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EXPERIMENTAL STUDY

The effects of electromagnetic radiation (2450 MHz wireless devices) on the heart and blood tissue: role of melatonin

Gumral N[1], Saygin M[1], Asci H[2], Uguz AC[3], Celik O[3], Doguc DK[4], Savas HB[4], Comlekci S[5]

Department of Physiology, Faculty of Medicine, Suleyman Demirel University, Isparta, Turkey.
fizyolog@gmail.com

ABSTRACT

OBJECTIVE: This study was designed to investigate the effects of 2450 MHz EMR on the heart and blood in rat and possible ameliorating effects of melatonin.

MATERIAL AND METHOD: Thirty-two female Wistar Albino rats were randomly grouped (by eight in each group) as follows: Group I: cage-control group (dimethysulfoxide (DMSO), 10mg/kg/day i.p. without stress and EMR. Group II: sham-control rats stayed in restrainer without EMR and DMSO (10mg/kg/day i.p.). Group III: rats exposed to 2450 MHz EMR. Group IV: treated group rats exposed to 2450 MHz EMR+melatonin (MLT) (10mg/kg/day i.p.).

RESULTS: In the blood tissue, there was no significant difference between the groups in respect of erythrocytes GSH, GSH-Px activity, plasma LP level and vitamin A concentration ($p > 0.05$). However, in the Group IV, erythrocytes' LP levels ($p < 0.05$) were observed to be significantly decreased while plasma vitamin C, and vitamin E concentrations ($p < 0.05$) were found to be increased when compared to Group III. In the heart tissues, MDA and NO levels significantly increased in group III compared with groups I and II ($p < 0.05$). Contrary to these oxidant levels, CAT and SOD enzyme activities decreased significantly in group III compared with groups I and II ($p < 0.05$) and increased in group IV compared with group I, however not significantly ($p > 0.05$). Besides, MLT treatment lowered the MDA and NO levels compared with group III.

DISCUSSION: In conclusion, these results demonstrated that contrary to its effect on the heart, the wireless (2450 MHz) devices cause slight oxidative-antioxidative changes in the blood of rats, and a moderate melatonin supplementation may play an important role in the antioxidant system (plasma vitamin C and vitamin E). However, further investigations are required to clarify the mechanism of action of the applied 2450 MHz EMR exposure *(Tab. 3, Fig. 1, Ref. 49)*. Text in PDF *www.elis.sk.*

KEY WORDS: 2450 MHz electromagnetic radiation, heart and blood of rat, oxidant-antioxidant system, melatonin.

Introduction

Nowadays, many electric and electronic devices facilitate our modern lifestyle, and produce a dense network of electromagnetic radiation (EMR). EMR penetrates all tissues of human body, and it is said that it can be a new pollutant. The electromagnetic fields (EMF) from several sources such as wireless network, microwave ovens, and mobile phones have been shown to influence the biological systems. The fact that the cumulative effect of EMR pollution by environment causes changes in biological parameters in biological systems of living organisms after their exposure has been demonstrated by several studies (1, 2, 3, 4). Many sources of non-ionizing radiation may affect people silently and continuously on daily basis. However, some people may be more vulnerable to the adverse effects of EMR due to individual susceptibility or occupational exposure.

The Council of Europe is recommending restrictions in enhanced digital cordless telecommunication (DECT) phones, Wi-Fi as well as wireless local area network (WLAN) in all schools to protect young children from potentially harmful radiation (5). Safety of such RF-EMFs is evaluated based on specific absorption rates (SARs). The SAR in a human body exposed to aRF-EMF is, however, very difficult to measure directly because SAR includes the internal strength of electric field and conductivity in the body. The International Commission on Non-Ionizing Radiation Protection recommended certain guidelines to protect the public from excessive exposure to radio frequency (RF) based upon the threshold for behavioral disruption at 4 W/kg SAR (6).

The rapid increases in the development and global deployment of wireless communication devices have a significant impact on life in modern society by making it easy. Conversely, exposure to EMR emitted from such devices has raised concerns about human health (7, 8).

