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Effects of folic acid on rat kidney exposed to 900 MHz electromagnetic radiation

Ömür Gülsüm Deniz*, Elfide Gizem Kıvrak, Arife Ahsen Kaplan, Berrin Zuhal Altunkaynak

Department of Histology and Embryology, Medical Faculty, Ondokuz Mayıs University, Samsun, 55139, Turkey

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ABSTRACT

Because of increased use of cell phones, the purpose of this study was to investigation of the oxidative damage caused by electromagnetic radiation (EMR) emitted by cell phones and histological and morphometrical determination of the possible protective role of folic acid (FA) in preventing the detrimental effects of EMR on the kidney. Twenty-four adult male *Wistar albino* rats were divided into control (Cont), EMR, EMR + FA and FA groups, each containing six rats. The EMR and EMR + FA groups were exposed to EMR for 60 min a day over a period of 21 days, while no EMR exposure was applied to the Cont and FA groups. The source of the EMR was an EMR device which emits a digital signal producing 900-MHz frequency radiation. The generator connected to a one-monopole antenna was used in this study and the rats were placed in the plexiglass restrainer at an equal distance from the monopole antenna. Following the experimental period, and after tissue processing, a physical disector-Cavalieri method combination was applied to the sections. The mean volume of the cortex, medulla, proximal and distal tubules increased significantly in the EMR groups compared to the Cont group ($p < 0.01$). Contrarily, the total number of glomeruli in the EMR group decreased compared to the Cont group ($p < 0.01$). The protective effects of FA was observed in the kidney ($p < 0.05$).

In conclusion, the 900-MHz EMR leads to kidney damage. FA may exhibit a protective effect against the adverse effects of EMR exposure in terms of the total number of glomeruli.

1. Introduction

The use of electromagnetic radiation (EMR) is increasing continuously in line with technological advances, leading to a much greater level of exposure to EMR in daily life than that which exists in nature. The recent rise in the use of cell phones and consequently in exposure to EMR has raised the question of its possible side-effects on the living organism and has caused some measure of concern [1–3]. The discovery of electricity resulted in major changes in human social life. The rapid development of industry and improvements in economic conditions has led to very considerable numbers of electronic devices being used [4]. In the current age, exposure to EMR emitted by various sources like power lines, radios, electrical home devices, computers and phones is an inescapable part of the daily life [5,6]. However, most people are unaware of the harmful effects on health of EMR which is emitted by these devices [4]. The 900-MHz and 1800-MHz radio waves are used frequently in mobile phone telecommunication [7,8]. For this reason, an intense exposure to EMR is usually possible in mobile phone usage. It is known that mobile phones emit EMR from 42 V/m at 0.1 m to 7 V/m at 1 m [9].

While the use of cellular phones is increasing very rapidly, this now seems to have become an important health problem, thanks to studies reporting that these radiation waves are harmful to human health. There has thus been considerable scientific focus on this and research into the biological effects of exposure to EMR [10–12]. A number of experimental studies have shown that EMR increases oxidative stress in various organs and tissues [13–16]. Furthermore, it was reported that EMR may have hazardous effects on tisues such as liver, testes, heart, lung, brain and kidney [11,12,17–20]. The close proximity of telephone antenna to the abdominal organs has raised various doubts regarding biological interactions between EMR and the kidneys in particular [21]. Of these, it is possible that 900-MHz radiation emitted from the mobile phone devices is absorbed by the kidneys with a higher ratio than the other internal organs because mobile phones are often carried on belts [13].

* Corresponding author.
E-mail address: omur.denizomu@gmail.com (Ö.G. Deniz).

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The use of mobile phones results in the production of free radicals, leading to subsequent damage in tissues [14,22]. At this point; kidney tissue is extremely sensitive to oxidative damage since it is one of the organs involving intense oxidation processes [21]. The continuity of cellular life depends on the balance in the execution of complex biological reactions. Endogenous or exogenous factors that might disrupt this balance lead to cellular damage. Of these, oxidative stress is particularly important because it causes various pathological conditions, and numerous studies have been performed on the subject. Radical reactions are a part of the homeostasis. Healthy cells eliminate free radicals by using antioxidants in a homeostatic manner. Cells are protected against the harmful effects of reactive oxygen species by their antioxidant defense systems [23–25]. An antioxidant defense system is developed by the living tissues to cope with the hazards exerted by the oxygen free radicals. The mentioned defense system includes the in situ production of antioxidants. The removal of the reactive species may be non-enzymatic or, as a more efficient way, enzymatic. The hazardous effects of reactive oxygen species (ROS) may be alleviated by the endogenous and exogenous antioxidant agents via their free radical scavenging activity and thus the immune defense can be boosted and the risk for the development of cancer and degenerative disorders may be reduced [26–30]. The system of antioxidant defense with the highest capability includes the stimulation of glutathione peroxidase (GPx), catalase (CAT) and superoxide dismutase (SOD) [31–33]. As it was reported by Martínez-Sámano et al. these enzymes function via the conversion of the free radicals into relatively harmless molecules or their removal from tissues [34].

