BASIC RESEARCH

Rat testicular impairment induced by electromagnetic radiation from a conventional cellular telephone and the protective effects of the antioxidants vitamins C and E

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OBJECTIVE: The aim of this study was to investigate the possible effects of electromagnetic radiation from conventional cellular phone use on the oxidant and antioxidant status in rat blood and testicular tissue and determine the possible protective role of vitamins C and E in preventing the detrimental effects of electromagnetic radiation on the testes.

MATERIALS AND METHODS: The treatment groups were exposed to an electromagnetic field, electromagnetic field plus vitamin C (40 mg/kg/day) or electromagnetic field plus vitamin E (2.7 mg/kg/day). All groups were exposed to the same electromagnetic frequency for 15, 30, and 60 min daily for two weeks.

RESULTS: There was a significant increase in the diameter of the seminiferous tubules with a disorganized seminiferous tubule sperm cycle interruption in the electromagnetism-exposed group. The serum and testicular tissue conjugated diene, lipid hydroperoxide, and catalase activities increased 3-fold, whereas the total serum and testicular tissue glutathione and glutathione peroxidase levels decreased 3-5 fold in the electromagnetism-exposed animals.

CONCLUSION: Our results indicate that the adverse effect of the generated electromagnetic frequency had a negative impact on testicular architecture and enzymatic activity. This finding also indicated the possible role of vitamins C and E in mitigating the oxidative stress imposed on the testes and restoring normality to the testes.

KEYWORDS: Electromagnetic Radiation; Testes; Infertility; Vitamins C and E.

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INTRODUCTION

Radiofrequency (RF) fields are part of the electromagnetic spectrum and are generally defined as covering the range of frequencies from 100 kHz to 300 GHz. Mobile phones communicate by transmitting radiofrequency waves, which are electromagnetic fields and, unlike ionizing radiation (e.g., X-rays and gamma rays) cannot break chemical bonds or cause ionization in the human body.

Given the large number of mobile phone users, investigating, understanding, and monitoring any potential public health impacts of mobile phone use are important. To date, the adverse health effects of mobile phone use are debatable. Partly in response to public concerns, a number of research studies on the potential health effects of electromagnetic fields (EMFs) have been performed in Europe, North America, and elsewhere. Studies to assess the potential long-term effects of mobile phone use are ongoing. The exact mechanism of the EMF interactions is not well understood, although a few studies have suggested the involvement of lipid peroxidation and free radical formation (1), as well as biochemically induced oxidative stress.

There have been many scientific studies warning about the adverse biological effects of this type of electromagnetic radiation (EMR) on the health of humans, animals, and birds (2-4). Although the radio waves of cellular phones do not have enough energy to cause the ionization of atoms and molecules, the recent concerns over long-term exposure to the electromagnetic radiation emitted by mobile phones should be taken more seriously given the growing trend toward deterioration of the male germ line (spermatogenesis and sperm maturation) (5). A high-quality, more recent epidemiological animal study was conducted by the Technical Working Group (TWG) on the health effects of EMFs (6).

No potential conflict of interest was reported.

Electromagnetic radiation is one of the environmental toxicants that are capable of compromising male fertility by inducing a state of oxidative stress in the testes.

Consequently, there is an urgent need to identify antioxidants that can supplement the tissue's own antioxidant strategies to rescue the testes from the consequences of a ROS attack. Therefore, this study has been performed to clarify the activities of some free radical scavenger enzymes in the blood and testicular tissue and the possible protective role of vitamins C and E after mobile phone-induced testicular impairment.

MATERIALS AND METHODS

Animals

The study population comprised 120 male Wister albino rats (body weight, 200±20 grams) that were obtained from the Experimental Animal Center of the College of Pharmacy, King Saud University in Riyadh, Saudi Arabia. The procedures used for animal care and housing were in accordance with the U.S. Department of Agriculture through the Animal Welfare Act (7USC 2131) of 1985 and the Animal Welfare Standards incorporated in 9 CFR Part 3, 1991. The rats were maintained under standard laboratory conditions in an air-conditioned room, where the temperature was maintained at 25℃ with constant humidity (40-50%), and kept on a 12/12-hour light/dark cycle throughout the experiment. They were provided with standard food pellets and water *ad libitum*. They were randomly divided into two main groups, the control and exposure groups, as follows:

Control groups

Control group (without stress and with the EMR exposure device turned off) (n = 10)