[1]Department of Physiology, Faculty of Medicine, Suleyman Demirel University, Isparta, Turkey, [2]Department of Pharmacology, Faculty of Medicine, Suleyman Demirel University, Isparta, Turkey, [3]Department of Biophysics, Faculty of Medicine, Suleyman Demirel University, Isparta, Turkey, [4]Department of Biochemistry, Faculty of Medicine, Suleyman Demirel University, Isparta, Turkey, and [5]Department of Electronics and Communication, Faculty of Engineering, Suleyman Demirel University, Isparta, Turkey.

Address for correspondence: M. Saygin, Suleyman Demirel University, Faculty of Medicine, Department of Physiology 32260 Isparta, Turkey.
Phone: +90.246.2113604, Fax: +90.246.2371165

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Exposure to EMR-related enhanced production of reactive oxygen species (ROS) and EMR could cause an increase in lipid peroxidation (LP) levels and decrease in the activity of antioxidant enzymes and vitamins (A, C, E) which prevent or protect against LP in humans (9, 10).

It is likely that carrying the device close to the chest could affect the heart. The heart functions can be compromised dangerously either directly by the impact of increased oxidative parameters within the myocardium or indirectly by those in the blood (11, 12).

On the contrary, enzymatic antioxidants neutralize the excessive ROS and prevent the cellular structure from damage. By using important active antioxidant ingredients, this damage will decrease significantly.

In mammals, melatonin (MLT) is synthesized by the pineal gland in a circadian rhythm and plays an important role in physiological activities including circadian rhythm regulation as well as visual, reproductive, cerebrovascular, neuroendocrine, and neuroimmunological actions. It is also a potent antioxidant that detoxifies a variety of ROS in many pathophysiological states (13, 14). MLT (N-acetyl-5-methoxytryptamine) is a neurohormone, also referred to as indoleamin. which functions as a potent and powerful endogenous free radical scavenger and antiapoptotic agent (15). In addition, it has been shown that MLT decreases DNA damage (16).

The present study was designed to determine the effects of 2450 MHz exposure on the heart and blood oxidant, antioxidant redox systems, as well as to investigate the role of vitamins and the possible protective effects of MLT on the blood and heart injury induced by EMR.

Materials and methods

Animals

Wistar Albino rats (n = 32) used in this study were four months old and weighed 237 ± 31 g. All experimental procedures were approved by Medical Faculty Experimentation Ethics Committee and animals were maintained and used in accordance with the Animal Welfare Act and Guide for the Care and Use of Laboratory Animals prepared by Suleyman Demirel University. The rats were housed individually in stainless-steel cages in a pathogen-free environment in our laboratory at + 22 °C ± 4 with light from 08:00 to 20:00 and free access to water, and fed with a commercial diet (Korkuteli Yem Ltd., Antalya, Turkey). Environmental average light intensity was 4000 lux and humidity was 40 ± 5 %.

Study groups

After one-week adaptation process, the animals were randomly divided into four equal groups as follows: cage-control group [dimethylsulfoxide (DMSO) 10 mg/kg/day, intraperitoneal (i.p.), without stress and EMR; Group I], sham-control group [rats stayed in restrainer without exposure to EMR and DMSO (10 mg/kg/day, i.p.); Group II], EMR group [rats exposed to 2450 MHz EMR 60 min/day for 30 days; Group III], and EMR+MLT [2450 MHz EMR exposed (60 min/day for 30 days;)+MLT (10 mg/kg/day, i.p.) treated group; Group IV]. The one-hour exposure to irradiation in groups III and IV took place between 9:00 and 12:00 each day. The first dose of MLT administration was performed 24 hours prior to exposure. Groups I and II rats received i.p. injections of DMSO in volume equal to that of MLT used in group IV. MLT was dissolved in a small (100 µl) amount of DMSO and then diluted with physiological saline solution. The MLT dose used in this study was chosen on the basis of our previously published experiment (17, 18).

Chemicals

All chemicals were of analytical grade, obtained from Sigma-Aldrich Chemical Inc. (St. Louis, MO, USA) and all organic solvents were from Merck Chemical Inc. (Istanbul, Turkey). All solutions, except phosphate buffers, were prepared daily and stored at +4.0 °C. The reagents were allowed to equilibrate at room temperature for at least 30 min before being used for analysis. The phosphate buffers were stable at +4.0 °C for one month.

