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Long-term effects of 900 MHz radiofrequency radiation emitted from mobile phone on testicular tissue and epididymal semen quality

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Abstract
The purpose of this study is to bridge this gap by investigating effects of long term 900 MHz mobile phone exposure on reproductive organs of male rats. The study was carried out on 14 adult Wistar Albino rats by dividing them randomly into two groups (n: 7) as sham group and exposure group. Rats were exposed to 900 MHz radiofrequency (RF) radiation emitted from a GSM signal generator. Point, 1 g and 10 g specific absorption rate (SAR) levels of testis and prostate were found as 0.0623 W/kg, 0.0445 W/kg and 0.0373 W/kg, respectively. The rats in the exposure group were subject to RF radiation 3 h per day (7 d a week) for one year. For the sham group, the same procedure was applied, except the generator was turned off. At the end of the study, epididymal sperm concentration, progressive sperm motility, abnormal sperm rate, all-genital organs weights and testis histopathology were evaluated. Any differences were not observed in sperm motility and concentration (p > 0.05). However, the morphologically normal spermatozoa rates were found higher in the exposure group (p < 0.05). Although histological examination showed similarity in the seminiferous tubules diameters in both groups, tunica albuginea thickness and the Johnsen testicular biopsy score were found lower in the exposure group (p < 0.05, p < 0.0001).

In conclusion, we claim that long-term exposure of 900 MHz RF radiation alter some reproductive parameters. However, more supporting evidence and research is definitely needed on this topic.

Keywords
900 MHz radiofrequency radiation, mobile phone, reproductive organs, sperm, testis

Introduction
Investigation of biological effects due to long-term electromagnetic field exposure is an unmet need for public health. There is no doubt that ever increasing use of wireless devices raise concerns on potential health effects. Especially, effects of RF emissions from mobile phones (MPs) have been the focus of many studies. Several adverse effects of mobile phone usage on different body organs such as brain (Hardell et al., 2011; Kesari et al., 2011a), ear (Colletti et al., 2011) and reproductive organs (Erogul et al., 2006; Falzone, 2011; Khillare & Behari, 1998) have been reported. Most of the MP users mention that they carry at least one MP device throughout the day, and they place it in a pocket or purse very close to their genitals (Lavranos, 2012). This, naturally, initiated a growing interest to investigate the effects of MP on reproduction organs. To understand whether MPs affect germ cells and testis tissue, potential adverse effects of cell phone exposure on male fertility were extensively investigated in the past decade by many researchers who reported significant adverse effects of RF on testis and seminal parameters, including motility, concentration and morphology (Agarwal et al., 2008; Fejes et al., 2005). Although studies in human highlighted adverse effect of MP on testis and semen quality, underlined mechanisms of these impacts have not been extensively addressed and therefore, detailed studies are still needed (Salama et al., 2010).

RF radiation may affect reproductive functions in one of three ways: either by a RF-specific non-thermal action, a thermal action or by a combination of these mechanisms (Derias et al., 2006). It is commonly accepted that RF emissions of cellular phones (CPs) are at non-thermal power density levels, but there is no consensus in the literature as to the non-thermal exposure levels of RF (Blackwell, 1979). Whether non-thermal microwave exposure affects reproductive ability is still controversial; epidemiological data have been inconclusive and animal tests were limited. Nakamura et al. (2000) suggested that the microwave exposure (2450 MHz, CW microwave at 2 mW/cm², power density...
for 90 min) produced uteroplacental circulatory disturbances, and ovarian and placental dysfunction during pregnancy, probably through non-thermal effects. However, in another study of Nakamura et al. (2003), they suggested that microwaves at 0.6 mW/cm² at 915 MHz, equal to a specific study of Nakamura et al. (2003), they suggested that probably through non-thermal effects. However, in another and ovarian and placental dysfunction during pregnancy, for 90 min) produced uteroplacental circulatory disturbances, DOI: 10.3109/15368378.2013.801850 Effects of mobile phone emissions on testis and sperms of rats the histology of reproductive organs.