Standard food pellets + vitamin C (40 mg/kg/day)-treated group (n = 10)

Standard food pellets + vitamin E (2.7 mg/kg/day)-treated group (n = 10)

Experimental EMR-exposed groups

EMR-exposed (n = 30)

EMR-exposed + vitamin C (40 mg/kg/day)-treated group (n = 30)

EMR-exposed + vitamin E (2.7 mg/kg/day)-treated group (n = 30)

Every ten rats were placed in a separate cage to avoid the stress of isolation or overcrowding. The duration of the exposure was 15, 30, and 60 min/day for 14 consecutive days for each of the six groups. At the end of each experimental period, ten animals from each group were sacrificed under mild diethyl ether anesthesia. Blood samples were taken from the retro-orbital venous plexus and centrifuged at 700 g for 30 min. The clear, non-hemolyzed supernatant sera were quickly removed and kept at −20℃ for further biochemical analysis. In addition, the testes were homogenized in an isotonic solution and kept at −20℃ for further biochemical investigations conducted to estimate the anti-oxidant parameters.

Whole-body exposure to electromagnetic radiation

In the EMR exposure room, there were no other metal or ferromagnetic materials around the clean benches that would change the structure of the electromagnetic field. The use of any EMR-emitting device (such as an extra cellular phone, centrifuge, fluorescent light ballasts, and computers) was not allowed so that the EMR generated by any of this equipment would not interfere with the experimental environment (7). The groups were separated from each other, with the control group isolated far from the source of the EMR. The EMR-exposed animal group was taken to the exposure room and then exposed to the EMR emitted by a commercially available GSM cellular phone (900/1800/1900 MHz, 2-W peak power, average power density of 0.02 mW/cm^2 at a specific absorption rate of approximately 0.9 W/kg). The distance between the phone and the rats was 50 cm. The rats were placed in Plexiglas cages with drilled ventilation holes of cm Ø that were attached with a mobile phone hand set (8). The control animals were exposed to a mobile phone without a battery in similar cages for the same period in a separate but similar room.

Biochemical assays to determine oxidative stress

Several oxidation-related analytes were assayed to investigate a range of oxidative changes potentially associated with EMR exposure. To estimate the different oxidative stress parameters and the anti-oxidant enzymes, a portion of the testes (0.25 g) was freeze-clamped, crushed at liquid nitrogen temperature, ice-cooled, homogenized in 2.5 ml of phosphate buffer (pH 7.4), and then centrifuged at 30,000 *g* for 15 min at 4℃. The supernatant was collected and preserved at −20℃ until use. Total glutathione (GSH; CS0260 Sigma-Aldrich, St. Louis, MO, USA), glutathione peroxidase (c-GPx; CGP1-1KT Sigma-Aldrich), and catalase (CAT; Cat100-1KT Sigma-Aldrich) levels were measured. In the course of our investigation of radiation-induced oxidation, we measured two indexes of peroxidation, namely conjugated dienes (CD; performed according to the method of Girotti et al. (9)) and hydroperoxides (LIP Di; Peroxi-Detect kit [PD1, Sigma-Aldrich]).

Conjugated diene determination

Conjugated dienes (CD) were assayed as a measure of early lipid oxidation, and thiobarbituric acid-reactive substances (TBARS) were assayed as an indicator of later aldehyde formation. The conjugated diene concentrations were determined with a modified assay (9).

Histopathology

Testis tissues were submitted for histological and morphologic examinations using conventional methods and were stained with hematoxylin and eosin.

Statistical analysis

The data were analyzed using analysis of variance (ANOVA; 30), followed by LSD analysis to evaluate the variations between groups and for multiple comparisons among different groups. The results are expressed as the means±SD. Values of $p>0.05$ were considered statistically insignificant, whereas values of $p<0.05$ were considered statistically significant.