Exposure system and design

Details of the exposure system have been described in detail elsewhere (19, 20). However, we reduced the power values to 1 mW/m2 in the experiments as described below. A "SET ELECO" generator from Set Electronic Co, Istanbul (Turkey), provided with a half-wave dipole antenna system, was used to irradiate the cells with a 2450 MHz radio frequency with 217 Hz pulses. The electric field density was set at 11 V/m in order to get a 0.1 W/kg whole-body average SAR. Radiation reflection and exposure were measured with a portable RF survey system (HOLADAY, HI-4417, Minnesota, USA with a standard probe. The EMR dose was calculated from the measured electric field density (V/m). SAR values were calculated by using electric properties of tissue sample and measured electric field intensities for every distance in certain frequency. We used eight rats in the exposure system at the same time (Fig. 1).

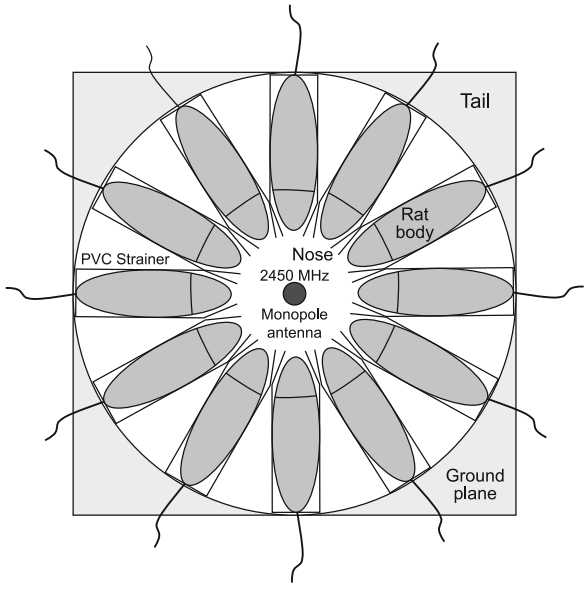

Fig. 1. Experimental setup for irradiation of the rats.

This device is organized with a special cylindrical strainer which is appropriate for exposure condition and physical size of one rat (15 cm in length; 5 cm in width). The noses of the rats were positioned in close contact to monopole antenna and the tube was ventilated from head to tail to decrease the stress of the rat while in the tube. The repetition time, frequency, and amplitude of the radio frequency energy spectrum were monitored by a satellite level meter (PROMAX, MC-877C, Barcelona, Spain). The distance between the head of the rat and antenna was calculated to be 1 m. Power rate was arranged as to1mW/m^2. The whole body average SAR values vary in range of 0.008–4.2 W/kg, representing average SAR value of 19 mW/kg for the whole body, with a value of 10.0 V/mat the closest point in the body. The whole exposure system was kept in a specific isolated room of Experimental Animal Research Center with a shielding effectiveness of 100 dB. By measuring the radio frequency, it was ensured that each of the rest of animals was kept out of contact with the radiation-generating device. The rats of group II were placed in a cylindrical restrainer with the radio frequency source switched off during periods similar to those used for irradiation. The animals from control group I were kept in their cage without any treatment or restraint of any kind and administered with 10 mg/kg/day DMSO.

Anesthesia and blood sampling

On day 30, the rats were anesthetized with a cocktail of ketamine hydrochloride (HCI) (90 mg/kg) and xylazyne HCI (10 mg/kg) which was administered i.p. before being sacrificed (17). After the abdominal incision, the blood was placed into ethylene diamine tetraacetic acid (EDTA) tubes and stored away from light. The heart samples were removed quickly and put into 5% phosphate buffer.

Preparation of heart and blood samples

Heart tissues were homogenized in a motor-driven tissue homogenizer (Janke & Kunkel Ultraturrax T-25, Germany) and sonicator (UW–2070 Bandelin Electronic, Germany) with phosphate buffer (pH 7.4). Unbroken cells, cell debris, and nuclei were separated by centrifugation at 10.000 g for 10 min at +4 °C.

The blood was separated into plasma and erythrocytes by centrifugation at 1,500×g for 10 min at +4 °C. The erythrocytes were washed three times in cold isotonic saline (0.9 %, v/w) and then hemolyzed with a nine-fold volume of 50 mM, phosphate buffer with pH 7.4. After addition of 4 mL butylhydroxytoluol per 1 mL of erythrocytes; the hemolysate and plasma samples were stored at –30 °C for not more than 3 months before measurement of enzymatic activity.