Preventive measures can be taken to alleviate the hazardous effects of EMR such as minimizing the duration of the phone calls, usage of EMR filters, avoidance of carrying the mobile device on the body, hands free usage of the phone and using the phone in airplane mode as long as possible [35].

Studies show that EMR has various effects on the living organism. While some studies have emphasized the damage caused by these waves, others have reported that EMR can be used for medical purposes. This discrepancy in the literature has led to an interest in the effects of EMR on the living organism and has shown that research on this subject is insufficient. The effects of these fields have therefore been investigated using various test methods [11,27]. It was reported that mobile phone usage has various biological effects on the kidney. At this point; it was proposed by Bedir et al. that the electromagnetic waves originating from the mobile phones may exert hazardous effects on the development of kidneys in rats [36]. Moreover; it was reported by Ulubay et al. that EMR can be counted among the stress factors which may cause extensive renal damage [12]. Besides; Odacı et al. indicated that exposure to EMR during the prenatal period may ensue pathological effects in the renal tissues of 21-days old rats because of oxidative stress and reduced levels of antioxidant enzymes [37]. Furthermore; it was indicated by Monfared et al. that there was no evident toxicity to the mice kidneys due to the cell phone exposure [38].

In the present study; folic acid (FA), which is reported to have a very potent free radical scavenging property against the potential effects of EMR and which is an important vitamin for human health was used [26]. Moreover, despite its water solubility, FA can prevent lipid peroxidation [39]. At this point; it was reported by Chauveau et al. that supplementation with the FA for a long term may decrease the elevated levels of plasma homocysteine level in chronic renal failure [40]. Also, it was reported by Sung et al. that FA may reduce the homocysteine levels in chronic kidney disease patients due to its property of being required in the metabolization of homocysteine and methionine [41]. Furthermore, it was indicated by Hwang et al. that FA usage has properties of renal protection against oxidative stress [42]. Nevertheless; chronic renal failure is counted among the risk factors for cardiovascular disease [43]. Qin et al. suggested that the risk for cardiovascular disorder in chronic kidney disease patients may be reduced by approximately 15% with FA therapy [44].

There are very limited amount of ways to estimate the structures in three-dimensional tissues and organs [45]. Also, several of the available methods are biased and give insufficient information. At this point, the analysis of the parameters such as cell number, volume, length and area of surface was widely estimated by using morphometric methods including stereology [46]. In the current study, the total number of glomeruli and kidney volume was estimated by using light microscopic morphometry (physical disector-Cavalieri method). The parameters like size, number, distribution of nephrons, cells and other elements gives crucial data concerning the organization and function of kidney under study. So, it is vital to measure the several structural components in a correct way. The estimation of these elements is also important in the examination of the way in which the kidneys react to trauma, disease and chemicals [47]. Some researches exist in the literature, concerning the utilization of stereological methods in renal disorders. Guo et al. used unbiased stereological methods and high-resolution light microscopy for the estimation of glomerular volume and dimensions of glomerular capillaries including the surface area and length in 7-month-old db/db diabetic mice. It can be deduced from their results that a connection exists between the glomerular hypertrophy in the diabetic neuropathy in this model and the elevated surface area and length of the glomerular capillaries [48]. Nevertheless, quantitative analysis of the kidney properties in a high- fat induced obesity model of rats was done by Altunkaynak et al. by using Cavalieri and physical disector method. It can be noted from the results that a diet rich on fats may ensue histopathological changes like dilatation, tubular defects, connective tissue enlargement and inflammation [49].

In the light of all the facts mentioned above; the purpose of this study was to determine the potential effects of EMR caused by cellular phones on rat kidneys, to investigate the possible protective effects of FA against 900-MHz EMR, to raise awareness of the issue in society and to make suggestions regarding means of protecting against the harm caused by this, or how to minimize it. For full clarifications of the effects of EMR on the renal tissue, there is a need for further studies at the especially cellular and molecular level.

