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EXPERIMENTAL STUDY

Modulatory effect of 900 MHz radiation on biochemical and reproductive parameters in rats

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ABSTRACT

In the present study, the effect of 900 MHz radiation exposure on blood biochemical and reproductive parameters was evaluated in adolescent rats. Male albino Wistar rats (8–10 weeks of age) were exposed to 900 MHz radiation (1hr/day, power density – 146.60 μW/cm^2) from a mobile phone for 28 days. On 29th day the animals were euthanized and malondialdehyde (MDA), total antioxidants (TA) levels and Glutathione-S transferase (GST) activity were studied in the blood. Reproductive parameters such as total sperm count, percentage of non-motile sperms, and sperm morphology were determined. Testes sections were stained with H&E staining and their cellular integrity was evaluated. Caspase-3 activity in the testes was also determined. MDA concentration was increased but TA levels and GST activity were not found to be different in 900 MHz group compared to controls. Sperm motility was found to be slightly reduced in 900 MHz group. Percentage of abnormal sperm was significantly elevated in 900 MHz group. Additionally, loss of germ cells particularly spermatocytes and spermatids was found in the testes of 900 MHz group. Testes caspase-3 activity was slightly elevated in 900 MHz exposed rats. Chronic 900 MHz exposure induced oxidative damage in the blood and lead to alterations in reproductive parameters in rats *(Fig. 4, Ref. 33).* Text in PDF *www.elis.sk.*

KEY WORDS: radiofrequency electromagnetic radiation, adolescent, mobile phone, reproduction, caspase-3.

Introduction

Radiofrequency electromagnetic radiation (RF-EMR) emitted by man-made sources are ubiquitous and swiftly increasing day by day in the developing world. The frequency band used for transmitting data varies from country to country. Global System for Mobile Communications (GSM) mobile phones employs 900/1800 MHz frequency bands for transmitting data. The 900 MHz band is utilized extensively even today by most of the mobile phone service providers in many countries (1). IARC recently classified RF-EMR emitting from mobile phones to be potentially carcinogenic (type-IIB) to humans (2) and this is a matter of concern for the general public. While the biological effects (brain tumors, glioma) RF-EMR may cause are not categorically established, WHO adviced the public to decrease the long-term continuous exposure to RF-EMR as there is a lack of data on long-term exposure (1). The council also urges various nations to conduct more research on this less explored problem as the mobile phone users are increasing over the globe exponentially.

The interaction of RF-EMR with various biological systems has been evaluated in various model organisms. A report demonstrates that intermittent exposure to RF-EMR (900MHz and 1800 MHz) 6 min/day for 5 days decreased reproductive potential and altered actin – cytoskeleton of the egg chambers of *Drosophila melanogaster.* The authors suggest that the observed effect might be due to DNA fragmentation (3). Reports indicate that electromagnetic fields can stress living cells. Blank & Goodman (4) have reported that electromagnetic fields (both extremely low frequency and radio frequency) induce cellular stress response mechanisms. The effect is differential as low energy fields directly interact with DNA to activate cellular stress response wherein the increasing electromagnetic field energy (radiofrequency range) lead to DNA strand breaks.

RF-EMR effects on blood and other tissues have been studied by researchers. Oktem et al (5) have reported that 900 MHz radiations induced renal impairments as demonstrated by an increase in tissue melondialdehide (MDA), urine N-Acetyl-Beta-D-Glucosaminidase (NAG) levels, decreased superoxide dismutase (SOD),

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catalase (CAT), and glutathione peroxidase (GSH-Px) activities but melatonin treatment ameliorated oxidative tissue injury in rat kidney. Additionally, 900 MHz pulse-modulated radiofrequency fields (SAR; 1.20 W/kg 20 min/day) for three weeks significantly increased the MDA and nitric oxide (NOx) levels in liver, lung, testis and heart tissues of the RF-EMR exposed rats compared to sham and control rats. Conversely, GSH levels were significantly reduced in exposed rat tissues (6).

