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Mobile phone radiation during pubertal development has no effect on testicular histology in rats

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Abstract

Mobile phones are extensively used throughout the world. There is a growing concern about the possible public health hazards posed by electromagnetic radiation emitted from mobile phones. Potential health risk applies particularly to the most intensive mobile phone users—typically, young people. The aim of this study was to investigate the effects of mobile phone exposure to the testes, by assessing the histopathological and biochemical changes in the testicular germ cells of rats during pubertal development. A total of 12 male Sprague Dawley rats were used. The study group ($n = 6$) was exposed to a mobile phone for 1 h a day for 45 days, while the control group ($n = 6$) remained unexposed. The testes were processed with routine paraffin histology and sectioned. They were stained with hematoxylin–eosin, caspase 3, and Ki-67 and then photographed. No changes were observed between the groups ($p > 0.05$). The interstitial connective tissue and cells of the exposed group were of normal morphology. No abnormalities in the histological appearance of the seminiferous tubules, including the spermatogenic cycle stage, were observed. Our study demonstrated that mobile phones with a low specific absorption rate have no harmful effects on pubertal rat testicles.

Keywords

Mobile phones, caspase 3, Ki-67, testes, puberty, rat

Introduction

We are continually exposed to electromagnetic radiation from numerous sources, including mobile phones. The use of mobile phones is now widespread and is on the rise (Repacholi, 2001). According to the data from the International Telecommunication Union of the United Nations, the number of mobile phone users exceeded 5.5 billion by the end of 2011. Excessive mobile phone use has encouraged interest in understanding the relationship between electromagnetic fields (EMFs) and human health (Fejes et al., 2007; Sage, 2000).

The effects of mobile phones on the reproductive system and on fetal development have attracted scientific scrutiny, and consequently, the number of studies investigating this issue has gradually increased (Celik et al., 2012).

Most animal studies on the topic have examined histopathological testicular changes due to the EMFs of mobile phones (Desai et al., 2009). Reported EMF-related alterations have been dependent on exposure conditions, species, and the histological parameters investigated (Dasdag et al., 2003). These changes are governed by the duration of mobile phone exposure, the specific absorption rate (SAR), and the energy level of the EMF radiation. Reduction of testicular size has been noted as an effect in various reports. Other reports have shown a decrease in the diameter of seminiferous tubules and epithelial thickness

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(Dasdag et al., 1999, 2003; Ozguner et al., 2005; Salama et al., 2010).

Leydig cells, seminiferous tubules, and spermatozoa are the primary targets of the damage caused by mobile phones on the male reproductive tract. Specifically, cellular phone exposure decreases testosterone biosynthesis, impairs spermatogenesis, and damages sperm DNA (Deepinder et al., 2007). Oxidative stress and scrotal hyperthermia are the main damaging mechanisms. It is well established that optimal spermatogenesis occurs at 35°C (Saikhun et al., 1998).

Yan et al. (2007) reported a significantly higher incidence of cell death and clumping in spermatozoa collected from the epididymis of rats exposed to EMF when compared with that collected from unexposed adult rats. By contrast, Dasdag et al. (2003) reported no statistically significant difference between exposed and unexposed rats for the parameters measured (sperm count, morphology, lipid composition, malondialdehyde, concentration, testicular histological structure, p53 immune reactivity, and rectal temperature). No significant harmful effects of EMF were found on the reproductive systems or developmental stages of adult experimental animals in numerous other studies (Cairnie and Harding, 1981; Celik et al., 2012; Forgacs et al., 2005, 2006; Lebovitz and Johnson, 1987; Kim et al., 2007; Repacholi, 2001; Ribeiro et al., 2007).

The relevant literature therefore provides inconsistent data, while some studies report that the male reproductive system sustains damage from EMFs, others report that EMFs have no effect or only a partial effect on testicular tissues and function. Among the possible reasons for this lack of consistency, reliable data are the frequency and amplitude of the implemented current, the intensity of the induced magnetic field, and the inconsistent exposure times employed across studies. As a result, the associated effects (if any) of cell phones on the male reproductive system cannot be accurately defined from the literature (Dasdag et al., 2008; Desai et al., 2009; Yan et al., 2007).

