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The effects of extremely low-frequency magnetic field exposure on apoptosis, neurodegeneration and trace element levels in the rat brain

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ABSTRACT

Aim: The aim of this study was to investigate the effects of 1mT, 1.5 mT, and 2 mT extremely low-frequency magnetic fields, which were within the limits for public environmental and occupational magnetic field exposure guidelines, on apoptosis, neurodegeneration and trace elements in rat brain cells.

Material and Method: A total of 35 adult male Wistar rats were allocated into four main groups: Group 1 (n=8) was healthy controls; Group 2 (n=9) was exposed to 1 mT extremely low-frequency magnetic field; Group 3 (n=9) was exposed to 1.5 mT extremely low-frequency magnetic field and Group 4 (n=9) was exposed to 2 mT extremely low-frequency magnetic field. All the rats in the exposure groups were exposed to 50 Hz extremely low-frequency magnetic field for 4 hours per day, 5 days per week for 30 days in the Helmholtz coils. After the exposure, rats were sacrificed and rat brains were evaluated for histopathological and immunohistochemical changes as well as about the trace element levels in the brain.

Results: Different levels of exposure to extremely low-frequency magnetic field doses caused increases in Ca levels and increased apoptosis in the rat brain. As the applied extremely low-frequency magnetic field levels increased, so did the apoptosis and Ca levels in the brain tissues.

Conclusion: Extremely low-frequency magnetic field exposure caused neurodegeneration in rat brain tissue, increased apoptosis, and increased Ca concentration. These changes may cause various biological damage, especially cancer in healthy tissues and measures should be taken to minimize extremely low-frequency magnetic field exposure in daily life in terms of protecting public health.

Keywords: Extremely low frequency, electromagnetic field, rat brain, apoptosis, trace elements, neurodegeneration

INTRODUCTION

As a result of the vast amount of currently developing technologies, humans are increasingly exposed to Electromagnetic Fields (EMF). The 0–300 Hz range of this spectrum is called Extremely Low Frequency (ELF). In daily life, almost all electrical devices used at homes and workplaces, particularly transformers and high voltage lines, are the major sources of exposure to ELF, so the general population is exposed to ELF-EMF involuntarily or accidentally (1).

It is now an accepted fact that ELF-EMF affects biological systems, although the mechanism has not yet been clarified. Despite the many studies investigating the biological effects of ELF-EMF, it cannot be claimed to be a potential human carcinogen based on side-effects as the results have been quite contradictory (2).

Programmed cell death or apoptosis, which is one of the most important factors in cancer control, plays an important role in regulating cell population in tissues, and a dysfunction in apoptosis can lead to tumor formation (2,3). Both epidemiological and laboratory studies including leukemia also indicate that, 50 Hz/ 60 Hz EMFs may lead to a number of different adult cancers including brain, lymphoma, nervous system, pharynx and breast

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cancers (4,5). Thus, clarifying the factors that may cause apoptosis, which plays a key role in neurodegenerative diseases and tumor pathogenesis, may be of guidance in the prevention and treatment of these diseases.

In vivo and in vitro studies on apoptosis induced by the effect of ELF-EMF have yielded conflicting results. While some studies have shown that ELF-EMF induces apoptosis susceptibility (6), others have shown that it decreases apoptosis (7,8). In a meta-analysis, it was reported that ELF-EMF significantly increased apoptosis in in-vitro studies (2). However, the effects of ELF-EMF on apoptosis in rat brain tissue in vivo have not been well studied.

ELF-EMF exposure may affect the levels of trace elements in serum and various organs by causing various biochemical changes in living cells. In studies with guinea pigs, changes in trace element levels have been found in the serum and other organs. In one study (9), magnesium levels were found to be increased, and Cu, Zn, and Ca levels remained unchanged, while another study (10) reported increased Cu, Zn, Ca and Mg levels in 50 Hz, 2 mT magnetic fields. Therefore, in the literature, there are conflicting results about the levels of trace elements in brain tissue.

In light of these informations, the aim of this study was to investigate the effects of 1mT, 1.5 mT, and 2 mT ELF-EMF. We tried to assess the effects of these magnetic fields which are within the limits for public environmental and occupational magnetic field exposure guidelines, on apoptosis, neurodegeneration, and trace elements in rat brain cells.

