In the liver and heart, NO levels were increased by the continuous exposure in both administration groups, however, intermittent exposure has reduced NO levels in the both of the experimental groups. Results of this study indicated that the responses of various tissues to magnetic field exposures differed according to intensity and exposure duration of magnetic fields.

Keywords: ELF-MF, Free radicals, Lipid peroxidation, GSH, NO.

ELF-EMF'in Beyin, Karaciğer ve Kalp Dokularında Oksidatif Parametreler Üzerine Etkilerinin Değerlendirilmesi

Öz

Bu çalışmanın amacı, sürekli ve kesikli aşırı düşük frekanslı manyetik alanların (ELF-EMF) beyin, karaciğer ve kalp dokularında; malondialdehit (MDA), nitrik oksit (NO) ve glutatyon (GSH) seviyeleri üzerindeki muhtemel etkilerini araştırmaktır. 18 gine domuzu üzerinde yapılan deneyler için hayvanlar üç gruba ayrılmıştır (her grup için n = 6). Deney grupları sürekli (4 saat / gün) veya kesikli olarak (2 saat açık / 2 saat kapalı / 2 saat açık) 7 gün boyunca 1.5 Hz frekansında ve 50 mT şiddetinde EMF'ye maruz bırakılmıştır. Dokulardaki MDA, NO ve GSH düzeyleri spektrofotometrik yöntemlerle belirlenmiştir. Kesikli maruziyet; kontrol ve sürekli maruziyet gruplarına kıyasla beyinde GSH seviyelerini arttırırken, MDA seviyelerini düşürmüştür. Her iki maruziyet grubunun GSH konsantrasyonlarının karaciğerde yükseldiği bulunmuştur. Karaciğerde ve kalpte, her iki uygulama grubunda da sürekli maruz kalma ile NO seviyeleri artmış, ancak kesikli maruziyet her iki deney grubunda da NO düzeylerini azaltmıştır. Bu çalışmanın sonuçları, çeşitli dokuların manyetik alan maruziyetlerine tepkilerinin, manyetik alanların yoğunluğuna ve maruz kalma süresine göre değiştiğini göstermiştir.

Anahtar Kelimeler: ELF-MF, Serbest radikal, Lipit peroksidasyonu, GSH, NO.

1. Introduction

Extremely Low Frequency (ELF) ($f<300$ Hz) Electromagnetic Fields (EMFs) emits a non-thermal effect of non-ionizing radiation and is sourced from extensive use of electrical and electronic appliances and power lines. In recent years, because of the increasing use of ELF-EMF producing devices, people are exposed to high levels of environmental electromagnetic fields and, potentially harmful side effects of EMFs have become an important public health issue. Therefore, some epidemiological investigations and experimental studies which effects of EMF on biological systems, have gained importance worldwide. Several studies have been indicated that exposure to ELF-EMF associated with brain tumors, neurodegenerative diseases, childhood leukemia, lymphoma, allergic and inflammatory disorders, some cardiovascular effects, chromosome abnormalities and miscarriages (Hardell and Sage, 2008).

Some in vitro studies have shown that ELF-EMF has effects on cell proliferation, apoptosis and differentiation however cellular responses of ELF-EMF on living organisms are uncertain. But there are different hypotheses to elucidate the effects of EMFs on biological systems. It has been suggested that EMF changes membrane structure and the permeability of small molecules, especially and Ca^{+2} transport system. Particularly ELF-EMF can affect many cellular functions, and an important effect is on signaling pathways.

One of the most assumed hypothesis is that ELF-EMF may be altered the normal balance of reactive oxygen species (ROS) by changing the production rate of molecules such as hydroxyl radical (OH), superoxide anion (O_2^-) and could modify on antioxidant pathways which scavenge ROS (Dröge, 2002). Increased ROS production is an important cause of oxidative damage that pioneering to irreversible or reversible tissue injuries, and lipids are especially susceptible to ROS attacks. Malondialdehyde (MDA) is produced by oxidation of polyunsaturated fatty acids with ROS in the cell membrane and used as a reliable oxidative stress marker (Moore and Roberts, 1998). Some experimental studies have shown that EMFs can enhance lipid peroxidation in various tissues by the increasing free radical production (Hashish et al., 2008; Goraca et al., 2010; Kiray et al., 2013). Nitric oxide (NO) which another indicator of oxidative and nitrosative stress, is a reactive molecule produced by L- arginine via the nitric oxide synthase (NOS) enzyme and plays a critical role in many physiological processes and in the pathogenesis of various diseases (Jelenković et al., 2006). Researchers studying EMF suggested that NO levels or NOS activity could have a prognostic value, pointing to the degree of EMF induced tissue damage (Noda et al., 2000; Ilhan et al., 2004; Ozguner et al., 2005).