Modulatory role of RF-EMR on reproductive capacity of an organism is a rising concern. Over a period of three decades, a substantial amount of reports have been published on this area but controversies remain in many of these reports. Tas et al (7) demonstrated that, 900 MHz radiation 3 h per day (7 d a week) for one year did not induce any change in sperm motility and concentration in exposed group but the morphologically normal spermatozoa counts were found higher in the exposure group compared to others. Nevertheless, tunica albuginea thickness and Johnsen testicular biopsy score were found decreased in the exposed group. In the meantime, Karaman et al (8) have found the presence of high number of immature cells in the lumen of the seminiferous tubule, reduction in the spermatogenic cell lines and tubules without lumen in rats exposed to RF-EMR on talk mode for 8 hours per day for 20 days. A study conducted by exposing rats to a mobile phone (1 h/day for 45 days) revealed no change in the architecture of seminiferous tubules and various stages of spermatogenesis cycle (9). Dasdag et al (10) have reported that when rats were exposed to cellular phones (20 min per day) for 1 month with average SAR of 0.52 W/kg (whole body), the testicular function or structure was not affected. The controversies in these reports warrant the need for further extensive research on this topic. During the adolescent period a variety of maturational processes take place in mammals. One of them is the sexual development and maturation. Mammalian spermatogenesis is an intricate process and is well regulated by several hormones but is influenced by environmental factors as well. Spermatogenesis occurs in the seminiferous tubules and the unique architecture of the seminiferous tubules renders a self-reliant environment providing all essential nutrients needed by a developing sperm. Whether exposure to RF-EMR during adolescent period affects blood biochemical indexes and reproductive parameters was evaluated in the present study.

Materials and methods

Animals & maintenance

Male albino Wistar rats (8–10 weeks of age; 120–150g) were obtained from Central Animal Research Facility (CARF), Manipal University, Manipal. Three rats were housed per each polypropylene cage (41 cm × 28 cm × 14 cm). They were kept in 12:12 hr L:D environment, in a temperature controlled room (23 ± 2 °C). Rats were given *ad libitum* access to food and water. All precautions were taken to use only the minimum required number of animals to generate significant data. All procedures used in the study were reviewed and ratified by the Institutional Animal Ethics Committee (IAEC).

Experimental groups

Animals were allocated into different groups (6/group) randomly. Group I (Control): They were kept undisturbed in the home cage for 28 days. Group II (Sham exposed): these rats were exposed to a GSM mobile phone (switch off mode, without battery) (1 hr/day) for 28 days. Group III (RF-EMR exposed): Animals of this group were exposed to 900 MHz radiation (Power density; 146.60 μW/cm^2) from a mobile phone (kept in silent mode without ring tone) for 1hr/day for 28 days. 48 hours after the last RF-EMR exposure all animals were euthanized and various parameters were studied.

Chronic 900 MHz radiation exposure and dosimetry

Mode of RF-EMR exposure and dose were replicated in the current study based on our earlier reports (11). Briefly, to expose the rats with chronic RF-EMR, a GSM mobile phone (2 W, SAR; 1.15 W/kg) operating in the 900 MHz band was used. During the exposure period it was kept in a wire mesh cage (made out of bamboo; 12 cm × 7 cm × 7 cm) in the center of the home cage. During the exposure period the phone was continuously activated (50 times/hr) by giving unattended calls. This was done by an auto dialer unit (indigenously made), which could dial four phones at a time. Phones kept in the cages had the same SAR specification and were purchased from the same manufacturer. The peak power density at 3 cm area near the cell phone was recorded by a spectrum analyzer (SPECTRAN HF-2025E, Aaronia AG, Germany). With the help of MCS Real-Time Spectrum Analyzer Software, (Aaronia AG, Germany) real time readings were recorded and it was analyzed for quantifying the peak power density to which the animals were exposed. The peak power density obtained was 146.60 μW/cm^2 in the vicinity of the phone (11).

