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RESEARCH ARTICLE

Effects of 4G mobile phone radiation exposure on reproductive, hepatic, renal, and hematological parameters of male Wistar rat

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Abstract

Background and objective Mobile phones have become a vital part of human life. Due to drastic increase in the number of mobile phone subscribers, exposure to radiofrequency radiation (RFR) emitted from these phones has increased dramatically. Hence, the effect of RFR on humans is an area of concern. This study was performed to determine the impact of 4G mobile phone radiation on the male reproductive system, liver, kidney, and hematological parameters.

Methods Seventy-day-old Wistar rats were exposed to 4G radiation (2350 MHz for 2 h/day for 56 days). Sperm parameters such as sperm count, viability, sperm head morphology, mitochondrial activity, total antioxidant activity, and lipid peroxidation of sperm were evaluated. Histopathology of the testis, prostate, epididymis, seminal vesicle, liver, and kidney was carried out. Complete blood count, liver and kidney function tests, and testosterone hormone analysis were done.

Results At the end of the experiment, results showed a significant ($p < 0.05$) decrease in sperm viability with alterations in the histology of the liver, kidney, testis, and other reproductive organs in the exposed group of rats. A reduced level of testosterone, total antioxidant capacity, and decreased sperm mitochondrial function were also observed in the exposed rats. Moreover, the exposed rats showed an increase in sperm lipid peroxidation and sperm abnormality. Hematological parameters like hemoglobin, red blood cells (RBC), and packed cell volume (PCV) showed a significant ($p < 0.05$) increase in the exposed rats.

Conclusion The results indicate that chronic exposure to 4G radiation may affect the male reproductive system, hematological system, liver, and kidney of rats.

Keywords Mobile phone radiation · Male infertility · ROS · Oxidative stress · Hematology · Hepatic · Renal

Background

Globally there are 8.6 billion mobile phone subscriptions (Statista 2022). These mobile phone technologies work on non-ionizing electromagnetic radiation. With the evolving technology from 1G to 5G (900 MHz to 28 GHz), there has been a substantial increase in the number of people being exposed to this radiation, thus becoming a major cause of concern to the public. In the context of mobile communication, the symbol "G" signifies "generation," with each succeeding generation adding improved features over its predecessor. The first generation, or 1G, operated on analog signals and was limited to voice communication only. Subsequently, 2G emerged, offering voice, data, and messaging services. Surpassing this technology came the third generation of mobile communication. 3G provided higher data transfer speed and video call feature. The evolution continued with the advent of 4G, characterized by enhanced data speeds surpassing 3G, along with improvements in voice calls, internet services, streaming capabilities, coverage, and applications. Consequently, 4G has become the prevailing technology in contemporary mobile communication. Addressing the limitations of 4G, the fifth generation (5G) was developed, with significantly faster speeds and reduced latency resulting in varied applications in every sector. This growth indicates a significant advancement in mobile communication technology. Currently, 5G is still in its growing phase whereas 4G has already established its wide-spread network in India. The existing 4G bands remain operational and used by vast majority of the population. Moreover, it is important to note that 5G also operates on pre-existing 4G bands for communication. Therefore, it is crucial to emphasize on this technology in

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order to understand its effects on the large population of users. Considering 4G, or the 4th generation technology, works on a frequency range from 850MHz, 1800MHz and 2300MHz to 2400MHz in India, in our studies, we have emphasized on 2300 MHz to 2400 MHz frequency LTE (Long Term Evolution Band), which is one of the most commonly used band. Previous reports suggest the adverse effect of electromagnetic radiation on different organs, viz., testis (Gaharwar et al. 2022; Pardhiya et al. 2022), liver (Alkis et al. 2021), kidney (Pardhiya et al. 2020; Hasan et al. 2021), brain (Delen et al. 2021; Hasan et al. 2022), parotid gland (Siqueira et al. 2016), and hormonal changes in the male and female reproductive systems (Kozlowska et al. 2022). Mobile phone usage may also increase the risk of early spontaneous abortions in pregnant women (Mahmoudabadi et al. 2018). Various in vitro, in vivo, and epidemiological studies have shown a higher risk of cancer upon a longer duration of exposure to these radiations (Cardis et al. 2011; Gupta et al. 2020). Epidemiological studies have shown the occurrence of sleep disorders, headaches, behavioral changes, and early spontaneous abortions (Hillert et al. 2008). Radiation emitted from mobile phones has been categorized as a possible carcinogen by the International Agency for Research on Cancer (IARC) (Miller et al. 2019).

Infertility is a global health problem among couples and approximately 50% of these cases are associated with male infertility (Barati et al. 2020). Generally, people tend to keep cell phones in their trouser pockets which makes reproductive organs and accessory glands like the testis, prostate, seminal vesicle, and epididymis in close vicinity to the phone and hence increased radiofrequency radiation (RFR) exposure to the reproductive system. Testis being a major organ for spermatogenesis, RFR exposure may be a possible threat to both the quality and quantity of sperms produced. In vitro studies on human semen have shown that exposure to RFR leads to an increase in oxidative stress (Agarwal et al. 2009); hence, sperms may be susceptible to oxidative stress-mediated damage (Shahin et al. 2018).

In about 30–80% of infertile men (Bui et al. 2018), the main cause of infertility is ROS-mediated sperm damage. Reactive oxygen species (ROS) such as superoxide anion ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($^{\bullet}OH$) are highly reactive molecules containing oxygen that are generated as natural byproducts of cellular metabolism. The balance between the production and removal of ROS is crucial for maintaining cellular health (Poljsak et al. 2013). Sperm cells require a small amount of ROS for normal functions like capacitation, hyperactivation, and acrosome reaction. The homeostatic balance between ROS and the antioxidant capacity of cells is important for sperm function (Singh et al. 2017). Excessive ROS levels create oxidative stress, overwhelming the body's antioxidant defense mechanisms. This imbalance leads to damage to lipids, proteins, and nucleic acids. ROS can oxidize lipids in cell membranes, leading to the production of lipid peroxides which disrupts the integrity of cell membranes and can lead to cell damage or death (Catala et al. 2016). For the generation of ROS in immature sperms, the two major sites are the sperm membrane and sperm mitochondria (Sabeti et al. 2016). Electromagnetic radiation exerts an adverse effect through ROS and other free radical generation that causes oxidative stress (Desai et al. 2009; Gautam et al. 2021; Garaj-Vrhovac et al. 2011). Excessive ROS may lead to oxidative damage of lipids in the membrane, DNA fragmentation, and apoptosis (Aitken and Baker 2013).

Blood plays a key role in the transport of oxygen and nutrients throughout our body. It also transports waste product generated in our body to the kidney and liver for subsequent disposal (Alghamdi and El-Ghazaly 2012). RFR exposure has shown alterations in blood cells and plasma enzymes (Alghamdi and El-Ghazaly 2012) which may be due to the stimulation of hemopoietic function within the bone marrow, leading to oxidative stress and potentially unregulated hemopoiesis. In turn, this may contribute to the development of anemia and bone marrow depression (Aberumand et al. 2016). RFR has been reported to elevate hemoglobin levels in earlier studies (Muehsam et al. 2013; Sani et al. 2018; Christopher et al. 2020). This increase in hemoglobin has been attributed to deoxygenation process in response to non-thermal effect of RFR. Another reason may be the adverse effect of free radicals on the membrane of red blood cells and cytoskeleton, causing leakage of hemoglobin from the cells (Jbireal et al. 2018). RFR has the potential to alter the activities of white blood cells. This can manifest as swift transformations, such as cell contraction, expansion, and rolling, along with a diminished capacity to move, leading to cell breakdown and a decrease in their count. Alteration to blood cells and disruption in the equilibrium of blood enzymes by RFR can lead to elevated cell apoptosis and functional disturbances in various cell types (Atasoy et al. 2009). Elevated white blood cell counts have been observed to correlate with tissue damage, disorders like leukemia, and alteration in bone marrow function. Consequently, the significant rise in white blood cell count in the exposed animals serves as a robust indication of stress-related effects. Earlier studies have also reported elevated levels of white blood cells because of RFR exposure (Alghamdi and El-Ghazaly 2012; Christopher et al. 2020). This underscores the need for additional research on the immune consequences of radiofrequency radiation exposure in the studied group (Kumari et al. 2016).

