



Biochemical, immunohistochemical and morphometrical investigation of the effect of thymoquinone on the rat testis following exposure to a 900-MHz electromagnetic field

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ABSTRACT

Long-term use of cell phones emitting electromagnetic fields (EMFs) have raised concerns regarding public health in recent year. We aimed to investigate the possible effects of 900 MHz EMF exposure (60 min/day for 28 days) on the rat testis. Another objective was to determine whether the deleterious effect of EMF radiation would be reduced by the administration of thymoquinone (TQ) (10 mg/kg/day). Twenty-four male adult *Wistar albino* rats were randomly selected, then assigned into four groups as follow Control, EMF, TQ and EMF + TQ. Testicular samples were analyzed using histological, stereological, biochemical and immunohistochemical techniques. Total numbers of primary spermatocytes and spermatids as well as Leydig cells were significantly decreased in the EMF group compared to the Control group ($p < 0.05$). In the EMF + TQ group, the total number of primary spermatocytes was significantly increased compared to the EMF group ($p < 0.05$). Superoxide dismutase (SOD) activity was significantly increased in the EMF group compared to the Control group ($p < 0.05$). Also, serum testosterone levels and wet weight of testes were significantly decreased in the EMF group compared to the Control group ($p < 0.05$). Our findings suggested that exposure to a 900 MHz EMF had adverse effects on rat testicular tissue and that the administration of TQ partially mitigated testicular oxidative damages caused by EMF radiation.

1. Introduction

Exposure to the electromagnetic field (EMF) emitted from cell phone has increased over the last decade (Sharma et al., 2015). Researchers are therefore interested in looking for the relationship between health risk and exposure to EMF emitted from cell phones.

The impact of EMF on living organisms depends on various factors. For example, body mass index, body weight, bone density, water and electrolyte levels can all modify conductivity and biological reactivity to EMF (Tabrah et al., 1998; Vesselinova, 2015). Previous studies have shown that EMF exposure causes a significant weakening of antioxidant enzyme activity in living organisms, followed by the formation of reactive oxygen species (ROS) (Narayanan et al., 2014). Overproduction of ROS can result in oxidative damages to protein, lipid and DNA in single nucleated cells of human peripheral blood (Lu et al., 2012). EMF radiation also can induce change in genomes, spatial memory, male and

female reproductive systems, kidney and hippocampal pyramidal cells (McCann et al., 1998; Guney et al., 2007; Bas et al., 2009; Hancı et al., 2018; Ulubay et al., 2015; Tang et al., 2015). Detrimental effects of EMF exposure have been observed in embryo organogenesis and brain cell development as well as the reproductive potential of female rat pups (Sonmez et al., 2010; Tenorio et al., 2011). Other studies have reported alteration of gene expression and single- and double-stranded deoxyribonucleic acid (DNA) breaks in response to EMF exposure (Svedenstal et al., 1999; Zalata et al., 2015). Testis can be one of the target organs affected by exposure to EMF (Oh et al., 2018). This possibly is due to the antioxidant capacity and susceptibility to oxidative stress of testis (Hancı et al., 2018).

Cell phones are usually carried by men on the belt close to the reproductive organs. In addition, occupational exposure to EMF radiation is also increasing in adults owing to working condition. Thus, the potential risks caused by EMF radiation can be the subject of considerable

Abbreviations: EMF, Electromagnetic Field; SOD, Superoxide Dismutase; CW, Continuous Wave; SAR, Specific Absorption Rate; DCXR, Dicarbonyl L-xylulose Reductase; AEC, 3-amino-9-ethyl Carbazyl; CE, Coefficient of Error

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research in adults. The Global System for Mobile Communications (GSM) standard operates on various carrier frequencies, but the standard for cell phones that operates in 900 MHz frequency is the most popular (Odaci et al., 2008). Therefore, our research was conducted on the rats at 900 MHz wave frequency.

Thymoquinone (TQ) (C₁₀H₁₀O₂, 2-isopropyl-5-methyl-1,4-benzoquinone) is a bioactive molecule of *Nigella Sativa* (Dehkordi and Kamkhah, 2008). The therapeutic roles of TQ are mainly related to its antioxidant, neuroprotective, anti-inflammatory, anti-viral, anti-allergic, anti-carcinogenic and anti-diabetic effects (Ashraf et al., 2011). Numerous studies have investigated the beneficial criteria of TQ (Goyal et al., 2017; Aras et al., 2015). In testicular tissues, TQ plays a protective role via its anti-inflammatory and antioxidant properties, which alleviates oxidative stress and histological damages (Fouad and Jresat, 2015; Gökçe et al., 2010). Ayan et al. (2016) reported that the administration of TQ caused a decrease in the apoptotic index in testicular cells exposed to oxidative stress. Literatures have been documented that TQ at a dose of 10 mg/kg/day has been considered as a safe substance against cellular toxicity (Goyal et al., 2017). Therefore, TQ is increasingly being investigated for its therapeutic properties.

The incidence of infertility after EMF exposure has gradually increased in recent years (Sharma et al., 2015; Kaplan et al., 2016; Yahyazadeh et al., 2018). We decided to investigate the effects of 900 MHz EMF on the rat testes and to determine the potential protective efficacy of TQ on testicular tissues after exposure to EMF.

