

Effect of electromagnetic fields and antioxidants on the trace element content of rat teeth

Mehmet Sinan Dogan¹

Mehmet Cihan Yavas²

Yasemin Yavuz³

Sait Erdogan⁴

İsmail Yener⁴

İbrahim Simsek¹

Zeki Akkus⁵

Veysel Eratilla⁶

Abdulsamet Tanik⁷

Mehmet Zulkuf Akdag²

¹Department of Pediatric Dentistry, Faculty of Dentistry, Harran University, Şanlıurfa, ²Department of Biophysics, Faculty of Medicine, ³Department of Restorative Dentistry, Faculty of Dentistry, ⁴Department of Chemistry, Faculty of Science, ⁵Department of Biostatistics, Faculty of Medicine, ⁶Department of Prosthodontics, ⁷Department of Periodontology, Faculty of Dentistry, Dicle University, Diyarbakir, Turkey

Abstract: The purpose of this study was to examine the possible effect of extremely low-frequency electromagnetic fields (ELF-EMFs), from a high-voltage source, on rat teeth in terms of changes in trace elements (TEs) and the effect of antioxidants (melatonin [MLT] and *Ganoderma lucidum* [GL]) in counteracting these effects. We used adult male Wistar albino rats with a mean weight of 250–300 g and divided the rats into eight groups. The groups were subjected to an ELF-EMF that was applied with a high-voltage line for 8 hours/day for 26 days (Groups I, II, and III) or 52 days (Groups V, VI, and VII). Groups IV and VIII were the 26- and 52-day control/sham groups, respectively. Groups II and VI were treated with GL, and Groups III and VII were treated with MLT. MLT and GL were administered daily based on the weight of the animals and appropriate standards. At the end of the study, the rats were euthanized, and their anterior teeth were extracted. The teeth were preserved in pure water before evaluating the major TEs. At the end of the study, TE concentrations (in mg/kg) were assessed in the control and test groups. Compared with Group V, statistically significant differences in the concentrations of zinc (Zn) and strontium (Sr) were found for Group VII (ELF-EMF + MLT) ($P < 0.05$). Therefore, ELF-EMF exposure can change the content of certain TEs in teeth and, after administering MLT and GL, the values of some of the TEs return to normal.

Keywords: ELF-EMF, tooth, trace element, melatonin, *Ganoderma lucidum*

Introduction

Recently, in both the workplace and the home, the usage of devices creating low- and medium-frequency electromagnetic fields (EMFs) has increased.¹ EMF causes harmful effects on human health. Exposure to EMF affects neural networks, body weight, tissue morphology and histology, biochemical parameters of the blood, hormones, the immune system, and the tissue repair system.² The modifications caused by EMF to cells and molecules depend on the length of the exposure, penetration, and the healing ability of the tissue.³ The EMFs created in home environments also have some negative effects such as leukemia and tumor formation in the central neural system.⁴

It has been determined that low-frequency EMF affects the cellular antioxidant defense mechanisms, which increases oxidative damage and causes DNA damage, thereby resulting in a carcinogenic effect.^{5–7} In addition, exposure to EMFs caused by mobile phones is reported to lead to lack of attention, damage in the inner ear, reduction of the speed of reflexes, blurred vision, and headaches.⁸ It is reported that high-frequency EMF has a genotoxic impact on tissues.⁹

Exposure to EMFs depresses melatonin (MLT; *N*-acetyl-5-methoxytryptamine) production in the pineal gland. MLT is an endogenous hormone that is released into the blood from the pineal gland.¹⁰ It stimulates the release of antioxidant enzymes and releases free radicals that are formed in the body.¹¹ Reductions in MLT production

Correspondence: Mehmet Sinan Dogan
Department of Pediatric Dentistry,
Faculty of Dentistry, Harran University,
Şanlıurfa 63300, Turkey
Email dtlider@hotmail.com

increase the risk of cancer.^{12–14} The level of serum MLT is reflected in the level of MLT in the saliva. The level of MLT in the saliva is one-quarter of the level of MLT in the body.¹¹ In addition, a type of fungi known as *Ganoderma lucidum* (GL) is effective for the prevention of free radical production and for the treatment of hypertension, diabetes, hepatitis, cancer, and human immunodeficiency virus (HIV) infections.¹⁵

Studies conducted on animals have demonstrated a relationship between exposure to EMFs, free radical production, and cancer formation.¹⁰ It has been reported that free radical production and tumor formation decrease when MLT is given to animals exposed to EMF under experimental conditions.¹⁰

EMFs also affect the balance of liquids and electrolytes and the concentration of trace elements (TEs).¹⁶ TEs play an important role in human health. The lack or abundance of TEs can have toxic effects.¹⁷ Measuring TE concentrations using tissue samples is critical in order to deduce these toxic effects and subsequently correct them. Generally, in TE studies, tissue samples such as blood, urine, tooth, nail, and hair are used.