Determinations of oxidant-antioxidant parameters assays

Heart assays

The malondialdehyde (MDA) levels in heart tissues were determined from the homogenate by following the double heating method of Drapper and Hadley (21). The concentration of MDA is expressed as micromoles per gram protein (mmol/mg protein) in the heart. Nitric oxide (NO) activities were determined by CAYMAN Chemical Nitrate/ Nitrite Colorimetric Assay (USA) kits. An auto analyzer (Olympus AU 2700, Melville, NY) and a spectrophotometry (Shimadzu UV-1601; Shimadzu, Kyoto, Japan) was used to estimate the levels of MDA and NO, the activities of superoxide dismutase (SOD) (22) and catalase (CAT) (23). Both of these antioxidant enzymes were expressed as U/gr. protein. The protein content in the blood and heart homogenate was measured by method of Lowry et al with bovine serum albumin as standard (24).

Blood assays

LP levels in the plasma were measured with the thiobarbituric-acid reaction by the method of Placer et al (25). The quantification of thiobarbituric acid reactive substances was determined by comparing the absorption to the standard curve of MDA equivalents generated by acid-catalyzed hydrolysis of 1,1,3,3 tetramethoxypropane.

The erythrocytes LP, glutathione (GSH) levels and glutathione peroxidase (GSH-Px) enzyme activities were analyzed. The GSH content of erythrocytes was measured at 412 nm using the method of Sedlak and Lindsay (26). The samples were precipitated with 50 % trichloracetic acid and then centrifuged at 1.000×g for 5 min. The reaction mixture contained 0.5 mL of supernatant, 2.0 mL of 0.2 M, pH 8.9 Tris–EDTA buffers and 0.1 mL of 0.01 M 5, 5′-dithio-bis-2-nitrobenzoic acid. The solution was kept at room temperature for 5 min and then read at 412 nm on a UV-Vis spectrophotometer. GSH-Px activities of the erythrocytes were measured spectrophotometrically at 37 °C and 412 nm according to the Lawrence and Burk (27). Hemoglobin values were measured by the cynamethemoglobin method of Cannan (28).

Plasma vitamins A, C and E analyses

Vitamins A (retinol) and E (α-tocopherol) were determined in the plasma samples by a modification of the method described by Desai (29) and Suzuki and Katoh (30). About 250 μL of plasma were saponified by adding 0.3 mL 60 % (w/v in water) KOH and 2 mL of 1 % (w/v in ethanol) ascorbic acid, followed by heating up to 70 °C for 30 min. After cooling the samples on ice, 2 mL of water and 1 mL of n-hexane were added and mixed with the samples and then put aside for 10 min to allow phase separation. An aliquot of 0.5 mL of n-hexane extract was taken and vitamin A levels were measured at 325 nm. Then reactants were added and the absorbance value of hexane was measured in a spectrophotometer at 535 nm. The calibration was performed using standard solutions of all trans retinol and α-tocopherol in hexane. The quantification of vitamin C (ascorbic acid) in the plasma was performed according to the method of Jagota and Dani (31). The absorbance of the samples was measured spectrophotometrically at 760 nm.

Statistical analyses

Variables were presented as frequencies, percentages, mean±standard deviations (Mean±SD). The Kolmogorov–Smirnov test was used to test for normal distribution of continuous variables and Levene test was used to for homogeneity of variance. Data characterized by normal distribution were expressed as mean±SD. Parameters without such distribution were expressed as median with range. The groups were compared by using p non-parametric Kruskal–Wallis test, and Mann–Whitney U test. Biochemical parameters were shown to fit the normal distribution

Tab. 1. The effects of melatonin and 2450 MHz EMR on lipid peroxidation (LP), glutathione (GSH) and glutathione peroxidase (GSH-Px) activity in erythrocytes of rats (mean ± SD).