2. Material and methods

2.1. Animals and experimental protocol

Twenty-four adult male *Wistar albino* rats (200 g in weight, 12 weeks old) were used in the present study. Ethical approval of our study was granted by the Ondokuz Mayıs University Laboratory Animal Ethical Committee (2013/66). Rats were obtained from the Ondokuz Mayıs University Medical Faculty Experimental Animal Research and Application Centre (Samsun, Turkey). The rats were randomly selected and housed in plastic cages. All rats were randomly allocated into four equal groups (n = 6 per group). During the experiment, all animals were kept in a 720:720-min day/night cycle at a temperature of 22 ± 2 °C and humidity of 50 ± 5 %. All animals had *ad libitum* access to water and food. The applications during the 28 day experimental period and the main study groups are described below:

- 1 Control group: Rats were not exposed to 900 MHz EMF or treated with antioxidant substances during the 28 day experimental period.
- 2 Electromagnetic field exposure (EMF) group: Rats were exposed to 900 MHz EMF for 60 min/day during the experiment period.
- 3 Thymoquinone (TQ) group: Rats were treated with TQ (10 mg/kg/day; via the oral route; Sigma-Aldrich, St. Louis, MO, USA) for 28 days (Aras et al., 2015).
- 4 Electromagnetic field exposure + Thymoquinone (EMF + TQ) group: Rats were exposed to 900 MHz EMF for 60 min/day and treated with TQ (10 mg/kg/day via the oral route; Sigma-Aldrich, St. Louis, MO, USA) during the experiment period.

At the end of the experiment, cardiac perfusion was applied to all rats under anesthesia by the injection of a 5:1 ratio of ketamine/xylazine (ketamine: 0.5 ml; 60 mg/kg body weight, Sigma Chemical Comp, St. Louis, MO, USA)(xylazine: 0.1 ml; 5 mg/kg Sigma Chemical Comp, St. Louis, MO, USA). After dissection, testes were immediately removed and weighed on an electric balance (Precisa, BJ 410C, Istanbul, Turkey).

2.2. Electromagnetic field exposure system and procedure

The procedure of EMF exposure was carried out as described by

Ulubay et al. (2015). A 900 MHz continuous wave (CW) electromagnetic energy generator [CW and GSM pulsed wave signal (CDMA 217 Hz pulse)] was used at a peak specific absorption rate (SAR) of 2 W/kg and average power density of 1 ± 0.4 mW/cm² (Koyu et al., 2005). The generator was manufactured by the Electromagnetic Compatibility Laboratory of Suleyman Demirel University, Turkey. The localized SAR values were determined by numerical calculations (Sirav and Seyhan, 2011). We also measured power density of generator using an EMF meter (Holaday Industry Inc., Adapazarı, Turkey). The monopole antenna connected to the generator was located perpendicular to the center of the round plastic cage to ensure a uniform distribution of electric field (Koyu et al., 2005). An 1 cm diameter air hole was devised on the lid of the plastic cage to reduce stress (Bilgici et al., 2013). Rats were arranged adjacent to each other at a 1 cm distance with their heads in the antenna direction. Subsequently, rats were exposed to EMF radiation.

2.3. Histological study

Testicular tissues were fixed in 10 % formalin, then subjected to routine tissue processing including dehydration, clearing, infiltration and embedding. A pilot study was performed on testicular samples to determine the sampling strategy. Under this strategy, testicular paraffin blocks were cut into a series of sections using the systematic random sampling technique via a rotary microtome (Leica RM2125RT). Every 7th 20-μm section was employed for stereological analysis. 7-μm sections also were obtained from the same testicular tissue blocks for histopathological and immunochemistry examination. Finally, all slides were stained with hematoxylin-eosin (Bancroft et al., 1994).

2.4. Anti-DCXR antibody

Dicarbonyl L-xylulose reductase (DCXR) has various functions in the body (Lee et al., 2013). DCXR protein as a marker of sperm maturation plays a critical role in fertilization (Ebert et al., 2015). An anti-DCXR antibody kit (ab154290; KiMERA Medical Laboratory Supplies, Istanbul, Turkey) was used to indicate the immunohistochemical staining of DCXR protein on sperms. First, sections were treated with 3 % H₂O₂ for 15 min, followed by the SuperBlock blocking buffer (ScyTek Laboratories, SotoGen Biomedikal Ltd., İstanbul, Turkey) for 7 min to eliminate non-specific immunoreactivity. These sections were incubated with anti-DCXR antibody (ab154290; Rabbit polyclonal, KiMERA Medical Laboratory Supplies, Istanbul, Turkey) diluted 1/1000 in citrate buffer at 37 °C for 2 h. Subsequently, sections were washed in PBS buffer three times for 5 min, then treated with biotinylated secondary antibody (ab93705; KiMERA Medical Laboratory Supplies, Istanbul, Turkey) for 30 min. Streptavidin peroxidase (ab93705; KiMERA Medical Laboratory Supplies, Istanbul, Turkey) was then applied for 20 min in order to observe the primary antibody-bound biotin. Sections were incubated with AEC (3-amino-9-ethyl carbazyl) chromogen (ab93705; KiMERA Medical Laboratory Supplies, Istanbul, Turkey). Finally, after counterstaining the sections with Mayer's hematoxylin, proportion of positive staining of sperms was analyzed using a light microscope (Leica, LDM 4000, Wetzlar, Germany) (Liu et al., 2002).

2.5. Stereological analysis

Stereological analyses were performed using the Stereo Investigator 9.0 software package (MFB Bioscience, Colchester, VT, USA) and a modified light microscope. Based on a pilot study, we determined the best sampling and counting strategy as well as the point density of the point-counting grid (Gundersen et al., 1988). Sections 20-μm thick were used to calculate the mean testicular volume and total number of cells.

