

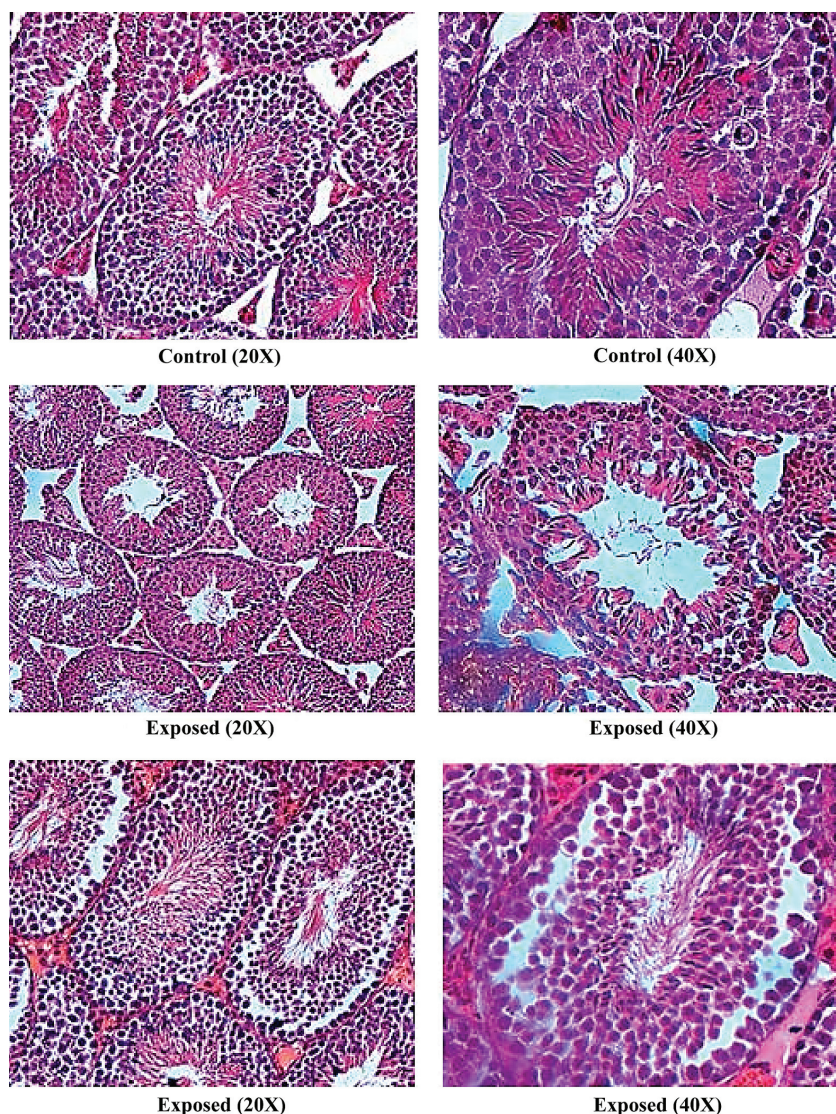


Figure 2. Transverse Section of testis of microwave-irradiated mice (H&E staining). Note significant decrease in the seminiferous tubule diameter and damaged seminiferous epithelium as well as degenerated Leydig cells in the testis of exposed mice (middle and lower panel) compared to that of control (upper panel). Germ cell of exposed mice have a degenerated appearance in the epithelium. Formation of gaps were also seen between the peripheral layers of spermatogonial cells in the seminiferous tubules of nonthermal low-level 2.45-GHz microwave-irradiated mice.

Catalase activity

Similar trend of decrement was also observed in CAT activity (Figure 7b). We found that CAT activity decreased significantly in all different tissues, that is, liver ($p < 0.001$), kidney ($p < 0.01$), hypothalamus ($p < 0.001$), and testis ($p < 0.001$) of MW-irradiated mice when compared to that of the control group.

GPx activity

GPx activity of both the isoforms (GPx1 and GPx2) was found to be decreased significantly ($p < 0.001$) in liver and kidney of MW-exposed mice as compared to that of the control; however, in the hypothalamus and testis GPx 1 and GPx 2, respectively, were found to be decreased significantly. On the other hand, in plasma, only one isoform (GPx1) was evident which did not

show any difference between the control and exposed mice (Figure 7c & d).

3 β HSD activity

It was found that 2.45-GHz MW radiation caused a statistically significant decrease in the activity of testicular 3 β hydroxysteroid dehydrogenase (3 β HSD) ($p < 0.01$), a crucial enzyme in the steroid biosynthesis pathway, compared to that of control (Figure 8A).

Plasma testosterone

Both oxidative and nitrosative stress has been shown to potentially reduce testosterone production *in vivo*. Therefore, we next measure the plasma testosterone concentration. It has been observed that plasma testosterone level decrease significantly ($p < 0.001$) in the MW-irradiated

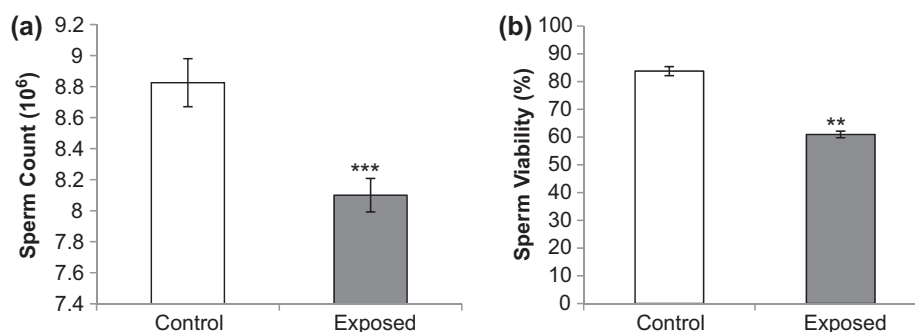


Figure 3. Effect of 2.45-GHz microwave (MW) irradiation on the (a) sperm count and (b) sperm viability of mice. MW irradiation significantly decrease the number of sperm along with increased number of dead sperm compared to that of control. Data are presented as mean $\pm$ standard deviations ($n = 15$). Significance of difference from control, ** $p < 0.01$, *** $p < 0.001$.

group of mice as compared to that of the control group (Figure 8B).

Immunohistochemistry of *i*-NOS

Nitric oxide (NO), which is a free radical gas as well as a ubiquitous signaling molecule produced by NO synthases, has been proposed to mediate many physiological functions. It has been proposed that the involvement of NO in stress is due to activation of testicular inducible *i*-NOS. To check whether the tissue-specific synthesis of NO is induced by the MW irradiation, immunohistochemical localization of *i*-NOS has been performed. Result shows a significant increase in *i*-NOS-immunoreactivity in the spermatogonial cells of seminiferous tubules and Leydig cells of MW-irradiated mice testis compared to that of control (Figure 9).

Immunofluorescence of 3β HSD

We also observed a significant decrease in the expression of 3β HSD, a key enzyme in steroidogenesis pathway, in the cytoplasm of testicular Leydig cells of exposed group of mice (Figure 10).

Discussion

Present study clearly elucidated that low-level 2.45-GHz MW radiation with power density = 0.029812 mW/cm² and SAR = 0.018 W/Kg has negative impact on male reproductive system. These experimental findings indicate that MW irradiation exerts a wide range of adverse effects on testicular functions. It affects the testicular morphology by decreasing the diameter of seminiferous tubules, degeneration of seminiferous epithelium, and formation of peripheral gaps in some tubules by the detachment of spermatogonial layer from the tunica propria. These changes including germ cell degeneration, reduced sperm counts, and viability may occur as the consequence of reduced testosterone concentrations in MW-exposed group of mice, since high level of testosterone is essential for normal spermatogenesis as well as for the maintenance of structural morphology and normal physiology of seminiferous tubules [51,52]. Yan and group also reported that long-term exposure to low-intensity MW radiation evidently increased the sexual dysfunction rate in humans [53]. During spermatogenesis, testosterone is also required for the attachment of

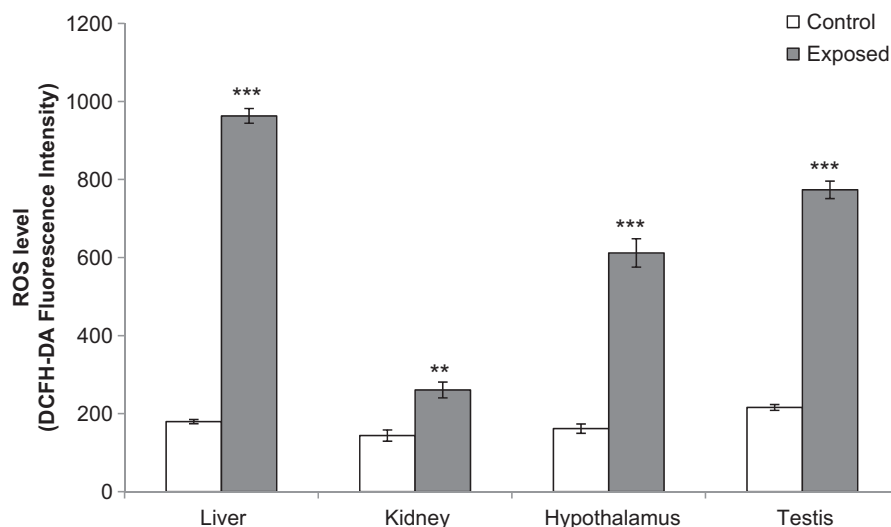


Figure 4. Increased DCFH-DA fluorescence (measure of ROS) was observed in liver, kidney, hypothalamus, and testis of microwave-irradiated mice as compared to that of control. Highly significant increase was observed in all the tissues. Results were expressed as mean $\pm$ standard deviations ($n = 15$). Significance of the difference was determined by using the two-tailed Student's *t* test. ** $p < 0.01$ and *** $p < 0.001$ significance of difference from control.

