

## Original Article

# The effects of 30 mT electromagnetic fields on hippocampus cells of rats

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## Abstract

**Background:** Despite the use of electromagnetic waves in the treatment of some acute and chronic diseases, application of these waves in everyday life has created several problems for humans, especially the nerve system. In this study, the effects of 30mT electromagnetic fields (EMFs) on the hippocampus is investigated.

**Methods:** Twenty-four 5-month Wistar rats weighing 150–200 g were divided into two groups. The experimental group rats were under the influence of an EMF at an intensity of 3 mT for approximately 4 hours a day (from 8 AM to 12 PM) during 10 weeks. After the hippocampus was removed, thin slides were prepared for transmission electron microscope (TEM) to study the ultrastructural tissue. Cell death detection POD kits were used to determine the apoptosis rate.

**Results:** The results of the TEM showed that, in the hippocampus of the experimental group, in comparison to the control group, there was a substantial shift; even intracellular organelles such as the mitochondria were morphologically abnormal and uncertain. The number of apoptotic cells in the exposed group compared to the control group showed significant changes.

**Conclusions:** Similar to numerous studies that have reported the effects of EMFs on nerves system, it was also confirmed in this lecture. Hence, the hippocampus which is important in regulating emotions, behavior, motivation, and memory functions, may be impaired by the negative impacts of EMFs.

**Key Words:** Electromagnetic fields, hippocampus, rats

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## INTRODUCTION

Electromagnetic radiation is a combination of an electric field and a magnetic field perpendicular to each other and in the direction perpendicular to the two fields which is distributed in space. Unlike other waves, electromagnetic waves pass through vacuum. In general, waves including electromagnetic radiation consist of four main characteristics of wavelength, frequency, speed, and amplitude. High-frequency photons have considerable energy to break chemical bonds, and this part of the spectrum is called as ionizing. A photon's energy to separate the chemical bonds is too low at

low frequency, which is called the non-ionizing part of the electromagnetic spectrum. Therefore, the biological effects of electromagnetic waves can be divided into

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ionizing and non-ionizing parts. Non-ionizing radiation can have adverse effects on human health such as headache, stress, fatigue, and anxiety.<sup>[26]</sup> Non-ionizing radiation includes the radiation radiated from medical equipment such as mammography, magnetic resonance imaging (MRI) and computed tomography (CT) scans or radiation from natural sources such as X-rays, gamma rays, ultraviolet rays, and infrared radiation. Non-ionizing radiation includes rays emitted from telecommunications equipment, cell phones and base transceiver station (BTS), disinfectant systems, laser productive resources, microwave ovens, infrared beams, as well as rays of home electric appliances such as hair dryers, vacuum cleaners, and washing machines. In the case of adverse effects of electromagnetic fields (EMFs), there are different views and opinions that result in further investigations associated with teratogenic, carcinogenic, hematological, immunological, and neurological effects. Epidemiology studies have shown the relationship between occupational and non-occupational exposure, especially exposure to extremely-low-frequency (ELF) fields and cancer risk including leukemia, brain tumors, and breast cancer.<sup>[2,8,16]</sup>

It's reported that exposure by electromagnetic fields in children may be led to the increased white blood cells and consequently the formation of leukemia.<sup>[11]</sup> According to some researchers, exposure to ELF fields may stop the secretion of melatonin, which is the hormone associated with sleep-wake rhythm. In addition, melatonin protects against breast cancer; if melatonin levels are reduced, it can result in an increase in the breast cancer incidence along with other primary factors.<sup>[24]</sup> By using a cohort study, an epidemiological investigation by Savitz was conducted on mortality from cardiovascular diseases (CVD) among electrical equipment workers exposed to ELF magnetic fields.<sup>[18]</sup> In addition to the risks of EMF on vital systems of the body, its applications should also be mentioned. Electricity with high frequency is applied for diathermy on the body and low frequency electricity is used to measure blood flow and skin resistance. Therapeutic applications of EMFs for hyperthermia (chemotherapy and radiotherapy) include brain surgery and cancer with high frequency, along with lung impedance measurement to determine the amount of fluid.<sup>[4]</sup> Diagnostic applications include MRI, magnetocardiogram, magnetoencephalogram, magnetocologram, and magnetortiongram.<sup>[1,14,15,24]</sup> Among the organs sensitive to the oxidative stress caused by EMF,<sup>[21]</sup> central nervous system tissues may be more susceptible to serious damage. One of the critical parts of the system is the limbic system because of its role in learning and memory. Limbic system is a part of the central nervous system located among the cerebral cortex and hypothalamus. We know that the limbic system plays an important role in emotions, behavior, motivation, and memory. Hippocampus formation (including the