MATERIAL AND METHOD

Experimental Animals

All procedures performed in the experimental animal unit were in accordance with the principles of the Guide for the Care and Use of Laboratory Animals (https://www.nap.edu/catalog/5140/guide-for-the-care-and-use-of-laboratory-animals). The Laboratory Animal Care Committee of Ankara Training and Research Hospital reviewed and approved the experimental protocol (Report no: 19.03.2020/609). A total of 35 adult male Wistar rats, each weighing 200-260 g, were used in this experimental study. All the animals were obtained from Gulhane Laboratory Animal Production and Research Center, and the animals were assigned randomly to one of four groups: Group 1 (n=8) was Healthy Controls; Group 2 (n=9) was exposed to 1 mT ELF-MFs; Group 3 (n=9) was exposed to 1.5 mT ELF-MFs and Group 4 (n=9) was exposed to 2 mT ELF-MFs. When selecting these magnetic field levels, the scaling factor was taken in to consideration. The level of magnetic field that a person would be exposed to was estimated by the equivalent animal exposure. One of these methods was the ellipsoid modeling performed when the animal is standing and on four legs in sweat (11). According to this model average value of human:guinea pig scaling factor was calculated as 3.1. According to the calculated scaling factor (3.1), 1 mT, 1.5 mT and 2 mT magnetic field values in the animal correspond to respectively 0,32 mT, 0,48 mT and 0,65 mT in humans.

All the rats in the exposure groups were exposed to 50 Hz ELF-MFs for 4 hours/day, 5 days per week for 30 days, and the exposures were applied between 9.00 am and 1.00 pm. During the exposure, 3 rats were placed in plexiglass cages and then positioned in the center of the Helmholtz coil at a controlled temperature (230C), 65–70% humidity, 12 hours light and 12 hours dark cycle and fed with standard food and water. All the rats were checked twice a day in respect to any changes in health. No restrictions were applied to the animals. During the study, all the rats were fed with standard chow and water ad libitum.

Anaesthesia with Ketamine (50 mg/kg) and Xylazine (5 mg/kg) was administered as intramuscular injections to all of the rats at the end of the last exposure, and all the animals were then sacrificed. Brain tissue samples were taken and frozen in liquid nitrogen and kept at -80°C until electron microscopy and histopathological examinations were performed.

Exposure systems

The ELF-EMF system was developed in Gazi University Faculty of Medicine, Biophysics Laboratory, and it has been described in detail in our previous study (12). EMF (1 mT, 1.5 mT, 2 mT) was produced in the center of an exposure system by means of a circular Helmholtz coils system. This system consisted of a combination of two parallel horizontal flat circular coils 42.75 cm in diameter with a common axis and 21.375 cm apart. Each coil had 154 turns and was constructed of insulated copper wires. EMFs were measured by a NARDA EFA 300 ELF EMFAnalyzer and a related probe at the center of the Helmholtz coils. These measurements were confirmed with a Gaussmeter (Yokogawa, Tokyo, Japan). The environment geomagnetic field in the measurement area was recorded as 0.04 μT.

Histopathologic Evaluation

Tissues were washed overnight in running water to remove the formalin. After routine pathological tissue monitoring, the samples were passed through graded alcohol (50%, 75%, 96%, 100%) and xylol series, and then were embedded in paraffin blocks. Paraffin sections in 5 μm thickness were prepared by Leica RM 2125 RT and the first, third and tenth sections were included in the slides. The preparations were passed through alcohol and xylol

series and were stained with hematoxylin-eosin (HE). All samples were examined under high-resolution light microscopy (Olympus DP-73 camera, Olympus BX53-DIC microscope; Tokyo, Japan). The neurodegeneration parameters evaluated included congestion, Purkinje cells with pyknotic nuclei, granular cells with pyknotic nuclei, neuronophagia, and gliosis. All changes detected in tissue structures were noted and scored according to the previously described grading systems as: 0= none, 1= mild, 2= moderate, and 3= severe (13).

Analysis of Apoptosis in the Brain Tissue

Immunohistochemical procedures

Caspase-3

Immunohistochemical staining was performed with the streptavidin-biotin-peroxidase complex (ABC) method according to the protocol of primary antibodies. Sections of 5μm thickness were taken from the previously prepared paraffin blocks onto the slides with poly-L-lysine and deparaffinized and dehydrated with alcohol and xylol. The sections were treated with citrate buffer (Ph 6.0) in a microwave oven (700 W) for 20 minutes to recover the antigen from the tissues. Then, in order to suppress the peroxidase activity, they were placed in a humid chamber in 0.3% H_2O_2 at room temperature for 15 minutes. Before applying the primary antibody, the tissues were incubated for 20 minutes with 5% normal goat serum for protein suppression. The sections were then incubated with Caspase-3 (1:200, Invitrogen, PA5-16335) primary antibody for 1 h at room temperature. The sections were washed with phosphate buffered saline (PBS) and incubated for 30 minutes in the secondary antibody, and then AEC (3-Amino 9-Ethyl Carbasole, Dako, Glostrup, Denmark) and were chromatographed for 10 minutes to ensure the visibility of the reaction. Hematoxylin was used for background contrast staining. Finally, the sections were passed through alcohol and xylol and closed with entellan.

To assess the caspase immunohistochemistry, 10 fields were chosen randomly and the cytoplasmic staining intensities in the cells were globally scored as 0 for no staining, 1 as weak, 2 as moderate, or 3 as strong staining. In each field, at each intensity, the total number of cells and the number of cells stained were counted. Staining indexes were calculated as follows based on the percentages of the stained nuclei for these three markers: negative: 0 (<1% positive); weak: 1 (1-25% positive); intermediate: 2 (>25–75% positive); and strong: 3 (>75% positive).