sperm concentration, sperm motility, sperm morphology and 900 MHz RF exposure on the rat testis including epididymal effect on testicular function or structure in rats (Dasdag et al., therm effects on blood estradiol and progesterone, on splenic normal killer cell activity, and on the uteroplacental circulation. Experimental animal studies addressing the same issue of the adverse effects of MP on testis and sperm quality were abundant than those with human studies (Dasdag et al., 1999; Jelodar et al., 2013; Otitoloju et al., 2010). However, results of these studies were conflicting. Some of these studies have not found any significant effect of MP on testicular histology, sperm count and morphology (Dasdag et al., 2003; Lee et al., 2010; Nisbet et al., 2012), others indicated that cell phone might have a wide spectrum of detrimental effects on sperm parameters (Kesari & Behari, 2010; Kesari et al., 2011b; Kumar et al., 2011). Therefore, results of these studies appeared contradictory and the question of adverse health effects provoked by MP is still unresolved to date. In one of the studies performed by Dasdag et al. (1999), the effects of microwaves emitted by mobile phones on male rats were investigated and, no detectable effects on sperm morphology were observed, but a significant decrease in seminiferous tubular diameter, and an increase in rectal temperature were found although there was an insignificant decrease in the epididymal sperm count. However, a subsequent study carried out to explore these results more thoroughly found that longer daily exposures to pulsed 800–915 MHz GSM microwave radiation at a whole-body average SAR of 0.52 W/kg had no effect on testicular function or structure in rats (Dasdag et al., 2003). Lee et al. (2010) concluded that subchronic exposure to 848.5 MHz with 2.0 W/kg SAR RF did not have any observable adverse effects on rat spermatogenesis. In a recent study by Lee et al. (2012), they also concluded that simultaneous exposure to RF electromagnetic fields at 4.0 W/kg SAR did not have any observable adverse effects on rat spermatogenesis. However, Erogul et al. (2006) reported that RF emissions by cellular phones affected human sperm motility. Akdag et al. (1999) concluded that epididymal sperm count and morphology, weight and morphology of testis and epididymes were affected by chronic prolonged microwave exposure (9450 MHz, SAR: 1.80 W/kg).

The interpretation of these studies is partially complicated by methodological variations such as lack of adequate thermal control, various species (or strains) of animals used and short exposure period. One particular deficiency in most studies is that they describe experiments with acute or short-term exposure of animals in an electromagnetic field (EMF). Experiments are needed to perform long-term exposure in order to demonstrate the chronic impact of EMF emitted from MPs.

Due to the uncertainties related to this topic, the aim of this study was to evaluate the possible effects of long-term 900 MHz RF exposure on the rat testis including epididymal sperm concentration, sperm motility, sperm morphology and the histology of reproductive organs.

Materials and methods

Subjects and animal care

Fourteen adult male Wistar Albino rats, 5 to 6 months of age with body weights between 250 and 350 g, were used in the experiment. The rats were separated into two groups equally such as sham (control) group and exposure group. All rats were housed in polycarbonate cages in a room with controlled temperature (21 °C to 23 °C) and humidity (50% to 55%) and a 12-h light–dark room conditions. All activities within the scope of this study were performed with the approval of the Dicle University Experimental Animal Ethics Committee in compliance with the provisions of the Strasbourg Universal Declaration of Animal Rights of 1986. The rats were fed laboratory pellet chow and water was given ad libitum. The experiment was performed after a stabilization period in the laboratory which lasted for couple of days.

Experimental design

A GSM signal generator (900PM10 type Everest Comp., Adapazari, Turkey), which produces 900 MHz band RF waveform identical to the one in mobile phones was used in the study to expose the rats. Emitted power (omnidirectional on the plane perpendicular to the antenna axis) of the generator was fixed during the exposure. The antenna of the generator was equivalent to that of a typical mobile phone. The rats were confined in a Plexiglas carousel and exposed to 900 MHz RF exposure emitted from the generator. Experimental setup is illustrated in Figure 1. For the exposure group, the rats were

Figure 1. Experimental setup, (a) top view, (b) side view for one carousel.
exposed to RF radiation 3 h per day (7 d a week) for 12 months. For the sham (control) group, the rats were placed in the carousel and the same procedure were applied to the rats (3 h per day, 7 d a week for 12 months), except that the generator was turned off, i.e. no RF signal was present. The antenna of the generator was placed at the center of the Plexiglas carousel to provide ideal exposure conditions. The distance of the antenna from the head of the rats was 1 cm.

All rats were kept under identical conditions for 12 months with free access to food and water. At the end of 12th month, the rats were intraperitoneally administered a combination of 6 mg/kg of 2% xylazine hydrochloride (Rompun) and 75 mg/kg ketamine hydrochloride (Ketalar) for anesthesia. Afterward, the testis of each rat was located and the testis, epididymis, seminal vesicles and ventral prostate were removed, cleared of adhering connective tissues. The testis, epididymis, seminal vesicles and ventral prostate weight were evaluated along with epididymal sperm concentration, sperm motility and sperm morphology. One of the testis was fixed in 10% Bouin fixative for histopathologic examinations. All measurements, analysis and evaluations were performed by persons who were unaware of the groups so that the subsequent analysis could be performed blind.