Puberty is the transitional period from childhood to adulthood that involves both physiological (e.g., testicular mass increase) (Coleman and Coleman, 2002; Prader, 1966) and psychological changes related to reproduction (Cohen et al., 1986; Wheeler, 1991). Alterations in germ cell actions and testicular hormone production can lead to phenotypic and morphological cell abnormalities, which contribute to impaired male reproductive health (Sharpe, 2001).

Testes play important endocrine roles at various phases of development, such as in sexual differentiation (Wilson et al., 1980) during both the fetal and pubertal maturation periods (Huhtaniemi, 1994). Testicular damage (e.g., nerve damage, mumps, cystic fibrosis, and radiotherapy) during the pubertal period may cause infertility later in life (Jewett et al., 1988; Masarani et al., 2006; Schover, 1987; Xu et al., 2012). Passage through puberty may cause testicular growth retardation, decreased testosterone levels, and low or no sperm count. In normal circumstances, the amount of sperm and the rate of daily sperm production increase during the pubertal and postpubertal phases of development (Cameron et al., 1993).

Apoptotic cell death is very important to the development and maturation for Leydig cells (Faria et al., 2003). Studies have shown that low testosterone production enhances the apoptosis of germ cells and may lead to infertility (Yuan and Xu, 2003). In pre- and early postnatal periods, apoptosis in spermatogenic cells determines the potential of male fertility and the degree of testicular growth. Therefore, it seems logical to question whether the EMFs from the increasing use of mobile phones by youths may have deleterious effects on their reproductive systems (Watilliaux et al., 2011).

While the testicular effects of prenatal and adult exposure to EMFs have been extensively examined in the scientific literature, there are limited studies investigating the effects of postnatal exposure and exposure during puberty. Therefore, to begin to fill this gap, we examined the histopathological and biochemical changes caused by the electromagnetic waves of mobile phones on the testes of rats during pubertal development.

Materials and methods

The study protocol was reviewed and approved by the Local Ethical Committee for Animal Experiments, School of Medicine, Recep Tayyip Erdoğan University, Turkey (Approval date 25 November 2011; meeting number 36).

Animals

A total of 12 male Sprague Dawley rats (Laboratory Animals of Experimental Surgery, Trabzon, Turkey), aged 45 days and weighing between 100 and 110 g, were used in the study. The rats were housed in plastic cages of size $36 \times 23 \times 21$ cm^3 (six rats in each cage) kept inside kept at room temperature (22 ± 2°C) with 50–55% relative humidity. Between 05:00 and 17:00, an automatic lighting control system with a timer and

photocells were used. All animals were fed with standard pelleted rat diet (Bayramoglu, Erzurum, Turkey) and tap water *ad libitum*. All procedures involving the test animals were designed and performed according to the National Institute of Health Guide for the Care and Use of Laboratory Animals.

Exposure

The study was performed on 12 rats divided into two groups ($n = 6$), one control and one exposed. The mobile phone used was conventional global system for mobile communications in this study, with a carrier frequency of 890–915 MHz, a modulation frequency of 217 Hz, and a maximal peak power of 2 W (Ragbetli et al., 2010). The mobile phone was kept in a wood cage sized $10.16 \times 5.08 \times 2.54$ cm^3 (Mailankot et al., 2009; Narayanan et al., 2010). The effect of the wooden cage, if any, on mobile phone radiation is not known. Animals were free to move in the cage during the exposure period and food and tap water were available *ad libitum*. The whole body average SAR was estimated using the finite-difference time-domain method (Kunz and Lubbers, 1993), and it was 0.48 W/kg. For the exposed group, the phones were used on talking mode for 1 h a day (19:00–20:00) for 45 days, and continued during postnatal period, while the control group remained unexposed. Control animals were exposed to a mobile phone without battery in a similarly sized cage for the same period in a separate, similar room (Mailankot et al., 2009). Lack of batteries ensured absence of potential electromagnetic radiation source. Both cages were brought back to the home room following phone exposure.

Histopathological evaluation

At the end of 45 days, the rats were intraperitoneally administered a combination of 6 mg/kg of 2% xylazine hydrochloride (Rompun) and 75 mg/kg ketamine hydrochloride (Ketalar). Following anesthesia, the animals were killed humanely by intracardiac perfusion with 4% formaldehyde. The testes were excised, and the specimens prepared for histopathological studies. In addition, testis length, width, and weight were measured. The testes were stored in buffer formaldehyde (10%) at 4°C for 24 h. The fixed tissues were dehydrated in graded ethyl alcohol and embedded in paraffin. The sections were cut at a thickness of 4–5 μm with a rotary microtome (RM2255; Leica, Solms, Germany) and prepared on glass slides for histopathological analysis. Sections were stained with haematoxylin–eosin (H&E) and then examined under a light microscope (BX51; Olympus, Tokyo, Japan) with digital camera (DP72; Olympus, Tokyo, Japan) and photographed at relevant magnifications.