Procedures

Determination of ROS, levels of TA and levels of GST

Rats were euthanized by cervical dislocation. Through direct cardiac puncture, blood was collected and various assays were performed. To determine the serum MDA levels Satoh's method (12) was followed. The method described by Koracevic et al (13) was used to determine total antioxidant levels and Beutler's (14) method was followed to determine Glutathione S-transferase activity in the serum.

Determination of epididymis weight

Epididymes were collected aseptically and weight of right and left epididymis was recorded separately using an electronic balance.

Total sperm count and calculation

Total spermatozoa count was performed using Neubauer counting chamber as per the earlier reports and the data was expressed in million/ml (15).

Sperm motility

Motility of the spermatozoa was assessed (motile and non-motile) using a light microscope at a magnification of 400×. A minimum of 200 spermatozoa were assessed and repeated again and the average value was taken.

Sperm morphology

Sperm suspension was mixed with 1 % aqueous eosin for 30 minutes. Following this, smears were made and coded for blind analysis. One thousand sperms were analyzed in each animal and they were classified into two broad categories such as normal and abnormal (both head and tail abnormality). The data was presented in percentage (16).

Testes cellular architecture (Hematoxylin & Eosin staining) and qualitative analysis of the sections

Paraffin embedded sections (6 µm thickness) were stained by H&E as reported earlier (17) and were evaluated qualitatively using a light microscope by an expert anatomist blinded to the experiments. Loss of normal histological cytoarchitecture, loss of spermatogonia, presence of vacuoles in the seminiferous tubules, tubules without lumen and sertoli cell damage were evaluated in all the groups. Images of these regions were captured by Olympus BX43 microscope attached with DP 21 microscope digital camera (Japan). These were further processed by CellSense software for various measurements.

Caspase-3 activity in the testes

The Caspase-3/CCPP32 Colorimetric Assay Kit (Biovision, USA) was used for assaying caspase-3 activity in the testis as reported earlier and the activity was expressed as U/µg of tissue protein (18).

Statistical analysis

The data is expressed as Mean ± S.E. One way analysis of variance test (ANOVA) followed by Tukey's post hoc test was done to determine statistical difference between various experimental groups. $p < 0.05$ was considered as statistically significant. GraphPad Prism statistical package (version 5.01) was used for data analysis.

Results

RF-EMR exposure on blood biochemical indexes

In comparison to controls, serum TBARS levels expressed as malondialdehyde (MDA) (nmol/L) was elevated in RF-EMR group (Fig. 1A) (Control/Sham exposed vs RF-EMR exposed; *** $p < 0.001$, δδδ $p < 0.001$; One way ANOVA and Tukey's tests). Total antioxidants (TA) expressed as uric acid equivalent (µmol/L) in all groups were not significantly different from each other (Fig. 1B). In comparison to controls, serum Glutathione-S transferase (GST) activity (expressed as IU) was not found to be significantly different in RF-EMR group (Fig. 1C).

RF-EMR effects on reproductive parameters

Epididymis weight, total sperm count, sperm motility, and sperm morphology

Both right and left epididymis weight in the RF-EMR group was found to be comparable with controls. One-way ANOVA test did not show any significant epididymis weight difference between various groups studied (Fig. 2A, B, C). RF-EMR exposure for a month did not affect the total epididymal sperm count (Fig. 2D). However, percentage of motile sperm was decreased in RF-EMR exposed group compared to others (Fig. 2E). Additionally, non-motile sperm was increased in the same group compared to controls but these differences were not found to be statistically significant. Percentage of abnormal sperm was observed to be significantly higher in RF-EMR exposed group when compared to controls (Fig. 2F, G3).

