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To cite this article: G Kerimoğlu, T Mercantepe, HS Erol, A Turgut, H Kaya, S Çolakoğlu & E Odacı (2016) Effects of long-term exposure to 900 megahertz electromagnetic field on heart morphology and biochemistry of male adolescent rats, Biotechnic & Histochemistry, 91:7, 445-454, DOI: [10.1080/10520295.2016.1216165](https://doi.org/10.1080/10520295.2016.1216165)

To link to this article: <https://doi.org/10.1080/10520295.2016.1216165>



Published online: 11 Aug 2016.



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Effects of long-term exposure to 900 megahertz electromagnetic field on heart morphology and biochemistry of male adolescent rats

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Accepted July 19, 2016

Abstract

The pathological effects of exposure to an electromagnetic field (EMF) during adolescence may be greater than those in adulthood. We investigated the effects of exposure to 900 MHz EMF during adolescence on male adult rats. Twenty-four 21-day-old male rats were divided into three equal groups: control (Cont-Gr), sham (Shm-Gr) and EMF-exposed (EMF-Gr). EMF-Gr rats were placed in an EMF exposure cage (Plexiglas cage) for 1 h/day between postnatal days 21 and 59 and exposed to 900 MHz EMF. Shm-Gr rats were placed inside the Plexiglas cage under the same conditions and for the same duration, but were not exposed to EMF. All animals were sacrificed on postnatal day 60 and the hearts were extracted for microscopic and biochemical analyses. Biochemical analysis showed increased levels of malondialdehyde and superoxide dismutase, and reduced glutathione and catalase levels in EMF-Gr compared to Cont-Gr animals. Hematoxylin and eosin stained sections from EMF-Gr animals exhibited structural changes and capillary congestion in the myocardium. The percentage of apoptotic myocardial cells in EMF-Gr was higher than in either Shm-Gr or Cont-Gr animals. Transmission electron microscopy of myocardial cells of EMF-Gr animals showed altered structure of Z bands, decreased myofilaments and pronounced vacuolization. We found that exposure of male rats to 900 MHz EMF for 1 h/day during adolescence caused oxidative stress, which caused structural alteration of male adolescent rat heart tissue.

Key words: adolescent, apoptosis, cellular phone, electromagnetic field, electron microcopy, heart, rat

The use of telecommunication devices that emit an electromagnetic field (EMF) including televisions, radios and mobile phones has increased dramatically. The effects on the human body of EMF emitted

by these devices are problematic (Valberg et al. 2007, World Health Organization 2010). Several reports link use of mobile phones to serious health problems in humans owing to their emission of EMF (Repacholi 2001, Fychting et al. 2005, Genuis 2008, Hardell and Sage 2008, Bas et al. 2009, Sonmez et al. 2010, Baş et al. 2013, Morgan et al. 2015, Odacı et al. 2008, 2015a,b,c), although contradictory results also have been reported (Aslan et al. 2011, 2013). Mobile phones have become the main source of exposure to EMF among adolescent children and teenagers. Because adolescents are exposed to EMF

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Biotechnic & Histochemistry 2016, **91**(7): 445–454.

at an early age, exposure during their lifetimes is extensive (Schüz 2005, Leitgeb 2008).

During prenatal and early postnatal development, the rat heart is particularly susceptible to injury from many agents including EMF (Türedi et al. 2015). We investigated the effects on male rat hearts of 900 MHz EMF for 1 h/day from postnatal day 21 to day 59, which is the adolescent period in rats, using histopathological and biochemical techniques, and electron microscopy. We used 900 MHz EMF, because this frequency is used in the Global System for Mobile Communications system in Europe and is virtually the standard for mobile phones worldwide (Hancı et al. 2013, 2015, Türedi et al. 2015, Topal et al. 2015).

Material and methods

Animals

All experiments and surgical procedures were conducted in the Karadeniz Technical University (KTU) Experimental Animals Surgical Research and Application Center and in the laboratories of the Medical Faculty Department of Histology and Embryology. All experimental procedures were performed in accordance with the *Guide for the Care and Use of Laboratory Animals* published by the U.S. National Institutes of Health. Approval was obtained from the KTU Animal Care and Ethics Committee.

We investigated the effects on heart tissue of exposure to 900 MHz EMF during adolescence (postnatal days 21–59). Twenty-four 28.3–43.9 g 21-day-old male Sprague-Dawley rats were obtained from the KTU Experimental Animals Surgical Research and Application Center, and housed in plastic cages under standard laboratory conditions at $22 \pm 2^\circ \text{C}$ and a 12 h light/12 h dark cycle. Commercial rat chow (Bayramoğlu Feed and Flour Industry Trading Corp., Erzurum, Turkey) and tap water were provided *ad libitum* for all animals except during EMF and sham EMF exposure.

The rats were divided randomly into three groups of eight. The control group (Cont-Gr) was not exposed to EMF during the study. The EMF group (EMF-Gr) was exposed to 900 MHz EMF in a Plexiglas cage 42 cm long $\times$ 30 cm wide $\times$ 50 cm high for 1 h at 11:00–12:00 AM every day between postnatal days 21 and 59. The sham group (Shm-Gr) comprised animals that were placed in the Plexiglas cage for 1 h at 10:00–11:00 AM every day between postnatal days 21 and 59 days, but without exposure to EMF. All groups were kept in the same room in

separate cages under the same environmental conditions, except during sham and EMF exposure.

Shm-Gr rats were placed together in the Plexiglas cage for 1 h each day without EMF exposure, then returned to their cages. EMF-Gr rats were placed in the Plexiglas cage for 1 h, exposed to EMF, then returned to their cages. The rats were permitted to move freely within the Plexiglas cage during both EMF and sham applications to minimize stress due to immobilization. The internal temperature and humidity in the Plexiglas cage were measured using a digital thermometer (YCOM KMN-303, Yu Yau Shuanghe Electron Instrument Co., Ltd., Zhejiang, China). The temperature within the Plexiglas cage during the sham treatment was $21.30 \pm 1.08^\circ \text{C}$ and during EMF exposure was $21.43 \pm 1.07^\circ \text{C}$. The humidity within the Plexiglas cage was $59.7 \pm 4.34\%$ during sham treatment and $59.8 \pm 4.11\%$ during EMF exposure.

