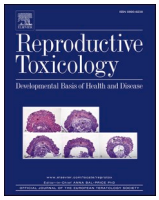
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Effects of 700 MHz radiofrequency radiation (5 G lower band) on the reproductive parameters of female Wistar rats

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ABSTRACT

The advent of 5 G technology has raised concerns about its potential biological effects, particularly reproductive health being one key area of focus. This study investigated the impact of 700 MHz, a lower 5 G frequency band, on the reproductive health of female Wistar rats. The experiment analyzed the effects of short-term and long-term exposure to 700 MHz mobile radiation on female Wistar rats. Rats were divided into three groups (control, sham-exposed, and exposed), with sample sizes of n = 6 for short-term and n = 8 for long-term exposure. For short-term exposure, rats were subjected to 6 hrs of radiation daily for 10 days, while for long-term exposure, rats exposed 4 hrs daily for 60 days. Physiological parameters, including estrous cyclicity, were monitored, and histopathological and biochemical analyses were conducted on harvested ovaries. Comet assay was performed to assess DNA damage. The results indicated no changes in estrous cycles or comet assay parameters in either exposure group. Serological hormone levels, including estradiol and progesterone, remained within normal ranges, but a slight yet significant increase in testosterone levels was observed in exposed groups. Oxidative stress markers revealed elevated malondialdehyde (MDA) levels and significant decreases in superoxide dismutase (SOD), total sulfhydryl content, and ferric reducing antioxidant power (FRAP) in exposed ovaries. Histopathological analysis showed no significant changes in ovarian morphology in short-term exposure but revealed alterations, including cystic follicles and abnormal vasculature, in long-term exposure. These findings suggest that 700 MHz radiation may induce oxidative stress and tissue changes in ovarian samples over prolonged exposure.

1. Introduction

In the last few decades humankind has experienced enormous advancement in the field of technology and ways of communication. Application of radiofrequency radiation (RFR) has revolutionized the way in which communication takes place globally. The large footprint of these radiation makes them ubiquitous in our environment and they form an indispensable part of our daily lives [18]. This widespread usage of radiofrequency and millimeter waves have made life convenient with various applications such as data transmission and communication, modern wireless devices, advancement in the surveillance systems for air travel, industrial applications, and improved diagnostic purposes in medical field, new sources of electromagnetic fields and radiofrequency radiation have emerged. This has resulted in the addition of another pollutant in the form of electropollution to an already large list of environmental contaminants that can have deleterious impacts and can impair human health and wellbeing [4]. The rollout of different 5 G frequencies globally represents the latest frontier in wireless communication. Offering unparalleled data transfer speeds and connectivity, 5 G technology has become integral to modern communication. However, alongside its benefits, concerns have emerged regarding the potential biological effects of prolonged exposure to radiofrequency radiation (RFR) emitted by 5 G systems. Among these concerns, these radiation on reproductive health has gained particular attention due to its implications for long-term population health. The list of bands for 5 G NR (5 G New radio) technology is divided into two unique frequency ranges (FR): FR1 and FR2. The FR1 comprises sub-6 GHz (450MHz-6GHz) frequency distributions, while the FR2 covers frequencies ranging from 24.25 GHz to 71.0 GHz [8]. In India, the rollout of 5 G is in three major bands based on the frequency; low band:

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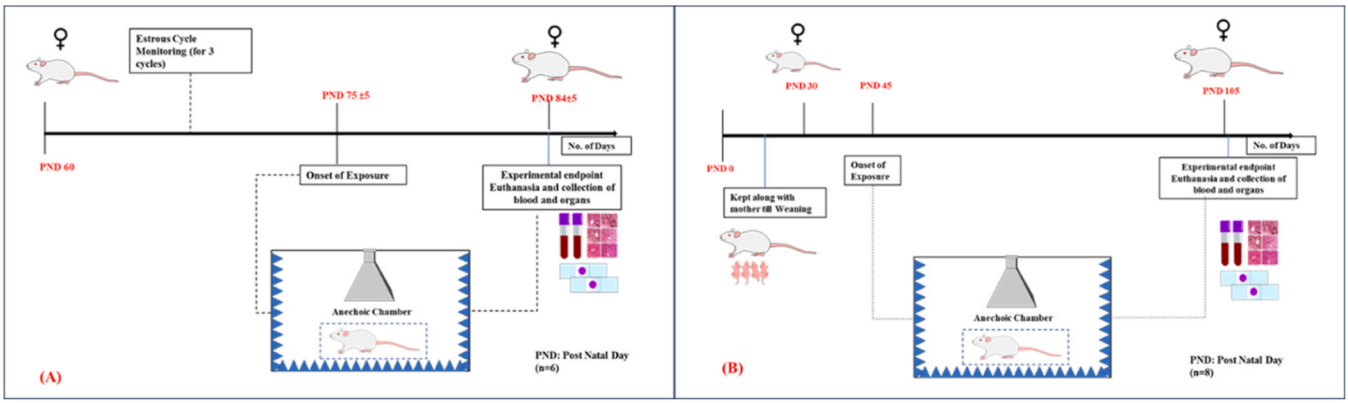


Fig. 1. Schematic representation of (A) Short-term and (B) Long-Term exposure set up.

600–900 MHz or < 1 GHz, mid band (1 GHz to 6 GHz) and high band/millimeter-Wave (mmWave) bands: 24–47 GHz. The Telecom Regulatory Authority of India (TRAI) has auctioned bands for 5 G frequencies, which include some existing bands such as 700 MHz, 800 MHz, 900 MHz, 1800 MHz, 2100 MHz, 2300 MHz, and 2500 MHz, as well as some new spectrum bands such as 600 MHz, 3300–3670 MHz, and 24.25–28.5 GHz (TRAI Annual Report 2022–23) [38].

Radiofrequency radiation (RFR) affects biological systems through various mechanisms. Since living tissue contains water, which consists of permanent dipoles, these molecules vibrate and contribute to energy absorption. low-level radiofrequency may induce biochemical effects. Proposed mechanisms include coherence, resonance, signal averaging, and non-linear effects, all of which generate weaker signals than those associated with normal physiological processes. A non-thermal mechanism identified by Challis [6] involves free radical formation in biomolecules with hyper-subtle structures, where external energy is concentrated if the RFR wavelength matches molecular energy level differences. However, energy dispersion through water molecules limits signal amplification. Another hypothesis suggests that RFR induces additional potentials on cellular membranes, affecting ion transport, though this occurs only at significantly higher voltages than those generated physiologically. Additionally, RFR may alter molecular bonds, affecting protein enzyme activity. Proteins, especially electrostatically bound ones, may undergo denaturation, aggregation, or structural instability due to RF exposure, influencing their enzymatic functions [41].

Recently the effects of 700 MHz were studied on zebrafish embryos. The study observed anxiolytic effects and slower habituative learning along with altered acetylcholinesterase due to exposure of 700 MHz [37]. The impact of RFR on fetal development remains controversial across various animal models [20]. Additionally, another study reported that RFR exposure inhibited ovulation and reduced the number of corpora lutea in rats [14]. Roshangar et al., [28] investigated the effects of low-frequency RFR exposure on oocyte differentiation and follicle growth, concluding that RFR exposure during the developmental period may disrupt both processes and reduce fertility by depleting ovarian reserves. Similarly, Türedi et al. [39] observed degeneration and vacuolization of granulosa cells toward the antrum in the ovarian tissue of rats exposed to 900 MHz RFR during the prenatal period. Conversely, some studies suggest an inconsistent relationship between mobile phone radiation and reproductive performance. In a study examining the effects of chronic 4 G radiation exposure on rats, demonstrated that 4 G radiation has the potential to adversely affect the male reproductive system, as well as the hematological, hepatic, and renal systems in rats [11].

