



Exposure to a 0.9-GHz electromagnetic field on postnatal days 21–45 may trigger the renin-angiotensin system in male rat: a histological and biochemical study

Ayşe İkinci Keleş¹ · Haydar Kaya² · Gökhan Keleş³ · Hüseyin Serkan Erol⁴ · Tolga Mercantepe⁵ · Ersan Odacı¹

Received: 6 August 2024 / Accepted: 17 September 2024 / Published online: 4 December 2024
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Abstract

The aim of this study was to examine the relationship between the renin-angiotensin system (RAS) and histological and biochemical changes occurring in the kidney tissue of male rats exposed to a 0.9 GHz electromagnetic field (EMF). Twelve male rats aged 21 days were randomly assigned to control (C-Gr) and EMF (EMF-Gr) groups. No procedure was performed on C-Gr, while the EMF-Gr rats were exposed to a 0.9 GHz EMF on postnatal days 21–45 (one hour a day for 25 days). Tissues were removed at the end of the experiment and evaluated using biochemical, and histopathological methods. Increased kidney tissue volume and weight and total body weight were determined in the group exposed to EMF. Lipid peroxidation, glutathione, catalase, and superoxide dismutase also increased in the kidney tissue of the EMF-Gr rats. Histopathological evaluation revealed cortical/medullary bleeding/obstruction and widespread fibrosis, dilatation, vacuolization, and degeneration in distal and proximal tubules, decreased and atypical parietal cells, and degeneration in epithelial cells. Additionally, dilated and degenerated glomeruli in the Malpighian body, Bowman's membrane degeneration and degeneration in the vascular pole, podocyte, pedicel and mesangial cells were also observed. As a result of exposure to EMF, oxidative stress, tissue volume and weight increased, and histopathological changes caused the formation of a pathway that triggers RAS in kidney tissues. In conclusion, long-term exposure to 0.9 GHz EMF can activate the renin-angiotensin system in the rat kidney, and we think that such activation may be associated with structural, histopathological, and biochemical changes occurring in renal tissue.

Keywords Electromagnetic field · Renin-angiotensin system · Kidney · Oxidative stress · Male rat

Deceased: The author Prof. Dr. Ersan Odacı has passed away after a heart attack.

✉ Ayşe İkinci Keleş
ayse_ikinci@hotmail.com; ayseikincikeles@aksaray.edu.tr
Haydar Kaya
hkaya@ktu.edu.tr
Ersan Odacı
eodaci@gmail.com

¹ Department of Histology and Embryology, Faculty of Medicine, Aksaray University, Aksaray, Türkiye

² Department of Electrical and Electronics, Faculty of Engineering, Karadeniz Technical University, Trabzon, Türkiye

³ He works freelance outside of university, Aksaray, Türkiye

⁴ Department of Biochemistry, Faculty of Veterinary Medicine, Kastamonu University, Kastamonu, Türkiye

⁵ Department of Histology and Embryology, Faculty of Medicine, Recep Tayyip Erdoğan University, Rize, Türkiye

Introduction

Rapid advances in technology are resulting in increased exposure to radiofrequency/microwave radiation deriving from devices that emit electromagnetic fields (EMFs), particularly cell phones. Cell phone systems in the world generally operate in the frequency range of 900 or 1800 MHz (Megahertz) and 2800 MHz (Universal Mobile Telecommunications System) (Kesari et al. 2013). In order to evaluate the renin-angiotensin system (RAS) effect, the study was planned to apply the lowest dose 900 Mhz. Several studies have investigated the biological effects of 900 Mhz (0.9 GHz) EMFs due to the rapid growth in cell phone use (İkinci et al. 2013; Odacı et al. 2016; Keleş et al. 2019a; Bodera et al. 2015; Vafaei et al. 2023). Kidney tissue is reported to be susceptible to EMF, and adverse effects are reported to emerge as a result of long-term exposure. Studies have reported that EMF not only causes cell death as a result of DNA injury, but also results in direct cell death and complex and dynamic interactions between glomerular, tubular, and interstitial cells (Cassady 1995). The application of EMF in the prenatal and postnatal period has been reported to cause deleterious effects, such as an increased total renal volume, a decreased glomerule count, apoptosis, increased connective tissue structures, tubular degeneration, and oxidative stress (Ulubay et al. 2015; Türedi et al. 2017; Bedir et al. 2015; Kilic et al. 2023).

Cells activate several mechanisms in order to cope with oxidative stress and repair damaged macromolecules. The primary defense mechanism is mediated by antioxidants, which have been shown to scavenge free radicals and reactive oxygen species (ROS) by enzymatic or non-enzymatic means. Antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT) and non-enzymatic antioxidants such as glutathione (GSH) are reported to be significantly affected under oxidative stress (Banerjee et al. 2001). Studies involving the kidneys have reported that EMF exacerbates oxidative stress (Bodera et al. 2015; Vafaei et al. 2023).

In addition to affecting blood pressure, RAS also affects a variety of processes, ranging from infection and immune responses to longevity (Sparks et al. 2014). Conditions such as decreased renal perfusion, increased parasympathetic system activity, low blood pressure perception, and ionic imbalance increase the release of renin from juxtaglomerular cells in the kidney (Lekshmi et al. 2014). Renin is produced by juxtaglomerular cells and the distal tubule in the kidney. Juxtaglomerular cells are the main source of renin in the circulation (Steglich et al. 2020). Angiotensin is produced by renin and angiotensin-converting enzyme (ACE) in a three-stage reaction (Angiotensin (Ang) I, II and III) (Sparks et al. 2014). The angiotensin II content in the kidney

is much higher than that in plasma, and increases through an unidentified mechanism in kidney diseases. Although there is some evidence that the majority of renal angiotensin is systemically produced by the liver under normal conditions, it is also manufactured by the proximal tubule of the nephron (Matsusaka et al. 2012). The enzyme ACE, responsible for the conversion of Ang I to Ang II is present in a similar manner along the kidney (Culver et al. 2017). Effects such as vasoconstriction, ROS generation, inflammation, Na⁺ and water retention, and fibrosis are reported as a result of the conversion of Ang I into Ang II in the kidney (Culver et al. 2017) (Fig. 1).

There is stated in the literature that since tissues and organs of children and adolescents have not yet reached adult structures, they will be absorb more microwave radiation than adults if they are exposed to EMF (Morgan et al. 2014). In the literature, the adolescence period in rats is defined as 21–34 (early), 34–46 (middle), and 46–59 days (late) (Tirelli et al. 2003). Comparing rats and humans; 21–32 days correspond to 2–12 years, and 33–55 days correspond to 12–16 years (Parker and Picut 2016). The purpose of this study was therefore to investigate the relationship between the RAS and histological and biochemical changes occurring in the kidney tissue of male rats exposed to 0.9 GHz EMF on postnatal days 21–45.

Methods

Ethics statements and laboratory conditions

Rats were purchased from Karadeniz Technical University (KTU) Experimental Animal Application Research Center. All animal procedures were approved by the KTU institutional ethical committee, Trabzon, Türkiye (Date: 19.06.2014, Protocol Number: 2014/30). All procedures were carried out in accordance with the principles of the Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85–23, revised 1996).