Electromagnetic field device, EMF exposure and intensity measurements

The 900 MHz EMF was generated by an ultra-high frequency oscillator (1218-BV, 900–2000 MHz lockable oscillator; General Radio Co., Concord, MA) with output power approaching 300 mW and frequency adjusted to 900 MHz. The oscillator was supported by a continuous power source (1267-B regulated power supply; General Radio Co.) and attached to a half-wave dipole antenna constructed of 1 mm wide $\times$ 15 cm long copper plate by a coaxial cable. The antenna was inserted into the central area of the Plexiglas cage, approximately 11 cm inside the opening (Fig. 1). Rats were placed in the Plexiglas cage for 1 h/day for exposure to 900 MHz EMF. The device was identical to those described earlier (Hancı et al. 2013, Türedi et al. 2015, Topal et al. 2015, Odacı and Özyılmaz 2015).

Electrical field intensities were measured at nine points as shown in Fig 1C), both on the inside and outside of the Plexiglas cage floor both with the cage empty and with rats inside. Electrical field intensities were measured a wide-range CA43 isotropic electrical field intensity meter (100 kHz–2.5 GHz, Chauvin Arnoux Group, Paris, France) during the sham and EMF applications. Measurements at nine different points revealed average electrical field intensities of approximately 7.0 V/m and 9.54 V/m outside the empty Plexiglas cage floor and on the inside of the empty Plexiglas cage floor, respectively (Table 1). With rats in the Plexiglas cage, average values were approximately 5.4 V/m and 8.4 V/m outside of the Plexiglas cage floor and on

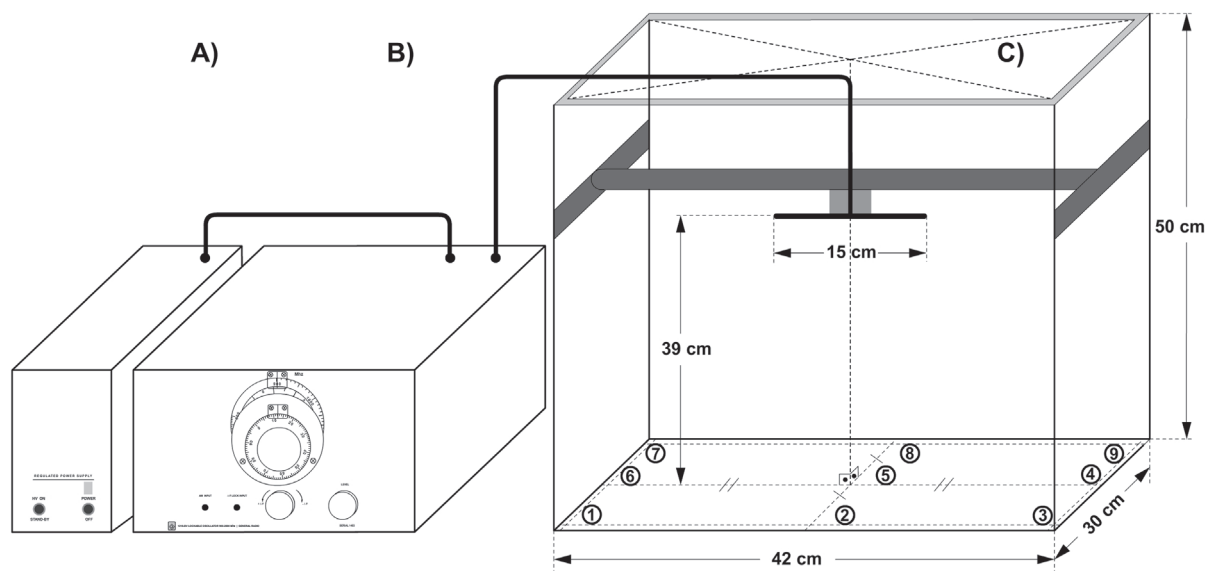


Fig. 1. Electromagnetic field exposure system and nine locations of electrical field intensity measurement points on the floor of the Plexiglas cage. A) Ultrahigh frequency oscillator. B) Continuous power source. C) Plexiglas cage with half-wave dipole antenna. The oscillator was supplied by a power source and connected to a half-wave dipole antenna by a coaxial cable. The antenna was inserted into the central region of the Plexiglas cage to a depth of 11 cm. During the experiment, electrical field intensity was measured at nine points inside and outside the Plexiglas cage when empty and with rats inside.

the inside of the Plexiglas cage floor, respectively (Fig. 2). These measurements indicated that EMF-Gr rats were exposed to a mean electrical field intensity of 8.4 V/m within the Plexiglas cage (0.187 W/m^2). Based on these data, the whole body specific absorption rate (SAR) was calculated to be 0.0093 W/Kg using an SAR calculator (RadHaz SAR Equivalency Calculator, Version 1.0.-2000; Richard Tell Associates, Inc., Colville, WA).

Tissue preparation

All rats were weighed and sacrificed by cervical dislocation under general anesthesia using 50 mg/kg Ketalar® (Eczacıbaşı Co., Istanbul, Turkey) on postnatal day 60 and the hearts were removed. The hearts were weighed (KERN PCB, Kern & Sohn GmbH, Balingen, Germany) and cut in half

by a vertical incision. One half of each heart was placed in a 1.5 ml Eppendorf tube and stored at -80°C for biochemical analysis. The remaining half of the heart was divided further into three parts. One part was used for transmission electron microscopy (TEM), another part was used for histological scoring and histopathological evaluation, and the final part was used for terminal deoxynucleotidyl transferase deoxyuridine triphosphate nick end labeling (TUNEL) assay for apoptosis (Kyrylkova et al. 2012).

