Protective effect of Melatonin and Ganoderma lucidum against the negative effects of extremely low frequency electric and magnetic fields in the periodontal tissues

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Abstract

Aim: The purpose of this study was to determine whether extremely low frequency electric and magnetic field (ELF-EMF) had any detrimental effects on periodontal tissue and to investigate, histologically and immunohistochemically whether melatonin (MLT) and Ganoderma lucidum (GL) had a protective role against these detrimental effects.

Methodology: A total of 56 adults, male Wistar Albino rats were used in this study. Rats were divided into 7 equal groups and exposed to ELF-EMF, which was produced by a high voltage source, 8 hours / day for 26 days. GL (20 mg / kg / day) and MLT (10 mg / kg / day) were administered by the oral gavage and intraperitoneal method.

Results: Fibrous degeneration in the periodontal membrane, inflammatory cell infiltration, connective tissue fiber organization, dilation, and hemorrhage in the blood vessels changes were determined in the periodontal tissue of the rats in groups I and IV. A statistically significant difference was determined in the groups treated with MLT and GL (p<0.05).

Conclusions: It has been determined that ELF-EMF exposure causes histological changes in the periodontal tissue of rats and that MLT and GL may protect against these effects.

Keywords: Extremely low frequency electric and magnetic field, Melatonin, Ganoderma lucidum

Introduction

Extremely low-frequency electromagnetic fields (ELF-EMF) are electromagnetic vibrating fields. They are very important from a public health viewpoint because of the common use of electrical power at 50 or 60 Hz in most countries (1).
relationship between ELF-EMF and an increased rate of some types of cancer(5). The main cause of these contradictory conclusions may emerge from the cellular and molecular alterations made by EMF, which differ with the duration of exposure of the tissue, penetration, the healing regeneration of the tissue, and some exposure parameters(6).

Some authors have also suggested that reactive oxygen species (ROS) can be active in the mechanisms of ELF-EMF effects(7,8). If cellular or plasma ROS levels were altered by exposure to ELF electromagnetic fields and radiofrequency radiation, the levels of oxidative DNA damage, cell death, and apoptotic processes in some tissues would change as well(7,9,10).

An increase in both reactive oxygen and nitrogen species during periodontal disease is responsible for the oxidative damage to periodontal tissues(11). Animal, laboratory, and epidemiological studies have suggested that exposure to ELF-EMF is related to many diseases(12). Tissues in the dental and jaws are also influenced by EMF. Periodontal tissues and the jawbone can also be affected(13). The obtained data is not enough to explain the interaction mechanisms between ELF-MF and oral tissues. The aim of the current study was to determine whether ELF-EMF had any harmful effects on periodontal tissue and examine histologically whether or not MLT and GL had a protective role against these harmful effects. The study focuses on changes in the histological parameters mainly in the periodontal tissue of male mice.

Materials and Methods

Animals and Experimental Procedures

This study was carried out by Prof. Dicle University. Dr. Sabahattin Payzin Health Units Research and Application Center. Experimental study protocol design (Diyarbakir, Turkey) was approved by the Dicle University Animal Ethics Committee (ethics committee approval no: 2013/13). In the study, 56 adult male Wistar Albino rats, each weighing between 250 and 300 gr, were used. After a 1-week adaptation period, the rats were randomly separated into 7 equal groups. The animals were kept in temperature-controlled cages and were exposed to 12-hour light and 12-hour dark cycles; they also had access to food and water. Group I (n = 8): ELF-EMF exposure for 26 days; group II (n = 8): ELF-EMF exposure for 26 days + GL; group III (n = 8): ELF-EMF exposure for 26 days + MLT; group IV (n = 8): ELF-EMF exposure for 52 days; group V (n = 8): ELF-EMF exposure for 52 days + GL; group VI (n = 8): ELF-EMF exposure for 52 days + MLT; group VII: (n = 8), control.

To create ELF-EMF, two transformers were used; each produced high voltage—10 kV (10,000 V). 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 and 5,000 VA. The rats in the 26-day and 52-day experimental groups (groups I, II, III, IV, V, and VI) were exposed to ELF-EMF for 8 hours each day (Figure 1).