SAR measurement

In our experimental setup, the electromagnetic field values were measured with an Electric-field probe, while the transmitter was operating, then, these measured values used in the electromagnetic field solver to find the field distribution inside the rat. Simulations were performed using CST Microwave Studio, an electromagnetic field solver based on finite integration technique (FIT). FIT is similar to a finite-difference time domain (FDTD) technique, but it employs discretization on general non-orthogonal grids using integral form of Maxwell’s equations rather than differential forms. Charge and energy conservation inherit to Maxwell’s equations are preserved with FIT, which leads to very stable numerical results in time-domain. The Voxel (volumetric pixel) rat model, which was formed using computerized tomography scans of a rat was used in the electromagnetic field simulations. The simulation model consists of electric field distribution inside and around the rat. Simulated field values were consistent with measured electric field data which were obtained with field probe. Point, 1 g and 10 g average SAR level of testis and prostate were found as 0.0623 W/kg, 0.0445 W/kg and 0.0373 W/kg, respectively (Figure 2). However, whole body (rms) and whole body maximum point SAR were found as 0.0369 (W/kg) and 2.023 (W/kg), respectively. In SAR calculations, a representative rat with 320 g weight which corresponds to average weight in the exposure group was used. Whole body maximum point SAR value is expected to exhibit a variation of $+/-0.121$ (W/kg) over the rats in the exposure group.

Epididymal sperm count

Spermatozoa in the left epididymis were counted by a modified method developed by Türk et al. (2007). Briefly, the epididymis was finely minced with anatomical scissors in 10 mL of physiologic saline, placed in a rocker for 10 min, and allowed to sit at room temperature for 2 min. After incubation, supernatant fluid was diluted 1:10 with a solution containing 5 g sodium bicarbonate, 1 mL formalin (35%), and 25 mg eosin per 100 mL of water. Total sperm number was determined using counting chambers. The cells were counted with the help of a light microscope (magnification, 200×).

Epididymal sperm motility evaluation

The fluid obtained from the cauda epididymis with a pipette was diluted to 2 mL with Tris buffer solution. A slide was placed on a phase-contrast microscope, and an aliquot of this solution was placed on the slide and percent motility was evaluated visually at a magnification of 400 times. Motility estimations were performed from three different fields in each sample. The mean of the three estimations was used as the final motility score. Samples for motility evaluation were kept at 37°C.

Epididymal sperm morphology evaluation

To determine the percentage of morphologically abnormal spermatozoa in the cauda epididymis, the slides stained with...
Hystopathologic analysis

The right testis from the rats were placed in 10% Bouin solution for 24 h for fixation and further pathologic examination. After fixation, the sections were subjected to routine histologic tissue preparation and dehydrated and embedded in paraffin. Paraffin blocks were sliced to 5 μm thickness with a microtome and the slices were subjected to routine hematoxylin and eosin (H&E) staining and were then examined under a light microscope (Nikon ECLIPSE 80i, Nikon, Tokyo, Japan) by a pathologist blinded to the groups. For each sample, 100 randomly selected seminiferous tubule diameters were measured. In addition, for each section, 100 randomly selected seminiferous tubules were evaluated using the Johnsen classification (Johnsen, 1970).

Statistical analysis

In this study, all analyses were performed using SPSS version 10.0 (SPSS, Inc., IBM, Armonk, NY). The results of epididymal sperm characteristics between the control and exposed group were analyzed via the Mann–Whitney U-test. Differences were considered statistically significant when calculated p values were less than 0.05.

Results

Epididymal sperm characteristics

The effects of 900 MHz RF on epididymal sperm concentration, progressive sperm motility and abnormal sperm rate are presented in Table 1. There was no statistically significant difference in the sperm concentration and the percentage of epididymal sperm motility among the groups. But, the morphologically normal spermatozoa rates were higher in the exposure group (p<0.05) than the sham group.

Table 1. Comparison of spermatological parameters between control and mobile phone group.

