



Biochemical and pathological changes in the male rat kidney and bladder following exposure to continuous 900-MHz electromagnetic field on postnatal days 22–59

Sibel Türedi, Gökçen Kerimoğlu, Tolga Mercantepe & Ersan Odacı

To cite this article: Sibel Türedi, Gökçen Kerimoğlu, Tolga Mercantepe & Ersan Odacı (2017) Biochemical and pathological changes in the male rat kidney and bladder following exposure to continuous 900-MHz electromagnetic field on postnatal days 22–59, International Journal of Radiation Biology, 93:9, 990–999, DOI: [10.1080/09553002.2017.1350768](https://doi.org/10.1080/09553002.2017.1350768)

To link to this article: <https://doi.org/10.1080/09553002.2017.1350768>



Published online: 27 Jul 2017.



Submit your article to this journal [↗](#)



Article views: 495



View related articles [↗](#)



View Crossmark data [↗](#)



Citing articles: 8 View citing articles [↗](#)

ORIGINAL ARTICLE



Biochemical and pathological changes in the male rat kidney and bladder following exposure to continuous 900-MHz electromagnetic field on postnatal days 22–59*

Sibel Türedi^a, Gökçen Kerimoğlu^a, Tolga Mercantepe^b and Ersan Odacı^{a†}

^aDepartment of Histology and Embryology, Faculty of Medicine, Karadeniz Technical University, Trabzon, Turkey; ^bDepartment of Histology and Embryology, Faculty of Medicine, Recep Tayyip Erdoğan University, Rize, Turkey

ABSTRACT

Purpose: To investigate the effect on male rat kidney and bladder tissues of exposure to 900-megahertz (MHz) electromagnetic field (EMF) applied on postnatal days 22–59, inclusive.

Materials and methods: Twenty-four male Sprague Dawley rats, aged 21 days, were used. These were divided equally into one of three groups, control (CG), sham (SG) or EMF (EMFG). CG was not exposed to any procedure. SG rats were kept inside a cage, without being exposed to the effect of EMF, for 1 h a day on postnatal days 22–59, inclusive. EMFG rats were exposed to continuous 900-MHz EMF for 1 h a day under the same conditions as those for the SG rats. Rats were sacrificed on postnatal day 60, and the kidney and bladder tissues were removed. Tissues were stained with hematoxylin and eosin (H&E) and Masson trichrome for histomorphological evaluation. The TUNEL method was used to assess apoptosis. Transmission electron microscopy (TEM) was also used for the kidney tissue. Oxidant/antioxidant parameters were studied in terms of biochemical values.

Results: The findings showed that tissue malondialdehyde increased in EMFG compared to CG and SG in both kidney ($p = 0.004$ and $p = 0.004$, respectively) and bladder tissue ($p = 0.004$, $p = 0.006$, respectively), while catalase and glutathione levels decreased compared to CG ($p = 0.004$; $p = 0.004$, respectively) and SG ($p = 0.004$; $p = 0.004$, respectively). In the EMF group, pathologies such as dilatation and vacuolization in the distal and proximal tubules, degeneration in glomeruli and an increase in cells tending to apoptosis were observed in kidney tissue. In bladder tissue, degeneration in the transitional epithelium and stromal irregularity and an increase in cells tending to apoptosis were observed in EMFG. Additionally, EMFG samples exhibited glomerular capillary degeneration with capillary basement membranes under TEM.

Conclusions: We conclude that continuous exposure to the effect of 900-MHz EMF for 1 h a day on postnatal days 22–59, inclusive, causes an increase in oxidative stress and various pathological changes in male rat kidney and bladder tissues.

ARTICLE HISTORY

Received 9 April 2017

Revised 9 June 2017

Accepted 20 June 2017

KEYWORDS

Electromagnetic field; kidney; bladder; oxidative stress; male rat

Introduction

While modern communication devices such as mobile phones that have become inseparable parts of daily life bestow numerous advantages, they are also raising health concerns due to the electromagnetic field (EMF) effects they create during use (Feychting et al. 2005; Davis et al. 2013; Hancı et al. 2013). Numerous studies have shown that exposure to EMF produced by mobile phones causes pathological effects (Salford et al. 2003; Bas et al. 2013; Racuciu et al. 2015). These studies have reported that exposure to EMF causes homological and cardiac disorders, irregularities in genital system functions, adverse effects on developmental stages and genetic disorders (Oral et al. 2006; Hardell & Sage 2008; Ghanbari et al. 2013; Lai et al. 2016). It has also been maintained that it can cause behavioral disorder in children,

memory and sleep problems, brain tumors and inner ear injury in association with long-term use (Ragy 2015).

The biological effects of EMF deriving from mobile phones depend on the duration of exposure, the organ affected, the characteristics of the tissue comprising the organ and the proximity of the organ to the source of EMF (Ahlbom et al. 2008; Odacı & Özyılmaz 2015; Ragy 2015). On the basis of this information, the effects of EMF deriving from mobile phones may vary depending on where the device is carried and the organ and tissue affected. For example, when the mobile phone is carried in an upper left shirt pocket, there is a strong probability that breast tissue or the heart close to that region will be affected. If carried inside a case attached to a belt, there is a strong possibility that the liver, kidney, bladder, ovary or testes will be exposed to the effects of EMF emitted by mobile phones (Odacı & Özyılmaz 2015;

CONTACT Sibel Türedi ✉ sibelturedi3361@hotmail.com Department of Histology and Embryology, Faculty of Medicine, Karadeniz Technical University, Trabzon, Turkey

*The biochemical results and histomorphological observations in this study appeared as poster presentations at the 6th World Congress of Oxidative Stress, Calcium Signaling and TRP Channels, 24–27 May 2016, Isparta, Turkey.

[†]Deceased.

Türedi et al. 2015, 2016). The kidneys play an important role in maintaining homeostasis in the body by helping regulate the amount of water and dissolved substances inside it eliminated from the body, the expulsion of metabolic wastes, controlling the volume of body fluid and the production of hormones such as renin, prostaglandin, erythropoietin and vitamin D. They are also known to influence erythrocyte production and the regulation of blood pressure (Dankers et al. 2011; Odacı et al. 2014). The kidneys filter 20% of the blood in the body in 1 minute, and are therefore at high risk of being affected by harmful substances. Studies have reported that the kidneys are highly susceptible to radiation (Cassady 1995), and that more radiation emitted by mobile phones operating at 900 MHz and carried on the belt is absorbed by the kidneys compared to other organs (Koca et al. 2013). Koca et al. (2013) reported that the EMF emitted by mobile phones causes glomerular damage, dilation and impairment in Bowman's capsule, tubular injury and inflammatory cell infiltration in kidney tissue.

There is wide consensus that since tissues and organs in children and adolescents have not yet achieved their adult anatomical structures, adolescents will be more affected by EMF than adults (Scientific Committee on Emerging and Newly Identified Health Risks [SCENIHR] 2007; Morgan et al. 2014). We encountered no previous studies investigating the potential effects in rat and bladder tissues of rats exposed to 900-MHz during the different stages of adolescence. In rats, adolescence begins on postnatal day 21 and ends on postnatal day 59 (Tirelli et al. 2003). The purpose of this study was therefore to investigate the effects of exposure to 900-MHz EMF in adolescence on the kidney and bladder tissue of male rats.

