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Protective effect of melatonin on the rat lung following exposure to 900-MHz electromagnetic field: a stereological and histopathological study

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Abstract

Long-term use of cell phone emitting electromagnetic field (EMF) has increased concerns regarding histopathological change in vital organs. Our present study was aimed to investigate the effect of 900 megahertz (MHz) EMF on the rat lungs, as well as the possible efficacy of melatonin (MEL) on lung tissues. Fifteen female Wistar albino rats were randomly selected and assigned into five groups as follow: control (CONT), SHAM, EMF, melatonin (MEL) and EMF+MEL group. Subsequently, female rats were then mated, and pregnant rats underwent the experimental application for 21 days. After parturition, 6 pups (2 pups from each mother) were randomly chosen and maintained for 4 weeks. At the end of the 4th week, the lung tissues of all groups were used for the stereological analysis and histopathological examination. We found that the mean volumes of the alveoli, bronchioles and blood vessels were significantly lower in the EMF group compared to the control group ($p<0.05$). In the EMF+MEL group, the mean volumes of the alveoli, bronchioles and blood vessels were significantly increased compared to the EMF group. Our histopathological results showed marked change in the lung tissues. We speculated that exposure to EMF caused the damage to the rat lung tissues, and that MEL administration alleviated 900 MHz EMF-induced complications.

Keywords: Electromagnetic field, melatonin, lung, stereology, rat

1. Introduction

The prolonged use of cell phones emitting electromagnetic field (EMF) has raised curiosity about its impact on body organisms. Besides, the number of mobile phone users are also increasing worldwide. Therefore, this condition can contribute to the enhance of the potential negative effect of EMF (Tang et al., 2015). There have been numerous studies that have reported a relationship between exposure to EMF and damage to biological tissues. EMF radiation can result in the complications in vital organs, which is a threat for public health (Ulubay et al., 2015; Yahyazadeh et al., 2019).

The body organism is equipped with antioxidant enzyme defense that plays an important role in oxidative balance. In fact, endogenous antioxidant enzymes can scavenge the free radicals and contribute to a decrease in reactive oxygen species (ROS). Imbalance of antioxidant enzyme activity and free radicals leads to oxidative stress. Oxidative stress can disturb the vital structures such as protein, DNA, carbohydrate, lipid, resulting in cellular damage (Moustafa et al., 2004; Hanukoglu, 2006; Yahyazadeh and Altunkaynak, 2019a;). Exposure to 900 megahertz (MHz) EMF as an environmental factor can causes overproduction of free radicals, resulting in oxidative stress (Yahyazadeh and Altunkaynak, 2019b). Earlier reports have shown that EMF induces oxidative damage to the liver and kidney (Inoue et al., 1985; Marron et al., 1986; Ames et al., 1993). It has been documented the harmful effect of EMF on the genetic materials, spatial memory, central nervous system, sleep, cardiovascular system, and immune function (McCann et al., 1998; Repacholi 2001; Tang et al., 2015). Lung tissues as a component of respiratory system may affected by EMF. Baltaci et al. (2012) reported that exposure to EMF had deleterious effect on the lung tissue.

Melatonin (MEL) that is synthesized and secreted by the pineal gland at night prevents lipid peroxidation (Reiter,

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1991). Because of high lipid solubility of MEL, it can pass through the cell membrane and all morpho-physiological barriers (Reiter, 1995). MEL that acts as antioxidant can scavenge the free radicals and enhance the activity of some antioxidant enzymes (Kaya et al., 1999; Okatani et al., 2001; Rodriguez et al., 2004)

There are numerous studies regarding the effect of EMF on various tissues and possible activity of antioxidants, but researches related to the lung tissues are limited. Therefore, we decided to investigate the adverse effect of EMF exposure on lung, as well as protective efficacy of MEL against EMF.