<table>
<thead>
<tr>
<th>Traits</th>
<th>Control</th>
<th>Mobile phone</th>
<th>Statistical significance (p=)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Motility (%)</td>
<td>74.7 ± 2.47</td>
<td>70.9 ± 5.90</td>
<td>0.720</td>
</tr>
<tr>
<td>Concentration (×10^9/mL)</td>
<td>34.2 ± 2.01</td>
<td>33.7 ± 1.80</td>
<td>1.000</td>
</tr>
<tr>
<td>Tail defects (%)</td>
<td>17.2 ± 3.14</td>
<td>10.9 ± 0.60</td>
<td>0.095</td>
</tr>
<tr>
<td>Head defects (%)</td>
<td>2.7 ± 0.42</td>
<td>2.0 ± 0.31</td>
<td>0.202</td>
</tr>
<tr>
<td>Total morphologic defects (%)</td>
<td>19.8 ± 3.00^a</td>
<td>12.9 ± 0.80^b</td>
<td>0.017</td>
</tr>
</tbody>
</table>

The values given for continuous variables are Mean ± S.D. Values with different superscripts in the same row are significantly different (^a,b; p < 0.05).

Table 2. Comparison of all genital organs weights between the control and the mobile phone group.

<table>
<thead>
<tr>
<th>Traits</th>
<th>Control</th>
<th>Mobile phone</th>
<th>Statistical significance (p=)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left testis (g)</td>
<td>1.52 ± 0.05</td>
<td>1.50 ± 0.02</td>
<td>0.161</td>
</tr>
<tr>
<td>Left epididymis (g)</td>
<td>0.55 ± 0.007</td>
<td>0.56 ± 0.01</td>
<td>0.097</td>
</tr>
<tr>
<td>Prostate (g)</td>
<td>0.63 ± 0.08</td>
<td>0.56 ± 0.05</td>
<td>0.158</td>
</tr>
<tr>
<td>Vescicula seminalis (g)</td>
<td>1.01 ± 0.05</td>
<td>1.14 ± 0.11</td>
<td>0.509</td>
</tr>
</tbody>
</table>

The values given for continuous variables are Mean ± S.D.

Prostat, vesiculasaeminalis, testicular and epididymal mass

The effect of RF on all-genital organs weights are presented in Table 2. Testis, epididymis, prostate and vesiculasaeminalis weights were similar in the sham and exposure groups (p > 0.05).

Histopathological evaluation of the testis

Results of histological analysis showed that the sham and exposure groups were found to be similar in general morphology, and there was no anomaly in intertubular microscopic blood vessels and endothelial cells (Figure 3). In addition, similar results were found in seminiferous tubule diameters (Table 3). However, according to the Johnsen testicular biopsy score, a significant decrease of spermatogenesis was found in the exposure group compared to the control group (p<0.0001, Table 3). We also found that the thickness of membrane tunica albuginia from the exposure group had significant thinning as compared to the tissue of animals from the sham group (p<0.05) (Figure 4, Table 3).

Discussion

Regarding mobile telephony, the first study conducted by Dasdag et al. (1999) investigated whether there were adverse effects due to RF exposure emitted by cellular phones in male Wistar albino rats. Dasdag et al. (1999) assessed the testis of rats irradiated with 900 MHz, with an exposure condition SAR 0.141 W/kg for 2 h/day for 1 month. The authors did not observe abnormalities regarding the sperm number and morphology. The same group of authors performed two more similar studies except the period of exposure time. The first study assessed the testis of rats exposed to 900 MHz in a waveguide, with an exposure condition SAR 0.52 W/kg for 20 min/day for 1 month (Dasdag et al., 2003). The second study assessed the testis of rats irradiated with 900 MHz, with an exposure condition SAR 0.57 W/kg for 2 h/day for 10 months (Dasdag et al., 2008). No differences were observed in the percentages of epididymal normal and abnormal sperms, the epididymal sperm count, motility and reproductive organ weights between control and experimental groups in these two studies. Thus, after the same group of authors completed previous studies they decided to perform a more detailed study examining the effects of 3 h/day for 12 months of exposure to 900 MHz RF emissions from cellular phones on sperm parameters (motility, concentration and morphologic defects), testis histology and reproductive organ weights in adult rats. However, this latest study also showed that...
3 h/day 12 months of cell phone exposure was not enough to show any effects on epididymal sperm count, sperm motility, reproductive organ weights. Previous animal studies have not found any significant effect of cell phone on epididymal sperm characteristic (Dasdag et al., 2003; Lee et al., 2010; Nisbet et al., 2012). But, surprisingly the morphologically normal spermatozoa rates were higher in the 900 MHz exposure group ($p<0.05$) than the sham group (Table 1). Nisbet et al. (2012) found similar results in their study and their findings also indicate that the morphologically normal spermatozoa rates were higher in the exposure group ($p<0.05$). They thought that this condition was due to a decline in melatonin concentration and an increase in testosterone concentration. Melatonin and testosterone hormone levels have not been tested in our study. However, more analysis and data are necessary on the higher morphologically normal spermatozoa rates observed in the exposure group of this study.