In physiological circumstances, organisms have enzymatic and non-enzymatic defenses against oxidative stress or oxidative damage. These systems, such as antioxidant vitamins (A, C and E vitamins), superoxide dismutase (SOD), catalase (CAT), Glutathione peroxidase (GSH-Px) and glutathione (GSH) protect cells against oxidative injuries such as lipid peroxidation. It has been shown that antioxidant defense systems can be altered by magnetic field exposures which lead to oxidative stress (Seyhan and Canseven, 2006; Coşkun et al., 2009; Goraca et al., 2010).

The liver which protects the body from dietary, environmental, metabolic and chemical toxins is the main organ responsible for detoxification. With it, the brain and heart have the highest oxygen consumption rates and the brain also contains high levels of unsaturated fatty acids. Then that of the liver, heart and brain are highly vulnerable to oxidative and nitrosative tissue damage. Moreover, antioxidant defense systems of these organs can also be easily affected by various stimulants.

The aim of this study is to establish a different biochemical approach in discontinuous and continuous EMF exposures. In this context, we examined the effects of continuous and intermittent exposure to 1.5mT, 50 Hz MF by biochemical assessment of MDA, GSH and NO levels in liver, heart and brain tissues of guinea pigs.

2. Materials and Methods

The necessary permissions for the study were obtained from the “Laboratory Animal Care Committee” of Gazi University. (Report no: 39–5858). Animal welfare and proper care were taken into consideration throughout all experiments.

2.1. EMF Exposure System

50 Hz 1.5 mT electromagnetic field exposure was designed according to the system developed by Canseven and Güler (2006). MF was produced with a spiral Helmholtz coil with a diameter of 42.75 cm, embedded in a circular wooden frame. Each spiral was formed with 154 turns of copper wire. The sinusoidal current at a frequency of 50 Hz was provided by a variable transformer (2,7 kilo Volt Amp-kVA) specially designed to feed the coils. The magnetic field at the center of exposure system was measured by a Hall-Effect Gaussmeter. The magnetic field's waveform and frequency have monitored by an oscilloscope. To avoid any degradation in the magnetic fields, the animals were kept in polycarbonate cages placed in the center of the energized coils during the experiments.

2.2. Animals

Eighteen adult male guinea pigs with body weights between 250 and 300 g were used in the experiment. All animals fed standard pellet food, they were housed in polycarbonate cages in a room with controlled temperature (22 °C), humidity (50 – 55 %), and 12-hour light-dark cycle. Animals were randomly separated into 3 groups of 6 animals each: 1) Control, 2) Continuous exposure group, 3) Intermittent exposure group. Continuous exposure group was exposed for 4 hours per day 50 Hz MF of 1.5 mT for a period of 7 days. Intermittent exposure group was exposed to per day 2 hours on, 2 hours off, 2 hours on (total 4 hours) 50 Hz MF of 1.5 mT for a period of 7 days. Control animals were not exposed to any magnetic field. Animals of all experimental groups were sacrificed under anesthesia at the end of the experiment. Tissues were immediately removed, frozen in liquid nitrogen, and stored at -80 ° C until biochemical analyses were performed.

2.3. Biochemical Analysis

2.3.1. MDA Levels

MDA levels in the tissues were determined by the spectrophotometric method of Casini et al. (1986). Briefly, tissue samples were homogenized in trichloroacetic acid (TCA) solution, and then the homogenates were centrifuged. An equal volume of 0.67% (m / v) thiobarbituric acid (TBA) and supernatant were mixed and boiled in a boiling water bath for 15 minutes at 100 ° C. The absorbance of the samples was measured at 535 nm. Lipid peroxide levels were expressed in MDA equivalents using an extinction coefficient of 1.56×10 5 mol^{-1} cm^{-1}.