Materials and methods

Animals and ethical procedures

Animals were obtained from the Karadeniz Technical University (KTU) Experimental Animals Surgical Research and Application Center. All the study procedures were approved by the KTU Animal Care and Ethics Committee (Date: 3 March 2015, Ethical Committee Decision Number: 4). All procedures were also consistent with the Guide for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health.

Experimental procedures

Twenty-four male Sprague Dawley rats (21 days old and weighing 28.3–43.9 g) were used. Rats were divided into three groups of eight rats each, control (CG), sham (SG) and electromagnetic field (EMFG) groups. All rats were maintained in the rat care room at $22 \pm 2^\circ\text{C}$ with $50 \pm 10\%$ humidity and under a 12 h light/12 h dark cycle. They were allowed access to commercially available standard rat chow (Bayramoğlu Feed and Flour Industry Trading Corporation Erzurum, Turkey) and tap water *ad libitum* throughout the experiment.

EMF application apparatus, sham and EMF applications

During sham and EMF applications, the SG and EMFG rats were taken to a separate room, known as the EMF exposure

room, containing only the apparatus used for EMF and sham administration. An apparatus used and described in detail in previous studies by the authors was also employed in the present study. This apparatus, designed by the authors and known as the EMF application apparatus, was placed inside the EMF exposure room (Hancı et al. 2013; Odacı et al. 2014; Odacı & Özyılmaz 2015; Türedi et al. 2015). Briefly, the EMF application apparatus used to establish a 900-MHz EMF consisted of three main components: an ultra-high frequency oscillator (1218-BV, ultra-high frequency lockable oscillator, 900–2000 MHz, General Radio Company, Concord, MA, U.S.A., Serial No. 1483) with an output power of approximately 300 mW and a frequency adjusted to 900 MHz, an uninterrupted power source providing constant electricity to the oscillator (1267-B Regulated Power Supply, General Radio Company, Concord, MA, U.S.A., Serial No. 903) and a cage made from Plexiglas (42 cm long $\times$ 30 cm wide $\times$ 50 cm high). The uninterrupted power source was attached by a cable to the city electricity grid and provided constant electricity for the oscillator through another cable. The oscillator outlet was attached to a semi-wave dipole antenna, 1 mm in diameter and 15 cm in length, by a coaxial cable. This copper antenna was placed approximately 11 cm beneath the open top and in the middle of the cage.

No procedure was performed on the CG rats, which were kept in the rat care room throughout the experiment. The EMFG rats were exposed to continuous 900-MHz EMF for 1 h a day inside the Plexiglas cage in the EMF exposure room from 11.00–12.00 h every day between postnatal days 22 and 59, inclusive. The SG rats were placed inside the same Plexiglas cage in the EMF exposure room from 10.00–11.00 h every day between postnatal days 22 and 59, inclusive, but were not exposed to EMF. After each sham and EMF application, all rats were transferred back to the rat care room.

Every day throughout the experiment, before the SG and EMF rats were taken to the EMF exposure room, the effect on application of the electrical fields and electromagnetic sources in the laboratory was determined by measuring the intensity of EMF inside the exposure room. A broadband EMF intensity meter (Chauvin Arnoux CA43 Isotropic Electrical Field Intensity Meter, Paris, France) with a 100–2.5 GHz measurement range was used for that purpose. Sham and EMF applications were initiated once we had ensured that there was no EMF effect inside the EMF exposure room. In addition, intensity of EMF was measured, using the same EMF intensity meter, during EMF application in EMFG, from nine separate points inside and beneath the Plexiglas cage, both before and after the rats were placed inside. Calculation from the measurements obtained showed that EMFG rats were exposed to a mean EMF intensity of 8.4 V/m (0.187 W/m^2) inside the cage. The whole-body specific absorption rate (SAR) was calculated at 0.0067 W/kg (RadHaz SAR Equivalency Calculator Version 1.0, Richard Tell Associates, Inc., Mesquite, NV, U.S.A.).

Mean temperature and humidity levels in the EMF exposure cage were measured using a digital temperature and humidity measuring device (YCOM KMN-303, Yu Yau Shuanghe Electron Instrument Co., Ltd, Zhejiang, China). Mean temperature within the EMF cage was $21.82 \pm 1.01^\circ\text{C}$ and

$21.9 \pm 1.09^{\circ}\text{C}$ during sham procedures and EMF applications, respectively. The mean temperature within the EMF exposure room and empty EMF exposure cage during sham procedure and EMF applications was $22 \pm 2^{\circ}\text{C}$. Mean humidity levels within the EMF exposure cage were $54.4 \pm 8.11\%$ and 51.8 ± 7.22 during sham procedures and EMF applications, respectively. The mean humidity within the EMF exposure room and empty EMF exposure cage during sham procedures and EMF applications was $50 \pm 10\%$.

Tissue preparation for analyses

At the end of the experimental period (postnatal day 60) all rats were sacrificed by cervical dislocation under deep anesthesia (Ketalar[®] 50 mg/kg, Eczacıbaşı Co., Istanbul, Turkey), and kidney and bladder tissues were removed. One part of the tissues was used for biochemical analyses. Kidney and bladder tissues were placed into separate tubes (1.5 ml, Eppendorf, Hamburg, Germany) for biochemical analyses and stored at -80°C in a deep freeze (U410 Premium; Ultra-Low Temperature Laboratory Freezers, New Brunswick Scientific Co., Inc., St. Albans, Herts, UK). Blocks of kidney tissue 1 mm^3 in size were used for transmission electron microscopy (TEM) investigation. Kidney and bladder tissues left over after these procedures were used for light microscopic procedures.

Light microscopic examination procedures

Kidney and bladder tissues were fixed in 10% neutral formaldehyde solution for light microscopic examinations. Following routine dehydration and transparency procedures, the tissues were embedded in paraffin blocks. Sections 5 micrometers (μm) in thickness were taken from these paraffin blocks using a rotary microtome (RM 2255; Leica Instruments, Nussloch, Germany). One part of these was stained with hematoxylin and eosin (H&E) and Masson trichrome. The other sections were used for apoptosis evaluations. Histopathological evaluations of sections stained with H&E and were performed by a histologist blinded to the study groups under a light microscope (BX51, Olympus, Tokyo, Japan). Microphotographs were taken at different magnifications using an Olympus DP 71 (Olympus, Tokyo, Japan) microscope camera.

TUNEL assay and apoptosis evaluation

The terminal deoxynucleotidyl transferase (TdT) deoxyuridine triphosphate nick end labeling assay (TUNEL) method was employed to assess apoptosis in kidney and bladder specimens, and DNA fragmentation was determined in cells from both tissues. Sections $5\text{ }\mu\text{m}$ in thickness were taken from the paraffin blocks described above and subjected to standard deparaffinization. TUNEL staining of sections was performed using an *in situ* cell Death Detection Kit (Roche, Mannheim, Germany), in accordance with the manufacturer's instructions. Apoptosis evaluation from the TUNEL-stained slides was performed using a light microscope (Olympus, BX51, Tokyo, Japan) at a magnification of $\times 400$. One hundred cells (tubular

epithelium cells in kidneys and epithelial cells from the bladder) in five different areas at $\times 400$ magnification, and the apoptotic index (AI) was calculated using the formula $\text{AI} = \text{number of TUNEL (+) cells} / \text{total cell number} \times 100$ (Türedi et al. 2016).

