

Oxidative stress and testicular damage induced by chronic exposure to 35.5 GHz millimeter wave radiation in male Wistar rats

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Abstract

Background: The widespread usage of millimeter waves (MMW) in different spheres of the public domain necessitates to have a better understanding of any possible health impacts caused by them. The latest example of this is the global implementation of 5G technology.

Objectives: The present investigation aimed to study the effects of 35.5 GHz frequency on reproductive parameters of male Wistar rats.

Methods: The study employed a randomized controlled design, with animals being assigned to control, sham-exposed, and exposed groups, with six animals in each group ($n = 6$). The experimental group was exposed to 35.5 GHz frequency for 2 h per day for 60 days. At the end of the exposure period, various sperm parameters such as sperm count, morphology, viability, and sperm mitochondrial activity were evaluated. The evaluation of oxidative stress was done by lipid peroxidation assay in testis homogenate. Superoxide dismutase, total sulfhydryl level, and total antioxidant capacity were assessed in testis homogenate. The DNA damage in the testis was assessed through the comet assay.

Results: The results showed a significant decrease in sperm viability and count, as well as morphometric changes in testis histopathology. Lipid peroxidation testis was significantly elevated in the exposed group, while superoxide dismutase (SOD), total sulfhydryl level, and total antioxidant capacity were significantly decreased in the exposed group. The comet assay revealed a significant increase in DNA damage in the exposed group, indicating genotoxic effects.

Discussion: The observed changes in sperm parameters, testicular morphology, and oxidative stress markers highlight the potential for testicular tissue damage via oxidative mechanisms. These results underscore the need for further research to elucidate the biological implications of chronic MMW exposure.

Rohit Gautam and Neha Jha contributed equally to this work.

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Conclusion: Chronic exposure to 35.5 GHz frequency may negatively impact male reproductive function and testicular tissue, primarily through mechanisms involving oxidative stress.

KEYWORDS

35.5 GHz, millimeter waves (MMW), oxidative stress, radio frequency radiation (RFR), testicular damage, Wistar rat

1 | INTRODUCTION

Over the last few decades, there has been a significant surge in the utilization of nonionizing part of the electromagnetic spectrum, particularly microwave and millimeter waves (MMW). The microwave band lies in the range of 3–30 GHz, and the millimeter band lies just above it, with the frequency range of 30–300 GHz and wavelength of 1–10 mm in free space.¹ Because of the wide range of applications, these frequency ranges have been utilized for numerous purposes, such as the Ka band (26.5–40 GHz), which supports higher bandwidth communication and is employed in various sectors such as satellite and radar communications.² Frequencies within the 35–36 GHz range are specifically used in Earth exploration-satellite services, including Global Precipitation Measurement, as well as in space research services.^{2,3} Additionally, electromagnetic radiation near 35 GHz is utilized by the United States military for both land-based and airborne radar systems.⁴ The health impacts of non-ionizing radiation (NIR) remain a subject of concern within the scientific community. Nonionizing radiations like microwave and millimeter waves (MMW) have photon energies ranging from 10^{-3} to 10^{-4} eV, which is much lower than the energy required for ionization (greater than 10 eV) or for breaking biomolecular bonds, including ionic, hydrogen, or van der Waals bonds. However, these radiations possess enough energy to cause the rotation of free molecules that have a dipole moment. These can affect biological systems via thermal and nonthermal mechanisms. Numerous studies and instances of occupational exposure have confirmed the adverse effects of these radiations on health.⁵ The impact of NIR on biological systems is influenced by various factors, including the specific absorption rate (SAR), radiation frequency, power, exposure duration, and environmental conditions.^{6,7}

Prolonged and chronic exposure to electromagnetic radiation, particularly at higher frequencies, has been shown to have detrimental effects on biological systems. The exact mechanism involved in the effects of MMW on biological systems remains to be elucidated. While most in vivo studies have reported an increased oxidative stress as a possible reason for the aberrations caused.⁸ These studies suggest a link between microwave radiation exposure and the generation of oxidative free radicals, leading to oxidative stress.⁷ Additionally, research has identified changes in the activity of enzymes such as ornithine decarboxylase (ODC) and calcium-dependent protein kinases after microwave exposure.⁹ People at workplaces where radar antennas were placed in proximity have increased occupational exposure to higher frequency microwave radiation and MMW. Recent

