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Effects of Mobile Phone Radiation (2100 MHz) on the Serology, Stereology, and Histopathology of the Kidney in Rats

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Abstract

Background: Electromagnetic Field (EMF) radiation is becoming increasingly relevant to public health concerns as mobile communication technologies evolve and expand. EMF induces apoptosis, mediated through an increase in free radicals in cells. In this study, for the first time, the effect of 2100 MHz (4G) mobile phone radiation on the serology, stereology, and histopathology of the kidney in Wister rats was observed.

Methods: Thirty-five healthy male Wistar rats were divided into five groups, with seven individuals in each. The four exposure groups received 15, 60, 120, and 180 minutes of daily radiation for 70 days. After euthanizing, blood samples were drawn from the heart for analysis of urea nitrogen (BUN), calcium, phosphorus, and creatinine. Kidneys were removed, routinely processed in paraffin blocks, and stained with hematoxylin and eosin. Stereological estimations of the proximal convoluted tubules (PCT), distal convoluted tubules (DCT), and glomeruli (G) were conducted via the Cavalier method.

Results: The macroscopic examination revealed consistent kidney findings without structural or color changes. Mean weight increased across all groups, with significantly higher gains in the 120- and 180-minute exposure groups ($P<0.05$). BUN showed a minor, non-significant increase ($P=0.116$), while creatinine levels were significantly elevated in all exposure groups ($P<0.001$). Calcium levels remained stable ($P=0.108$), but phosphorus decreased significantly in the 15-minute exposure group ($P=0.043$). Histopathological examination detected no structural changes. Stereological analysis showed reduced proximal convoluted tubule volume in exposure groups ($P=0.009$), lower distal tubule volume ($P<0.001$), and consistently reduced glomerular volume ($P<0.001$).

Conclusion: Studies show that EMF radiation with a lower frequency (920 MHz) will damage renal tissues than higher frequency (2100 MHz) waves. In conclusion, this study found no macroscopic or histopathological evidence of kidney damage due to mobile phone radiation exposure. However, significant alterations in creatinine levels and renal structural parameters in certain exposure groups suggest potential effects on renal function and structure. Further research is essential to elucidate these mechanisms and their clinical relevance.

Keywords: Cell phone, Kidney, Serology test, Pathology

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Introduction

Radiofrequency (RF) radiation has become a predominant part of life in the modern era, drawn from a wide variety of sources, both natural and human-made, resulting in a continuous influx of this electromagnetic energy (1). RF radiation is becoming more prevalent in the world due to the rapid development of technology, which has resulted in an environment that has become saturated with electromagnetic waves (2). Global Positioning System (GPS) that guides our travels or the endless television programs we watch, these frequencies have become an integral part of our everyday lives (3). The vast majority of RF radiation in the world comes from the technology that people rely on to remain connected, some of which is the result of natural phenomena (4, 5).

As a result of the complex relationship between these diverse electromagnetic frequencies and their impact on biological systems, understanding the intricate relationship between them poses a multifaceted challenge (6, 7). There are several frequencies in the RF spectrum

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that have found applications in global settings, and a nuanced exploration of the entire spectrum is necessary to gain a complete understanding of the RF spectrum (8, 9). It is becoming increasingly apparent that the implications of mobile networking for public health are significant, necessitating a thorough and rigorous investigation (10). The multifaceted nature of this research extends to animal studies, investigating whether RF radiation causes cancer to develop, how it functions in the brain, and how it affects the reproductive system as well (11). As an example, immunohistochemistry was used in a study to detect the presence of C-caspase, an enzyme that contributes to apoptosis, in the nervous system exposed to mobile RF radiation (12, 13).

Neuroscientists research the potential adverse effects of RF radiation and its benefits, both of which have been intriguing and contradictory, fueling debate as to whether RF radiation can have both adverse and beneficial effects (14). Upon closer exploration of this area, it is evident that electromagnetic fields may significantly impact protein structures, macromolecules, and even the integrity of the DNA in some cases (15, 16). The human body absorbs frequencies above 15 MHz and low-frequency radiations, operating at 50/60 Hz, emitted by household appliances, because of cellular stress due to the alteration of membrane fluidity in cells (17-19). Radiation from RF devices can also affect specific organ systems in a wide range of ways. For instance, rat renal tissue exposed to frequencies between 850 and 1900 MHz exhibits a number of pathological changes, such as mononuclear leukocyte infiltrations between tubules, dilated and vacuolated tubules, and glomeruli that are atrophying and congested (20, 21). Additionally, rats exposed to decametric electromagnetic waves (0.3 to 3 GHz) have been found to experience interstitial hemorrhaging, hyperemia, and constrictions in the glomeruli after exposure to frequencies with intensities exceeding those of mobile phones (22).