Histological staining scores and histopathological evaluation

Heart specimens were fixed in 10% formalin (Sigma-Aldrich, St. Louis, MO) for 10 days, dehydrated through graded ethanols (Merck, Darmstadt,

Table 1. Electric field strength measurements from nine points beneath and within the empty Plexiglas cage (PC) and during electromagnetic field application

PC condition	PC measurement areas	Measurements points									Mean $\pm$ SD
		1	2	3	4	5	6	7	8	9	
Empty (V/m)	Beneath PC	9.3	6.5	5.5	3.4	7.4	9.7	7.1	7.1	7	7 ± 1.87
	Within PC	10.8	10.8	7.5	5.6	10.5	9.1	11.2	9.3	11.1	9.54 ± 1.91
Rats within (V/m)	Beneath PC	6.7	3.9	5.1	3	6.3	7	4.8	6.4	5.7	5.43 ± 1.35
	Within PC	11.2	7.8	7.2	4.8	8.7	9.4	9.3	8.5	9.1	8.44 ± 1.76

V/m = volts/meter.

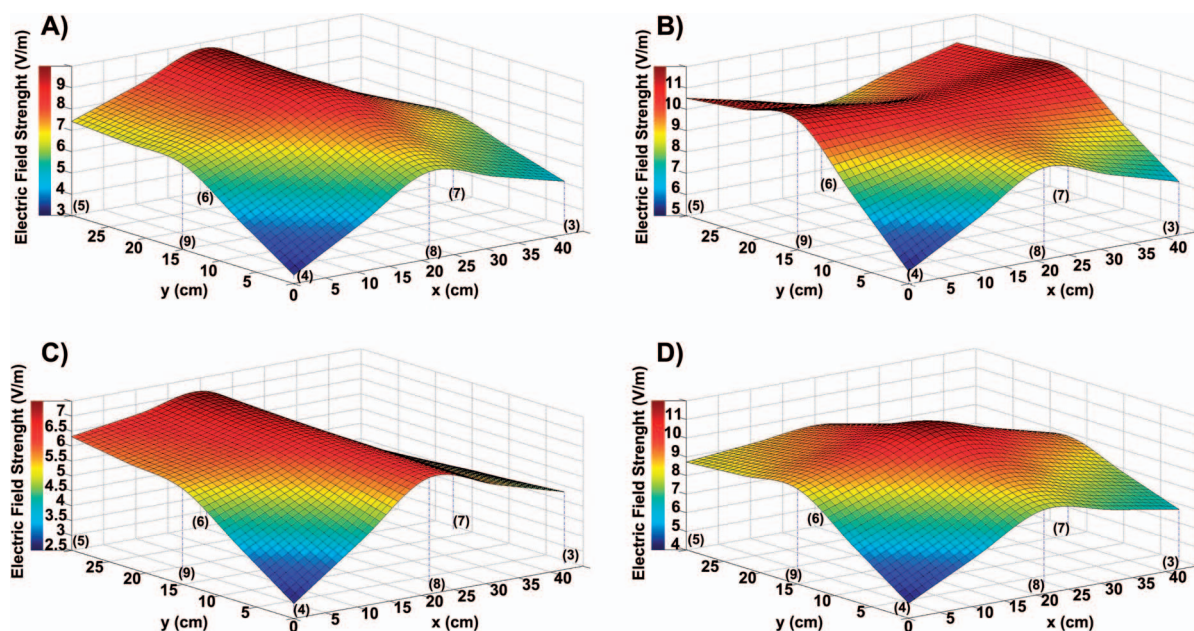


Fig. 2. Positional distributions of mean EMF intensities (MEMFI) on the x-y axes at nine points of the Plexiglas cage. A) MEMFI along the x-y axes beneath the Plexiglas cage with no rats inside. B) MEMFI along on the x-y axes on the floor of the Plexiglas cage with no rats inside. C) MEMFI along the x-y axes beneath the cage with rats inside. D) MEMFI along the x-y axes on the floor of the Plexiglas cage with rats inside.

Germany), cleared in xylene (Sigma-Aldrich), then embedded in paraffin (JT Baker, Deventer, Holland). Serial sections were cut at 5 μm using a microtome (Leica RM 2255, Leica Instruments, Nussloch, Germany) and collected slides coated with a gelatin-formaldehyde mixture. After rehydration through graded alcohols, sections were stained with hematoxylin and eosin (H & E) (Bancroft et al. 1996). Qualitative assessment was performed by a histologist using a research light microscope (Olympus BX 51; Olympus Co., Tokyo, Japan). Photographs of heart tissue sections were taken using the same microscope with a camera attachment (Olympus DP 71; Olympus Co.). Histopathological changes including myocardial vascular congestion, cytoplasmic eosinophilia, vacuolization and irregularity of muscle fibers, and loss of cross striations were recorded.

TUNEL assay for apoptosis evaluation

We assessed myocardial apoptosis using the TUNEL method. Paraffin blocks of heart tissue were prepared as for histology. For TUNEL staining, serial sections were cut at 4 μm , mounted on glass slides and subjected to standard histological deparaffinization protocols. Sections then were stained by the TUNEL method using a cell death detection kit (In Situ Cell Death Detection Kit,

POD, 11684817910; Roche Diagnostics, Mannheim, Germany) according to the manufacturer's instructions. TUNEL stained slides were examined using a light microscope at $\times 400$. To do this, five fields were chosen randomly in each section and 100 cells were counted in each field. Cells with brown nuclei were considered apoptotic. The apoptotic index (AI) was calculated using the formula: $\text{AI} = (\text{TUNEL positive cells} / \text{total cells}) \times 100$ (Türedi et al. 2015, Odacı and Özyılmaz 2015). All counting procedures were performed by one of the authors who was blinded to the identity of the section.