studies have shown that exposing rats to 35 GHz MMW leads to changes in protein expression in the rat alveolar macrophage cell line.¹⁰ In another study, exposure to 35 GHz millimeter waves with the power density 75 mW/cm², skin samples showed aggregation of neutrophils in vessels, degeneration of stromal cells, and damage in collagen.¹¹ In a study on people who have routinely worked with radar systems (18 GHz), alterations of visual reaction time and short-term memory are observed.⁴ Another recent study indicates that MMW exposure can lead to temperature increases in the cornea, particularly in regions adjacent to the eyelashes.¹² However, the presence of eyelashes may mitigate some of these thermal effects by altering the electric field distribution and the absorption characteristics of the cornea. Further studies also emphasized that the thermal effects of millimeter waves can lead to localized heating, which may cause tissue damage and pain.¹³ Understanding the thresholds at which these effects occur is essential for developing safety regulations that protect users from adverse health effects associated with prolonged exposure to millimeter waves.

Recent animal studies showed an increase in oxidative stress with adverse effects on sperm viability, indicating negative effects on male fertility.¹⁴ Exposure to lower frequency (2.4 GHz) has also shown an effect on sperm parameters and testicular structure with increased oxidative stress.¹⁵ While a small amount of reactive oxygen species (ROS) is necessary for normal sperm function, such as capacitation and hyperactivation,¹⁶ excessive ROS production led to oxidative stress, disrupting the cell's redox balance.¹⁷ The sperm cell membrane is rich in polyunsaturated fatty acid, and sperm cell have less cytoplasm antioxidant enzymes, which makes it more prone to oxidative damage.¹⁸ The oxidative stress causes damage to membrane lipids and DNA damage in spermatozoa via DNA base adduct formation and cell death.¹⁹ Based on the above studies and the hypothesis that 35.5 GHz impacts biological functions and affects male reproductive health, we aimed to assess the effects of 35.5 GHz radiation on reproductive parameters, including sperm count, morphology, viability, and mitochondrial activity, as well as testis morphometry and histopathology.

2 | MATERIALS AND METHODS

2.1 | Experimental design

Male Wistar rats of 45days (weighing approximately 190–210 g) were used for the study. Animals were divided into three groups: control, sham-exposed, and exposed, with $n = 6$ rats in each group.

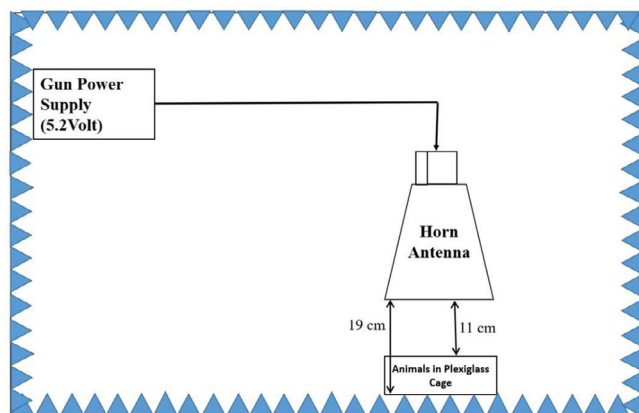


FIGURE 1 Schematic diagram of exposure set-up for exposing rats to 35.5 GHz radiation.

Animals were exposed to 35.5 GHz radiation, 2 h daily for 60 days consecutively in a specially designed anechoic radiation chamber. The animals and study protocol were approved by the Institutional Animal Ethics Committee (IAEC, Code no 1/2021). Animals were obtained from the Central Laboratory Animal Resources, Jawaharlal Nehru University, New Delhi, and housed in groups of three animals per cage in an experimental animal house. Ambient environment was maintained at 25°C room temperature, 12:12 h day/night cycle, food and water were provided ad libitum. Animals were kept per the Control and Supervision of Experiments on Animals (CCSEA) guidelines.

2.2 | Exposure setup

Animals were exposed to 35.5 GHz frequency radiation in an anechoic chamber. A gun power supply with 5.2 DC volts was used as a source, which was fed to the Gun Oscillator. It was connected to the horn antenna through which 4.67 W/m² power density fell on the Plexiglass cage (Figure 1). The Power density was calculated using a monopole probe, which was 3.8 W/m² inside the Plexiglass cage where experimental animals were kept. Specific absorption rate (SAR) was calculated by the method of Durney, which was 0.07 W/kg at the beginning of the experiment and 0.0564 W/Kg at the end of the experiment.²⁰

2.3 | Sample collection and tissue somatic index

After the exposure period concluded, the male reproductive organs (testis, epididymis, prostate, and seminal vesicle) were excised and weighed. The tissue somatic index was determined by calculating the organ-to-body weight ratio. The left testis and epididymis were fixed for histopathological study, and the right testis sample was homogenised in phosphate buffer saline (PBS) for various biochemical assays for evaluating oxidative stress.