2.3.2. GSH Levels

The GSH levels were determined by a modified method of Ellman (Aykaç et al., 1985). Tissues were homogenized in cold TCA solution, and homogenates were centrifuged. After centrifugation, 0.5 ml of supernatant was added on to the mixing of the 0.3 M disodium hydrogen orthophosphate dihydrate (Na_2HPO_4 $2H_2O$) solution and dithiobisnitrobenzoate (DTNB) solution. The absorbance of samples was measured at 412 nm. The GSH levels were calculated using an extinction coefficient of 13.600 mol^{-1} cm^{-1}.

2.3.3. NOx Levels

NOx levels in tissues were determined by the Griess reaction (Green et al., 1982). Briefly, tissue samples were homogenized in phosphate buffer saline (pH = 7.4) and centrifuged. Then the supernatant was added to sodium hydroxide solution (NaOH) and incubated at room temperature. After incubation, 10 % (w/v) zinc sulfate ($ZnSO_4$) was added for deproteinization, and this mixture was then centrifuged. Obtained supernatants were used for subsequent analysis. Nitrate levels of tissues were assayed by spectrophotometric method (Miranda et al., 2001). Nitrite levels were detected by the Griess reaction. Various concentrations of sodium nitrite and nitrate solutions were used as standards.

2.4. Statistical Analysis

One Way ANOVA test was used to evaluate the findings. All values were given as arithmetic mean ± standard deviation, and $p < 0.05$ was considered significant.

3. Results

3.1. MDA levels

MDA values in the brain, liver, and heart tissues are summarized in Figure 1. MDA levels of brain tissue in the intermittent magnetic field exposure group were significantly decreased when compared with control and continuous exposure groups. However continuous magnetic field exposure caused a slight decrease in MDA levels versus controls but the difference was not significant. Heart tissue MDA values revealed significant increases in the continuous exposure group when compared with controls. Also, in intermittent exposure group's MDA levels were found to be elevated than in control group but this rise was not statistically significant. Liver MDA levels increased with both intermittent and continuous EMF exposure, but only the increment in the intermittent exposure group was statistically significant.

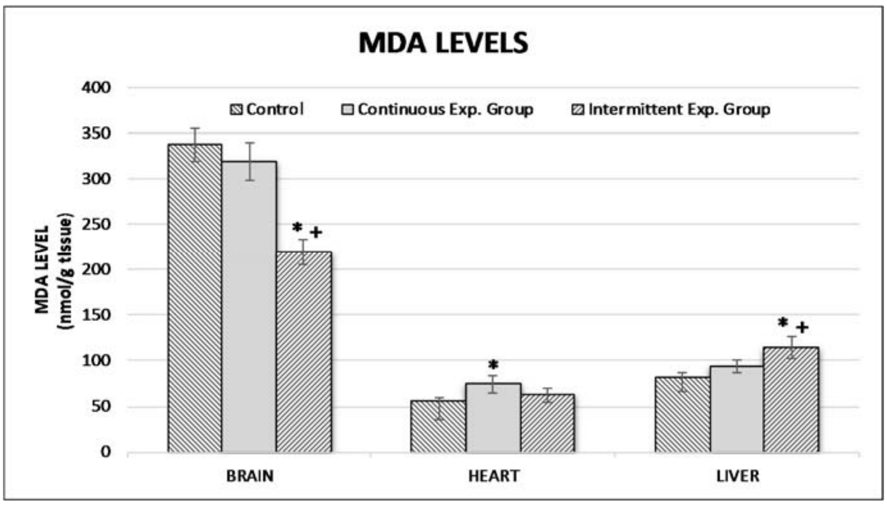

Figure 1. Effects of continuous and intermittent EMF exposure on MDA levels
 $p < 0.05$ significantly different control
+ $p < 0.05$ significantly different experimental groups

3.2. GSH levels

The results of GSH levels in the brain, liver, and heart tissues are shown in Figure 2. The GSH level of brain tissue in the intermittent exposure group was found to be significantly increased compared with both control and continuous exposure groups. In the continuous exposure group, the GSH level was found to be higher than in controls however the change was not significant. Heart tissue GSH value revealed significant increases in the intermittent exposure group when compared with continuous exposure group. Whereas GSH levels in the liver were found to be statistically significantly increased in both experimental groups compared to the control group.