In addition, teeth are reported to be a suitable indicator of overall TE concentrations.^{17,18} TEs are found at different concentrations in the tooth enamel. Changes in the density of some TEs affect the health of teeth and general human health. Some elements may cause tooth decay. The relationship between the concentrations of elements such as calcium (Ca), zinc (Zn), phosphorus (P), and magnesium (Mg) in the structure of decaying teeth has been reported.¹⁷ Fluorine (F) and phosphorus (P) are elements that prevent tooth decay, whereas selenium (Se), magnesium (Mg), cadmium (Cd), platinum (Pt), lead (Pb), and silicon (Si) can cause tooth decay.¹⁷

Mg is a key element for the functioning of important biological polyphosphate compounds, such as ATP, DNA, and RNA. Mg is also used in the mineralization of teeth and bones. Ca²⁺ is a key factor for neurotransmitter secretion, oxidative stress, and apoptosis. Zn is necessary for DNA synthesis, RNA transcription, cell division, and cell activation. Copper (Cu) is an essential component of the respiratory enzyme complex, cytochrome c oxidase. Zn and Cu play protective roles against oxidation by acting as cofactors for antioxidant enzymes. Iron (Fe) plays an essential role by combining with oxygen molecules in hemoglobin and myoglobin. Se is found combined with several antioxidant enzymes such as glutathione peroxidase and thioredoxin reductase. Manganese (Mn) is necessary for the regulation of blood sugar and cellular energy levels, immune system

functioning, bone growth, defense against free radicals, and blood coagulation (along with vitamin K).¹⁹

The impact of EMFs on TEs in enamel and dentin can be explained by the changes that they induce in chemical binding exhibited by TEs.²⁰ The purpose of this study was to examine the impact of extremely low-frequency (ELF) EMFs, from a high-voltage source, on TE concentrations in rat teeth. In addition, the impact of MLT and GL on the unwanted effects of the EMFs was also evaluated.

Materials and methods

This study was performed at the Prof Dr Sabahattin Payzin Health Sciences Research and Application Center at Dicle University, Diyarbakır, Turkey. All animal procedures were conducted in accordance with the Principles of Laboratory Animal Care and the rules of the Scientific and Ethics Committee of Dicle University Health Research Center (ethics committee approval no: 2013/13).

Adult male Wistar albino rats with a mean weight of 250–300 g were used in this study. After a 1-week adaptation period, the rats were randomly divided into eight groups. These groups were treated as follows: Group I: 26 days of high-voltage ELF-EMF exposure; Group II: 26 days of high-voltage ELF-EMF exposure + GL; Group III: 26 days of high-voltage ELF-EMF exposure + MLT; Group IV: control/sham group for 26 days; Group V: 52 days of high-voltage ELF-EMF exposure; Group VI: 52 days of high-voltage ELF-EMF exposure + GL; Group VII: 52 days of high-voltage ELF-EMF exposure + MLT; and Group VIII: control/sham group for 52 days.

To produce the ELF-EMF, two transformers were used, which produced a high-voltage EMF (up to 10 kV was used). For transformer 1, the input was 220 V and the output was 10 kV. For transformer 2, the input was 10 kV and the output was 220 V. The rats in both the 26-day and the 52-day experimental groups (Groups I, II, III, V, VI, and VII) were exposed to ELF-EMF for 8 hours/day. We measured the mean magnetic field intensity (2.48 μ T) and the electric field strength (80.3 V/m) in the Plexiglas cages. The EMF was measured with the aid of a Spectran device NF5035 (AARONIA AG, Strickshaid, Germany), using the 6-minute measurement method (International Commission for Non-Ionizing Radiation Protection [ICNIRP]).