hippocampus, dentate gyrus, and parahippocampal gyrus) is a part of the limbic system which impacts the different aspects of emotional behavior through the hypothalamus and its communication with autonomic nervous system, as well as its effects on the endocrine system. The effect on reaction to fear and anger as well as emotions associated with sexual behavior is evident.

Moreover, according to some evidence, the hippocampus plays a role in converting recent memory to long-term memory, and any damage to it causes severe memory impairment. Referring to the previous studies, neurological disorders caused by electromagnetic waves involve memory loss, lethargy, insomnia, and depression. Previous investigations have shown mild side effects such as headaches, dizziness, temporary amnesia, sleep disorders, and reduction of learning ability, as well as remarkable effects such as leukemia, and gene mutation, chromosomal abnormalities in humans and animals.<sup>[19,16,22]</sup> Major effects of acute exposure to electric fields have been reported on the mood and behavior of humans and laboratory animals. Relevant reports indicate that weakening of individuals' reaction time, especially their attention, is due to the exposure to these fields. The major effect of chronic exposure to electric field has been reported on growth, often slowing the growth process. The evidence also shows that chronic exposure acts as a biological stimulus. In many cases, free radicals produced by electromagnetic waves are reported to be the cause of a variety of diseases and cancers.<sup>[7]</sup> Electromagnetic waves with an increase in free radicals level can disturb homeostasis systems such as the cardiovascular and nervous systems.<sup>[5-7,20]</sup> Furthermore, studies show that less vulnerability to electromagnetic radiation is caused in cells with higher antioxidant activities. The apoptosis process is one of the important pathways induced by these waves. A brief reference to the process of apoptosis is provided below. Perhaps the turning point for research on apoptosis is attributed to Kerr Wyllie and Currie studies in 1972.<sup>[4]</sup> Apoptosis is a physiological mechanism of cell death and the controller of development and homeostasis multicellular organisms. The cells usually die when they receive mixed development messages. Apoptosis is responsible for removing cells in abnormal tissues such as tumors. Apoptosis is controlled by the genetic system and is induced by external or internal factors such as hormones, cytotoxic lymphocytes, anti-cancer drugs, cytokines, and gamma or ultraviolet rays.<sup>[16]</sup> Despite the major differences between apoptosis and necrosis, sometimes morphological changes are used to distinguish them from one another. Necrosis occurs in acute pathological conditions. Nuclear chromatin compresses and disappears during the last stages. Swollen cytoplasm and membrane organelles and cells are gradually destroyed and the cell is removed by phagocytes. On apoptosis, the cells are fragmented with all contents

and swallowed by macrophages. There are two pathways for apoptosis. The extrinsic pathway induces apoptosis by proapoptotic ligands such as APO21/TRAIL and CD95L/FASL as well as binding receptors. The second pathway is the internal or mitochondrial route observed in cells with homeostasis disorders. This route is induced by protein family of BCL2. Apoptosis is mediated by a group of proteases called caspases. Damaging elements of cell DNA lead to an increased protein level of P53 and preapoptotic protein activity of P53 leads to the growth stop at G1 or apoptosis induction. By considering apoptosis, the effects of EMFs on the central nervous system tissue can be investigated. In this regard, the current research aims to examine the effects of EMF (3 mT power) on the hippocampus part of the nervous system.