Biochemistry

The heart tissues stored in tubes at -80°C were used for biochemical analysis. Before the experiments, tissues were homogenized with liquid nitrogen in a mortar. Next, 15 mg of tissue from each group was treated with 1.5 ml of suitable homogenate buffer: lipid oxidation, 10% KCl; superoxide dismutase, 50 mM KH_2PO_4 with 10 mM EDTA, pH 7.8; catalase, 50 mM KH_2PO_4 containing 0.5% Triton-X 100, pH 7; glutathione, 50 mM Tris-HCl, pH 7.4. The mixture was homogenized on ice in Eppendorf tubes with steel balls using a homogenizer (Tissuelyser LT, Qiagen N.V., Hilden, Germany) for 10 min. Homogenates were filtered and centrifuged using a refrigerated centrifuge at 4°C . Supernatants were

used to determine superoxide dismutase (SOD) and catalase (CAT) enzyme activity, and lipid oxidation and glutathione (GSH) levels. All chemicals were obtained from Sigma-Aldrich Co. All assays were performed at room temperature and all samples were analyzed using software (Gen 5; Bio-Tek Instruments Inc., Winooski, VT) using a spectrophotometer (Epoch; Bio-Tek Instruments Inc.).

Catalase activity

The heart tissues were homogenized in 1.5 ml 50 mM KH_2PO_4 buffer containing 0.5% Triton X-100, pH 7.8. The homogenate was centrifuged (Universal 320R; Hettich GmbH & Co., Kirchenger, Germany) at $10,000 \times g$ for 60 min at 4°C . Decomposition of H_2O_2 in the presence of CAT was observed at 240 nm in a quartz cuvette (Hellma GmbH & Co., Mulheim, Germany). The amount of enzyme required to decompose 1 mmol H_2O_2 /min at 25°C and pH 7.8 was defined as CAT activity. Results are expressed as mmol/min/mg heart tissue (Aebi 1984).

Superoxide dismutase activity

Estimations of SOD enzyme activities were based on the superoxide radicals produced by xanthine and xanthine oxidase reacting with nitro blue tetrazolium to form formazan dye. The heart tissues were homogenized in 1.5 ml of 50 mM KH_2PO_4 buffer containing 10 mM EDTA, pH 7.8. The homogenate was centrifuged at $5,000 \times g$ for 60 min at 4°C . SOD activity was measured at 560 nm and activity is expressed as mmol/min/mg heart tissue (Sun et al. 1988).

Amount of glutathione

The amount of GSH in the heart tissues was calculated using 5, 5'-dithiobis (2-nitrobenzoic acid) using the method described by Sedlak and Lindsay (1968). The heart tissues were homogenized in 1.5 ml of 50 mM Tris-HCl, pH 7.4. The homogenate was centrifuged at $3,000 \times g$ for 30 min at 4°C . The standard curve was obtained by using reduced GSH (Sigma-Aldrich Co.). The recovery rate exceeded 95%. The absorbance was measured at 412 nm and tissue GSH levels were expressed as nmol/mg heart tissue.

Determination of lipid oxidation

LPO in heart tissues was calculated by estimating malondialdehyde (MDA) levels using the

thiobarbituric acid test (Ohkawa et al. 1979). The heart tissues were scraped from the mortar, weighed and homogenized in 1.5 ml of 100 g/l KCl. To 0.5 ml of the homogenate was added a solution containing 0.2 ml of 80 g/l sodium lauryl sulfate, 1.5 ml 200 g/l acetic acid, 1.5 ml 8 g/l 2-thiobarbiturate and 0.3 ml distilled water. The mixture was incubated at 98°C for 1 h. Upon cooling, 5 ml 15:1 n-butanol:pyridine was added. The mixture was vortexed (MS3 Digital; IKA-Werke GmbH & Co., Staufen, Germany) for 1 min and centrifuged for 30 min at $1,000 \times g$. The absorbance of supernatant was measured at 532 nm. The standard curve was obtained using 1,1,3,3-tetramethoxypropane. The recovery rate was $>90\%$. The results were expressed as nmol/g tissue.

Transmission electron microscopy

Heart samples, 1 mm^3 , were fixed in 1% glutaraldehyde (Merck, Hohenbrunn, Germany) in phosphate buffer, pH 7.3, for 2 h and postfixed in 1% OsO_4 for 1 h. Samples were washed in phosphate buffer, dehydrated through a series of graded ethanols, cleared with propylene oxide and embedded in epoxy resin (Araldite CY212; Agar Scientific, Essex, UK). Semithin 700–750 nm sections were cut using an ultramicrotome (LKB Nova, Bromma, Sweden) and stained with toluidine blue. Sections then were viewed using a Leica DM 6400 microscope (Leica Microsystems, Wetzlar, Germany). Ultrathin 30–40 nm sections were cut from areas identified in semithin sections using an ultramicrotome (LKB Nova, Bromma, Sweden) and mounted on 200-mesh copper/nickel grids. Sections were stained with uranyl acetate and Reynold's lead citrate; sections were viewed using a JEOL 100SX electron microscope (JEOL Ltd., Akishima, Tokyo, Japan) and photographed at $\times 8,000$ using photographic image capture (Kodak 4489, Eastman Kodak Company, Rochester, NY).

Statistical analysis

Statistical analyses were performed using SPSS software (Statistical Package for the Social Sciences, version 13.1, SSPS Inc., Chicago, IL). Kruskal Wallis analysis of variance was used to compare differences between groups. Dual comparisons between groups that exhibited significant differences were evaluated using the Mann-Whitney U test with corrected Bonferroni. Results are given as means $\pm$ SD. Values for $p \leq 0.05$ were considered statistically significant.

Results

Body and heart weights

There were no statistically significant differences in body or heart weights among groups at postnatal day 60.