TUNEL assay

TUNEL (Terminal Deoxynucleotidyl Transferase mediated Deoxyuridine Triphosphate-dUTP Nick end Labeling) assays were performed according to the manufacturer's catalog procedure (In situ Cell Death Detection Kit, POD, Roche, Germany) to determine apoptosis-related DNA breaks in the rat brain cells. Routine deparaffinization and dehydration with alcohol and xylol were applied to standard 5-μm thick tissue sections on the poly-L-lysine-coated glass slides. Subsequently, sections were incubated for 15 minutes at room temperature in 0.3% H_2O_2 to suppress peroxidase activity. Sections were washed with PBS and incubated with Protein K (20 mg/ml; Roche, Mannheim, Germany) at room temperature and in a humid chamber for 15 minutes. Washed sections were then incubated for 1 h at 37°C in a dark environment in freshly prepared 50 μl of TUNEL reaction mixture (TdT & dUTP) solution. The sections were then coated with 50 μl of anti-fluorescein antibody conjugate POD enzyme homogeneously and incubated for 30 minutes at 37°C. Finally, the washed sections were treated with AEC (3-Amino 9-Ethyl Carbasole, Dako, Glostrup, Denmark) for 10 minutes and then covered with a slide after staining with hematoxylin for background contrast. Immunohistochemical and TUNEL assay scoring were evaluated according to previous studies (14-16).

In the TUNEL assay, which stains all the nuclei, 10 randomly chosen areas under the microscope were used. In each area of observation, the total number of nuclei and the highlighted nuclei were counted. Staining indexes were calculated as follows based on the percentages of the stained nuclei for these three markers: negative: 0 (<1% positive); weak 1 (1-25% positive); intermediate: 2 (>25–75% positive); and strong: 3 (>75% positive).

Detection of Trace Elements in the Brain

Reagents

All aqueous solutions were prepared with deionized water obtained using an ultra-pure water system (Aqua Nova Hepta Distillated, resistivity 0.34 MΩ-cm, Kristianstad, Sweden). HNO_3 (65%) and H_2O_2 (30%) from Merck supra-pure grade (Darmstadt, Germany) were used for digestion of the samples and dilution. Plastic bottles, autosampler cups, Teflon vessels, vials for collecting samples, and glassware were cleaned by soaking in HNO_3 (10% v/v) for a day, rinsing four times with ultra-pure water, and drying in an oven at 40°C. All prepared solutions were stored in high-density polypropylene bottles. Stock standard solutions of analytes (1 g/L each) were obtained from Merck. Mixed standard solutions were freshly prepared by diluting the stock standard solutions to the desired calibration ranges in 0.2% HNO_3.

Apparatus

An Inductively Coupled Plasma Mass Spectrometry (ICP-MS) method with a Thermo Scientific ICAP Q Series (Bremen, Germany) was used for element

analysis. Digestion was carried out using Milestone Ethos Up, High-Performance Microwave Digestion System (Serisole, Italy).

Analytical procedures

The samples were analyzed using an in-house validated and accredited method based on ICP-MS using microwave acid digestion. Briefly, 30 mg tissue samples were weighed and then the samples were dried using a magnetic mixer with a heating plate. The dried samples were transferred into a Teflon vessel and digested with 3 mL 65% HNO_3 and 1 mL 30% H_2O_2. Digestion conditions for the microwave system were applied as 15 minutes at 200°C rising (max power), 15 min at 200°C waiting (max power), and 15 min cooling to 80°C. After the cooling process, the total volume of the digested samples were made up to 10.0 mL with 0.2% HNO_3. Digested samples were introduced to ICP-MS for element determination. The operation parameters for the investigated elements were set as recommended by the manufacturer (**Table 1**).

Table 1. Instrument operating parameters for ICP-MS.

Parameter	Value
Peristaltic pump speed	40 rpm
Pump tubing	orange/green tubing for both carrier (sample) and internal standard
Nebulizer	PFA-ST
Interface cones	Nickel
RF Power	1550 W
Cool gas flow	14 L/min
Auxiliary gas flow	0.8 L/min
Nebulizer gas flow	0.97 L/min
Number of sweeps	20
Dwell Times	0.001 - 0.02 ms
Replicates per analysis	3

A mixed standard solution of Mg, Ca, Cu, Zn, and Se was used for calibration procedures (**Table 2**). The investigated element levels in the brain samples were expressed as µg/g for Mg, Ca, Cu, and Zn and as ng/g for Se (**Table 3**).