2.4 | Histopathology of organs

Post-exposure, the left testis and epididymis samples were fixed overnight in 10% formalin and subsequently embedded in paraffin wax. Following this, blocks were prepared, and 5 µm tissue sections were cut. Sections were further mounted on slides and stained with hematoxylin and eosin.²¹ The samples were then examined under a light microscope at 4×, 10×, and 20× magnification.¹⁴

2.5 | Testis morphometry and Johnson scoring

Testis dimensions (length and width), seminiferous tubule diameter, germinal epithelial height, and tunica albuginea thickness were measured. A single-blinded researcher visualized six nonconsecutive testicular sections of the testis for each rat. Testicular damage was quantified using Johnsen's scoring method.²² Around 20 random seminiferous tubules from each section were evaluated and scored from 1 to 10 based on spermatogenesis and the presence or absence of Sertoli cells, spermatogonia, spermatocytes, spermatids, and spermatozoa. A higher Johnson's score represents spermatogenesis with no or less damage, while a lower score shows damaged seminiferous tubules with alteration in spermatogenesis.

2.6 | Sperm parameters

Sperm were collected from the cauda region of the epididymis via mincing the cauda in 5 mL of Ham's F12 media for 15–20 min at 37°C temperature. Thereafter, sperm were filtered with an 80 µ nylon mesh and stored at –80°C for further analysis. Sperm count was evaluated by the method of the World Health Organisation (WHO) manual.^{23,24} Sperm morphology was assessed via staining with 0.5% Eosin Y, and around 300 sperm per sample were observed for sperm head morphology.²⁵ Sperm viability was evaluated by the Eosin Y staining method. Light pink and colourless cells were considered viable, while dark pink cells were non-viable. Around 100 cells per sample were observed.²⁶

2.7 | Scanning electron microscope analysis of sperm

To analyse any morphological alterations induced by radiation, a scanning electron microscope (SEM) was used on a rat sperm sample. Briefly, Sperm samples were fixed in Karnovsky's fixative (2.5% glutaraldehyde and 2% paraformaldehyde) and washed with 0.1 M phosphate buffer (pH 7.4). Postwashing, the samples were centrifuged at 300g at 37°C and the pellet was collected. Samples were dehydrated in an acetone graded series. After that, sperm were put on clean microscope slides, air-dried, and then the slide was mounted on the SEM stub. It was sputtered with chromium particles and analyzed under SEM (TESCAN CLARA Serial No 120-0062) at 3 kV.

2.8 | Sperm mitochondrial activity

Sperm mitochondrial activity was measured via staining with 3,3'-diaminobenzidine (DAB), which gets deposited at the Cytochrome-c complex²⁷. In Brief, sperm were stained with DAB and kept at 37°C for 1 h. Smear was prepared on glass slides, fixed for 10 min in 10% formaldehyde, and air-dried. Sperm were visualized under a light microscope, and 200 sperm per sample were analyzed.

2.9 | Oxidative stress markers

The excised right testis was homogenized at a ratio of 1:10 (w/v) in ice-cold PBS. The crude suspension was centrifuged at 10,000g for 10 min at 4°C, after which the supernatant was collected and used for the oxidative stress analysis. Oxidative stress markers play a crucial role in assessing lipid peroxidation and DNA damage. Malondialdehyde (MDA) level provides an indication of lipid peroxidation and is considered an oxidative stress marker. MDA levels were measured via the thiobarbituric acid reactive substances (TBARS) assay.²⁸ The MDA level was measured as mmol MDA per milligram protein. The protein content was estimated via Bradford assay. Further SOD activity in the testis was evaluated via photoreduction of NBT.²⁹ Total sulfhydryl (SH) level was measured using 5,5'-dithiobis-2-nitrobenzoic acid (DTNB) reagent. FRAP assay for total antioxidant capacity was performed using ferric chloride and TPTZ.³⁰