The animals were housed in standard Type III rat cages at a mean temperature of 22 ± 2 °C and humidity of $50\% \pm 5$ controlled by an automated system, in an automatically adjusted 12-h light and dark cycle. Drinking water and standard rat pellet chow were provided.

Experimental groups and study design

The 12 male 21-day-old Sprague Dawley rats used in the study were obtained from the KTU Surgical Application and Research Center. These were randomly assigned into two groups, control (C-Gr) ($n=6$) and EMF (EMF-Gr) ($n=6$). C-Gr was not exposed to EMF, while EMF-GR was exposed

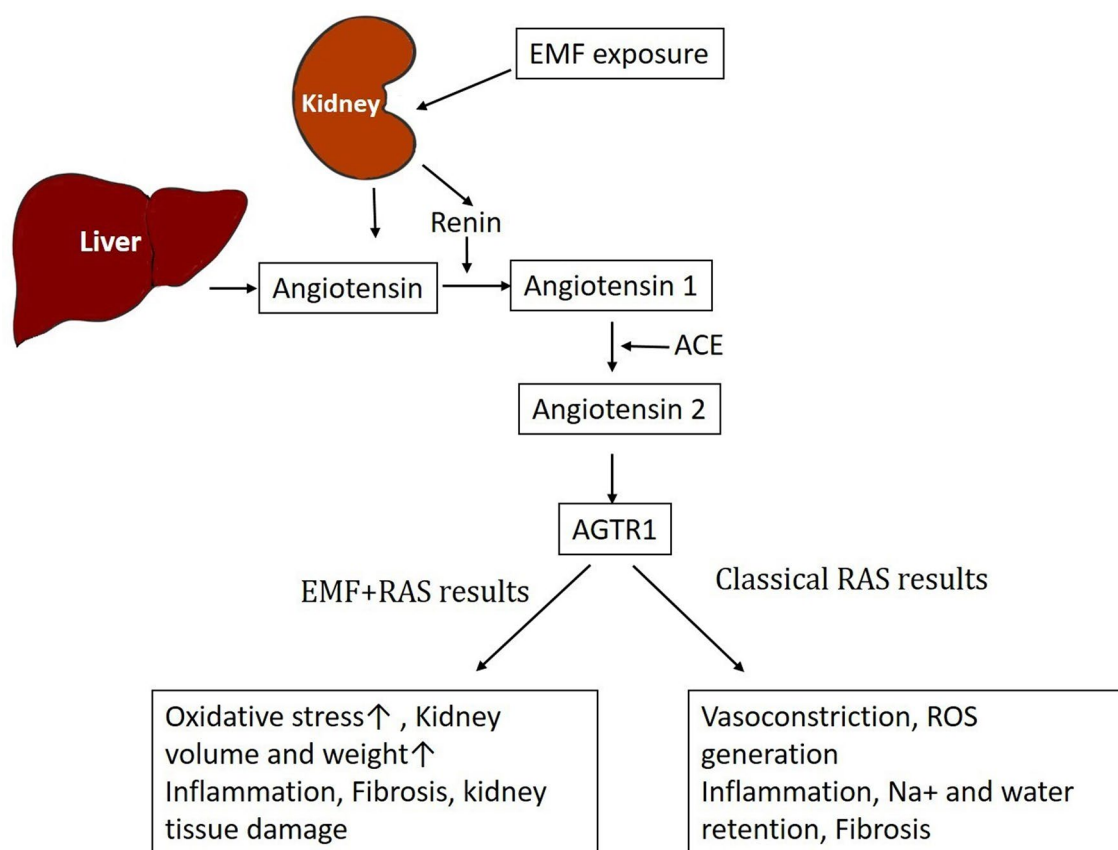


Fig. 1 Flowchart showing the mechanism of the renin-angiotensin system (RAS). ACE: Angiotensin-converting enzyme, AGTR1: Angiotensin II receptor type 1, EMF: electromagnetic field

to 900 MHz EMF for 25 days (seven days per week/one hour each day (11–12 a.m.)). The EMF application commenced with weaning (day 21) and continued for 25 days (days 21–4).

The EMF application system was established and applied as described in previous studies (Keleş 2020; Keleş and Süt 2021; Keleş et al. 2021; Gur et al. 2021). The intensity of EMF was also measured using the same device (C.A 43 Isotropic Electrical Field Intensity Meter, Chauvin Arnoux Group, Paris, France) with a range of 100 kHz–2.5 GHz every day during the study period (Fig. 2a).

Body weight measurement

The rats were weighed using a digital sensitive scale (KERN & SOHN GmbH, D- 72336 Balingen, Germany) at the end of the experiment.

Kidney volume and weight measurement

At the conclusion of the experiment, all animals were sacrificed by cervical dislocation. Right and left kidney tissues were removed and weighed with a digital sensitive scale

(KERN & SOHN GmbH, D- 72336 Balingen, Germany). Archimedes' principle was employed to measure the kidney volumes (Duebel et al., 1984; Keleş et al. 2020, 2022). The right and left kidneys were separately immersed in a graduated cylinder filled with water. The volume of each kidney was calculated as equal to that of the displaced water.

Histological procedures

Right and left kidney tissues were collected from the experimental animals. Section 1 mm³ in volume were taken from the right kidney for electron microscopic examination and were placed into 100 mM phosphate buffer containing 2.5% glutaraldehyde. The left tissues were placed in 10% formalin for light microscopic examination.

Light microscopic procedures

The left kidney tissues were stored in 10% formalin for one week and then subjected to routine histological procedures. Briefly, the kidney tissues were blocked, and 5-μm thick sections were taken using a microtome (Thermo Scientific Shandon Finesse 325 microtome, UK). These were then