We used the parameters of histological score according to the method described by Cosentino et al. (1986). These parameters are the degeneration of germ cells, the disorganization and disarray of germ cells and the vacuolization, edema, degeneration, and defects in the seminiferous tubules (Cosentino et al., 1986).

Two histologists blindly graded Ki-67 and caspase-3 immunoreactivity levels of the specimens with a three-point scoring system, grading them as weak (1 point [+], or 15% of the region), moderate (2 points [++], or 30% of the region), and intense (3 points [+++], or 55% of the region).

Ki-67 and apoptosis immunoreactivity levels were detected using the streptavidin–biotin peroxidase method. Testis tissue samples were deparaffinized in xylene and rehydrated in an ethanol series. The sections were incubated in 3% hydrogen peroxide for the inhibition of endogenous peroxidase activity. Nonspecific binding sites of antibodies were blocked with normal bovine serum (1–10%). Primary antibodies anti-Ki-67 (i.e., rabbit polyclonal antibody to Ki-67, ab66155, Abcam, Cambridge, UK) were diluted to 1/300 and applied for 75 min, and anti-caspase 3 (abcam13847, Abcam, Cambridge, UK) was diluted to 1/50 and applied for 60 min. The slides were rinsed and a biotinylated secondary antibody (Universal LSAB Kit-K0690, Dako, Denmark) was used for 30 min. The sections were incubated with streptavidin–horseradish Peroxidase (Universal LSAB Kit-K0690, Dako, Denmark).

Bound antibodies were identified by staining with 3,3′ diaminobenzidine and washed in phosphate-buffered saline. Germ cell nuclei were stained with hematoxylin (EnVision™ FLEX Code K8018, Dako, Denmark), dehydrated through an increasing ethanol series, cleared in xylene, and mounted with Entellan (Code 107960, Merck, Darmstadt, Germany). Antibody binding was examined using a light microscope, and the sections were photographed.

Biochemical processes

Blood samples (3.0 ml) were removed from the left ventricle of the animals to measure the amount of electrolytes such as calcium (Ca^{+2}), chloride (Cl^{-}), potassium (K^{+}), magnesium (Mg^{+2}), and sodium (Na^{+}) using a standard autoanalyzer technique

Table 1. Effects on testicular morphometric size and weight of mobile phone.

Groups ($n = 6$)	Testicular weight		Testicular morphometric size	
	Right (g)	Left (g)	Length (cm)	Width (mm)
	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
Control	1.08 ± 0.02	0.99 ± 0.01	2.18 ± 0.03	9.29 ± 0.04
Exposed	1.03 ± 0.02[a]	0.95 ± 0.01[a]	2.15 ± 0.01[a]	9.22 ± 0.02[a]

[a]$p > 0.05$ aganist control.