2.5.1. Estimation of mean testicular volume

The Cavalieri method was used to estimate the mean testicular

volume (Altunkaynak et al., 2016a,b, 2019). First, the contour of the regions of interest was traced at a magnification of $5 \times$. We then superimposed the point-counting grid over the delineated area. The area of each section was automatically computed using the following formula:

$$\text{Area}(A) = a(p) \times \sum P$$

where “a(p)” is the point interval area, and “ $\sum P$ ” is the total number of hitting points on each section. The mean volume of each section then was calculated as:

$$\text{Volume}(V_1) = t \times A$$

Where “ V_1 ” is the mean volume of each section, “t” is the thickness of the section plus the determined interval, and “A” is the sectional area. Finally, the total volume of each testis was computed using the formula below:

$$\text{Total } V = V_1 + V_2 + \dots V_n$$

2.5.2. Estimation of total numbers of spermatids, primary spermatocytes, spermatogonia, Leydig and Sertoli cells

The total number of cells was estimated by means of the optical fractionator (Yahyazadeh and Altunkaynak, 2019a). All sampling fractions were selected using the systematic random sampling method. The optical fractionator sampling scheme was also determined based on a pilot study. According to the counting rule of the optical fractionator, the unbiased estimation of the total cell numbers was performed as follow:

$$N = \sum Q \cdot \frac{1}{SSF} \cdot \frac{1}{ASF} \cdot \frac{1}{TSF}$$

where “N” is the total number of cells, “Q” is the total number of counted dissector cells, “ASF” is the area sampling fraction, “SSF” is the section sampling fraction, and “TSF” is the thickness sampling fraction.

A coefficient of error value was calculated to determine the adequate number of cells in each animal (Gundersen and Jensen, 1987; Yahyazadeh and Altunkaynak, 2019b). CE was also validated with a pilot study. Additionally, statistical power analysis indicated that the number of animals in each group was adequate for this experimental study. The power was calculated using G*power software (version 3.1.9.4).

2.6. Biochemical analysis

2.6.1. SOD assay

Superoxide dismutase (SOD) is an endogenous antioxidant enzyme in biological systems. We used SOD assay kit (Cayman Chemical Co., Ann Arbor, MI, USA) to measure the serum levels of SOD using the colorimetric method. All biochemical test procedures were applied in accordance with the manufacturers' instructions. Briefly, 200 μ l of diluted radical detector and 10 μ l of SOD standard were added to the wells of a plate. Subsequently, 10 μ l of each sample was placed into the sample wells, followed by adding 10 μ l of xanthine oxidase to initiate the reaction. The plate was shaken, then incubated for 30 min at room temperature. Finally, the wells were read at 440 nm using a UV-vis spectrophotometer (Shimadzu UV-MINI 1240; Shimadzu; Istanbul, Turkey).

2.6.2. Testosterone assay

Serum testosterone level was measured using a testosterone EIA kit (Cayman Chemical Co., Ann Arbor, MI, USA) according to the manufacturer's instructions. Briefly, all reagents and samples were added to the wells of the ELISA plate, then covered with plastic film to develop in the dark for 60–90 min. Finally, plate was read at a wavelength of 420 nm.

Table 1

Quantitative values of wet weight and mean volume of testes in all groups.

Groups	Testis weight (g)	Mean testicular volume (cm ³)
Control	1.78 $\pm$ 0.07	2.6 $\pm$ 0.08
EMF	1.54 $\pm$ 0.03*	2.2 $\pm$ 0.12
TQ	1.77 $\pm$ 0.05	2.5 $\pm$ 0.11
EMF + TQ	1.68 $\pm$ 0.04	2.3 $\pm$ 0.09

Data: means $\pm$ SEM.

* significantly different from the Control group, $p < 0.05$, the number of subjects (N) for each group: N = 6.

2.7. Statistical analysis

Statistical analysis was performed using IBM version 21.0 SPSS software (SPSS Inc., Chicago, IL, USA). Since distribution of data was normal in each group, One-Way ANOVA and the Bonferroni Post-hoc test were applied to analyze the data. The results were expressed as mean $\pm$ SEM. P values less than 0.05 were deemed statistically significant.

3. Results

3.1. Testicular wet weight

The wet weights of testes for all groups are summarized in Table 1. We found a significant decrease in the testicular wet weight in the EMF group compared to the Control or TQ groups ($p < 0.05$). By contrast, there was no significant difference between the EMF group and the EMF + TQ group.

3.2. Biochemical analysis results

Biochemical results are listed in Table 2. SOD level was significantly increased in the EMF ($p < 0.01$) or EMF + TQ group ($p < 0.01$) compared to the Control group. We found no significant difference between the EMF and EMF + TQ groups. Also, SOD level was significantly decreased in the TQ group compared to the EMF group ($p < 0.01$).

We found a significant decrease in serum testosterone level in the EMF group compared to the Control ($p < 0.01$) or TQ groups ($p < 0.01$). To the contrary, there was no significant difference between the EMF and EMF + TQ groups.

3.3. Stereological results

The stereological data are listed in Tables 1 and 3. There was no significant difference in the mean volumes of testes in the EMF group compared to the Control, EMF + TQ and TQ groups.

We found no significant difference in the total number of spermatogonia between the EMF and Control groups or the EMF + TQ and EMF groups.

The total number of primary spermatocytes was significantly decreased in the EMF group compared to the Control or TQ group.

Table 2

Quantitative values of SOD enzyme activity and testosterone level in all groups.

Groups	SOD activity (U/ml)	Testosterone (pg/ml)
Control	0.10 $\pm$ 0.009	438 $\pm$ 17
EMF	0.19 $\pm$ 0.013*	271 $\pm$ 25*
TQ	0.11 $\pm$ 0.011	523 $\pm$ 27
EMF + TQ	0.17 $\pm$ 0.018	387 $\pm$ 28

Data: means $\pm$ SEM.

* significantly different from the Control group, $p < 0.05$, the number of subjects (N) for each group: N = 6.

Table 3Quantitative values of the total number ($\times 10^4$) of spermatogenic cells, Sertoli and Leydig cells in all groups.