RESULTS

Histopathological alterations

The testis sections in the three control groups revealed that the seminiferous tubules consist of several layers of epithelial cells (Figure 1). The cuboidal spermatogonia cells

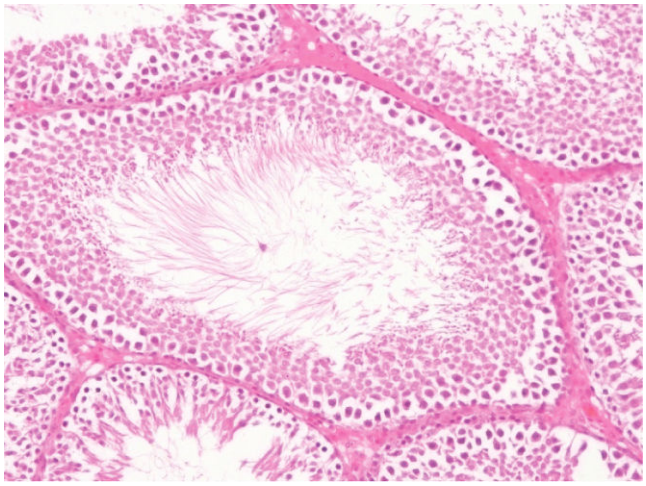

Figure 1 - Section of rat testis showing the normal seminiferous tubules, interstitium, spermatids and spermatogenic cells at different stages of development. H&E ×400.

have clear cytoplasm and rounded nuclei, which may show division. Sertoli cells are large epithelial cells that have a vesicular nucleus with a large nucleolus. Next to the spermatogonia cells is a zone of spermatocytes, the nuclei of which are usually in mitotic division. A large number of small cells, the spermatids, are seen external to the spermatocytes. The spermatids then further develop into spermatozoa. The latter usually lie in groups with their heads projecting between the deeper cells and are connected with one of the Sertoli cells of the lining epithelium.

In contrast, in the sections of testis exposed to EMR, the histopathological changes in the testis exhibited degenerative changes and high levels of mitotic division after 14 days of exposure (Figures 2 and 3). The sections show degeneration in the seminiferous tubules with the complete absence of spermatozoa, and some spermatogonic nuclei also show karyomegaly and a high incidence of mitotic divisions. In the EMF group, the diameter of the seminiferous tubules was significantly increased compared with the normal unexposed EMR rat testicular architecture, whereas the mean height of

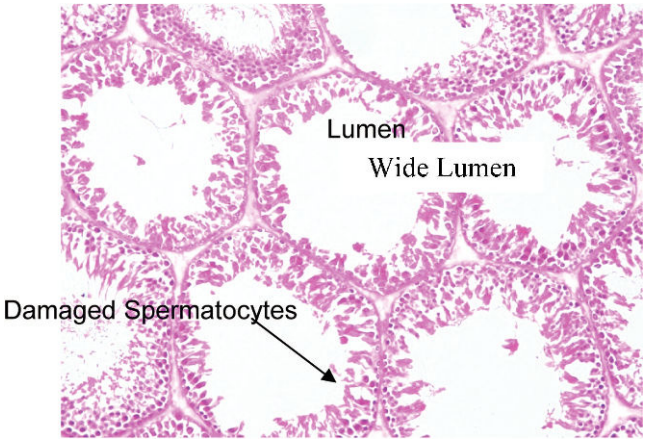

Figure 2 - Photograph of a section of rat testis exposed to EMR for 60 minutes showing the damaged spermatocytes and spermatids, marked mitotic divisions and pyknotic nuclei of some spermatogonia, widening of the seminiferous tubular lumen and absence of spermatozoa within the lumen. H&E ×100.

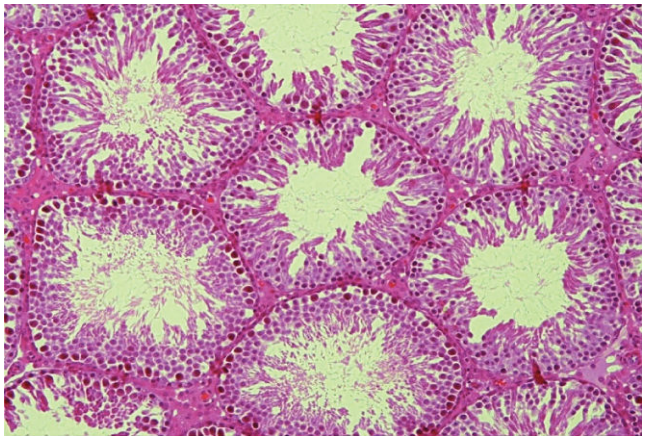

Figure 3 - Photomicrograph of the marked regenerative effect of protective treatment with vitamin C showing normal seminiferous tubules, spermatocytes and spermatids. H&E ×100.

the germinal epithelium was significantly decreased. A reduction in the spermatid numbers within the lumen of the seminiferous tubules, marked mitotic divisions and pyknosis of some spermatogonic nuclei were observed in the testis sections after a two-week exposure to EMR.