## SUBJECTS AND METHODS

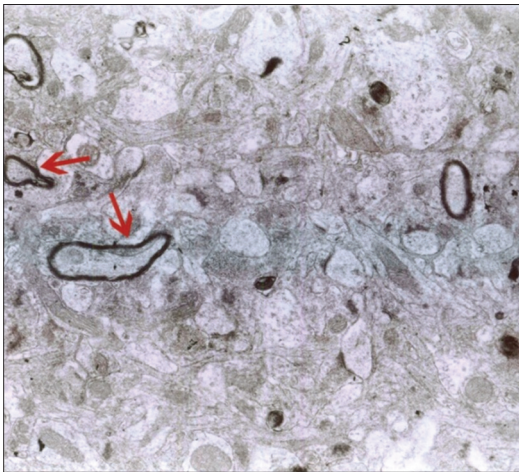
Twenty-four 5-month Wistar rats weighing 150–200 g were used in this experimental study. The rats were collectively kept in a standard condition at a temperature of  $2 \pm 24^{\circ}\text{C}$ , 12 h light and 12 h of darkness, and humidity of 50–70 in a room with continuous ventilation inside metal cages. Food and water were given to the rats without any restriction. The rats were assigned to two groups, that is, experimental and control groups, with 12 rats in each group. The experimental group rats were exposed to EMF at an intensity of 3 mT for approximately 4 h a day (from 8 AM to 12 PM) for 10 weeks. An EMF generator device, producing a field of 3 mT, designed and manufactured in Medical Physics, was used. The generator was made of according to the Helmholtz theory. With regard to this selection, issues such as the need for achieving a uniform field and with certain intensity and different constraints like the need for a suitable place for rats living inside the machine were the determinative. Considering to the generator ampere consumption and its long time use during the day, to avoid overheating the device proper ventilation, the ventilation system was installed on to. Generally, the generator consists of 2 windings of opposite direction which produce a uniform field in the center of the device where animals are located. A uniform field was produced between the windings due to passing electricity with alternating current and frequency of 50 Hz. At the end of the experimental period, at first, the rats were anesthetized by a piece of cotton soaked in chloroform in a plastic container in the package. After stopping the heartbeat, we proceeded to remove the head and skull. After the brain became visible, we took the brain out of the skull hole seamlessly and carefully. Then, the hippocampus was removed and washed with 0.1 M phosphate buffer at a pH of 7.4. Next, secondary stabilization was performed by 1% osmium tetroxide. After dehydration using ethanol, the samples were made transparent by using propylene oxide. In the last phase, the samples were embedded in resin. Uranyl acetate

and lead nitrate using transmission electron microscopy (TEM) (LEO, 200 Germany) were applied for coloring of toluidine blue semi-thin slices as well as thin slices. Cell death detection POD kits (Roche Company made in Germany) were used for determining the apoptosis rate. Coloring was performed by toluidine blue. Using these kits, apoptotic cell nucleus turned brown, while the normal cell nuclei retained the toluidine blue color. In this section, the hippocampus samples were stained by TUNEL techniques, therefore, apoptotic cells became visible as golden brown.