Parameters	Group I (n=8)	Group II (n=8)	Group III (n=8)	Group IV (n=8)
LP (μmol/g protein)	16.71±0.94	15.95±1.30	17.30±0.99	15.81±1.99[a]
GSH (μmol/g protein)	8.34±1.20	8.35±1.41	8.55±0.93	8.45±1.18
GSH-Px (IU/g protein)	23.58±1.98	23.16±3.01	23.19±1.53	25.27±3.65

Values are presented as means±SD. The relationships between groups and results of biochemical markers are assessed by one-way ANOVA, a – $p < 0.05$ compared with EMR group (Group III).

Tab. 2. The effects of melatonin and 2450 MHz EMR on lipid peroxidation (LP) and antioxidant vitamin concentrations in plasma of rats (mean ± SD).

Parameters	Group I (n=8)	Group II (n=8)	Group III (n=8)	Group IV (n=8)
LP (nmol/mL)	1.18±0.12	1.25±0.14	1.22±0.23	1.17±0.07
Vitamin A (nmo/l)	2.02±0.15	1.94±0.17	1.98±0.10	1.99±0.15
Vitamin C (μmol/l	33.36±4.74	36.19±9.57	31.94±6.02	41.16±7.89[a]
Vitamin E (μmol/l)	5.00±0.33	5.01±0.27	4.81±0.34	5.23±0.33[a]

Values are presented as means±SD. The relationships between groups and results of biochemical markers are assessed by one-way ANOVA. a – $p<0.05$ compared with EMR group (Group III).

and repeated measures, ANOVA and *post hoc* Least Significant Difference (LSD), Bonferroni, and Tukey tests were used to compare groups. Calculations were made using the SPSS 15.0 program pack. The value of $p < 0.05$ was set as the value for significance.

Results

Animals' weight did not change in the experiment. The difference in the erythrocytes GSH levels, GSH-Px activity, plasma LP levels and vitamin A concentration were not found statistically significant between the groups ($p > 0.05$). Parameter of oxidative stress erythrocytes LP levels were increased in group III but the latter increase was not significant. However, erythrocytes LP levels decreased significantly in the group IV ($p < 0.05$). GSH-Px levels increased in group IV compared with group III, but the latter increase was not significant ($p > 0.05$) (Tab. 1).

Parameters of oxidative stress plasma LP levels were increased in group III compared with group I but the latter increase was not significant. Plasma LP levels decreased in group IV but the decrease was not significant ($p > 0.05$). Plasma vitamin A, C and E concentrations decreased in group III compared with group I, but the latter decrease was not significant ($p > 0.05$). Plasma vitamin C and E concentrations increased significantly in the group IV compared with group III ($p < 0.05$) (Tab. 2).

In heart tissues, MDA levels increased significantly in group III compared with group II and decreased in group IV compared with group III ($p < 0.05$). NO levels increased significantly in group III compared with group I and decreased in group IV compared with group III ($p < 0.05$). Contrary to these oxidant levels, CAT enzyme activity decreased significantly in group III compared with group I ($p < 0.05$) but it was increased insignificantly in group IV compared with group I ($p > 0.05$). Other antioxidant enzyme SOD activity decreased significantly in group III and IV compared with group I and II ($p < 0.05$). Also SOD activities increased in group IV compared with group III but the latter increase was not significant ($p > 0.05$) (Tab. 3).

Discussion

The use of wireless internet has rapidly increased due to wide usage of Wi-Fi access points' infrastructure in workplaces, public places, houses, and especially individual computers. The majority of all users of these Wi-Fi access points all around are possibly the young population. As a result of close frequent usage of mobile phones, several abnormalities may be caused by release of EMR in human body as in blood and heart.

EMR can also cause brain damage that leads to a decrease in MLT secretion from pineal gland. This situation induces EMR-related damage and thus the body is vulnerable to it. Kesari et al studied 900-MHz microwave radiation effects on the brain and found an overproduction of ROS and a significant decrease in level of GSH-Px and SOD as observed in the rat brain under mobile phone radiation exposure. In addition, they found a significant decrease in the level of pineal MEL (32). Quin et al, exposed male rats to 1800 MHz EMR in for 32 days (2 h/day), and evaluated MLT and testosterone levels related to circadian rhythm. These rhythms were disturbed after exposure to RF, with the effect being more pronounced on MLT rather than on testosterone. These findings showed that the regulation of testosterone is controlled by MLT, while MLT is more sensitive to RF exposure. This situation explained the MLT decrease after exposure to EMR which caused ROS production in the systems (33).