Histological Examination

Tissue preparation for light microscopy

After the research, the rats were sacrificed by anesthetic overdose. The periodontal tissue was removed from the rats and the was conserved with neutral buffered 10% formalin solution and decalcified with 5% EDTA. After preservation, samples were directly dehydrated in a graded series of ethanol and then placed in paraffin wax; 4.6 mm thick sections were cut with a microtome (Rotatory Microtome, Leica, RM 2265, Germany) and were mounted on coated slides. The sections were stained with hematoxylin and eosin (H-E).

Immunohistochemical staining

Antigen retrieval was performed in a citrate buffer solution (pH: 6.0) twice—first 7 min, then 5 min—in a microwave at 700 W. Samples were allowed to cool to room temperature for 20 minutes and then washed twice in distilled water for 5 minutes. Endogenous peroxidase activity was blocked for 10 minutes with 0.1% hydrogen peroxide. Primary antibodies were...
injected into the Ultra V block. For 10 minutes prior to administration of Vascular Endothelial Growth Factor, 1/200, Santa Cruz, CA) and then kept overnight. The secondary antibody (Histostain Plus Kit, Invitrogen, Carlsbad, CA) was applied for 20 minutes. The slides were then exposed to streptavidin-peroxidase for 20 minutes. Diaminobenzidine was used as a chromogen.

The control slides were prepared as described above but with the skipping of the primary antibodies. After counterstaining with hematoxylin, the slides were washed in tap water for 5 minutes and washed in distilled water for 2 and 5 minutes; then, the slides were mounted.

### Statistical Analysis

Statistical analysis of the data in our study was done using the SPSS (IBM®; 21.0 Windows, Chicago, USA) statistical program. Histological data; mean (M) and standard deviation (SD). Mann Whitney U test was used to compare the data of the non-normal distributions of the two groups, and the Kruskal Wallis test was used to compare the two groups. The Bonferroni corrected Mann-Whitney U test was performed in comparison to multiple groups. The differences between the groups were considered statistically significant at p<0.05.

### Results

Through histological and immunohistochemical studies, at the end of the study period, it was detected that exposure to ELF-EMF had caused degeneration to the epithelial basal layer cells, dilation of connective tissue vessels and hemorrhage, inflammatory cell infiltration, and connective tissue filamentary organization.

### Histopathological results

As a result of the H-E staining procedure, results were obtained. In group I, degeneration and desquamation were observed in the cells of the basal layer of epithelium; dilatation and hemorrhaging were observed in the connective tissue vessels, and deterioration was observed in the fibrous structures of connective tissue (Fig. 2a). In group II, desquamated cell rashes were observed in the epithelium basal layer; freely distributed erythrocytes, due to minor bleeding, was observed in the vessels, and fibrosis was observed in the connective tissue areas (Fig. 2b). In group III, proliferation was observed in the basal part of the gingival epithelial layer, and protection was observed in the fibrous and cellular structure of connective tissue. The cells and fibers in the periodontium layer were tightly ordered; they were sticking firmly to the dentinal-cement area (Fig. 2c).
In group IV, thinning and degeneration of the dental epithelium in the neck region of the tooth, degeneration and occasional edema in the periodontal area hyalinization fibrous structure, veins dilated and hemorrhagically observed (Fig. 2d). In group V, wide desquamation and pycnotic nucleus cell infiltrate in the gingival epithelium, mononuclear cell infiltrations in the connective tissue area, hemorrhages in the veins between the fibers in the periodontal membrane and erythrocytes in the free state were observed (Fig. 2e). In group VI, regular organization of cells in the basal layer of the gingival epithelium, connective tissue and periodontal membranes with vein dilatation were observed. Hemorrhaging was not observed (Fig. 2f). Group VII was the control group. The cells in the basal layer in the gingival epithelium were observed regularly, and the regular organization of connective tissue and periodontal membrane fibers was observed (Fig. 2g).

Figure 2d. In Group IV, 52 days high voltage (ELF-EMF) application group (H-E staining Bar 100 mm).

Figure 2e. In Group V, Group ELF-EMF + GL applied on day 52 (H-E staining Bar 100 mm).

Figure 2f. In Group VI, Group ELF-EMF + MLT applied on day 26 (H-E staining Bar 100 mm).