Testes cellular architecture

H&E stained testes sections of control and sham groups' displayed normal cytoarchitecture of seminiferous tubules, which consisted of various stages of germ cells (Fig. 3D). As depicted in Figure 3A and D (red arrow) many cuboidal spermatogonia cells with rounded nuclei were seen resting on the basement membrane. Healthy sertoli cells (large epithelial cells) were also seen in control and sham exposed groups (green arrow). Layers of normal spermatocytes (adjacent to the spermatogonia) are also found with control group (blue arrow in Figure 3D). In contrast to all these, the testes sections of test group showed some histopathological changes such as loss of germ cells particularly spermatocytes and spermatids associated with absence of spermatozoa (yellow arrow head in Figure 3F). In addition to that, exfoliation of spermatogonia and Sertoli cell damage was observed (red arrow head in Figure 3F). Few seminiferous tubules displayed hyaline mate-

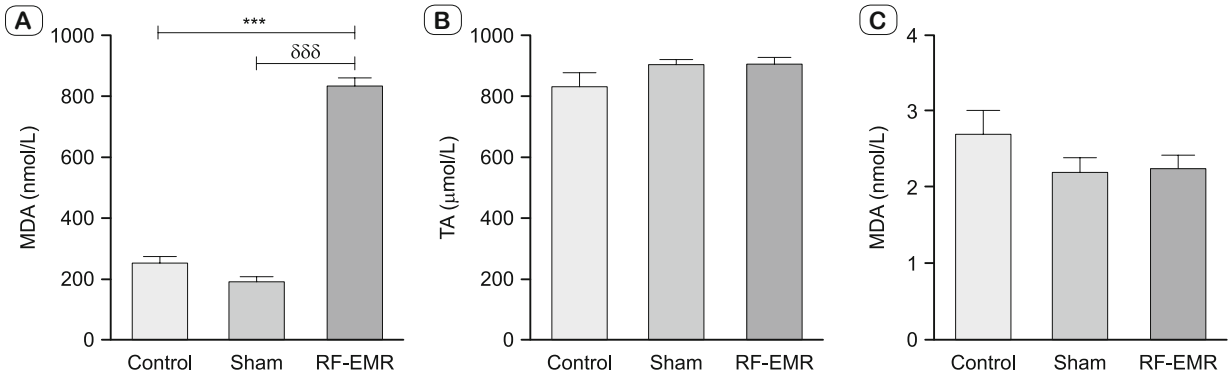

Fig. 1. RF-EMR effects on levels of thiobarbituric acid reactive substances (A), total antioxidants (B), and Glutathione S-transferase activity (C) in the blood (* $p < 0.001$, $^{\delta\delta\delta}$ $p < 0.001$).**