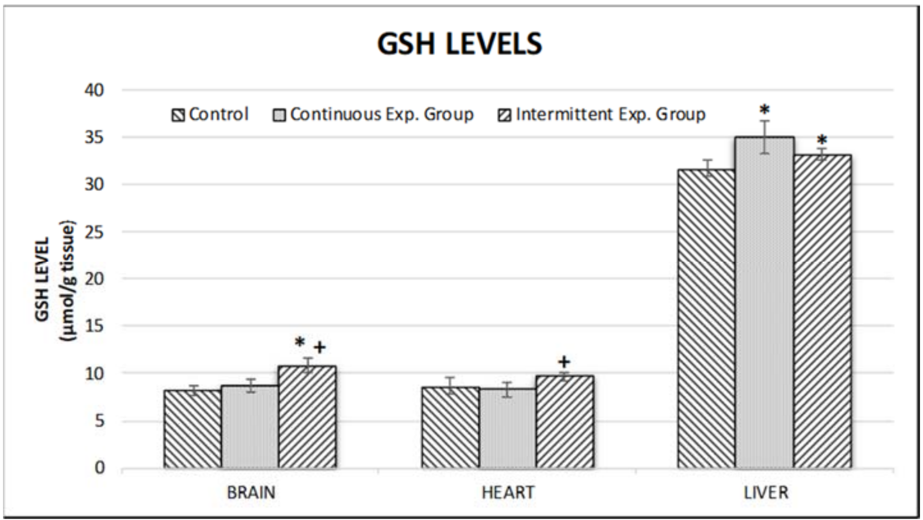

Figure 2. Effects of continuous and intermittent EMF exposure on GSH levels
 $p < 0.05$ significantly different control
+ $p < 0.05$ significantly different experimental groups

3.3. NOx levels

NOx results in the brain, liver, and heart tissues are shown in Figure 3. There was no significant change in NOx levels in brain tissue of either the experimental or control groups. NOx values in heart tissue were significantly decreased in the continuous exposure group compared to controls. However intermittent magnetic field exposure caused the significant increase in NOx levels of heart tissue versus continuous exposure group and controls. In the liver like the heart tissue, NOx levels were found to be increased in the group of intermittent exposures while being reduced in the group of continuous exposures.