2.10 | Comet assay

Single-cell suspension from the testis was prepared, and DNA damage was assessed using the Comet assay.³¹ Briefly, slides were precoated with a thin layer of 1% agarose. The cell samples were then mixed with 0.5% low-melting-point agarose (LMPA), transferred to the slides, and covered with a coverslip to ensure a uniform layer. Further and a third layer of agarose was added over the solidified LMPA-sample mixture. The agarose "sandwich" containing the sample was then submerged in a lysis buffer to lyse the cell membranes and incubated overnight in an ice-cold lysis buffer. Subsequently, the slides were immersed in electrophoresis buffer for 30 min to facilitate DNA unwinding, followed by electrophoresis at 25 V and 300 mA for 30 min. After electrophoresis, the slides were neutralized by coating them with a neutralization buffer for 10 min. The slides were then drained, exposed to 100% cold ethanol, and allowed to dry. Next, the slides were stained with 0.02% ethidium bromide (EtBr) and visualized under a fluorescence microscope (LMI) at 20× magnification. Various parameters of DNA damage were quantified using Comet Score software (TriTek Comet Score Pro).

2.11 | Statistical analysis

All experiments were conducted at least three times for each experimental group ($n = 6$). Statistical analysis was performed by one-way ANOVA followed by Tukey's post hoc test, using GraphPad Prism 9

TABLE 1 Organ (testis, seminal vesicle, prostate, and epididymis) to body weight ratio for control, sham-exposed, and exposed groups.

Organs	Control ($\pm$ SD)	Sham exposed ($\pm$ SD)	Exposed ($\pm$ SD)
Testis (g)	1.58 $\pm$ 0.2	1.68 $\pm$ 0.15	1.20 $\pm$ 0.45
Seminal vesicle (g)	1.20 $\pm$ 0.21	1.34 $\pm$ 0.23	1.16 $\pm$ 0.22
Prostate (g)	0.42 $\pm$ 0.09	0.45 $\pm$ 0.05	0.44 $\pm$ 0.09
Epididymis (g)	0.55 $\pm$ 0.07	0.59 $\pm$ 0.06	0.51 $\pm$ 0.09

Note: Data represented as mean $\pm$ SD.

software. Data were represented as mean $\pm$ SD. The significance level was denoted by p -values, where $p < 0.05$ *, $p < 0.01$ **, $p < 0.001$ *** and $p < 0.0001$ **** were considered statistically significant at increasing levels.

3 | RESULTS

3.1 | Organ to body weight ratio

A decrease in tissue somatic index ratio was observed. Significant changes were not seen in organs of exposed group animals when compared with the control and sham-exposed (Table 1). This suggests that organ weight alone may not be a sensitive indicator of MMW-induced reproductive damage. However, subtle effects that are not visible in overall anatomical measurements could still play a role in functional impairments.

3.2 | Histopathological analysis and testis morphometry

Histopathology analysis of the epididymis section in the control and sham-exposed group showed normal histological appearance, packed with sperm, whereas the exposed group showed lower density of spermatozoa and vacuolisation (Figure 2A). In the Testis, the exposed group showed a decrease in seminiferous tubular diameter with a decrease in germinal epithelial height and tunica albuginea thickness compared with control and sham-exposed (Figure 2C). Johnson scoring of the testis showed a significant decrease in score in the exposed group, indicating alteration and deformities in testis histopathology of the exposed group rats compared with the sham-exposed group. Johnson scoring confirmed a notable decline in testicular health and functionality in the exposed group. These findings suggest that MMW radiation disrupts spermatogenesis, potentially by damaging the microarchitecture of the testis (Figure 2B).

3.3 | Sperm parameters

Results showed a significant decrease in sperm count in the exposed group as compared with the control and sham-exposed groups.