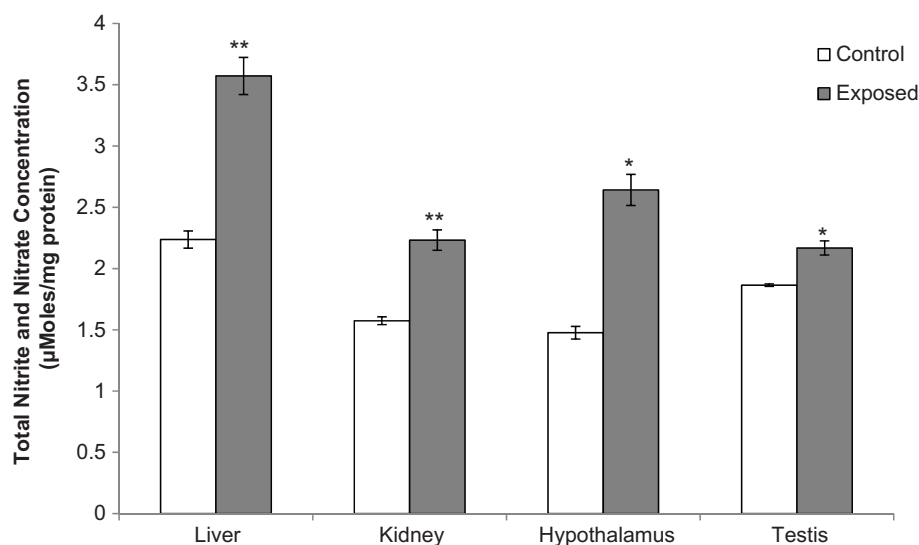


Figure 5. MW-irradiated mice showed a significant increase in total nitrite and nitrate concentration in liver, kidney, hypothalamus, and testis ($n = 15$). Values are expressed as mean $\pm$ standard deviations. * $p < 0.05$ and ** $p < 0.01$ significance of difference from control.

different generations of germ cells in seminiferous tubules and therefore decreased testosterone concentration may lead to detachment of germ cells from seminiferous epithelium and cell death by initiating germ cell apoptosis [54,55]. Our findings are consistent with previous studies which suggest that MW radiation can negatively affect the quality of sperm which can potentially impair the reproduction in the humans and rodents [56,57]. Although, some researchers have reported that acute MW exposure can have direct effect on the seminiferous tubule epithelium through increase in testicular temperature but, the power density and SAR values used in these studies were too high [18,19,58] and greater than the present study. MW exposure at power density = 0.029812 mW/cm^2 and SAR = 0.018 W/Kg does not cause any elevation in the ambient temperature of the animal cage as well as rectal temperature of mouse and hence eliminates the possibility of thermal effects of MW exposure on spermatogenesis and sperm count and viability.

With a significant elevation in ROS, RNS, and MDA level in liver, kidney, hypothalamus, and testis, we also observed a significant decrease in ROS-scavenging enzymes (SOD, CAT, and GPx) in exposed group of mice. These findings are consistent with our previous study in female mice which demonstrated that mice exposed to 2.45-GHz (CW) MW irradiation is responsible for generating extensive free radical load as evident from increased ROS/RNS and reduction in antioxidant enzyme activities [16]. The kidney is a major potential route for the absorption of hazardous materials encountered in the environment. It helps in the excretion of waste products of metabolism and drugs. It regulates the homeostasis of body water as well as performs endocrine (hormonal) functions. As a multifunctional organ, liver takes part in uptake, metabolism and excretion of nutrients. It helps in the excretion of endogenous wastes and xenobiotics, synthesis and secretion of plasma proteins, clotting factors. It also maintains metabolic homeostasis (glucose and lipids) and assists in the digestion of fat (via bile). The alteration/

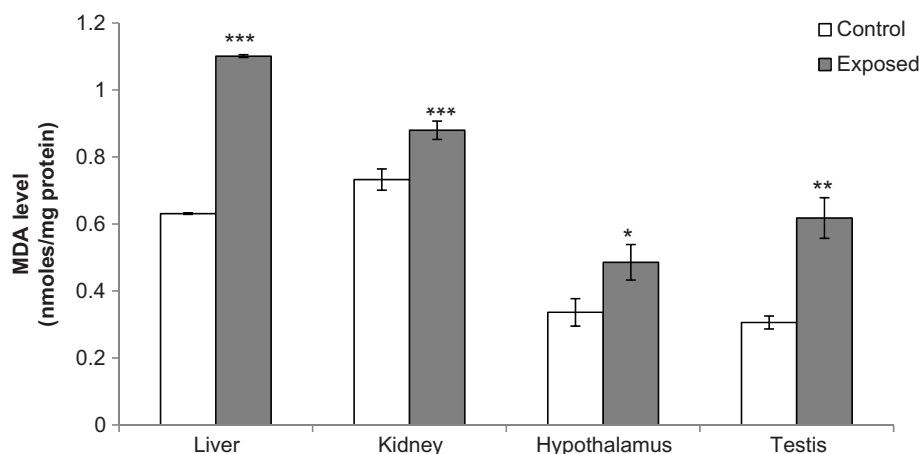


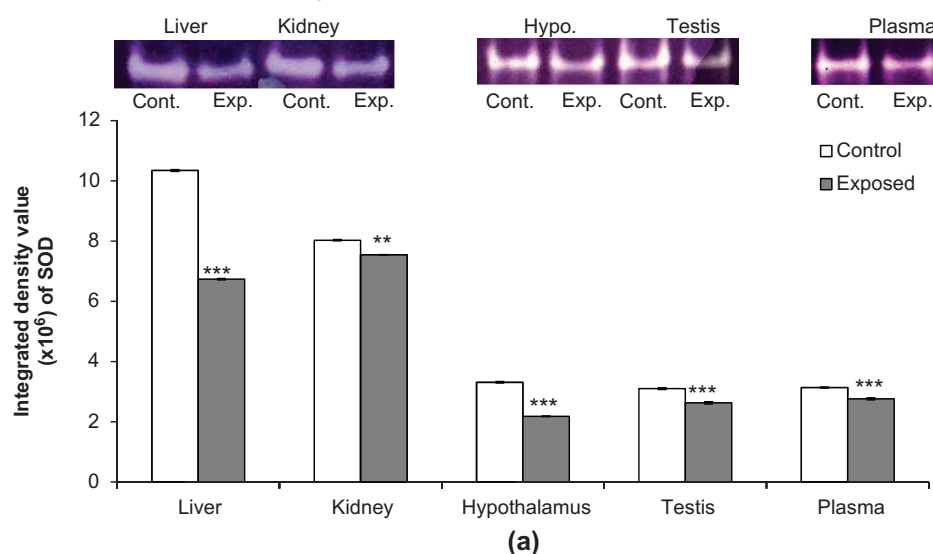
Figure 6. 2.45-GHz MW irradiation result in increased lipid peroxidation. Significant increase in malondialdehyde (MDA) level, end product of lipid peroxidation, was observed in different tissues of MW-exposed mice. Values were expressed as mean $\pm$ standard deviations ($n = 15$). Data were analyzed using the two-tailed Student's t-test. Significance of difference from control, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

significant increase in oxidative and nitrosative stress parameters (free-radical load, ROS, NO, and MDA level) and significant decrease in the activities of antioxidant enzymes in kidney and liver suggests the ill effects of MW at the organ as well as the system level. These continuously produced ROS are scavenged by SOD, GPx, and CAT. Under some circumstances, these endogenous antioxidant defenses are likely to be perturbed as a result of overproduction of oxygen radicals, inactivation of detoxification systems, consumption of antioxidants, and failure to adequately replenish antioxidants in tissue. It has been demonstrated in numerous studies that ROS are directly involved in oxidative damage of cellular macromolecules such as lipids, proteins, and nucleic acids in tissues. The exact mechanism of MW-interactions is not yet clearly understood, although few studies have suggested the involvement of lipid peroxidation, free radical formation, and biochemically induced oxidative stress. Since, in

addition to its reproductive effects MW radiation also affects liver and kidney, present study suggests that MW exposure may interfere with the overall functions of the organ system.