Transmission electron microscopy procedures

Sampling, pre-fixation, washing, post-fixation, washing, dehydration, saturation, embedding, polymerization, ultrathin sectioning, observation and photographic image capture were used in processing kidney tissue samples for electron microscopy. For this purpose, 1-mm kidney specimens were first fixed in 100 mM phosphate buffer containing 2.5% glutaraldehyde for 1.5 h at 4°C . The kidneys were then washed in phosphate buffer and stored at 4°C for later processing. The kidneys were post-fixed in 1% osmium tetroxide, dehydrated in an ethanol series, and then embedded in epoxy resin (Araldite CY212) kit (Agar Scientific, Essex, UK). Sagittal sections 750 nanometers in thickness were cut and stained with toluidine blue for light microscopy analysis. Stained sections were visualized and imaged by camera (Leica DM 6400, Leica Microsystems, Wetzlar, Germany). For electron microscopy analysis, ultrathin sections 40–70 nm thick were cut using an ultramicrotome (LKB Nova, Bromma, Sweden) set on 200-mesh copper or nickel grids. Ultra-thin sections at the top of the nickel grid were stained with uranyl acetate and with Reynold's lead citrate for TEM analysis. Images were obtained using a $1005 \times$ TEM (JEOL Ltd, Akishima, Tokyo, Japan) and recorded on photographic image capture film (Kodak 4489, Eastman Kodak Co., Rochester, NY, U.S.A.).

Biochemical tissue analyses

Superoxide dismutase (SOD) and catalase (CAT) enzyme activities and levels of glutathione (GSH) and lipid peroxidation (LPO) were determined in the kidney and bladder tissues. In order to prepare the tissue homogenates, the tissues were ground with liquid nitrogen in a mortar. In the next step, 0.5 g of tissue was treated with 4.5 ml of appropriate buffer (SOD: pH 7.4/0.2 mM Tris-HCl buffer, CAT: pH 7/50 mM phosphate buffer, GSH: pH 7.4/50 mM Tris-HCl buffer, LPO: %10 KCl solution). The tissue mixtures were homogenized on ice for 15 min using a homogenizer (Ultra Turrax T25; Rose Scientific Ltd, Edmonton, Canada). Homogenates were filtered and centrifuged at 4°C . These supernatants were subsequently used for biochemical measurements. All biochemical measurements were carried out using a UV-Vis spectrophotometer (UV-1601; Shimadzu Co., Kyoto, Japan). Kidney and bladder tissue LPO levels were measured by estimating malondialdehyde (MDA) levels using the thiobarbituric acid test (Ohkawa et al. 1979). The standard curve was obtained by using 1, 1, 3, 3-tetramethoxypropane. The results were expressed as nmol/g tissue. Estimations of SOD activities were based on the manufacture of superoxide radicals generated by xanthine and xanthine oxidase. SOD activity was measured at 560 nm by the degree of inhibition of this reaction. Activity was expressed as mmol/min/mg kidney and

bladder tissues (Sun et al. 1988). Decomposition of H_2O_2 in the presence of CAT was observed at 240 nm. CAT activity was defined as the amount of enzyme required to decompose 1 mmol of hydrogen peroxide (H_2O_2) per minute, at 25 °C and at pH 7.8. Results were expressed as mmol/min/mg of tissue (Aebi 1974). GSH levels were measured using 5, 5'-dithiobis (2-nitrobenzoic acid) following the modifying method described by Sedlak and Lindsay (1968). The absorbance was measured at 412 nm, and tissue GSH levels were expressed as nmol/mg of tissues.

Statistical analysis

All statistical analyses were performed on SPSS (Statistical Package for the Social Sciences, version 13.1, SPSS Inc., Chicago, IL, U.S.A.) software. Data are expressed as mean $\pm$ standard deviation (SD). Kruskal Wallis analysis of variance and the Mann-Whitney U test with Bonferroni correction were used for statistical analysis. Statistical significance was set at $p < 0.05$ and $p < 0.001$.

Results

Biochemical analysis results

The study findings showed that tissue MDA levels increased in EMFG compared to CG and SG in kidney ($p = 0.004$ and $p = 0.004$, respectively) and bladder tissue ($p = 0.004$, $p = 0.006$, respectively). CAT and GSH levels in EMFG decreased compared to CG ($p = 0.004$; $p = 0.004$, respectively) and SG ($p = 0.004$; $p = 0.004$, respectively). However, an increase was observed in SOD values in kidney tissues in EMFG ($p = 0.006$), but not in bladder tissue ($p > 0.05$) (Table 1).

Histopathological findings

Kidney tissues in H&E stained sections from CG and SG had a normal histological appearance, and no pathology was observed (Figure 1(A,B)). However, dilatation and vacuolization in the distal and proximal tubules, tubular epithelial cell loss and glomerular degeneration and edema were determined in kidney tissue from EMFG (Figure 1(C,D)). No pathology was observed at morphological assessment of bladder tissue sections stained with H&E and Masson's trichrome

from CG and SG (Figures 2(A,B) and 3(A,B)). However, EMFG Masson trichrome stained sections exhibited histopathological injury such as degeneration in the transitional epithelium, followed by desquamation and stromal irregularity (Figures 2(C,D), and 3(C,D)).

Immunohistochemical analysis results

Apoptotic characteristics were further confirmed by TUNEL stained sections under light microscopy at a magnification of $\times 400$. Immunohistochemical evaluation was based on the intensity of TUNEL (+) cells in the kidney and bladder tissues. Analysis of kidney and bladder sections stained using the TUNEL method from CG and SG revealed few apoptotic cells in epithelial tissues (Figure 4(A,B,D,E)). Sections from the EMF group exhibited significant quantities of apoptotic cells in the proximal-distal tubular epithelium and transitional bladder epithelium (Figure 4(C,F)). The AI in kidney and bladder tissue increased significantly in EMFG compared to CG and SG ($p < 0.001$). Additionally, AI increased significantly in SG compared to CG ($p < 0.001$) (Table 1).

Transmission electron microscopy observation results for kidney tissue

Typical normal appearances in glomerular capillary and basal membrane structures and the lamina densa and lamina rara interna and externa were observed in CG samples at TEM investigation. Typical podocytes and foot processes were observed in CG (Figure 5(A)). SG samples exhibited a similar morphological structure in kidney tissue to the CG specimens, with typical glomerular capillary, podocytes and foot processes at TEM analyses (Figure 5(B)). Additionally, glomerular capillary degeneration with capillary basement membrane, and loss of lamina densa, lamina rara interna and externa were observed in EMFG samples under TEM. Atypical podocytes, pedicel degeneration and narrowing in Bowman capsule space were also observed in EMFG samples (Figure 5(C,D)).

Discussion

Numerous studies have reported that the EMF emitted by mobile phones causes injury at the cellular and molecular

Table 1. Biochemical analysis results and apoptotic index values of kidney and bladder tissues for control, sham and electromagnetic field (EMF) groups.