2. Materials and methods

2.1. Experimental design

Kidney tissues used in this study were obtained from the rats of a previous study (project no: PYO·TIP. 1904.13.025) focusing on a different organ, approved by the Ondokuz Mayıs University Ethical Committee for Animal Experiment (HADYEK/23, 27.08.2014). During the experiments, the conditions set by the Ethical Board were adhered to, and the animals were cared for in the research center during the experimental stage of the study carried out in this center. The calculation of sample size with power analysis is the conventional method with the highest scientific properties. At this point, power analysis was used to calculate the sample size for the present study. Finally, we have used need 6 rats per group based on the power analysis. In this context; this study involved 24 male *Wistar albino* rats obtained from the Ondokuz Mayıs University, Faculty of Medicine, Experimental Animals Research and Practice Center. These were 11–12 weeks old and weighed 200-250 g. Other histological procedures were carried out in the Department of Histology and Embryology. During the experiment, all rats were kept in standard plastic cages with a circadian rhythm of 12 h of light and 12 h of darkness at a room temperature of $20 \pm 24\,^{\circ}C$ in the

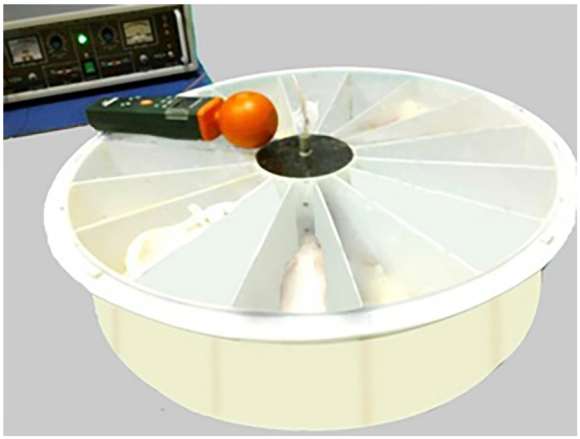

Fig. 1. The system used to expose rats to EMR in the round plastic EMR cage around the monopole antenna.

Experimental Animals Research and Practice Center. The animals were given pellet feed and tap water. The *Wistar albino* rats in this study were assigned randomly and divided into four groups of six animals each.

- **Cont group**: The rats in this group were fed for 21 days with no restriction of pellet feed. They were given tap water and were not exposed to EMR.
- **EMR group**: The animals in this group were exposed to a 900-MHz EMR for an hour at specific hours of the day (13:30-14:30) for 21 days and did not receive any treatment. EMR exposure was applied using a special mechanism.
- **EMR + FA group**: The animals in this group received 50 mg/kg FA via gavage once a day 1 h before [20] is being exposed to EMR for 60 min for 21 days.
- **FA group**: The animals in this group were not exposed to EMR, but were given 50 mg/kg FA (dissolved in 2 ml distilled water) at a specific time of day for 21 days via gavage.

All rats were anesthetized with intraperitoneal ketamine (10 mg/100 g body weight; Sigma Chemical Comp., St. Louis, MO, USA) and xylazine (50 mg/100 g body weight; Sigma Chemical Comp., St. Louis, MO, USA.) at the end of the experiment. After cardiac perfusion, the kidney tissues of all rats were removed.

2.2. EMR exposure system

An EMR generator with an output of 1–2 W (PW = Pulse Wave) operating at 900–1800 MHz was used as a source of EMR. Rats were exposed to EMR with a 900-MHz half-wave monopole antenna. The antenna used was equivalent to mobile phone antenna with circular polarization and direction. The exposure device used consisted of a round plastic cage with 16 separate parts (diameter: 5.5 cm, length: 12 cm), the monopole antenna of the exposure system and an EMR meter (Fig. 1). The signal generator was activated at 2 W, and the mean electric field density (mW/cm^2) in the vicinity of the monopole antenna was measured accurately using an electrical field probe (EXTECH RF EMR strength meter) during the experiment. These measurements were recorded every six minutes starting from minute 0. Eleven measurements were recorded at the end of each day.

2.3. Exposure to EMR

For exposure to EMR, the system's monopole antenna was embedded in the center of the round plastic cage, which was divided into 16 segments in order to ensure that the electric field was evenly distributed. All the rats were placed in such a way as to be in close contact with the monopole antenna [28,29]. The rats were then exposed to a 900-MHz EMR for 60 min a day over 21 days. The monopole antenna was placed in a vertical position within the plastic cage, and the rats were positioned within 1 cm of each other. The rats' heads were placed facing the antenna [30]. Fig. 1 shows the EMR exposure apparatus.