Statistical Analyses

Data obtained in the study were analyzed statistically using the Statistical Package for Social Sciences (SPSS) v.15.0 software (SPSS Inc, Chicago, IL, USA). Mean and standard deviation values were calculated. Data were analyzed using the Kruskal-Wallis test, One-way analysis of variance (ANOVA) and non-parametric tests (Mann Whitney U test). Bonferroni correction was used for the Kruskal Wallis and Mann Whitney U-test post hoc. Descriptive statistics were used to determine the continuous variables and frequency distributions for categorical variables. In all analyses, a value of $p<0.05$ was considered statistically significant.

RESULTS

Histopathological Results

The percentage of histopathological grading of neurodegeneration parameters including congestion, Purkinje cells with pycnotic nuclei, granular cells with pycnotic nuclei, neuronophagia, and gliosis are presented on the graphs (**Figure 1**). The congestion rate was significantly higher in groups 2 (1mT), 3 (1.5 mT) and 4 (2 mT) compared to the control group ($p < 0.01$). The levels of Purkinje cells with pycnotic nuclei, granular cells with pycnotic nuclei and neuronophagia were similar in groups 1(control) and 2 (1 mT) ($p > 0.05$) while they were significantly higher in groups 3 (1.5 mT) and 4 (2 mT) ($p < 0.01$). The histopathological grades were increased as the ELF-EMF dose exposure increased (**Figure 1**).

Table 2. Concentrations of elements used for calibration procedures.

	Mg (mg/L) concentration	Ca (mg/L) concentration	Cu (mg/L) concentration	Zn (mg/L) concentration	Se (µg/L) concentration
Calibration Blank	0.0	0.00	0	0	0
Standard 1	0.4	0.25	1	2	20
Standard 2	0.6	0.50	2	4	40
Standard 3	0.8	1.00	4	8	60
Standard 4	1.2	2.00	8	16	80
Standard 5	1.5	4.00	16	32	100
Correlation Coefficient	0.996	0.998	0.998	0.999	0.999

Table 3: The effects of ELF-EMF exposure on Mg, Ca, Cu, Zn and Se levels in the rat brains

	Group 1 (Control) (n=8)	Group 2 (10G) (n=9)	Group 3 (15G) (n=9)	Group 4 (20G) (n=9)
Tissue Dilution (mg/10ml)	63.0±23.88	44.62±20.17	53.75±19.17	56.58±16.29
Mg (µg/g)	446.51±237.88	580.56±67.42	456.61±84.76	674.25±345.17
Ca (µg/g)	278.19±110.04	490.16±179.91	298.53±130.54	628.58±210.98
Cu (µg/g)	8.47±3.95	15.65±13.1	10.38±3.74	11.14±5.76
Zn (µg/g)	14.46±10.68	18.69±4.0	17.74±7.04	19.53±8.19
Se (µg/g)	293.40±98.46	402.13±167.12	327.59±110.84	350.98±170.52

For Mg, Cu, Zn, Se, $p>0.05$ for all groups. For Ca, Group 1-2 p=0.011, Group 1-3 p=0.963, Group 1-4 p=0.002, Group 2-3 p=0.011, Group 2-4 p=0.190, Group 3-4 p=0.008

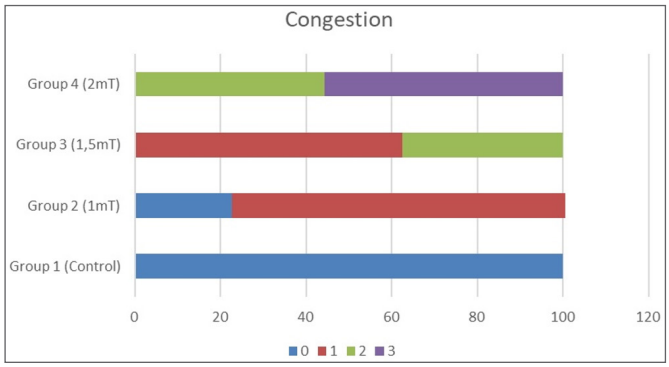

Figure 1a. The level of congestion of the groups

Group 1-2 p=0.002, Group 1-3 p<0.001, Group 1,4 p<0.001, Group 2-3 p=0.074, Group 2-4 p<0.001, Group 3-4 p=0.002,

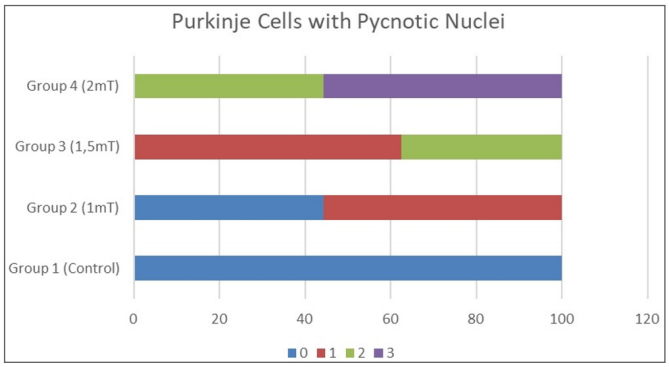

Figure 1b: The levels of Purkinje cells with pycnotic nuclei of the groups

Group 1-2 p=0.059, Group 1-3 p<0.001, Group 1-4 p<0.001, Group 2-3 p=0.021, Group 2-4 p<0.001, Group 3-4 p=0.002.