Along with the increasing use of mobile phones, electromagnetic emissions in human environments have notably increased (23, 24). Despite extensive research, the implication of these newly emerged technologies is still surrounded by controversy (25-27). Many contradicting results still exist in the literature, which calls for experimental animal studies (28, 29). Many studies have provided evidence for the cytotoxic effects of mobile range electromagnetic waves in laboratory conditions on organs such as the brain, liver, and kidney (30-33).

In a study conducted by Yavas et al the researchers aimed to explore the potential histopathological effects of exposure to Radio-frequency Radiation (RFR) from mobile phones on rat heart and spleen tissue. They exposed one group of rats to 2100 MHz GSM-like RFR for 5 hours a day for 14 days and compared them to a sham-control group. The results showed no significant changes in the heart tissue, but the spleen exhibited trabecular irregularity and sinusoid enlargement in the histopathological examination. However, immunohistochemical staining for p53 did not indicate significant differences. The conclusion suggested that short-term RFR exposure did not lead to major morphological changes in heart and spleen tissue, possibly due to the body's protective mechanisms against RFR (34).

Bedir et al conducted a study investigating the effects of long-term exposure to 2100 MHz electromagnetic fields on the kidneys of Sprague Dawley rats. In conclusion, this research revealed that exposure to 2100 MHz for 6 and 12 hours induced oxidative stress-mediated acute renal injury, with the severity depending on the duration of exposure and dosage (35).

According to the study by Berköz et al in 2018, chronic exposure to 1800 MHz radio-frequency electromagnetic radiation (RF-EMR) harms rats' liver, kidney, and brain tissue. The results showed that the RF-EMR exposure, liver, kidney, and brain tissues showed increases in malondialdehyde and nitric oxide, as well as decreased levels of reduced glutathione and reduced superoxide dismutase, and catalase activities. RF-EMR has been shown to cause oxidative and nitrosative damage to these tissues, suggesting that it may be an environmental stressor (36).

In a study by Okatan et al., they investigated the impact of a 900-megahertz (MHz) electromagnetic field (EMF) exposure on the kidneys of female rats during mid-late adolescence. Three groups were established: a control group, a sham group, and an EMF group. The EMF group was exposed to the 900-MHz EMF for 1 hour daily from postnatal days 35 to 59. The results showed significant kidney changes in the EMF group, including hemorrhage in the glomerulus, tubular epithelium irregularities, glomerular degeneration, and more. Oxidative stress indicators were also altered in the EMF and sham groups compared to the control group. This study suggests that exposure to this EMF during adolescence may have adverse effects on the kidneys of female rats (37).

In summary, Kilic et al's study suggests that exposure to 900 MHz EMF can activate the renin-angiotensin system in the brain and kidneys of both male and female offspring, possibly linked to increased inflammation and oxidative stress (38). Zosangzuali et al conducted a study on the effects of 1800-MHz RF-EMR from mobile phone base stations on Swiss albino mice. The studied mice were exposed to RF-EMR for various durations, and it was found that 12 hr/day and 24 hr/day exposures significantly reduced brain antioxidants while increasing lipid peroxidation. However, other organs (heart, kidney, liver) showed no significant changes in oxidative stress parameters. Liver enzyme activities and creatinine levels remained unchanged. The study also noted a decrease in red blood cell count and an increase in white blood cell count in mice exposed for 12 hr/day and 24 hr/day. This

suggests that RF-EMR from MPBS may induce oxidative stress in the brain, characterized by reduced antioxidants and increased lipid peroxidation, potentially linked to the generation of reactive oxygen species (39).

The present study aimed to evaluate for the first time the serological, stereological, and histopathological implications of 2100 MHz (4G) electromagnetic radiation in rat models.

Methods

Weighing Rats

The animals were weighed using an electronic digital scale (Tanita Corporation, Tokyo, Japan). Their weight was recorded for 10 days during the study period.