2.4. Histological procedures

If the surface of a hemisected fresh kidney specimen in examined, it can be seen that there are two different regions as cortex and medulla. Cortex is located on the outer side and is reddish brown in colour, while medulla is located on the inner side and lightly coloured [50]. At this point; for light microscopic examination, freshly obtained kidney samples were fixed in 10% buffer formalin, dehydrated in a graded alcohol series, and cleared in xylene. Following dehydration, samples were embedded in paraffin. Each paraffin block was cut at 10 μm sagittally using a rotary microtome (Leica, Istanbul, Turkey). These sections were then stained with a Masson's trichrome staining procedure for histopathological and stereological examination. Following the processes of deparaffinization and rehydration, the sections underwent the procedure of staining with Weigert iron haematoxylin for 5 min and then they were washed with tap water for 5 min at room temperature. After that, they underwent staining with acid fuchsin and washed one more time with tap water for 5 min. Then, the concerning sections were treated with mordant phosphotungstic acid and washed with tap water for 5 min. Lastly, the sections underwent staining with aniline blue and washed with tap water for 5 min at room temperature. Sections underwent dehydration in consecutive grades of alcohol (70%, 80%, 96% and 100%) for 2 min in each one. Xylene was used to clear the sections and then was mounted in entellan [51]. Regarding these procedures, collagen is stained as blue with aniline blue while the nuclei are stained as black with Weigert's iron haematoxylin and cytoplasm is stained red with acid fuchsin. At this point; following the routine histological procedures, parts of the kidney to be estimated were easily identified in the Masson's trichrome sections under the light microscope. In this context; it was seen that the renal corpuscles and their tubules are features of the cortex. The renal corpuscles are seen as the structures with spherical shapes and they include the glomeruli, the capillary networks with unique properties. The outer layer of the Bowman's capsule (parietal layer) is enveloped by a simple squamous epithelium. Besides; the inner layer of the Bowman's capsule (visceral layer) consists of the specialized epithelial cells [50,52]. Furthermore; the straight tubules, collecting ducts and a capillary network with special features are characteristics of the medulla. Glomeruli are not seen in the medulla. Moreover, proximal and distal tubules display some properties specific to them as the proximal tubules have larger diameter than the distal tubules and that the proximal tubules possess lumen with irregular shape while the lumen of the distal tubules is round in shape. Also, the distal convoluted tubule has low cuboidal epithelium and do not display a brush border while the proximal convoluted tubule possess low columnar epithelium and displays a brush border [50,53].

2.5. Stereological analysis

2.5.1. Volume estimation with the cavalieri principle

Stereological methods are used to elicit quantitative descriptions of three-dimensional structures using two-dimensional images [54]. The most commonly used method in stereological volume calculations is the Cavalieri principle. In this study were also estimated using the Cavalieri principle [49,55]. At this point;

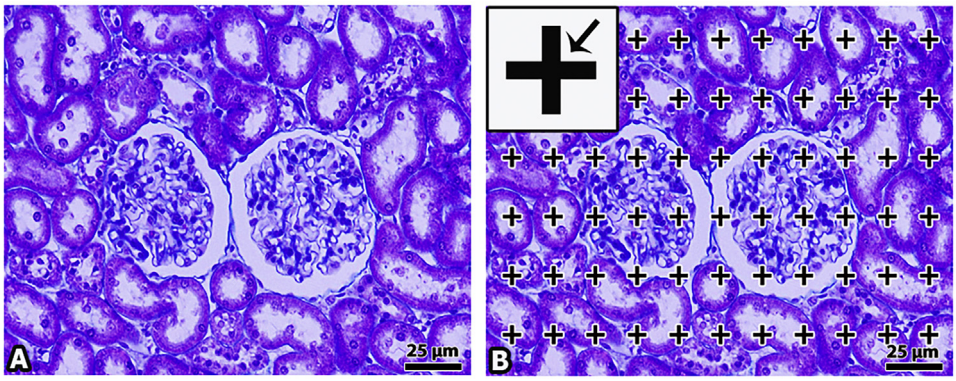

Fig. 2. Light microscopy image showing the stereological procedure for applying the Cavalieri principle. Selected sections (A) stained with Masson's trichrome staining method were used for the point counting grid method (B) under a light microscope with camera attachment. The black arrow shows the left upper corner of the cross taken as a point. The number of the points touching the structure was used in order to determine the volume density of the kidney, proximal and distal tubule and cortex and medulla.

according to our pilot study kidney sections were taken at 1/18 sampling fraction for the Cavalieri method. Moreover; the distance between the points of grid was 0.5 cm. Grid size (x-y axes) was 0.25 cm^2 [56]. When using the Cavalieri principle, a coefficient of error (CE) is obtained in order to calculate the reliability of the point density of the grids and sectioning intervals [57]. Previously published formulae were used to calculate the CE and coefficient of variation (CV) [58]. Sections 10 μm in thickness were obtained at intervals of 330 μm. An appropriate point per area of interest (total kidney areas) was calculated for each section. Finally, kidney volumes were calculated using the formula [49] (Fig. 2)

$$\boldsymbol{V} = \boldsymbol{t} \cdot \frac{\boldsymbol{a}}{\boldsymbol{p}} \cdot \sum_{\boldsymbol{i}=1}^{\boldsymbol{m}} \mathbf{P}i$$

where V is the mean volume of the kidney; t, the mean section thickness; a/p, the inter-point area; and $\sum$P, the total number of points superimposing whole serial sections of the kidney.