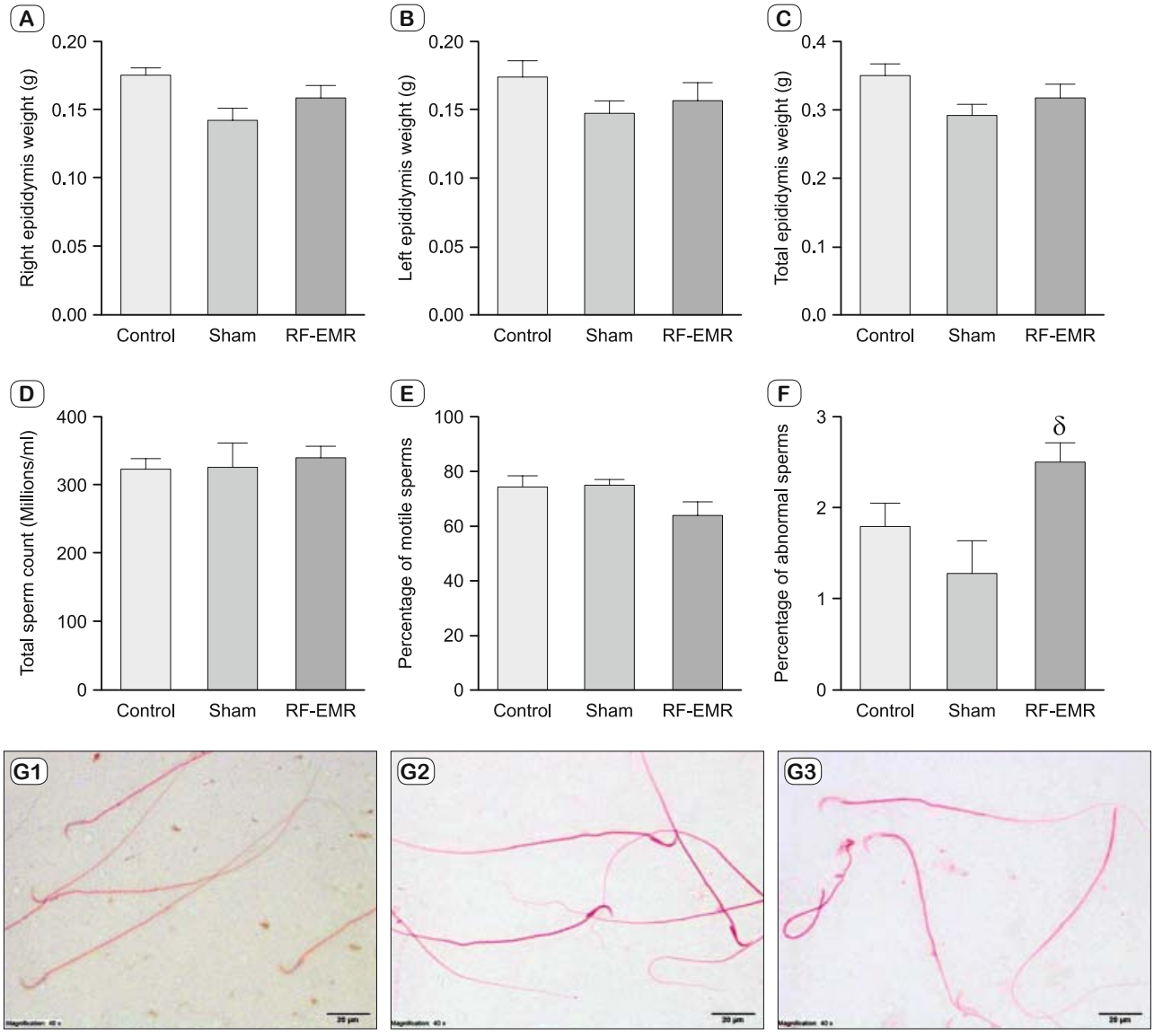

Fig. 2. RF-EMR effects on reproductive parameters. Epididymis weight (A, B, C), Total sperm count (D), Percentage of motile sperms (E) Percentage of abnormal sperms (F), and sperm morphology in various groups studied (G1–G3). (δ $p < 0.05$), Scale bar is 20 µm & magnification is 400× in panels G1–G3. G1 – control, G2 – Sham exposed, G3 – EMR exposed.

rial/mass in their lumen, which suggests necrosis (green dots in Figures 3C and F).

Caspase-3 activity in the testes:

Caspase-3 activity was determined to examine the possible activation of apoptotic pathway in the testes. As presented in Figure 4, when compared to other groups, caspase-3 activity was observed to be elevated in RF-EMR exposed rats. However, this change was not observed to be statistically significant.

Discussion

The term "biological effect" is used to refer to changes of a physiological, biochemical, or behavioral nature which are induced in an organism, tissue or cell, in response to external stimulation (19). Critical analysis of the results revealed possible modulatory role of RF-EMR on reproductive parameters of rats. It is clear from the results that epididymis weight and total sperm count were not different in the test group compared to controls. However, motile sperm count was observed to be decreased in the RF-EMR group compared to controls. Although these findings are not statistically significant, this mild change is a matter of concern. Additionally, sperm abnormality was also observed to be significantly high in the RF-EMR exposed rats. This indicates that, even in the presence of exposure to RF-EMR, spermatogenesis process is occurring but certain factors that help in the maturation of sperm could have been influenced or affected by RF-EMR. The histological