However, supplemental treatment with vitamin C or E for two weeks had a marked regenerative effect on the histopathological features of the seminiferous tubules (Figures 3 and 4), causing a significant decrease in the effects of EMR.

Biochemical changes

The concentrations of the oxidative markers in the blood and testicular tissue at the end of each treatment period are shown in Tables 1 and 2. The changes in the levels of blood GSH, c-GPx, CD, LIP Di, and CAT are reported in Table 1. The levels of GSH and GPx were significantly elevated in all of the EMR-exposed groups compared with the control group, whereas the levels of CD, LIP Di, and CAT were decreased. In contrast, the levels of GSH, c-GPx, CD, LIP Di, and CAT in the vitamin C- and E-supplemented groups were significantly ($p<0.05$) lower than those in the EMR-exposed groups. Vitamins C and E seemed to provide

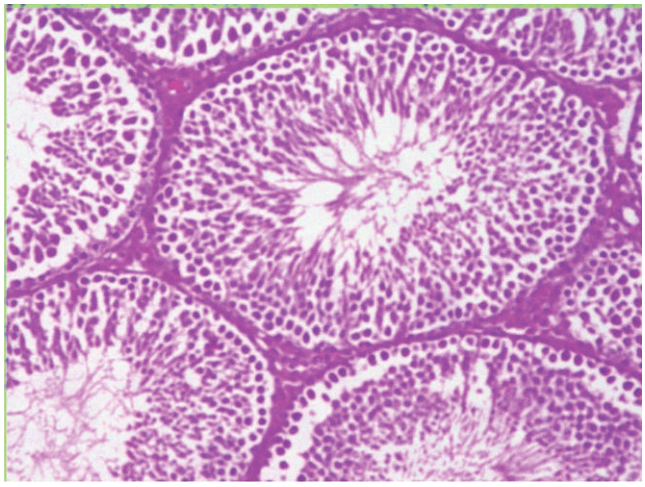

Figure 4 - Magnified photograph of a section of testis after the protective treatment with vitamin E for two weeks showing remarkable regenerative features. H&E ×400.

Table 1 - Effects of vitamin C and vitamin E pretreatment on the biochemical parameters in the serum of control and EMR-exposed rats (2-week exposure time).

Treatments	Duration of EMR exposure														
	15 min/day					30 min/day					60 min/day				
	Enzymes					Enzymes					Enzymes				
	GSH (µmol/g)	c-GPx (Units/ml)	CD (µmol/mg)	LIP Di (nmol/ml)	CAT (µmol/ml)	GSH (µmol/g)	c-GPx (Units/ml)	CD (µmol/mg)	LIP Di (nmol/ml)	CAT (µmol/ml)	GSH (µmol/g)	c-GPx (Units/ml)	CD (µmol/mg)	LIP Di (nmol/ml)	CAT (µmol/ml)
1	3.90 ±0.3	15.80 ±1.1	2.18 ± 0.2	3.76 0.5	0.49 ±0.1	3.90 ±0.3	15.80 ±1.1	2.18 ±0.2	3.76 ±0.5	0.49 ±0.1	3.90 ±0.3	15.80 ±1.1	2.18 ±0.2	3.76 ±0.5	0.49 ±0.1
2	3.55 ±0.3	15.58 ±1.1	2.06 ±0.2	3.70 ±0.4	0.54 ±0.1	3.55 ±0.3	15.58 ±1.1	2.06 ±0.2	3.70 ±0.4	0.54 ±0.1	3.55 ±0.3	15.58 ±1.1	2.06 ±0.2	3.70 ±0.4	0.54 ±0.1
3	3.48 ±0.3	15.01 ±0.8	1.82 ±0.2	3.70 ±0.5	0.56 ±0.1	3.48 ±0.3	15.01 ±0.8	1.82 ±0.2	3.70 ±0.5	0.56 ±0.1	3.48 ±0.3	15.01 ±0.8	1.82 ±0.2	3.70 ±0.5	0.56 ±0.1
4	2.11 * ±0.1	11.38 * ±1.0	3.40 ±0.4	5.25 ±0.7	1.01 ±0.1	1.45 ±0.1	9.00 * ±0.7	4.60 ±0.5	5.70 * ±0.6	2.13 * ±0.2	1.08 * ±0.1	6.60 * ±0.5	6.00 * ±0.6	6.63 * ±0.7	3.39 * ±0.3
5	3.11 ±0.2	14.25 * ±1.1	2.40 ±0.2	5.03 ±0.4	0.72 ±0.6	3.03 * ±0.2	12.88 * ±0.8	2.66 ±0.3	5.05 ±0.7	0.71 * ±0.1	2.76 * ±0.2	10.63 * ±0.5	3.50 * ±0.1	5.51* ±0.7	0.94 * ±0.1
6	2.96 ±0.2	12.91* ±1.0	2.25 ±0.2	4.63 ±0.4	0.74 ±0.0	3.01 * ±0.2	13.30 * ±0.8	3.08 ±0.3	4.96 ±0.6	0.79 * ±0.0	2.76 * ±0.3	10.60 * ±0.5	3.71* ±0.0	5.28 * ±0.5	1.38 * ±0.1