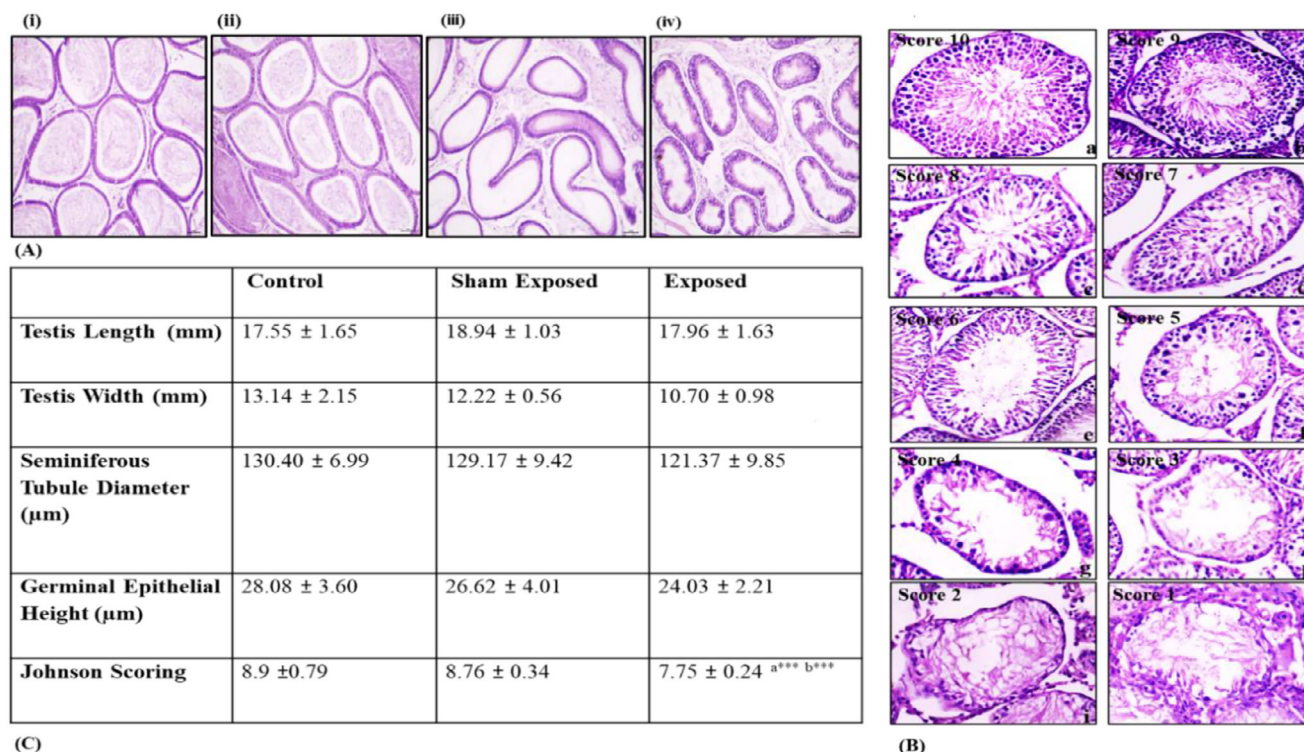


FIGURE 2 Histopathology analysis (A) Epididymis (40×). Control (i) and sham-exposed (ii) showed normal structure filled with sperm, while exposed groups (iii and iv) showed alterations. (B) Standardized Johnson scoring in cross sections of seminiferous tubules of control, sham-exposed, and exposed groups using hematoxylin-eosin (HE) staining. (C) Table: Testis morphometry and Johnson scoring. Data are represented as mean ± SD (a* exposed with respect to control, b* exposed with respect to sham).

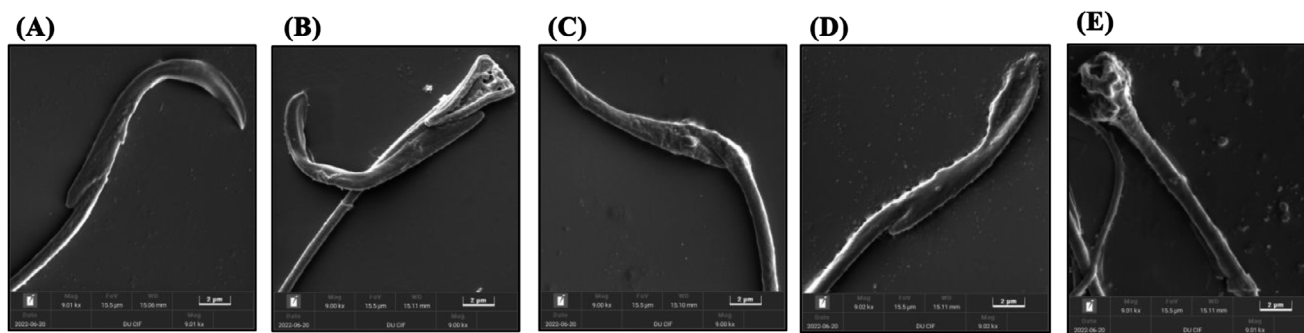


FIGURE 3 SEM analysis of sperm. The control group showed a normal hook-shaped (A) structure, while the exposed group showed alterations like bent neck (B), banana (C, D), and amorphous (E).

Sperm morphology showed an increase in abnormal sperm percentage in the exposed group compared with the sham-exposed and control groups (Table 2). Ultrastructure analysis via SEM images also confirmed normal hook-shaped sperm in the control group animals, while an increase in sperm head abnormalities, such as bent neck, banana, and amorphous, was observed in the exposed group (Figure 3).