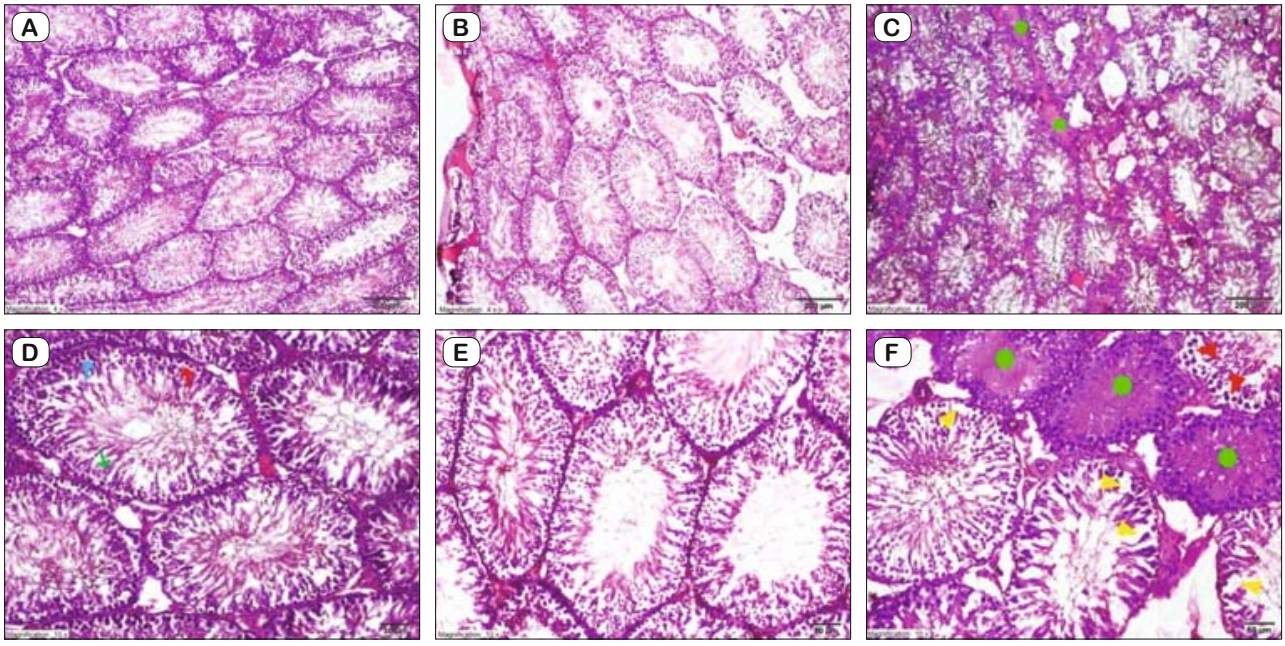

Fig. 3. RF-EMR effects on testes morphology. Scale bar is 200 µm & magnification is 40× in panels A–C, Scale bar is 50 µm & magnification is 100× in panels D–F. A, D – control, B, E – Sham exposed, C, F – EMR exposed.

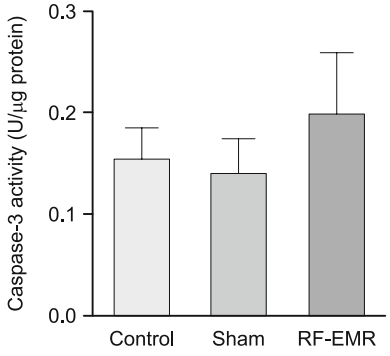

Fig. 4. RF-EMR effects on testes caspase-3 activity.

changes seen in the current study also suggest that there is a biological effect or impact due to RF-EMR on rat testes. Some of the seminiferous tubules in the RF-EMR exposed group showed necrotic cells indicating possible cell death or degeneration in the seminiferous tubules. Caspase-3 activity was also slightly elevated in RF-EMR group suggesting that the spermatogenesis is occurring in a hostile environment.