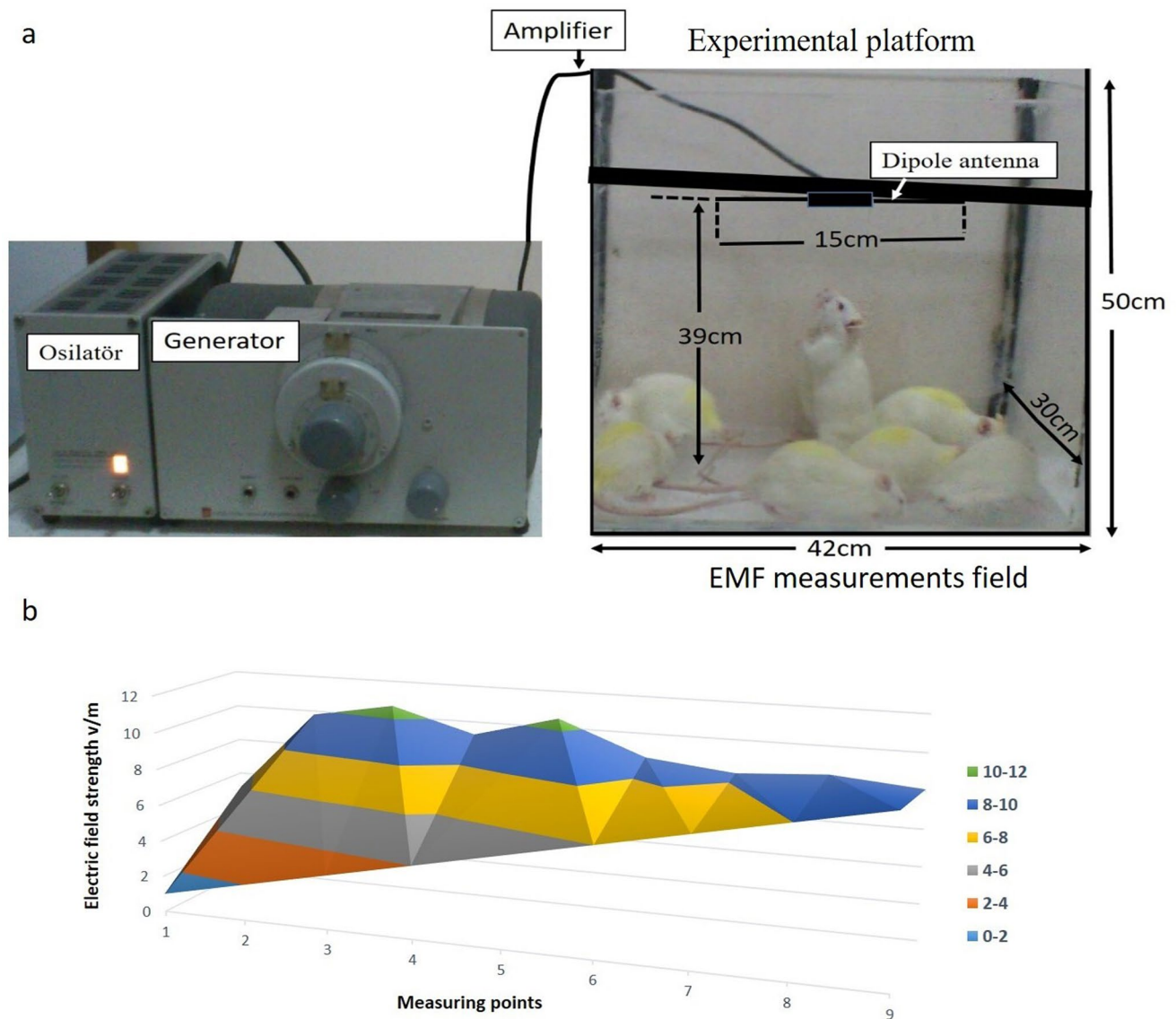


Fig. 2 The electromagnetic field (EMF) application platform (**a**). Results showing the distribution of mean EMF intensities measured from nine different points on the floor of the EMF-application cage during application, with the rats inside the cage (**b**)

stained with hematoxylin and eosin, Masson's trichrome, and periodic acid-Schiff for histopathological assessments. The evaluations made with the light microscope (BX53; Olympus Optical Co. Ltd., with Olympus DP72 camera attached, Tokyo, Japan) (Yahyazadeh 2024a, b; Yahyazadeh et al. 2024) were performed by two histologists blinded to the study groups was employed for histopathological examination. The kidney tissue sections (medulla and cortex) were examined for the presence of damage to the tubules, Bowman's membrane, glomeruli, and vascular pole, as well as for vascular congestion, inflammatory, edema, fibrosis, and cells (tubule epithelial cells, podocytes, and mesangial cells). During histopathological scoring, each sample was recorded as absent (0), minimal (+), moderate (++) or severe (+++) in 10 different areas (Bedir et al. 2015).

Tubular damage in the sections was also examined under light microscopy and was scored depending on the cortical tubule percentage exhibiting epithelial necrosis. The scoring system employed was as follows: 0 - normal, 1 - <10%, 2 -10-25%, 3-26-75%, and 4 ->75%. The scoring was based on tubular necrosis, loss of the proximal tubule brush border, apical membrane bulging, detachment of tubular epithelial cells from the basal membrane, and intraluminal aggregation of cells and proteins (Pan et al. 2014). Eight areas from each section were evaluated by two separate individuals.

Transmission electron microscopic procedures

The kidney samples were pre-fixed in 100 mM phosphate buffer containing 2.5% glutaraldehyde. They were then

post-fixed in 1% osmium tetroxide (Merck Darmstadt, Germany), dehydrated in an ascending ethanol series, and finally embedded in an epoxy resin (Araldite CY212) kit (Agar Scientific, Essex, UK). For electron microscopy analysis, ultra-thin Sects. 40–70 nm thick were cut using an ultramicrotome (LKB Nova, 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 Reynold's lead citrate for transmission electron microscopy analysis. Images were prepared using a JEOL SX1010 transmission electron microscope (JEOL Ltd., Akishima, Tokyo, Japan) for photographic image capture (Kodak 4489, Eastman Kodak Company, Rochester, NY, USA) (Odacı et al. 2015; Keleş et al. 2021).

ACE, renin, and AGTR1 protein level image analyses

ACE, renin, and AGTR1 protein levels were evaluated using immunohistochemistry staining images of kidney tissue samples available on the Human Protein Atlas. (<https://www.proteinatlas.org>). All immunohistochemistry staining images and quantitative data from patients were analyzed from the information on the site.

To access the images, the ACE, renin, and AGTR1 protein titles were entered one by one into the search motor and the kidney was selected from the tissues that appeared. All the tissue samples were analyzed histologically using immunohistochemistry staining images (ACE: Antibody CAB002426; RENIN Antibody HPA005131; AGTR1 Antibody HPA003596).

Biochemical tissue analyses

A tissue sample (0.5 g) was taken from the remaining part of the right kidney for biochemical analysis, placed in

Eppendorf tubes, and stored at -80°C . Lipid peroxidation (LPO), GSH, SOD, and CAT level activities in kidney tissues were examined. The thiobarbituric method was applied for LPO measurement (Ohkawa et al. 1979). GSH activity was measured using Sedlak and Lindsay's method (Sedlak and Lindsay 1968), and SOD activity was determined in accordance with the method developed by Sun et al. (Sun et al. 1988). CAT activity was determined using the Aebi method, which measures via hydrogen peroxide dissociation at 240 nm (Aebi 1974).

Statistical analysis

Statistical analysis was performed on SPSS version 22 statistical software. Data distributions were assessed using the Shapiro-Wilk test and a histogram chart. The t-test was applied since the groups were found to be normally distributed. Data were expressed as mean $\pm$ standard deviation, and p values < 0.05 were considered statistically significant.

Results

Electromagnetic field measurements

Specific absorption rate (SAR) values were calculated using special software (RadHaz SAR Equivalence Calculator Version 1.0, © 2000 Richard Tell Associates, Inc., Mesquite, NV, USA) (Keleş et al. 2018, 2019a, 2019b). The measurement results showed that the EMF-Gr male rats were exposed to an average electric field intensity of 8.8 V/m (0.242 W/m^2) within the exposure cage (Fig. 2b).