*Significant at the level of $p<0.05$.
1 = without stress and EMR **(control)**.
2 = without stress and EMR + vitamin C (40 mg/kg/day) **(control)**.
3 = without stress and EMR + vitamin E (2.7 mg/kg/day) **(control)**.
4 = EMR-exposed.
5 = EMR-exposed + vitamin C (40 mg/kg/day).
6 = EMR-exposed + vitamin E (2.7 mg/kg/day).
GSH = Total glutathione.
c-GPx = Cellular glutathione peroxidase.
CD = Conjugated dienes.
CAT = Catalase.

Table 2 - Effects of vitamin C and vitamin E pretreatment on the antioxidant levels in the testicular tissue of control and EMR exposed rats for two weeks.

Treatments	Duration of EMR exposure														
	15 min/day					30 min/day					60 min/day				
	Enzymes					Enzymes					Enzymes				
	GSH (µmol/g)	c-GPx (Units/ml)	CD (µmol/mg)	LIP Di (nmol/ml)	CAT (µmol/ml)	GSH (µmol/g)	c-GPx (Units/ml)	CD (µmol/mg)	LIP Di (nmol/ml)	CAT (µmol/ml)	GSH (µmol/g)	c-GPx (Units/ml)	CD (µmol/mg)	LIP Di (nmol/ml)	CAT (µmol/ml)
1	4.58 ±0.4	9.00 ±0.6	4.58 ±0.5	5.38 ±0.4	3.70 ±0.4	4.58 ±0.4	9.00 ±0.6	4.58 ±0.5	5.38 ±0.4	3.70 ±0.4	4.58 ±0.4	9.00 ±0.6	4.58 ±0.5	5.38 ±0.4	3.70 ±0.4
2	4.20 ±0.4	9.43 ±0.6	5.61 ±0.5	4.55 ±0.4	3.86 ±0.4	4.20 ±0.4	9.43 ±0.6	5.61 ±0.5	4.55 ±0.4	3.86 ±0.4	4.20 ±0.4	9.43 ±0.6	5.61 ±0.5	4.55 ±0.4	3.86 ±0.4
3	4.49 ±0.4	9.51 ±0.5	4.90 ±0.6	4.31 ±0.4	3.86 ±0.4	4.49 ±0.4	9.51 ±0.5	4.90 ±0.6	4.31 ±0.4	3.86 ±0.4	4.49 ±0.4	9.51 ±0.5	4.90 ±0.6	4.31 ±0.4	3.86 ±0.4
4	2.96 * ±0.3	5.81* ±0.5	6.33 * ±0.7	8.93 * ±0.7	5.40 * ±0.4	1.71* ±0.2	4.40 * ±0.5	9.21 * ±0.5	12.03 * ±0.7	7.48 * ±0.4	1.03 * ±0.3	2.34 * ±0.4	12.20 * ±0.6	15.18 * ±0.9	10.34 * ±0.4
5	3.59 ±0.4	7.75 * ±0.5	5.25 ±0.7	6.36 * ±0.5	4.25 ±0.5	4.19 * ±0.5	7.28 * ±0.6	6.68 * ±0.6	7.56 * ±0.4	4.65 * ±0.4	2.79 * ±0.4	7.66 * ±0.5	7.72 * ±0. 8	8.74 * ±0.4	5.96 * ±0.5
6	3.81 ±0.5	9.08 * ±0.6	5.20 ±0.6	5.80 * ±0.7	4.36 ±0.6	3.91* ±0.4	7.68 * ±0.5	5.75 * ±0.6	6.95 * ±0.4	5.21* ±0.5	3.36 * ±0.5	7.34 * ±0.6	6.94 * ±0.7	7.78 * ±0.5	5.74 * ±0.6