With reference to the reproductive system, it appears that in testis, over production of free radicals and decreased anti-oxidant status are responsible for causing toxic effects on the testicular physiology. SOD and CAT are enzymatic antioxidants which inactivate the superoxide anion ($O_2^{\bullet-}$) and peroxide (H_2O_2) radicals by converting them into water and oxygen. GPx is the final member of the antioxidant triad which is involved in the reduction of hydrogen peroxides using glutathione as an electron donor. Therefore, as a consequence of MW radiation-induced decrease in ROS-scavenging enzymes: SOD, CAT, and GPx, there is spontaneous increase in toxicity due to elevation of free radicals superoxide ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($\bullet OH$), nitric oxide ($NO\bullet$),

1. Superoxide dismutase activity



2. Catalase activity

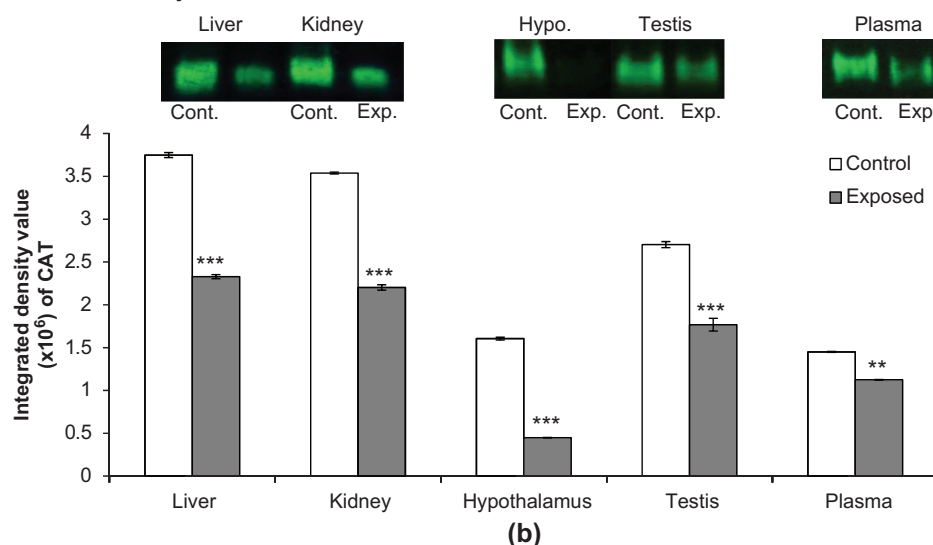


Figure 7. Changes in Antioxidant enzymes activities—SOD (a), CAT (b) and two isoforms of GPx (GPx 1-c and GPx 2-d) of 2.45-GHz microwave-irradiated mice demonstrated by native gel. The intensity of bands in all the cases is quantified by gel densitometry. Values are expressed as mean $\pm$ standard deviation. Significance of difference from control * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

3. Glutathione peroxidase activity

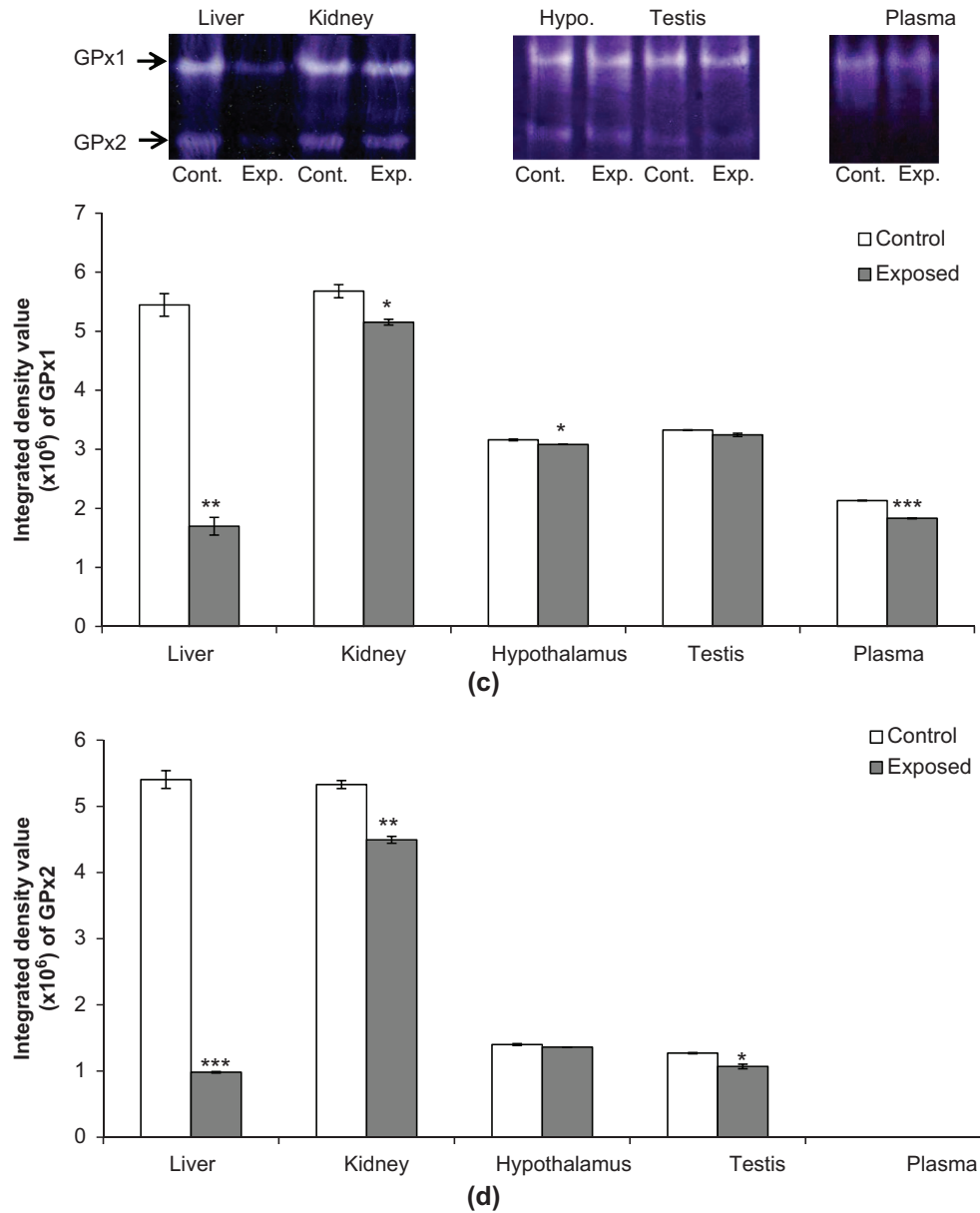


Figure 7. (Continued)

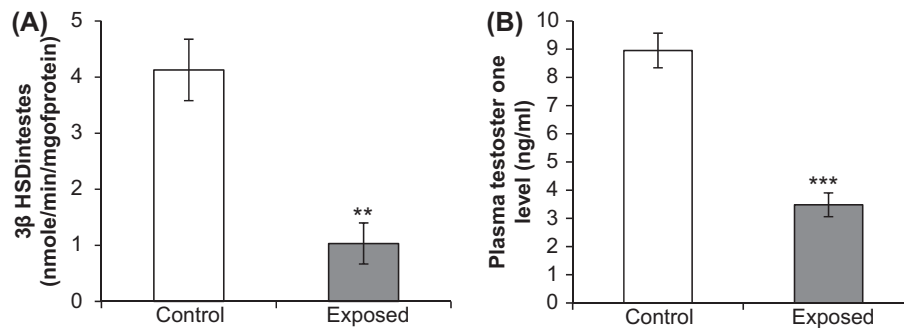


Figure 8. Effect of 2.45-GHz microwave irradiation on (A) testicular 3β HSD and (B) Plasma Testosterone concentration. Highly significant decrease was observed in 3β-HSD activity, a key enzyme in steroidogenesis pathway. Plasma testosterone level is also found to be significantly reduced in MW-exposed group. Values are expressed as mean ± standard deviation. Significance of difference from control, **p < 0.01, ***p < 0.001.

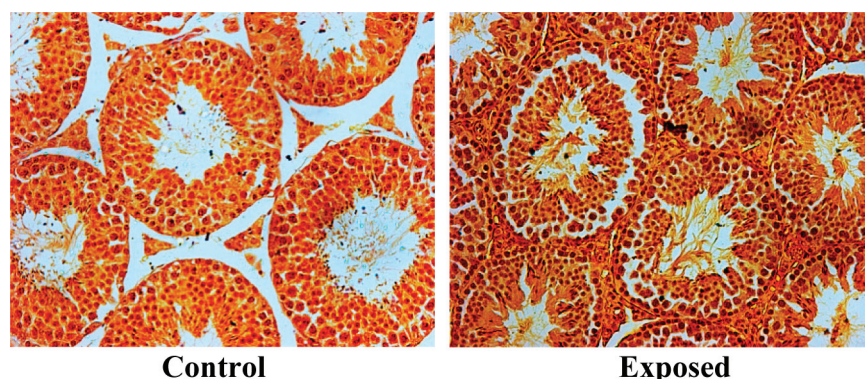


Figure 9. Immunohistochemistry of i-NOS in mice testis. Note significantly increased i-NOS immunostaining in the spermatogonial cells of seminiferous tubules and Leydig cells of microwave (MW)-irradiated mice testis compared to that of control. 2.45-GHz nonthermal MW exposure induce the activation of testicular i-NOS and subsequently raising the production of NO which is a free radical gas as well as main causative agent of nitrosative stress, resulting in free radical load.

etc. in the body. This results in increased lipid peroxidation in different tissues. Desai et al. [59] and Agarwal et al. [60] showed a possible mechanism for increased ROS generation in various cellular targets. MW radiation stimulates plasma membrane NADH oxidase. SOD and CAT remove $O_2^{\bullet-}$ generated by NADPH oxidase and play a major role in decreasing lipid peroxidation and protecting spermatozoa against oxidative damage [61]. Evidences show a link between deficient SOD and CAT activity, and male infertility [62–66]. GPx must play an important protective role against oxidative attack since its specific inhibition *in vitro* using mercaptosuccinate leads to a large

increase in sperm lipid peroxidation [67]. Male factor infertility linked with a reduction in seminal plasma [68] and spermatozoa GPx activity [69] further supports an important role for this enzyme in male fertility.