Groups	Spermatogonia	Primary spermatocytes	Spermatids	Sertoli cells	Leydig cells
Control	40.97 $\pm$ 0.97	44.70 $\pm$ 1.29	50.19 $\pm$ 1.25	22.96 $\pm$ 0.93	29.04 $\pm$ 0.94
EMF	33.87 $\pm$ 0.81	35.37 $\pm$ 1.09*	42.38 $\pm$ 0.91*	19.09 $\pm$ 0.50	22.83 $\pm$ 1.13*
TQ	41.32 $\pm$ 1.05	42.17 $\pm$ 1.68	49.31 $\pm$ 1.31	21.36 $\pm$ 1.05	30.57 $\pm$ 1.48
EMF + TQ	36.37 $\pm$ 1.03	37.60 $\pm$ 9.53**	46.68 $\pm$ 0.87	19.97 $\pm$ 0.58	27.50 $\pm$ 0.99

Data: means $\pm$ SEM.

* significantly different from the Control group.

** significantly different from the EMF group, $p < 0.05$, the number of subjects (N) for each group: N = 6.

($p < 0.01$). We also found a significant increase in the total number of primary spermatocytes in the EMF + TQ group compared to the EMF group ($p < 0.05$). By contrast, there was no significant difference between the EMF + TQ group and the TQ and Control groups.

The total number of spermatids was significantly less in the EMF group than the Control or TQ groups ($p < 0.05$). We also found no significant difference between the EMF + TQ group and the EMF group.

We found a significant decrease in the the total number of Leydig cells in the EMF group compared to the Control ($p < 0.05$) or TQ ($p < 0.01$) group. To the contrary, there was no significant difference in the total number of Leydig cells the EMF + TQ group compared to the EMF group.

Our findings showed no significant difference in the total number of Sertoli cells between the EMF and the Control or EMF + TQ groups.

3.4. Histopathological results

Testicular architectures appeared normal in the Control and TQ groups (Figs. 1a, b and 2 a, b). To the contrary, remarkable structural changes were observed in the testicular tissues of the EMF group. Some regions of seminiferous tubules exhibited vacuoles, separated

spermatogenic cells from the tubule wall and absence of spermatogonia. Numerous irregularly shaped and occasionally necrotic seminiferous tubules were also detected. We also observed an absence of spermatogenic cells, as well as disturbance in the interstitial tissue (Fig. 1c–f). We found structural damages to testicular tissue in the EMF + TQ group, but these were less than those of the EMF group (Fig. 2c–f).

3.5. Immunohistochemical analysis results

Positive immunohistochemical staining of spermatozoa was evaluated. Positive-stained spermatozoa were reddish-brown. Immunohistochemical findings exhibited strong positive staining of spermatozoa in the Control and TQ groups. By contrast, slight positive immunohistochemical staining of spermatozoa was observed in the EMF group. In the EMF + TQ group, TQ treatment partially improved the proportion of DCXR-positive spermatozoa (Fig. 3).

4. Discussion

Exposure to EMF emitted by cell phones has a significant effect on living organisms (Yahyazadeh and Altunkaynak, 2019b; Mokarram

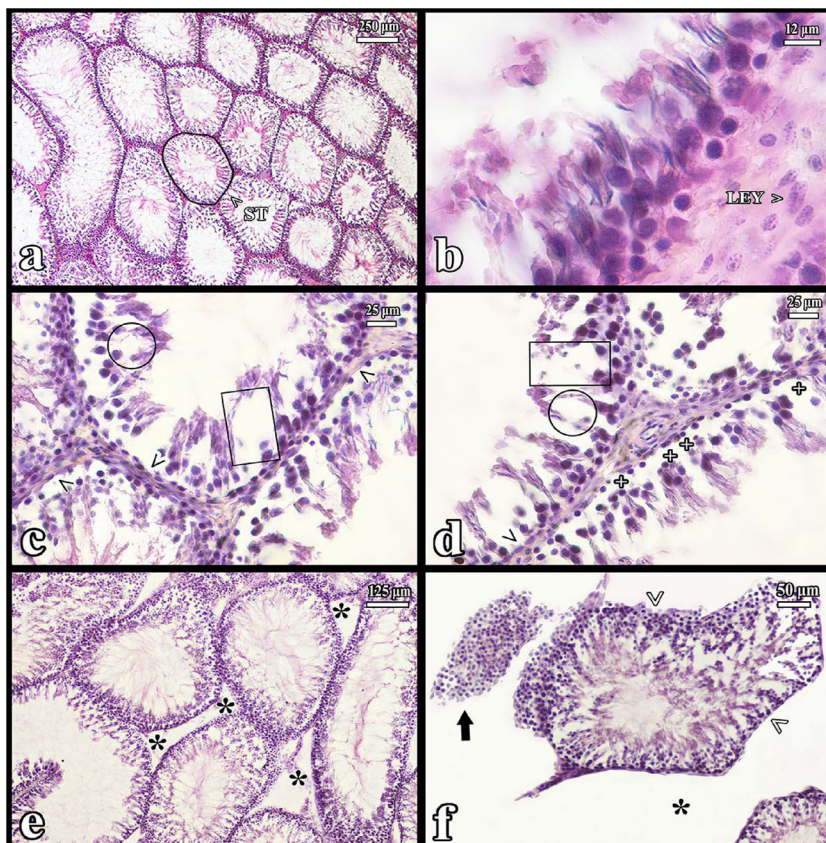


Fig. 1. Light microscopic images of the testes in the Control (a and b) and EMF (c–f) groups. ST; seminiferous tubule, LEY, Leydig cells; rectangles; region of spermatogenic cell absence, black arrowhead; absence of spermatogonia, +; seminiferous epithelium separated from the tubule wall, *; seminiferous tubules separated from each other, arrow; necrotic seminiferous tubule, circles; vacuoles, white arrowhead; irregular shapes of seminiferous tubules, rectangles; absence of spermatogenic cells. Magnification: a and e, 50x; b, 400x; c and d, 200x; f, 100 $\times$.