In this study, we examined the impact of one of the lower 5 G bands, 700 MHz, on the female mammalian reproductive system, using Wistar rats as a model organism. A variety of assays, including reproductive hormone analysis, histopathological examination of the ovaries, and ROS detection methods, were utilized to evaluate changes in reproductive function and oxidative stress levels. By focusing on these specific parameters and using rigorous methodologies, this research aims to provide a comprehensive understanding of the physiological effects of RFR on key reproductive parameters of female Wistar rats. The findings not only contribute to the field of reproductive toxicology but also have broader implications for public health, particularly as wireless technology continues to expand in everyday life.

2. Materials and method

2.1. Animal care

Young female Wistar rats aged 4 weeks were obtained from Central Laboratory Animal Resources (CLAR) at the Jawaharlal Nehru University (JNU). The animals were housed under standardized conditions and were acclimatised to lab environment and handling. Ambient temperatures (25°C) with relative humidity (40–50 %) were maintained. Animals were provided with food pellet and water ad libitum and their health and weight was monitored throughout the experiment. A 12-hour light-dark cycle was maintained. All experimental procedures were conducted in compliance with the guidelines set by the Institutional Animal Ethics Committee (IAEC, Code No. 09/2023) Jawaharlal Nehru University (JNU), New Delhi, and the Control and Supervision of Experiments on Animals (CCSEA).

2.2. Study design

This study utilized a controlled experimental design to investigate the impacts of 700 MHz 5 G radiation on the reproductive health of female Wistar rats. Rats were divided into three experimental groups namely: control (no exposure), sham-exposed (exposed to the experimental setup without actual radiation), and exposed (subjected to 700 MHz radiation). The control group remained in standard home cages under laboratory conditions throughout the study. The sham-exposed and exposed groups were also housed in home cages except during the daily exposure period. During the exposure period, both the sham-exposed and exposed rats were placed in plexiglass cages within specialized anechoic chambers. The exposed group received 700 MHz radiofrequency radiation (RFR) via a horn antenna inside the anechoic chamber. Two types of exposures were performed: short-term and long-term. In the short-term exposure (n = 6 per group), 75-day-old female rats (exposed group) were exposed to 700 MHz radiation for 6 hrs daily over 10 days. For long-term exposure (n = 8 per group), 45-day-old female rats were exposed to 700 MHz radiation for 4 hours daily over 60 days (Fig. 1). Estrous cyclicity was monitored throughout the experiment. Post completion of the exposure period of rats which was 10

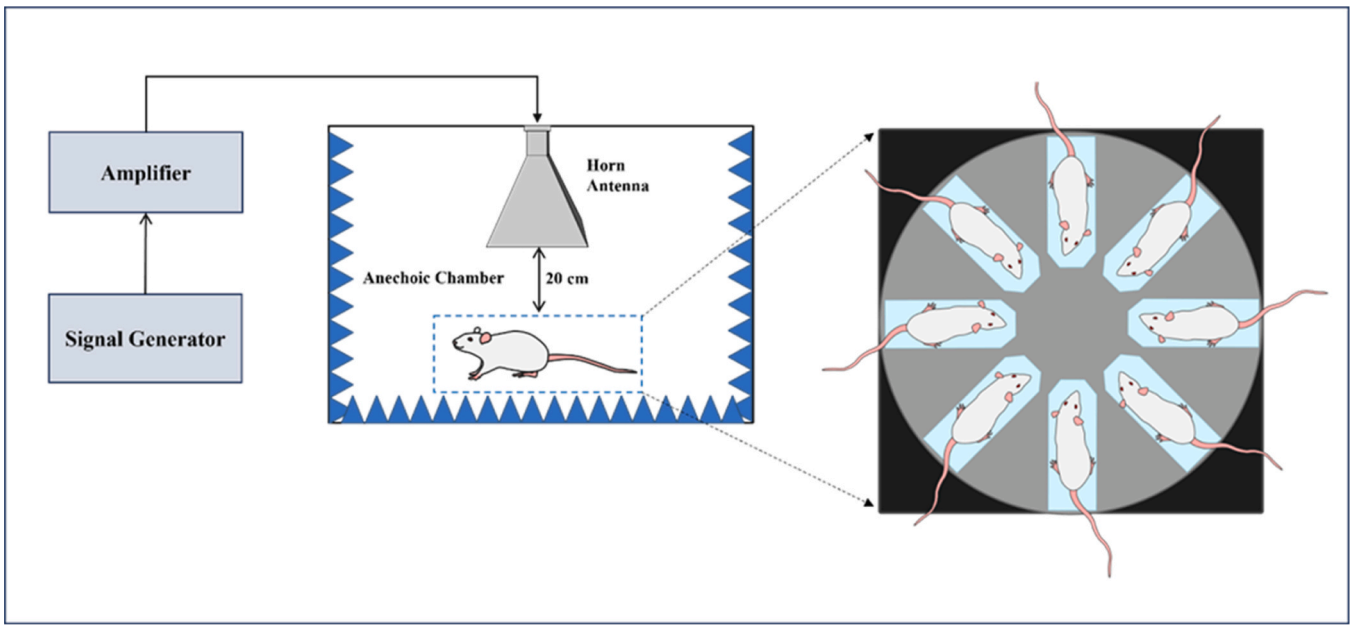


Fig. 2. Schematic representation of the exposure setup.

days for short-term and 60 days for long-term exposure, experimental endpoint was reached and rats were euthanized as per ethical guidelines and samples were collected for various biochemical, serological, and histopathological analysis as discussed in the subsequent sections.

2.3. Exposure set up

The desired frequency of 700 MHz was obtained by transmitting carrier signal with the help of signal generator (Aeroflex IFR 2023B). Experimental rats were place in plexiglass glass cage which was further kept in the anechoic chamber as previously described by Gautam et al., [11]. The power of the generated signal was amplified by amplifier (AR Model No 1S1G4) and was transmitted to pyramidal waveguide horn antenna (WR-1150) which radiated the signal to the plexiglass cage containing rats. The distance between the antenna and the plexiglass cage was 20 cm (Fig. 2). Power density inside the cages was measured with help of Narda measurement system (SRM-3006 Isotropic probe model G 0648). The animals were exposed to power density 0.7192 mW/cm$^{2.}$ Average whole body specific absorption rate (SAR) was calculated to be 0.375 W/Kg by Durney's method [9].

2.4. Estrous cycle monitoring

Post vaginal opening, estrous monitoring was done daily in the morning at the same time for all three experimental groups for both short-term and long-term exposures. The monitoring continued until the experimental endpoint of the exposures was reached. Rats were monitored for their estrous cycle with the help of non-invasive techniques such as visual identification vaginal cytology [10]. The vaginal lavage method was employed for cytological examination. Briefly, 100 μL of phosphate-buffered saline (PBS) was introduced into the vaginal opening of the rats using a blunt-tip pipette. The resulting vaginal smear was transferred onto glass slides and observed under a microscope to identify different cell types, such as nucleated epithelial cells, cornified anucleate epithelial cells, and leukocytes. The vaginal smear cells were analyzed both unstained and stained (using crystal violet) to confirm their cellular morphology and identification [1].