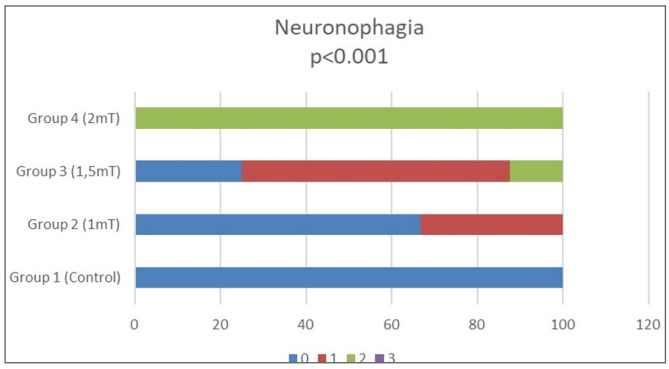

Figure 1c: The level of granular cells with pycnotic nuclei of the groups

Group 1-2 p=0.059, Group 1-3 p<0.001, Group 1-4 p<0.001, Group 2-3 p=0.046, Group 2-4 p<0.001, Group 3-4 p=0.004,

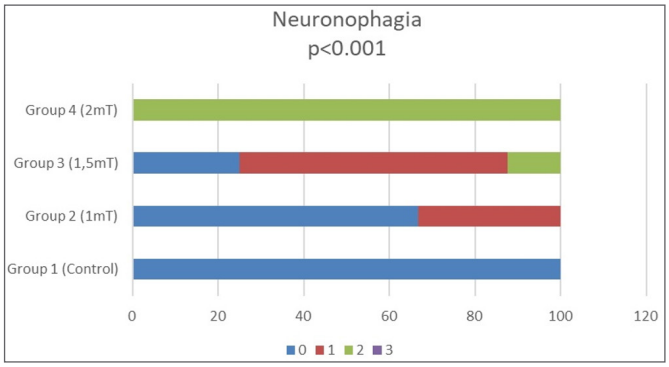

Figure 1d: The level of the neuronophagia of the groups

Group 1-2 p=0.277, Group 1-3 p=0.01, Group 1-4 p<0.001, Group 2-3 p=0.114, Group 2-4 p<0.001, Group 3-4 p=0.001,

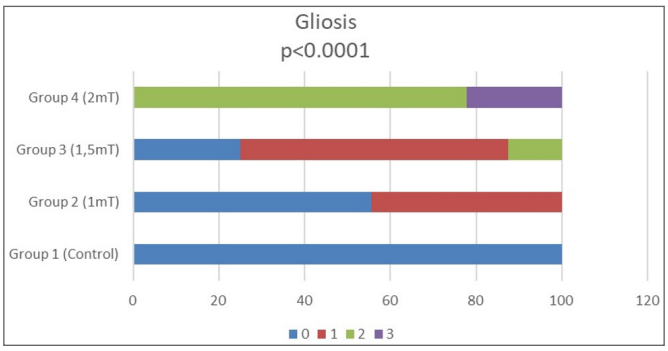

Figure 1e: The gliosis level of the groups

Group 1-2 p=0.139, Group 1-3 p=0.01, Group 1-4 p<0.001, Group 2-3 p=0.236, Group 2-4 p<0.001, Group 3-4 p=0.001,

Figure 1: The percentage of histopathological grading of neurodegeneration parameters were shown on the graphs. The neurodegeneration parameters of the histopathological analysis of brain tissues with 0 indicating no staining, 1 weak, 2 moderate, and 3 strong staining.

Immunohistochemical analysis

The immunohistochemical analysis including the percentage analysis of caspase-3 and TUNEL positivity are presented on the graphs for all groups, with 0 for no staining, 1 as weak, 2 as moderate, or 3 as strong staining (**Figure 2**). Caspase-3 positivity was statistically higher in groups 3 (1.5mT) and 4 (2mT) than in group 1 (control) and group 2 (1mT) and TUNEL positivity was statistically higher in group 2 (1mT), group 3 (1.5mT) and 4 (2mT) than in group 1 (control) (**Figure 2**). Caspase-3 and TUNEL positivity was determined to increase as the ELF-EMF dose exposure increased.

Histopathological sections of brain tissues of the groups are shown in **Figure 3** (a, b, c, d showing HE; e, f, g, h showing Caspase-3 staining and i, j, k, l showing TUNEL staining)

Brain Mg, Ca, Cu, Zn, and Se Levels

The Mg, Ca, Cu, Zn, and Se levels in the brain samples are shown in **Table 3**. There was a significant increase in Ca levels in Group 2 (1mT), Group 3 (1.5mT), and Group 4 (2mT) than in the control group. Brain Ca levels increased as the ELF-EMF dose exposure increased. There were no significant differences between the control and ELF-EMF-exposed groups in terms of Mg, Cu, Zn, and Se levels.