Low amount of controlled endogenous ROS generation by spermatozoa play a significant role in inducing sperm capacitation/acrosome reaction and acquisition of sperm-fertilizing ability [28,70]. Thus, although physiological levels of ROS are required for spermatogenesis, an excessive production of ROS resulting from low-level 2.45-GHz MW radiation appears to have deleterious effects on testicular function. High generation of ROS accompanied by

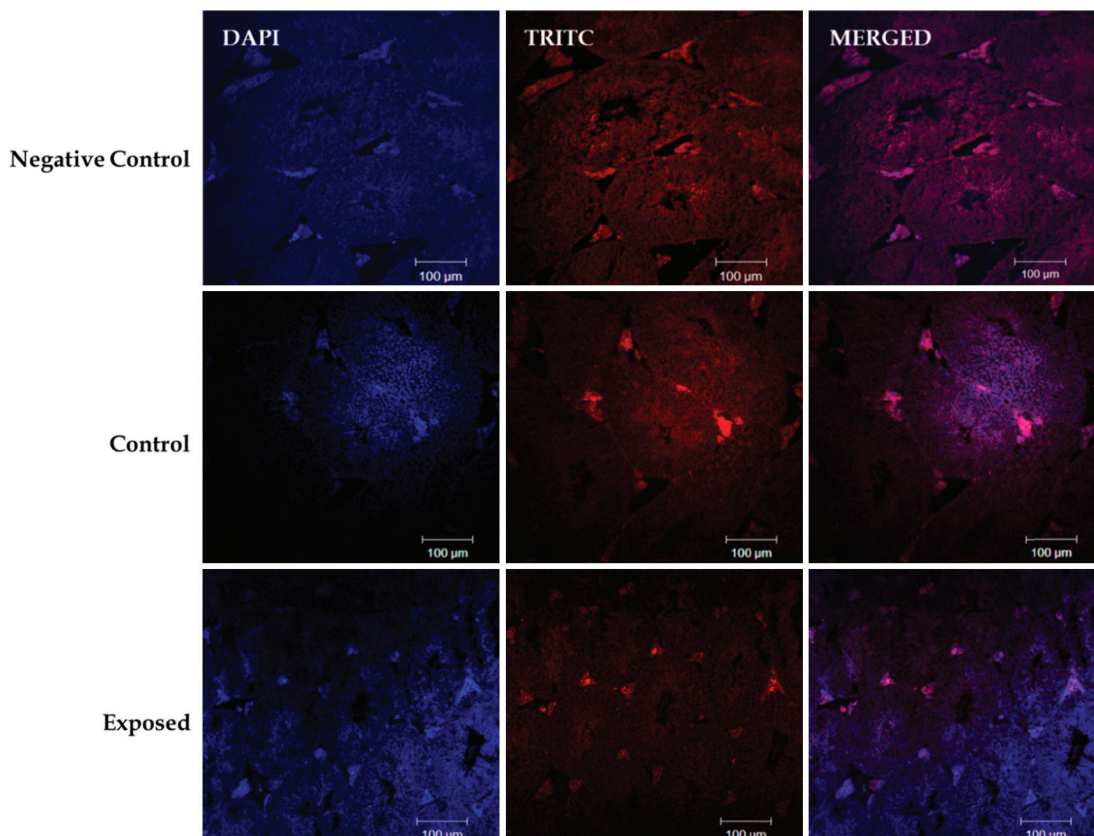


Figure 10. Confocal microscopy showing immunofluorescence of 3β-HSD in testicular Leydig cells of MW-irradiated mice. Note significantly decreased expression of 3β-HSD enzyme, one of the prime enzyme in the steroidogenesis pathway, which play a key regulatory role in testicular androgenesis, in the cytoplasm of testicular Leydig cells of low-level 2.45-GHz MW-exposed mice compared to that of control.

low scavenging antioxidant levels in different tissues will induce a state of oxidative stress. Since testicular membranes are rich in molecules such as fatty acids (i.e., lipids), which are prone to oxidative injury, it is reasonable to consider that lipid peroxidation (i.e., damage to the cell membranes) may contribute to the gonadal dysfunction that occurs as a result of low-level MW irradiation. Since, overproduction of free radicals by MW exposure results in lipid peroxidation, which may cause rupture of the cell as well as nuclear membrane, it is suggested that degeneration of seminiferous epithelium and disarray in cellular organization of seminiferous tubules is the outcome of MW-induced oxidative/nitrosative stress in the testicular environment. Free radicals generated after MW irradiation also react with the polyunsaturated fatty acid-rich spermatozoa resulting in peroxidation which finally leads to sperm destruction causing reduced motility and viability. Studies also suggest that elevated ROS level endanger sperm motility, viability, and function by interacting with membrane lipids, proteins and nuclear and mitochondrial DNA [71–74]. Falzone et al. [75] demonstrated that mitochondrial membrane potential of the human sperm cells was decreased after 900 MHz mobile phone EMR. Further, Eroglu and group [12] reported that semen samples from 27 healthy human volunteers exposed to MW radiation emitted from an active GSM cell phone at 10 cm distance caused a statistically significant decrease in forward sperm movement, as well as a reduction in total number of motile sperm. It has been observed that such radiation can reduce the fertilizing potential of men [10,11,13] and may lead to oxidative stress via increasing ROS level and decreasing total antioxidant capacity (TAC) score in human semen [14].

2.45-GHz MW irradiation at SAR = 0.018 W/Kg significantly decreased the steroidogenic enzyme β -HSD in the testis as well as plasma testosterone level. We suggest that MW radiation-induced oxidative and nitrosative stress adversely affects steroidogenesis and thus spermatogenesis which may lead to infertility in chronic condition. Since testicular functions and the process of spermatogenesis is hormonally regulated by a negative feedback loop, adverse effects of MW radiation on HPG axis cannot be ruled out. MW radiation-induced elevation in hypothalamic ROS and RNS may disrupt the hormonal communication of the neuroendocrine axis which disturbs the steroidogenesis and spermatogenesis process of testis. Present findings are consistent with other studies which reported that MW exposure disrupts the histological architecture of seminiferous tubules, reduces the Leydig cell population, and testosterone concentration in rats [76]. In addition to deleterious effects of ROS and NO at the hypothalamus level it is also possible that increased NO level in testis following 2.45-GHz MW irradiation may decrease steroidogenesis by affecting Leydig cells directly and thus spermatogenesis in male. NO has been also shown to potentially reduce testosterone production both *in vivo* [77] and *in vitro* [78,79]. Thus, similar to ROS, although optimum level of NO has beneficial effects on reproductive performance, its over production may affect steroidogenesis/spermatogenesis adversely [80,81].

Based on adverse testicular morphology and physiology as well as increased ROS and RNS at both the central and peripheral level, we suggest that 2.45-GHz MW irradiation with power density = 0.029812 mW/cm² and SAR = 0.018 W/Kg has a negative effect on testicular function (testicular steroidogenesis and spermatogenesis) through the induction of oxidative and nitrosative stress. It is well known that mice are metabolically more active than humans, providing an indication of possible effects on human reproductive system at similar power density and SAR value. Since these findings may be extrapolated to humans, it is high time to have the awareness based on experimental reports regarding the possibility of long-term deleterious effects of MW radiation.

Conclusion

Present experimental findings clearly indicate that MW irradiation impairs male reproductive system via inducing oxidative as well as nitrosative stress. It is obvious that low-level 2.45-GHz MW irradiation produces degenerative changes in the spermatogonial cells and inhibits testosterone production by increasing ROS and RNS production and disturbing testicular antioxidant status. We also suggest that MW irradiation acts as an environmental toxicant that stimulates lipid peroxidation. Further, oxidative and nitrosative stress induced at the central and peripheral level as a consequence of low-level 2.45-GHz MW exposure diminishes the steroidogenic capacity of the testis by decreasing β -HSD activity as well as decreased plasma testosterone level, resulting in decreased spermatogenesis. This experimental report also suggests that chronic exposure to nonthermal MW radiation may include deleterious changes in reproductive performance, which may lead to infertility following long-term exposure. Further, similar to IR, MW-induced nonionizing radiation may also impose health risk. Hence, long-term continuous exposure to man-made nonionizing MW radiation even at low level should also be minimized.

Declaration of interest

The authors report no declarations of interest. The authors alone are responsible for the content and writing of the paper.