Biochemical analysis

We found higher MDA levels in EMF-Gr than in Cont-Gr ($p=0.004$) and Shm-Gr ($p=0.006$) animals. SOD levels in EMF-Gr also were higher those of Cont-Gr ($p=0.004$) animals, while GSH and CAT levels in EMF-Gr animals were lower than in Cont-Gr ($p=0.004$ and $p=0.006$, respectively). GSH and CAT levels in Shm-Gr animals were lower ($p=0.004$ and $p=0.013$, respectively) while SOD levels higher than in Cont-Gr animals ($p=0.004$) (Table 2).

Histology and histopathology

H & E stained sections of Cont-Gr hearts exhibited normal myocardial architecture including striated, branching and anastomosing muscle fibers. Cont-Gr cardiomyocytes exhibited acidophilic cytoplasm with central oval basophilic nuclei. Occasional connective tissue cells and capillaries were observed among the myocardial cells (Fig. 3A). The Shm-Gr animals generally exhibited normal cardiomyocytes (Fig. 3B). H & E stained sections from EMF-Gr animals exhibited structural changes and capillary congestion in the myocardium. Some muscle fibers exhibited increased cytoplasmic eosinophilia, loss of striation and single or clustered cytoplasmic microvacuoles were observed in the cardiomyocytes (Fig. 3C).

TUNEL staining

TUNEL stained sections revealed only a few apoptotic cells in Cont-Gr animals (Fig. 3a). We

found numerous apoptotic cells in Shm-Gr animals (Fig. 3b), however. The number of TUNEL positive cells EMF-Gr animals was greater than in Shm-Gr animals (Fig. 3c) ($p=0.001$). The AI of myocardial cells in EMF-Gr animals was significantly greater than in Shm-Gr ($p=0.001$) and Cont-Gr ($p=0.001$) animals (Table 2).

Transmission electron microscopy

Transmission electron microscopy of Cont-Gr samples exhibited normal myocardial ultrastructure and characteristic organized myofilaments in the myocardial cell cytoplasm. Normal Z bands and sarcomeres were evident in Cont-Gr sections (Fig. 4A). Sections from the hearts of the Shm-Gr were examined under a TEM and characteristic organized myofilaments and typical myocardial cell cytoplasm fine structure were observed (Fig. 4B). The Shm-Gr findings were similar in appearance to the Cont-Gr findings, i.e., the sham treatment did not affect the Shm-Gr rats. The EMF-Gr sections exhibited Z bands with interrupted structural integrity and indistinct sarcomeres. Myofilaments exhibited structural impairment and reduction. Considerable vacuolization was evident in EMF-Gr myocardial cells. In addition, diffuse edema was observed among the myofibrils (Fig. 4C, D).

Discussion

It has been reported that exposure to EMF prior to adulthood may have pathological effects. Children appear to be more susceptible to the effects of EMF than adults (Gandhi et al. 1996). During the adolescent period, some organs have not yet attained the anatomical structures of adulthood (Kim et al. 2013, Morgan et al. 2014). Also, the use of mobile phones is increasing during childhood and adolescence (International Telecommunication Union 2014). The literature includes reports concerning some effects of exposure to EMF during

Table 2. Apoptotic index and biochemical parameters for the study groups

Parameters	Cont-Gr	Shm-Gr	EMF-Gr	p^1	p^2	p^3
Apoptotic index (%)	3.25 ± 1.38	11.75 ± 2.05	31.00 ± 5.78	0.001	0.001	0.001
MDA (nmol/mg heart tissue)	44.48 ± 6.57	47.68 ± 4.53	59.28 ± 5.19	0.423	0.004	0.006
SOD (mmol/min/mg heart tissue)	0.44 ± 0.03	0.53 ± 0.01	0.52 ± 0.03	0.004	0.004	0.261
GSH (nmol/mg heart tissue)	0.80 ± 0.04	0.66 ± 0.04	0.63 ± 0.02	0.004	0.004	0.294
CAT (mmol/min/mg heart tissue)	122.95 ± 10.35	98.42 ± 14.80	97.57 ± 7.07	0.013	0.006	0.462

$n=8$ for each group; data are means $\pm$ SD.

Cont-Gr, control group; Shm-Gr, sham group; EMF-Gr, EMF group; MDA, malondialdehyde; SOD, superoxide dismutase; GSH, glutathione; CAT, catalase; p^1 , Shm-Gr versus Cont-Gr; p^2 , EMF-Gr versus Cont-Gr; p^3 , EMF-Gr versus Shm-Gr.