Body and kidney weight results

The rats' body and kidney weights are shown in Table 1. Analysis revealed a significant weight gain in the EMF-Gr rats compared to C-Gr ($p=0.001$). A significant increase was also observed in kidney tissue weights in EMF-Gr relative to C-Gr ($p=0.001$).

Kidney volume results

Rats' kidney tissue volumes were compared between EMF-Gr and C-Gr. The analysis revealed a significant increase in volume in EMF-Gr tissues ($p=0.001$) (Table 1).

Biochemical analysis results

SOD, CAT, LPO, and GSH values in C-Gr and EMF-Gr tissues are shown in Table 2. EMF-Gr kidney tissue LPO,

Table 1 Control and EMF group body and kidney weights and kidney volumes

		Control group ($n=6$)	EMF group ($n=6$)
Measured Parameters		Mean $\pm$ standard deviation	
Body weight/g		273.833 ± 58.945	$341.833 \pm 41.701^*$
Kidney weight /g	Left	1.134 ± 0.406	$1.323 \pm 0.123^*$
	Right	1.139 ± 0.94	$1.294 \pm 0.132^*$
	Total weight	2.388 ± 0.108	$2.617 \pm 0.253^*$
Kidney volume/ml	Left	1.183 ± 0.160	$1.700 \pm 0.214^*$
	Right	1.200 ± 0.089	$1.683 \pm 0.190^*$
	Total volume	1.183 ± 0.160	$2.850 \pm 0.659^*$

Data presented as mean $\pm$ standard deviation. $*p < 0.01$ compared to the control group. Statistically significant compared to the control group. EMF: electromagnetic field

Table 2 Kidney tissue biochemical analysis results ($n = 6$)

	Control group	EMF group
Measured Parameters	Mean $\pm$ standard deviation	
LPO (nmol/mg tissue)	18.110 $\pm$ 1.229	51.041 $\pm$ 0.860 ^a
CAT (mmol/min/mg tissue)	0.194 $\pm$ 0.002	0.539 $\pm$ 0.022 ^a
GSH (nmol/mg tissue)	0.946 $\pm$ 0.025	1.838 $\pm$ 0.051 ^a
SOD (mmol/min/mg tissue)	5.955 $\pm$ 0.569	7.383 $\pm$ 0.334 ^a

Data presented as mean $\pm$ standard deviation. ^a $p = 0.001$ statistically significant compared to the control group. EMF: electromagnetic field, LPO: lipid peroxidation, CAT: catalase, GSH: glutathione, SOD: superoxide dismutase

GSH, CAT, and SOD values exhibited a statistically significant increase in comparison to C-Gr ($p < 0.05$).

Morphological changes in the kidney

ACE, renin, and AGTR1 protein levels image results

Tissues specimens were evaluated in terms of ACE, AGTR1, and renin protein levels. No ACE protein expression was

detected in Bowman's capsule, glomerular cells, or collecting ducts. Low levels were detected in distal tubules (50%) and high levels in proximal tubules (microvilli) (75%). Renin protein expression was not detected in Bowman's capsule, glomerular cells, or collecting ducts. Small amounts were detected in tubular cells (in the distal and proximal tubules) (25%). No AGTR1 protein expression was determined in glomerular or tubular cells (Fig. 3).

Electron microscopy results

Under transmission electron microscopy, C-Gr sections exhibited typical glomerular capillaries together with normal basal membranes. Typical podocytes and foot processes were also observed. The capillary basement membrane, lamina densa, lamina rara interna and externa exhibited a regular ultrastructure (Fig. 4). However, EMF-Gr specimens exhibited glomerular capillary degeneration, and distortion was present in the capillary basement membrane lamina densa, and lamina rara interna and externa. Electron

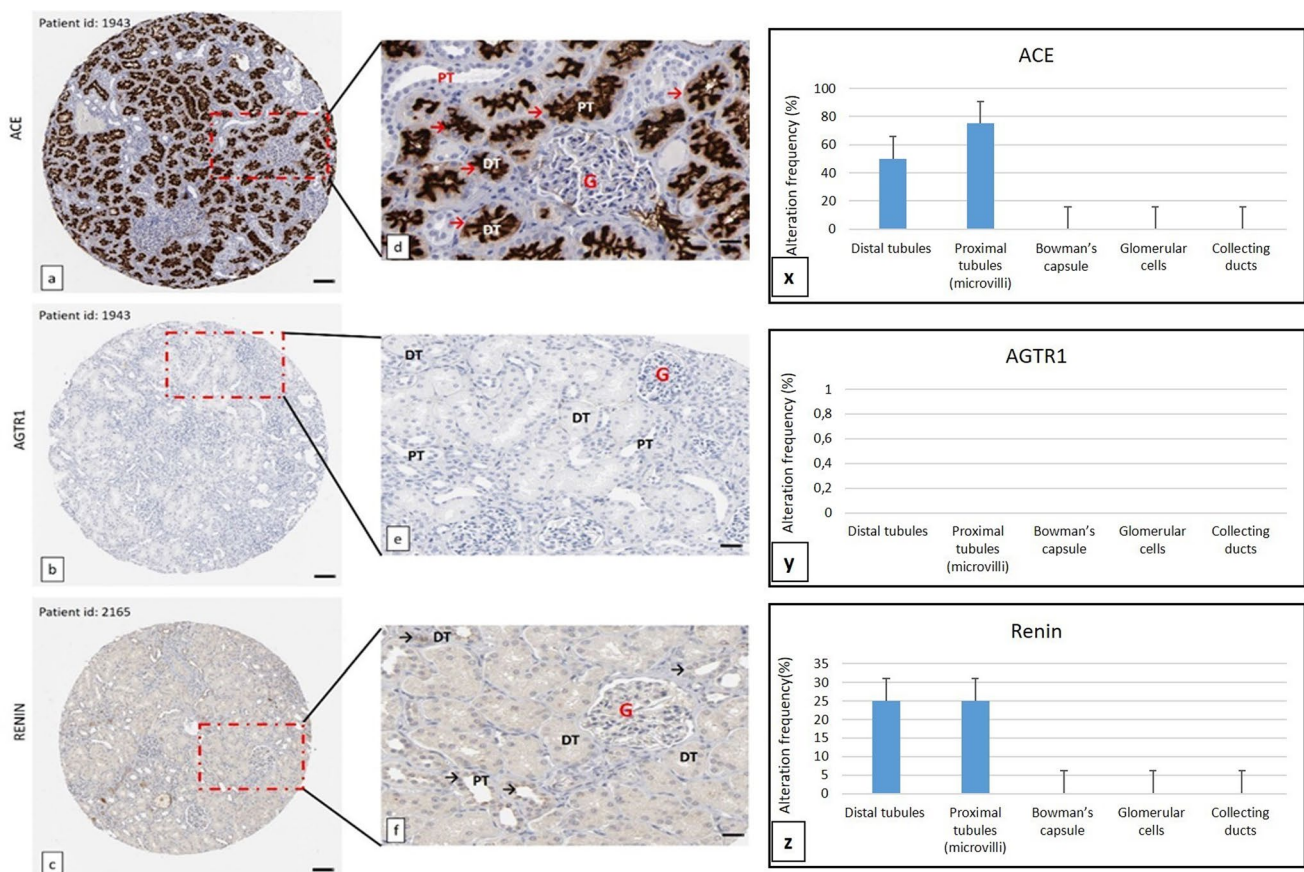


Fig. 3 Representative images of ACE, AGTR1, and RENIN protein expression levels based on immunohistochemistry staining in kidney tissues (a-f). (→) proteins levels indicator; DT: Distal tubule, PT: Proximal tubule G: Glomerulus, ACE: Angiotensin-converting enzyme, AGTR1: Angiotensin II receptor type 1. Rat IDs are provided