2.5. Sample collection

Post completion of the exposure period (10 days for short-term and 60 days for long-term), rats were euthanized immediately as per ethical protocol. Under the influence of anaesthesia (sodium thiopentone), cardiac blood was collected in centrifuge tubes. Post euthanising the rats, major organs such as brain, liver, kidney, spleen, ovaries, uterine horns were excised out and weighed post saline washing. In this study rat ovaries were the target organs and were used in subsequent biochemical assays and histopathological examinations. The left ovary was preserved for histopathological slides and the right ovary was homogenised in phosphate buffer saline (PBS) for various biochemical assays.

2.6. Reproductive steroidal hormones

The obtained blood was centrifuged for 15 min at 2200 g at 4 °C to obtain serum. The blood serum was stored at −20 °C till further analysis [2]. The collected serum was used to perform quantification of steroidal hormones namely estradiol, progesterone, and testosterone. These hormones were quantified by performing a delayed one-step immunoassay using a chemiluminescent microparticle immunoassay (ARCHITECT Chemiluminescent Microparticle Immunoassay). The assays were performed as per the standards of the manual. Testosterone levels were measured using ARCHITECT 2nd Generation Testosterone (2P13) with limit of detection 0.0144 ng/mL. Estradiol was quantified using ARCHITECT Estradiol (7K72) with a detection limit of 0.77 pg/mL, while progesterone level was determined using ARCHITECT Progesterone (7K77) with a detection limit of 0.1 ng/mL. The intra-assay coefficient of variation (CV) for all assays was $\leq$ 6 %, and the inter-assay CV was $\leq$ 10 %.

2.7. Histopathology of ovary

The excised ovaries were fixed in 10 % formalin to ensure tissue preservation. Following fixation, the tissues were embedded into paraffin blocks, and 5 μm thick sections were cut using a microtome. These sections were mounted onto glass slides and stained with Hematoxylin and Eosin (H&E) to highlight the cellular and structural details of the tissue. The stained samples were examined under a light microscope

Table 1
Body and organ weight(s) of short-term and long-term exposure. Data represented as mean ± SD. Where, a* indicates statistically significant difference compared to the control group and b* indicates a statistically significant difference compared to sham-exposed group (ANOVA, Tukey's multiple comparison tests).

Short- Term Exposure				Long-Term Exposure		
Body Weight (g)	**Control**	**Sham**	**Exposed**	**Control**	**Sham**	**Exposed**
Initial	207 ± 17.33	213.66 ± 20.02	213 ± 11.82	122.06 ± 6.46	123.25 ± 3.10	122.62 ± 6.09
Final	228.83 ± 13.65	228.5 ± 20.21	228.3 ± 6.10	232.83 ± 9.93	240.5 ± 10.17	231.3 ± 9.12
Organ Weight				**Organ Weight**		
Brain	1.806 ± 0.09	1.876 ± 0.11	1.805 ± 0.11	1.88 ± 0.17	1.9 ± 0.23	1.16 ± 0.22
Liver	9.205 ± 1.94	9.335 ± 0.723	8.881 ± 0.521	9.17 ± 1.2	9.24 ± 1.27	7.25 ± 0.45^{a**b**}
Spleen	1.035 ± 0.166	0.97 ± 0.20	1.001 ± 0.177	0.613 ± 0.123	0.59 ± 0.07	0.57 ± 0.14
Kidney	0.82 ± 0.1	0.92 ± 0.07	0.82 ± 0.19	0.85 ± 0.09	0.89 ± 0.08	0.77 ± .07^{b*}
Left Ovary	0.08 ± 0.005	0.07 ± 0.013	0.078 ± 0.009	0.08 ± 0.01	0.07 ± 0.01	0.06 ± 0.01^{a*}
Right Ovary	0.08 ± 0.005	0.08 ± 0.005	0.076 ± 0.005	0.07 ± 0.006	0.07 ± 0.009	0.07 ± 0.01

(LMI) at magnifications of 4x, 20x and 40x allowing for detailed histological assessment of the ovarian architecture. The ovarian analysis focused on evaluating the histological structure of the organ and quantifying the number of follicles at various stages of follicular development. For follicular quantification, assessment was conducted according to the methodologies outlined by de Melo et al., [7]. Briefly, ovaries were analyzed in multiple sections with different histological planes. The sections were cut and visualized in a manner that maintained a 35 μm distance between two consecutive visualized sections. This was achieved by leaving seven microtome ovarian slices between each observed section to avoid double counting of follicles since the average diameter of oocyte is 20–30 microns. On an average ten sections were analyzed per ovary for each rat in all the three groups for both short-term and long-term exposure. The obtained number of follicles were divided by number of sections analyzed to obtain an average number of follicles per section.

2.8. Biochemical assays for oxidative stress

The right ovary tissue homogenate was used to perform various biochemical assays to estimate oxidative stress in the ovarian samples from the three groups, for both short-term and long-term exposure. Briefly, the right ovary was thoroughly rinsed with ice-cold phosphate-buffered saline (PBS). The ovarian tissue was crushed and homogenized in ice-cold PBS to prepare a 10 % (w/v) homogenate using a tissue homogenizer. The obtained crude homogenate was then centrifuged to obtain the supernatant for the estimation of protein content, lipid peroxidation, ferric reducing antioxidant power (FRAP), total sulfhydryl (SH) content and Superoxide dismutase activity. Pardhiya et al., [25].

2.8.1. Lipid peroxidation

Malondialdehyde (MDA), an indicator of lipid peroxidation, was estimated following the method described by Uchiyama and Mihara [40]. Tissue homogenate was mixed with Tris-KCl buffer (pH 7.4), 30 % trichloroacetic acid (TCA), and 52 mM thiobarbituric acid (TBA). This mixture was vortexed and incubated in a water bath at 90°C for 60 minutes, followed by cooling to room temperature. TBA reactive substances (TBARS) were extracted by (Eppendorf Centrifuge 5418 R) centrifugation at 3000 × g for 10 minutes. The amount of TBARS was estimated by measuring the absorbance at 532 nm (UV-1800 SHIMADZU) and calculated as micromoles of MDA equivalents per milligram of protein.

2.8.2. Total antioxidant capacity by FRAP

The total antioxidant capacity (TAC) of the tissue homogenate was estimated using the Ferric Reducing Antioxidant Power (FRAP) method, as described by Benzie and Strain [3]. The assay solution was prepared by mixing 0.3 M acetate buffer (pH 3.6) with 10 mM TPTZ in 40 mM HCl in a 10:1 ratio. To this solution, 1 mL of $FeCl_3$ and 1.5 mL of FRAP reagent were added, followed by 50 μL of 10 % tissue homogenate. The reaction mixture was incubated at 37°C for 10 minutes, resulting in the formation of a blue-colored Fe^{2+}/TPTZ complex. The optical density of the solution was recorded at 593 nm. A standard curve of Fe^{2+} concentration was used to determine the FRAP values, which were expressed as mM Fe^{2+} equivalents per gram of tissue.