Oxidative stress may be a potential route for reproductive dysfunction. Reactive oxygen species (ROS) have been thought to be involved in various cellular functions and they can be both crucial and toxic to cells (20). ROS induces peroxidation of phospholipids in the membrane leading to loss of membrane integrity (21). There are multiple conditions known to disrupt the equilibrium between ROS production and cellular defense mechanisms. This results in cell dysfunction and destruction leading to tissue damage. Several reports suggest that RF-EMR could alter the free radical metabolism in tissues and induce oxidative stress (22–24). In the current study, blood oxidative stress indicators were found to be altered by RF-EMR exposure. Increased MDA concentration in the serum indicates the possible lipid peroxidation in the blood. However, it is interesting to note that TA and GST were not much altered in the same group. Determining TA will help to assess the redox status in an organism. In an ideal situation an increase in lipid peroxidation decreases the TA level, but in the current study we could not find such a change. At present we cannot explain this finding and further research is needed to answer this. The glutathione transferase (GST; otherwise called, glutathione S transferase) is a phase II detoxification enzyme found mainly in the cytosol. In addition to its role in catalyzing the conjugation of electrophilic substrates to glutathione (GSH), it has peroxidase and isomerase activities. Reports suggest that, GST can inhibit the Jun N-terminal kinase (accordingly guarding cells against H_2O_2-induced cell death) and is able to bind non-catalytically to an extensive variety of endogenous and exogenous ligands (25). GST activity was found to be slightly reduced in 900 MHz exposed rats in comparison to controls, but not significantly. We believe that the generalized oxidative stress caused by RF-EMR could be the possible reason for reproductive changes in RF-EMR exposed group. Additionally, in comparison to control rats, caspase-3 activity in the testes were observed to be elevated in 900 MHz exposed rats. Although caspase-3 activity were not significantly different in RF-EMR group, the observed change suggests a possible activation of apoptotic pathway in RF-EMR exposed rats. With the present data we cannot categorically suggest this, as we have not done any special staining to detect the apoptotic/necrotic cells in the current study. Further research is required to confirm this or support this argument.

Reports suggest that, sperm function and male infertility are influenced by ROS-induced oxidative stress (26–28). ROS can directly attack unsaturated fatty acids on the sperm membrane, induce lipid peroxidation, damage membrane integrity, destroy axoneme structure, and eventually reduce sperm activity and fertility (28, 29). Several reports throw light into the possible adverse effects of electromagnetic or static magnetic fields on reproductive system. A report demonstrated that RF-EMR exposure enhanced mitochondrial reactive oxygen species (ROS) generation in human spermatozoa that caused decreased sperm motility and viability along with stimulating DNA base adduct development and eventually fragmentation of sperm DNA (30). Another study suggests that RF-EMR exposure did not have any effect on acrosome reaction, but significantly influenced sperm morphology and its binding to hemizona (31). A recent review on the effect of RF-EMR on sperm quality found a relationship between RF-EMR exposure and reduced sperm motility and viability (32). Dasdag et al, reported that RF-EMR exposure significantly decreased seminiferous tubule diameter and height of the germinal epithelium in rats (33). Mobile phone radiation can have several effects such as thermal, non-thermal (specific), cumulative (include both thermal and non-thermal). Despite extensive research on the subject we are unable to categorically rule out the possible biological effects of RF-EMR on humans and other animals. In the present scenario there is a need of extensive research in this area as the technology is advancing and spreading over the globe at a far quicker pace.

Conclusion

Chronic whole body RF-EMR exposure induced oxidative damage in the blood and caused alterations in reproductive parameters in RF-EMR exposed rats as demonstrated by increased percentage of abnormal sperm and decreased motile sperm counts. It also induced alterations in the cellular architecture of seminiferous tubules in rats.

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