*Significant at the level of $p<0.05$.
1 = without stress and EMR **(control)**.
2 = without stress and EMR + vitamin C (40 mg/kg/day) **(control)**.
3 = without stress and EMR + vitamin E (2.7 mg/kg/day) **(control)**.
4 = EMR-exposed.
5 = EMR-exposed + vitamin C (40 mg/kg/day).
6 = EMR-exposed + vitamin E (2.7 mg/kg/day).
GSH = Total glutathione.
c-GPx = Cellular glutathione peroxidase.
CD = Conjugated dienes.
CAT = Catalase.

significant protection against the oxidative stress induced by the exposure of the rats to EMR.

The changes in the levels of GSH, c-GPx, CD, LIP Di, and CAT in the testicular tissue homogenates are provided in Table 2. The GSH level and GPx activity decreased significantly ($p<0.05$) in the EMR-exposed rats compared with the control group. The percentage decrease ranged from 64.6 to 26.0%. In contrast, the levels of CD, LIP Di and CAT increased significantly in the EMR-exposed rats compared with the control group. However, the protective effect of vitamins C and E was evident from the increased activities of GSH and c-GPx in the irradiated testicular tissue, which reached normal levels. Moreover, the levels of CAT and the two markers of lipid peroxidation, CD and LIP Di, were significantly decreased in the irradiated testicular tissue of the supplemented vitamin groups, which reached normal levels. The protective effect of vitamins C and E was evident from the increased activity of GSH and c-GPx and decreased levels of CD, LIP Di, and CAT to approximately normal values in the testicular tissue.

DISCUSSION

Mobile phones create an EMF around them when in use, thus increasing the electromagnetic contamination, also known as ''electrosmog'', in the vicinity. In response to public and governmental concern, the WHO established the International Electromagnetic Fields (EMF) Project in 1996 to assess the scientific evidence of the possible adverse health effects of the electromagnetic fields and to conduct a formal health risk assessment of radiofrequency field exposure by 2012 to fill these knowledge gaps (10). Biological systems are affected by microwave radiation primarily due to the resulting increase in temperature, i.e., thermal damage (11), although non-thermal effects have also been studied (12). The cellular target of EMF is still controversial. Recent studies on human semen have suggested an increased production of ROS in human semen due to cell phone radiation (13,14). An analysis of antioxidant enzymes glutathione peroxidase and superoxide dismutase showed a decrease, while an increase in catalase was observed. Malondialdehyde showed an increase and histone kinase showed a significant decrease in the exposed group. Micronuclei also show a significant decrease in the exposed group. A significant change in sperm cell cycle and were recorded. Generation of free radicals was recorded to be significantly increased. The findings of Kesari et al. (15) on antioxidants, malondialdehyde, histone kinase, micronuclei, and the sperm cell cycle are clear indications of an infertility pattern that is initiated due to an overproduction of reactive oxygen species. They also concluded that radiofrequency electromagnetic waves from commercially available cell phones may affect the fertilizing potential of spermatozoa.

There is evidence from several high-quality studies published before 2000 suggesting a lack of effects on testicular function in experimental animals provided that the exposures do not induce hyperthermia. The responses to RF fields are identical to those induced by heating using conventional means. Very few recent studies have addressed this issue, and these studies mostly support the conclusion that male fertility and testicular function are not affected in the absence of hyperthermia (16-19).

In contrast, several authors stated that electromagnetic waves have a wide range of damaging effects on the male reproductive system and sperm parameters and cause significant changes in the sperm cell cycle (15-24).

ROS are continuously neutralized by antioxidants present in body tissues (25). Whenever the production of ROS exceeds the scavenging capacity of antioxidants, oxidative stress (OS) will result (26). In 1992, researchers found that electromagnetic fields increase the free radical activity in cells (27). Within the last decade, *in vivo* animal studies have shown that OS develops in response to cell phone radiation (28-32). EMR may disturb ROS metabolism by increasing the production of ROS or by decreasing the activity of antioxidant enzymes. The capability of EMR to induce oxidative stress in the testes strongly suggests that the testis is a vulnerable tissue that is highly dependent on oxygen to drive spermatogenesis and yet highly susceptible to the toxic effects of reactive oxygen metabolites; in this context, the testis is very similar to the brain.