It has also been reported that brain cells in rats exhibited escalated breakage in the single strand of DNA on chronic exposure of non-thermal microwave radiation. Furthermore, impairment of hepatic, renal, and splenic tissues is also advocated to be caused by RFR. A study revealed that the enzymatic activity in the liver of rat is altered when exposed to microwave radiation of 900 MHz every day for 1 h for a period of 3 and 6 months (El-kott and Saad 2011). RFR causes cell damage in the liver causing leakage of enzymes in the bloodstream such as aspartate aminotransferase, alanine

aminotransferase, and alkaline phosphatase (Moradpour et al. 2020). RFR was also reported to significantly increase lipid peroxidation and nitric oxide levels in liver tissue and decrease antioxidant enzymes such as superoxide dismutase, glutathione peroxidase, and myeloperoxidase (Ozgur et al. 2010). Histopathological alterations also occurred in liver tissue upon RFR exposure (Pardhiya et al. 2020). Previous study has reported that RFR causes histopathological changes in the kidney and testis of male mice when exposed to isothermal non-ionizing radiation (Ishaq Khayyat 2011). Also, with increase in the exposure time, these histopathological effects intensified. Contemporary studies validate that apart from generating oxidative stress in different tissues, RFR exposure also leads to substantial alterations in the antioxidant marker level in blood (Kivrak et al. 2017).

Due to the adverse effects of 4G RFR radiation and gap in the effect of unmodulated carrier 4G waves, the present work was conducted with the aim to study the possible impact of unmodulated 4G mobile frequency on reproductive, renal, hepatic, and hematological parameters in male Wistar rats. The research questions aimed to answer with the study were whether the unmodulated 4G carrier mobile frequency could have an ill effects on the male reproductive system (testis, epididymis, vas deferens), kidney, liver, and hematological system.

Methodology

Biophysical parameters and exposure details

Adult male Wistar rats (7 weeks old) were grouped in two sets of sham-exposed (Control) and exposed with 6 rats per group. Animals were kept under standard conditions of temperature (25 °C ± 2 °C) and relative humidity (40–50%) with 12-h light and dark cycles. Water and food pellets were given ad libitum to the animals. Experimental protocols were done according to the Institutional Animal Ethics Committee guidelines (IAEC, Code No. 1 /2021), JNU, New Delhi, and Committee for Purpose of Control and Supervision of Experiments on Animals (CPCSEA). The experimental group of rats was kept in plexiglass cages and placed inside the anechoic chamber (Fig. 1). The desired power signal was obtained by transmitting carrier signal from a signal generator (Keysight model no -N9310A) at 4G frequency (LTE Band 40) 2350 MHz. The power of the signal was low, thus was amplified by the amplifier (Amplifier Research Model number-1S1G4) before being sent to the WR-340 waveguide horn antenna (pyramidal shaped and 15dBi gain), which radiates the signal on the experimental animals (kept at 20 cm distance from the horn antenna). Animals were kept in plexiglass cone-shaped cages (dimensions length 25 cm × minimum and maximum width 3.5 and 8.5 cm × height 6 cm) exposed to Carrier signal 4G frequency of 2350 MHz (2 h/day) for 56 days. Power density was measured inside the cage by Narda measurement system NBM-520 (probe model 5092). Animals were exposed to the average power density of 0.2488 mW/cm^2, and the average specific absorption rate (SAR) calculated for exposed group animals was 0.0625 Watt/kg (Durney et al. 1979).

Organ to body weight ratio

At the completion of the exposure period, rats were euthanized as per animal ethics protocol. Various organs such as the testis, prostate, epididymis, seminal vesicle, liver, and kidney were excised and weighed. Organ to body weight ratio was calculated.

Morphometry and histopathology of organs

The testis, epididymis, vas deferens, liver, and kidney were fixed in 10% formalin, and paraffin blocks were prepared.

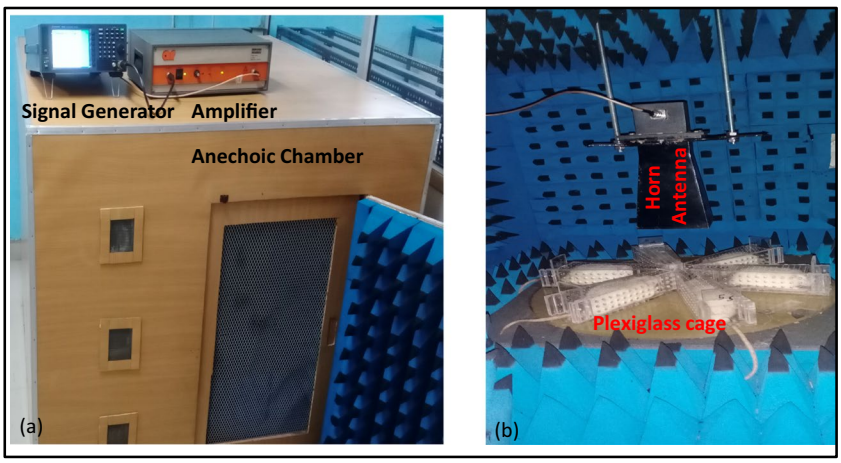


Fig. 1 **a** Exposure set up of 4G (2350 MHz frequency) exposure showing anechoic chamber, signal generator, and amplifier. **b** Horn antenna and plexiglass cage with the exposed group of animals

Sections of 5 µm thickness were cut by microtome which was further stained using H&E (hematoxylin–eosin).

The testis dimension (length and width) of each animal from both groups was measured using a vernier caliper. Seminiferous tubule diameter, germinal epithelial height, and tunica albuginea thickness were measured from histopathological images of the testis. Twenty random round seminiferous tubules were selected to measure the seminiferous tubule diameter and germinal epithelial height under the light microscope (400×) (Mehraein and Negahdar 2011). Epididymis, vas deferens, liver, and kidney from both groups were also examined for any histopathological alterations.

Protein estimation

Protein estimation was done according to Bradford (1976). Briefly, Bradford reagent was prepared by mixing 100 mg of Coomassie Brilliant Blue G-250 mixed in 50 ml ethanol (95%), followed by addition of 100 ml phosphoric acid 85% (w/v), the final volume was made to 1000 ml, filtered, and stored in the dark. Ten microliters of sperm sample was incubated with 990 µl Bradford reagent for 10 min at room temperature. At the end of the incubation period, the absorbance of the solution was measured at 595 nm in UV–Visible spectrophotometer. Bradford assay was also done on a series of BSA (bovine serum albumin) standard solutions with known concentrations and the absorbance values of these concentrations were used to create a standard curve. The protein concentration of the sperm samples was determined using the BSA standard curve.

Hormone analysis

Blood was obtained from rats by heart puncture in centrifuge tubes and centrifuged for 15 min at 2200 g at 4 °C and serum was collected for the testosterone assay. Testosterone in serum was quantified by using a chemiluminescent immunoassay kit (Access Immunoassay Systems REF 33560, Beckman Coulter). The assay was performed as per the user manual.