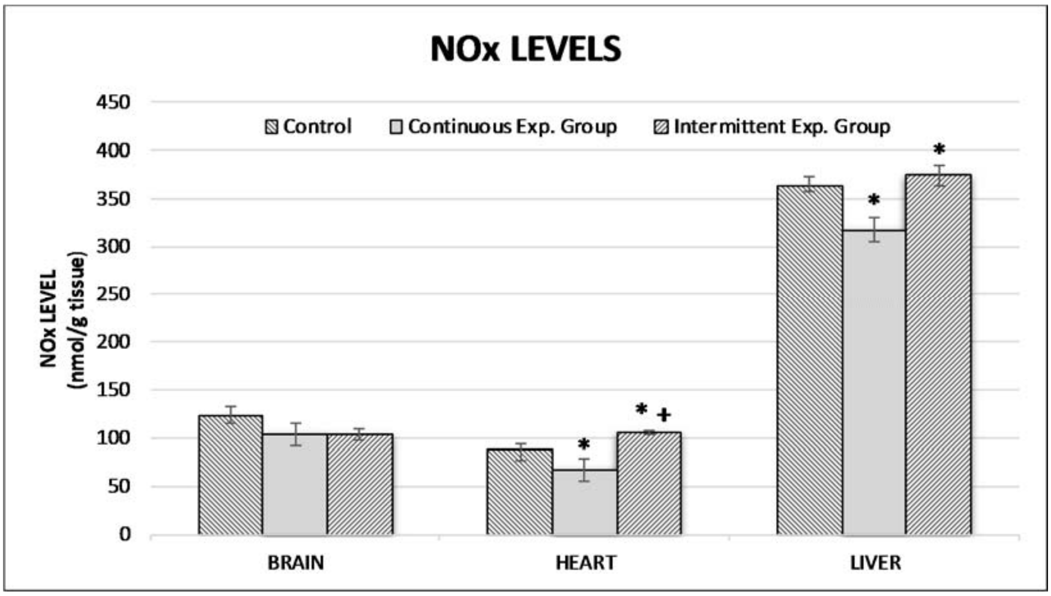

Figure 3. Effects of continuous and intermittent EMF exposure on NOx levels
 $p < 0.05$ significantly different control
+ $p < 0.05$ significantly different experimental groups

4. Discussion

Oscillating external electric fields, such as magnetic fields, move the free ions on both sides of the plasma membrane through membrane by creating an oscillatory force on them. This movement of ions leads to the deterioration of the ion channels in the membrane, the biochemical change in the membrane, and thus the deterioration of the whole cell function (Panagopoulos et al., 2002; Hardell and Sage, 2008). For this reason, it is important to investigate the cellular sources of free radicals in order to understand the pathological processes occurring via EMF effect in biological systems.

Our previous study showed that 50 Hz, 1.5 mT intermittent and continuous EMF exposure for 4 days increased the MDA levels in the serum and liver tissues of the guinea pigs but decreased the MDA levels in the brain. However, 50 Hz, 1.5 mT intermittent and continuous EMF exposure for 4 days had not affect NOx levels in brain and liver tissues (Coşkun et al., 2009).

In this paper, we evaluated whether 50 Hz magnetic field of 1.5 mT continuous or intermittent exposure periods for 7 days could affects MDA, NOx and GSH levels of brain, liver and heart tissues in guinea pigs.

MDA levels in liver were found to be increased for intermittently (2h on/ 2h off/ 2h on) applied 50 Hz, 1.5 mT ELF-EMF during 7 days compared to the control and the continuous exposure groups. In the liver, ROS primarily produced in the endoplasmic reticulum and mitochondria of hepatocytes via cytochrome P450 enzymes. Under the physiological conditions, cells control the level of oxidative stress and sustain a balance between antioxidant and oxidant particles (Urtasun et al., 2008; Li et al., 2010). ELF-EMF exposure could be lead to increased lipid peroxidation by caused excessive ROS production due to altered mitochondrial electron transport chain in hepatocytes. Consistent with our findings Seyhan and Canseven (2006) showed that liver MDA levels were increased when exposed to 1, 2, 3 mT (50 Hz) by 4h/day over five days. Also, exposure to 50 Hz, 1.4 mT for 30 days, was resulted that increased hepatic lipid peroxidation as having reported by Hashish et al. (2008). In the heart tissue MDA level was found to be significantly increased by 50 Hz, 1.5 mT continuous magnetic field exposure. Similar to the results of this study heart TBARS levels were increased by 40Hz, 7 mT, for 2 weeks EMF stimulation in rats (Goraca et al., 2010). Another investigator has demonstrated that 50 Hz, 3mT electromagnetic field exposure increased lipid peroxidation in myocardial tissue of rats (Kiray et al., 2013). During the abnormal circumstances, toxic oxygen metabolites can be produced by cardiomyocytes, endothelium, and fibroblasts in the heart. Elevated MDA levels in the heart suggest that EMF exposure may induce production of superoxide anions which formed by cardiomyocytes, fibroblast, and endothelium. Superoxide anions dismutate to hydrogen peroxide that could be able to react with membrane lipids and cause their peroxidation.