2.8.3. Total sulfhydryl content

The total sulfhydryl (SH) content was estimated using 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB). Tissue homogenates were mixed with DTNB reagent in phosphate buffer, resulting in the formation of a yellow-colored product. The absorbance of this solution was measured at 412 nm. The SH content was calculated using the molar extinction coefficient of the DTNB-SH adduct (13,600 $M^{-1}cm^{-1}$) as described by Canabady-Rochelle et al. [5].

2.8.4. SOD activity

Superoxide dismutase (SOD) activity was evaluated following the protocol of Rukmini et al. [29], with slight modifications. The reaction mixture was prepared by combining 0.067 M phosphate buffer (pH 7.8), 0.12 mM riboflavin, 1.5 mM nitro-blue tetrazolium (NBT), 0.01 M methionine, and the tissue sample. The reaction mixture was exposed to a fluorescent lamp for 20 minutes to generate radicals via riboflavin activation. After the incubation period, the absorbance of the reaction mixture was measured at 560 nm. SOD activity was calculated as the percentage inhibition of NBT reduction compared to the blank.

2.9. DNA damage by comet assay

DNA damage in isolated ovary cells was assessed using the alkaline Comet assay to detect single-strand breaks and alkali-labile sites, following the protocol of Tice et al. (2000) and Singh et al. [35,36]. Briefly, cell suspensions were embedded in low melting point agarose (LMPA) on pre-coated slides with a layer of normal melting point agarose (NMPA). An additional layer of LMPA was applied, and the slides were lysed overnight in ice-cold lysis buffer. DNA was unwound in an alkaline electrophoresis buffer for 30 minutes to denature double-stranded DNA and expose strand breaks. Electrophoresis was conducted at 25 V and 300 mA for 30 minutes. The slides were then neutralized, fixed with 100 % ethanol, and stained with 0.02 % ethidium bromide (EtBr). DNA damage was visualized under a fluorescence microscope (LMI) at 20 × magnification, and various parameters of DNA damage were analyzed using Comet Score software (TriTek Comet Score 2.0).

2.10. Statistical analysis

All experiments were conducted in triplicate for each experimental group (control, sham-exposed, and exposed) across both short-term and long-term exposure. For short-term exposure, samples from each group ($n = 6$), and for long-term exposure, samples from each group ($n = 8$)

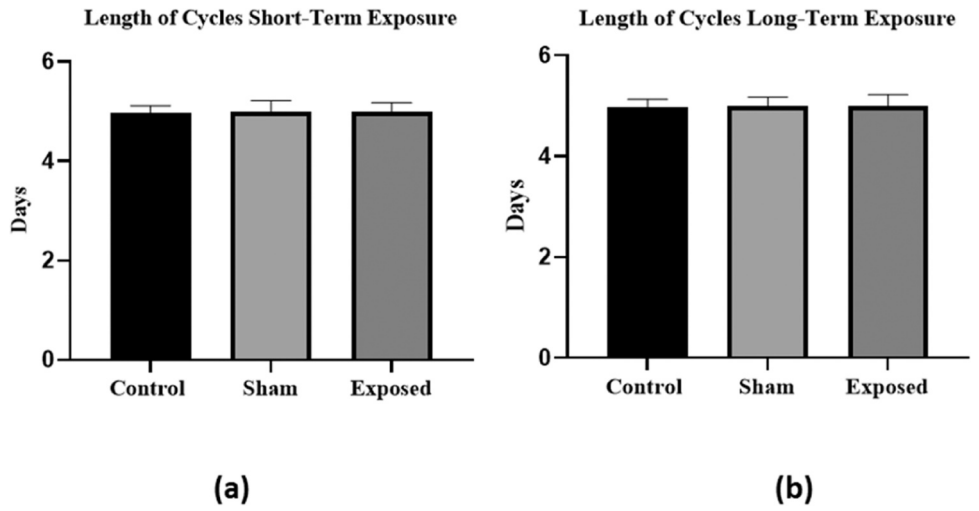


Fig. 3. Length of estrous cycle for the three groups in (a) short-term and (b) long-term radiation. Data represented as mean ± SD.

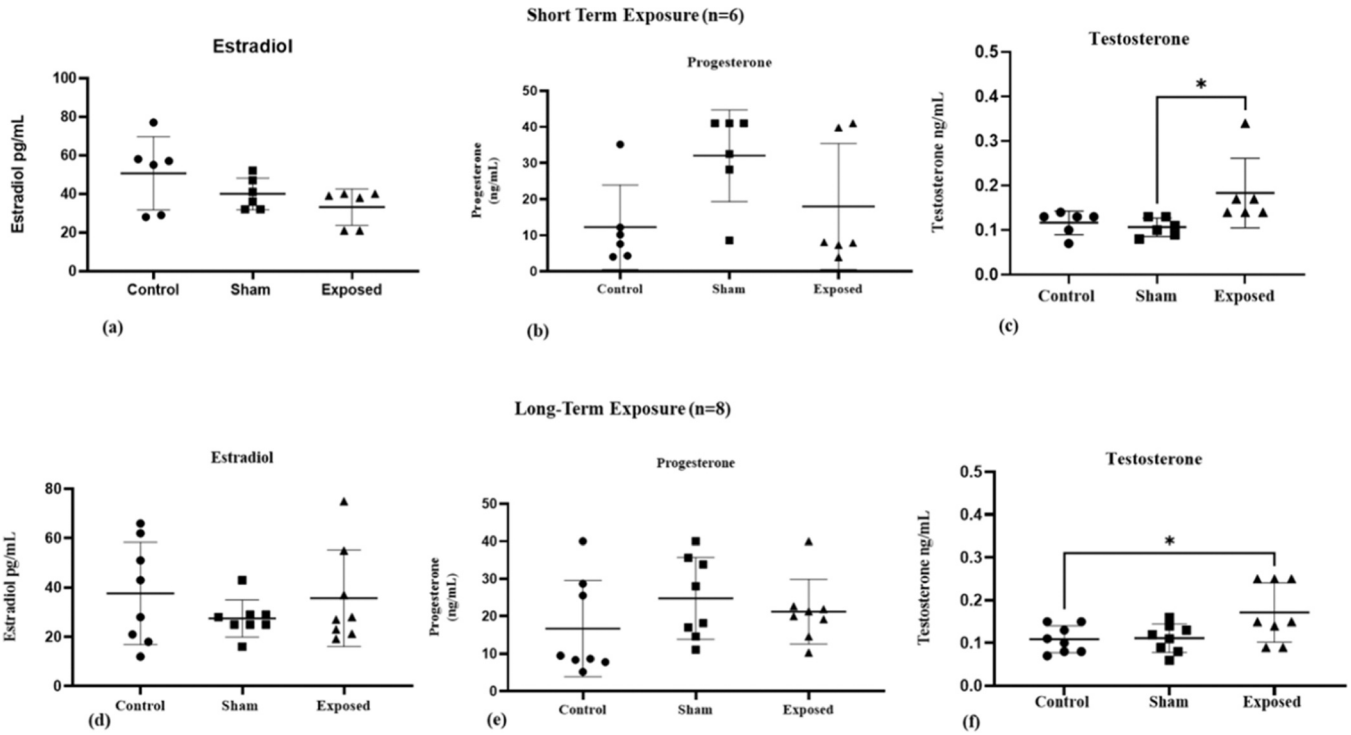


Fig. 4. Serum levels of estradiol, progesterone and testosterone level measured in control, sham and exposed group (a, b, c for short-term exposure and d, e and f for long-term exposure). Significant elevated level of testosterone observed in exposed group compared to control (for both exposures). Data represented as mean ± SD. Where, $*p < 0.05$ between control, sham exposed and exposed (ANOVA, Tukey's multiple comparison tests).

were analyzed. Data were presented as mean ± standard deviation (SD). Statistical analysis was performed using one-way and two-way analysis of variance (ANOVA). Post hoc comparisons were conducted using Tukey's and Sidak's tests with GraphPad Prism 9 software. Statistical significance was determined based on *p*-values, where $p < 0.05$ * is significant, $p < 0.01$ * *, and $p < 0.001$ * ** were considered highly significant.