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Immunohistopathologic demonstration of deleterious effects on growing rat testes of radiofrequency waves emitted from conventional Wi-Fi devices

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Abstract *Objective:* To investigate effects on rat testes of radiofrequency radiation emitted from indoor Wi-Fi Internet access devices using 802.11.g wireless standards.

Methods: Ten Wistar albino male rats were divided into experimental and control groups, with five rats per group. Standard wireless gateways communicating at 2.437 GHz were used as radiofrequency wave sources. The experimental group was exposed to radiofrequency energy for 24 h a day for 20 weeks. The rats were sacrificed at the end of the study. Intracardiac blood was sampled for serum 8-hydroxy-2'-deoxyguanosine levels. Testes were removed and examined histologically and immunohistochemically. Testis tissues were analyzed for malondialdehyde levels and prooxidant–antioxidant enzyme activities.

Results: We observed significant increases in serum 8-hydroxy-2'-deoxyguanosine levels and 8-hydroxyguanosine staining in the testes of the experimental group indicating DNA damage due to exposure ($p < 0.05$). We also found decreased levels of catalase and glutathione peroxidase activity in the experimental group, which may have been due to radiofrequency effects on enzyme activity ($p < 0.05$).

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Conclusions: These findings raise questions about the safety of radiofrequency exposure from Wi-Fi Internet access devices for growing organisms of reproductive age, with a potential effect on both fertility and the integrity of germ cells.

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Introduction

There has been continuing public anxiety about the potential health consequences of wireless communication tools using radiofrequency (RF), despite authorities on the subject having set safety boundaries to protect the community against RF exposure [1–3]. Therefore, the World Health Organization advised research in this area. Animal studies involving rats have been conducted to analyze the effects of Wi-Fi signals on health parameters and stress markers [4]. The environmental wireless 802.11.g device (also called Wi-Fi device, or wireless internet access device or WIAD) has in general higher frequency ranges and longer exposure times than wireless phones [5]. Thus, the level of health hazards associated with Wi-Fi devices might be different from, and possibly higher than, mobile phones (MB). Additionally, Wi-Fi devices commonly expose the whole body to RF, unlike MB which generally irradiate some parts of the body, e.g. cranium, more than others.

Exposure of animals to RF electromagnetic radiation (EMR) may lead to a variety of changes in tissues. The observed changes vary depending on the wireless exposure characteristics, species studied, and histological methodology used for the detection of effects [6,7]. Human beings have also been shown to be adversely affected by prolonged and repeated exposure to RF EMR [8,9]. One adverse effect is on the male reproductive system. Both the number and fertilizing capacity of mouse and human sperm were shown to be decreased after RF exposure [10,11]. Another important issue about the effects of RF EMR is induction of carcinogenesis [12–14]. The mechanism underlying the induction of infertility and carcinogenesis by RF EMR emitted from wireless devices appears to be DNA damage due to oxidative stress (OS) [15,16]. DNA oxidation could occur when guanine, the most electron-rich DNA base, is subjected to the oxidizing influence of RF waves [17]. The purpose of this study was to demonstrate, by measuring serum 8-hydroxy-2'-deoxyguanosine (8OHdG) and testis tissue 8-hydroxyguanosine immunohistochemistry, whether the commonly used indoor WIADs cause DNA damage due to OS, and could be a contributing factor for infertility and the induction of carcinogenesis in rat testes.

Materials and methods

Subjects and animal care

The local ethics committee for the use of laboratory animals approved the study. Ten 8-week-old inbred healthy male Wistar albino rats, weighing 215–285 g, were used in the experiment. The rats were housed in acrylic-glass

cages, 20 × 30 × 40 cm in size. The rats were separated into two groups of five: one experimental group and one control group. They were kept in well-ventilated cages and rooms, not restrained. The experimental group was exposed to WIAD RF of 2437 megahertz (MHz), while the control group was shielded against RF radiation.

Wireless Internet access device – whole body exposure

Two commercially available wireless ADSL 2 + gateways (USR9108 MAXg, US), operating at 802.11.g standards, were used in this study. The output power was 95 mW, average, as equivalent isotropically radiated output power. The maximum specific absorption rate (SAR) of the wireless gateways in the conformity assessment test was 0.091 W/kg.

A network storage link was attached to each wireless device, by which a 250 GB capacity Universal Serial Bus hard disk was connected. Gateways were wirelessly bridged to each other. Rats' cages were placed in between the gateways so that the distance between the cages and the gateways was 25 cm, as suggested by the regulatory information in the producer's user guide according to the FCC (Federal Communications Commission) Radiation Exposure Statement [18]. Data transmission was performed after each hard disk was assigned as a network drive. Exposure was continued for 24 h a day for 20 weeks (Fig. 1).

At the end of the 20 weeks all rats were sacrificed by decapitation under anesthesia and intracardiac blood samples were obtained. Sera were separated by

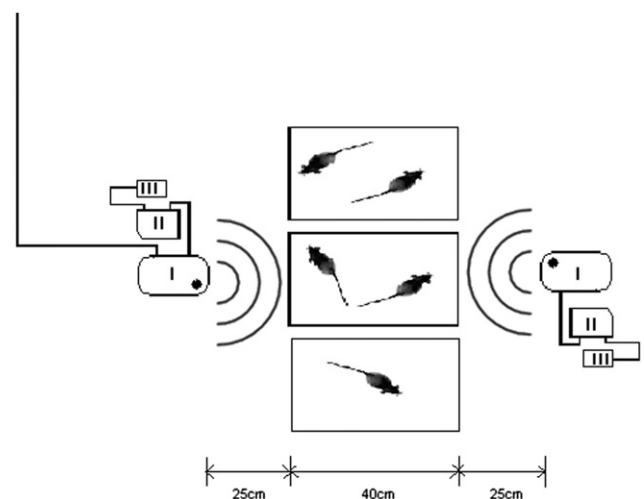


Figure 1 Experimental design of the study: (I) hard disk, (II) network storage link, (III) wireless gateway.

centrifugation (at 1250 g for 15 min at 25 °C) and stored at –80 °C. Testes were removed, one of the testes snap-frozen in liquid nitrogen (–196 °C) and stored at –80 °C until biochemical analyses, and other testis tissues fixed in a solution of 10% formaldehyde for histopathologic examination.

Determination of 8-hydroxy-2'-deoxyguanosine serum levels

8OHdG was measured with the highly sensitive 8OHdG competitive sandwich enzyme immunoassay kit (Catalog No. 839320, Cayman Chemical Co., MI, USA). The test principle is based on competition between 8OHdG and 8OHdG acetylcholinesterase conjugate via 8OHdG monoclonal antibodies. Plates were read by a Bio-Rad Benchmark Plus microplate reader at 420 nm (Bio-Rad Laboratories Inc., CA, USA). Results were expressed in nanograms per milliliter.

Histologic examination of testes

After fixation, the testes were embedded in paraffin, sectioned at 4 µm, deparaffinized, and stained with hematoxylin & eosin. The testicular tissues were evaluated histopathologically by using Johnsen's testicular biopsy score (TBS) count [19] (Table 1).

8-Hydroxyguanosine immunohistochemistry of testes

Tissue sections from every paraffin block were immunostained with antibodies for 8-hydroxyguanosine, which were mouse-raised monoclonal antibodies (ab62623; 1 mcg/mL) obtained from AbCAM. Nuclear or perinuclear cytoplasmic staining was considered as 'positive'. Intensity was graded as 0 (no staining), 1 (weak staining), 2 (strong staining) or 3 (very strong staining). On each slide, the intensity of staining in 10 seminiferous tubules was

counted and averaged. The modified immunohistochemical histologic score (HSCORE) was used for semiquantitative estimation of 8OHdG antibody staining of the testicular tissue. This is a score used for the evaluation of intensity and percentage of cells that stain at each intensity [20]. The HSCORE is formularized mathematically as follows:

$$\text{HSCORE} = \sum (\text{Pi} \times \text{I}/100)$$

where Pi is the percentage of stained cells, ranging from 1% to 100%, and I is the intensity of staining, ranging from 0 to 3. Staining intensities were calculated as shown in Table 2.

Biochemical analysis of prooxidant–antioxidant parameters of testis tissues

The tissues were removed from the freezer (–80 °C) and disaggregated thoroughly with physiological saline (1 g in 5 mL) in a homogeniser (Heidolph Diox 900, Germany). After centrifugation at 4000 g for 20 min (Harrier 18/80 MSB080.CR2.K, UK), the supernatants were removed to be used in the analyses.

Malondialdehyde (MDA) level was measured by the thiobarbituric acid reactive substances method. MDA reacts with thiobarbituric acid to form a colored complex that has maximum absorbance at 532 nm. Xanthine oxidase (XO) activity was determined by measuring uric acid formation from xanthine at 293 nm. Superoxide dismutase (SOD) activity was measured by a method based on the nitroblue tetrazolium reduction rate. Catalase (CAT) activity was determined by measuring decrease in absorbance of hydrogen peroxide (H₂O₂) at 240 nm. Glutathione peroxidase (GPX) activity was measured by following changes in NADPH absorbance at 340 nm. In the activity calculations, extinction coefficients of H₂O₂, NADPH and uric acid were used for CAT, GPX and XO, respectively. Absorbances were read by a Unicam Helios α (UV–VIS) spectrophotometer (Spectronic Unicam, Cambridge, UK).