2.5.2. Numerical density and total number of glomeruli

The physical disector method, based on pairs of sampled sections, was used to estimate the total number of glomeruli obtained from kidney samples [12]. One of these two-dimensional plane sections is referred to as the "*reference*" section and the other as the "*look up*" section. At this point; according to our pilot study the sampling fraction was 1/6 for the physical disector. The essential concept in the disector technique is counting the tip of the particle, which is found only in the reference section, and not in the look up section. Such an approach involves counting the "tips" of those particles with any "tips" between two plane sections. Since each particle generally has only one tip facing a particular direction, this type of counting allows for an unbiased calculation of the number of particles in a given volume [57]. The dimension of the unbiased counting frame [49] on the computer screen was $8 \times 8\ cm^2$. In this context; a digital camera was used to take photos of each slide at X100 magnification. The estimated reference section area of each kidney profile was divided into equal fields in the x and y-axes of the microscope. Lastly, photos of *all the fields in each step* were collected by using a digital camera, as it was estimated via motorized microscopy stages previously. The same procedure was used for the other, look up section [12,49]. Following these, a proper unbiased counting frame was put manually on both the reference and look-up sections [59] as it is seen on the computer screen to perform the process of counting in accordance with the disector method. Then; glomeruli could be encountered in the reference section but not in the look-up section. Finally, the method of disector counting was utilized for the pairs of sections [12,49]. CV and CE of the estimated number values for glomeruli in each group were within acceptable ranges. The generally accepted highest limit of CE is 5% [58].

In order to estimate total number of glomeruli, we calculated the numerical density of glomeruli per volume unit by applying the formula

$$\boldsymbol{N_v} = \frac{\boldsymbol{\Sigma Q^-}}{\boldsymbol{\Sigma V_{Disector}}}$$

where ΣQ is the total number of glomeruli counted and ΣV (t × A) is the total volume of the reference disectors used.

Finally, the total number of kidney glomeruli was calculated using the formula

$$\mathrm{TN(total\ glomeruli)} = \mathrm{N_V \cdot V_{ref}}$$

Where TN is total number of kidney glomeruli, $\mathbf{N}_V$ the numerical density of glomeruli and $\mathbf{V}_{ref}$ the total kidney volume estimated using the Cavalieri principle.

2.6. Statistical analysis

For the statistical analysis, SPSS Ver. 21 software was used. Data were expressed as mean ± SEM and were subjected to statistical analysis. Moreover; data were normally distributed in the present study. So; differences among groups were assessed using ANOVA which is a parametric test followed by the Tukey's HSD test with post-hoc analysis to identify individual group differences. Differences were regarded as statistically significant at $p < 0.05$.

3. Results

Stereological and histopathological examinations were performed on Masson's trichrome stained sections which were taken in accordance with the systematic random sampling and the appropriate stereological method to be applied.

3.1. Stereological results

3.1.1. Mean kidney volumes

Mean kidney volumes were estimated using the Cavalieri principle on a series of sections. The estimated volumes of relevant areas from all groups are shown in Fig. 3. A significant increase was observed in the EMR group compared with the Cont, EMR + FA and FA groups ($p < 0.05$). A significant change was also observed between EMR + FA and FA ($p < 0.05$). CE and CV values for all groups are shown in Table 1.

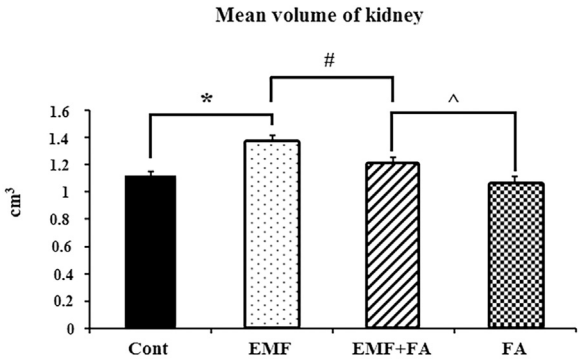

Fig. 3. Bar diagram showing the mean kidney volumes in all groups. (*), (#), and (^) indicate differences between the matched groups at the 0.05 level ($p < 0.05$). The error bars indicate standard error mean (SEM).

Table 1
The mean CE and CV values of the mean kidney volume for each group.