Haematological and liver and kidney function test

For the assay of complete blood count (CBC), the heart was punctured, and blood was collected in EDTA-coated tubes. CBC was done using Nihon Kohden Automated Blood Cell counter. For the liver function test and kidney function test, blood was collected in centrifuge tubes and serum was obtained by centrifugation at 2200 g for 15 min at 4 °C. Liver and kidney function tests were done with fully automated Mindray BS/430.

Sperm parameters

Cauda epididymis was excised out and minced in Ham F12 media (5 ml) for 15–20 min at 37 °C. Sperm count was done via hemocytometer as per the WHO manual. Sperm viability was calculated by staining sperm with 1% eosin Y. Light pink and colorless sperm were considered viable and dark pink-colored sperm were considered non-viable. Around 100 sperm per animal were observed (Cao et al. 2011). Sperm morphology analysis was done using a dye eosin Y. Staining of the sperm was done with 0.5% eosin Y. A smear was prepared on a clean glass slide and then fixed in 70% methanol. Around 300 sperm per animal were observed (Narayana et al. 2001).

Sperm mitochondrial activity

The mitochondrial activity of sperm was tested with 3,3-diaminobenzidine (DAB). DAB donates electron to cytochrome c and gets oxidized, polymerized, and deposited at cytochrome c of mitochondria (Blumer et al. 2008). Based on the percentage staining of sperm midpiece, the cells were classified as class I, class II, class III, and class IV (100% stained, > 50% stained, < 50% stained, and unstained), respectively.

FRAP (ferric reducing antioxidant power) assay and lipid peroxidation

FRAP in the sperm sample was assessed according to Benzie and Strain, (1996). Briefly, 50 µl of sperm sample was added to 1.5 ml of FRAP reagent. The mixture was kept for 8 min at 37 °C, and the absorbance of blue-color complex formed was measured against a blank reagent at 593 nm. The data were expressed as FRAP value (mM of ferric ions reduced to ferrous form per mg sample protein). Malondialdehyde (MDA) level is a marker for lipid peroxidation (Ohkawa et al. 1979). Briefly, a 125-µl sperm sample was mixed with 30% TCA (trichloroacetic acid) and 0.15 M Tris KCl buffer and vortexed. Thereafter, 52 mM TBA (thiobarbituric acid) was added and vortexed. The MDA level was evaluated using its extinction coefficient 155 mM^{-1} cm^{-1} and represented as µmoles MDA/mg protein.

Statistical analysis

Mean ± standard error of means (SEM) was calculated. GraphPad Prism 5.03 was used for performing statistical

analysis by Student's *t*-test and one way ANOVA. The significance level was denoted by *p* values where the $p < 0.05$ is significant, $p < 0.01$, and $p < 0.001$ were considered as highly significant.

Table 1 Organ to body weight ratio of control and exposed group of animals

	Control	Exposed
Relative weight (g)		
Testis	0.463 ± 0.013	0.390 ± 0.010
Seminal vesicle	0.385 ± 0.015	0.418 ± 0.021
Prostate gland	0.145 ± 0.013	0.133 ± 0.010
Epididymis	0.182 ± 0.006	0.181 ± 0.004
Liver	3.13 ± 0.104	2.81 ± 0.108
Kidney	0.308 ± 0.026	0.27 ± 0.005
Testicular morphometry		
Testis length (mm)	20.61 ± 0.37	19.96 ± 0.34
Testis width (mm)	13.13 ± 0.10	12.83 ± 0.17
Seminiferous tubule diameter (μm)	142.18 ± 2.21	141.54 ± 0.84
Germinal epithelium height (μm)	30.08 ± 1.37	30.14 ± 0.61
Tunica albuginea thickness (μm)	16.68 ± 0.99	14.96 ± 0.75
Hormonal assay		
Testosterone (ng/ml)	4.98 ± 1.50	3.61 ± 1.13

Data represented are mean ± SEM, $n = 6$

Results

Organ to body weight ratio

Results showed no significant change in the organ to body weight ratio (testis, liver, kidney, seminal vesicle, prostate, and epididymis) of exposed group animals as compared to the sham-exposed group (Table 1).

Morphometry and histopathology of organs

Testis

The measured dimensions of the testis (length and width) showed no significant changes (Table 1). After H&E staining, testis histopathology showed no significant changes in seminiferous tubule diameter and germinal epithelial height (Table 1). Tunica albuginea thickness showed an insignificant decrease in the exposed group of rats (Table 1). Histopathology analysis of testis showed the distorted shape of seminiferous tubules, vacuolization, and altered spermatogenesis in the exposed rats (Fig. 2).

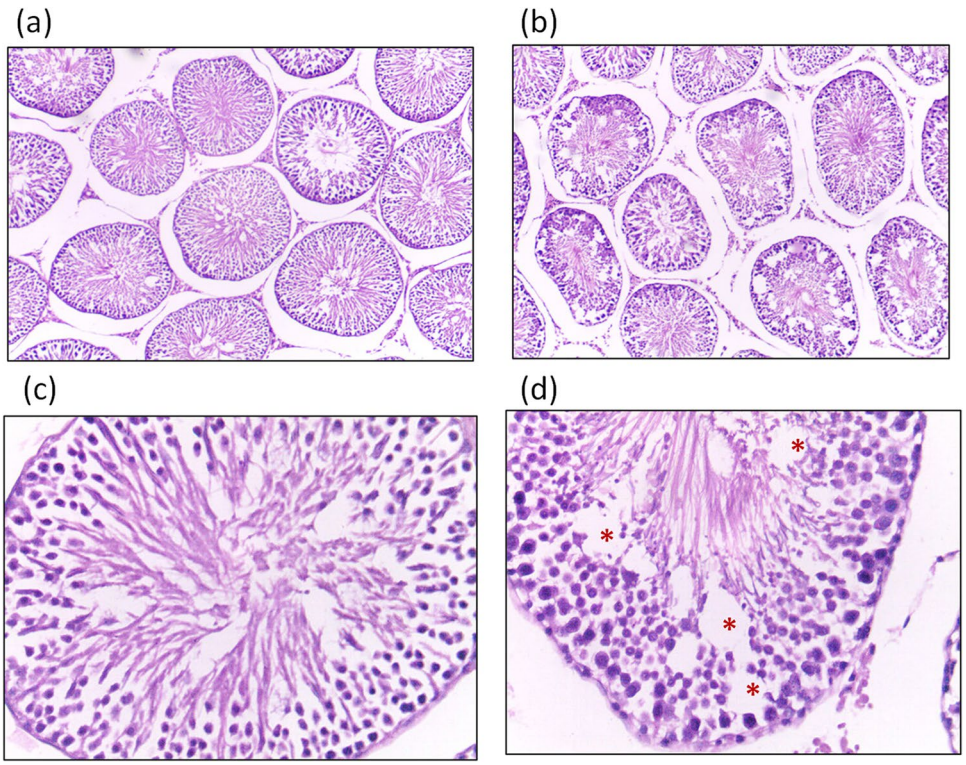


Fig. 2 Histopathology image of the testis showing seminiferous tubule of (**a**) control and (**b**) exposed at 100 × magnification, (**c**) control, and (**d**) exposed at 400 × magnification. The asterisk (*) represents vacuolization in the seminiferous tubule of exposed rats