Tab. 3. The effects of melatonin and 2450 MHz EMR on malondialdehyde (MDA) and nitric oxide (NO) levels, catalase (CAT) and superoxide dismutase (SOD) activities in cardiac tissues of rats (mean ± SD).

Parameters	Group I (n=8)	Group II (n=8)	Group III (n=8)	Group IV (n=8)
MDA (micromol/g prt)	0.118±0.00	0.112±1.02	0.138±0.01[b]	0.110±0.01[c]
NO (mikroM/mg protein)	7.92± 1.87	11.61±2.19[a]	11.86±0.89[a]	7.45±1.53[c]
CAT (U/g protein)	26.07±8.17	17.78±6.16	15.45±1.54[a]	18.49±5.41
SOD (U/g protein)	707.41±56.81	631.64±90.20	441.89 ±119.60[a,b]	485.05±65.39[a,b]

Values are presented as means±SD. The relationships between groups and results of biochemical markers are assessed by one-way ANOVA, a – $p < 0.05$ compare with control group (Group I), b – $p < 0.05$ compare with sham group (Group II), c – $p < 0.05$ compared with EMR group (Group III).

In addition, the lack of MLT levels may cause the decline in the antioxidant defense system and induce ROS production-related oxidative stress. Adverse effects of MLT reduction is reversed by external MLT intake. Exogenous intake of MLT has several beneficial effects against oxidative stress inducers especially EMR and other environmental pollutants. MLT promotes antioxidant defense systems, such as vitamins C and E which are the most frequently used antioxidants. Vitamin C is a nonenzymatic antioxidant, able to scavenge several radicals within cells, and plasma, hydroxyl radical inclusive (34). It is possible that vitamins C and E act in a synergistic manner. Vitamin E is primarily oxidized to the tocopheroxyl radical and then reduced back to tocopherol by vitamin C and GSH (35). According to this relationship, MLT may be also used to ameliorate the oxidative stress induced by 2450 MHz EMR. Meral et al exposed guinea pigs to 900 MHz EMR (217-Hz pulse rate, 2-W maximum peak power, SAR 0.95 w/kg) from a cellular phone for 12 h/day (11-h 45-min stand-by and 15-min spiking mode) for 30 days, and evaluated their blood MDA, GSH, vitamin A, D3 and E levels, and CAT enzyme activity. They showed that levels of MDA, vitamins A, D3 and E, and CAT enzyme activity increased, and GSH activity decreased in the blood tissue (36). Elhag et al found out that the exposure to EMR [(15 min day (-1) for four days) (EMR-F) and acute dose of EMR (EMR-A)] produced oxidant and antioxidant status in rats, and resulted in a significant decrease in the levels of vitamin E in EMR-F and EMR-A groups compared with control group (37). Guney et al exposed rats to 900 MHz mobile phone-induced endometrial impairment and investigated the role of vitamins E and C. They showed that vitamins E and C significantly increased the activities of antioxidant enzymes and decreased the powerful production of ROS (38). Naziroglu and Gumral investigated effects of EMR from wireless devices (2450 MHz EMR 60 min/day for 28 days), and evaluated their effect on the brain antioxidant redox system. Researchers found that the values of vitamins E and C concentration in the brain cortex were lower in EMR group compared with controls (39). All findings of these studies related to EMR-caused oxidative damage and production of LP from many tissues were similar to the results in this study. EMR caused LP production in the blood tissue. MLT showed ameliorative effect on this LP production. In line with these studies, we found that vitamin C and E levels were higher in MLT-treated group than in the others. This indicates that by increasing their levels, vitamins protect the blood against EMR exposure-induced oxidative stress by engaging their antioxidant properties.