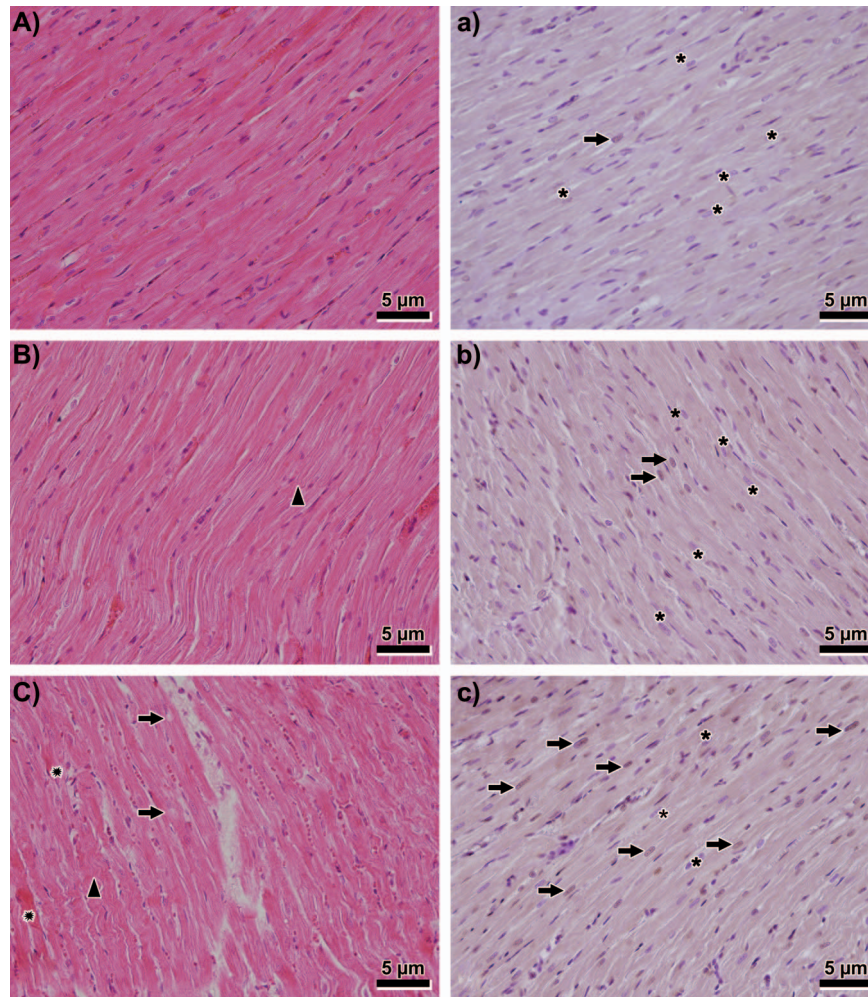


Fig. 3. H & E (A, B, C) and TUNEL (a, b, c) staining of myocardial sections from Cont-Gr, Shm-Gr and EMF-Gr rats. Normal myocardial structure can be seen in Cont-Gr tissue (A) in H & E stained sections. Muscle fibers (Δ) can be seen in Shm-Gr animals (B). Vacuoles (arrow), muscle fibers (Δ) and increased cytoplasmic eosinophilia (star) can be seen in muscle fibers of EMF-Gr animals (C). Normal cells (star) and rare apoptotic cells (arrow) can be seen in Cont-Gr animals in TUNEL stained sections (a). More apoptotic cells were observed in Shm-Gr (b) than in Cont-Gr animals. Apoptotic cells in EMF-Gr animals (c) were significantly more numerous than in the other groups. All panels $\times 400$.

adolescence (Schüz 2005, Leitgeb 2008); however, there have been few studies concerning the effects of exposure to EMF on the adolescent heart, which is regarded as a post-mitotic organ during adolescence.

Structural alterations of heart tissue caused by EMF can be detected by both light microscopy and TEM. Türedi et al. (2015) investigated on postnatal day 21 the hearts of male pups from pregnant rats exposed to EMF throughout pregnancy. These investigators reported vacuolization and structural impairment of heart tissue and pyknotic nuclei in the muscle fibers. Capillary congestion also was observed in EMF-Gr heart tissue. By contrast to the earlier report (Türedi et al. 2015), the capillary congestion that we observed may have been due to

continuing exposure to EMF until one day before sacrifice. Also, the vacuolization and altered tissue morphology in EMF-Gr and Shm-Gr in the heart tissue were consistent with their AIs, which indicates that the damage observed by light microscopy was due to apoptosis caused by EMF exposure.

Others have investigated the effects of exposure to EMF on the heart using TEM. Kiray et al. (2013) reported fragmentation of myofibrils, vacuoles in the perinuclear region and structural alteration of mitochondria, while structural alteration of myofibrils and Z bands was reported by Türedi et al. (2015). We also observed significant pathology in EMF-Gr animals. The structural integrity of Z bands in EMF-Gr was disrupted and sarcomeres were not clearly defined. We observed reduced

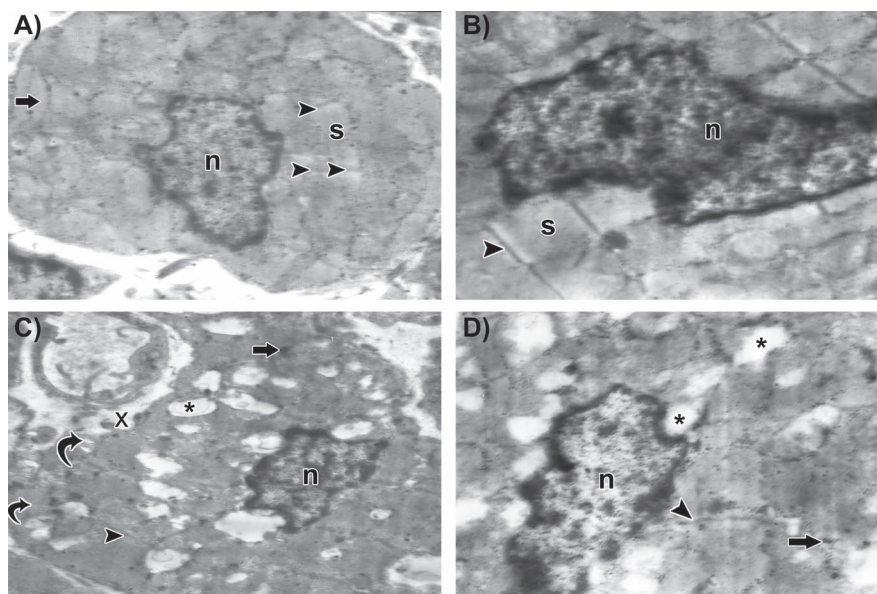


Fig. 4. Transmission electron microscopy of the myocardium of the Cont-Gr (A), Shm-Gr (B) and EMF-Gr (C and D) rats. Normal structure consisting of Z bands (arrowhead), nucleus (n), myofilaments (tailed arrow) and sarcomeres (S) can be seen in Cont-Gr and Shm-Gr animals. Disrupted Z bands (arrowhead), pyknotic nuclei (n), vacuoles (*), edematous areas (cross), structurally altered myofilaments (curled arrow) and electron dense particles (arrow) can be seen in EMF-Gr animals. $\times 8,000$.

myofilaments and increased vacuolization in myocardial cells of EMF-Gr animals. Also, we observed diffuse edema among the myofibrils.