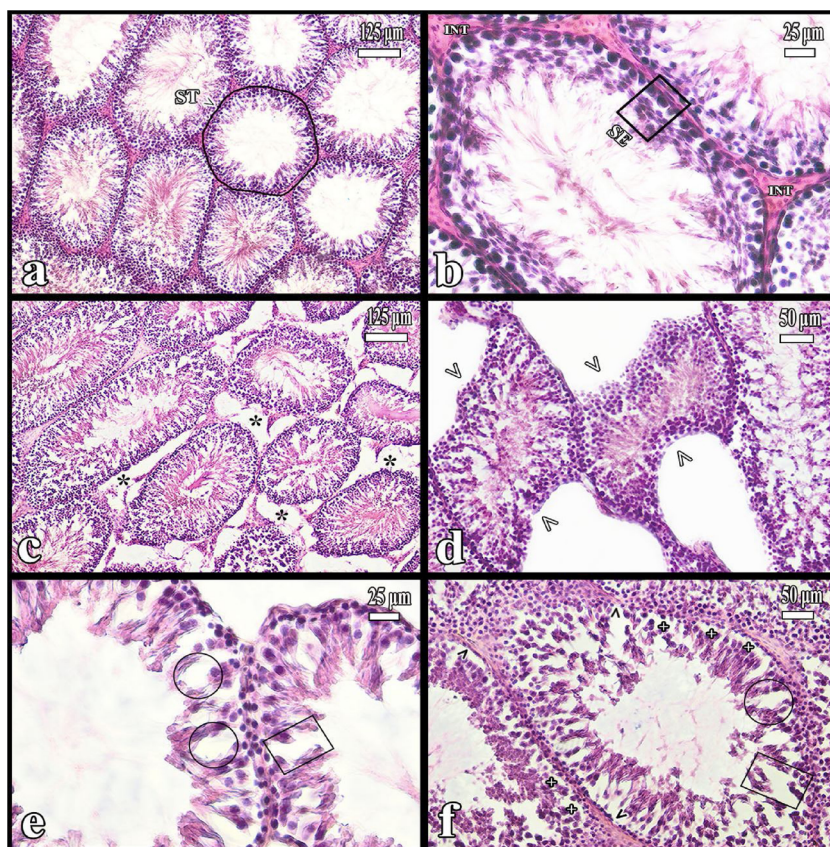


Fig. 2. Light microscopic images of the testes in the TQ (a and b) and EMF + TQ (c–f) groups. ST; seminiferous tubule, SE; seminiferous epithelium, INT; interstitial space, *; seminiferous tubules from each other, circles; vacuoles, white arrowhead; irregular shapes of seminiferous tubules, rectangles; absence of spermatogenic cells, +; seminiferous epithelium separated from the tubule wall, black arrowhead; absence of spermatogonia. Magnification: a and c, 50x; b and e, 200x; d and f, 100 × .

et al., 2017; Kaplan et al., 2016). Also, EMF radiation can be harmful to animal and human fertility (Altun et al., 2018; Çetkin et al., 2017; La Vignera et al., 2012). The impact of EMF depends on various factors such as frequency, exposure duration and power. Cell phones have been

legally limited to 2 W/kg SAR (International Commission on Non-Ionizing Radiation Protection, 2010), and many have SAR values of 1.4 W/kg (Agarwal et al., 2011).

Our findings revealed that testicular wet weight was decreased in

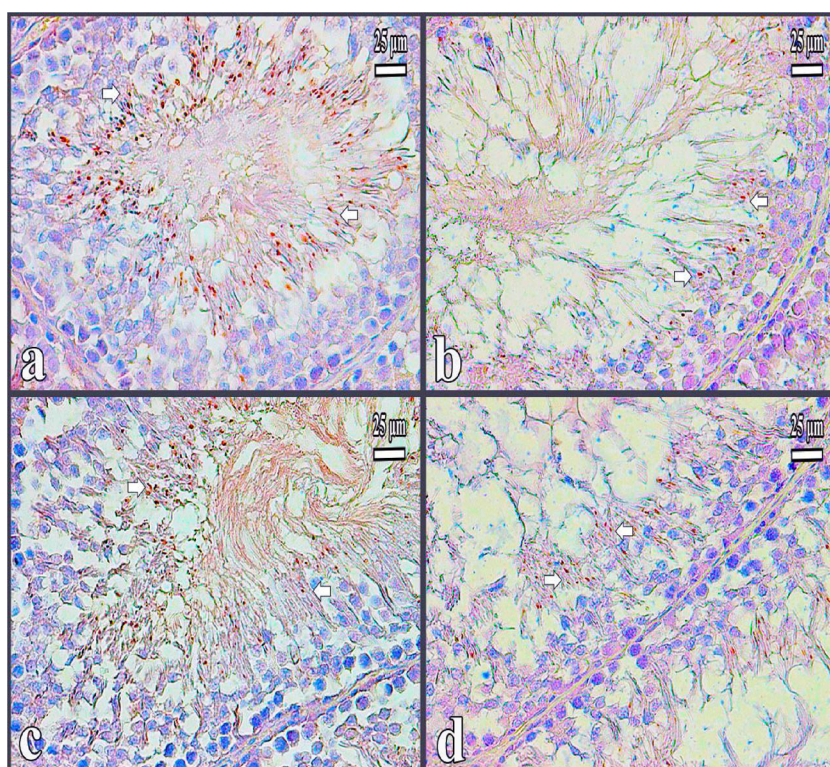


Fig. 3. Immunohistochemistry with anti-DCXR antibody in the Control (a), EMF (b), TQ (c) and EMF + TQ (d) groups. Arrow, positive immunostaining of the spermatozoa; a and c, strong immunostaining of the spermatozoa; b, slight immunostaining of the spermatozoa; d, moderate immunostaining of the spermatozoa. Magnification: a–d, 200 × .

the EMF group compared to the Control group. This may be due to a decrease in spermatids and testosterone levels as well as change in endogenous antioxidant enzymes (Sadik, 2008; Cormier et al., 2018). Also, there is a relationship between testicular weight and function. The complex processes of spermatogenesis and production of mature male gametes may be affected by pathogenic agents (Agarwal et al., 2004). Agarwal et al. (2011) reported that cell phones enhanced the production of free radicals in the organism, then impaired testicular functions. In agreement with our findings, Çetkin et al. (2017) documented that exposure to an 890–915 MHz EMF affected testicular tissue in the *Wistar albino* rats, leading to testicular weight loss. The administration of TQ also did not improve testicular weight loss in the EMF + TQ group compared to the EMF group.