3.4 | Sperm mitochondrial activity

Results showed a significant decrease in Class-I (fully functional mitochondria in the mid-piece) and an increase in Class-III (less than 50% functional mitochondria in mid-piece) in the exposed group rats compared with the sham-exposed. The difference in observed values of sperm mitochondrial function tests was significant compared

TABLE 2 Sperm parameters (sperm count, viability, and morphology) and mitochondrial function test for control, sham-exposed, and exposed groups.

	Control	Sham exposed	Exposed
Sperm count million/gram of cauda	45.51 ± 12.93	46.41 ± 16.49	21.69 ± 9.60 ^{a,b*}
Sperm viability (%)	68.13 ± 5.90	58.87 ± 6.98	49.69 ± 9.43 ^{a,b*}
Sperm morphology (%)	1.11 ± 0.40	1.06 ± 0.49	15.33 ± 0.56
Bent	0.33	0.22	1.88
Banana	0	0.11	0.77
Amorphous	0.22	0.27	1.05
Hookless	0.05	0.11	0.44
Headless	0.5	0.33	11.16
Mitochondria function test			
Class I	98.58 ± 0.58	99.16 ± 0.40	97.83 ± 1.03 ^{b*}
Class II	0.91 ± 0.49	0.66 ± 0.60	1.41 ± 0.91
Class III	0.41 ± 0.37	0.08 ± 0.20	0.58 ± 0.37 ^{b*}
Class IV	0.083 ± 0.20	0.08 ± 0.20	0.16 ± 0.25

Note: Results showed a significant decrease in sperm count and sperm viability in the exposed group compared with the control and sham-exposed groups. Results also showed a significant decrease in functional mitochondria in the exposed group compared with the sham-exposed group. (data are represented as mean ± SD, a exposed with respect to control, b* exposed with respect to sham).

with the control and sham-exposed group. As mitochondria play a crucial role in providing energy for sperm motility and function, this dysfunction likely contributes to impaired reproductive capacity. These results emphasize the sensitivity of mitochondrial structures to MMW radiation and their role in mediating reproductive toxicity.³²

3.5 | Oxidative stress marker

In the lipid peroxidation assay, the MDA level obtained in the testis of the exposed group was significantly higher than that of the control and sham-exposed group. The antioxidant enzyme SOD levels, total Sulfhydryl level, and total antioxidant capacity measured via FRAP assay were also found to be significantly decreased in the exposed group compared with sham-exposed and control groups (Table 3). These findings suggest that MMW radiation disrupts the balance between reactive oxygen species (ROS) production and antioxidant defenses, leading to oxidative damage in reproductive tissues. Oxidative stress is a well-established mechanism of testicular damage and impaired spermatogenesis, further corroborating the observed histological and functional alterations.

3.6 | Comet assay

Results from the comet assay have shown an elevated comet length in the exposed group compared with the control. The tail DNA% and olive tail movement in the exposed group animals have increased in the exposed group compared with the control and sham-exposed groups (Figure 4). This indicates that MMW radiation induces genotoxic effects in germ cells, potentially compromising genetic integrity and raising concerns about long-term reproductive health and offspring viability. DNA damage in sperm cells is a critical factor in male infertility and could have far-reaching consequences for reproductive success.