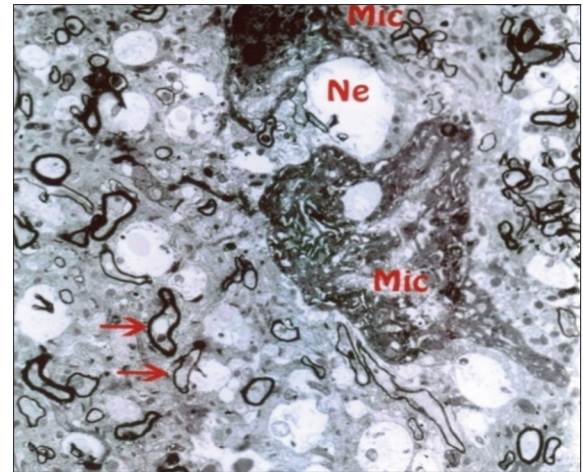
## RESULTS

As mentioned previously, the aim of this study was to evaluate the ultrastructural and histochemical changes resulting from electromagnetic waves at a frequency of 50 Hz and an intensity of 3 mT on the hippocampus of rats' brain after exposure to the EMF. Therefore, findings of the effects of EMFs on hippocampal tissue among the groups exposed to the EMF, considering the mentioned terms and conditions, and the control group without exposure to EMFs have been investigated. The results are presented in two parts, that is, ultrastructural changes and histochemical examination. Ultrastructural changes: As seen in Figure 1, which displays the electron micrograph of rats' brain hippocampal tissue section in the control group, the cross-section contains some sections of myelinated and free nerves along with glial cells and some mitochondria [Figure 1]. Figure 2 shows the electron micrograph of adult rats' brain hippocampal tissue section in experimental groups under the influence of EMF. The presented cross-section contains some sections of myelinated and free nerves along with some glial cells of dense core and compact chromatin. Moreover, the mitochondria are not in their natural state, compared with the control group mitochondrial; crystals are erratic and vague. Figure 3 presents a micrograph of a nerve cell in the control group rats' hippocampus parenchymal tissue. As shown in the figure, nerve cells with normal nucleus and organelles inside the cells including the mitochondria and natural endoplasmic reticulum as well free ribosome particles are clear. Myelinated and free nerves sections are clearly visible among the nerve cells. Figure 4 shows two nerve cells for the group under the influence of EMF. As is clear from the figure, the nerve cells are damaged. Chromatin is clearly visible compactly with clear marginalization, and the amount of intracellular organelles is decreased; further, vacuoles are visible with a subtle membrane inside the cytoplasm. Moreover, intracellular organelles such as the mitochondria are morphologically abnormal and uncertain. Photomicrography from the hippocampus parenchyma of rats after the TUNEL method in the control group shows red blood cells (RBCs), nerve cell nucleus, and loss of apoptotic cells [Figure 5]. Finally,

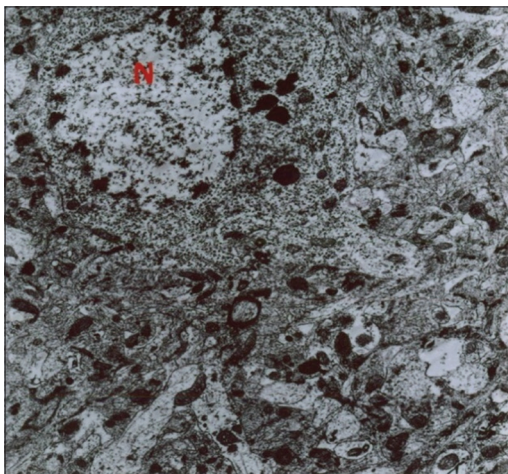




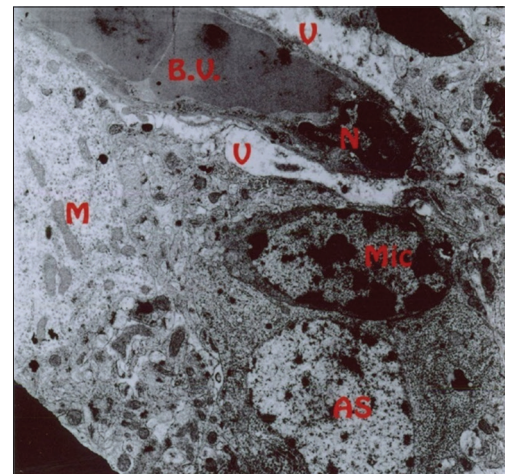
**Figure 1: Electron micrograph of rats' brain hippocampal tissue section in the control group (red arrows show myelinated and free nerves)**



**Figure 2: Electron micrograph of adult rats' brain hippocampal tissue section in the experimental group (arrows show myelinated and free nerves). Ne: Nucleus, Mic: Mitochondria**