in the upper right corner of each image. Quantification (%) of ACE, AGTR1, and RENIN protein expression levels based on immunohistochemistry staining in kidney tissue samples (x, y, z). (ACE: Antibody CAB002426; RENIN Antibody HPA005131; AGTR1 Antibody HPA003596). Scale bar = 200 μ m, x4 (a, b, c); 50 μ m, x20 (d, e, f)

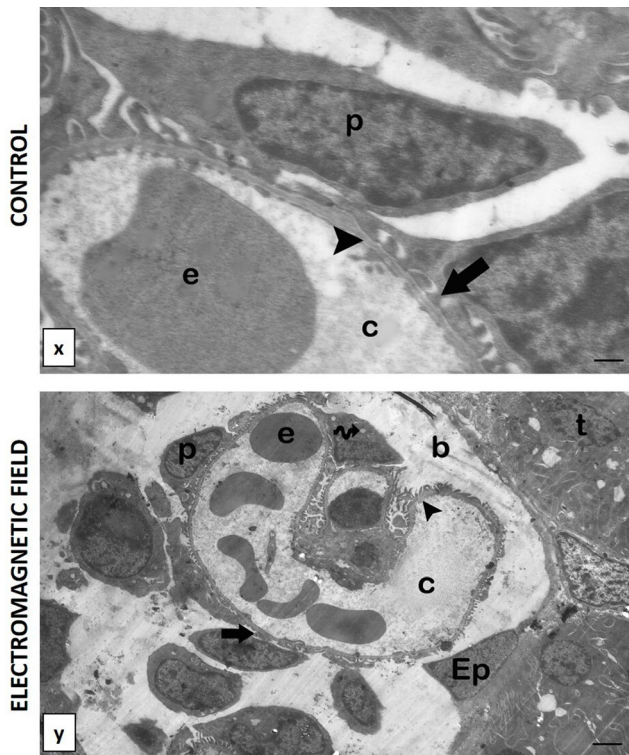


Fig. 4 Transmission electron microscope images of kidney tissues from the control (C-Gr) and electromagnetic field (EMF-Gr) groups. Electron micrograph from C-Gr showing a glomerular capillary together with normal basal lamina (arrowhead). The capillary basement membrane lamina densa and lamina rara interna and externa exhibit a typical structure (arrowhead). Electron microscopic findings show typical podocytes (p) and foot process (arrow). Electron micrograph from EMF-Gr showing glomerular basement membrane degeneration. The capillary basement membrane lamina densa, lamina rara internal and external have disappeared (arrowhead). Electron microscopic findings show an atypical parietal epithelial cell (Ep). Pedicel degeneration and podocyte infolding (arrow). Podocyte cytoplasm vacuoles can be seen (spirally arrow). Erythrocyte (e). Capillary (c). Podocytes (P). Tubules (t). Bowman capsular space (b). Scale bar = $\times 10,000$ (x); $\times 2000$ (y)

microscopic findings revealed atypical parietal epithelial cells, and pedicel degeneration and podocyte infolding. Podocyte cytoplasm vacuoles were also observed (Fig. 4).

Light microscopy results

No renal pathology was detected in C-Gr sections stained with hematoxylin and eosin, Masson's trichrome, or period acid-Schiff (Figs. 5a, b and e, 6a, b and e and 7a, b and e). However, examination of cortex tissue from EMF-Gr revealed dilatation in distal and proximal tubules, and degeneration and decreases in epithelial cells. In addition, dilated and degenerated glomeruli, and degeneration in Bowman's membrane (parietal membrane), the vascular pole, and podocytes and mesangial cells were observed in Malpighian bodies. Cortical/medullary bleeding/obstruction

and widespread fibrosis were also present (Figs. 5c, d and f, 6c, d and f and 7c, d and f).

Histopathological scoring revealed significant increases in tubules and Malpighian bodies (glomeruli, Bowman's membrane, the vascular pole, podocytes, and mesangial cells), cortical/medullary bleeding/obstruction, and fibrosis in EMF-Gr compared to C-Gr ($p < 0.05$) (Table 3). Histological scoring of tubular damage in C-Gr and EMF-Gr revealed values of 0.33 ± 0.52 and 6.33 ± 2.42 , respectively. Analysis revealed 75% tubular injury in EMF-Gr (Figs. 4, 5, 6 and 7).

Discussion

Previous studies have observed that exposure to EMF in the pre- and postnatal periods leads to kidney tissue damage, although no research has to date addressed the mechanism involved (Ulubay et al. 2015; Türedi et al. 2017, 2022; Bedir et al. 2015; Borzoueisileh et al. 2020). The deleterious effects of EMF on DNA and tissue is an established fact. However, the effect mechanism involved needs to be investigated in detail. The application of EMF is thought to trigger the RAS system. This raises the question of whether the RAS is triggered as a consequence of the damage occurring in tissue, or whether this damage in tissue develops because the RAS is triggered. The results of the present study were focused around these two questions.

Examination of the group exposed to EMF in this study detected an increase in kidney tissue volume and weight. A previous study reported that the application of 900-MHz EMF in the prenatal period led to an increase in renal volume and a decrease in glomerule numbers (Ulubay et al. 2015). An increase in rats' body weights was also detected in the group exposed to EMF. These increases in kidney tissues and body weights suggest that this is related to water and salt retention as a result of impairment of renal functions. This retention resulting from long-term exposure may have resulted in a volume increase in kidney tissue. Exposure to EMF may have increased the sequential production of angiotensin I and II by triggering renin and ACE production. Angiotensin also causes the release of aldosterone from the adrenal glands. These two effects lead to sodium and water retention in the kidney and an increase in blood pressure and intraglomerular pressure (Culver et al. 2017). Normally, RAS is the main regulator of sodium and water homeostasis (Luo et al. 2015). However, continuous repetition of exposure resulted in increases in weight and volume in kidney tissue in association with water and salt retention. This may have led to an increase in rats' body weights in consequence.