Groups		CE and CV values
Cont	CE	0.03
	CV	0.06
EMR	CE	0.02
	CV	0.08
EMR + FA	CE	0.04
	CV	0.11
FA	CE	0.03
	CV	0.09

3.1.2. Mean cortex and medulla volumes

The results are shown in Fig. 4. The mean cortex and medulla volumes in the EMR exposed group were significantly higher than those of the Cont group ($p < 0.01$). A significant decrease was also observed in terms of mean cortex and medulla volumes in the EMR + FA and FA groups ($p < 0.05$). No significant difference was determined between Cont and FA groups ($p > 0.05$). CE and CV values for all groups are shown in Table 2.

Table 2
The mean CE and CV values of the mean cortex and medulla volumes for each group.

Groups		CE and CV values
Cont	CE	0.02
	CV	0.05
EMR	CE	0.04
	CV	0.12
EMR + FA	CE	0.05
	CV	0.09
FA	CE	0.02
	CV	0.07

3.1.3. Mean proximal and distal tubule volumes

The estimated volumes for all groups are shown in Fig. 5. The mean volumes of proximal and distal tubules of the EMR only group were significantly higher than in the Cont group ($p < 0.01$). No significant differences were determined among the other groups ($p > 0.05$). CE and CV values for all groups are shown in Table 3.

3.1.4. Total number of glomeruli

Total number of glomeruli was estimated using the physical disector method on each Masson's trichrome stained section. The estimated values for all groups are shown in Fig. 6. There was a significant decrease in the EMR and the EMR + FA groups in terms of total number of glomeruli compared with the Cont and FA groups ($p < 0.05$). FA treatment also caused a significant increase in the total number of glomeruli in EMR + FA compared to the EMR

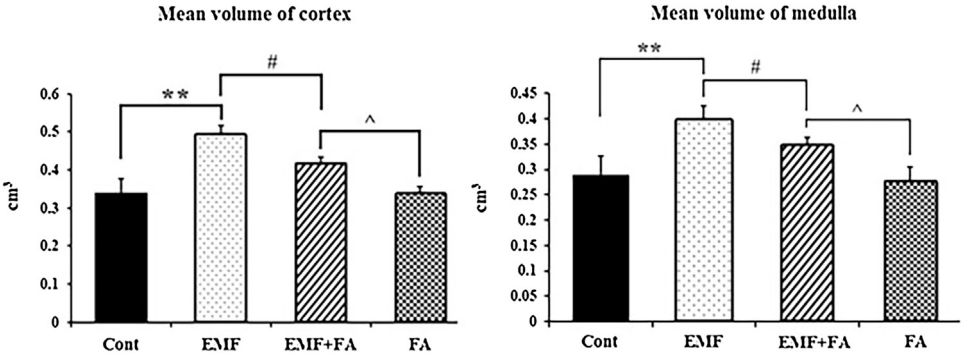

Fig. 4. Bar diagram showing the mean cortex and medulla volumes in all groups. (**) indicates the differences between groups at the 0.01 level ($p < 0.01$). (#), and (^) indicate the differences between relevant groups at the 0.05 level ($p < 0.05$). The error bars indicate standard error mean (SEM).

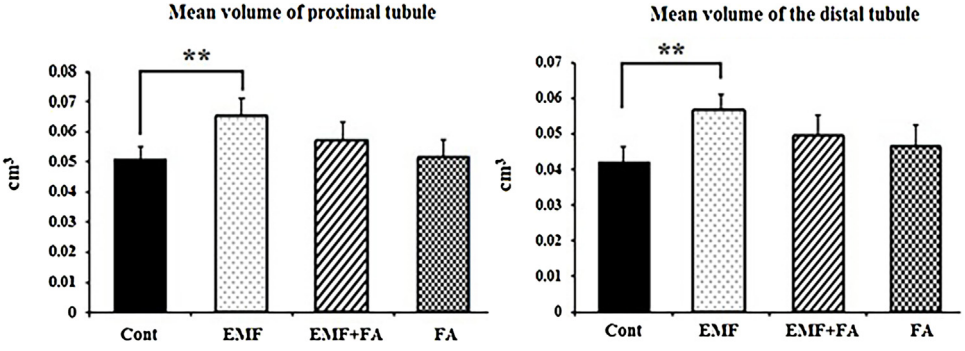

Fig. 5. Bar diagram showing the mean volumes of proximal and distal tubules in all groups. (**) indicates the differences between relevant groups at the 0.01 level ($p < 0.01$). The error bars indicate standard error mean (SEM).

Table 3
The mean CE and CV values of the mean proximal and distal tubule volumes for each group.