Parameters	Control group		Sham group		EMF group	
	Kidney	Bladder	Kidney	Bladder	Kidney	Bladder
MDA (nmol/mg tissue)	48.84 $\pm$ 1.82	14.66 $\pm$ 0.37	46.80 $\pm$ 0.80	16.17 $\pm$ 1.04	85.20 ^a $\pm$ 4.75	20.31 ^a $\pm$ 1.85
CAT (mmol/min/mg tissue)	574.73 $\pm$ 42.24	114.21 $\pm$ 11.05	565.14 $\pm$ 30.58	119.85 $\pm$ 14.53	463.33 ^b $\pm$ 20.84	73.88 ^b $\pm$ 11.65
GSH (nmol/mg tissue)	0.91 $\pm$ 0.04	0.62 $\pm$ 0.02	0.72 $\pm$ 0.03	0.73 $\pm$ 0.03	0.6 ^b $\pm$ 0.06	0.53 ^b $\pm$ 0.02
SOD (mmol/min/mg tissue)	0.45 $\pm$ 0.02	0.25 $\pm$ 0.01	0.47 $\pm$ 0.01	0.24 $\pm$ 0.01	0.49 ^c $\pm$ 0.0	0.25 $\pm$ 0.01
Apoptotic index (%)	13.2 $\pm$ 1.85	15.89 $\pm$ 2.92	23.2 ^d $\pm$ 2.71	23.07 ^d $\pm$ 2.64	39.28 ^e $\pm$ 3.55	42.19 ^e $\pm$ 5.43

Data are expressed as mean $\pm$ SEM; $n = 8$ for each group; MDA: malondialdehyde; SOD: superoxide dismutase; CAT: catalase; GSH: glutathione. CG: control group; SG: sham group; EMFG: EMF group.

^aMDA levels increased in EMFG compared to CG and SG in kidney ($p = 0.004$ and $p = 0.004$, respectively) and bladder tissue ($p = 0.004$, $p = 0.006$, respectively).

^bCAT and GSH levels decreased in EMFG compared to CG and SG in kidney ($p = 0.004$; $p = 0.004$, respectively) and bladder tissue ($p = 0.004$; $p = 0.004$, respectively).

^cSOD level increase in EMFG compared to CG and SG in kidney ($p = 0.006$; $p = 0.006$, respectively).

^dApoptotic index value increased in SG compared to CG in kidney and bladder tissue ($p < 0.001$).

^eApoptotic index value increased in EMFG compared to CG and SG in kidney and bladder tissue ($p < 0.001$).

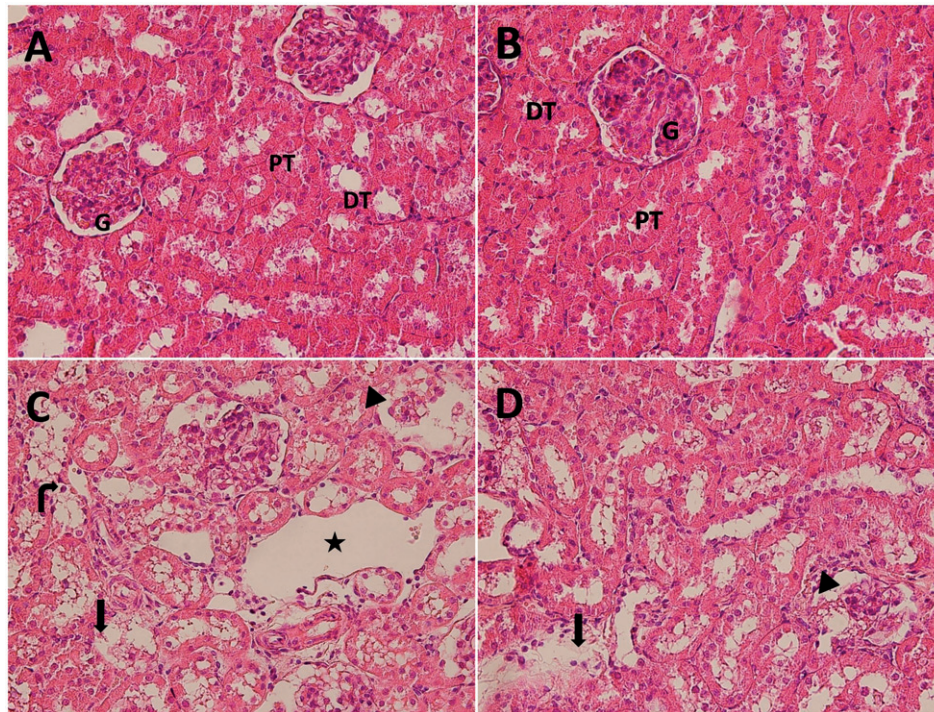


Figure 1. Representative photomicrographs of kidney tissues for the control (A), sham (B) and electromagnetic field (C and D) groups. While regular morphologies were observed in the control (A) and sham groups (B), widespread tubular (arrow) and glomerular damage (arrow head), dilatation of spaces between the tubules (star), and vacuolization of tubule epithelial cells (curved arrow) were observed in the electromagnetic field group (C, D) (G, glomerulus; DT, distal tubule; PT, proximal tubule) (H&E staining, Panel A, B, D and C $\times 400$ original).

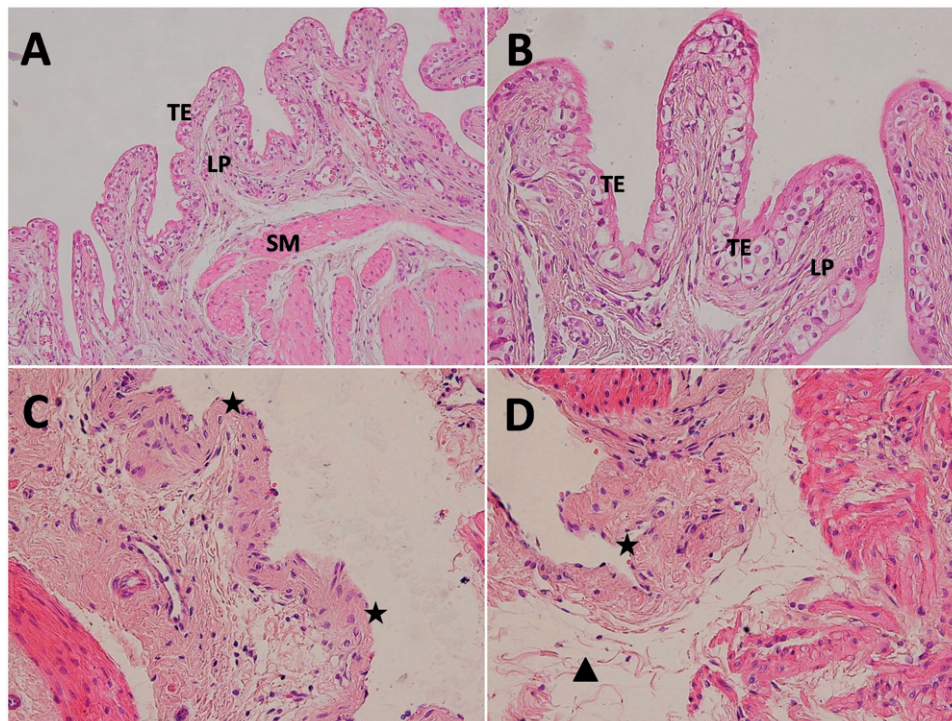


Figure 2. Representative photomicrographs of urinary bladder tissues for each group. No pathology was observed in the control (A) and sham groups (B) at histopathological evaluation. Widespread degeneration and desquamation in the transitional epithelium (stars) and stromal irregularity (arrow head) were observed in the electromagnetic field group (C and D) (TE, transitional epithelium; LP, lamina propria; SM, smooth muscle) (H&E staining, Panel A, B $\times 200$; Panel C, D $\times 400$ original).