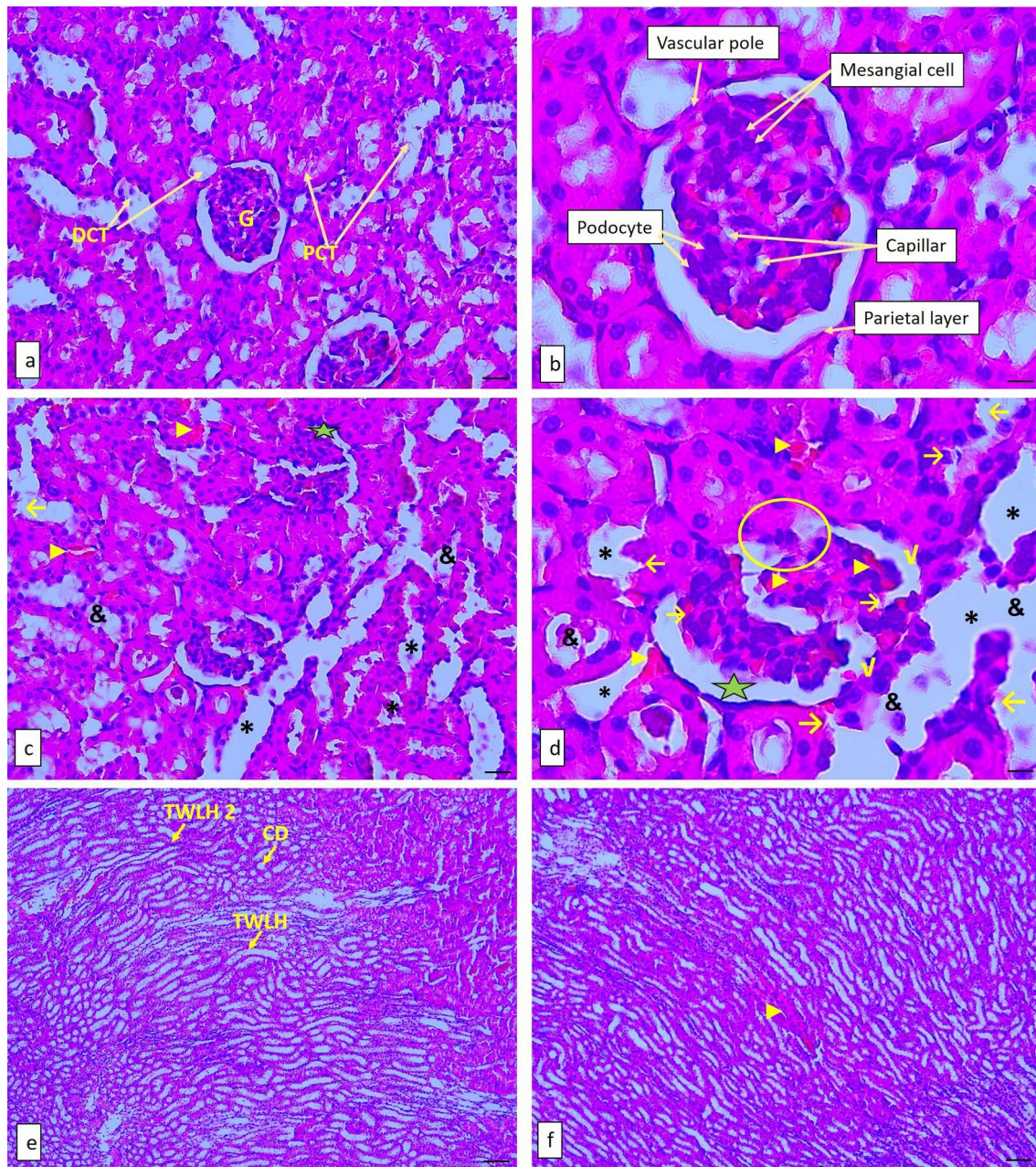


Fig. 5 Histology photomicrographs showing hematoxylin & eosin staining of the rat kidney. Normal histology can be seen in the control group (a, b, e). (c, d, f) Electromagnetic field group; (*) tubular dilatation, (&) tubular degeneration, (▶) vascular cortical/ medullary hemorrhage/congestion, (→) cell degenerations, (←) epithelium desquamation, (☆) dilatation of Bowman's capsule and glomerular damage,

(√) parietal layer degeneration, (in circle) degeneration at the vascular pole. G: Glomerulus; PCT: Proximal convoluted tubule; DCT: Distal convoluted tubule, CD: collecting duct, TWLH: a thick-walled loop of Henle, TWLH 2: a thin-walled loop of Henle. Scale bar = 100 μ m, x10 (e, f); 50 μ m, x20 (a, c); 10 μ m, x100, immersion oil (b, d)

Biochemical analyses of kidney tissues from male rats exposed to EMF revealed an increase in GSH, LPO, SOD, and CAT values. High LPO values are an indicator of oxidative stress in kidney tissues. The antioxidant enzymes SOD, CAT, and GSH are components of the cellular defense system (Urso et al. 2003). The findings of this study show that oxidative stress in kidney tissues increases as a result of

exposure to EMF, and that this activates antioxidants for the elimination of that stress. Previous studies have reported that EMF can also cause damage to DNA, leading to an increase in oxidative stress (Cassady 1995; Türedi et al. 2017). Studies have reported a close association between the RAS and oxidative stress. RAS suppression will result in a decrease in oxidative stress (de Cavanagh et al. 2007). The increased