In the present study, the Johnsen testicular biopsy score indicated that the exposure group had a significantly higher morphologically normal spermatozoa rates than the control group ($p<0.05$). The values given for continuous variables are Mean ± S.D. Means within line with different superscripts differ significantly.

Table 3. Comparison of morphological parameters between the control and the mobile phone group.

<table>
<thead>
<tr>
<th>Traits</th>
<th>Control</th>
<th>Mobile Phone</th>
<th>Statistical significance ($p=$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seminiferous tubules diameter ($\mu$m)</td>
<td>283.7 ± 3.84</td>
<td>292.9 ± 3.05</td>
<td>0.826</td>
</tr>
<tr>
<td>Tunica albuginea thickness ($\mu$m)</td>
<td>24.0 ± 0.50$^a$</td>
<td>22.1 ± 0.32$^b$</td>
<td>0.004</td>
</tr>
<tr>
<td>Johnsen’s biopsy score (/10)</td>
<td>9.50 ± 0.04$^a$</td>
<td>9.42 ± 0.05$^b$</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

The values given for continuous variables are Mean ± S.D. $^a,b$Means within line with different superscripts differ significantly.

Figure 3. Light micrographs of testicular tissue. Control group (a) and mobile phone exposed group (b). Testis shows normal seminiferous tubule morphology (a, b). Diameters of seminiferous tubules were found same in statistical analysis (a, b Hematoxylin - eosin).

Figure 4. Microscobical visualizations of tunica albuginea thickness in the control group (a) and mobile phone exposed group (b). Tunica albuginia thickness of exposed rat was found lower than the control group (a,b Staining Masson Trichrome).
decreased score compared to the control group. When the results of this study were first evaluated at a glance, it was found that the mobile phone application did not affect the epididymal semen quality negatively, but after the histopathologic examination it was found that testicular tissue was affected negatively and the exposure group had a lower biopsy score than the control group ($p<0.0001$). These results implied that the testicular tissue in the exposure group gradually got affected negatively at an unknown time after the onset of the experiment; however, these negative effects had not occurred yet at the semen quality level, because of the spermatogenic cycle.

In this study, long-term exposure of RF did not result in alteration of seminiferous tubule diameter of testis ($p>0.05$). The seminiferous tubule diameter results differ from the results of our previous study (Dasdag et al., 1999). In the previous study, Dasdag et al. (1999) found reduced seminiferous tubule diameters in exposed group rats. Similarly, several researchers reported decreased seminiferous tubule diameter of testis from animals exposed to cell phone radiation (Erpek et al., 2007; Ozgüner et al., 2005). However, we believe that the difference between the results of the previous study of Dasdag et al. and this study may have originated from different SAR levels and exposure time periods because these two important parameters may have different effects on the morphology.

In this study, the membrane tunica albuginea thickness of exposed rats was found lower than the sham group ($p<0.05$) (Table 3). Earlier findings suggest that the tunica albuginea exerts contractile properties and the tunica may have physiological functions such as (1) promotion of the transport of spermatozoa out of the testis into the epididymis; (2) maintenance of the interstitial pressure inside the testis; and (3) control of the blood flow through the testis (Davis & Langford, 1969; Middendorff et al., 2002). Testicular tunica albuginea could be distinguished in three connective tissue layers named outer, middle and inner layers (Arenas et al., 1997). Our results showed a decrease in outer layer of tunica albuginea thickness. We suggest that thinning of tunica albuginea is a result of less synthesis of Type I, Type III, Type V collagens, outer layer’s main structures, with aging. Dermis degenerations which occurs in connective tissue with aging, shows itself with collagen irregularity and decrease of fibroblast proliferation capacity (Varani et al., 2006). In addition, a previous study for 1–24 months period, gradual increase of collagen degradation was demonstrated for most tissues in rats (Mays et al., 1998). Arenas et al. (1997) showed that decrease of tunica albuginea occurred only at outer layer, although there was no change in mean thickness of total tunica albuginea with aging. As a result of our study, we suggest that degenerative changes, which occur in tunica albuginea with chronologically, may have been accelerated by 900 MHz RF exposure during 12 months.

**Conclusion**

In the present study, we demonstrated that the membrane tunica albuginia thickness and the Johnsen testicular biopsy score decreased in the exposed rat testis compared to those of the sham group. But, there is definitely a need for further research to determine whether testis histology, sperm function and sperm quality are affected from mobile phone exposure.

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**Declaration of interest**

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

**References**