Figure 2g. In Group VII, Control group; (H-E staining Bar 100 mm).

**Immunohistochemical results**

As a result of the VEGF immune-staining, the following results were obtained. VEGF-positive expression was observed vascular endothelial cells along the gingival and periodontal membrane in the cervical of the tooth (Fig. 3a). VEGF positive was observed in cell infiltrations under the basal layer; VEGF-positive expression was observed in the endothelial cells of the expanded vessel wall (Figure 3b). Inflammatory cells decreased under gingival epithelium, and VEGF-positive expression was observed in small vessels (Fig. 3c). VEGF expression also increased in the intensive inflammatory cells and in the hypervaried veins in the connective tissue area under the epithelium (Fig. 3d). VEGF-positive cells were observed around the vein wall (Fig. 3e). VEGF-positive cells were observed in the gingival and periodontal membrane (Fig. 3f). VEGF-negative cells were also observed in the gingival and periodontal membrane (Fig. 3g).
The statistical results of the scores in relation to histopathological examinations are shown in Tables 1 and 2. A statistically significant difference was determined among the control group and groups I, II, and III in fibrous degeneration in the periodontal membrane, inflammatory cell infiltration, fiber organization of connective tissue, dilation, and hemorrhage in the blood vessels created as a result of ELF-EMF (p<0.05, Table 1). A statistically significant difference was determined between group I and group III in fibrous degeneration in the periodontal membrane, inflammatory cell infiltration, fiber organization of connective tissue, dilation, and hemorrhage in the blood vessels (p<0.05, Table 1). While a statistically significant difference was determined between group I and group II in fibrous degeneration in the periodontal membrane (p<0.05), no statistically significant difference was determined among inflammatory cell infiltration, fiber organization of connective tissue, dilation, and hemorrhage in the blood vessels (p>0.05, Table 1). A statistically significant difference was determined among the control group and groups IV and V in fibrous degeneration in the periodontal membrane, inflammatory cell infiltration, fiber organization of connective tissue, dilation, and hemorrhage in the blood vessels created as a result of ELF-EMF (p<0.05, Table 2). A statistically significant difference was determined between the control group and Group VI in fibrous degeneration in the periodontal membrane, but no statistically significant difference was determined inflammatory cell infiltration, fiber organization of connective tissue, dilation, and hemorrhage in the
blood vessels (Table 2). A statistically significant difference was determined among the group IV, V, and VI in fibrous degeneration in the periodontal membrane, inflammatory cell infiltration, fiber organization of connective tissue, dilation, and hemorrhage in the blood vessels created as a result of ELF-EMF (p<0.05, Table 2). A statistically significant difference was determined between Groups V and VI in fibrous degeneration in the periodontal membrane, inflammatory cell infiltration, fiber organization of connective tissue, dilation, and hemorrhage in the blood vessels created as a result of ELF-EMF (p<0.05, Table 2).

**Table 1.** Comparison of Histopathological Values of Rats on Day 26

<table>
<thead>
<tr>
<th></th>
<th>Group 1 (mean±SD)</th>
<th>Group 2 (mean±SD)</th>
<th>Group 3 (Control) (mean±SD)</th>
<th>P</th>
<th>P1-2</th>
<th>P1-3</th>
<th>P2-3</th>
<th>P2-7</th>
<th>P3-7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibrous degeneration in the periodontal membrane</td>
<td>3.29±0.76</td>
<td>2.43±0.54</td>
<td>1.71±0.48</td>
<td>0.003*</td>
<td>0.040</td>
<td>0.003*</td>
<td>0.030*</td>
<td>0.001*</td>
<td>0.001*</td>
</tr>
<tr>
<td>Inflammatory cell infiltration</td>
<td>2.88±0.69</td>
<td>2.57±0.53</td>
<td>1.29±0.49</td>
<td>0.002*</td>
<td>0.424</td>
<td>0.028*</td>
<td>0.032*</td>
<td>0.001*</td>
<td>0.001*</td>
</tr>
<tr>
<td>Fiber organization of connective tissue</td>
<td>3.00±0.82</td>
<td>2.29±0.75</td>
<td>1.86±0.69</td>
<td>0.043*</td>
<td>0.133</td>
<td>0.022*</td>
<td>0.266</td>
<td>0.002*</td>
<td>0.001*</td>
</tr>
<tr>
<td>Dilation and hemorrhage in blood vessels</td>
<td>2.86±0.69</td>
<td>2.00±0.58</td>
<td>1.14±0.69</td>
<td>0.003*</td>
<td>0.030</td>
<td>0.003*</td>
<td>0.030*</td>
<td>0.002*</td>
<td>0.001*</td>
</tr>
</tbody>
</table>