High MDA and low GSH levels in rat liver, lung, testis and heart tissues (Esmekaya et al. 2011), and high MDA and low CAT and GSH levels in guinea pig brain (Meral et al. 2007) have been reported following exposure to EMF. Consistent with the literature, we found that the MDA level in EMF-Gr heart tissue was increased compared to both Shm-Gr and Cont-Gr animals. GSH and CAT levels in EMF-Gr animals decreased compared to Cont-Gr tissue. We attribute the increased MDA levels in EMF-Gr animals to lipid oxidation caused by EMF induced oxidative stress. The increased MDA levels in EMF-Gr animals was not observed in Shm-Gr animals, which suggests that EMF caused greater oxidative stress than being kept in the cage without EMF.

SOD levels in the EMF group were higher than those reported by Ozguner et al. (2005) and Kiray et al. (2013); we used a longer exposure to EMF than these two earlier studies. Türedi et al. (2015) reported high MDA and SOD, and low GSH levels that are comparable to our findings in the heart tissue of 21-day-old male rats exposed to a 900 MHz EMF for 1 h/day on days 13–21 of the prenatal period. By contrast to our investigation, however, these authors reported high CAT levels in the EMF

group. Based on our biochemical findings, it appears that exposure to EMF during adolescence increased oxidative stress in heart tissue.

Apoptosis can be initiated by both physiological and pathological agents (Elmore 2007); it plays an important role in the pathogenesis and progression of nearly all heart diseases (Gill et al. 2002, Takemura et al. 2013). EMF also can cause apoptosis in heart tissue. Tayefi et al. (2010) reported increased apoptosis in the heart tissue of rats exposed to EMF from the prenatal period through postnatal day 20. Kiray et al. (2013) reported increased apoptosis in adult rat heart tissue exposed to EMF in adulthood. Exposure to EMF during the prenatal period alone has been reported to cause increased apoptosis in male rat pup heart tissues during adolescence (Türedi et al. 2015).

We conclude that exposure to continuous 900 MHz for 1 h/day during adolescence may cause oxidative stress in the male adolescent rat, which then may produce morphological alterations in male adolescent rat heart tissue.

Acknowledgment

Our work was supported by the Karadeniz Technical University Research Fund, Project number: 12222.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

- Aebi H (1984) Catalase in vitro. *Methods Enzymol.* 105: 121–126.
- Aslan A, Aydoğan NH, Atay T, Çömlekçi S (2011) The effects of electromagnetic field exposure at short and long term 900 MHz frequency emitted from mobile phones on rat bone tissue. *Dicle Med. J.* 38: 452–457.
- Aslan A, Atay T, Gülle K, Kırdemir V, Özden A, Çömlekçi S, Aydoğan NH (2013) Effect of 900 MHz electromagnetic fields emitted from cellular phones on fracture healing: an experimental study on rats. *Acta Orthop. Traumatol. Turc.* 47: 273–280.
- Bancroft JD, Stevens A, Turner DR (1996) *Theory and Practice of Histological Techniques*. 4th ed., Churchill Livingstone, London. pp. 99–113.
- Bas O, Odacı E, Kaplan S, Acer N, Ucok K, Colakoglu S (2009) 900 MHz electromagnetic field exposure affects qualitative and quantitative features of hippocampal pyramidal cells in the adult female rat. *Brain Res.* 1265: 178–185.
- Baş O, Sönmez OF, Aslan A, İkinci A, Hancı H, Yıldırım M, Kaya H, Akça M, Odacı E (2013) Pyramidal cell loss in the cornu-ammonis of 32-day-old female rats following exposure to a 900 megahertz electromagnetic field during prenatal days 13–21. *NeuroQuantology* 11: 591–599.
- Elmore S (2007) Apoptosis: a review of programmed cell death. *Toxicol. Pathol.* 35: 495–516.
- Esmekaya MA, Ozer C, Seyhan N (2011) 900 MHz pulse-modulated radio frequency radiation induces oxidative stress on heart, lung, testis and liver tissues. *Gen. Physiol. Biophys.* 30: 84–89.
- Fychting M, Ahlbom A, Kheifets L (2005) EMF and health. *Ann. Rev. Publ. Health* 26: 165–189.
- Gandhi OP, Lazzi G, Furse CM (1996) Electromagnetic absorption in the human head and neck for mobile telephones at 835 and 1900 MHz? *IEEE Trans. Microw. Theory Tech.* 44: 1884–1897.
- Gandhi OP, Morgan LL, de Salles AA, Han YY, Herberman RB, Davis DL (2012) Exposure limits: the underestimation of absorbed cell phone radiation, especially in children. *Electromag. Biol. Med.* 31: 34–51.
- Genuis SJ (2008) Fielding a current idea: exploring the public health impact of electromagnetic radiation. *Publ. Health* 122: 113–124.
- Gill C, Mestrlil R, Samali A (2002) Losing heart: the role of apoptosis in heart disease—a novel therapeutic target? *FASEB J.* 16: 135–146.
- Hancı H, Odacı E, Kaya H, Aliyazıcıoğlu Y, Turan İ, Demir S, Çolakoğlu S (2013) The effect of prenatal exposure to 900-MHz electromagnetic field on the 21-old-day rat testicle. *Reprod. Toxicol.* 42: 203–209.
- Hancı H, Türedi S, Topal Z, Mercantepe T, Bozkurt I, Kaya H, Ersöz Ş, Ünal B, Odacı E (2015) Can prenatal exposure to a 900 MHz electromagnetic field affect the morphology of the spleen and thymus, and alter biomarkers of oxidative damage in 21-day-old male rats? *Biotech. & Histochem.* 90: 535–543.
- Hardell L, Sage C (2008) Biological effects from electromagnetic field exposure and public exposure standards. *Biomed. Pharmacother.* 62: 104–109.
- International Telecommunication Union (2014) *The World in 2014, ICT Facts and Figures*. Retrieved June 02, 2016 from <http://www.itu.int/en/ITU/Statistics/Documents/facts/ICTFactsFigures2014-e.pdf>.
- Kim SK, Choi JL, Kwon MK, Choi JY, Kim DW (2013) Effects of 60 Hz magnetic fields on teenagers and adults. *Environ. Health* 24: 12–42.
- Kiray A, Tayefi H, Kiray M, Bagriyanik HA, Pekcetin C, Ergur BU, Ozogul C (2013) The effects of exposure to electromagnetic field on rat myocardium. *Toxicol. Ind. Health* 29: 418–425.
- Kyrylkova K, Kyryachenko S, Leid M, Kioussi C (2012) Detection of apoptosis by TUNEL assay. *Methods Mol. Biol.* 887: 41–47.
- Leitgeb N (2008) Mobile phones: are children at higher risk? *Wien Med. Wochenschr.* 158: 36–41.
- Meral I, Mert H, Mert N, Deger Y, Yoruk I, Yetkin A, Keskin S (2007) Effects of 900-MHz electromagnetic field emitted from cellular phone non-brain oxidative stress and some vitamin levels of guinea pigs. *Brain Res.* 1169: 120–124.
- Morgan LL, Kesari S, Davis DL (2014) Why children absorb more microwave radiation than adults: the consequences. *J. Microsc. Ultrastruct.* 2: 197–204.
- Morgan LL, Miller AB, Sascio A, Davis DL (2015) Mobile phone radiation causes brain tumors and should be classified as a probable human carcinogen (2A) (review). *Int. J. Oncol.* 46: 1865–1871.
- Odacı E, Özyılmaz C (2015) Exposure to a 900 MHz electromagnetic field for 1 hour a day over 30 days does change the histopathology and biochemistry of the rat testis. *Int. J. Radia. Biol.* 91: 547–554.
- Odacı E, Bas O, Kaplan S (2008) Effects of prenatal exposure to a 900 MHz electromagnetic field on the dentate gyrus of rats: a stereological and histopathological study. *Brain Res.* 1238: 224–229.
- Odacı E, Hancı H, İkinci A, Sönmez OF, Aslan A, Şahin A, Kaya H, Çolakoğlu S, Baş O (2015a) Maternal exposure to a continuous 900-MHz electromagnetic field provokes neuronal loss and pathological changes in cerebellum of 32-day-old female rat offspring. *J. Chem. Neuroanat.* <http://dx.doi.org/10.1016/j.jchemneu.2015.09.002>
- Odacı E, Hancı H, Yuluğ E, Türedi S, Aliyazıcıoğlu Y, Kaya H, Çolakoğlu S (2015b) Effects of prenatal exposure to a 900 MHz electromagnetic field on 60-day-old rat testis and epididymal sperm quality. *Biotech. & Histochem.* 90: 93–101.
- Odacı E, Ünal D, Mercantepe T, Topal Z, Hancı H, Türedi S, Erol H, Mungan S, Kaya H, Çolakoğlu S (2015c) Pathological effects of prenatal exposure to a 900 MHz electromagnetic field on the 21-day-old male rat kidney. *Biotech. & Histochem.* 90: 93–101.