3. Results

3.1. Body, ovarian, and vital organ weight

Throughout the experiment, no general signs of stress were observed among the three experimental groups for both short-term and long-term exposures. Body weight and organ weights recorded at the end of the experimental periods are presented in (Table 1). For short-term exposure no significant alterations in organ and body weight was observed, however for long-term exposure significant changes were observed in organ weight of liver and left ovary of exposed groups compared to control and sham.

3.2. Estrous cycle

The length of the estrous cycle remained unaffected in both short-term and long-term exposures, with no alterations observed among the three groups, as shown in Fig. 3. Rats in all three groups completed

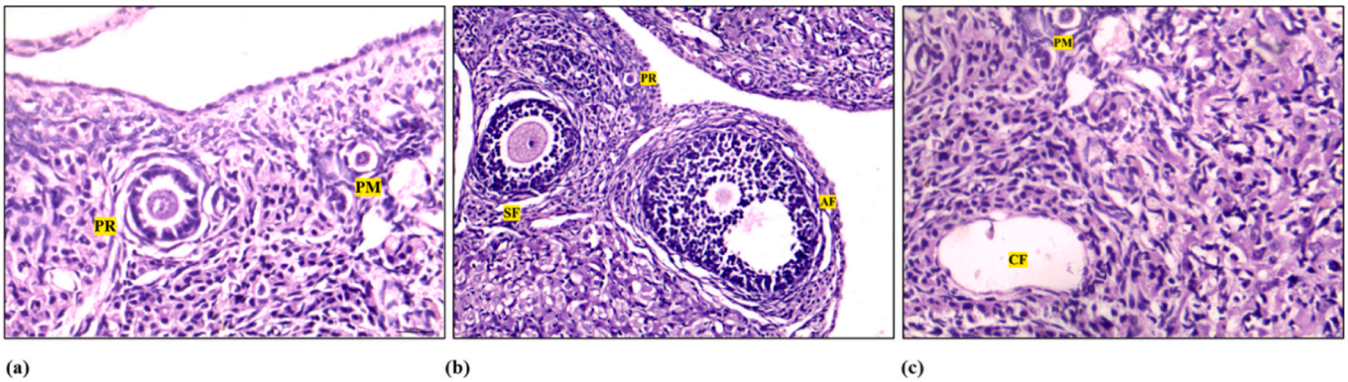


Fig. 5. Representative images of rat ovarian follicles at different stages: (a) primordial (PM) and primary (PR) follicles; (b) secondary (SF) and antral (AF) follicles; (c) cystic follicle (CF).

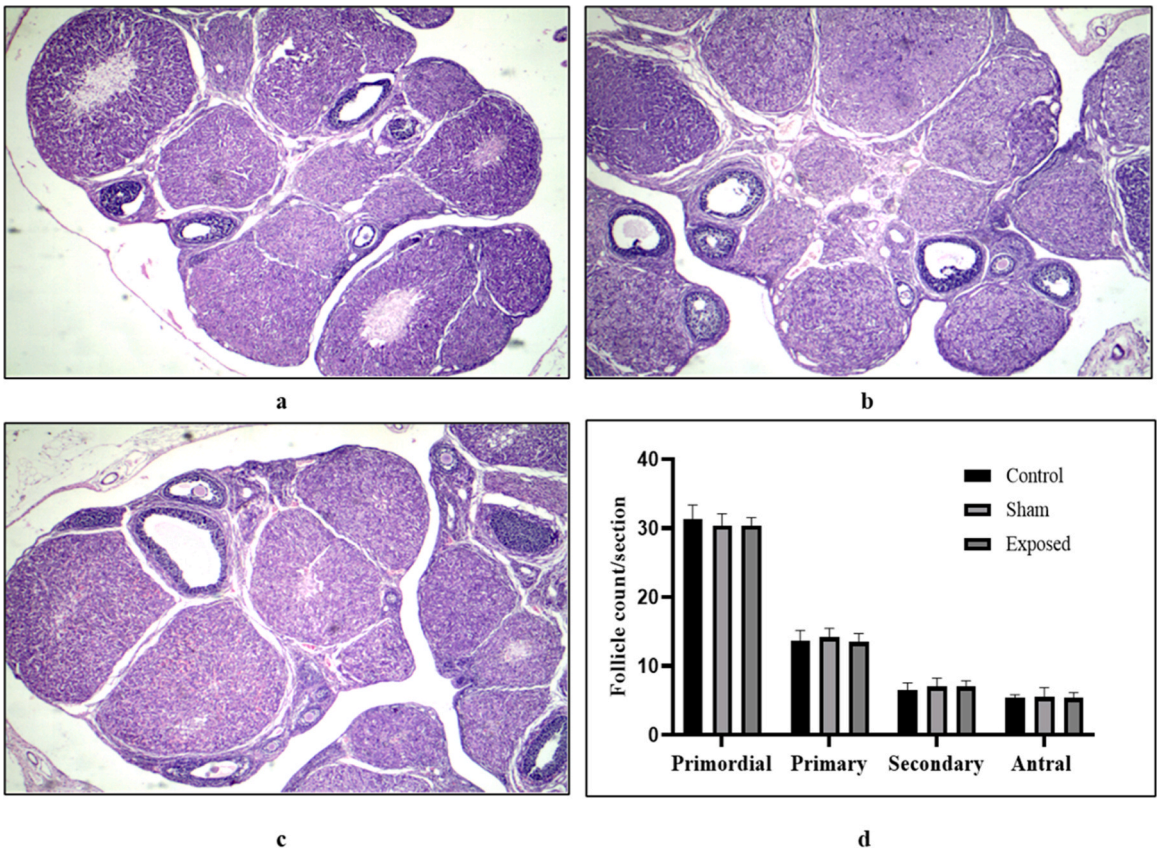


Fig. 6. Photomicrographs (4x magnification) showing Wistar ovary histology (a) control, (b) sham-exposed and (c) exposed group with no major differences observed in the three group for short-term exposure. (d) follicular count per ovarian section of control, sham and exposed for short-term term exposure (n = 6).

one estrous cycle within 4.8–5 days. Visual examination was confirmed by vaginal smear examination and different stages was identified based on presence of different kind of epithelial, cornified epithelial and leukocytes.

3.3. Steroidal hormonal assay

Serological levels of estradiol, progesterone, and testosterone were quantified among the three groups following the exposure period for both short-term and long-term exposures, as shown in (Fig. 4). The values of all hormones remained within the healthy range for rats. However, a significant elevation in testosterone levels was observed for both short-term and long-term exposures. In the short-term exposure group, a significant difference was noted between the exposed group and the sham group ($p = 0.0409$). For long-term exposure, a significant difference was observed between the exposed group and the control group ($p = 0.0422$).