Statistical analysis

Five animals per group were required for the study with a power of 87% and error type I of 5% to detect a one unit difference between the groups in nonparametric unpaired tests. For central tendency and dispersion of data we used median and quartile deviation (QD), respectively. The Mann–Whitney U-test was used for group comparisons. Multivariate analysis of variance was used to search for any significant relationship with dependent variables of 8OHdG and parameters of biochemical OS in groups. Pillai's trace significance test was used for significance of the overall relationship between dependent variables and RF groups. A

Table 1 Evaluation of testicular biopsy score (TBS) count [19].

Score	Description
1	No cells in tubular section
2	No germ cells, but Sertoli cells present
3	Spermatogonia are the only germ cells present
4	Only a few spermatocytes (<5), but no spermatids or spermatozoa present
5	No spermatozoa or spermatids, but many spermatocytes present
6	No spermatozoa and only a few spermatids present (<5–10)
7	No spermatozoa, but many spermatids present
8	Only a few spermatozoa present (<5–10)
9	Much spermatogenesis, but germinal epithelium disorganized with marked sloughing or obliteration of lumen
10	Complete spermatogenesis with many spermatozoa

Table 2 Evaluation of HSCORE count [20].

Score	Description
0	Percentage of unstained cells × 0
1	Percentage of weakly stained cells × 1
2	Percentage of moderately stained cells × 2
3	Percentage of intensely stained cells × 3

p value less than 0.05 was considered as significant. PASW version 17 (Chicago, IL, USA) was used for the statistical procedures.

Results

There was no difference between the weights of the control and experimental groups before and after the experiment. Median values of TBS, as evaluated using Johnsen's scale, were lower in the experimental than the control group (9 ± 0 vs 10 ± 0). The difference in the TBS between the control and experimental groups was statistically significant ($p < 0.01$). 8OHdG levels in the experimental group were significantly higher than in the control group. Routine histological examinations of the experimental and control group were not different. 8-Hydroxyguanosine immune reactivity, which shows oxidative damage to DNA, was positive in both groups. The HSCOREs of the experimental group were significantly higher than those of the control group ($p < 0.01$) (Table 3). GPX and CAT activities of the experimental group were statistically lower than those of the control group ($p < 0.05$ and $p < 0.01$, respectively). SOD and XO activities and MDA levels of the experimental group were lower than in the control group, but the difference was not statistically significant ($p > 0.05$) (Table 4). The histological and immunohistochemical changes in the experimental and control groups are shown in Fig. 2. Multivariate analysis of variance showed a significant relationship between RF group and the OS parameters of 8OHdG, MDA, GPX, CAT, XO and SOD (F value 19.66; $p = 0.017$).

Discussion

Various studies have investigated the effects on the human body of RF EMR from mobile phones, with inconclusive results, but there have been no studies as yet on the health effects of WIADs, which are associated with higher frequency radio waves and longer exposure times than MBs. Currently, the most popular wireless technology employs the 802.11.g protocol, which is used in many wireless electronic devices, including baby video monitors, Wi-Fi MBs and television devices. A wireless 802.11.g RF device has unique properties, e.g. with regard to frequency range, output power and exposure time, which may have an untoward influence on health. It uses the 2400-MHz band, which is higher than the frequencies used by GSM (Global

Table 3 Clinical and laboratory characteristics of rats exposed to WIAD RF radiation and control group.

	Control group	Experimental group	p
No. of rats	5	5	
Weight (g)	230 (30)	250 (9)	0.456
8OHdG (ng/mL)	1.15 (0.67)	3.00 (0.44)	0.021 ^a
HSCORE	30 (5)	250 (30)	0.008 ^a
TBS	10 (0)	9 (0)	0.003 ^a

Values are medians, with QD in parentheses.

^a Statistically significant difference.

Table 4 MDA levels and prooxidant–antioxidant enzyme activities in testicular tissues of experimental and control groups.

	Control group ($n = 5$)	Experimental group ($n = 5$)	p
MDA (nmol/mg)	0.23 (0.025)	0.21 (0.005) ^a	0.166
XO (mIU/mg)	0.0083 (0.0010)	0.0064 (0.0005)	0.209
SOD (U/mg)	0.84 (0.02)	0.76 (0.01)	0.074
GPX (IU/mg)	0.0040 (0.0003)	0.0024 (0.0009)	0.036 ^a
CAT (IU/mg)	5.57 (0.115)	2.65 (0.180)	0.009 ^a

Values are medians with QD in parentheses.

^a Statistically significant difference.

System for Mobile Communications) or 3G (third generation) phones. The output power of Wi-Fi devices ranges from 20 mW to 4 W, and is similar to or greater than that of MBs, which range from 1 mW to 2 W [21,22]. WIADs are generally 'on' while transceiving data from the net and they cause longer human exposure than MBs. Furthermore, they commonly expose the whole body to RF EMR, unlike MBs, which generally cause a more uneven and local irradiation of some parts of the body, e.g. the head and pelvic organs.

The reason why young growing rats were used in this study is that their growing organs may be more prone to the effects of RF EMR, like children and adolescents who spend a lot of time using wireless computers at home and school. We studied the effects on testes since there have been several studies analyzing the effects of wireless phones on male fertility [23,24], and the reproductive system is more sensitive than other organs to RF devices such as laptop computers. Whole body irradiation with WIADs was employed in this experiment, and thus the methodology used in this study differs significantly from that of the previous investigators, and is unique because it explores the effects of the commonly used conventional WIADs on the health of living organisms.

Previous animal studies have not found any significant effect of RF EMR on testicular histology, sperm count and morphology. Cairnie and Harding [25] studied the effects of RF in the mouse testis and found no significant differences in sperm count and no increase in the percentage of morphologically abnormal sperm in microwave-exposed animals. They used a 2.45-GHz continuous wave for shorter periods of up to 30 days (16 h/day). Ono et al. [26] could not find any mutagenic effect of 2.45-GHz intermittent whole body RF exposure in the testes of mouse embryos. The methodological differences compared to those studies may account for the different outcomes we observed in our study. Since we suspected that the continuous exposure to Wi-Fi devices may have more pronounced effects compared with the shorter and intermittent exposure to MBs, we used a longer and continuous exposure as the test environment in this study. One may suspect that this does not reflect real life conditions, where the duration and intensity of exposure may be more variable. However, the growing technological involvement of the modern human being (with wireless modems, wireless

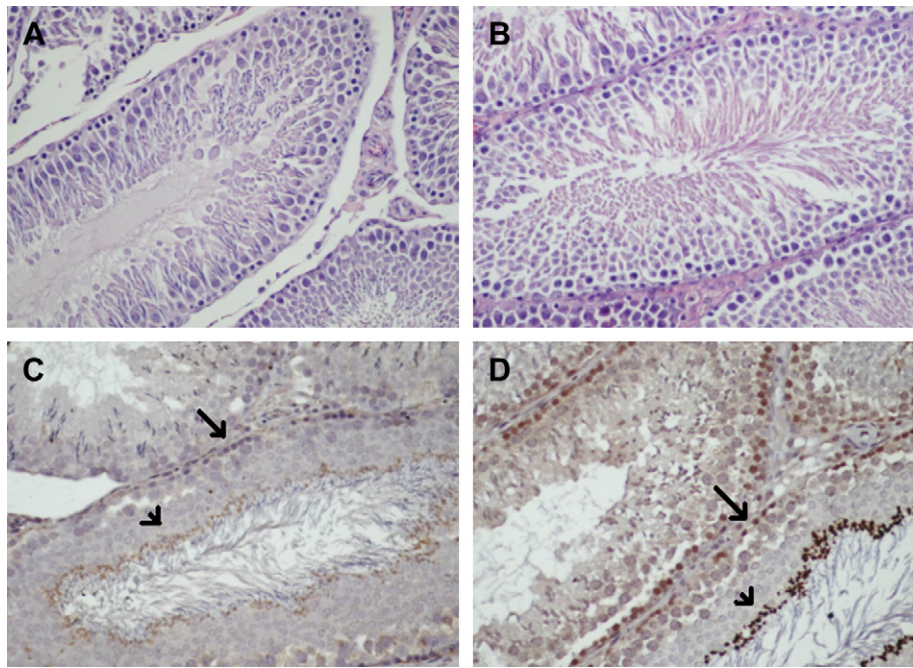


Figure 2 Hematoxylin & eosin (A, B) and immunohistochemical (C, D) staining of testicular tissues of control and Wi-Fi RF-exposed rats. (A,B) No difference was detected in routine histological examinations of the control and Wi-Fi groups (original magnification $\times 200$). (C) In the control group, 8-hydroxyguanosine nuclear positivity is nearly undetectable (original magnification $\times 200$). (D) Diffuse and intense bead-like nuclear immunopositivity (short and long arrows) is seen in the spermatocytic cells of the Wi-Fi group (original magnification $\times 200$).

routers, wireless home monitoring systems, wireless baby monitoring systems, wireless data bridges in between buildings, multiple Wi-Fi mobile phones that interact with wireless routers, use of wireless facilities for various entertainment purposes, etc.) probably exposes many children and adults to RF energy levels comparable to those used in this study. We also used a highly sensitive method to demonstrate any histopathologic changes that might have occurred.