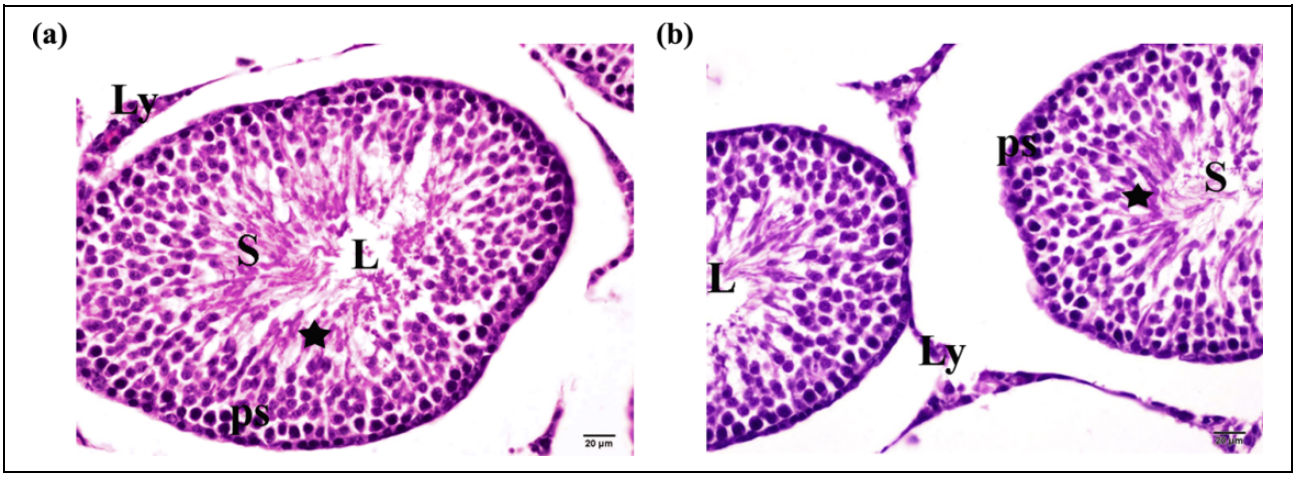

Figure 1. Histopathological changes of testis tissue visualized under light microscopy (a,b). A: Control group, B: exposed groups, S: spermium, L: lumen, Ly: Leydig cells, star: spermatids, ps: primary spermatocytes; H & E; ×40, Bar: 20 µm. H&E: hematoxylin–eosin.

(Architect c16000 Autoanalyzer, Abbott Diagnostics, Waltham, Massachusetts, USA).

Statistical analysis

Data were analysed using the Statistical Package for Social Sciences (SPSS; version 18 for Windows, IBM, Chicago, Illinois, USA). Data for normally distributed variables were examined by parametric tests. Student's *t* test was used to compare the values of Ca^{+2}, Cl^{-}, K^{+}, Mg^{+2}, and Na^{+} between the control and exposed groups.

The immunoreactivity scores of the groups showed nonnormal distribution according to the Kolmogorov–Smirnov and Shapiro–Wilk tests ($p < 0.05$). Group score data were separately compared using the *post hoc* Mann–Whitney *U* test for pairwise comparisons of independent samples.

Results

The testicular weights and morphometric sizes of testes were similar in both groups ($p > 0.05$; Table 1). H&E staining showed no histopathological changes in the control group, but there was slight vacuolization during specific stages of the spermatogenic cycle of the exposed group. Distribution range and the amounts of interstitial Leydig cells were the same in both groups. In addition, the interstitial capillaries in the exposed group were slightly dilated and the connective tissue was mildly swollen. However, these were not clinically significant histopathological changes ($p > 0.05$). Sperm production was not affected, as determined from the accumulation of spermium within the lumen (Figure 1).

The primary spermatocytes were intensely stained with Ki-67 (+++). A moderate (++) to weak (+) immunopositive reaction was found in early and late spermatids. The intense staining observed was annular in the histone regions of late spermatids. Slight positive immunostaining (+) was observed in the tails and heads of the spermium. Sertoli cells were moderately positive for Ki-67, and both the cells and the apical cytoplasm picked up the stain. The apical cytoplasm of the Sertoli cells and the end sections approaching the lumen were mildly stained (Figure 2).