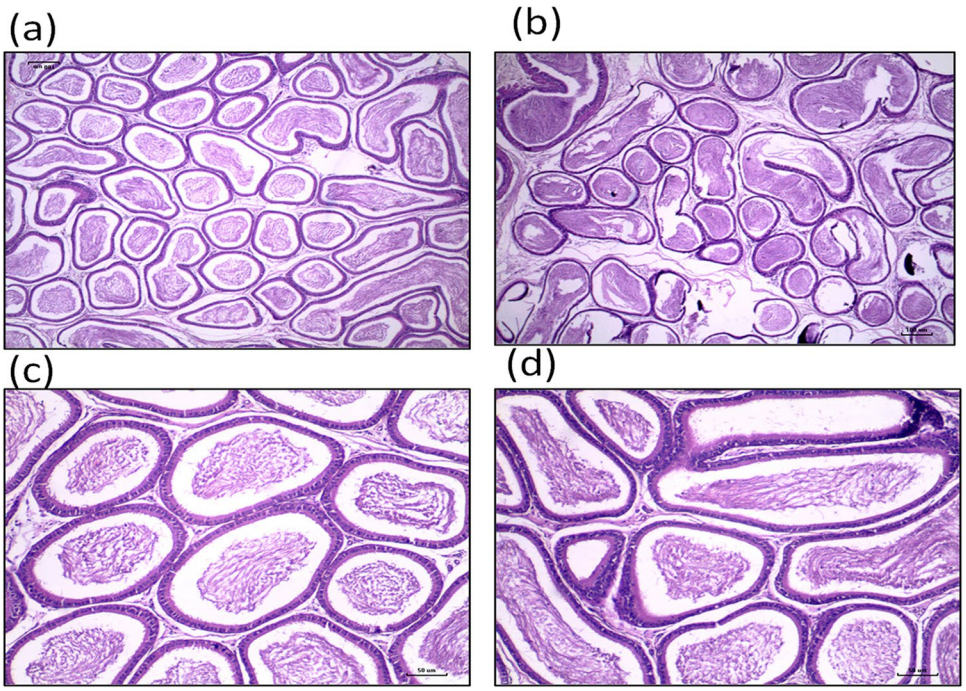


Fig. 3 Histopathology image of epididymis of (**a**) control and (**b**) exposed rats at 100× magnification and (**c**) control and (**d**) exposed rats at 400× magnification

Epididymis

H&E staining of the control group showed a normal appearance with sperm mass of densely packed spermatozoa, while the exposed group showed an irregular-shaped structure (Fig. 3).

Vas deferens

Histopathological image of vas deferens showed a normal structure in sham-exposed group with lumen, muscular wall, epithelial lining, and stereocilia, while the exposed group

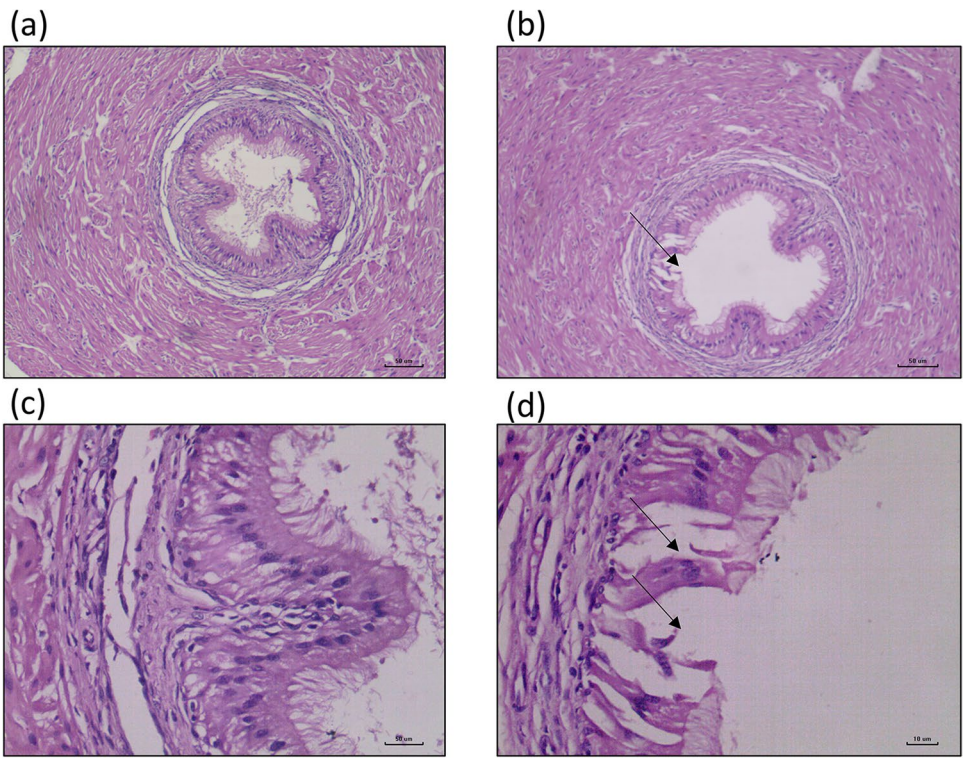


Fig. 4 Histopathological images of vas deferens (**a**) and (**c**) control showing normal structure with lumen (L), muscular wall (M), epithelial lining (E), and stereocilia (S) at 100× and 400×, respectively. In the exposed group, the arrow shows alterations in the epithelial lining in (**b**) and (**d**) at 100× and 400×, respectively

showed structural changes in epithelial lining and stereocilia (Fig. 4).

Liver

The control (Fig. 5a) showed a normal structure of the liver with clear central vein, normal nucleus, healthy hepatocytes, and clear and normal size of sinusoids. However, exposed rats showed various abnormalities such as sinusoid dilatation, presence of pyknotic nuclei, degenerated nuclei, apoptotic bodies, infiltration of inflammatory cells, cytoplasmic vacuoles, focal necrosis, congested central vein and sinusoids, and degenerated hepatocytes (Fig. 5b–i).

Kidney

Histological analysis of the kidney showed no pathological findings in the control group of rats. Kidney tissues showed normal morphology of the glomerular and tubular structures (Fig. 6a). Histopathological assessment of the exposed kidney showed few alterations such as tubular dilatation, degenerated capsule, vacuolization, and dilatation of the capsule (Fig. 6b and c).

Hormonal assay

Testosterone levels in serum decreased insignificantly in the exposed group of rats as compared to the sham-exposed group.