Groups		CE and CV values
Cont	CE	0.05
	CV	0.11
EMR	CE	0.03
	CV	0.07
EMR + FA	CE	0.02
	CV	0.04
FA	CE	0.06
	CV	0.14

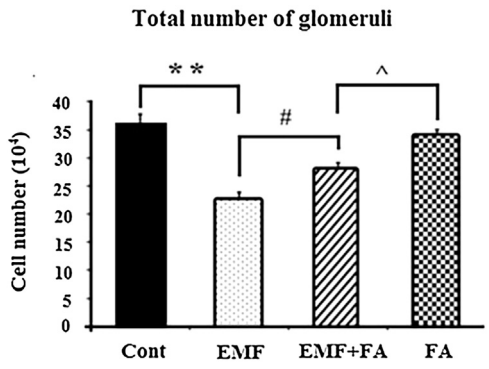

Fig. 6. Bar diagram showing the total numbers of glomeruli in all groups. (**) indicates the differences between relevant groups at the 0.01 level ($p < 0.01$). (#), and (^) indicate the differences between groups at the 0.05 level ($p < 0.05$). The error bars indicate standard error mean (SEM).

Table 4
The mean CE and CV values of the total number of glomeruli for each group.

Groups		CE and CV values
Cont	CE	0.03
	CV	0.05
EMR	CE	0.06
	CV	0.09
EMR + FA	CE	0.04
	CV	0.12
FA	CE	0.05
	CV	0.15

group ($p < 0.05$). A highly significant difference was also observed between the EMR group and the Cont group ($p < 0.01$). No significant difference was determined between the Cont and the FA groups ($p > 0.05$). CE and CV values for all groups are shown in Table 4.

3.2. Histopathological results

All Masson's trichrome stained renal specimens were examined histopathologically under a light microscope. The kidneys of the rats in the Cont group exhibited normal mammal renal histology. Furthermore; there was increased degeneration including tubular defects, glomerulosclerosis, and hydrophic degeneration of tubule cells in the EMR groups (Fig. 7). The administration of FA reduced these degenerative effects of EMR.

4. Discussion

The effects of EMR emitted by cell phones and base stations on human health are now subjects of serious scientific discussion [1–3]. So; various researchers have sought to investigate the potential effects of EMR using different methods [11,12]. At this point; stereology, a scientific methodology, involves the estimation of the three-dimensional properties of histological structures based on two-dimensional images and has grown rapidly in recent decades. Due to properties such as objectivity and the avoidance of any systematic deviation from real values, these techniques make it possible to work with low levels of error and to obtain highly reliable results within a short time. Therefore; stereology now plays a central role and is an essential element in morphometric studies [60,61]. In this context; our stereological results show that the mean volume of the cortex and medulla and proximal and distal tubules were significantly increased in the EMR group compared to the Cont group ($p < 0.01$). The increase in the kidneys' volume following EMR exposure may have resulted from oedema because of mononuclear cell infiltrations among the tubules. And it is clearly understandable that dilatation may cause an increase in the kidney volume [49]. Moreover, the total number of glomeruli in the EMR group decreased compared to the Cont group ($p < 0.01$). At this point; apoptosis is a major mechanism for the controlling the glomerular population's size [62–64]. Also; it is responsible for the progressive cell loss emerging in the glomerular sclerosis' pathogenesis [64]. Hattori et al. reported that reduction in the glomerular cells's number is related to apoptosis which causes a decrease in the renal function [65]. Furthermore; histopathological evaluation in the present study revealed that the kidneys of the rats in the Cont group exhibited a normal mammal renal histology. Images from the EMR groups showed increased degeneration including tubular defects, glomerulosclerosis, and hydrophic degeneration of tubule cells. The administration of FA again reduced these degenerative effects of EMR. From this perspective, these histopathological changes in the tubules may be attributed to the decrease in glomerular blood flow and filtration and the constriction in peritubular capillary walls in interstitial connective tissue caused by oxygen radicals in the kidneys through the release of bioactive lipids. This constriction may result in insufficient nutrition and oxygenation of the proximal and distal tubules and consequently in degeneration [66]. It may also be concluded that vacuolization takes place due to a decrease in toxicity caused by recurrent EMR exposure in smooth endoplasmic reticulum and starts with an increase in the density of the organelle. This organelle plays a role in cell detoxification, the regression thereof and its manifestation in the form of vacuolizations [66]. In addition, mononuclear infiltration can be said to be associated with the massing of the mononuclear and polymorphonuclear cells that occurs with chemotaxis.

The fact that standard cell phones emit electromagnetic radiation means that they increase oxidative stress and apoptosis in several tissues in the body [67,68]. The kidney is particularly sensitive to oxidative damage as oxidative processes occur in more intensely within it [12]. At this point; the major effect of antioxidants on human health occurs through their radical scavenging mechanism. Increasing numbers of studies are focusing on the harmful effects of EMR and on the use of antioxidants in order to minimize these [69]. One study in the current literature [12] reported that prenatal exposure of rat kidneys to a 900-MHz EMR results in an increase in total kidney volume and decreased glomeruli numbers. Another study showed that exposure to 900-MHz mobile phones reduced renal SOD, CAT, and GPx activities [13]. Exposure to 900-MHz EMR has also been shown to cause congested blood vessels, vacuolated and degenerated renal tubules with necrosis in the renal epithelium, dilatation of Bowman's capsules with atrophied glomeruli, and infiltration of leucocytes [66].