Our volumetric results showed no significant change in the mean testicular volume between the EMF group and the Control group. This suggests that exposure to 900 MHz EMF has no adverse effect on the mean testicular volume.

We found that the total numbers of primary spermatocytes, spermatids and Leydig cells was lower in the EMF group compared to the Control group. This may be attributed to oxidative stress and hormonal changes caused by 900 MHz EMF. Oxidative stress caused by EMF exposure may have resulted in genotoxicity and damage to spermatogenic cells, disturbing spermatogenesis (Solek et al., 2018). Moreover, the reduction in the production of testosterone level may cause death in testicular cells (Hardy et al., 2005). Spermatogenesis is regulated by neuroendocrine activity along the hypothalamus-pituitary-testis axis. Environmental pollutants such as EMF can interfere with the neural and endocrine system functions, then cause subfertility or infertility due to impaired spermatogenesis (Gye and Park, 2012). Khillare and Behari (1998) reported that EMF radiation may affect Leydig cells in testicular tissue. Kesari et al. (2011) found that exposure to 900 MHz EMF for 2 h daily led to an increase in ROS levels and sperm apoptosis in the male rat. Guler et al. (2010) reported that EMF exposure also causes DNA damage to germ cells in the mice testicular tissue and other studies have reported that EMF induces spermatogenic cell death via activating apoptosis in male rats (Luo et al., 2013; Bin-Meferij and El-Kott, 2015). Gorpichenko et al. (2014) documented that the rate of DNA fragmentation was increased in the first 2 h of exposure to EMF. They also reported a decrease in Leydig cell number and an increase in apoptotic cell number in *Wistar* rats. We observed a significant increase in the total number of primary spermatocytes following administration of TQ in the EMF + TQ group compared to the Control group. By contrast, TQ treatment exhibited no protective effect on the total number of spermatids and Leydig cells. We found that TQ had a weak ameliorative effect against cellular damage caused by EMF radiation.

Our biochemical analysis showed an increase in SOD activity in the EMF group compared to the Control group. EMF radiation can cause overproduction of free radicals, leading to oxidative stress (Kerimoğlu et al., 2018). The ability of EMF to induce oxidative stress may be due to the high demand for oxygen in testicular tissue for spermatogenesis. However, testicular tissue is sensitive to the toxic effects of reactive oxygen metabolites. Several studies have reported a relationship between exposure to EMF and levels of SOD enzyme activity. Oyewopo et al. (2017) reported that chronic exposure to EMF reduced SOD levels, which results in disturbance in testicular function caused by oxidative stress. Irmak et al. (2002) reported that SOD enzyme activity was significantly increased after exposure to 900 MHz EMF in rabbits. We found no significant difference in SOD level between the EMF group and the TQ + EMF group; however, TQ did not improve SOD levels in testicular tissue.

Our findings also revealed a decrease in testosterone levels in the EMF group compared to the Control group. Testosterone levels can be affected by various factors, such as oxidative stress and impairment of Leydig cells. Hales et al. (2005) reported that high level of testicular oxidative stress can disrupt the steroidogenic capacity of Leydig cells, then lead to complications in production of testosterone levels. Also,

reductions in testosterone level may have resulted from a failed response to luteinizing hormone in damaged Leydig cells caused by EMF (Sepehrmanesh et al., 2014). Some studies have suggested that exposure to cell phones for 60 min/day for 3 months and 2 h/day for 45 days significantly reduce serum testosterone levels in *Wistar albino* rats (Meo et al., 2010; Kesari and Behari, 2012). We also found that TQ treatment did not improve serum testosterone levels in the EMF + TQ group compared to the EMF group.

Our histological and histopathological examination of testicular tissues showed no structural alterations in the Control or TQ groups. In the EMF group, histopathological abnormalities were most evident in the interstitial tissue, seminiferous epithelium and tubules. Our finding that exposure to EMF caused structural damages to testicular tissues is consistent with the study reported by Al-Damegh (2012). Holdcraft and Braun (2004) suggested a high level of testosterone that is essential for maintaining the normal structures of seminiferous tubules. Accordingly, a decrease in testosterone level may have led to morphological change in testicular tissue. Administration of TQ in the EMF + TQ caused a slight improvement in structural abnormalities compared to the EMF group.

Our immunohistochemical study determined the activity of DCXR proteins by applying anti-DCXR antibody to testicular tissue specimens. Our finding that EMF caused slight positive staining of spermatozoa in the EMF group is consistent with earlier report that exposure to EMF impacted on the expression of DCXR protein and gene (Zalata et al., 2015). Numerous studies have documented agents that play a crucial role in altering the genetic material of the cell. Overproduction of ROS can induce oxidative damage to the structure of the DNA bases and sugars (Guler et al., 2010). Accordingly, impairment of the oxidative balance due to ROS formation may lead to change in DCXR protein expression (Fujii and Tsunoda, 2011). Kim et al. (2009) suggested that 60 Hz EMF caused apoptosis in spermatogenic cells in the mouse testis. We also found that TQ slightly increased the number of positive stained spermatozoa in the EMF + TQ group than the EMF group. This possibly was due to weak antioxidant activity of TQ.