It has been accepted that EMF exposure could increase lipid peroxidation in the brain, which is a susceptible organ against oxidative damage, due to its high oxygen consumption rate and high content of oxidizable lipid (Luo et al., 2016; Yin et al., 2016). Falone at al. (2008) showed that 50 Hz magnetic field stimulation had increased production of ROS in rat brains. Therewithal 60 Hz magnetic field caused that enhancement of lipid peroxidation in the midbrain, cerebellum and cortex of mice as was reported by Lee et al. (2002). In another study, in the Wistar albino rats, ELF-EMF exposure was shown to increase TBARS levels in the brain and alter the lipid profile (Martínez-Sámano et al., 2018). On the other hand, some investigators have found that magnetic field exposure did not change lipid peroxidation in the brain (Yoshikawa et al., 2000; Seyhan and Canseven, 2006). In the present study contrary to findings in the literature we observed that intermittent exposure to 50 Hz, 1.5 mT 4h/day for 7 days significantly decreased MDA levels in brain tissue. Also, in the intermittent exposure group, GSH level was found to be significantly increased compared to controls. This result suggests that decreases in MDA levels may have been a consequence of elevated levels of GSH having a protective effect on brain tissue against free radical damage. This results on MDA and GSH levels are consistent with our previous report (Coşkun et al., 2009).

Antioxidants are considered to have a preventive action on biological macromolecules towards free radical damage. It is assumed that GSH is a primary defense line for free radical production as a cofactor and a sweeper in the metabolic elimination of ROS. The level of GSH can be raised on account of an adaptive response against low intensities oxidative stress owing to an increment in its synthesis; however, strong oxidative stress may depress GSH levels due to the oxidation of GSH to GSSG and the deficiency of the adaptive response skills (Martínez-Sámano et al., 2010). In the several studies, it has been reported that exposures of different intensity and frequency MF can alter the antioxidant conditions of various animals and tissues (Lee at al., 2002; Seyhan and Canseven, 2006; Martínez-Sámano et al., 2010; Kiray et al., 2013). The present study demonstrated that intermittent exposure to 50 Hz, 1.5 mT ELF-MF 4h/day during 7 days increased GSH levels in brain and heart tissues. It has been also observed that both intermittent and continuous exposure augmented GSH levels in the liver. It is conceivable that elevated GSH levels may develop as a protective response to cell damage resulting in increased free radical production resulting from EMF exposure.

Nitric oxide is a substantial messenger molecule which plays a crucial role in an extensive variety of physiological processes inclusive of cytotoxicity, vasodilation, immune modulation, and neurotransmission (Moncada, 1992). It was reported that 0.1 mT 60Hz magnetic field administration unchanged NO levels in the liver (Yoshikawa et al., 2000). Our observation also demonstrated that both continuous and intermittent exposure of 60 Hz, 1.5 mT ELF-MF did not alter NOx levels in brain tissue. It has been well-known that the entity of NOS in neuronal cells is associated with calmodulin and calcium. The mechanism to explain this situation might be that EMF may suppress NOS activation due to effects the level of intracellular calcium, and consequently no change in NO production. In the heart and liver tissues, continuous exposure has been causing a reduction in NOx levels. This result can be elucidated by reduced catabolism of NO or declined production of NO with a decrease in activation of NOS as a consequence of EMF administration. On the other hand, in the intermittent exposure group, NOx levels of heart and liver were found to increase. Kim et al. (2017) indicated that ELF-MF might have cause calcium-dependent NOS activation which enhances NO synthesis. As pointed in previous reports, a possible mechanism for explaining variable NO levels may be that ELF-MF changes calcium ion flux across the cell membrane, the consequence of affected by iNOS activation associated with calcium and calmodulin (Noda et al., 2000; Jelenković et al., 2006; Seyhan and Canseven, 2006; Sirmatel et al., 2007).

In this study, we also compared the effects of continuous and intermittent exposure to 50 Hz ELF-MFs. Boland et al. (2002) reported that 50 Hz intermittent exposure at high intensities enhanced NO-induced oxidative cell damage in brain cell culture more than continuous exposure did. However, the exposure protocols used in this study show that biochemical parameters were almost equally affected by continuous and intermittent exposures. This result possibly proposes that the effects depend on the total amount of daily exposure rather than the intervals.

In conclusion, we found that both continuous and intermittent magnetic field exposure can alter oxidant and antioxidant balance of liver, brain and heart tissues. Results of this study indicated that the responses of cells to oxidative damage are tissue-specific and different from one another. The differences observed between brain, heart and liver tissues could be based on the duration of stimulation, exposure period or field intensities of magnetic fields. Further studies with different exposure periods, intensities and types of MFs might clarify the specific mechanisms involved and assess the precautions against EMF induced oxidative stress.

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