4 | DISCUSSION

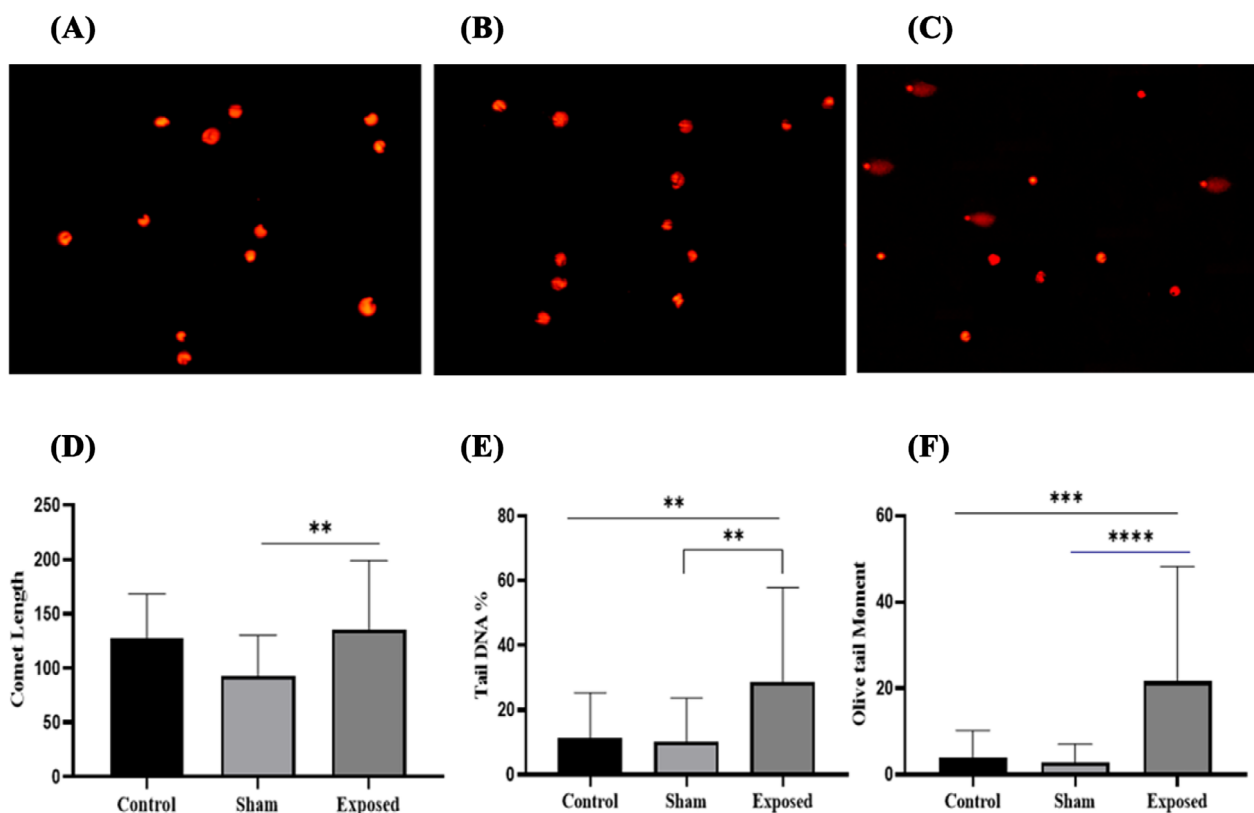
NIR exposure is increasing rapidly, and the effects of microwave and radio frequency radiation have been debated worldwide. In the current investigation, we found remarkable changes in the morphometry of the testis. Earlier studies showed that non-thermal exposure to microwave and millimeter wave radiations can affect testis morphometry. Testes are a sensitive organ as spermatogenesis occurs in the testis; therefore, any alteration may lead to affect the sperm quality.³³ Previous research has also demonstrated that 2 h of exposure to microwave frequencies in the 2.1 GHz region can affect the histology of the testis and spermatogenesis.³⁴ The results were further corroborated in a study using 2.45 GHz microwave exposure.³⁵ In different studies, it has been reported that exposure to RF-EMF (radio-frequency electromagnetic field) of mobile phones leads to increased oxidative stress, alteration in testicular function, and decreased gonadotropic hormonal profile.³⁶ Additionally, various sperm parameters were evaluated, and a decrease in sperm count, viability, and alteration in sperm head morphology was observed in the exposure group. Likewise, findings have shown that exposure to 27 GHz affects the sperm quality in the marine mussel, *Mytilus galloprovincialis*. A significant decline was observed in sperm motility after only 10 min of exposure.³⁷

ROS plays a vital role in sperm function. Earlier reports suggest the role of mitochondrial dysfunction in RFR-induced oxidative damage to sperm via free radical and ROS generation. Additionally, Research has demonstrated that low-level microwave irradiation induces chromatin condensation in human cells, which subsequently causes damage to cell membranes.³⁸ Further studies have proven long-term exposure to RFR can increase ROS generation, which can induce DNA damage, micronuclei formation, base adduct formation, etc.^{39,40} Findings from our study also showed a significant increase in lipid peroxidation and decrease in SOD levels, total Sulfhydryl level and total antioxidant capacity in exposed group animals as compared with control groups, which were corroborated with previous findings.⁴¹ From various in vitro and in vivo studies, it has also been shown that electromagnetic radiation can be genotoxic, mutagenic, and can increase the frequency of micronuclei formation, chromosome aberrations, and DNA single and double strand breaks.^{42,43} Genotoxic study of 35.5 GHz radiation was done on DNA damage by the comet assay showed DNA damage in

TABLE 3 Oxidative stress markers, lipid peroxidation, superoxide dismutase, total sulfhydryl, and total antioxidant capacity via FRAP assay measured in the control, sham-exposed, and exposed groups.