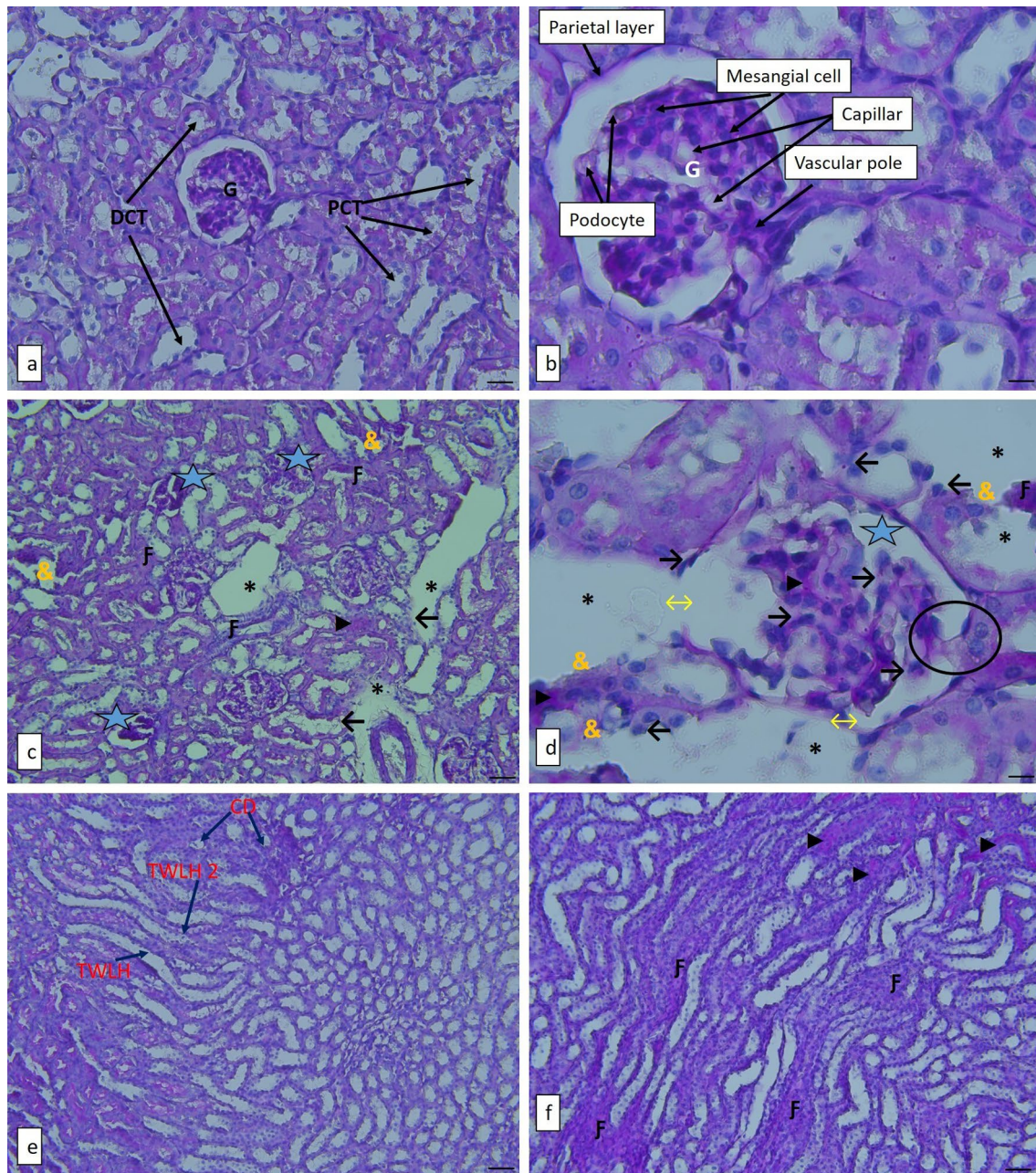


Fig. 6 Histology photomicrographs showing periodic acid-Schiff staining of the rat kidney. Normal histology of the kidney tissue can be seen in the control group (**a, b, e**). (**c, d, f**) Electromagnetic field group; (*) tubular dilatation, (&) tubular degeneration, (▶) vascular cortical/ medullary hemorrhage/congestion, (→) cell degenerations, (←) epithelium desquamation, (☆) dilatation of Bowman's capsule

and glomerular damage, (↔) Parietal layer degeneration, (in circle) degeneration at the vascular pole and (F) fibrosis. G: Glomerulus; PCT: Proximal convoluted tubule; DCT: Distal convoluted tubule, CD: collecting duct, TWLH: a thick-walled loop of Henle, TWLH 2: a thin-walled loop of Henle. Scale bar = 50 μ m, x20 (**a, c, e, f**); 10 μ m, x100, immersion oil (**b, d**)

oxidative stress in the findings of the present study may suggest that it can trigger the RAS. Our study indicates that the rise in oxidative stress may have occurred due to the increase in the effects of Ang II as a result of the triggering effect of EMF exposure on renin and ACE. Indeed, a previous study reported an increase in renin and angiotensin levels in kidney tissue from groups exposed to EMF as a

result of pre- and postnatal 900-MHz EMF exposure (Kilic et al. 2023). This study confirms our hypothesis. However, there is no information about the mechanism between EMF and RAS.

Another finding of this study was the observation of histopathological changes in kidney tissues from EMF-Gr. Histopathological changes occurring in kidney tissue may

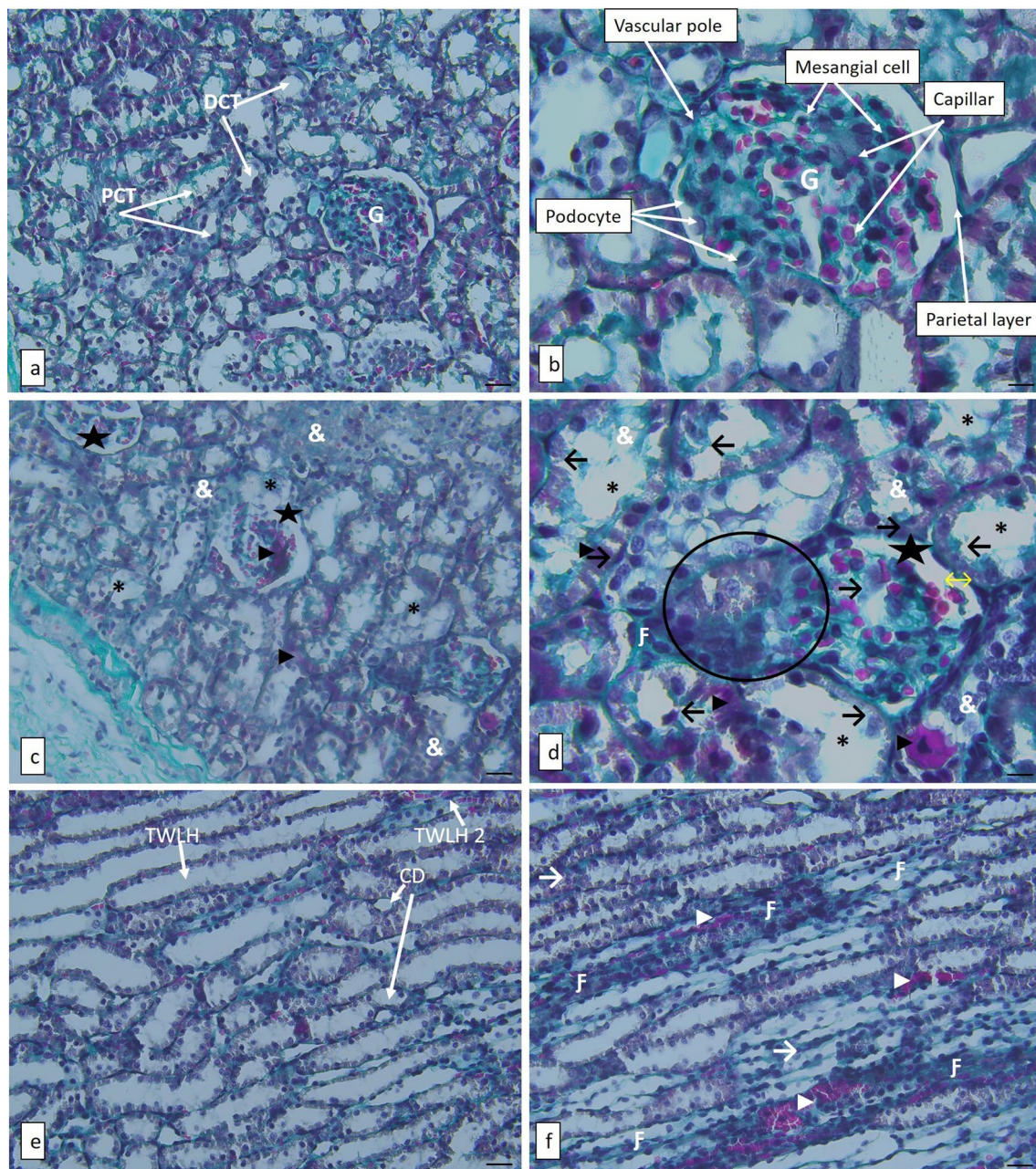


Fig. 7 Histology photomicrographs showing Masson's trichrome staining of the rat kidney. Normal histology of the kidney tissue can be seen in the control group (**a,b,e**). (**c,d,f**) Electromagnetic field group; (*) tubular dilatation, (&) tubular degeneration, (★) vascular cortical/ medullary hemorrhage/congestion, (→) cell degenerations, (←) epithelium desquamation, (☆) dilatation of Bowman's capsule and