In the present study; we concluded that EMR is a stress factor due to the tissue damage resulting from exposure to it [23]. FA also exhibited statistically significant beneficial effects on the mean volumes of the kidney and cortex and medulla and on total number

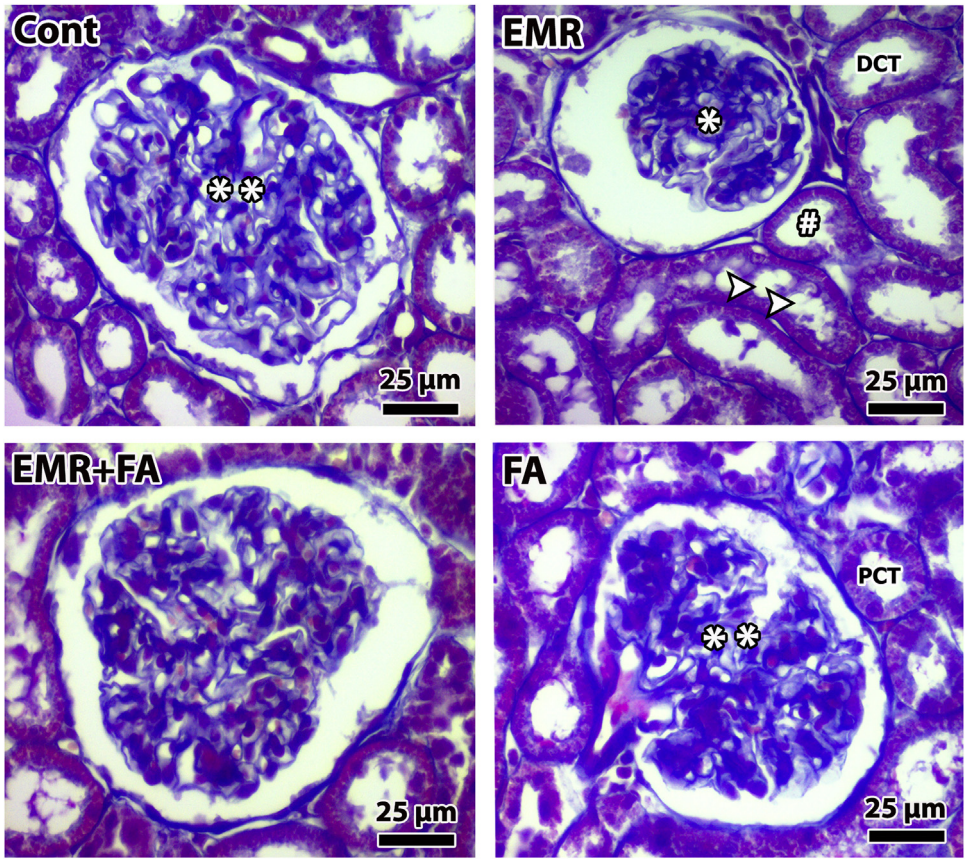

Fig. 7. Photomicrograph of kidney tissue from the Cont, EMR, EMR + FA and FA groups showing a normal glomerulus (square), glomerular sclerosis (asterisk), degeneration in tubules (white arrow), and increased hydrophic degeneration of tubular cells (arrowheads). It should be noted that such images may not be informative since their appearance may be altered by the section plane, tissue shrinkage or swelling, or the area of the images taken. The biological comments are based on stereological data. PCT, proximal convoluted tubule and DCT, distal convoluted tubule.

of glomeruli ($p < 0.05$). In this study, FA acted as a free radical scavenger and eliminated the potential harmful effects of EMR. This is the first study to use FA against renal damage caused by EMR exposure using the stereological methods.

Finally, this study represents the first report of the protective effect of FA against kidney damage induced by EMR using stereological methods. The modification of antioxidants is important in terms of preventing the harmful effects of EMR on biological systems and minimizing the resulting damage. Moreover; further studies are needed in order to better understand the deleterious mechanisms associated with EMR and to develop protective or supportive treatments to protect the health of the community.

Ethics statements

The Animal Ethics Committee of Ondokuz Mayıs University approved the protocol, and appropriate measures were taken by our study group to minimize pain or discomfort in the animals. The experimental part of this study and the stereological and histopathological examinations were performed at the Ondokuz Mayıs University Department of Histology and Embryology.

Conflict the interest

The authors declare that they have no conflicts of interest.

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