	Control	Sham exposed	Exposed
Lipid peroxidation-testis (μM MDA/mg protein)	0.3909 ± 0.1088	0.4601 ± 0.06521	$0.8952 \pm 0.1659^{a***b***}$
SOD-testis (unit SOD/mg protein)	9.03 ± 1.48	8.55 ± 0.78	$5.49 \pm 1.8^{a***b***}$
Total sulfhydryl (mmol/mg protein)	35.02 ± 2.5	34.10 ± 1.5	$27.40 \pm 6.9^{a*b*}$
FRAP (mM/mg protein)	8.52 ± 0.48	8.40 ± 0.3	$6.24 \pm 0.72^{a**b**}$

Note: Exposed group showed a significant increase in oxidative stress (data are represented as mean $\pm$ SD, a* exposed with respect to control, b* exposed with respect to sham). Values were considered significant at p -value < 0.05 , where asterisk (*) represents the level of significance, * ($p < 0.05$), ** ($p < 0.01$), *** ($p < 0.001$) and **** ($p < 0.0001$).

**FIGURE 4** Comet assay control (A) and sham-exposed (B) with normal DNA, no comet tail was observed. Exposed group (C) with damaged DNA showing a comet tail (20 $\times$). The exposure group showed an increase in comet length, tail DNA%, and olive tail movement compared with the control group.

the exposed group compared with the control. DNA damage leads to apoptosis or other forms of cell death, and it might further affect fertility and reproductive outcomes.⁴⁴ Moreover, the present study shows that millimetre waves affect the reproductive system via an increase in oxidative stress, which leads to alterations in sperm parameters, testicular histopathology, and DNA damage in testicular cells. Studies have also hypothesized that EMF can alter the membrane potential by affecting ion channels.⁴⁵ Studies have shown that a highly coherent electric field, which is similar to the original electric field of the EMF but less intense, is produced when millimeter waves are exposed to the body. These rapidly fluctuating, highly penetrating magnetic

fields generate significant forces on ions dissolved in the body's aqueous environments, causing movement of these ions within both the extracellular fluid and the intracellular aqueous compartments, which may cause thermal and normal effects on biological tissues.^{46,47} When the voltage sensor's charges are applied with force by the regenerated EMF, the voltage-gated calcium channels (VGCCs) are activated, which affect membrane permeability and alter sperm capacitation and fertilization capacity.⁴⁸ Further biological effects of radiation may vary with different exposure parameters, such as frequency, emitting power, polarization, and duration of exposure (intermittent or continuous).

5 | CONCLUSION

Based on the findings, exposure to 35.5 GHz radiation was shown to impact different parameters related to male reproductive health. While no statistically significant changes were observed in the tissue somatic index, detailed histopathological, biochemical, and functional assessments revealed significant reproductive alterations. Marked histological alterations were observed in testicular and epididymal tissues, accompanied by a significant reduction in Johnson's score, indicating impaired spermatogenesis. Further, sperm analysis showed a decline in count and viability, coupled with increased morphological abnormalities and mitochondrial dysfunction, which are critical indicators of compromised fertility. Ultrastructure analysis through SEM also revealed the changes in sperm head morphology. Additionally, the study highlighted oxidative stress as a key mechanism of MMW-induced toxicity. Elevated lipid peroxidation levels, coupled with reduced antioxidant enzyme activity (SOD, sulfhydryls, and FRAP), underscore the oxidative imbalance induced by radiation. Importantly, genotoxic damage evidenced by the comet assay reinforces concerns over DNA integrity in germ cells.

Collectively, these findings demonstrate that MMW radiation can disrupt structural, functional, and molecular aspects of the male reproductive system, raising important concerns regarding prolonged or repeated exposure to such non-ionizing radiation sources. This underscores the need for further mechanistic studies and regulatory policies to evaluate and mitigate potential risks associated with increasing environmental and occupational exposure to MMW radiation.

AUTHOR CONTRIBUTIONS

Rohit Gautam: Original manuscript; experimentation; conceptualization; data analysis. **Neha Jha:** Manuscript editing; experimentation; data collection; data analysis. **Anuj Kumar Tomar:** Manuscript editing; experimentation; data analysis. **Jay Prakash Nirala:** Resources; data analysis. **Taruna Arora:** Supervision; formal analysis. **Paulraj Rajamani:** Supervision; visualization; resource management; validation.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

All data generated or analyzed during this study are included in the article. Further inquiries can be directed to the corresponding author.

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