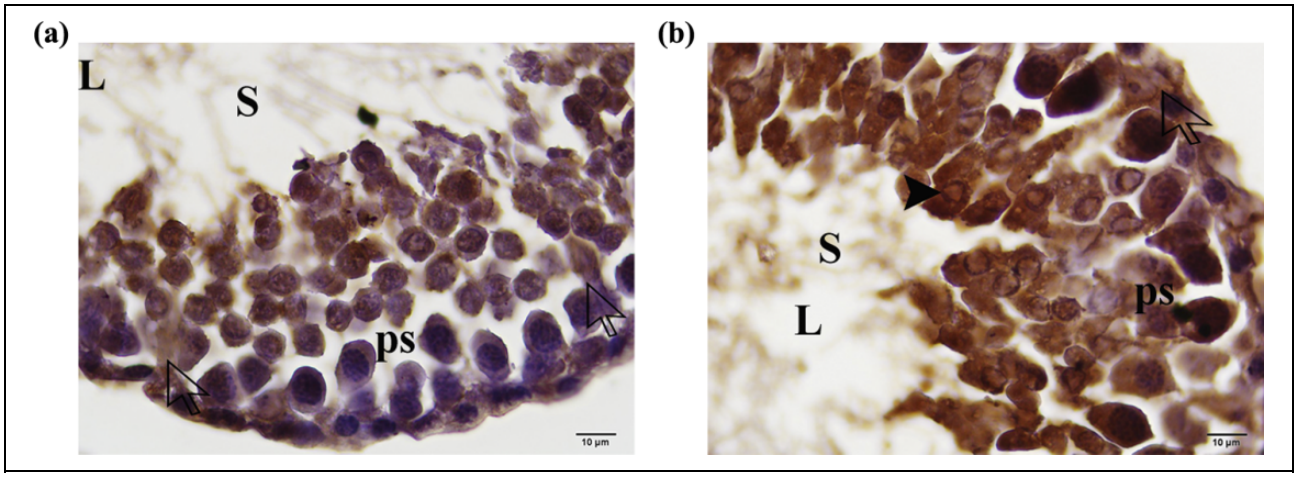

Figure 2. Localization of immunoreactive Ki-67 in testicular sections from rat (a, b). A: Control group, B: exposed groups, S: spermium, L: lumen, White arrow: Sertoli cell immunopositivity; Black arrowhead: histon immunopositivity. Immunoperoxidase (IHC) stain, ×100, Bar: 10 µm. IHC: immunohistochemical.

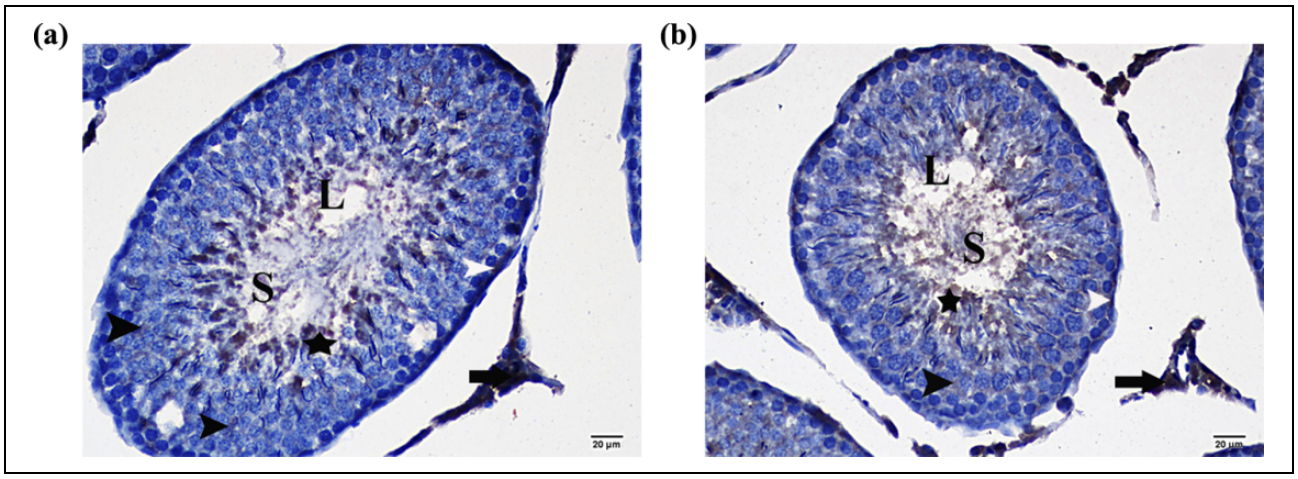

Figure 3. Localization of immunoreactive caspase 3 in testicular sections from rat (a,b). A: Control group, B: exposed groups, S: spermium, L: lumen, Black arrow: Leydig cells, White arrowhead: spermatogonia immunopositive cells, Black arrowhead: primary spermatocytes immunopositive cells, star: late spermatid immunopositive cells, IHC stain, ×40, Bar: 20 µm. immunohistochemical.

Caspase-3 staining was intense in the spermatogonium series and in late spermatids in areas close to the lumen in both groups. The immunostaining was weak in the primary spermatocytes and in the apical cytoplasmic parts of the Sertoli cells close to the lumen in both groups. No positive immunostaining was observed in the adult spermium. The Leydig cells were similarly stained in both groups (Figure 3). Both Ki-67 and caspase-3 immunopositivity were no significantly different between the two groups ($p > 0.05$).