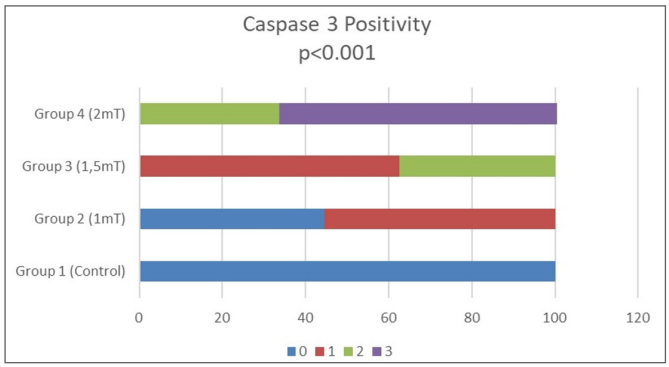

Figure 2a: The caspase 3 positivity of the groups

Group 1-2 p=0.059, Group 1-3 p<0.001, Group 1-4 p<0.001, Group 2-3 p=0.01, Group 2-4 p<0.001, Group 3-4 p=0.001

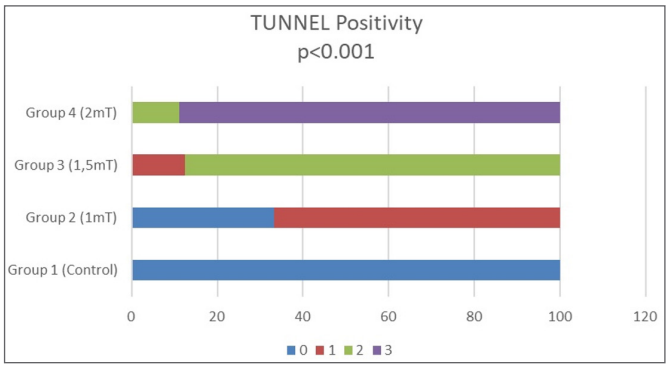

Figure 2b: The TUNNEL Positivity of the groups

Group 1-2 p=0.021, Group 1-3 p<0.001, Group 1-4 p<0.001, Group 2-3 p=0.001, Group 2-4 p<0.001, Group 3-4 p<0.001,

Figure 2: Immunohistochemical procedures; caspase 3 and TUNNEL positivity.