Natural antioxidants are thought to reduce the effects of oxidative stress. TQ is capable of attenuating oxidative stress in the testicular tissue (Ayan et al., 2016). To the best of our knowledge, our study is the first to evaluate the antioxidant activity of TQ against EMF exposure in the rat testis. There are numerous studies that investigate the protective effects of TQ, but their findings are inconclusive (Kanter, 2011). Hewita et al. (2016) showed that TQ had no significant effect on testicular toxicity in an animal model. Hossain et al. (2012) also reported that a low level concentration of TQ caused growth stimulation in cancer cells. Hence, further studies are required to investigate the possible effect of other natural antioxidants on EMF-induced damages.

5. Conclusion

The present study investigated the protective effect of TQ on the rat testis exposed to 900 MHz EMF. Our stereological and histopathological findings showed that EMF radiation caused a deleterious effect on testicular tissue. In addition, the administration of TQ exhibited partial protection against testicular damages caused by 900 MHz EMF radiation.

CRedit authorship contribution statement

Ahmad Yahyazadeh: Conceptualization, Methodology, Software, Visualization, Investigation, Data curation, Writing - original draft. **Berrin Zuhal Altunkaynak:** Conceptualization, Methodology, Validation, Visualization, Supervision, Writing - review & editing. **Suleyman Kaplan:** Validation, Writing - review & editing.

Declaration of Competing Interest

No potential conflict of interest was reported by the authors.