MLT and GL were prepared according to the weight of the animals and appropriate standards. For each rat in the two MLT groups, 10 mg/kg MLT (Merck KGaA, Darmstadt, Germany) was dissolved in pure ethanol and then diluted with distilled water. The MLT was then administered intraperitoneally on a daily basis. For each rat in the two

GL groups, 20 mg/kg GL (Gano Excel Industries Sdn. Bhd., Kedah, Malaysia) was prepared (by dilution with distilled water) and administered by oral gavage. At the end of the study, the rats underwent anesthesia (0.1 cc xylazine + 0.9 cc ketamine per rat). Then, the rats were sacrificed by intracardiac lethal injection and exsanguination euthanasia, and their anterior teeth were extracted. To preserve their quality, the teeth samples were stored at ambient temperature in sealed plastic bags prior to processing.

Microwave digestion

All teeth samples were washed with water and then dried in an oven at 65°C. All the samples weighed between 0.2 g and 0.5 g (according to precise measurement in a Teflon vessel) prior to being digested with 6 mL of concentrated ultrapure nitric acid (65%) and 2 mL of ultrapure hydrogen peroxide (30%). The acid digestion of the teeth samples was performed using a commercial high-pressure laboratory microwave oven (Ethos One; Milestone Srl – Via Fatebenefratelli 1/5, 24010 Sorisole (BG), Italy). For each sample, a one-step microwave program was used, increasing for 15 minutes to 200°C at 1,000 W and maintenance at 200°C and 1,000 W for 10 minutes. Any undissolved material was removed with filtered 25 mm polyethylene syringes.

Instruments

An inductively coupled plasma mass spectrometry (ICP-MS) system (model 7700X; Agilent, Santa Clara, CA, USA) was used for the simultaneous detection of Li, B, Mg, Ca, V, Mn, Fe, Co, Zn, As, Se, strontium (Sr), Ag, Cd, Pb, and P.

Reagents and solutions

The nitric acid (Merck) and hydrogen peroxide (Merck) were analytical-grade reagents. Ultrapure water was used in all the experiments.

Statistics

The between-group differences in the experimental data were compared statistically. For this purpose, Mann–Whitney *U* and Wilcoxon signed-rank tests were used.

Results

At the end of the study, the concentrations (in mg/kg) of Li, B, Mg, Ca, V, Mn, Fe, Co, Zn, As, Se, Sr, Ag, Cd, Pb, and P were obtained from the control and test groups, as shown in Table 1. Compared with the value of other TEs, according to the Mann–Whitney *U* test, the concentrations of Sr and Zn displayed significant differences ($P < 0.05$).

In Group I, the concentrations of Li, B, Zn, As, V, Cd, Pb, and Se increased, and the concentrations of Mg, Fe, Co, Ca, and P decreased. However, in Group V, the concentrations of Li, As, and Se increased, and the concentrations of B, Ca, Mn, Co, V, Sr, Cd, and Pb decreased.

In Group II, GL returned the concentrations of Li, B, Mg, Ca, Fe, Zn, As, P, and Se to the levels in the 26-day control group. In Group III, MLT returned the concentrations of B, Mg, Ca, Fe, Zn, V, Cd, Pb, P, and Se to the levels in the 26-day control group.

In Group VI, GL returned the concentrations of Co, V, and Se to the approximate concentrations in the 52-day control group. In Group VII, MLT returned the concentrations of Li,