levels in adult living tissue (Salford et al. 2003; Bas et al. 2013; Racuciu et al. 2015). Some studies of adult experimental animals have reported that EMF applied at different frequencies and for varying durations can cause pathological effects in kidney tissue, including glomerular atrophy,

pyknotic nuclei in the renal tubule epithelium and cytoplasmic vacuolization (Ozturk et al. 2003; Fahmy et al. 2015). Odacı et al. reported degeneration in the kidney tubule epithelium on postnatal day 21 in rat pups exposed to 900-MHz applied between days 13 and 21 of pregnancy

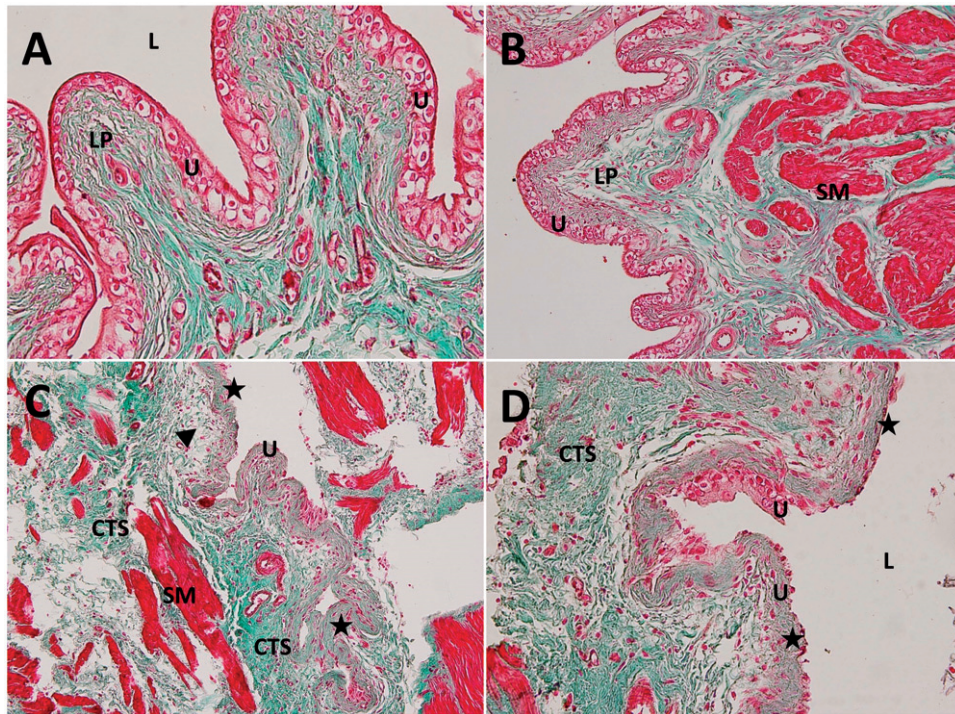


Figure 3. Representative images of Masson trichrome-stained sections of urinary bladder tissues. The lumen (L), urothelium (U), lamina propria (LP), connective tissue structures (CTS) and smooth muscle (SM) layers are indicated. Normal morphology was observed in the control (A) and sham groups (B), while widespread degeneration and desquamation in the transitional epithelium (stars), and stromal irregularity (arrow head) were observed in the electromagnetic field group (C and D) at histopathological evaluation (Masson Trichrome staining, Panels A and D $\times 400$; Panels B and C $\times 200$ original).

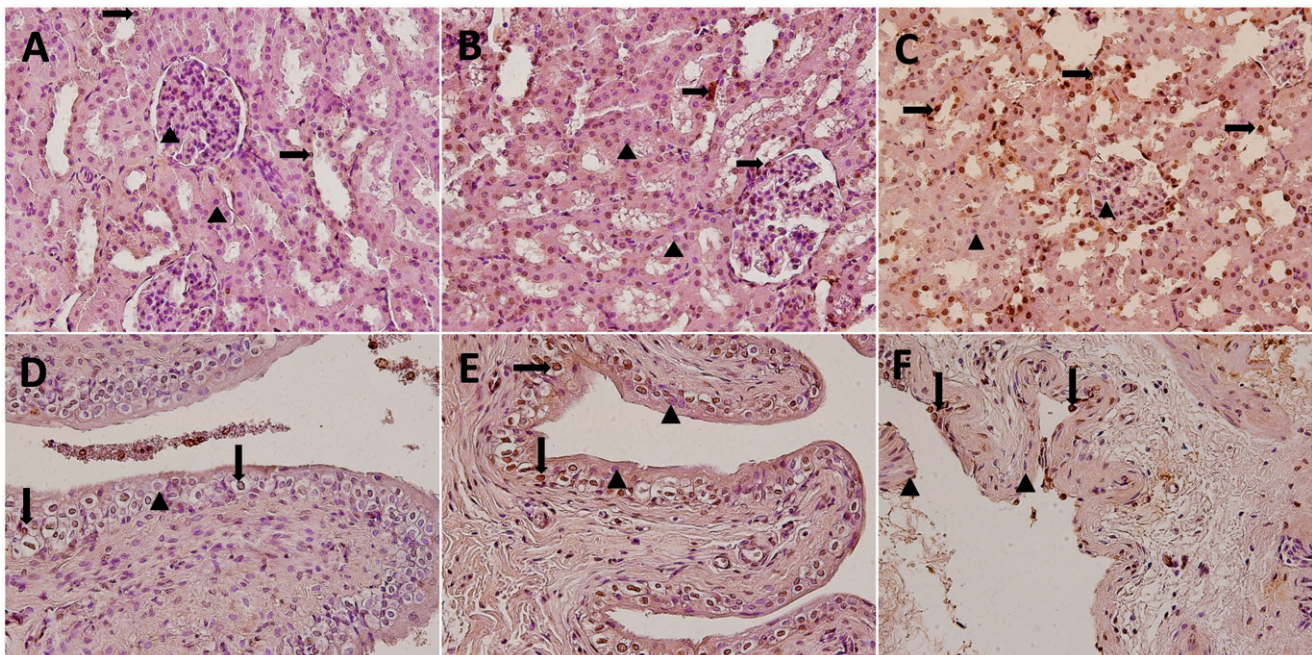


Figure 4. Representative photomicrographs of TUNEL-stained sections from kidney and bladder tissues for the control (A and D, respectively), sham (B and E, respectively) and electromagnetic field (C and F, respectively) groups. Normal cells (arrow heads) in addition to a smaller number of TUNEL (+) apoptotic cells (arrow) were observed on control and sham group sections. High levels of apoptotic cells (arrow) were observed in the proximal-distal tubular epithelium (C) and transitional epithelium of the urinary bladder (F) in electromagnetic field group sections (TUNEL staining, all panels $\times 400$ original).