3.4. Ovarian histopathology

The identified follicular stages of the rat ovarian system are depicted in (Fig. 5). The histoarchitecture of ovaries in the short-term exposure across all three experimental groups showed no significant differences, with normal ovarian morphology observed in all groups (Fig. 6). However, for the long-term exposure, certain abnormalities were identified in ovarian morphology, including cystic follicles and an abnormal blood

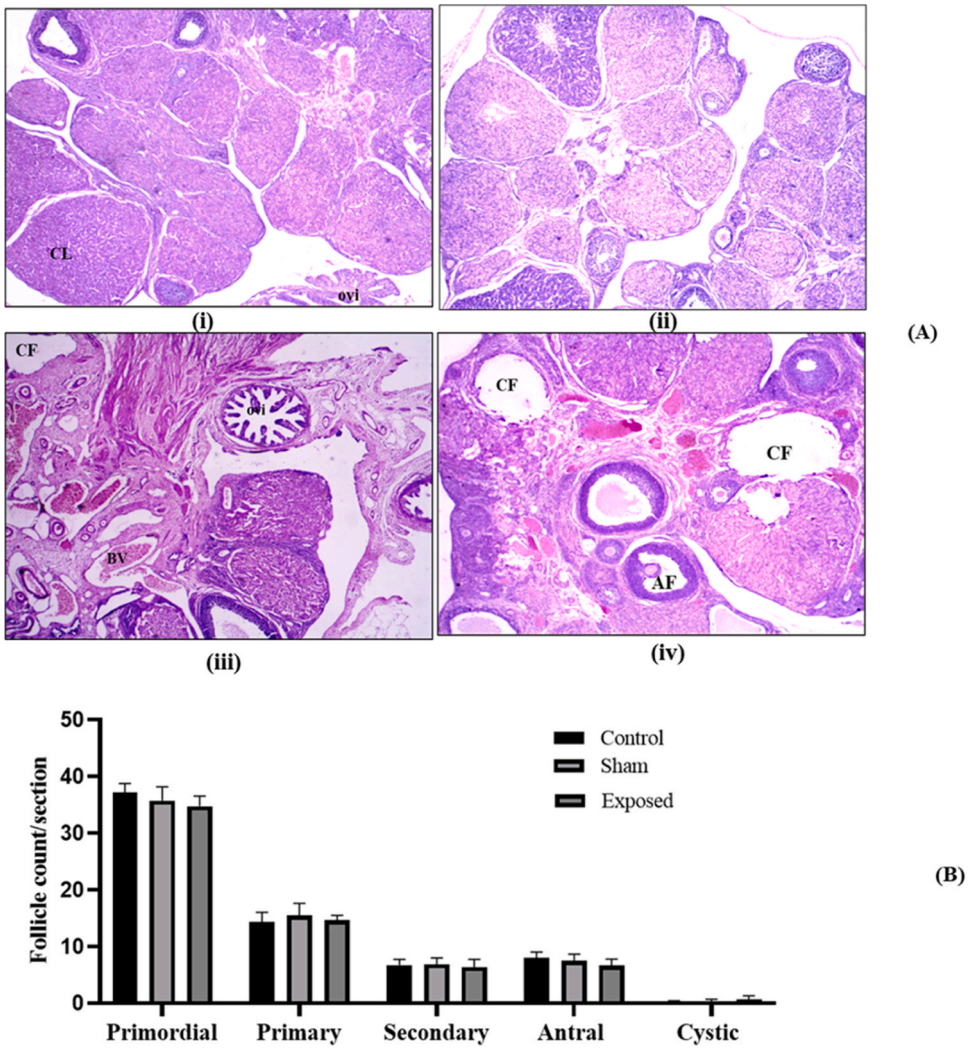


Fig. 7. (A) H&E-stained ovarian sections at 20x magnification showing (a) the control group and (b) the sham-exposed group, both exhibiting normal histo-architecture, with normal blood vascularity (BV) and various stages of ovarian follicle development, along with clear corpora lutea (CL). (c) and (d) show the exposed group, with abnormal blood vascularity (BV) and the presence of cystic follicles (CF). (B) Follicular counts per ovarian section of control, sham, and long-term exposed groups (n = 8).

vascular system. Despite these observations, the quantification of follicles in the long-term exposure did not reveal any significant differences among the three groups (Fig. 7).

3.5. Oxidative stress assay

For oxidative stress parameters significant results were obtained in both short-term and long-term exposure which corroborates with the previously reported studies from literature where RFR is shown to induce oxidative damage by the generation of oxidative free radicals and subsequent induction of oxidative stress [12]. In lipid peroxidation assay, the MDA level obtained in the ovarian of the exposed group was significantly higher than the control and sham-exposed group. The antioxidant enzyme SOD levels, total Sulfhydryl level and total antioxidant capacity measure via FRAP assay were also found to be significantly decreased in exposed group as compared to sham exposed and control groups (Fig. 8).

3.6. Comet assay

For both short-term and long-term exposure to 700 MHz, results from the comet assay showed no significant increase in comet length in ovarian samples from the exposed group compared to the control and sham groups. Similarly, the tail DNA % and olive tail moment did not exhibit notable changes in the exposed group relative to the control and sham-exposed groups (Fig. 9). The results of comet are indicative of non-damage of DNA in both short term and long-term exposure. These findings suggest that 700 MHz radiation does not induce significant genotoxic effects in ovarian cells, thereby posing minimal risk to genetic integrity. However, it is worth noting that the number of comets observed in the exposed group was visibly higher (Figure A), warranting further investigation into potential subtle or cumulative effects.

4. Discussion

Health impacts of RFR are widely debated and over the years many research studies performed with different frequencies have corroborated with the negative impacts caused by them. According to Sanyal and Behari [30], the effects of RF radiation on biological systems are influenced by several factors, such as the SAR value, radiation frequency, power, exposure duration, and other environmental conditions. According to the National Research Council [24], the transition from basic physical interactions involving radiofrequency radiation (RFR) with molecules or ions to biological changes, which may lead to potential