Table 1 TE contents in each group at the end of the study

TE (mg/kg)	Group I	Group II	Group III	Group IV	Group V	Group VI	Group VII	Group VIII
⁷ Li	0.2617	0.1695	0.0834	0.1693	0.1735	0.1013	0.1495	0.1443
¹¹ B	0.0417	0.0277	0.0275	0.0264	0.0216	0.0187	0.0166	0.0352
²⁴ Mg	0.6803	0.8939	0.8814	0.8768	0.8505	0.8191	0.7801	0.8464
⁴⁴ Ca	0.4895	0.5342	0.5240	0.5767	0.5436	0.5537	0.5624	0.6148
⁵⁵ Mn	0.0015	0.0018	0.0013	0.0016	0.0013	0.0012	0.0013	0.0016
⁵⁶ Fe	0.1330	0.2026	0.2493	0.2606	0.2645	0.2587	0.2358	0.2518
⁵⁹ Co	0.0061	0.0088	0.0042	0.0074	0.0031	0.0053	0.0065	0.0050
⁶⁶ Zn	0.0682	0.0532	0.0585	0.0550	0.0616	0.0991	0.1775	0.1335
⁷⁵ As	0.0859	0.0402	0.0000	0.0310	0.0215	0.1174	0.0441	0.0094
⁵¹ V	0.1041	0.1001	0.0494	0.0683	0.0325	0.0715	0.0911	0.0765
⁸⁸ Sr	0.1473	0.1215	0.1322	0.1422	0.1632	0.1663	0.1735	0.1816
¹⁰⁷ Ag	0.0013	0.0012	0.0011	0.0011	0.0010	0.0012	0.0011	0.0012
¹¹¹ Cd	0.0042	0.0096	0.0017	0.0013	0.0029	0.0018	0.0038	0.0056
²⁰⁸ Pb	0.1166	0.1688	0.0820	0.0861	0.0810	0.0967	0.1156	0.1258
¹⁵ P	0.6900	0.9770	0.9470	0.8200	0.9130	0.8170	0.8620	0.9840
⁷⁸ Se	0.2016	0.0534	0.0472	0.0411	0.3113	0.1167	0.1120	0.1524

Notes: Group I, 26 days of high-voltage ELF-EMF exposure; Group II, 26 days of high-voltage ELF-EMF exposure + GL; Group III, 26 days of high-voltage ELF-EMF exposure + MLT; Group IV, control/sham group for 26 days; Group V, 52 days of high-voltage ELF-EMF exposure; Group VI, 52 days of high-voltage ELF-EMF exposure + GL; Group VII, 52 days of high-voltage ELF-EMF exposure + MLT; and Group VIII, control/sham group for 52 days.

Abbreviation: TE, trace element.

Ca, V, Cd, Pb, and Se to the approximate concentrations in the 52-day control group. The concentrations of Ag in the various groups changed very little.

Discussion

EMFs affect a wide area, and they can be caused by low and high currents. Due to the development of electromagnetic technology, EMFs are now widely used in various fields, including military applications, medical devices, and security systems. Concerns regarding the hazardous biological effects of EMFs on human health are on the rise.¹⁹

High-frequency magnetic fields affect superficial tissues, whereas it is reported that low-frequency magnetic fields have hazardous impacts on deeper tissues.²¹ The impact of magnetic fields on cellular functions has been examined, and damage to DNA, the production of oxidation products, and effects on intracellular Ca^{2+} signals have been found.²² Exposure to EMFs affects the formation of free radicals and some elements. Lack or abundance of certain metals leads to multiple clinical impacts. A previous study conducted on rats exposed to EMFs found that the Zn plasma concentration increased while the Fe concentration decreased.²² In this study, a statistically significant difference was found in the concentration of Zn in Groups I and V and, after antioxidant usage (MLT or GL), the Zn concentration approximated that in the control group. In addition, it was found that high-voltage ELF-EMFs increased the concentrations of Li, B, and Se and decreased the concentrations of Mg, Ca, and Fe. The values returned to normal after administering an antioxidant (MLT or GL).

Another animal study of EMFs reported that they affect the liquid and electrolyte balance.¹⁶ At the same time, EMFs also affect the concentration of some TEs and the electrolyte balance (Na^+ , K^+ , Ca^{++} , and Mg^{++}). Lack or abundance of TEs also causes toxic effects.¹⁷ There are several TEs in the calcified tissue of the teeth. Measurements showing lack or abundance of TEs provide information about diseases. The causes of decayed/healthy tooth structure can be understood in terms of decreases in matrix elements such as Ca and P and increases in non-matrix elements such as Sr, Na, K, Mg, Zn, and C.^{23,24} In this study, the impact of EMFs and antioxidants on the TE concentrations in rat teeth was examined.

In order to deduce and subsequently correct these impacts, it is important to measure the TE concentrations using a tissue sampling method. Generally, in TE studies, tissue samples such as blood, urine, nail, and hair samples are used. Nevertheless, teeth have also been reported to be suitable indicators of overall TE concentrations, as teeth consist of a diverse range of elements, and the concentrations of these elements are affected by overall TE concentrations in the body.^{17,18}

TEs are found at different concentrations in the tooth enamel. Previous studies have reported the relationship between elements such as Ca, Zn, P, and Mg in decaying teeth.^{21,24} In addition, lack of Zn causes reductions in thrombocyte aggregation, T lymphocyte concentrations, and the levels of phytochrome.²⁵ Zn plays an essential role as a structural, catalytic, and regulatory factor in biological organisms. In addition, Zn ensures that cells are protected from oxidative stress.²⁵ In our study, the level of Zn in the teeth of rats exposed to EMFs for 52 days was lower than the level in the 52-day control group, and the Zn level in the groups that were treated with an antioxidant (MLT or GL) increased only compared to the group exposed to EMFs. In addition, according to Groups VII–VIII, in the teeth of rats exposed to EMFs for 52 days, there was a significant decrease in the level of Zn ($P < 0.05$).