*P<0.05, **P<0.001, P; Kruskal Wallis test; P1-2, P1-3, P1-7, P2-3, P2-7 ve P3-7, Mann Whitney U test

**Table 2.** Comparison of Histopathological Values of Rats on Day 52

<table>
<thead>
<tr>
<th></th>
<th>Group 4 (mean±SD)</th>
<th>Group 5 (mean±SD)</th>
<th>Group 6 (mean±SD)</th>
<th>Group 7 (Control) (mean±SD)</th>
<th>P</th>
<th>P4-5</th>
<th>P4-6</th>
<th>P5-4</th>
<th>P5-6</th>
<th>P5-7</th>
<th>P4-7</th>
<th>P5-7</th>
<th>P6-7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibrous degeneration in the periodontal membrane</td>
<td>3.71±0.49</td>
<td>2.71±0.48</td>
<td>1.71±0.49</td>
<td>0.57±0.54</td>
<td>P&lt;0.001**</td>
<td>0.006*</td>
<td>0.001*</td>
<td>0.006*</td>
<td>0.001*</td>
<td>0.001*</td>
<td>0.001*</td>
<td>0.001*</td>
<td>0.005*</td>
</tr>
<tr>
<td>Inflammatory cell infiltration</td>
<td>3.43±0.54</td>
<td>2.29±0.76</td>
<td>1.00±0.58</td>
<td>0.57±0.54</td>
<td>P&lt;0.001**</td>
<td>0.011*</td>
<td>0.001*</td>
<td>0.008*</td>
<td>0.001*</td>
<td>0.003*</td>
<td>0.003*</td>
<td>0.174</td>
<td></td>
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<tr>
<td>Fiber organization of connective tissue</td>
<td>3.29±0.76</td>
<td>2.29±0.76</td>
<td>1.00±0.58</td>
<td>0.43±0.53</td>
<td>P&lt;0.001**</td>
<td>0.036*</td>
<td>0.002*</td>
<td>0.008*</td>
<td>0.001*</td>
<td>0.002*</td>
<td>0.002*</td>
<td>0.081</td>
<td></td>
</tr>
<tr>
<td>Dilation and hemorrhage in blood vessels</td>
<td>3.43±0.54</td>
<td>2.57±0.53</td>
<td>0.86±0.69</td>
<td>0.29±0.49</td>
<td>P&lt;0.001**</td>
<td>0.018*</td>
<td>0.001*</td>
<td>0.002*</td>
<td>0.001*</td>
<td>0.001*</td>
<td>0.001*</td>
<td>0.100</td>
<td></td>
</tr>
</tbody>
</table>

*P<0.05, **P<0.001, P; Kruskal Wallis test; P1-2, P1-3, P1-7, P2-3, P2-7 ve P3-7, Mann Whitney U test
Discussion

The increased use of electric power artificially increased low-frequency electromagnetic fields (ELF-EMF) has resulted in poor results in the home and workplace. Epidemiological studies suggested that the ELF-EMF is related to childhood leukemia, cancer of the nervous system, and cancers in workers. Several studies have shown that immune cells are subject to ELF-EMF changes resulting in molecular and cellular changes in immune system activation(14). Anisian et al. suggested that EMF exposure may cause major changes in gingival tissue and may cause reduction to the integument layers. Therefore, exposure to EMF may cause pathological alterations leading to tooth and gum diseases(15).