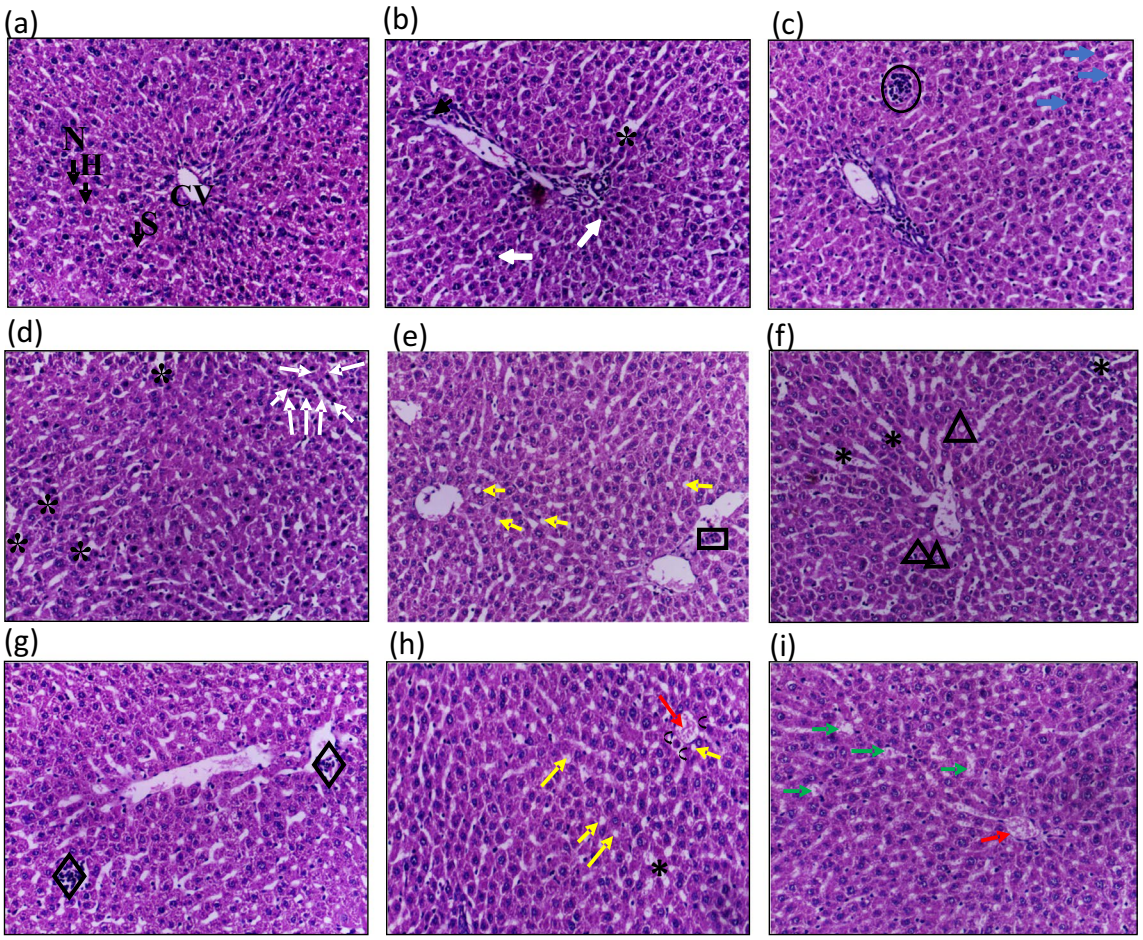


Fig. 5 Histology of the liver of control (**a**) and RFR-exposed rats (**b**–**i**). N, nucleus; H, hepatocytes; S, sinusoids; CV, central vein; *dilatation of sinusoids; white arrow: pyknotic nuclei; blue arrow: apoptotic bodies; circle: infiltration of inflammatory cells; square: infiltration of inflammatory cells in centrilobular hepatocytes; yellow arrow: cytoplasmic vacuoles; triangle: degenerated nuclei; diamond: focal necrosis and infiltration of inflammatory cells; red arrow: congested central vein; curved arrow: degenerated hepatocytes; yellow arrow: vacuolated cytoplasm; green arrow: sinusoidal dilation and congestion. Magnification 40×

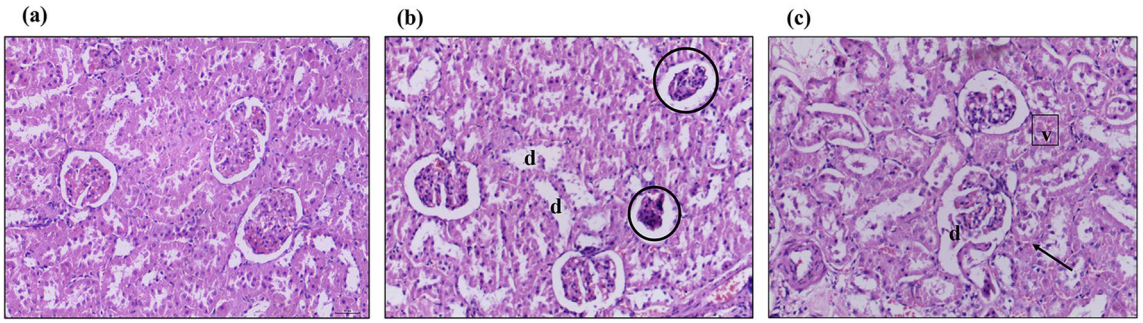


Fig. 6 Histology of the kidney of (**a**) control and (**b**, **c**) RFR-exposed rats where d, tubular dilatation; circle, degenerated capsule; v, vacuolization; arrow: dilatation of Bowman's capsule

Table2 Complete blood profile parameters of control and exposed group of animals

S. No	Parameters	Control	Exposed
1	Hemoglobin (g/dl)	14.13 ± 0.94	15.366 ± 0.66*
2	Total leucocyte (thou/mm^3)	3.81 ± 6.3	6.3 ± 4.56
3	Neutrophils (%)	44.16 ± 8.18	42 ± 5.17
4	Lymphocytes (%)	37.66 ± 11.21	40.833 ± 7.46
5	Eosinophils (%)	2.33 ± 0.81	1.666 ± 0.81
6	Monocytes (%)	15.83 ± 8.23	15.33 ± 6.86
7	Basophils (%)	0 ± 0	0.166 ± 0.40
8	RBC (mill/mm^3)	7.83 ± 0.73	8.465 ± 0.36*
9	PCV/ hematocrit (%)	37.53 ± 3.11	40.91 ± 1.68*
10	MCV (fL)	47.91 ± 0.95	48.366 ± 0.66
11	MCH (picogram)	18.08 ± 0.83	18.15 ± 0.28
12	MCHC (g/dL)	37.73 ± 1.01	37.55 ± 0.28
13	Platelet count (thou/mm^3)	658.83 ± 181.64	832.66 ± 177.48
14	RDW-CV (%)	12.43 ± 0.32	12.5 ± 0.57

Data is represented as mean ± SEM, $n = 6$ and *represents ($p < 0.05$)

Table 3 Liver function test parameters of control and exposed group of animals

S. No	Parameters	Control	Exposed
1	Bilirubin total (mg/dL)	0.096 ± 0.39	0.0683 ± 0.007
2	Bilirubin direct (mg/dL)	0.061 ± 0.02	0.0483 ± 0.011
3	Bilirubin indirect (mg/L)	0.035 ± 0.01	0.02 ± 0.008
4	AST (SGOT) (U/L)	141.016 ± 84.30	119.81 ± 68.71
5	ALT (SGPT) (U/L)	62.35 ± 12.97	49.88 ± 4.87
6	Alkaline phosphatase (U/L)	279.35 ± 51.38	198.54 ± 46.28 *
7	Total protein (g/dL)	5.7 ± 0.28	5.93 ± 0.43
8	Albumin (g/dL)	3.21 ± 0.09	3.2 ± 0.14
9	Globulin (g/dL)	2.48 ± 0.21	2.73 ± 0.43
10	A/G Ratio	1.30 ± 0.08	1.19 ± 0.19
11	Gamma GT (IU/L)	0.61 ± 0.21	0.63 ± 0.32

Data is represented as mean ± SEM, $n = 6$ and *represents ($p < 0.05$)

Hematological parameters

Hemoglobin, red blood cells (RBCs), and PCV (packed cell volume) showed a significant ($p < 0.05$) rise in the exposed group with respect to the control group (Table 2). While other parameters, like mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), red blood cell distribution width (RDW-CV%), and mean corpuscular hemoglobin (MCH), showed no significant changes in the exposed group with respect to control. The total leucocyte and platelets counts increased but non-significantly in the exposed group of animals.

Effect on liver function markers

Liver function parameters have been enlisted in Table 3. Enzymes like alkaline phosphatase (ALP), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) are related to liver function. Insignificant differences in total bilirubin, direct bilirubin, indirect bilirubin, total protein, albumin, globulin, A/G ratio, and gamma GT between the control and exposed rats were observed. AST and ALT levels in the exposed group decreased; however, the difference was insignificant. A significant decline in ALP level was observed in the exposed group in comparison to the control rats.