Ohkawa H, Ohishi H, Yagi K (1979) Assay for lipid peroxide in animal tissues by thiobarbituric acid reaction. *Anal. Biochem.* 95: 351–358.

Ozguner F, Altinbas A, Ozaydin M, Dogan A, Vural H, Kisioglu AN, Cesur G, Yildirim NG (2005) Mobile phone-induced myocardial oxidative stress: protection by a novel antioxidant agent caffeic acid phenethyl ester. *Toxicol. Ind. Health* 21: 223–230.

Repacholi MH (2001) Health risks from the use of mobile phones. *Toxicol. Lett.* 120: 323–331.

Schüz J (2005) Mobile phone use and exposures in children. *Bioelectromagnetics* 7: S45–S50.

Sedlak J, Lindsay RH (1968) Estimation of total, protein-bound, and non-protein sulfhydryls groups in tissue with Ellman's reagent. *Anal. Biochem.* 25: 192–205.

Sonmez OF, Odaci E, Bas O, Kaplan S (2010) Purkinje cell number decreases in the adult female rat cerebellum following exposure to 900 MHz electromagnetic field. *Brain Res.* 1356: 95–101.

Sun Y, Larry WO, Ying L (1988) A simple method for clinical assay of superoxide dismutase. *Clin. Chem.* 34: 497–500.

Takemura G, Kanoh M, Minatoguchi S, Fujiwara H (2013) Cardiomyocyte apoptosis in the failing heart—a

critical review from definition and classification of cell death. *Int. J. Cardiol.* 167: 2373–2386.

Tayefi H, Kiray A, Kiray M, Ergur BU, Bagriyanik HA, Pekcetin C, Fidan M, Ozogul C (2010) The effects of prenatal and neonatal exposure to electromagnetic fields on infant rat myocardium. *Arch. Med. Sci.* 6: 837–842.

Topal Z, Hancı H, Mercantepe T, Erol HS, Keleş ON, Kaya H, Mungan S, Odacı E (2015) The effects of prenatal long-duration exposure to 900 megahertz electromagnetic field on the 21-day old newborn male rat liver. *Turk. J. Med. Sci.* 45: 291–297.

Türedi S, Hancı H, Topal Z, Ünal D, Mercantepe T, Bozkurt İ, Kaya H, Odacı E (2015) The effects of prenatal exposure to a 900-MHz electromagnetic field on the 21-day-old male rat heart. *Electromag. Biol. Med.* 34: 390–397.

Valberg PA, van Deventer TE, Repacholi MH (2007) Workgroup report: base stations and wireless networks-radiofrequency (RF) exposures and health consequences. *Environ. Health. Perspec.* 115: 416–424.

World Health Organization (2010) WHO research agenda for radiofrequency fields. Retrieved May 23, 2015 from <http://www.vodafone.com.au/doc/who-rf-2010.pdf>