2. Materials and methods

2.1. Experimental procedure and animals

Ethical approval for our investigation was granted by the Laboratory Animal Ethics Committee of Ondokuz Mayıs University. Fifteen female and ten male Wistar Albino rats (body weight of 180-200 g, 8-10 weeks old) were used in the present study. All animals were purchased from the Experimental Animal Research and Application Centre of the Medicine Faculty of Ondokuz Mayıs University (Samsun, Turkey). Two male and three female rats were randomly selected for each group, then placed in the plastic cage. Rats were mated overnight, and males were separated in the presence of vaginal plug. Female rats accepted as pregnant were designated as gestation day 1. During the 21-day gestation stage, rats were maintained in plastic cage under a 12 h light:12 h dark cycle at 22 ± 2°C and 50 ± 5% humidity and allowed *ad libitum* access to food and water. Experimental applications on the female rats of each group were performed as follows:

1. Control (CONT) group: Rats were not exposed to electromagnetic fields and any substances.
2. SHAM group: Rats were exposed to much stress but not EMF and any substances.
3. Electromagnetic field (EMF) group: The rats were exposed to the 900 MHz EMF for 21 days.
4. Melatonin (MEL) group: Rats was treated intraperitoneally with 50 mg/kg/day MEL (Sigma-Aldrich, St Louis, MO, USA) for 21 days (Jahnke et al., 1999).
5. Electromagnetic field + Melatonin (EMF+MEL) group: The rats were exposed to the 900 MHz EMF for 21 days and treated intraperitoneally with 50 mg/kg/day MEL for 21 days.

After delivery, groups of six male pups (two male pups from each mother) were created. The pups were maintained in suitable conditions for four weeks without any treatment. At the end of 28-day experimental period, all animals were perfused under anesthesia by intraperitoneal injection of 60 mg/kg 5:1 ketamine (Sigma-Aldrich, USA) and 5 mg/kg xylazine (Sigma- Aldrich, USA). The lung tissues were immediately dissected and used for stereological and histopathological analysis.

2.2. Histological procedure

The lung tissues were a solution of 10% for two weeks. All samples underwent to routine tissue processing involved in dehydration, clearing, and embedding. The paraffin blocks were cut at 7 µm thickness based on the systematic random sampling method using microtome (Leica RM2125RT, Leica Instruments, Germany). The sections were then mounted onto slides and then stained with triple.

2.3. Electromagnetic field exposure system

Procedure of this EMF exposure was according to our previous study (Ulubay et al., 2014). A 900 MHz continuous wave electromagnetic energy generator was used with a peak specific absorption rate (SAR) of 2 W/kg and an average power density of 1 ± 0.4 mW/cm^2 (Koyu et al., 2005). This generator was manufactured by the Electromagnetic Compatibility laboratory of Suleyman Demirel University. Estimation of localized SAR values were done as described by Sirav and Seyhan (2011). We also measured the power density of EMF using an EMF meter (Holaday Industry Inc., Adapazarı, Turkey). The monopole antenna of the exposure system was located perpendicular in the center of the round plastic cage to ensure that electric field distributed uniformly (Koyu et al., 2005). A 1 cm diameter air holes was devised on the cage to attenuate stress in rats. Animals were arranged adjacent to each other at a 1 cm distance with their heads in direction of the antenna.

2.4. Stereological analyses

The mean volumes of the regions of interest were estimated by means of the Cavalieri method and point-counting methods (Altunkaynak and Altunkaynak, 2007; Dursun et al., 2010). Briefly, the series of selected sections were photographed using light microscope (Leica RM 2135, Leica Instruments, Nussloch, Germany) and transferred to the private computer. We also used point-counting method with a regular grid of test points randomly positioned on the light micrographs. We then counted the sum of points hitting the regions of interest. Finally, the mean volumes of the alveoli, bronchioles and blood vessels in the lungs have calculated using the following formula (Yahyazadeh and Altunkaynak, 2019b): Volume = a(p) x ∑P x t; where, “a(p)” is the area of point interval, “ΣP” is the number of points hitting the regions of interest and “t” is the section thickness plus interval.

Estimation of the coefficient of error and the coefficient of variation showed that the number of the points counted in each animal and group was enough, respectively (Gundersen and Jensen, 1987; Yahyazadeh et al., 2017).

2.5. Statistical analysis

Statistical analysis was performed using IBM version 20.0 SPSS software (SPSS Inc., Chicago, IL, USA). We used one-way ANOVA and the Bonferroni post hoc test to analyze the

data. Results were expressed as means ± SEM. Values for $p \leq 0.05$ were considered statistically significant.

3. Results

3.1. Stereological results

Stereological results are given in Fig. 1-3 and Table 1.