glomerular damage, (↔) Parietal layer degeneration, (in circle) degeneration at the vascular pole and (F) fibrosis. G: Glomerulus; PCT: Proximal convoluted tubule; DCT: Distal convoluted tubule, CD: collecting duct, TWLH: a thick-walled loop of Henle, TWLH 2: a thin-walled loop of Henle. Scale bar = 50 μ m, x20 (**a, c**); 20 μ m x40 (**e, f**); 10 μ m, x100, immersion oil; **b, d**)

play an RAS-triggering role. Previous studies have reported that EMF causes damage in tissue in addition to DNA damage (Cassady 1995; Türedi et al. 2017, 2022; Borzoueisileh et al. 2020). Evaluation showed cortical/medullary bleeding/obstruction and widespread fibrosis. When organs are exposed to different pathogenic factors, such as injury and inflammation, this can alter the cell phenotype and gradually

give rise to fibrosis (Zhang et al. 2020). EMF is thought to be one such pathological agent. Our findings show that fibrosis can develop in kidney tissue as a result of exposure to 0.9 GHz EMF.

Dilatation, vacuolization and degeneration in the distal and proximal tubules, degeneration and a decrease in epithelial cells, and atypical parietal cells were also detected in

Table 3 Control and EMF group histopathological scoring ($n=6$)

Histological Parameters	Mean $\pm$ Standard Deviation	
	Control Group	EMF Group
Tubular damage	0.67 $\pm$ 0.52	2.33 $\pm$ 1.03 ^a
Degeneration of tubular epithelial cells	0.83 $\pm$ 0.75	1.83 $\pm$ 0.75 ^a
Dilatation of Bowman's capsule	0.00 $\pm$ 0.00	1.33 $\pm$ 0.52 ^a
Glomerular damage	0.33 $\pm$ 0.52	2.17 $\pm$ 0.41 ^a
Degeneration of Bowman's membrane	0.00 $\pm$ 0.00	1.67 $\pm$ 0.52 ^a
Degeneration in podocyte and mesangial cells	0.17 $\pm$ 0.41	1.83 $\pm$ 0.75 ^a
Degeneration in the vascular pole	0.17 $\pm$ 0.41	2.33 $\pm$ 1.03 ^a
Cortex/medullary hemorrhage/congestion	0.00 $\pm$ 0.00	2.17 $\pm$ 0.41 ^a
Fibrosis	0.17 $\pm$ 0.41	2.67 $\pm$ 0.82 ^a
Perivascular edema	0.00 $\pm$ 0.00	0.17 $\pm$ 0.41

Data presented as mean $\pm$ standard deviation. ^a $p < 0.01$ compared to the control group

kidney tissues in this study. In addition, dilated and degenerated glomeruli, and degeneration in Bowman's membrane (parietal membrane), the vascular pole, and podocytes and mesangial cells were observed in Malpighian bodies. Although angiotensin is normally produced by the liver, it is also known to be produced by the proximal tubule of the nephron (Matsusaka et al. 2012). Our histological examinations revealed low levels of the enzyme ACE, responsible for the conversion of Ang I to Ang II in the RAS, in the distal tubules and high levels in the proximal tubules (microvilli). One of our findings, the presence of atypical giant parietal cells, reveals the sloughing of tubular epithelia as a result of severe necrosis. This will in turn result in decreased ACE protein production. That decrease will conclude with the transmission of signals for increased production of ACE. Moreover, damage was detected in the present study in the juxtaglomerular cell, macula densa and its extension, the distal tubule, in the Malpighian body. Renin produced in the kidney is known to be manufactured in the juxtaglomerular cells and distal tubule (Steglich et al. 2020). Damage occurring in both areas will result in renin enzyme insufficiency. This will in turn result in the transmission of a signal to increase renin production. In conclusion, we think that the histopathological changes directly caused in tissues by EMF may emerge as a trigger resulting from an insufficiency of RAS components (ACE gene expression and renin) whose production is essential. Excessive increases in renin, angiotensin, and renin will occur as a result of EMF triggering the RAS. Effects such as vasoconstriction, ROS generation, inflammation, salt and water retention, and fibrosis will be observed as a result. Prolongation of EMF exposure will also cause the stimulus to continue and lead to an increase in kidney tissue damage. This damage in renal tissue resulting from EMF will trigger the RAS, and findings emerging

due to the RAS will lead to impairments in kidney tissue. This cycle will be capable of causing severe kidney injury. Studies have reported that over-activation of the RAS may result in cardiovascular diseases, diabetes, stroke, and kidney failure (Dzau 2005; Hussain and Awan 2018).

Conclusion

In conclusion, we think that exposure to an 0.9-GHz EMF may activate the rat kidney RAS and that such activation may be associated with histological and biochemical changes in renal tissue. The trigger effect mechanism of EMF on the RAS is addressed for the first time in this article. In the light of the study details, we think that further research is now warranted to elucidate the cellular and molecular mechanisms involved in the effects of EMF on kidney tissue and in order to contribute to human health in this area. Although there are many animal studies showing that EMF has negative effects on kidney tissue, human studies do not seem to be given any importance. In the literature, a human survey study shows that prolonged phone calls and higher EMF exposure during pregnancy increase the risk of congenital anomalies of the kidney and urinary tract in the offspring (Çeleğen et al. 2024). Another study states that EMF may increase the risk of bladder cancer (Band et al. 2005). The limited number of studies conducted provide us with show us that EMF may have effects on the urinary system and more studies need to be done on this subject.

Author contributions AIK and EO designed the study. HK EMF installed the system. AIK and TM performed the histological procedures. HSE made biochemical analyses. GK made a statistical analysis. AIK wrote the paper. All authors approved the final version of the manuscript.

Funding No financial support is provided for the research.

Data availability No datasets were generated or analysed during the current study.

Declarations

Ethical approval All procedures applied to animals were approved by the Institutional Ethics Committee at Karadeniz Technical University (KTU) (Date: 19.06.2014, Protocol Number: 2014/33).

Competing interests The authors declare no competing interests.

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