Effect on kidney function markers

Kidney function parameters have been mentioned in (Table 4). Urea, uric acid, and creatinine level was measured to find out the normal functioning of the kidney. In the exposed group, urea and uric acid concentration decreased and creatinine level increased, but changes were not significant as compared to the control group of rats. In addition, no significant changes were found in sodium, potassium, phosphorus, calcium, and chloride level in the exposed group.

Table 4 Kidney function test parameters of control and exposed group of animals

S. No	Parameters	Control	Exposed
1	Urea (mg/dL)	29.26 ± 4.45	22.73 ± 2.24
2	Blood urea nitrogenase (mg/dL)	13.67 ± 2.08	10.62 ± 1.04
3	Creatinine (mg/dL)	0.43 ± 0.05	0.45 ± 0.04
4	Uric acid (mg/dL)	2.89 ± 0.51	2.79 ± 0.99
5	Calcium (mg/dL)	10.31 ± 0.42	10.01 ± 0.49
6	Phosphorus (mEq/L)	7.94 ± 0.91	6.85 ± 1.43
7	Sodium (mEq/L)	147.15 ± 5.87	145.83 ± 4.3
8	Potassium (mEq/L)	8.05 ± 1.96	7.89 ± 2.07
9	Chloride	108.93 ± 7.29	108.5 ± 4.43

Table 5 Sperm parameters (count and viability), percentage of sperm head abnormality, and FRAP and MDA levels in control and exposed group of rats

Parameters	Control	Exposed
Sperm count (million/ml)	44.26 ± 9.64	39.17 ± 7.52
Percentage viability	72.5 ± 1.33	60.83 ± 2.21**
Sperm head morphology (%)		
Amorphous	0.5	0.06
Banana	0.33	1.44
Bent	0	0.06
Hookless	0.5	0.22
Total abnormality (%)	1.33	1.78
FRAP (mM/mg protein)	38.98 ± 3.68	38.30 ± 4.22
MDA (μmoles/mg protein)	4.03 ± 0.79	4.84 ± 0.80

Data is represented as mean ± SEM, $n = 6$ and **represents ($p < 0.01$)

Sperm parameters

Sperm count and sperm viability

Results showed a decrease in the sperm count of the exposed group of rats in comparison to the control; however, the decrease was statistically insignificant (Table 5). After staining the sperm with eosin (Fig. 7), the viability result showed a significant decrease in the exposed group of animals as compared to the control group (Table 5).

Sperm morphology

Upon analysis of sperm morphology, the sperm head alterations observed in the exposed group of rats increased insignificantly from sham-exposed rats (Table 5). Figure 8a shows the normal hook-shaped head. Abnormal shapes of the head were observed in sperms with altered morphology such as amorphous head (Fig. 8b), banana head (Fig. 8c), bent head (Fig. 8d), and hookless-shaped head (Fig. 8e).

Sperm mitochondrial activity

After staining sperm mitochondria with DAB, the result showed an insignificant increase in non-functional mitochondria of the exposed group (Fig. 9). The result showed a reduction in class I type (sperm with 100% functional mitochondria) in the exposed group in comparison to control. Class II (> 50% functional mitochondria), class III (< 50% functional mitochondria), and class IV (0% functional mitochondria) types of mitochondria increased in the exposed group relative to control animals.

Biochemical assay of sperms (FRAP assay and lipid peroxidation)

There was an insignificant rise in lipid peroxidation of sperms in the exposed group as compared to the control (Table 5). Significant changes were not observed in the total antioxidant level (FRAP assay) of the exposed group as compared to control (Table 5).

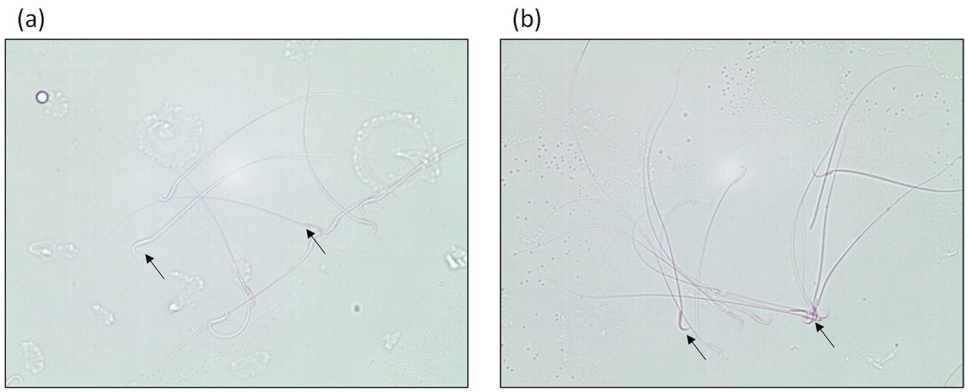


Fig. 7 Sperm viability after eosin staining (**a**) control with colorless and light pink-stained sperm head (**b**) exposed group with dark pink-colored head

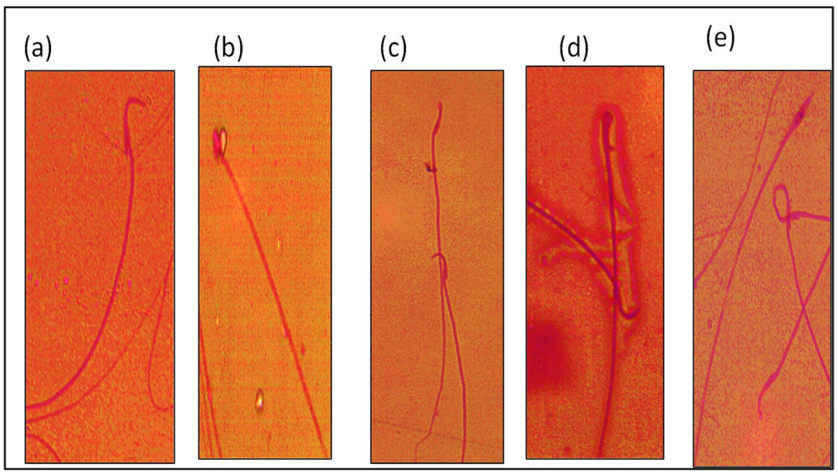


Fig. 8 Sperm morphology. **a** Normal sperm with hook shape, (**b**) amorphous, (**c**) banana, (**d**) bent, and (**e**) hookless head of abnormal sperm