Exposure to EMFs affects the functions of biological systems due to increases in oxidative stress.²⁶ A previous study examined the impact of magnetic field exposure on oxidative stress created by EMFs and identified protective impacts.²⁷ In our study, we found that treatment with an antioxidant (MLT or GL) caused the TE concentrations (that were changed by high-voltage ELF-EMFs) to return to their normal values.

Zn is both an antioxidant and a factor involved in the structure of superoxide dismutase. Oxidative stress (which can increase after repeated infections and surgical operations) creates an increased metabolic demand for antioxidants.^{25,28} For this reason, the Zn concentration in the body decreases.²⁸ In our study, the concentration of Zn increased after 26 days of exposure to high-voltage ELF-EMFs (Group I), and 52 days of exposure to high-voltage ELF-EMFs decreased the concentration of Zn (Group V). In addition, the Zn concentration was found to return to normal after usage of antioxidants (MLT or GL) in Groups II, III, and VII.

Living organisms are constantly being exposed to natural geomagnetic fields ranging between 20 and 70 μT . Previous studies have shown that EMFs increase the concentration of reactive oxygen products and cause pro-inflammatory changes in biological systems. Magnetic field exposure (128 mT for 1 hour/day for 5 days) has been found to decrease the amount of Se in the kidneys, muscles, and brain and decrease glutathione peroxidase activity in the kidneys and muscles.²⁹ In our study, no statistically significant difference was found in terms of the Se levels. However, numerically, the concentration of Se increased in Groups I and V.

In a previous study conducted in rats, TE concentrations in the serum were examined.²² No significant change was found in the ratio of Na, K, Ca, P, and Se in the serum. In contrast, an increase in the Zn ratio and a decrease in the

Fe concentration were found. Lack or abundance of these TEs can lead to clinical impacts.²² In our study, although a statistically significant change was not observed in the level of Fe, in terms of numerical values, the Fe level returned to normal in the group exposed to ELF-EMF for 26 days after treatment with an antioxidant (MLT or GL).

In another study, it was found that serum Na, K, Mg, Ca, Zn, Cu, Fe, Se, and Mn values in rats exposed to electrical fields of 50, 100, and 200 kV/m for 48 hours remained unchanged.¹⁹ A further study conducted on rats examined the impact of a 2.45 GHz Wi-Fi-induced EMF on teeth, and the study found that the development of rat teeth and surrounding tissues was not affected by 2.45 GHz Wi-Fi. In addition, no statistically significant differences were found in the concentrations of Ca, K, Mg, Na, P, or Cd in the rat teeth.³⁰ However, the concentrations of Zn, Ag, and B decreased and the concentrations of Fe and Sr increased.³⁰ In our study, an increase was detected in the concentration of B in rat teeth exposed to ELF-EMF for 26 days (Group I).

It has been reported that Se, Mg, Cd, Pt, and Pb are carcinogenic, while F and K are cariostatic.³¹ In our study, as a result of exposure to 26 days of high-voltage ELF-EMFs, an increase was found in the concentrations of Se, Cd, and Pb and a decrease was detected in the concentration of K (compared to the 26-day control/sham group).

Conclusion

The data showed that exposure to high-voltage ELF-EMFs causes changes in TE concentrations in rat teeth. Increases and decreases in TE concentrations can have toxic effects on human health. There were changes in the values of cariogenic elements such as Se, Mg, Cd, and Pb. ELF-EMFs increased the values of Se, Cd, Pb, and Mn. The use of antioxidants, especially MLT, was found to reduce these values to their normal levels. However, we believe that further epidemiological, histological, and chemical studies should be conducted in this area.

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The authors declare that there were no other contributors involved in this work.

Disclosure

The authors report no conflicts of interest in this work.

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