Study design and grouping

In this experimental study, 35 healthy male Wistar rats (4-5 months old, weighing 350-400 g) were obtained from the laboratory animal center of the Ferdowsi University of Mashhad. Animals were acclimated for seven days upon arrival, and housing ensured standard temperature and humidity, while water and laboratory diet were available ad libitum. For exposure to EMF, we used special cages. The rats were divided into five groups (one control and four exposure), with 7 per group. The four exposure groups were irradiated by EM for 15, 60, 120, and 180 minutes per day, lasting 70 days. The control group rats were also transferred to the irradiation chamber daily while the frequency emitter was turned off. After 70 days, the rats were killed by carbon dioxide gas, and the weight of the rats and the surface characteristics of the kidneys were observed.

Electromagnetic wave generator

An electromagnetic wave generator was designed and manufactured, running at a frequency of 2100 MHz, to simulate the Universal Mobile Telecommunications System (UMTS), Fourth-Generation (4G) broadband based on the global system for mobile communication standards. During the experiments, a spectrum analyzer was used to ensure the reliability and consistency of the generated EM wave. Furthermore, temperature stability was ensured during the irradiation.

Serological evaluations

Upon the 70th day of experiments, the rats were euthanized via CO_2. After opening the abdominal cavity, blood samples were taken directly from the heart and left undisturbed at room temperature for 15 minutes; subsequently, samples were centrifuged at 3000 g for 10 minutes, and the serum portion was transferred to clean tubes, maintained at -70°C for later serological analysis. Using a Hitachi 902 auto-analyzer (Roche Diagnostic, Mannheim, Germany) and utilizing Pars Azmoon kits (Pars Azmoon commercial kits, Tehran, Iran), serum urea nitrogen and creatinine, calcium, phosphorus, and BUN levels were measured according to the International Federation of Clinical Chemistry (IFCC) rules.

Histologic evaluations

Immediately after the dissection, the right kidney was removed and fixed in 10% formalin to be analysed by light microscopy. After routine tissue processing (Dehydration, Clearing, and embedding), samples were embedded in paraffin, and 6 µ sections were made via a microtome (Leica/RM 2145). The tissue samples were then routinely stained by haematoxylin and eosin (H&E) and observed by light microscopy (Olympus MVX10, Olympus, Hamburg, Germany) for histopathologic findings.

Stereological evaluations

To compute stereological estimations, a camera-equipped microscope was used, and accurate analysis was performed utilizing the DP2-BSW software (Olympus) and the Cavalier method. A 161-point grid was used to count points of the desired structures according to a previous study (10). To avoid counting errors, the points were counted if the upper right corner of the point was on the target structure. This method was used to estimate the volume of the proximal convoluted tubules (PCT), distal tubules (DCT), and glomeruli (G). In total, 1650 areas were measured from 165 prepared slides. Stereological studies were done by the Cavalier principle to estimate the epithelial height, follicular thickness, follicular volume, and volume of collagen fiber in septa (8). For this purpose, 10 sections were selected from each specimen and photographed by a camera attached to a light microscope (Olympus CX22). A point grid was used for point counting. Grid was cited on the figures, and each parameter was counted, and the following formula blindly estimated the thyroid gland:

$$V\ (mm^3) = d \times \Sigma p \times a\ (p)$$

Where d is the interval between sections and section thickness; Σp is the total number of points considered on the area of sections; and $a\ (p)$ is the area represented by each point in the grid.

Statistical analysis

The average biochemical parameters, including phosphorus, calcium, creatinine, and BUN, and stereological variables, including proximal, distal, and glomerulus, are compared in control and experimental groups (15, 60, 120, and 180 minutes).

Analysis of variance was performed using a randomized complete design experiment. All data were analysed by One-Way ANOVA using the GLM procedure of the SPSS version 26 software. Tukey's multiple range test ($P < 0.05$) was used for comparison of the means. Otherwise,

the non-parametric Kruskal-Wallis test was used. The presumption of homogeneity of the variance of the variables in the groups was investigated by Levene's test.

Results

Animal weights

The results showed that the rats exposed to high-frequency electromagnetic waves exhibited increased weight volume compared with the control group following the enhanced exposure periods. However, only the weight of animals in experimental groups 3 and 4 (120 and 180 minutes) showed a significant increase ($P<0.05$) compared to the control group. Notably, the significant increase ($P<0.05$) in the 120-minute group was higher than that in the 180-minute group (Figure 1).