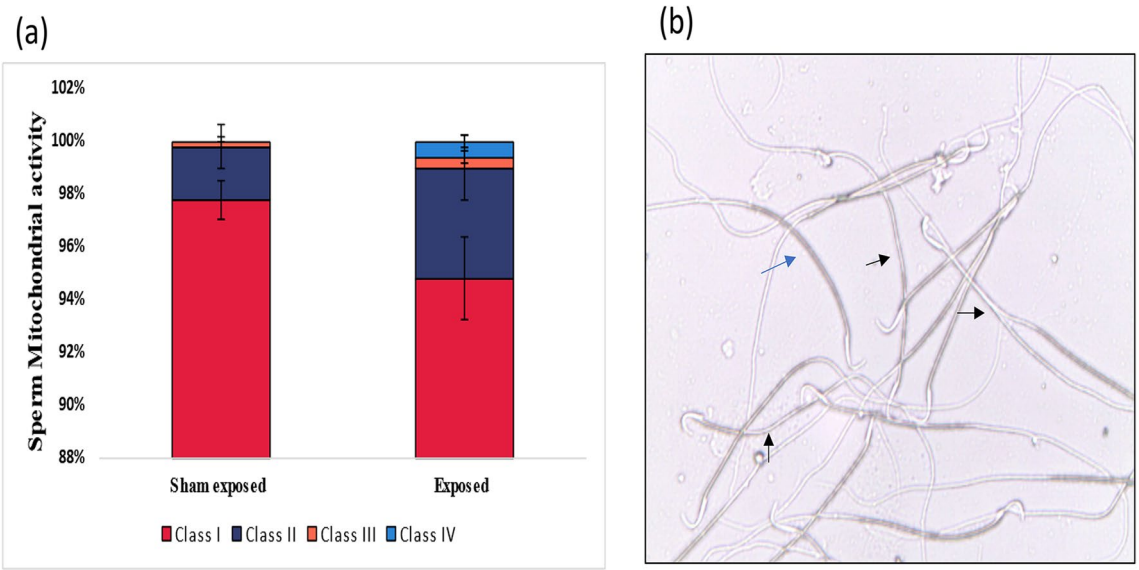


Fig. 9 Sperm mitochondria function test. **a** The exposed group showed a decrease in the percentage of sperm with functional mitochondria as compared to control. Data represented are mean ± SEM, $n = 6$. **b** Sperm with a full midpiece stained in the functional mitochondria (blue arrow), and sperm with an unstained midpiece in non-functional mitochondria (black arrow)

Discussion

With the increase in mobile phone users, it is crucial to find out the effect of electromagnetic radiation on human health. Mobile phone radiation exposure has been shown to affect the male reproductive system. In vitro and in vivo studies have indicated that sperm quality is negatively affected by mobile phone exposure (Yahyazadeh et al. 2020; Gautam et al. 2019). Previous studies conducted on 2G and 3G frequencies have shown adverse effects such as neuronal DNA damage, impaired neurogenesis (Singh et al. 2020), reproductive changes such as alteration in sperm parameters (Gautam et al. 2019), changes in the liver (D'Silva et al. 2017), brain, and induction of oxidative stress. Further, recent findings have shown that on exposing the animals to 4G frequency, the animals show anxiety-like behavior,

change in hippocampus morphology, blood hemostasis, and histopathological changes in the kidney and testis (Hasan et al. 2021, 2022). However, there has been no study on animals exploring the effect of 4G carrier frequency without modulation. Therefore, our research is focused on understanding the potential health effects associated with the 4G carrier frequency on sperm parameters, reproductive, hepatic and renal organs, and hematology of male Wistar rats.

The present study show no significant changes in organ (testis, epididymis, prostate, seminal vesicle) to body weight ratio of the exposed group of rats as compared to the control. Morphometric analysis of testis (seminiferous tubular diameter, germinal epithelial height, and tunica albuginea thickness) showed no significant changes in the exposed group. However, histopathological analysis of the testis showed irregularly shaped seminiferous tubules with vacuolization in the exposed group animals. Evaluation of changes in sperm parameters like sperm count and morphology showed no significant changes. Furthermore, sperm viability showed a significant decrease in exposed group of animals. These findings are in agreement with other studies which show long-term exposure of 2.45 GHz on Wistar rats for 24 h/day for 12 months that resulted in decreased organ weight. Testicular analysis showed a decrease in the seminiferous tubule diameter and thickness of tunica albuginea (Dasdag et al. 2015). The vacuolization observed in the seminiferous tubules in the testis of exposed rats was a qualitative study; hence, its extent could not be quantified.

The epididymis is an essential part of the male reproductive system because it creates an ideal micro-environment for spermatozoa to acquire their fertilizing capacity, motility, storage, and survival. In the present study, noticeable changes in the histoarchitecture of the epididymis and vas deferens were observed.

Kidney function test was performed in order to evaluate the impact of RFR on the kidney. However, we did not observe any significant changes in urea, uric acid, and creatinine levels. Histological analysis of the kidney showed no pathological findings in the control group of rats. Kidney tissues showed normal morphology of the glomerular and tubular structures (Fig. 6). Histopathological assessment of the 4G radiation-exposed kidney showed few alterations such as tubular dilatation, degenerated capsule, vacuolization, and dilatation of the capsule. The renal histopathological injury was not severe so as to alter the function of the kidney. Severe histopathological changes in the kidney include necrotic and apoptotic tubular cells, interstitial inflammation, vascular sclerosis, glomerulosclerosis, tubulointerstitial fibrosis, etc. which were absent in the kidney of exposed rats. Hence, a mild histopathological alteration did not affect kidney function markers. Histological alterations have also been observed by other researchers in the kidney of RFR exposed rats (Bedir et al. 2015; Deniz et al. 2017). Exposure of male Wistar rats for 60 min a day for 21 days to 900 MHz radiation resulted in the sclerosis of glomeruli, tubular degeneration, and increase in hydrophic degeneration in the tubular cells of the kidney (Deniz et al. 2017).

Insignificant differences in total bilirubin, direct bilirubin, indirect bilirubin, total protein, albumin, globulin, A/G Ratio, and gamma GT between the control and exposed rats were observed. AST and ALT levels in the exposed group decreased; however, the difference was insignificant. The only significant alteration in liver function markers was that of ALP. Even though elevated level of ALP is commonly associated with liver disease and low serum, ALP levels are a rare finding, a study has documented the prevalence of low serum ALP levels on patients with chronic liver disease (Iluz-Freundlich et al. 2021). They suggested that low ALP levels may be a possible contributor to disease pathogenesis. Apoptotic bodies, inflammation, and sinusoidal dilatation are all histopathological alterations in the condition of chronic liver disease (Malhi et al. 2006, Krishna 2017). Low ALP levels have also been reported in blood serum as a result of exposure to both non-ionizing (Adebayo et al. 2019) and ionizing radiation (Abdelhalim and Moussa 2013) and have been suggested to indicate liver damage. Hence, this alteration in ALP enzyme level in the exposed group of rats may be indicative of liver function alteration and hence correlates with our histopathological findings in liver such as sinusoidal dilatation, pyknotic nuclei, apoptotic bodies, focal necrosis, and infiltration of inflammatory cells. Though a rarely reported marker for liver function, decrease in ALP levels do indicate an alteration in liver function and is hence indicative of the alteration in liver function due to 4G radiation exposure. The possible mechanism for low levels of ALP may be that ALP in liver tissue itself may have been inhibited by excessive ROS production. Li et al. (2023) showed that ROS inhibits the activity of ALP. Hence, decreased ALP levels may have been induced by inhibition of ALP activity by ROS.

Various histopathological alterations were observed in the liver such as hepatocyte swelling, vacuolization, and focal necrosis in the liver. A study found that GSM mobile phone (850–1900 MHz) exposed mice showed infiltration of inflammatory cells in interlobular areas and vacuolated hepatocytes with denser nuclei as a result of 1 h exposure in answering mode for 10 days. Longer duration of 18 h exposure per day in standby mode resulted in intense inflammation around the central vein with swollen hepatocytes and vacuolated cytoplasm (Al-Glaib et al. 2008). In another study, newborn mice exposed to mobile phone emitting RFR (1900–2200 MHZ) for 48 min per day for 1 to 6 months showed structural changes in the liver (Mugunthan et al. 2019). One month exposure showed cytoplasmic vacuolation and pyknotic nuclei. Two months exposure showed additional alterations such

as sinusoid dilatation and parenchymal hemorrhages. Exposure results of 5 and 6 months showed inflammatory infiltrations around the portal vein and central vein. However, no significant differences were observed in AST and ALT levels between the control and exposed groups in any duration of exposure, which is in line with our findings. Another mobile phone exposure (900 MHz) study on infant mice for 45 min per day for 1 month showed alterations like vacuolated cytoplasm, deep staining of nuclei, and accumulation of fibrous structure around the portal vein (Hanafi et al. 2012). Oxidative stress is suggested as one of the mechanisms contributing to organ damage (Korolczuk et al. 2016), and electromagnetic radiation has been shown to cause oxidative stress through ROS and other free radical generation (Gautam et al. 2021). In this study, the potential cause of sinusoid dilatation in liver may be due to liver injury caused by oxidative stress as a result of radiofrequency exposure. Sinusoidal dilatation in the liver because of RFR has also been observed in earlier studies (Moradpour et al.2020; Sharma et al. 2021). Pyknosis involves the shrinking of chromatin and the nucleus, a process that can occur in both apoptosis and necrosis (Liu et al. 2023). Electromagnetic radiation has been indicated to induce cell damage, apoptosis, and necrosis in liver cells (Ma et al. 2015; Hasan and Islam 2020). Hence, induction of apoptosis and necrosis may be the potential cause of the presence of pyknotic nuclei in the liver of exposed rats.