3.2. The mean volume of bronchioles

The mean volume of bronchioles is summarized in Fig. 1 and Table 1. We found that the mean volume of bronchioles was significantly decreased in the EMF group compared to the CONT group ($p < 0.01$). There was a significant increase in the EMF+MEL groups compared to the EMF group ($p < 0.05$). We found no significant difference in the mean volume of bronchioles in the SHAM, MEL, and EMF+MEL groups compared to the CONT group.

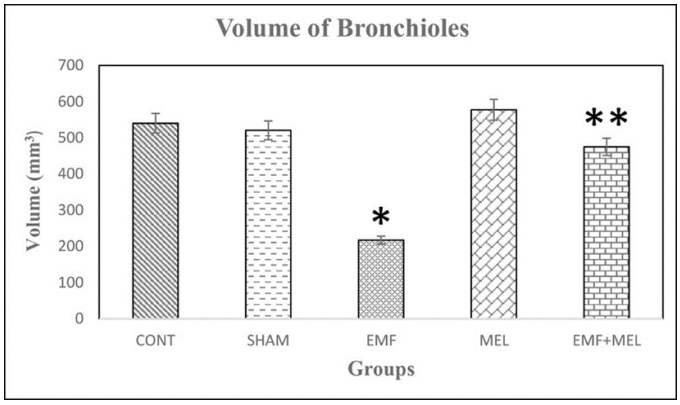

Fig. 1. The graph of bronchiole volume in all groups. *, significantly different from the CONT group at 0.05 level; **, significantly different from the EMF group at 0.01 level

3.3. The mean volume of alveoli

The mean volume of alveoli is given in Fig. 2 and Table 1. The mean volume of alveoli was significantly decreased in EMF group compared to the CONT ($p < 0.01$). To the contrary, we found a significant increase in the EMF+MEL group compared to the EMF group ($p < 0.05$). The mean volume of alveoli was not significantly different in the SHAM, MEL, and EMF+MEL groups compared to the CONT group.

3.4. The mean volume of blood vessels

The mean volume of blood vessels is given in Fig. 3 and Table 1. We found that the mean volume of blood vessels was significantly lower in the EMF group than the CONT group ($p < 0.01$). By contrast, the mean volume of blood vessels was significantly higher in the EMF+MEL groups compared to the EMF group ($p < 0.05$). We found no significant difference in the mean volume of bronchioles in the SHAM, MEL, and EMF+MEL groups compared to the CONT group.

3.5. Histopathological results

The structures of the lung tissues appeared normal in the CONT and SHAM groups (Fig. 4). By contrast, histopathological changes were evident in the EMF group; however, we observed irregular border of epithelium and dispersion of connective tissue in bronchiole (Fig. 5E and F). There was no marked change in the MEL group than the CONT group (Fig. 5G and H). In the EMF+MEL group, structural changes were detected, but these alterations were fewer than the EMF group (Fig. 5I and J).

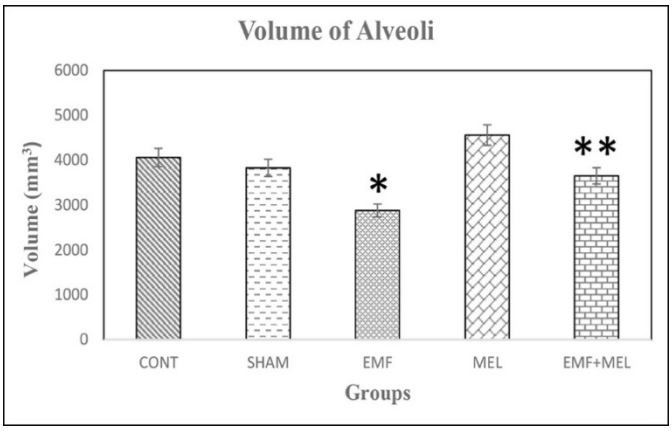

Fig. 2. The graph of alveolus volume in all groups. *, significantly different from the CONT group at 0.05 level; **, significantly different from the EMF group at 0.01 level