Macroscopic results

The kidneys of all studied groups were reddish-brown with a smooth and shiny surface, with no anatomical changes observed. It was found that the mean weight had increased in all groups, including the control group, at the end of the study. The 120- and 180-minute exposure groups were statistically different from the control group, with significantly higher weight gain ($P<0.05$).

Serological results

The control group displayed stable baseline values for the assessed parameters, laying the foundation for comparisons with the exposure groups. BUN levels exhibited a minor increase across the 180-minute observation period, although this change was not statistically significant ($P=0.116$). Contrary to that, creatinine levels in the exposure groups showed significant differences compared to the control group, with the 15, 60, 120, and 180-minute groups demonstrating elevated levels ($P<0.001$). Calcium levels remained relatively stable in the exposure groups, with no significant differences compared to the control group ($P=0.108$). The differences observed among the exposure groups indicate a potential time-dependent effect of radiation exposure on renal serology. The results of the analysis of variance, including Fisher's test statistic and its significance level for each biochemical parameter, are reported in Table 1.

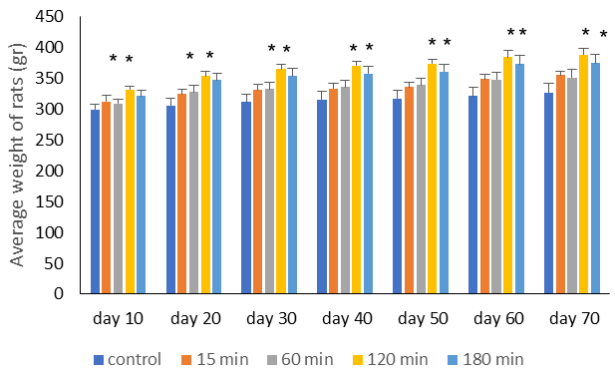

Figure 1. The weight of animals every 10 days in different groups of rats exposed to high-frequency (N = 7) ($P\leq0.05$)

The results of pairwise comparisons between biochemical parameters in the graph show that the mean creatinine parameter in the test groups is significantly higher than in the control group, and there is no significant difference between the test groups. The mean phosphorus parameter in the 180-minute group is significantly lower than in the control group (Figure 2).

In Figure 2, the results of Tukey's post-hoc test are presented to compare the mean of biochemical parameters between the groups. If there is no significant difference between the mean variables, the bars will have a common Latin letter.

Histopathological results

As a result of the light microscopy analysis of H&E-stained kidney sections, no histopathological changes, including interstitial hemorrhaging, hyperemia, and glomerular congestion, were observed in any of the groups under examination (Figure 3).

Stereological results

For the mean volume of the proximal convoluted tubules, a statistically significant reduction was observed in the mean volume of the exposure groups compared to the control group ($P=0.009$). This suggests that radiation exposure led to a notable alteration in PCT mean volume over time. In the case of the mean volume of the distal tubules, the mean values in all exposure groups were significantly lower than in the control group, showing a significant difference ($P<0.001$), with the most pronounced effects observed in the 120- and 180-minute exposure groups. The mean volume of the glomeruli also exhibited significant differences, with mean values consistently lower in all exposure groups than in the control group ($P<0.001$). Longer exposure durations resulted in more pronounced effects, suggesting a dose-response relationship. Table 2 presents the results of the variance analysis for each stereological variable.

To compare the mean of significant stereological variables between the groups, Tukey's post-hoc test is presented in Table 2. If there is no significant difference between the mean variables, then the bar will display a common Latin letter.

According to the results of paired comparisons between stereological variables in Figure 4, PCT volume in the 120- and 180-minute groups is significantly lower than in the control group, while there is no significant difference between exposure groups. Compared to the control group, the 120- and 180-minute groups exhibited significantly lower mean volumes of DCT, as well as lower mean variables. There is no significant difference between the 120- and 180-minute groups in terms of glomerular volume, but the 120-minute group showed a greater mean

Table 1. Statistical analysis (Fisher's test) of biochemical parameters for control and exposure groups.