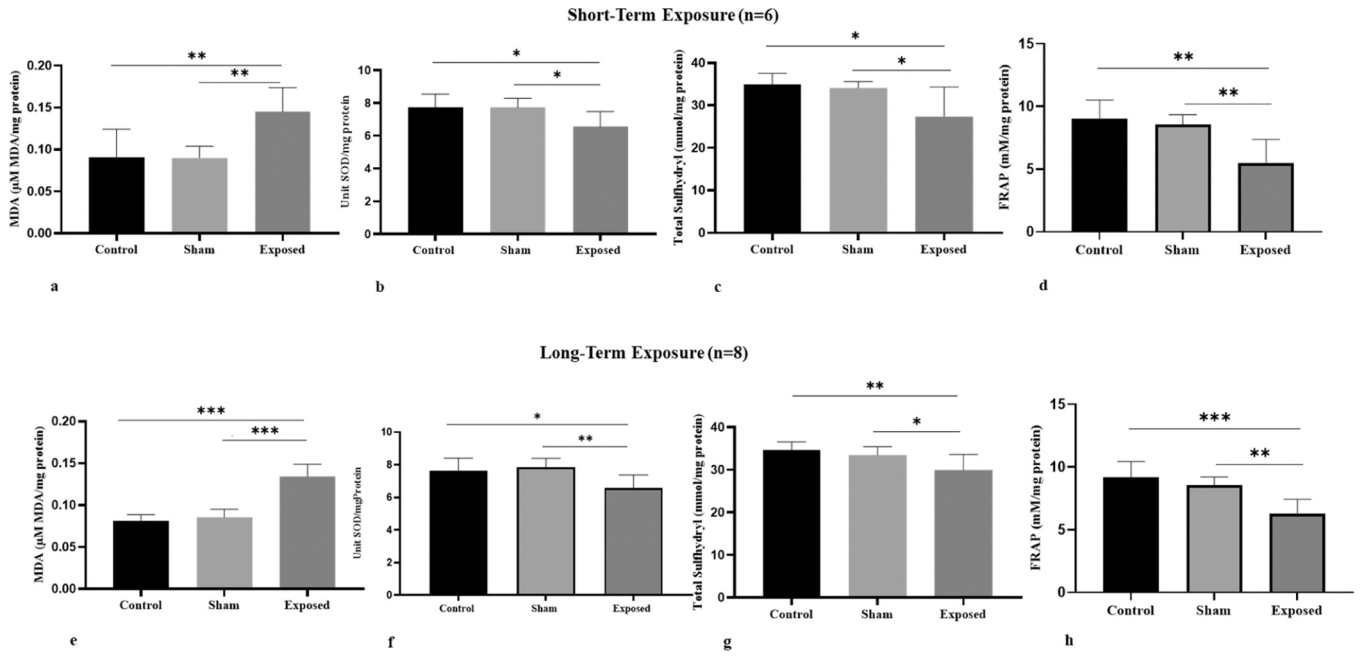


Fig. 8. Different oxidative assays performed for the three groups. Significant difference in oxidative parameters i.e., Lipid Peroxidation, SOD, Total Thiol and FRAP observed (a-d for short-term exposure and e-h for long-term exposure). Data represented are mean ± SD, n = 6 (for short-term exposure) and n = 8 (for long-term exposure) where, $*p < 0.05$, * $*p < 0.01$ and * $**p < 0.001$ between control, sham exposed and exposed (ANOVA, Tukey's multiple comparison tests).

health effects, involves a complex sequence of events. These interactions may modulate chemical reaction rates and influence molecular attachment to cellular membranes, potentially altering cellular function. Such modifications at the cellular level may subsequently affect the physiological processes of the organism. RFR-induced biological effects can be mediated by either thermal or non-thermal interactions [6]. According to the International Commission on Non-Ionizing Radiation Protection [16], thermal effects become more pronounced when the temperature increase exceeds 1°C. For the general public, thermal impacts due to mobile phone radiation and RFR are minimal due to strict thermal regulations enforced worldwide. These regulations establish exposure limits for electromagnetic radiation, including wireless communication technologies, to ensure that radiation levels remain below thresholds known to cause significant thermal effects that could negatively impact human health [17]. Therefore, non-thermal impacts of RFR and its biological implications become important to study. Our study was based on the hypothesis that the non-thermal effects of RFR disrupt the reproductive functioning of the mammalian female reproductive system. We assessed impacts of 700 MHz on ovarian health, hormonal changes, oxidative stress, and estrous cycle regularity. The rationale for selecting 700 MHz was based on the fact that it is widely prevalent in India as part of the lower 5 G band and has a broader reach.

The findings revealed some interesting insights into how prolonged exposure to 700 MHz radiation may affect the reproductive health of female rats, but also highlighted the lack of significant changes in certain parameters, similarly a study found no significant differences between male Wistar rats of different ages (juvenile, adult, and pre-senile) exposed to 2.4 GHz RF-EMF from a simulated 5 G base station and control animals. Parameters such as body weight, temperature, and performance in the Morris water maze were unaffected by the exposure [21]. Lipid peroxidation significantly increased in both short-term and long-term exposure groups. Antioxidants like the enzyme SOD, sulfhydryl group and total antioxidant capacity significantly decreased in the exposure groups. This is indicative of oxidative stress induced by both the acute as well as chronic exposures in the ovary. Oxidative stress and reactive oxygen species (ROS) formation are natural processes that occur during physiological activities such as phagocytosis and mitochondrial function. However, if ROS production is not regulated by antioxidants, it can lead to oxidative damage in lipids, nucleic acids, and proteins within reproductive cells. Radiofrequency radiation (RFR) exposure is known to increase ROS production, particularly when water the primary component of cells is subjected to RFR, triggering ROS generation through multiple pathways [22]. Although cells possess enzymatic and non-enzymatic antioxidant defenses, excessive oxidative stress induced by electromagnetic radiation (EMR) can surpass these protective mechanisms, depleting antioxidants and ultimately resulting in cellular damage [19,23].

According to Shahin et al. [34] microwave-induced oxidative stress may lead to DNA strand breakage in the brain cells and implantation failure and reproductive abnormalities. One potential mechanism by which radiofrequency radiation (RFR) may impact cells and tissues independent of heat-related effects is through the generation of reactive oxygen species (ROS). ROS include molecules such as superoxide ($\bullet O^{2-}$), hydroxyl radicals ($\bullet OH$), hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$), and various organic compounds. While ROS are naturally produced during normal cellular processes and play essential roles in cellular signaling, elevated ROS levels can disrupt cellular homeostasis, damage cellular structures, and impair function, ultimately leading to oxidative stress [31]. To mitigate this, organisms rely on a balance maintained by antioxidants and enzymatic defense systems [32]. Key enzymes involved in ROS regulation include superoxide dismutase (SOD), catalase, and glutathione peroxidase. SOD neutralizes superoxide radicals, while catalase and glutathione peroxidase break down hydrogen peroxide and hydroperoxides into harmless byproducts like water, oxygen, and alcohol [15]. However, RFR exposure has been shown to induce ROS production and impair the function of antioxidant enzymes such as SOD, catalase, and glutathione peroxidase, leading to oxidative stress [25]. This oxidative imbalance, combined with RFR-induced disruptions in ion channel function, activates cellular receptors and signaling pathways. These pathways can stimulate secondary messenger systems, influencing processes such as gene expression, peptide synthesis, protein production, and enzyme activity, all of which are critical for protein function. Altered protein levels due to RFR exposure can affect vital cellular processes, including cell replication, cell cycle regulation, and apoptosis. Such disruptions can cause damage to key biomolecules, including lipids, DNA, and proteins, which may