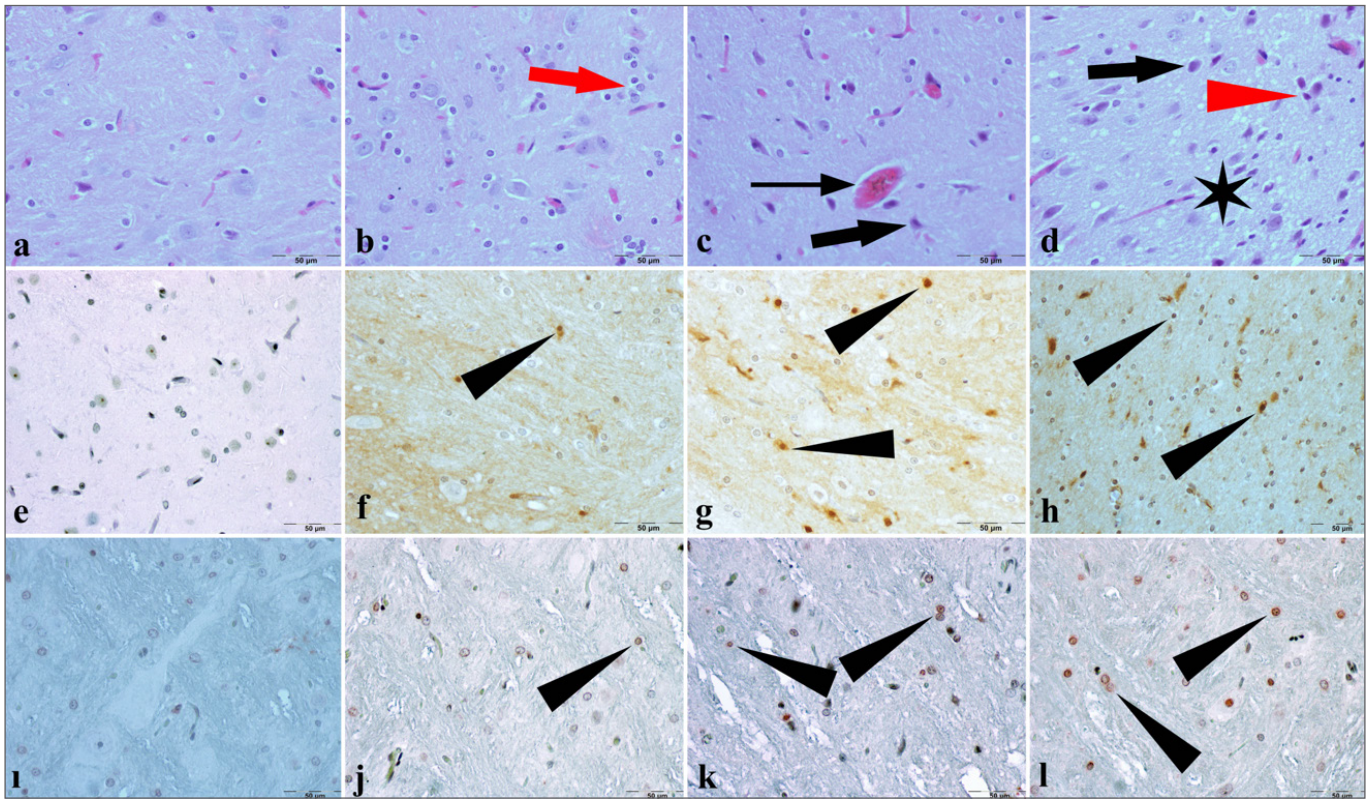

Figure 3: Representative photomicrographs for all groups stained with HE and immunohistochemistry.
a, e, i: control (group 1), b, f, j : group 2 (10G), c, g, k : group 3 (15G), d, h, l : group 4 (20G)
(a), (e) : immunonegative
(b): red arrow shows gliosis
(c): thin arrow shows congestion, thick arrow shows picnosis
(d): arrow shows picnosis, red arrowhead shows neuronophagia, star shows spongiosis (demyelination)
(f), (g), (h), (j), (k), (l): black arrowheads show immunopositive apoptosis
a, b, c, d showing HE, e, f, g, h showing Caspase-3 staining and i, j, k, l showing TUNEL staining. Scale bar: 50 µm.

DISCUSSION

The results of this study demonstrated that different levels of exposure to ELF-EMF doses applied 4 hours a day for 30 days caused increases in Ca levels and increased apoptosis in the rat brain. As the applied ELF-EMF levels increased, the neurodegeneration levels, apoptosis levels and Ca levels in the brain tissues also increased. But there was no significant effect in terms of Mg, Cu, Zn, and Se levels in the rat brain.

The caspases are essential molecules of cellular processes such as inflammation, proliferation, and differentiation, and they can be activated both by intrinsic and extrinsic pathways (17). The TUNEL assay method has been utilized to show the apoptosis-related DNA strand breaks produced by DNA fragmentation (19). Yumusak et al. (18) reported that caspase-3 and TUNEL levels were significantly increased in stressed cells, which confirmed the pathological mechanism underlying apoptosis. In the current study, considering the significant increase

in TUNEL and caspase-3 levels, DNA breaks and damage and increased apoptosis were found in cells exposed to ELF-EMF. It was shown that ELF-EMF caused neurodegeneration in rat brain tissue, and this neurodegeneration increased in severity as the ELF-EMF dose increased. Caspase-3 and TUNEL levels increased as the ELF-EMF dose increased, indicating apoptosis.

Apoptosis plays a vital role in normal tissue homeostasis throughout the life of multicellular organisms, starting with embryogenesis. There are conflicting results in studies about the effects of ELF-EMF on apoptosis in cells. Basille et al. (19) reported that 50 Hz ELF-EMF decreased the rate of apoptosis in melanoma cells by increasing the levels of anti-apoptotic factors (BAG3). Kurian et al. (20) showed that 60 Hz. ELF-EMF decreased the rate of apoptosis in rat myocardial cultures by increasing the anti-apoptotic protein (Bcl-2) levels, leading to a decrease in caspase-3 activity. In a study by Kim et al. (21), it was reported that in TUNEL staining, germ cells showed a significantly higher apoptotic rate in exposed mice than sham controls and in another study (22), apoptotic cells were reported to increase as the duration and dose increased.

ELF-EMF can cause various biochemical changes in cell membranes and tissues by causing changes in ion permeability. Various animal experiments have investigated how trace elements are affected by ELF-EMF in the brain and various organs. In one of these studies, Erdem et al. (9) applied intermittent or continuous 50 Hz and 1.5 mT ELF-EMF to guinea pigs and found an increase in Mg levels in the brain, but no difference in Cu, Zn, and Ca levels. In another study, Ülkü et al. (23) reported that exposure of 500 μT ELF-EMF for 10 months at 2 hours per day caused a decrease in Ca, Mg, and Zn levels in the ribs of rats. Canseven et al. (10) showed that when guinea pigs were exposed to ELF-EMF at 50 Hz, 2mT, 5 days, 4 hours a day, Ca concentration increased in the brain tissue and the plasma. Gmitrova et al. (24) reported that when guinea pigs were exposed to ELF-EMF at 50 Hz, 3 mT, 5-6 days, 1 hour a day, the Ca concentration increased and the Mg concentration decreased in the brain. In these studies, the effect of EMF can be seen to vary according to the frequency and amplitude of the EMF, and properties of the applied areas. These differences in results may be due to experimental parameters, exposure period, application period during the day, and analysis methods (25). In the current study, it was observed that the Ca concentration increased in the brain tissue of the groups exposed to 50 Hz, 1 mT, and 2mT ELF-EMF for 4 hours a day for 30 days, but even though there was an increase in Mg, Cu, Zn, and Se levels, they did not reach a statistically significant level. It can be considered that these ELF-EMF levels may cause changes in the amounts of trace elements in the brain tissue, but they did not reach statistically significant levels due to the small number of rats in the groups. As far as can be observed from other studies and the current study, it can be speculated that Ca can cause an imbalance in the element composition in the tissues through Ca channels. This may be a risk factor that can cause impaired brain function.