**Figure 3: Micrograph of a nerve cell in control group rats' hippocampus parenchymal tissue**



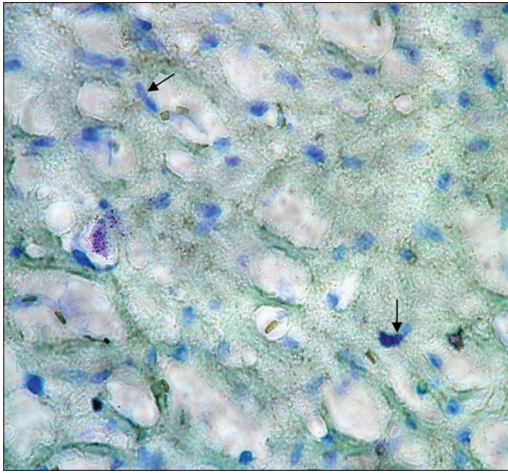
**Figure 4: Micrograph of nerve cells in the experimental group. N: Nucleus cell, Mic: Mitochondria, AS: Astrocyte cell, V: Vacuole**

photomicrograph of hippocampus parenchyma after EMF exposure by TUNEL method shows the number of apoptotic cells, which compared to the control group has a significant increase [Figure 6].

## DISCUSSION

The results of the Bio Institute group research in 2009 showed that electromagnetic radiations from cell phones' high-pressure lines, telecommunications stations, and many other sources emitting electromagnetic radiation are acceptable, permissible, and compatible to the standards of Federal Communications Commission (FCC) in daily lives of humans. These radiations do not have health risks. However, when humans are exposed for hundreds and thousands of times, and sometimes permanently exposed to electromagnetic radiation, absorption level of radiations exceed the standard limit, and thus their adverse effects develop. The results of this study showed that exposure to electromagnetic fields of

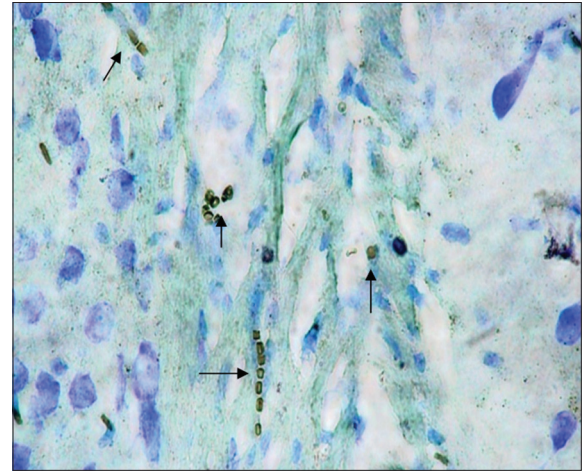
50 Hz with 3 mT intensity leads to a significant increase in apoptotic cells. It should be noted that these cells are mainly visible in the hippocampus glial cells. TUNEL technique in the present study clearly showed apoptotic glial cells. It seems that reduction in the number of glial cells and neurons in confirming the occurrence of apoptosis in neurons and glial cells, which can be due to the field impact on cell programmed death and its incidence, should be considered. These findings are consistent with studies conducted by Salford to examine the relationship between pathological leakage of albumin from the blood-brain barrier and neuronal damage by pulsed microwaves emitted from mobile. The results of this research revealed a significant destruction of neurons in the cortex, hippocampus, and basal ganglia of rats that were exposed to mobile radiation for 2 hours. Perhaps the cell death observed in this study is due to the production of free radicals in brain tissue neurons of the irradiated mice.<sup>[19]</sup>



**Figure 5: Normal cell nuclei became toluidine blue in color and most cells have a normal shape. (Arrows show normal cells)**