In the biochemistry analyses, we observed no significant differences for blood Ca^{+2}, Cl^{-}, K^{+}, Mg^{+2}, or Na^{+} levels between the groups ($p > 0.05$; Table 2) (Harkness and Wagner, 1995).

The intensity and duration of magnetic field radiation used in this study were found to be insufficient to cause a change in temperature in testicles (Dasdag et al 2003; Yan et al 2007). None of the temperature differences between two groups were statistically significant.

Discussion

The results of this study demonstrate that mobile phone exposure for 45 days may not cause pathological changes in the testicular tissue of rats. Histopathological examination of rats receiving EMF showed normal organization and no degeneration in germ

Table 2. Blood Ca, Mg, K, Na, and Cl levels in both groups.

	Ca (mg/dL)	Mg (mg/dL)	K (mmol/L)	Na (mmol/L)	Cl (mmol/L)
Groups ($n = 6$)	Means ± SD	Means ± SD	Means ± SD	Means ± SD	Means ± SD
Control	10.68 ± 0.58	2.94 ± 0.13	6.20 ± 0.22	149.50 ± 1.38	100.83 ± 1.94
Exposed	10.35 ± 0.38[a]	2.84 ± 0.12[a]	6.15 ± 0.23[a]	149.67 ± 1.03[a]	100.33 ± 1.37[a]

Ca: calcium; Mg: magnesium; K: potassium; Na: sodium; Cl: Chloride.
[a]$p > 0.05$ aganist control.

cells, slight vacuolization, a mild degree edema, and no decrease in sperm concentration. Neither Ki-67 nor caspase-3 immunopositivity was significantly different between the two groups ($p > 0.05$). The testicular weight and morphometric size of the testes were similar in both groups. There were no significant differences between the two groups in terms of Ca^{+2}, Cl^{-}, K^{+}, Mg^{+2}, and Na^{+} in the blood ($p > 0.05$).

Research on the bioeffects of mobile phones has concentrated on DNA damage, cell proliferation, and apoptosis. Environmental stress is the activation of an apoptotic pathway leading to an increase in cell death. Apoptosis could also be an important mechanism for removing damaged cells and thus preventing the proliferation of potential cancer cells (Jaattela, 1999; Merola et al., 2006).

Caspases play an important role in male infertility (Said et al., 2004). Caspase 3 has been detected within both the cytoplasm and the perinuclear area of germ cells (Paasch et al., 2004). The proper regulation of caspase plays an important role in sperm differentiation and testicular maturity (Said et al., 2004). In this study, the detection of active caspase 3—a well-known feature of typical apoptosis—has evidenced that 1 h a day of mobile phone exposure in rats may not be enough to induce apoptosis in testes tissue within 45 days. These findings seem to be consistent with those of other studies.

Dasdag et al. (2003) reported that 900 MHz microwave radiation emitted from mobile phones did not affect apoptotic parameters such as p53 in rat testes. In another study, these same authors showed that exposure to long-term 900 MHz radiation lasting for 2 h a day, 7 days a week over a period of 10 months did not affect caspase 3 levels in rat testes (Dasdag et al., 2008).

Lee et al. (2010) examined the testicular histologic changes in rats whose whole bodies were exposed to electromagnetic radiation (848.5 MHz, 2.0 W/kg) for 90 min a day for 12 weeks. The authors then investigated the frequency of spermatogenesis stages, germ cell counts, and the appearance of apoptotic cells in the testes. Finally, they measured p53, p21, caspase 3, and the Bcl-2 protein (an anti-apoptotic protein) of the testes in both control and exposed animals. They concluded that subchronic exposure to 848.5 MHz of radiofrequency and electromagnetic radiation did not have any noticeable effects on rat spermatogenesis (Lee et al., 2010).

In another study, Yilmaz et al. (2008) evaluated Bcl-2 in rat testes. The experimental group was exposed to mobile phones for 20 min a day for 1 month. The testes were then investigated by means of immunohistochemistry. No difference was observed between the testes sections of the sham-control and experimental groups in terms of Bcl-2 staining. These results denote that mobile phones did not alter the anti-apoptotic protein in the testes of rats (Yilmaz et al., 2008).