Ca ion is involved in various immunological, endocrinological, and neurological events. Ionic balance is vital, especially in the microenvironment of the central nervous system. Calcium, a key mediator of intracellular signaling and an important factor in determining cell fate, is influenced by EMF. ELF-EMFs have been reported to increase the expression of presynaptic calcium channels in the presynaptic terminal which promotes the release of synaptic vesicles (26). Disruption in intracellular homeostasis of Ca ion is also the first step of lethal injury caused by acute oxidative stress. Ca ions are also very important in the modulation of the effects of the magnetic field in cells. It is thought that magnetic field exposure can cause free ion movement, especially Ca movement, and oscillations, and the occurrence of fluctuations in this normal ionic balance changes normal cell behavior and cell membrane behavior (27). Alterations in Ca homeostasis affect many cellular processes, including apoptosis. Sheikh et al. (28) reported that perturbation in intracellular Ca concentrations, especially increased mitochondrial Ca levels, is responsible for apoptotic triggering. Stratton et al. (29) reported that pulsed ELF-EMF causes transient plasma membrane damage in human monocytic leukemia cells, which leads to calcium influx, and as a result, it increases the tendency to apoptosis by increasing microvesicle release. The changes in Ca level can be considered to have contributed to the neurodegeneration and increased apoptosis that was determined histopathologically in this study.

The mammal brain is protected from harmful molecules by the blood-brain barrier. There is evidence that EMF exposure increases the permeability of the blood-brain barrier (30). However, while in one study it was reported that ELF-EMF had an effect on the blood-brain barrier in diabetic rats and had no effect on an intact barrier, in another study (32), it was observed that the amount of Ca was increased in the cerebrospinal fluids of cows exposed to 60 Hz, 30μT ELF-EMF, which could be associated with increased permeability in the blood-brain barrier. Similarly, in the current study, it was thought that the increased Ca levels in the rat brain tissue may be associated with increased blood-brain barrier permeability.

Lai et al. (32) found that a magnetic field of 60 Hz. for 2 hours at 0.1-0.5 mT caused single- and double-strand breaks in DNA, and the severity of these breakages increased as the exposure time increased. Acute

magnetic field exposure also increases apoptosis and necrosis in rat brain cells. Akdağ et al. (33) reported that for 10 months and 2 hours per day, 100-500 μT did not increase apoptosis, evaluated with active-caspase-3 immunohistochemical staining, but it created toxic effects in the rat brain by increasing oxidative stress and reducing the antioxidant defense system. In addition to these controversial studies on oxidative stress and apoptosis, it is necessary to draw attention to two more studies that investigated why ELF-EMF increases apoptosis in some cells and reduces or does not affect others. One of these studies was conducted by Oda et al., (34) investigating the effect of 50 Hz ELF-EMF on immature cerebellar granule neurons, and it was seen to have prevented apoptosis and increased survivability. It was concluded that ELF-EMF could be a potential tool by which neuronal death or survival could be manipulated. In another study conducted by Falone et al., (35) it was stated that a 50 Hz magnetic field could be a risk factor for oxidative stress-based nervous system pathologies by weakening antioxidant defense mechanisms in the aged rat brain. It is an interesting finding that ELF-EMF protects immature neurons from apoptosis while leading mature neurons to apoptosis. Furthermore, in immature and developing neurons, Ca has a mainly apo-protective role, whereas in fully mature and established neurons, Ca plays an apo-inducing role (36). In the light of these pieces of information, the use of mature rats in the current study may explain their predisposition to apoptosis and neurodegeneration in the presence of high Ca level caused by the applied ELF-EMF. However, a limitation of this study is that oxidative stress was not evaluated in the rat brain tissue.

CONCLUSION

The results of this study showed that ELF-EMF exposure caused neurodegeneration in rat brain tissue, increased apoptosis, and increased Ca concentration. Although it is not possible to adapt the results of this study directly to humans, it can be considered that these changes may cause various biological damage, especially cancer, in healthy tissues and measures should be taken to minimize ELF-EMF exposure in daily life in terms of protecting public health.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of University of Health Sciences Ankara Training and Research Hospital The Laboratory Animal Care Ethics Committee (Date: 19.03.2020, Decision No:609)

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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