ROS is one of the important factors behind the oxidative stress dependent damage to the blood cells (Gaharwar and Paulraj 2015). RFR exposure generates free radicals or ROS which is responsible for an increase in oxidative stress which further damages the biomolecules like protein, DNA, lipid, etc. which effect cell growth and proliferation. In the current study, we observed a significant increase in hemoglobin, RBC, and PCV level. An increase in RBC in the RFR-exposed samples in in vitro (Kumari et al. 2016) and in vivo studies (Sedehi Esfahani et al. 2007) has been shown. This increase in RBC upon RFR exposure may be due to the creation of hypoxia like condition which is associated with increased oxidative stress. This hypoxic condition may induce kidneys to produce more erythropoietin and subsequently more erythrocytes. Thus, the circulating RBCs increase to fulfil the oxygen demand and deliver oxygen efficiently to organs. This increase in RBC may have also resulted in increase in Hb and PCV of blood.

In the current findings, sperm mitochondrial activity decreased in the radiation-exposed group of animals, with reduction in a number of total active mitochondria. The changes were insignificant, but mitochondria are considered as the main source for the generation of ROS via mobile phone radiation exposure. Studies have reported sperm mitochondrial ROS to be a major contributor for infertility in males and sperm mitochondria as a prominent target, due to its high energy requirements (Brohi et al. 2017; Koppers et al. 2008). Further, increased oxidative stress in sperms is linked with reduced sperm count, poor motility, and morphology (Agarwal et al. 2014). Sperm plasma membrane is highly vulnerable to ROS, as it is made up of high polyunsaturated fatty acids (PUFAs) like docosahexaenoic acid (DHA), which are necessary for sperm membrane fluidity required for zona pellucida binding and fertilization. High level of ROS can affect sperm-oocyte interaction significantly (Agarwal et al. 2008). The alteration in spermatogenesis could be reflected from the reduction in sperm count in the exposed group of rats. These alterations have a potential impact on fertility. With fewer sperm available, the likelihood of a sperm reaching and fertilizing an egg decreases. A lower sperm count means there are fewer chances of sperm successfully navigating through the female reproductive system to fertilize the egg. In addition to a decrease in sperm count, the exposed group of rats also showed an increase in sperm abnormality and a significant decrease in sperm viability. Sperm with abnormal shapes might not be able to penetrate the egg properly. Morphologically, abnormal sperm may have difficulty binding to the egg's surface and fertilizing it. Sperm with low viability may have a shorter lifespan and decreased ability to survive within the female reproductive tract. As a result, they might not survive long enough to reach and fertilize the egg when it is released during ovulation. Additionally, sperm with reduced viability might not have the necessary strength and endurance to penetrate the protective layers surrounding the egg. This reduces the likelihood of successful fertilization.

In this study, the primary reproductive hormone, testosterone level decreased in the exposure group. This may be due to the vulnerability of Leydig cells to electromagnetic radiation. Exposure to non-ionizing RFR induces oxidative stress, alteration in testosterone level, protein kinase C, injury, apoptosis, and reduction of function in Leydig cells (Lwow et al. 2017; Oyeyipo et al. 2011; Kumar et al. 2011). However, the decrease in the testosterone levels in the exposed group of rats was insignificant in the present study. A probable reason for this may be the use of unmodulated continuous radiofrequency wave for exposure. Literature shows that modulation of waves is crucial for induction of significant alterations in organs (Huber et al. 2005). A comparative study of an unmodulated and modulated radiofrequency radiation showed a highly significant effect from modulated waves than the unmodulated waves (Mohammed et al. 2013).

Oxidative stress markers, i.e., lipid peroxidation increased, and total antioxidant level decreased (FRAP assay) in the sperm of exposed group of animals, but changes were not significant, whereas earlier exposure studies from

1G to 3G radiation showed significant changes in reproductive parameters and oxidative stress markers and other antioxidant enzyme levels (Ghanbari et al. 2013; Negi and Singh 2021). In the current study, animals were exposed to continuous 4G (2350 MHz) signal exposure without any modulation. This variation in results may be due to differences in study design, duration, sources (different wireless signals have different carrier frequencies and types of modulation), variations in SAR value due to conductivity or permittivity of biological tissue, and other environmental factors (temperature and humidity).

Conclusion

After exposure of rats to 4G frequency (2350 MHz) for 56 days, results obtained have showed alterations in sperm viability, count, head abnormality, and sperm mitochondrial activity of rats. A decrease in testosterone level may be associated with Leydig cell injury or its reduced function. RFR also induced histopathological alterations in the male reproductive organs, liver, and kidney. Some of the hematological parameters and liver function parameters showed significant changes while no significant changes in kidney function parameters were observed after the exposure. The results indicate that 4G radiation increases oxidative stress, induces peroxidation of membrane lipids, and decreases total antioxidant capacity in the testis. In the present experiment, among the sperm parameters studied, only sperm viability decreased significantly in the exposed group. However, long-term exposure may decrease sperm count and quality as well. Significant alterations in kidney and liver function tests may also result upon the longer duration of exposure. Thus, it may be concluded that 4G radiation (Carrier frequency 2350 MHz) can affect the reproductive, renal, and hepatic system of male Wistar rat. In order to further explore the impact of 4G radiation on reproductive health, cellular and molecular studies could be done in reproductive cells. In vitro as well as in vivo studies with varying time duration could be conducted to understand the underlying mechanism and cell signaling pathways involved in the alterations induced by 4G radiation. The relationship between the dosage of 4G radiation and reproductive health outcomes with variation in exposure duration could be done to determine the thresholds at which adverse effects may occur. Further, effects of 4G radiation could be examined among different age groups of animals. Young, mature, and aged animals may respond differently, and understanding age-dependent effects is important for assessing long-term implications. Behavioral aspects of reproductive health in animals after long-term exposure to 4G radiation could be examined. Future work can be done to check if 4G radiation can induce genetic or epigenetic changes such as DNA integrity, gene expression and epigenetic modifications in reproductive cells and tissues.

Author contribution Rohit Gautam: experimentation, visualization, conceptualization, data analysis; Sonali Pardhiya: experimentation, conceptualization, data analysis; Jay Prakash Nirala: resources, data analysis; Priyanka Sarsaiya: data analysis; Paulraj R: supervision, resources, project administration, funding acquisition, validation.

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Data availability Data will be made available on request.

Declarations

Ethics approval The study was approved by the Institutional Animal Ethics Committee (IAEC, Code No. 1/2021).

Consent to participate Not applicable.

Consent for publication Not applicable.

Conflict of interest The authors declare no competing interests.

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