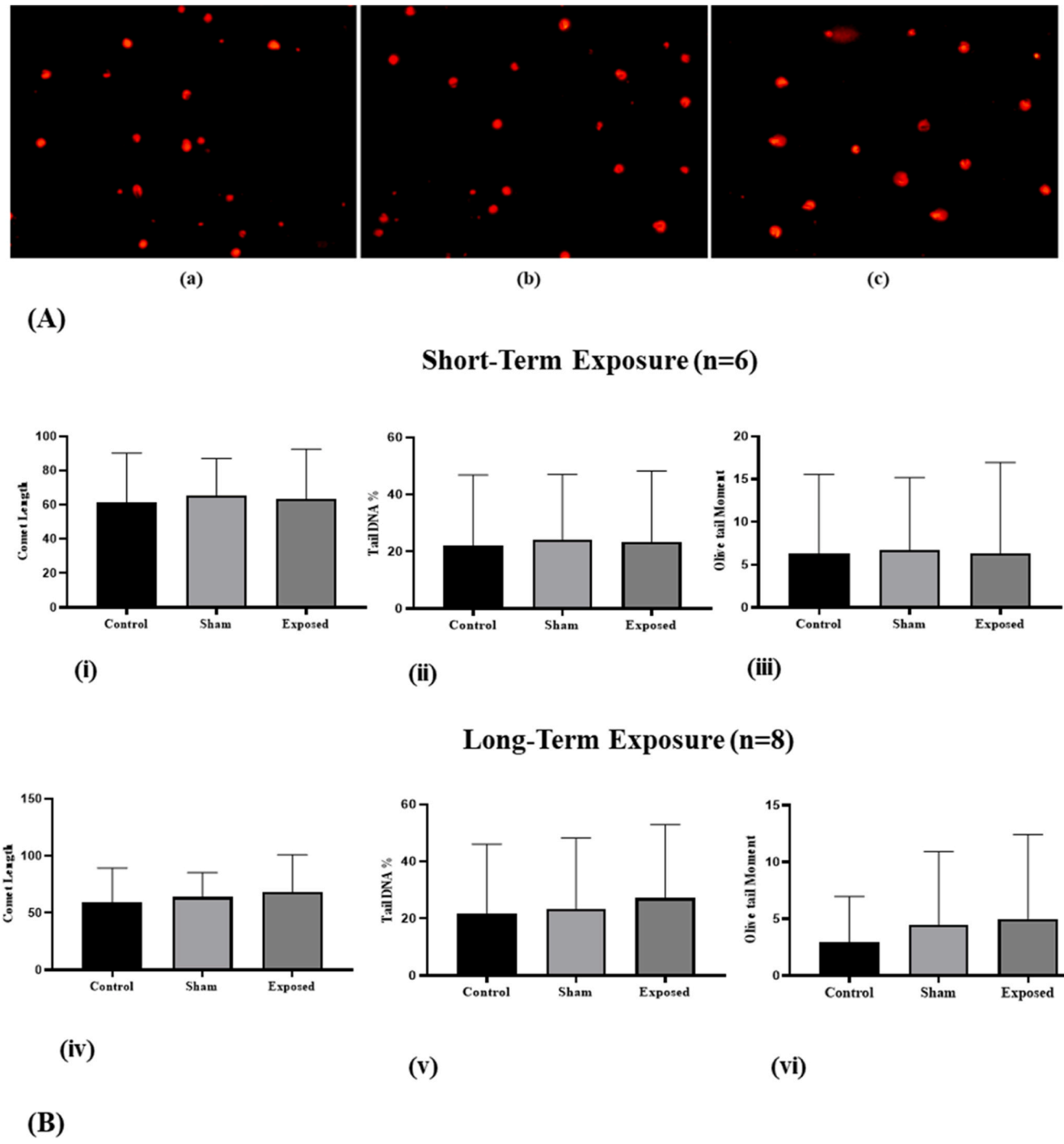


Fig. 9. (A) Representative images of Control (a), Sham (b) and Exposed group (c) to evaluate DNA damage obtained from fluorescence microscope analysis (20X). Whole cell heads without any comet pattern were considered undamaged. (B) Graphs showing analysis of different parameters obtained from the comet assay with no major difference obtained among the three groups: (i- iii) for short-term and (iv- vi) for long-term exposure.

trigger programmed cell death (apoptosis). Furthermore, studies have uncovered variations in the activity of ornithine decarboxylase (ODC) and calcium-dependent protein kinase subsequent to exposure to RFR [27,26].

In our study the estrous cycle remained unaffected by 700 MHz radiation exposure, serum testosterone levels showed a slight but significant increase in the exposed groups. Testosterone is a critical hormone for both reproductive and metabolic functions in females, and while the observed increase is within the normal range of rats, it still potential influence of RFR on female system. Elevated androgen levels have been associated with ovarian dysfunction in some contexts [13]. However, the absence of major changes in estrous cyclicity indicates that the hormonal changes did not significantly impact the overall reproductive cycle within the study period. Histopathological examination of ovarian tissue revealed subtle changes in the long-term exposure group, while no significant alterations were noted in the short-term exposure group. The long-term exposure group exhibited changes in ovarian architecture, possibly indicating the cumulative effects of prolonged radiation exposure. In a study, mice exposed to 1800 MHz mobile phone radiation exhibited significant alterations in the histoarchitecture of ovarian and

uterine morphology. The ovaries of irradiated mice showed a reduced number of developing and mature follicles, fewer corpus lutea, and an increased number of atretic or degenerative follicles. Based on the findings it was deduced that mobile phone radiation may lead to ovarian and uterine dysfunction by increasing reactive oxygen species (ROS) and reactive nitrogen species (RNS) production, thereby disrupting antioxidant balance. The oxidative and nitrosative stress generated at both the hypothalamic and peripheral levels (ovary and uterus) as a consequence of long-term mobile phone exposure may severely impair reproductive function [33]. In our study, the subtle changes observed in the ovarian histoarchitecture of exposed group of long-term exposure suggest that the ovaries may be more susceptible to radiation-induced damage over extended periods, which could have implications for fertility and reproductive health. However, the lack of major histopathological changes in the short-term exposure group indicates that the immediate effects of 700 MHz radiation may be less pronounced. The comet assay, used to assess DNA damage in the ovarian cells, did not show any significant changes in DNA integrity between the exposed and control groups. This lack of a significant increase in comet scoring suggests that while oxidative stress was elevated, the radiation exposure did not result in detectable DNA damage in ovarian cells within the time frame of the study. This could imply that the oxidative damage caused by the 700 MHz radiation may not be severe enough to cause direct DNA strand breaks, or that the DNA repair mechanisms in ovarian cells may be sufficient to mitigate the damage.

5. Conclusion

Based on the findings, exposure to 700 MHz radiation did not affect the estrous cycle in either short-term or long-term exposure. Serological hormone levels, including estradiol, progesterone, and testosterone, remained within the healthy range for rats, although a slight but significant increase in testosterone levels was observed. Ovarian homogenates from both short-term and long-term exposure groups showed evidence of oxidative stress, as indicated by significant changes in oxidative stress markers such as superoxide dismutase (SOD), total sulfhydryl levels, ferric reducing antioxidant power (FRAP), and elevated malondialdehyde (MDA). These findings align with studies linking oxidative stress to such radiation exposure. Histopathological analysis revealed that while no major significant changes were observed in ovarian tissues following short-term exposure, long-term exposure showed certain alterations. These results suggest that 700 MHz radiation may induce oxidative stress and tissue changes in ovaries over prolonged exposure periods, emphasizing the importance of further investigation into the long-term effects of such radiation on reproductive health.

CRediT authorship contribution statement

Jha Neha: Writing – original draft, Visualization, Methodology, Investigation, Data curation, Conceptualization. **Rajamani Paulraj:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Project administration, Formal analysis, Conceptualization. **Tomar Anuj Kumar:** Writing – review & editing, Software, Methodology, Visualization. **Sarsaiya Priyanka:** Writing – review & editing, Software, Methodology, Visualization. **Nirala Jay Prakash:** Resources. **Pardhiya Sonali:** Writing – review & editing. **Gupta Surabhi:** Writing – review & editing, Validation, Supervision, Formal analysis. **Chaturvedi P K:** Supervision, Resources.

Ethics approval statement

The animals and study protocol were approved by the Institutional Animal Ethics Committee (IAEC, Code no 09/2023) and maintained as per Control and Supervision of Experiments on Animals (CCSEA) guidelines.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

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