In an analysis of antioxidant enzymes, Kesari et al. (15) found a decrease in GSH and an increase in catalase. The group exposed to EMR exhibited an increased level of malondialdehyde, a result that was also evident in our study. However, studies designed to measure malonaldehyde (MDA) levels have shown conflicting results (16,18,29,31,33).

Our findings demonstrate that the exposure of male rats to EMR emitted by mobile phones alters the enzymatic activity in the blood and testicular tissue. Bediz et al. (34) suggested that long-term exposure to low-frequency EMR increases lipid peroxidation in the brain and other body organs. Our results are in partial agreement with the findings of other studies (34-36).

Some authors have shown that electromagnetic fields (EMFs) penetrate living organisms and alter the cell membrane potential (37). This alteration may affect free-radical processes within the cell and alter the activities of antioxidant enzymes, particularly CAT and c-GPx, in different organs. At the level of the testes, oxidative stress is capable of disrupting the steroidogenic capacity of Leydig cells (38) and the capacity of the germinal epithelium to differentiate normal spermatozoa (39). Many studies have indicated that EMR decreases the size of the testicular organs and the diameter of the seminiferous tubules (16,33,40-42). However, a study by Ribeiro et al. (18) and a follow-up study by Dasdag et al. (43) could not find any significant adverse effects of cellular phones (1835-1850 MHz) on the rat testis. Wang et al. (44) suggested that RF-EMR may change the permeability of the blood-testis barrier. RF-EMR-mediated ROS formation can lead to heat shock protein (hsp) production and phosphorylation, which can alter the secretion of growth factors and mediate an increase in the blood-testis barrier permeability, as suggested by Desai et al. (26).

Although the testes clearly do possess highly specialized antioxidant defensive enzymes, there are clear benefits to be gained by treating susceptible individuals with exogenous antioxidants. A variety of antioxidants have been assessed for their ability to counteract EMR-induced oxidative stress in the testes. To determine the relative potential of different antioxidants to counteract oxidative stress in the testes, the exposure of the rats to EMR for different periods and the use of vitamins C and E as defensive agents involved the application of antioxidant therapy prior to or in conjunction with the creation of a brief period of oxidative stress and the subsequent comparison of various testicular attributes (lipid peroxidation, histopathology, and antioxidant enzyme status) with those of the controls. In the present study, vitamins

C and E, when given as a supplement in conjunction with the EMR exposure, were shown to effectively protect the testes from the oxidative damage created by the exposure to EMR and significantly suppress the oxidative DNA damage induced in the rat testicular cells.

Vitamins C and E are powerful antioxidants that are vital for the maintenance of mammalian spermatogenesis and that counteract the testicular oxidative stress induced by pro-oxidant exposure (45-51). Vitamin E has also been shown to suppress lipid peroxidation in testicular microsomes and mitochondria (52,53) and to reverse the detrimental effects of oxidative stress on testicular function (45,49,54-59). Studies have demonstrated that antioxidants, such as melatonin, caffeic acid phenyl ester, vitamin C and vitamin E, prevent the oxidative stress and apoptosis caused by EMR in animal tissues (28,29,41,60,61). This prophylactic treatment could thus be used to counteract the observations recorded by Mailankot et al. (62), who found that rats exposed to mobile phone emissions for 1 hour a day for 28 days had impaired semen quality that may impair male fertility because the RF-EMR exposure resulted in a significant increase in lipid peroxidation and a low GSH content in the testis and epididymis. Notably, pulsed electromagnetic field (PEMF) therapy was recently used as a scavenging agent to combat oxidative stress (63). PEMF is generated by many medical devices used in medical therapies. Therefore, the issue of whether vitamins C and E could be used along with PEMF to relieve the adverse effects of EMR remains to be elucidated.

In summary, EMR has a negative effect on testicular function through the induction of oxidative stress and the concomitant disruption of the testicular antioxidant status. Given the importance of vitamins C and E in this defensive strategy, the ability of antioxidants, such as vitamins C and E, to ameliorate this pathology confirms their importance in overcoming oxidative stress in this context. Vitamins C and E ameliorate the EMR-induced oxidative stress in the testes, thus facilitating the restoration of testicular tissue morphology and function by suppressing testicular lipid peroxidation and restoring the levels of GST and GSH to normal physiological levels.

AUTHOR CONTRIBUTIONS

The author was solely responsible for the entire experiment, which included planning and performing the experiments and writing and revising the manuscript.

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