Variable	Group	Num.	Mean	Stand. dev.	Coefficient of var.	Fisher's statistics	*P* value
	Control	7	25.800	1.837	0.071		
	15 min	7	27.000	1.716	0.064		
BUN	60 min	7	26.571	1.813	0.068	2.029	0.116
	120 min	7	25.571	1.440	0.056		
	180 min	7	24.714	1.380	0.056		
	Control	7	0.540	0.055	0.101		
	15 min	7	0.636	0.069	0.109		
Creatin	60 min	7	0.649	0.049	0.075	9.355	<0.001
	120 min	7	0.693	0.053	0.077		
	180 min	7	0.727	0.076	0.104		
	Control	7	9.140	0.336	0.037		
	15 min.	7	8.871	0.423	0.048		
Calcium	60 min	7	8.600	0.258	0.030	2.129	0.108
	120 min	7	9.057	0.351	0.039		
	180 min	7	8.757	0.556	0.064		
	Control	7	6.020	0.687	0.114		
	15 min	7	5.629	0.637	0.113		
Phosphate	60 min	7	5.471	0.538	0.098	2.808	0.043
	120 min	7	5.886	0.546	0.093		
	180 min	7	5.071	0.512	0.101		

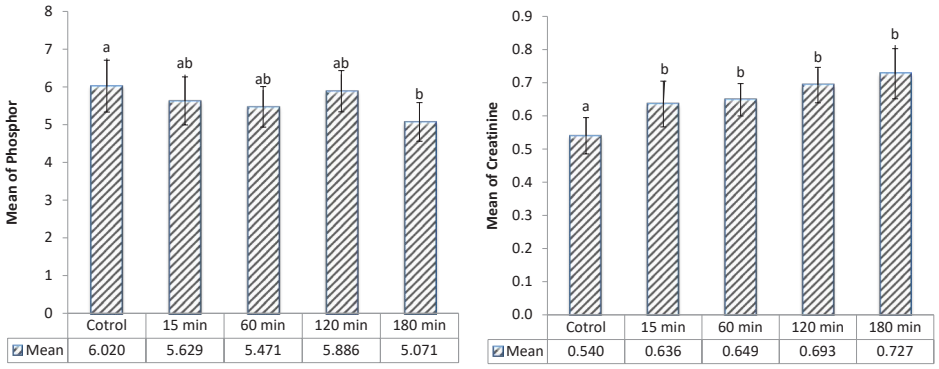

Figure 2. Comparison of the mean of biochemical parameters

volume of glomeruli (Figure 4).

Discussion

The results of the present study show that exposure to electromagnetic field (EMF) waves at a frequency of 2100 MHz (4G) for 15, 60, 120, and 180 minutes daily for 70 days has significant effects on serological and stereological parameters of Wistar rat kidneys. These findings emphasize the importance of investigating the mechanisms through which EMF waves affect biological tissues, especially the kidneys.

Despite differences in serological markers for markers of renal function, such as bilirubin and calcium, between groups exposed to RF for different durations daily, the group exposed to 15 min had lower phosphorus levels than the control group, and creatinine levels were significantly increased in all exposed groups. There is no evidence for an effect of short-term radiation that is observed in longer exposures. The current evidence for this effect is insufficient, and future studies should confirm its existence. Reactivation of renal parenchymal cells may have prevented the deviation of biochemical factors, even in the groups exposed to 180 min of daily radiation, even though some cells are normally inactive. According to current stereological estimates, tubular and glomerular volumes were not similar between rats exposed for different durations. Based on stereological analysis, the radiation-exposed groups showed decreased proximal convoluted tubule volume, decreased distal tubule volume, and a persistent decrease in glomerular volume.