In our study, ELF EMF was applied at 26 days according to the control group. There were significant differences in the fibrous degeneration in the periodontal membrane, inflammation, fiber organization of connective tissue, dilation, and hemorrhage in the blood vessels. However, there was also a decrease in values due to applied GL. This shows us that the ELF-EMF causes the cellular changes in the tissues, and when the GL substances are applied, the negative effects of the ELF-EMF are reduced. We think that this is caused by the anti-inflammatory effects of GL. ELF-EMF was applied at 52 days according to the control group. There were significant differences in the fibrous degeneration in the periodontal membrane, inflammation, fiber organization of connective tissue, dilation, and hemorrhage in the blood vessels. However, there was also a decrease in values due to applying MLT and GL. This shows us that the ELF-EMF causes the cellular changes in the tissues, and when MLT and GL substances are applied, the negative effects of ELF-EMF are reduced. We think that this is caused by the anti-inflammatory effects of MLT and GL.

Many types of research has supported the use of magnetic fields to help bone healing, but their application for soft tissue healing is still ambiguous, as some authors have showed that they reduce wound healing duration but increase the strength of scars(16,17). In our study, ELF-EMF was applied at 26 and 52 days according to the control group. There was a significant increase in fiber organization of the connective tissue. Thus, ELF-EMF can reduce the wound healing duration and increase strength of scars.

It has been reported that exposure to EMF formed from 50 Hz has a stimulating effect on cells and tissues, and can, therefore, help in recovery from periodontitis(18). Vianale et al. suggested that ELF-EMF inhibits the production of RANTES, MCP-1, MIP-1a, and IL-8 through the inhibition of the NF-kB signaling pathway. They supposed that ELF-EMF may inhibit inflammatory processes(19). However, in our study, ELF-EMF was determined to have raised the inflammatory cell infiltration.

Miyakoshi suggested that exposure to the magnetic field for 48 hours has no effect on normal human gingival fibroblast growth(20). Soda et al. confirmed that exposure to ELF-EMF (60 Hz, 3mT) significantly increased the collagen synthesis in the cells. The effect was more pronounced than cells cultured for 14 days in long-term cultured cells for 21 days(21). Similar to the results of that study, the current study determined, as a result of the histologic and immunohistochemical staining, that ELF-EMF raised the fibrous degeneration in the periodontal membrane, fiber organization of connective tissue, dilation, and hemorrhage in the blood vessels at 52 days compared with 26 days.

When MLT is administered in the case of inflammation, the cell protector stimulates bone formation as it promotes the production of type 1 collagen; it also has regulatory effects on osteoblast and osteoclast cell activation(22). Dundar et al. suggested that local melatonin application during implant surgery may improve bone implant connection (BIC) (23). In our study, MLT was applied at 52 days according to the ELF-EMF group. There was significant reduction in the fibrous degeneration in the periodontal membrane, inflammation, fiber organization of connective tissue, dilation, and hemorrhage in the blood vessels as compared to the ELF-EMF applied group. This shows us that when MLT substances are applied, the negative effects of ELF-EMF are reduced. This demonstrates effectively support the host immune defense and the anti-inflammatory effect of MLT.

GL has been used in the treatment of various diseases, such as allergy, arthritis, bronchitis, gastric ulcer, hyperglycemia, hypertension, chronic hepatitis, insomnia, nephritis, nerve weakness, inflammation, and cancer(24).

In our study, GL was applied at 26 and 52 days according to the ELF-EMF group. There was a significant reduction in the fibrous degeneration in the periodontal membrane, inflammation, fiber organization of connective tissue, dilation, and hemorrhage in the blood vessels, as compared with the ELF-EMF applied group. This shows us that when GL substances are applied, the negative effects of ELF-EMF are reduced. This demonstrates that GL protects the body and contributes to a long life. It also has anti-inflammatory effects.

Conclusions

According to the present study, exposure to ELF-EMF results in the degeneration of the periodontal structure and damage to important periodontal structures. There have been changes in inflammatory cells and connective tissue, it is important for periodontal and oral health. In periodontal tissues, vessel dilatation change, and hemorrhaging occur, which are very important for the immune system. The use of MLT and GL reduces the damage that can occur in the periodontal tissue, as a result of ELF-EMF exposure. There is a need for further studies on this subject.
Ethical Approval: Ethics committee approval was received for this study from Dicle University (No:2013/13).

Peer-review: Externally peer-reviewed.


Conflict of Interest: No conflict of interest was declared by the authors.

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