The current theory is compatible with the studies conducted by Lai and *et al.* They examined the effects of a magnetic field of 0.01 mT with a frequency of 60 Hz for 24 hours to assess the level of DNA breaks in rats' brain cells. Results have shown that high-intensity electromagnetic waves lead to increase in apoptosis and necrosis in the brain cells of rats. This increase in mortality may be due to the free radicals produced by electromagnetic waves that results in breaking DNA and nerve cells death.<sup>[3]</sup> Simonian *et al.* reported that hydroxyl radicals and nitric oxide particularly in brain cells may lead to apoptosis and cell death.<sup>[22]</sup> These results are in line with the findings of Soleimani *et al.*<sup>[23]</sup> They are also compatible with the present research findings that investigated the effects of EMFs of 3mT on central nervous system (brain, cerebellum, spinal cord). Results indicated that cerebellar Purkinje cells' size decreased compared to the controlled cerebellum, which could be because of cells activity reduction.<sup>[22]</sup> Numerous studies have shown that EMF could also affect cells genome and results in decreasing DNA repair or apoptosis. EMF also affects the cell membrane and through the impact on glycoproteins affects intracellular processes such as intracellular enzyme operation, cytoskeleton, and the cell nucleus. In confirming these findings, it has been shown that the contents of the light and transmitters such as gamma-Aminobutyric acid (GABA) are reduced in Purkinje cells under the influence of EMF.<sup>[9]</sup>

Other findings of this study on cerebellum reveal significant decrease in the number of Purkinje cells under the influence of EMF that can be due to their effect on programmed cell death and apoptosis. In addition, some findings show the effect of EMF on calcium homeostasis changes of membrane and in stimulating the synthesis of ornithine decarboxylase as well as an increase in activity that alters the expression of DNA synthesis.<sup>[17]</sup> Some studies refer to the relationship between the intensity



**Figure 6: Apoptotic cell nucleus turned brown and shows an increased number of after exposed to electromagnetic field (arrows show apoptotic cells)**

and duration of exposure to electromagnetic waves and the number of damaged neurons and its biological effects.<sup>[12]</sup> Wei *et al.* examined the effects of sinusoidal EMFs with an intensity of 1.2–0.3 gauss for 3–72 hours on the proliferation of human astrocyte cells. The results showed that electromagnetic waves with a frequency of 60 Hz depending on the time and studied dose may increase the number of human astrocytes. However, the waves had no effect on DNA synthesis of rat cortical astrocytes.<sup>[28]</sup> Furthermore, Joubert *et al.* evaluated the apoptosis level of neurons cultured in *in vitro* environment of rats' embryo cortex that were exposed to mobile radiation for 24 hours. According to the results, there was no significant difference in the apoptosis level of cultured neurons between the experimental and control groups.<sup>[27]</sup> In addition, Takahashi examined the effects of induced mutation via electromagnetic waves emitted by mobile. In this study no increase was observed in the number of astroglia in the affected areas of the central nervous system and glial cells apoptosis among the mice exposed to mobile waves with a frequency of 1.5 MHz for 2–4 weeks. Moreover, no evidence of brain DNA damage and histopathological degeneration in brain was reported.<sup>[25]</sup> The results of the study by Takahashi are consistent with the findings of previous researches in which the lack of mutation increase in brain cells DNA is mentioned; however, these are in contrast with the findings of our research. Perhaps the contrast is due to the short duration of exposure to EMFs compared to the duration of the current research (10 weeks). Heterochromatic and shrinking of neural and glial cells through TEM microscopic examination are other findings of this study. Thus, neural and glial cells exposed to electromagnetic fields were damaged and had a dense core and compact chromatin with clear marginalization. Heterochromatic and shrinking of cerebellar Purkinje cells as well as neural and glial cells of brain and spinal



cord are our other findings that were recognized by light microscopic and TEM examination. To further confirm these findings, morphometric analysis showed that the average of the nucleus diameter and the nucleus axial ratio decreased in the experimental group. All the changes may represent reduction of metabolic activity of cells affected by EMF. Previous studies have revealed that EMF breaks up DNA in rats' brain cells. The adverse effects of EMF on chromosomes in metaphase and chromosomes failure in culture medium and chromatin structure changes have been previously reported. In addition, EMF causes cross-linking of DNA, increasing apoptosis and necrosis in brain cells. We also found out changes in organelles such as mitochondria and endoplasmic reticulum. Dilatation of the endoplasmic reticulum and irregularity and ambiguity of the mitochondrial crystals are some of those changes. Organelle changes may be due to the adverse impacts of field on their membrane.