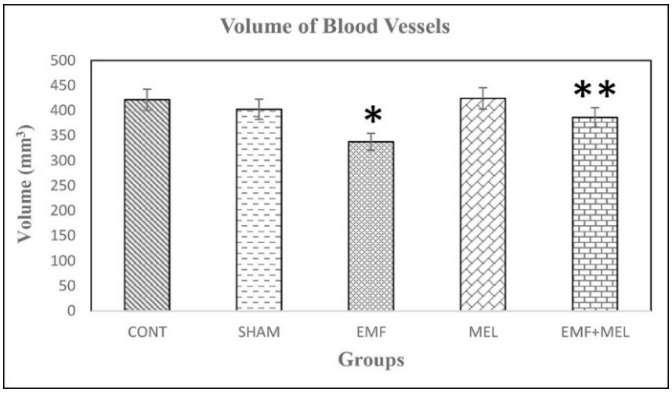

Fig. 3. The graph of blood vessel volume in all groups. *, significantly different from the CONT group at 0.05 level; **, significantly different from the EMF group at 0.01 level

Table 1. The mean volumes of bronchiole, alveoli, and blood vessels in the rat lung

	Groups				
	CONT	**SHAM**	**EMF**	**MEL**	**EMF+ MEL**
Mean volume of bronchioles	540±49	520±20	217±14*	577±30	474±29**
Mean volume of alveoli	4057±15	382±111	2876±147*	4556±96	3648±157**
Mean volume of blood vessel	422± 54	403±13	338±17*	424 ±29	386±9**

Data indicate means ± SEM. *, Significantly different from control group at 0.05 level; **, significantly different from EMF group at 0.01 level

4. Discussion

Due to the increasing use of microwave devices and mobile phones emitting EMF, researchers have been investigating the impact of EMF on the health of the body, as well as the growth and development of living organism. We also decided to survey whether administration of MEL would alleviate the adverse effect of EMF. Previous studies reported that EMF

exposure caused cellular toxicity via overproduction of free radicals, resulting in DNA damage (Wolf et al., 2005).

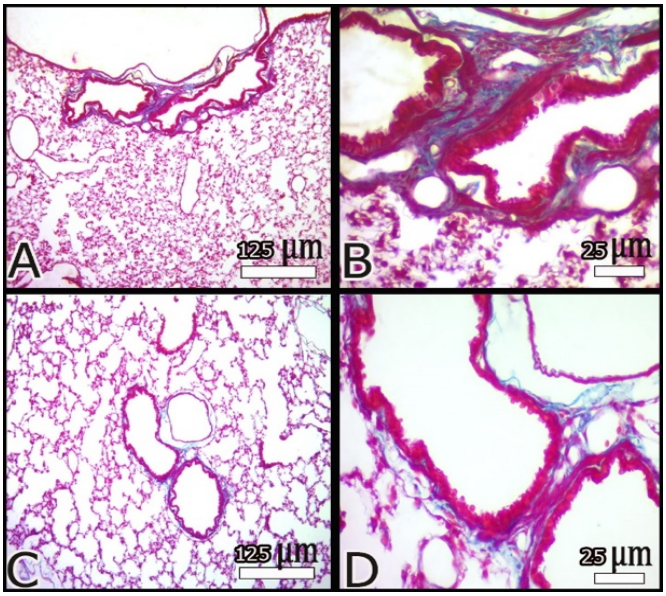

Fig. 4. Images obtained from the lung of the CONT (A and B) and SHAM (C and D) groups