Mechanism of action of electromagnetic waves: EMF

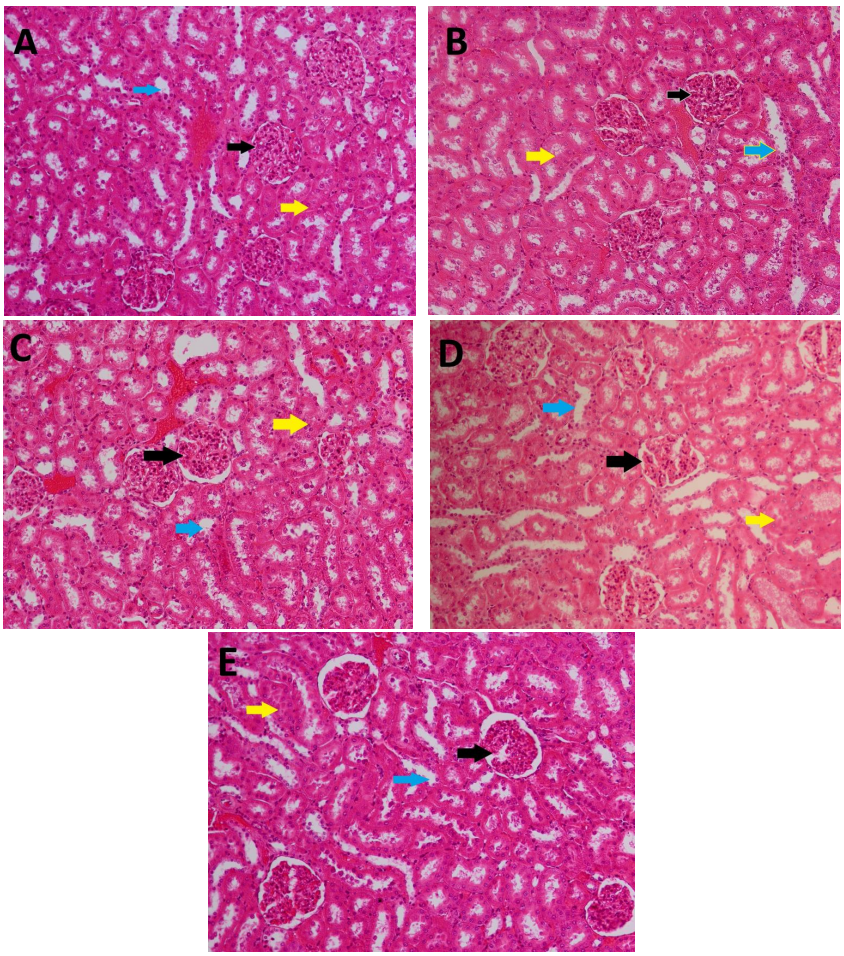

Figure 3. Histologic view of the renal cortex in the kidney, A: Control, B: 15 min, C: 60 min, D: 120 min, E: 180 min exposure. Black arrow: glomerulus, blue arrow: distal convoluted tubule, yellow arrow: proximal convoluted tubule, H&E staining, X100

Table 2. Statistical analysis (Fisher's test) of stereological variables for control and exposure groups, $P \leq 0.005$).

Variable	Group	Num.	Mean	Stand. dev.	Coefficient of var.	Fisher's statistics	*P* value
	Control	7	337.622	18.294	.054		
	15 min	7	324.490	17.515	.053		
Proximal	60 min	7	321.356	17.928	.056	4.136	0.009
	120 min	7	308.707	18.065	.059		
	180 min	7	302.721	17.484	.058		
	Control	7	196.482	15.464	.078		
	15 min	7	203.250	15.971	.079		
Distal	60 min	7	215.396	16.482	.077	33.103	<0.001
	120 min	7	152.617	17.170	.113		
	180 min	7	127.821	16.825	.132		
	Control	7	79.610	7.126	.090		
	15 min	7	80.211	5.742	.072		
Glomerulus	60 min	7	83.550	5.884	.070	11.207	<0.001
	120 min	7	68.056	6.799	.100		
	180 min	7	65.479	6.175	.094		

waves at a frequency of 2100 MHz can exert their effects through two main mechanisms: thermal and non-thermal. The thermal effect is due to the absorption of wave energy by water molecules and other polar molecules in the tissue, which leads to a local increase in temperature. This thermal stress can alter cellular metabolism and, over time,