(Odaci et al. 2014). In addition, Bedir et al. (2015) emphasized that exposure to EMF emitted from mobile phones in the prenatal period can lead to moderate histopathological injury, and that this can result in kidney injury in the postnatal period. Ulubay et al. (2015) reported a decrease in total glomerulus numbers and in cortex-medulla volume in

experimental group rats. Koca et al. (2013) reported that 900-MHz EMF emitted by mobile phones caused injury in glomeruli and tubules in kidney tissue and dilation and impairment in Bowman's capsule. In addition, they reported irregularities in the capillary endothelium, thickening in the basal membrane and damaged connections among tubules at electron

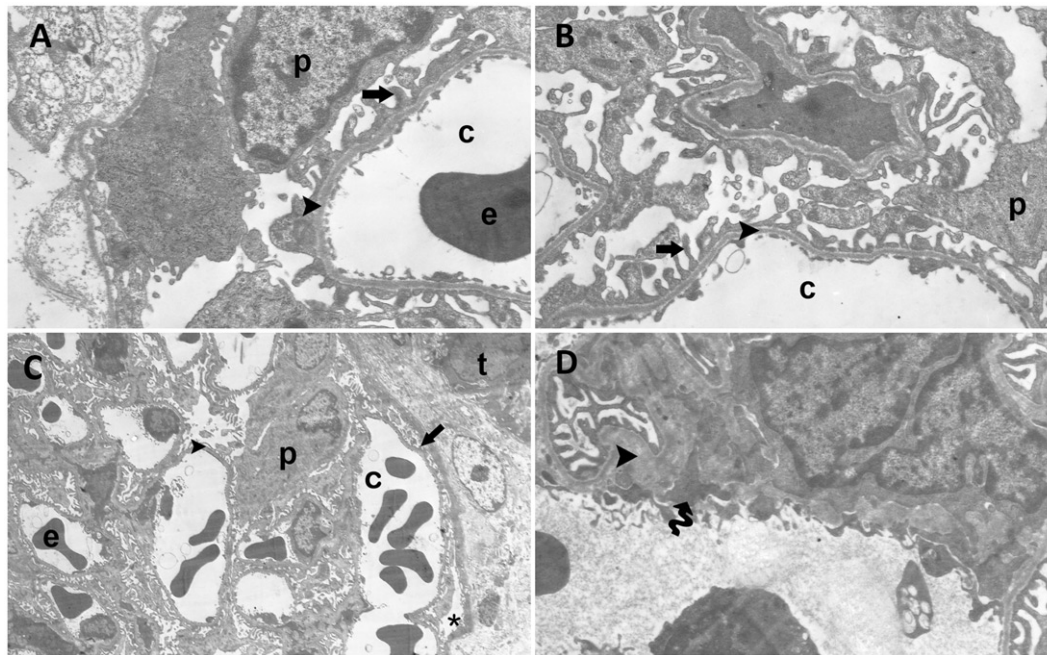


Figure 5. Transmission electron micrographs of kidney tissues from the control (A), sham (B) and electromagnetic field (C, D) groups. A normal capillary (C) basement membrane with lamina densa and lamina rara interna and externa (arrow head) were observed in control (A) and sham (B) group sections (panels A and B $\times 10,000$). Additionally, glomerular capillary (C) basement membrane with loss of lamina densa and lamina rara were disappear (arrow) were observed in electromagnetic field group (C and D). Narrowing in Bowman capsule spaces (asterisk), podocyte degeneration (p), podocyte foot process folding (arrow) and pyknotic nuclei were also observed in (C) and (D). Additionally, dense subendothelial electron deposits along the glomerular basement were seen in the glomerular capillary of electromagnetic field group animals (spiral arrow). p, podocyte; e, erythrocyte, pedicel (arrow), tubules (t) (Panel C, D $\times 2000$ original).

microscopic examination. Under light microscopy, we observed severe glomerular degeneration in the kidney tissue of rats exposed to 900-MHz EMF in adolescence, and diffuse tubular dilation and vacuolization in tubular epithelium cells, together with a low level of edema. Our TEM findings indicated glomerular degeneration, loss of lamina rara interna and externa, atypical podocytes and pedicel degeneration together with narrowing in Bowman's capsule in the experimental group. Tubule damage in the kidney plays an important role in acute renal disorders. The most important marker demonstrating the severity of that damage is glomerular injury (Koca et al. 2013). In the light of our findings and the information in the literature, it may be concluded that EMF applied for different periods and at different developmental stages can give rise to serious histopathological changes in kidney tissue.

The bladder consists of four main layers, the urothelium, the suburothelium, detrusor smooth muscle and an external serous membrane. The urothelium serves as a permeable barrier, protecting tissues in other layers from harmful compounds in urine. Injury in the structure of the bladder endothelium will result in vasodilation, the deposition of immune complexes and infiltration. This gives rise to oversecretion of inflammatory mediators and erythematous swelling and bleeding in the bladder. Dysfunctional pathology of the smooth muscle in the bladder wall results in thickening of the wall as a result of an increase in fibrotic tissue and hyperplasia or hypertrophy in the detrusor smooth muscle. The bladder is unable to adapt to this, and pathological injury develops in the muscle layer (Tyagi et al. 2010; Qiao et al. 2014). In addition to kidney tissue, we also investigated the

effects of exposure to 900-MHz EMF on rat bladder tissue. Our findings revealed degeneration and subsequent desquamation in the bladder epithelium, fragmentation in the lamina propria and cells tending to apoptosis. Band et al. (2005) investigated the incidence of bladder cancer in a case-control study and suggested that exposure to EMF may involve an increased risk of the disease. Gurbuz et al. (2010) investigated the genotoxic effect of pulse-modulated 1800-MHz GSM radio frequency radiation (RFR) on bladder cells. Although an increase in exfoliated bladder cells was observed compared to the control group, the difference was not statistically significant. We encountered no previous studies investigating potential injury to rat bladder tissue caused by exposure to 900-MHz EMF. From that perspective, the current study is the first to report that exposure in adolescence to the effect of 900-MHz emitted by mobile phones can result in damage to bladder tissue.

EMF emitted by mobile phones causes a change in transmembrane signaling, which affects the electrical structure of cell membrane proteins and ion passage. It is also known to exhibit genotoxic effects leading to an increase in free radicals and oxidative stress together with changes in antioxidant defense systems (Al-Akhras et al. 2001; Consales et al. 2012; Kumar et al. 2013). Lipids are generally the biological target of oxidative stress (Ciejka et al. 2011). Several studies have reported that EMF leads to LPO (Koyu et al. 2009; Ciejka et al. 2011). MDA is an important product of impairment of chain reactions causing the oxidation of unsaturated fatty acids, and is a marker of LPO. In addition, it is known to be a marker of oxidative stress mediated by lipid peroxidation in kidney tissue (Ozguner et al. 2005a). MDA levels increased

significantly in kidney and bladder tissue of rats exposed to the effect of a 900-MHz EMF in adolescence compared to the control group. Ozguner et al. (2005a, 2005b) investigated the effect on kidney tissue of exposure to 900-MHz EMF in 8-week-old rats and reported an increase in MDA levels. Ragy (2015) determined an increased MDA level and a decrease in total antioxidant capacity in kidney tissue from adult rats (16–18 weeks). Another study reported a marked increase in MDA in kidney tissue of 21-day-old male rat pups exposed to the effect of 900 MHz EMF on days 13–21 of the prenatal period (Odacı et al. 2014). Our study findings and those of other research are mutually corroborative, and indicate that oxidative damage occurring under the effect of EMF emerges as a result of free oxygen radicals.