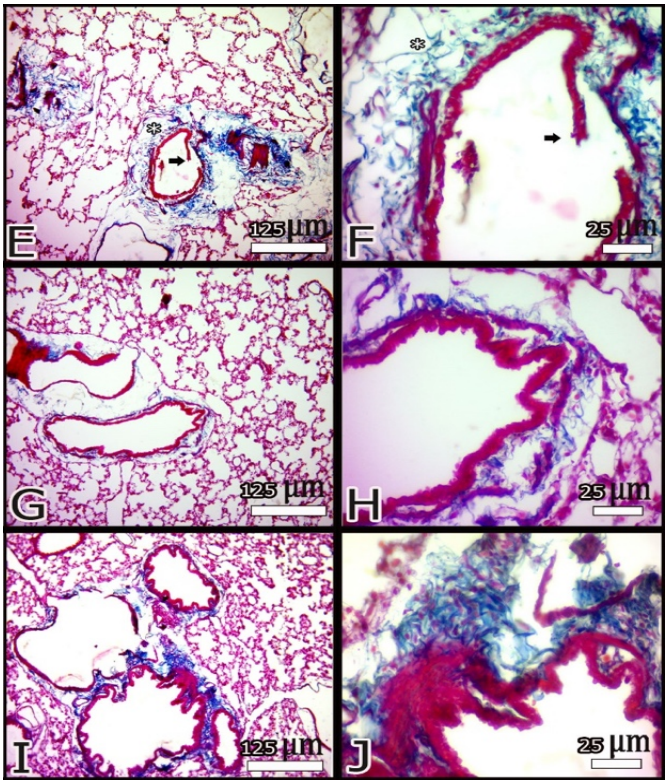

Fig. 5. Images obtained from the lungs of the EMF (E and F), MEL (G and H) and EMF+MEL (I and J) groups. Arrow; irregular border of epithelium in bronchiole, Asterisk; dispersion of connective tissue

Also, the rate of this cytotoxicity depends on the stress level, tissue type, duration of EMF exposure, and dose intensity (Davies 1999). Free radicals can damage biological membranes by attacking lipids and proteins, changing their nature and breaking protein connections (Selmaoui et al., 1999). Researchers has found that EMF can affect vital phenomena such as cell growth and differentiation, apoptosis, changes in hormone levels and change some proteins of membrane and intracellular (Selmaoui et al., 1997; Fuenta et al., 2008; Tenuzzo et al., 2009). Baltaci et al. (2012) documented that exposure to EMF led to cellular damage in the lung tissues associated with biochemical change such as a significant increase in malondialdehyde (MDA). The present study estimated the mean volumes of alveoli, bronchioles and blood vessels using stereological methods. This method in fact provides three-dimension data from a kind of two-dimension section, as well as accurate and valid results (Altunkaynak et al., 2012). Our quantitative results showed that mean volumes of alveoli, bronchioles and blood vessels were significantly decreased in EMF group compared to the CONT group. This possibly was due to adverse effect of exposure. Also, our findings were consistent with earlier report that EMF radiation induced cellular damage and biochemical alteration in the lung tissues (Baltacı et al., 2012). In the EMF+MEL group, administration of MEL caused a significant increase in the mean volumes of alveoli, bronchioles, and blood vessels. This possibly was due to antioxidant effect of MEL, which protected the lung tissue from oxidative stress (Baltaci et al., 2012).

On the other hands, MEL may have contributed to the direct elimination of hydrogen peroxide (H_2O_2) (Brzezinski 1997; Tan et al., 2000). Previous studies reported that MEL acted a protective role against lipid peroxidation in the lung tissue. Besides, MEL not only plays an immunomodulatory by stimulating the production of cytokine but also enhances antioxidant enzyme activity in cells (Brennan et al., 2002; Farías et al., 2012; Tahamtan et al., 2015). Takhtfooladi et al. (2006) suggested that MEL decreased the vascular permeability, then attenuated damage to the lung tissue. Takhtfooladi et al. (2006) suggested that MEL decreased the vascular permeability, then attenuated damage to the lung tissue. Recently, researchers reported that MEL could increase lumen diameter and provide a slight reduction in the wall of the pulmonary arteries (Torres et al., 2015). Accordingly, MEL as a safe antioxidant ameliorates pulmonary vascular function via reduction of oxidative stress. In fact, there are a relationship between administration of MEL and decrease in toxic effect of EMF.

Our histopathological results exhibited that structures of the lung tissues in the CONT and MEL groups were in normal appearance. We also observed marked histological change in the lung tissues of the EMF group. Exposure to EMF caused irregularity of the epithelial border and dispersion of connective tissue in bronchiole. This may have resulted from harmful effect of EMF on the lung tissues. To the contrary, amelioration in the structural alterations of lung tissues was detected in the EMF+MEL group compared to that the EMF group. These results revealed the protective efficacy of MEL against EMF-induced damage. To the best of our knowledge, our study is the first to investigate the possible effect of MEL on the rat lung tissue exposed to 900

MHz EMF.

In conclusion, our quantitative data displayed that exposure to EMF had hazardous effect on the lung tissues. This subject has not only interested researchers to obtain more details but also provided concerns with respected to risks threatening the public health. Moreover, we suggested that MEL improved the EMF-induced damage to the lung tissues. However, further studies should be carried out to clarify the unknown information.

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Declaration of interest

No conflict of interest was declared by the authors.

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