A similar examination conducted on the kidney structure of rats for 52 days and 1 hour in a day. The rats were exposed to a field of low frequency (9450 MHz). Due to light microscopic examination and vacuolization, epithelium kidney tubules were observed in the apical and degenerative changes in the glomerulus were realized. Inflation of organelles with membranes of mitochondria and endoplasmic reticulum were observed through the examination by an electron microscope.<sup>[13]</sup> Another results supporting the findings of the present study have been reported via histochemical methods, in which the enzyme level of succinate dehydrogenase (SDH) and the menu amino oxidase (MAO) are decreased under the influence of the field. It was also reported that in rats under the influence of a field of 30 Mw/cm<sup>2</sup> damage to the mitochondria of the brain cortex lead to changes in transcription A-factor, mitochondrial, and mRNA.<sup>[29]</sup> Permeability of brain-blood barrier under the influence of the field has also been reported. This permeation is attributed to the thermal effects of EMF. Because increased vascular permeability is due to the effects of histamine, basement membrane changes are likely because of the effects of increased histamine secretion. There are many studies suggesting that myelinated nerve fibers such as motor neurons are vulnerable to electromagnetic waves. These findings are in line with the results of this study. At the same time, Terada conducted a study for investigation of electromagnetic waves' effects emitted from mobile devices on the human motor cortex motor. His results suggested that exposure to electromagnetic waves for 30 minutes do not cause changes in the motor cortex neurons or interneurons. McNamee *et al.* demonstrated that a field of 60 Hz for 2 hours is not capable to damage DNA and increase the apoptosis in cerebellar granular cells in the mouse brain.<sup>[10]</sup> According to the mechanism of EMF impact, in

this case it can be assumed that EMF is an iron-mediated process that increases the formation of hydroxyl free radical in the cell and causes breaking of DNA bands and cell death. Cells with high iron absorption, for example, proliferating cells, and cells with high metabolic activity, such as brain cells, are the most vulnerable cells to the EMF effects.

Examination of the proliferating cells shows that the most vulnerable time is during the phase G/S of the cell cycle when transferrin receptors are expressed and too much iron is penetrated. Free radicals produce large amounts of hydrogen peroxide in the presence of iron via the Fenton reaction. Cells with high metabolic activity produce large amounts of hydrogen peroxide through the mitochondrial electron transport, and therefore become more vulnerable to the effects of EMF. On the other hand, the negative effects of EMF depend on the cells iron storage ability in the form of ferritin. For example, liver cells can be less susceptible to the effects of EMF even when they are having a lot of iron input because they contain lots of ferritin. Cancer cells are realized by transferrin receptors on the surface, thus they have high iron absorption. The effects of free radicals are very extensive, however, there are four reactions that are associated specifically with cell harassment: Membrane lipids peroxidation, mitochondrial damage, creating crosslinking in proteins, and DNA damage. However, perhaps damaging effects of electromagnetic waves are because of the body's rising temperature and producing free radicals that both agents could be considered as damaging elements to the tissues of the body, especially the nervous system. Therefore, in order to eliminate the negative impacts of these waves on the nervous system, it is recommended that unnecessary and prolonged use of devices generating such waves be avoided.

## CONCLUSION

This study concluded that EMFs with any frequency and intensity can be damaging to the nervous system. In this study we found that EMFs by increasing structural changes in cells and apoptosis in them can alter the cells function at every position of it. Therefore, by avoiding the usage of these stimulators, their negative effects can be reduced.

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Nil.

## Conflicts of interest

There are no conflicts of interest.

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