Under normal conditions, cells are protected against oxidative injury by several mechanisms and antioxidant systems. Antioxidant molecules include SOD, CAT and GP-x, and cellular antioxidant defense mechanisms include low molecular weight GSH, vitamin C and vitamin E (Meral et al. 2007; Imge et al. 2010; Tkalec et al. 2013). EMF causing oxidative stress is thought to be capable of compromising the antioxidant defense system (Ciejka et al. 2011). SOD is one of the most important antioxidant defense systems against ROS. SOD converts superoxide anion radicals into H_2O_2 . H_2O_2 is broken down by CAT in peroxisomes into oxygen and water (Celino et al. 2011). GSH has been described as the prime defense system, acting as a scavenger and co-factor in the metabolic detoxification of ROS (Goraca et al. 2010). On the basis of our study findings, CAT and GSH activities decreased significantly in rat kidney and bladder tissue. In addition, SOD activity increased significantly in kidney tissue in EMFG compared to CG, but no significant difference was determined in bladder tissue. Ozguner et al. (2005a, 2005b) investigated the effect of exposure to 900-MHz EMF on kidney tissue of 8-week-old rats, and reported a decrease in SOD, CAT and GSH activities. In another study, Odacı et al. (2014) noted a decrease in SOD and CAT activities in tissue from 21-day-old male rat pups exposed to the effect of 900 MHz EMF on days 13–21 of the prenatal period. Ozturk et al. (2003) reported an increase in GSH activity. GSH plays a protective role in the scavenging of radicals and in molecular repair. Additionally, this scavenging process can result in a chain production dependent on H_2O_2 that causes oxidative stress to enter the cell and on oxidized GSH. Increased SOD activity may therefore be a response intended to balance or suppress high chain oxidation of GSH or decreased GSH concentrations (Yurekli et al. 2006). The kidneys have relatively weak enzymatic antioxidant defense systems capable of producing high levels of ROS through blood perfusion and high anaerobic metabolism (Odacı et al. 2014; Fahmy et al. 2015). Although our study differs from previous research in terms of antioxidant enzymes, our findings support the idea of an imbalance in the antioxidant defense system. In addition, our study is the first in the literature in terms of clarifying the relation between the effect of 900-MHz EMF on rat bladder tissue and oxidant/antioxidant systems.

Apoptosis, or programmed cell death, provides tissue homeostasis and plays an important role in normal development. However, stress-based apoptosis is triggered by external factors such as radiation, deficient nutrition, temperature

and chemicals (Akdağ et al. 2010; Hancı et al. 2013). Numerous studies have reported that exposure to EMF can result in apoptosis (Chavdoula et al. 2010; Odacı & Özyılmaz 2015). Hancı et al. (2013) reported that 900-MHz EMF applied on prenatal days 13–21 causes diffuse apoptosis in spermatogenic cells in the 21-day rat testis. Kerimoğlu et al. (2016) reported diffuse apoptosis in the heart tissue of male rats exposed to 900-MHz EMF in adolescence. Özyurt et al. (2014) reported an increase in caspase 3 activity and 8-OHdG, an index of oxidative DNA injury in kidney and bladder tissues exposed to radiation in 3-month-old rats. When our findings and those of other studies are considered together, they suggest that the increase in apoptosis in kidney and bladder tissue caused by EMF may be associated with the endothelial cells lining the intestinal and urogenital systems being mitotically very active and to a significant increase in MDA levels. In conclusion, our findings suggest that the effect of 900-MHz applied throughout adolescence leads to morphological injury as a cause of increased oxidative stress in rat kidney and bladder tissue.

Disclosure statement

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

Notes on contributors

Sibel Türedi, Ph.D., Karadeniz Technical University, Faculty of Medicine, Department of Histology and Embryology, Trabzon, Turkey.

Gökçen Kerimoğlu, Asst. Prof. M.D., Karadeniz Technical University, Faculty of Medicine, Department of Histology and Embryology, Trabzon, Turkey.

Tolga Mercantepe, Asst. Prof. Ph.D., Department of Histology and Embryology, Faculty of Medicine, Recep Tayyip Erdogan University, Rize, Turkey.

Ersan Odacı, Ph.D. M.D., Karadeniz Technical University, Faculty of Medicine, Department of Histology and Embryology, Trabzon, Turkey.