Erythrocytes are the most common type of blood cell, while approximately 2.4 million new erythrocytes are produced per second in humans (40). The main role of erythrocytes is to transport oxygen to the body tissues via blood flow. Another role of erythrocytes is to promote normal blood flow when vessels are constricted due to shear stress. They release ATP and cause the vessels to relax (41). Recently, it has been demonstrated that similarly to endothelial cells erythrocytes too produce NO (42) and this may also contribute to the regulation of vascular tonus. This physiological function is very important for homeostasis. In a previous study focused on exposure of blood tissue of rats to EMR and protective effects of selenium and L carnitine, they found that the LP levels in plasma and erythrocytes were significantly higher in EMR group. Erythrocyte reduced GSH activities while erythrocyte GSH-PX activities in EMR group were found to be lower than in EMR+L-carnitine group. The frequency of 2450 MHz induced blood toxicity by inhibiting free radicals supporting antioxidant redox system although selenium has no effect on the investigated values (43). Da Silva et al aimed to assess antioxidant effects of MLT on the sickle cell suspension. They showed that the effect of MLT on LP, and combined antioxidant treatments also on MDA levels were dose-dependent (44). Vijayalaxmi et al studied the effect of 2450 MHz radiofrequency (SAR: 10.9 W/kg) on four healthy volunteers whose peripheral blood samples were investigated. Samples exposed *in vitro* to an acute dose of 1.5 Gy ionizing gamma-radiation were used as positive controls and treated with MLT. The authors evaluated lymphocytes to determine the extent of genetic damage assessed from the incidence of micronuclei (MN), and showed that MLT had no effect on cells exposed to EMR, but MLT significantly reduced the frequency of MN in gamma-radiation-exposed cells (45). Irmak et al exposed rabbit serum to EMR (900 MHz), and evaluated oxidant and antioxidant levels. They found that compared to the sham group, the serum SOD activity in EMR-exposed animals increased, whereas their NO levels decreased. Researchers suggest that this finding may indicate that EMR disrupts the pathophysiology of blood by increasing levels of oxidative stress (46). All of these findings mentioned above were similar to the results in this study. Erythrocyte LP levels in EMR group of this study increased significantly whereas in the MLT-treated group it significantly decreased. GSH-Px levels in erythrocytes increased in MLT-treated group but this was not significant. It was shown that EMR caused oxidative damage in erythrocytes and MLT was shown to ameliorate the effect of this damage. MLT started to protect erythrocytes from oxidative stress effects of 2450 MHz radiation but more time is needed, or it requires an increased dose of MLT.

Furthermore, frequent exposition to EMR may cause heart problem because the effect is continual. Olgar et al found that EMF of 2.1 GHz does not change the contractility and intracellular Ca2+ transients but decreases β-adrenergic responsiveness through NO signaling in rat ventricular myocytes while EMF exposure led to a significant increase in NO levels in rat heart (47). A study by Zhu et al, showed that an exposition to 2450 MHz for 20 min caused an edema in myocardial cells, malalignment in muscle fibers, obvious injury in cells, and significant increase in expression of Caspase-3 and HSP 70 protein in EMR group (48). Contrariwise, Colak et al investigated effects of EMR (40 min/day/20 days) on the heart rate, blood pressure and ECG parameters and also evaluated exogenous MLT. Results of the study showed that there were no changes among the groups both before and after the experiment, and MLT exhibited no additional effects, i.e. neither beneficial nor hazardous (49). In their study, they did not evaluate the oxidative stress markers of this damage, which reflects the basic mechanism of heart damage. Therefore, the damage may occur silently, but clinically, it cannot become more conspicuous. In this study, the increase in MDA and NO levels, and decrease

in CAT and SOD enzyme activities showed that oxidative stress occurred in heart tissues of EMR-administered groups. MLT could increase antioxidant enzyme activities slightly by external intake. Increased levels of NO in the sham group can be explained by a study conducted by Chang et al. They found that that DMSO improves the expression of recombinant COX-1 via NOS and/or the transcription factor Kr-h1 (48). Due to this, DMSO could induce NO levels in only EMR-administered group by improving NOS activity as shown in the present study.

In conclusion, studies about EMR-induced heart injuries related to oxidative stress are scarce. All of the results of this study demonstrated that wireless (2450 GHz) devices cause slight oxidative-antioxidative changes in blood and heart of rat while moderate melatonin supplementation may play an important role in the oxidant system (LP in erythrocyte and on heart tissue) and antioxidant system (plasma vitamin C and vitamin E). However, further investigations are required to clarify the mechanism of action induced by exposure to EMR of 2450 MHz.

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