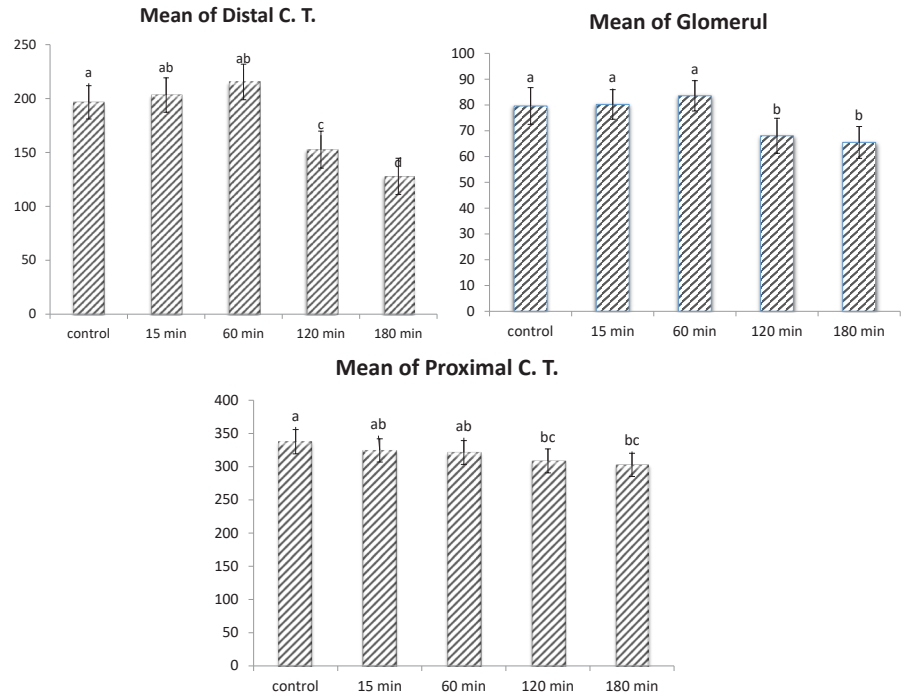

Figure 4. Comparison of mean stereological variables

induce oxidative stress and cellular damage. In this study, a significant increase in creatinine levels ($P < 0.001$) in all exposed groups indicated impaired glomerular filtration function, potentially related to the thermal stress induced by the waves. The increase in creatinine, a key indicator of renal function, may be associated with the observed decrease in glomerular volume ($P < 0.001$) according to stereological analysis.

The non-thermal effect of EMF waves includes changes in cell membrane potential and activation of intracellular signaling pathways. 2100 MHz waves may induce weak electrical currents in glomerular and tubular cell membranes, alter membrane permeability, and disrupt ionic balance (e.g., calcium and sodium). These changes could be responsible for the decrease in proximal and distal tubule volume ($P < 0.009$ and $P < 0.001$, respectively), as observed in stereological findings.

Previous studies have shown that EMF waves at lower frequencies (e.g., 920 MHz) may cause more pronounced renal tissue damage. However, this study showed that 2100 MHz waves, while not causing significant structural or histopathological changes, produced significant functional and stereological effects. This difference may be attributed to the higher frequency, which could limit tissue penetration and thermal effects while enhancing non-thermal effects.

Clinical Relevance and Limitations: The dose-dependent increase in creatinine and the reduction in glomerular and tubular volumes, especially in the 120- and 180-min exposure groups ($P < 0.05$), highlight the potential impact of EMF exposure. These findings may have important clinical implications for humans, given the widespread use of 4G technology. However, the lack of significant changes in bilirubin ($P = 0.116$) and calcium levels ($P = 0.108$) suggests that these effects may be limited to specific metabolic pathways. One limitation of this study is the lack of oxidative stress markers (such as MDA or ROS) that could have elucidated the underlying molecular mechanisms.

Conclusion

In conclusion, our findings indicate that 2100 MHz electromagnetic irradiation for more than 120 minutes per day may increase tubular volumes in rat models. Furthermore, only short-term daily radiation resulted in altered serological states. The observed consequences of 2100 MHz were notably less than previously reported for lower frequency waves (900 MHz and less). Future studies are indicated to evaluate the effects of various RF frequencies simultaneously.

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Authors' Contribution

Conceptualization: Ahmadreza Raji, Mohsen Maleki, Morteza Kaffaee Razavi.
Data curation: Alireza Sanati Fas.
Formal analysis: Ahmadreza Raji, Mohsen Maleki, Mehrdad Mohri.
Funding acquisition: Ahmadreza Raji, Mohsen Maleki.
Investigation: Alireza Sanati Fas.
Methodology: Morteza Kaffaee Razavi.
Project administration: Ahmadreza Raji, Mohsen Maleki.
Resources: Ahmadreza Raji, Mohsen Maleki.
Software: Morteza Kaffaee Razavi.

Supervision: Ahmadreza Raji, Mohsen Maleki.
Validation: Ahmadreza Raji, Mohsen Maleki.
Visualization: Alireza Sanati Fas.
Writing–original draft: Ahmadreza Raji, Alireza Sanati Fas.

Competing Interests
The authors declare no conflict of interest.

Data Availability
Raw data were generated at the Department of Basic Sciences, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Iran. Derived data supporting the findings of this study are available from the corresponding author, Ahmadreza Raji, on request.

Ethical Approval
The experiments were conducted according to the Ferdowsi University of Mashhad Animal Care Committee guidelines (Ethical code: IR.UM.REC.1401.056).

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