In this study, 8-hydroxyguanosine immune reactivity was observed in both the control and study group. We think that the immune reactivity of the control group may have resulted from endogenous DNA damage caused by background electromagnetism emitted by the Earth or electronic devices or the metabolic rate of animals [27]. We avoided using reverberating or shielding chambers since we did not want to eliminate the effects caused by the background electromagnetism of the Earth on both groups. If we had eliminated this interference for the control group, the significant difference we found in our laboratory findings would not have reflected the differential real effect of RF from our wireless gateways, and would have yielded a falsely higher statistical difference between the two groups.

De Iuliis et al. [9] also showed a correlation between increasing SAR and decreased sperm motility and vitality, and increased OS and 8OHdG markers, stimulating DNA base adduct formation and ultimately DNA fragmentation. Although their results are in very good accordance with ours, they studied purified human spermatozoa ex vivo and used one night's exposure time with lower RF frequency and higher SAR values. To the best of our knowledge, our

study is the first in the literature to show oxidative DNA damage in the reproductive tissues of living organisms caused by conventional Wi-Fi devices.

OS is a well-established cause of male infertility and carcinogenesis [28,29]. Reactive oxygen species (ROS) from spermatozoa and invading leukocytes cause infertility by affecting sperm motility [30]. Physiologically, ROS are kept at low levels by intracellular antioxidant mechanisms of spermatozoa. Glutathione (GSH), a major thiol in mammals, is an important antioxidant, and plays a major role in the antioxidant defense mechanism against prooxidant offenders. Tissue GSH reflects the capability of tissues to scavenge the radicals, preserve the cellular reduction–oxidation balance, and defend the cells. The depletion of GSH in RF EMR-exposed animals could be responsible for the low percentages of motile sperm by affecting the spindle microtubule formation. Though we did not measure the GSH level of testis tissues, the decreased activity of GPX in the experimental group reflects a disturbance in the antioxidant defense system, and one possible mechanism for this may be the reduction in the availability of its substrate, GSH. The decreased activity of CAT, which is also an important part of the cellular antioxidant defense mechanism, may result in increased levels of prooxidants such as superoxide radicals and hydrogen peroxide. These findings support the theory that the decreased activities of antioxidant enzymes leading to OS in testis tissues might be responsible for the increased DNA oxidation as demonstrated by increased serum levels of 8OHdG and staining of spermatocytic cells by nuclear and perinuclear 8-hydroxyguanosine antibodies. The decreased SOD and XO activities, though insignificant, could imply a generalized

RF-induced decrease in enzyme activities. Similar findings were reported for human ejaculate exposed to RF EMR from MBs [31]. As MDA levels did not differ between our RF groups, we suggest that RF EMR from WIADs induces OS leading to DNA oxidation but not lipid peroxidation which disturbs testicular function and structure in rats exposed to these devices. The decrease in enzyme activities in general together with the increased 8OHdG serum levels and immunohistochemical staining observed are of great interest, since both would appear to result from WIAD RF.

The association of parameters used in our study with fertility is not certain. Even so, recent studies have demonstrated the relationship between oxidative DNA damage and infertility [32,33]. Others have found decreased CAT and GPX testicular activities to be responsible for fertility problems in animal models and humans [34–36]. Very similar to these studies, we have immunohistologically demonstrated DNA damage and found decreased CAT and GPX activities in the testicular tissues of rats, which may cause infertility. It should be noted that the DNA damage that occurred in the rat testes may be heritable. In order to demonstrate how this DNA damage affects the fertility and organ histology of offspring from mated rats, further functional and structural studies about fertility should be performed.

Due to their height, small children are more likely to be affected by wireless devices positioned close to the floor. Also, children using wireless computers or staying between two wireless devices are exposed to higher levels of radiation than the dose used in our study. The model we have used may not be the perfect one for demonstrating the deleterious effect of Wi-Fi devices, which would be a limitation. However, achieving a model 100% compatible with humans is nearly impossible in experimental animal studies.

Studies on the effects of MBs have used RF sources in direct or very close contact to the organs studied, while in real life this is hardly the case [37]. Unlike in previous MB studies where animals were restrained during the exposure or shielded against background Earth or exogenous electromagneticism, we allowed the animals to move freely, did not shield the control group from background radiation, and did not radiate the experimental group in direct contact with the RF antennae, in order to set up an experimental design more akin to real world conditions.

Our study is in agreement with previous studies that showed the role of MBs in male infertility and carcinogenesis [23,38]. Although the energy levels used in this study were derived from the parameters suggested by the manufacturer, such as distance, power output and duration, they were not safe for rat testicles. The question of whether tissues or organs other than the testicles are affected by these energy levels was not within the scope of this study and requires further research to be resolved. Obviously, extrapolating our findings to humans may not be justified, since the human body may have a more effective shielding capacity against RF energy compared to rats. Further studies are required to evaluate the long-term effects of the commonly used current (g type) and newer (n type) versions of WIADs on male infertility and germ cell mutagenesis in other species and human beings.

Conclusion

The results of the present study demonstrate that continuous long-term WIAD exposure oxidatively affects the testes in growing rats. Further human studies are needed to answer the question of whether RF waves emitted from Wi-Fi devices affect fertility.

Conflict of interest

None.

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Author's themselves.

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Smart Meters; Correcting the Gross Misinformation,
Letter by 54 Scientists and MDs; 2012

See this article: <https://maisonsaine.ca/sante-et-securite/electrosmog/smart-meters-correcting-gross-misinformation.html>

Smart Meters: Correcting the Gross Misinformation

[agfauteux](#) | 11 juin 2012 | [13 Commentaires](#)

Quebec-based magazine [La Maison du 21e siecle](#) asked physician [David O. Carpenter](#), former founding dean of the University at Albany (NY)'s School of Public Health, to comment an open letter published in the Montreal daily *Le Devoir* on May 24 2012. This letter claimed wireless smart meters pose no risk to public health. More than fifty international experts contributed to the following rebuttal.



Dr David O. Carpenter, founder, University at Albany (NY) School of Public Health

We, the undersigned are a group of scientists and health professionals who together have coauthored hundreds of peer-reviewed studies on the health effects of electromagnetic fields (EMFs). We wish to correct some of the gross misinformation found in the [letter regarding wireless “smart” meters that was published in the Montreal daily *Le Devoir* on May 24.](#)

Submitted by a group [Quebec engineers, physicists and chemists](#), the letter in question reflects an obvious lack of understanding of the science behind the health impacts of the radiofrequency (RF)/microwave EMFs emitted by these meters.

The statement that « Thousands of studies, both epidemiological and experimental in humans, show no increase in cancer cases as a result of exposure to radio waves of low intensity... » is [false \(1\). In fact, only a few such studies — two dozen case-control studies of mobile phone use](#), certainly not thousands, have reported no elevations of cancer, and most were funded by the wireless industry. In addition, these reassuring studies contained significant experimental design

flaws, mainly the fact that the populations followed were too small and were followed for a too short period of time.

Non industry-funded studies have clearly demonstrated a significant increase in cancer cases among individuals who have suffered from prolonged exposure to low-level microwaves, transmitted notably by radio antennas. The effects were best documented in meta-analyses that have been published and that include grouped results from several different studies: [these analyses](#) consistently showed an [increased risk of brain cancer](#) among regular users of a cell phone who have been exposed to microwaves [for at least ten years](#). Children and youths are especially vulnerable (2). For example, the [2009 Hardell-Carlberg study](#) reported a consistent association between use of mobile or cordless phones and two types of head tumors, astrocytoma grade I-IV and acoustic neuroma. The authors »found an especially high risk for persons that started use of mobile or cordless phones before the age of 20 years, although based on low numbers «.

Brain Cancer Rates

Furthermore, the argument that brain cancer rates do not indicate an overall increase in incidence is not evidence that cell phones are safe: the latency for brain cancer in adults after environmental exposure can be long, up to 20-30 years. Most North Americans haven't used cell phones extensively for that long. The evidence of the link between long-term cell phone use and brain cancer comes primarily from Northern Europe, where cell phones have been commonly used since the 1990s. Nevertheless, the [most recent collection of primary brain tumors mined from pathology units in Australia](#) showed brain cancer incidence rose by about 35% between 2000 and 2008 in the Australian Capital Territory and New South Wales (total population : more than 7 million).

In May 2011, after reviewing the published scientific literature regarding cancers affecting cell phone users, [the International Agency for Research on Cancer \(IARC\) classified radiofrequency radiation as a 2B, possible human carcinogen](#). Despite the absence of scientific consensus, the evidence is sufficiently compelling for any cautious parent to want to reduce their loved one's exposure to RF/microwave emissions as much as possible, as recommended by [various countries](#) such as Austria, Belgium, [Germany](#), [Russia](#) and the [United Kingdom](#).