References

- Aebi H. 1974. Catalase. In: Bergmeyer HU, editor. *Methods of enzymatic analysis*. New York: Academic Press; pp. 673–684.
- Ahlbom A, Bridges J, de Seze R, Hillert L, Juutilainen J, Mattsson MO, Neubauer G, Schüz J, Simko M, Bromén K. 2008. Possible effects of electromagnetic fields (EMF) on human health-opinion of the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR). *Toxicology*. 246:248–250.
- Akdağ MZ, Dasdağ S, Ulukaya E, Uzunlar AK, Kurt MA, Taşkın A. 2010. Effects of extremely low-frequency magnetic field on caspase activities and oxidative stress values in rat brain. *Biol Trace Elem Res*. 138:238–249.
- Al-Akhras MA, Elbetieha A, Hasan MK, Al-Omari I, Darmani H, Albiss B. 2001. Effects of extremely low frequency magnetic field on fertility of adult male and female rats. *Bioelectromagnetics*. 22:340–344.
- Band PR, Le ND, MacArthur AC, Fang R, Gallagher RP. 2005. Identification of occupational cancer risks in British Columbia: a population-based case-control study of 1129 cases of bladder cancer. *J Occup Environ Med*. 47:854–858.
- Bas 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.
- Bedir R, Tunkaya L, Şehitoğlu İ, Kalkan Y, Yılmaz A, Şahin OZ. 2015. The effect of exposure of rats during prenatal period to radiation spreading from mobile phones on renal development. *Ren Fail*. 37:305–309.
- Cassady JR. 1995. Clinical radiation nephropathy. *Int J Radiat Oncol Biol Phys*. 31:1249–1256.
- Celino FT, Yamaguchi S, Miura C, Ohta T, Tozawa Y, Iwai T, Miura T. 2011. Tolerance of spermatogonia to oxidative stress is due to high levels of Zn and Cu/Zn superoxide dismutase. *PLoS One*. 6:e16938.
- Consales C, Merla C, Marino C, Benassi B. 2012. Electromagnetic fields, oxidative stress, and neurodegeneration. *Int J Cell Biol*. 2012:683–697.
- Ciejka E, Kleniewska P, Skibska B, Goraca A. 2011. Effects of extremely low frequency magnetic field on oxidative balance in brain of rats. *J Physiol Pharmacol*. 62:657–661.
- Chavdoula ED, Panagopoulos DJ, Margaritis LH. 2010. Comparison of biological effects between continuous and intermittent exposure to GSM-900-MHz mobile phone radiation: detection of apoptotic cell-death features. *Mutat Res*. 700:51–61.
- Dankers PY, Boomker JM, Meijer EW, Popa ER, van Luyn MJ. 2011. From kidney development to drug delivery and tissue engineering strategies in renal regenerative medicine. *J Control Release*. 152:177–185.
- Davis DL, Kesari S, Soskolne CL, Miller AB, Stein Y. 2013. Swedish review strengthens grounds for concluding that radiation from cellular and cordless phones is a probable human carcinogen. *Pathophysiology*. 20:123–129.
- Fahmy HM, Mohammed FF, Abdelrahman RT, Abu Elfetoh MM, Mohammed YA. 2015. Effect of radiofrequency waves emitted from conventional WIFI devices on some oxidative stress parameters in rat kidney. *J Drug Metab Toxicol*. 6:195.
- Feychting M, Ahlbom A, Kheifets L. 2005. EMF and health. *Annu Rev Public Health*. 26:165–189.
- Ghanbari M, Mortazavi SB, Khavanin A, Khazaei M. 2013. The effects of cell phone waves (900 MHz-GSM Band) on sperm parameters and total antioxidant capacity in rats. *Int J Fertil Steril*. 7:21–28.
- Goraca A, Ciejka E, Piechota A. 2010. Effects of extremely low frequency magnetic field on the parameters of oxidative stress in heart. *J Physiol Pharmacol*. 61:333–338.
- Gurbuz N, Sirav B, Yuvaci HU, Turhan N, Coskun ZK, Seyhan N. 2010. Is there any possible genotoxic effect in exfoliated bladder cells of rat under the exposure of 1800 MHz GSM-like modulated radiofrequency radiation (RFR)? *Electromagn Biol Med*. 29:98–104.
- Hancı H, Odacı E, Kaya H, Aliyazıcıoğlu Y, Turan I, 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.
- Hardell L, Sage C. 2008. Biological effects from electromagnetic field exposure and public exposure standards. *Biomed Pharmacother*. 62:104–109.
- Imge EB, Kiliçoğlu B, Devrim E, Cetin R, Durak I. 2010. Effects of mobile phone use on brain tissue from the rat and a possible protective role of vitamin C: a preliminary study. *Int J Radiat Biol*. 86:1044–1049.
- Kerimoğlu G, Mercantepe T, Erol HS, Turgut A, Kaya H, Çolakoğlu S, Odacı E. 2016. Effects of long-term exposure to 900 Megahertz electromagnetic field on heart morphology and biochemistry of male adolescent rats. *Biotech Histochem*. 11:1–10.
- Koca O, Gökçe AM, Öztürk MI, Ercan F, Yurdakul N, Karaman MI. 2013. Effects of intensive cell phone (Philips Genic 900) use on the rat kidney tissue. *Urol J*. 10:886–891.
- Koyu A, Ozguner F, Yilmaz HR, Uz E, Cesur G, Ozcelik N. 2009. The protective effect of caffeic acid phenethyl ester (CAPE) on oxidative stress in rat liver exposed to the 900 MHz electromagnetic field. *Toxicol Ind Health*. 25:429–434.
- Kumar S, Behari J, Sisodia R. 2013. Influence of electromagnetic fields on reproductive system of male rats. *Int J Radiat Biol*. 89:147–154.
- Lai J, Zhang Y, Zhang J, Liu X, Ruan G, Chaugai S, Tang J, Wang H, Chen C, Wang DW. 2016. Effects of 100-µT extremely low frequency electromagnetic fields exposure on hematograms and blood chemistry in rats. *J Radiat Res*. 57:16–24.
- 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 on 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.
- 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 Radiat Biol*. 91:547–554.
- Odacı E, Ünal D, Mercantepe T, Topal Z, Hancı H, Türedi S, Erol HS, Mungan S, Kaya H. 2014. The pathological effects of prenatal exposure to a 900-MHz electromagnetic field on the 21-day-old male rat kidney. *Biotechn Histochem*. 90:93–101.
- Ohkawa H, Ohishi H, Yagi K. 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem*. 95:351–358.
- Oral B, Guney M, Ozguner F, Karahan N, Mungan T, Comlekci S, Cesur G. 2006. Endometrial apoptosis induced by a 900-MHz mobile phone: preventive effects of vitamins E and C. *Adv Ther*. 23:957–973.
- Ozguner F, Oktem F, Ayata A, Koyu A, Yilmaz HR. 2005a. A novel antioxidant agent caffeic acid phenethyl ester prevents long-term mobile phone exposure-induced renal impairment in rat. *Mol Cell Biochem*. 277:73–80.
- Ozguner F, Oktem F, Ayata A, Koyu A, Yilmaz HR. 2005b. A novel antioxidant agent caffeic acid phenethyl ester prevents long term mobile phone exposure-induced renal impairment in rat. Prognostic value of malondialdehyde, N-acetyl-beta-D glucosaminidase and nitric oxide determination. *Mol Cell Biochem*. 277:73–80.
- Oztürk A, Baltacı AK, Mogulkoc R, Oztekin E. 2003. Zinc prevention of electromagnetically induced damage to rat testicle and kidney tissues. *Biol Trace Elem Res*. 96:247–254.
- Özyurt H, Çevik Ö, Özgen Z, Özden AS, Çadırcı S, Elmas MA, Ercan F, Gören MZ, Şener G. 2014. Quercetin protects radiation-induced DNA damage and apoptosis in kidney and bladder tissues of rats. *Free Radic Res*. 48:1247–1255.
- Qiao Z, Xia C, Shen S, Corwin FD, Liu M, Guan R, Grider JR, Qiao LY. 2014. Suppression of the PI3K pathway *in vivo* reduces cystitis-induced bladder hypertrophy and restores bladder capacity examined by magnetic resonance imaging. *PLoS One*. 9:e114536.
- Racuciu M, Miclaus S, Creanga D. 2015. On the thermal effect induced in tissue samples exposed to extremely low-frequency electromagnetic field. *J Environ Health Sci Engineer*. 13:85.
- Ragy MM. 2015. Effect of exposure and withdrawal of 900-MHz electromagnetic waves on brain, kidney and liver oxidative stress and some biochemical parameters in male rats. *Electromagn Biol Med*. 34:279–284.
- Salford LG, Brun AE, Eberhardt JL, Malmgren L, Persson BR. 2003. Nerve cell damage in mammalian brain after exposure to microwaves from GSM mobile phones. *Environ Health Perspect*. 111:881–883.
- Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR). 2007. Possible effects of electromagnetic fields (EMF) on human health.
- 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.
- Sun Y, Larry WO, Ying L. 1988. A simple method for clinical assay of superoxide dismutase. *Clin Chem*. 34:497–500.
- Tirelli E, Laviola G, Adriani W. 2003. Ontogenesis of behavioral sensitization and conditioned place preference induced by psychostimulants in laboratory rodents. *Neurosci Biobehav Rev*. 27:163–178.
- 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. *Electromagn Biol Med*. 34:390–397.
- Türedi S, Hancı H, Çolakoğlu S, Kaya H, Odacı E. 2016. Disruption of the ovarian follicle reservoir of prepubertal rats following prenatal exposure to a continuous 900-MHz electromagnetic field. *Int J Radiat Biol*. 92:329–337.
- Tkalec M, Stambuk A, Srut M, Malarić K, Klobučar GI. 2013. Oxidative and genotoxic effects of 900 MHz electromagnetic fields in the earth worm *Eisenia fetida*. *Ecotoxicol Environ Saf*. 90:7–12.

- Tyagi P, Barclay D, Zamora R, Yoshimura N, Peters K, Vodovotz Y, Chancellor M. 2010. Urine cytokines suggest an inflammatory response in the overactive bladder: a pilot study. *Int Urol Nephrol.* 42:629–635.
- Ulubay M, Yahyazadeh A, Deniz ÖG, Kıvrak EG, Altunkaynak BZ, Erdem G, Kaplan S. 2015. Effects of prenatal 900 MHz electromagnetic field exposures on the histology of rat kidney. *Int J Radiat Biol.* 9:35–41.
- Yurekli AI, Ozkan M, Kalkan T, Saybasili H, Tuncel H, Atukeren P, Gumustas K, Seker S. 2006. GSM base station electromagnetic radiation and oxidative stress in rats. *Electromagn. Biol Med.* 5:177–188.