On the other hand, caspase activity depends on the concentration of the cytosolic Ca^{+2} ion. Ca^{+2} is an essential ion, participating in many biological processes including hormone secretion and mitotic division (Giorgi et al., 2008; Rizzuto et al., 2003). Ca^{+2} levels can also promote apoptotic cell death in response to pathological conditions such as necrosis (Choi, 1995). Increase in the concentration of Ca^{+2} occurs both at the early and late stages of the apoptotic pathway (Kruman et al., 1998; Lynch et al., 2000; Tombal et al., 1999). It has been shown that while severe Ca^{+2} dysregulation can promote cell death through necrosis, more controlled intracellular Ca^{+2} concentration increases induced by milder insults promote cell death through apoptosis (Pinton et al., 2008). Our study showed that rats exposed to a mobile phone had no statistically significant total plasma Ca^{+2} values.

In accordance with these findings, the results of the present study seem to support those of previous studies. Therefore, longer or more powerful exposures may yield apoptosis-inducing effects, which might warrant further study.

Ki-67 is a very important proliferation-associated nuclear antigen. Ki-67 expression is of great

significance for the regulation of the cell cycle (Schluter et al., 1993). The Ki-67 antigen is expressed in the nuclear matrix of cells during the G_1, S, G_2, and M stages of the cell cycle, with a maximum expression during the G_2 stage and early mitosis. Its biological half-life is approximately 1 h. The Ki-67 protein is not identified in G_0 resting cells or in cells during the early G_1 phase nor is it related to the DNA repair process (Angelopoulou et al., 2008; Gerdes et al., 1984; Steger et al., 1998). In this study, Ki-67 and H&E have shown active spermatogenesis and a well-arranged spermatogenic germ cell series in all groups.

Imai et al. (2011) evaluated the effects of EMF on young, developing male rats. Five-week-old rats were exposed to 1.95 GHz (SAR 0.4–0.08 W/kg), with a whole body exposure, for 5 h a day for 5 weeks, matching the period of reproductive maturation in these rats. There were no differences in the weights of the testes among the groups. No abnormalities in sperm motility, sperm morphology, or the histologic appearance of the seminiferous tubules (including the stages of the spermatogenic cycle) were observed. Interestingly, the testicular sperm count increased significantly following exposure to 0.4–W/kg SAR (Imai et al., 2011).

Ribeiro et al. (2007) examined the testicular function of rats after subchronic exposure to radiofrequency emitted from a conventional mobile phone. The animals were exposed to a cellular phone (1835–1850 MHz) for 1 h a day for 11 weeks. Neither total body weight nor absolute or relative testicular or epididymal weights were significantly affected (Ribeiro et al., 2007).

Ji Yoon et al. (2007) subjected rats to 2.45-GHz EMFs for 1–2 h a day for 8 weeks; they reported no significant changes in testis weight, seminiferous tubule diameter, sperm counts, or sperm morphology.

Since three studies are development related, they are in complete agreement with ours. However, the rats are exposed to EMF and the effects found were those observed in the puberty–adolescent transition period. They are also in agreement with our study that covered the neonatal–puberty period.

Ozguner et al. (2005) exposed rats to 900 MHz, 30 min a day, 5 days a week for 4 weeks. They did not find any significantly different values in terms of testis weight, testicular biopsy score count, or percentage of interstitial tissue.

Even though the above one study was conducted in adults, the findings were similar to ours. Our structural findings are also supported by electron microscopic study (Celik et al., 2012).

Celik et al. (2002) reported on rats that were continuously exposed to a mobile phone (SAR 1.58 W/kg) for 7 days a week for 12 weeks. The authors did not observe any apparent degeneration in the seminiferous epithelium during the histopathological examination. Although spermiogenesis was observed to be normal in the study, they found some abnormal sperm morphologies during the electron microscope examinations of the group that was subjected to the EMF (Celik et al., 2012).

All the above discussed literature supports the results obtained in our study. As mentioned, most of the studies in the field of apoptosis and EMF have indicated that EMF does not affect the apoptotic process (Dasdag et al., 2003, 2008; Yilmaz et al., 2008).

In conclusion, the results of most of the studies are in agreement with the findings of the present study, which show that exposure to 900 MHz radiation for 1 h a day over a period of 45 days does not affect caspase-3 levels in rat testes. In addition, no abnormality in the histologic appearance of the seminiferous tubules (including the stages of the spermatogenic cycle) was observed. Therefore, our study demonstrates that a mobile phone with a low SAR has no harmful effects on the pubertal rat testicles.

Conflict of interest

The authors declared no conflicts of interest.

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