Electrosensitivity

Public fears about wireless smart meters are well-founded. They are backed by various medical authorities such as those of the [Santa Cruz County](#)(California) Public Health Department. These authorities are worried about the growing number of citizens who say they have developed [electrohypersensitivity](#) (EHS), especially since for many of them, the symptoms developed after the installation of such meters (it takes some time for most people to link the two events).

Since the turn of the millennium, people are increasingly affected by ambient microwaves due to the growing popularity of wireless devices such as cell phones and Wi-Fi Internet. Therefore, the mass deployment of smart grids could expose large chunks of the general population to alarming risk scenarios without their consent. According to [seven surveys done in six European countries between 2002 and 2004, about 10% of Europeans have become electrosensitive](#). The most

famous person to [publicly reveal her electrosensitivity is Gro Harlem Brundtland](#), formerly Prime Minister of Norway and retired Director of the World Health Organization (WHO).

[While there is no consensus on the origins and mechanisms of EHS](#), many [physicians and other specialists around the world](#) have become aware that EHS symptoms (neurological dermatological, acoustical, etc.) seem to be triggered by exposure to EMF levels well below current international exposure limits, which are established solely on short-term thermal effects (3). Organizations such as the [Austrian Medical Association](#) and the [American Academy of Environmental Medicine](#) have recognized that the ideal way to treat of EHS is to reduce EMF exposure.

Therefore, caution is warranted because the growing variety of RF/microwave emissions produced by many wireless devices such as smart meters have never been tested for their potential biological effects.

Well-known bioeffects

While the specific pathways to cancer are not fully understood, it is scientifically unacceptable to deny the weight of the evidence regarding the increase in cancer cases in humans that are exposed to high levels of RF/microwave radiation.

The statement that « there is no established mechanism by which a radio wave could induce an adverse effect on human tissue other than by heating » is incorrect, and reflects a lack of awareness and understanding of the scientific literature on the subject. In fact, [more than a thousand studies](#) done on low intensity, high frequency, non-ionizing radiation, going back at least fifty years, show that some biological mechanisms of effect do not involve heat. This radiation sends signals to living tissue that stimulate biochemical changes, which can generate various symptoms and may lead to diseases such as cancer.

Even though RF/microwaves don't have the energy to directly break chemical bonds, unlike ionizing radiation such as X-rays, there is scientific evidence that this energy can cause DNA damage indirectly leading to cancer by a combination of biological effects. [Recent publications](#) have documented the generation of free radicals, [increased permeability of the blood brain barrier](#) allowing potentially toxic chemicals to enter the brain, induction of genes, as well as altered electrical and metabolic activity in human brains upon application of cell phone RF/microwaves similar to those produced by smart meters.

These effects are cumulative and depend on many factors including RF/microwave levels, frequency, waveform, exposure time, biovariability between individuals and combination with other toxic agents. Clear evidence that these microwaves are indeed bioactive has been shown by the fact that low-intensity EMFs have proven clinically useful in some circumstances. Pulsed EMFs have long been used to successfully [treat bone fractures](#) that are resistant to other forms of therapy. More recently, frequency-specific, amplitude-modulated EMFs have been found useful to treat [advanced carcinoma](#) and chronic pain.

High frequency EMFs such as the microwaves used in cell phones, smart meters, Wi-Fi and cordless “DECT” phones, appear to be the most damaging when used commonly. Most of their

biological effects, including symptoms of electrohypersensitivity, can be seen in the damage done to cellular membranes by the [loss of structurally-important calcium ions](#). Prolonged exposure to these high frequencies may eventually lead to cellular malfunction and death.

Furthermore, malfunction of the parathyroid gland, located in the neck just inches from where one holds a cell phone, may actually cause electrohypersensitivity in some people by reducing the background level of calcium ions in the blood. RF/microwave radiation is also known to [decrease the production of melatonin](#), which protects against cancer, and to [promote the growth of existing cancer cells](#).

Early warning scientists attacked

In recommending that the Precautionary Principle be applied in EMF matters, the European Environment Agency's Director [Jacqueline McGlade wrote in 2009](#): "We have noted from previous health hazard histories such as that of lead in petrol, and methyl mercury, that 'early warning' scientists frequently suffer from discrimination, from loss of research funds, and from unduly personal attacks on their scientific integrity. It would be surprising if this is not already a feature of the present EMF controversy... » Such unfortunate consequences have indeed occurred.

The statement in the *Le Devoir* letter that « if we consider that a debate should take place, it should focus exclusively on the effects of cell phones on health » is basically an acknowledgement that there is at least some reason to be concerned about cell phones. However, while the immediate exposure from a cell phone is of much greater intensity than the exposure from smart meters, cell phone use is temporary.

Smart meters

[As Australian Associate Professor of neurosurgery Vini G. Khurana reports](#), adverse neurological effects have been reported in people who sustain close proximity to wireless meters, especially under 10 feet (3 metres).

A wireless smart meter produces radiofrequency microwave radiation with two antennas in approximately the same frequency range (900 MHz to 2.4 GHz) as a typical cell tower. But, depending on how close it is to occupied space within a home, a smart meter can cause much higher RF exposures than cell towers commonly do. If a smart meter is located on a common wall with a bedroom or kitchen rather than a garage wall, for example, the RF exposure can be the same as being within 200 to 600 feet distance of a cell tower with multiple carriers. With both cell towers and smart meters, the entire body is immersed by microwaves that go out in all directions, which increases the risk of overexposure to many sensitive organs such as the eyes and testicles. With a cell phone, people are exposed to microwaves primarily in the head and neck (unless using speaker mode), and [only when the device is turned on or in standby mode](#).

Wireless smart meters typically produce atypical, relatively potent and very short pulsed RF/microwaves whose biological effects have never been fully tested. They emit these millisecond-long RF bursts on average 9,600 times a day with a maximum of 190,000 daily transmissions and a peak level emission two and a half times higher than the stated safety signal, as the California utility [Pacific Gas & Electric recognized](#) before that State's Public Utilities

Commission. Thus people in proximity to a smart meter are at risk of significantly greater aggregate of RF/microwave exposure than with a cell phone, not to mention the cumulative exposure received by people living near multiple meters mounted together, pole-mounted routers or utility collector meters using a third antenna to relay RF signals from 500 to 5,000 homes.

[A technical study performed by Sage Associates](#) in California indicates that RF levels from various scenarios depicting normal smart meter installation and operation may violate even the out-of-date US public safety standards which only consider acute thermal effects. This can happen when a person stands close to the meter to read the power consumption, or touches it, or shades the meter face with a hand to better read it. Emissions are also increased by reflective materials, such as stainless steel, other metals and mirrors, which can re-radiate stronger than the otherwise unaltered background. Microwaves are absorbed and dissipated by partially conductive materials, such as cement and special RF shielding paints and fabrics.

In addition to the erratic bursts of modulated microwaves emitted by wireless smart meters transferring usage data to electric, gas and water utilities, wireless as well as wired smart (powerline communication) meters are also a [major source](#) of ‘‘dirty electricity’’ (electrical interference of high frequency voltage transients typically of kilohertz frequencies). Some scientists, such as [American epidemiologist Sam Milham](#), believe that many of the health complaints about smart meters may also be caused by dirty electricity generated by the « switching » power supply activating all smart meters. Since the [installation of filters to reduce dirty electricity](#) circulating on house wiring has been found to relieve symptoms of EHS in some people, this method should be considered among the priorities aimed at reducing potential adverse impacts. Indeed, the Salzburg State (Austria) Public Health Department confirms its concern about the potential public health risk when in coming years almost every electric wire and device will emit such transient electric fields in the kilohertz-range due to wired smart meters.

Rather be safe than sorry

The apparent adverse health effects noted with smart meter exposure are likely to be further exacerbated if smart appliances that use wireless communications become the norm and further increase unwarranted exposure.

To date, there have been few independent studies of the health effects of such sources of more continuous but lower intensity microwaves. However, we know after decades of studies of hazardous chemical substances, that chronic exposure to low concentrations of microwaves can cause equal or even greater harm than an acute exposure to high concentrations of the same microwaves.

This is why so many scientists and medical experts urgently recommend that measures following the Precautionary Principle be applied immediately — such as using wired meters — to reduce biologically inappropriate microwave exposure. We are not advocating the abolishment of RF technologies, only the use of common sense and the development and implementation of best practices in using these technologies in order to reduce exposure and risk of health hazards.

(1) • [Scientific papers on EMF health effects](#)

(2) On Nov. 19 2012, we struck from this letter an error propagated in the media claiming that « In May 2012, the [U.K.'s Office of National Statistics reported a 50 percent increase in incidence of frontal and temporal lobe tumors in children between 1999 and 2009.](#) »

(3) [Explanation and studies on electrosensitivity](#)

(4) [Governments and organizations that ban or warn against wireless technology](#)

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- [Franz Adlkofer](#), M.D., Chairman of the [Pandora Foundation](#), Coordinator of the [European Reflex Report](#) on [DNA-damage by cellphone radiation](#), Neuendorf, Germany
- [M. S. H. Al Salameh](#), PhD, Professor of Electrical Engineering, University of Science & Technology, Irbid, Jordan
- [Jennifer Armstrong](#), MD, Past President, American Society for Environmental Medicine, Founder, Ottawa Environmental Health Clinic, Ontario, Canada
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