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References

- Agarwal, A., Nallella, K.P., Allamaneni, S.S., Said, T.M., 2004. Role of antioxidants in treatment of male infertility: an overview of the literature. *Reprod. Biomed. Online* 8, 616–627.
- Agarwal, A., Singh, A., Hamada, A., Kesari, K., 2011. Cell phones and male infertility: a review of recent innovations in technology and consequences. *Int. Braz. J. Urol.* 37, 432–454.
- Al-Damegh, M.A., 2012. Rat testicular impairment induced by electromagnetic radiation from a conventional cellular telephone and the protective effects of the antioxidants vitamins C and E. *Clinics Sao Paulo (Sao Paulo)* 67, 785–792.
- Altun, G., Deniz, Ö.G., Yurt, K.K., Davis, D., Kaplan, S., 2018. Effects of mobile phone exposure on metabolomics in the male and female reproductive systems. *Environ. Res.* 167, 700–707.
- Altunkaynak, B.Z., Akgül, N., Yahyazadeh, A., Altunkaynak, M.E., Türkmen, A.P., Akgül, H.M., Üna, L.B., 2016a. Effect of mercury vapor inhalation on rat ovary: stereology and histopathology. *J. Obstet. Gynaecol. Res.* 42, 410–416.
- Altunkaynak, B.Z., Altun, G., Yahyazadeh, A., Kaplan, A.A., Deniz, O.G., Türkmen, A.P., Önger, M.E., Kaplan, S., 2016b. Different methods for evaluating the effects of microwave radiation exposure on the nervous system. *J. Chem. Neuroanat.* 75, 62–69.
- Altunkaynak, B.Z., Akgül, N., Yahyazadeh, A., Makaracı, E., Akgül, H.M., 2019. A stereological study of the effects of mercury inhalation on the cerebellum. *Biotech. Histochem.* 94, 42–47.
- Aras, S., Gerin, F., Aydin, B., Ustunsoy, S., Sener, U., Turan, B.C., Armutcu, F., 2015. Effects of sodium arsenite on some laboratory signs and therapeutic role of thymoquinone in the rats. *Eur. Rev. Med. Pharmacol. Sci.* 19, 658–663.
- Ashraf, S.S., Rao, M.V., Kaneez, F.S., Qadri, S., Al-Marzouqi, A.H., Chandranath, I.S., Adem, A., 2011. Nigella sativa extract as a potent antioxidant for petrochemical-induced oxidative stress. *J. Chromatogr. Sci.* 49, 321–336.
- Ayan, M., Tas, U., Sogut, E., Cayli, S., Kaya, H., Esen, M., Erdemir, F., Uysal, M., 2016. Protective effect of thymoquinone against testicular torsion induced oxidative injury. *Andrologia* 48, 143–151.
- Bancroft, J.D., Cook, H.C., Stirling, R.W., 1994. *Manual of Histological Techniques and Their Diagnostic Applications*. Churchill Livingstone, London.
- Bas, O., Odaci, E., Mollaoglu, H., Ucok, K., Kaplan, S., 2009. Chronic prenatal exposure to the 900 megahertz electromagnetic field induces pyramidal cell loss in the hippocampus of newborn rats. *Tox. Ind. Health.* 25, 377–384.
- Bilgici, B., Akar, A., Avci, B., Tuncel, O.K., 2013. Effect of 900 MHz radiofrequency radiation on oxidative stress in rat brain and serum. *Electromagn. Biol. Med.* 32, 20–29.
- Bin-Meferij, M.M., El-Kott, A.F., 2015. The radioprotective effects of *Moringa oleifera* against mobile phone electromagnetic radiation-induced infertility in rats. *Int. J. Clin. Exp. Med.* 8, 12487–12497.
- Cormier, M., Ghoulil, F., Roumaud, P., Bauer, W., Touaibia, M., Martin, L.J., 2018. Influences of flavones on cell viability and cAMP-dependent steroidogenic gene regulation in MA-10 Leydig cells. *Cell Biol. Toxicol.* 34, 238–308.
- Çetkin, M., Kızılkhan, N., Demirel, C., Bozdağ, Z., Erkiş, S., Erbağcı, H., 2017. Quantitative changes in testicular structure and function in rat exposed to mobile phone radiation. *Andrologia* 49.
- Dehkordi, F.R., Kamkha, A.F., 2008. Antihypertensive effect of *Nigella sativa* seed extract in patients with mild hypertension. *Fundam. Clin. Pharmacol.* 22, 447–452.
- Ebert, B., Kisiela, M., Maser, E., 2015. Human DCXR - another 'moonlighting protein' involved in sugar metabolism, carbonyl detoxification, cell adhesion and male fertility. *Biol. Rev. Camb. Philos. Soc.* 90, 254–278.
- Fouad, A.A., Jresat, I., 2015. Thymoquinone therapy abrogates toxic effect of cadmium on rat testes. *Andrologia* 47, 417–426.
- Fujii, J., Tsunoda, S., 2011. Redox regulation of fertilisation and the spermatogenic process. *Asian J. Androl.* 13, 420–423.
- Gorpinchenko, I., Nikitin, O., Banyra, O., Shulyak, A., 2014. The influence of direct mobile phone radiation on sperm quality. *Cent. European J. Urol.* 67, 65–71.
- Goyal, S.N., Prajapati, C.P., Gore, P.R., Patil, C.R., Mahajan, U.B., Sharma, C., Talla, S.P., Ojha, S.K., 2017. Therapeutic potential and pharmaceutical development of thymoquinone: a multitargeted molecule of natural origin. *Front. Pharmacol.* 8, 656.
- Gökçe, A., Oktar, S., Koc, A., Gonenci, R., Yalcinkaya, F., Yonden, Z., Duru, M., 2010. Protective effect of thymoquinone in experimental testicular torsion. *Urol. Int.* 85, 461–465.
- Guler, G., Tomruk, A., Ozgur, E., Seyhan, N., 2010. The effect of radiofrequency radiation on DNA and lipid damage in non-pregnant and pregnant rabbits and their newborns. *Gen. Physiol. Biophys.* 29, 59–66.
- Gundersen, H.J., Jensen, E.B., 1987. The efficiency of systematic sampling in stereology and its prediction. *J. Microsc.* 147, 229–263.
- Gundersen, H.J., Bagger, P., Bendtsen, T.F., Evans, S.M., Korbo, L., Marcussen, N., Møller, A., Nielsen, K., Nyengaard, J.R., Pakkenberg, B., et al., 1988. The new stereological tools: disector, fractionator, nucleator and point sampled intercepts and their use in pathological research and diagnosis. *APMIS* 96, 857–881.
- Güney, M., Ozguner, F., Oral, B., Karahan, N., Mungan, T., 2007. 900 MHz radio-frequency-induced histopathologic changes and oxidative stress in rat endometrium: protection by vitamins E and C. *Toxicol. Ind. Health* 23, 411–420.
- Gye, M.C., Park, C.J., 2012. Effect of electromagnetic field, exposure on the reproductive system. *Clin. Exp. Reprod. Med.* 39, 1–9.
- Hales, D.B., Allen, J.A., Shankara, T., Janus, P., Buck, S., Diemer, T., Hales, K.H., 2005. Mitochondrial function in Leydig cell steroidogenesis. *Ann. N. Y. Acad. Sci.* 1061, 120–134.
- Hanci, H., Kerimoğlu, G., Mercantepe, T., Odacı, E., 2018. Changes in testicular morphology and oxidative stress biomarkers in 60-day-old Sprague Dawley rats following exposure to continuous 900-MHz electromagnetic field for 1 h a day throughout adolescence. *Reprod. Toxicol.* 81, 71–78.
- Hardy, M.P., Gao, H.B., Dong, Q., Ge, R., Wang, Q., Chai, W.R., Feng, X., Sottas, C., 2005. Stress hormone and male reproductive function. *Cell Tissue Res.* 322, 147–153.
- Holdcraft, R.W., Braun, R.E., 2004. Hormonal regulation of spermatogenesis. *Int. J. Androl.* 27, 335–342.
- Hossain, S., Sikes-Thurston, E., Leppla, S.H., Wein, A.N., 2012. Thymoquinone as a novel antibiotic and chemotherapeutic agent: a natural therapeutic approach on *Staphylococcus aureus*, *Bacillus anthracis*, and four NCI-60 cancer cell lines. *J. Exp. Second. Sci.* 19, 1–4.
- International Commission on Non-Ionizing Radiation Protection, 2010. Guidelines for limiting exposure to time-varying electric and magnetic fields (1 Hz to 100 kHz). *Health Phys.* 99, 818–836.
- Irmak, M.K., Fadilloğlu, E., Güleç, M., Erdoğan, H., Yağmurca, M., Akyol, O., 2002. Effects of electromagnetic radiation from a cellular telephone on the oxidant and antioxidant levels in rabbits. *Cell Biochem. Funct.* 20, 279–283.
- Kanter, M., 2011. Thymoquinone reestablishes spermatogenesis after testicular injury caused by chronic toluene exposure in rats. *Toxicol. Ind. Health* 27, 155–166.
- Kaplan, S., Deniz, O.G., Önger, M.E., Türkmen, A.P., Yurt, K.K., Aydın, I., Altunkaynak, B.Z., Davis, D., 2016. Electromagnetic field and brain development. *J. Chem. Neuroanat.* 75, 52–61.
- Kerimoğlu, G., Güney, C., Ersöz, Ş., Odacı, E., 2018. A histopathological and biochemical evaluation of oxidative injury in the sciatic nerves of male rats exposed to a continuous 900-megahertz electromagnetic field throughout all periods of adolescence. *J. Chem. Neuroanat.* 91, 1–7.
- Kesari, K.K., Kumar, S., Behari, J., 2011. Effects of radiofrequency electromagnetic wave exposure from cellular phones on the reproductive pattern in male wistar rats. *Appl. Biochem. Biotechnol.* 164, 546–559.
- Kesari, K.K., Behari, J., 2012. Evidence for mobile phone radiation exposure effects on reproductive pattern of male rats: role of ROS. *Electromagn. Biol. Med.* 31, 213–222.
- Khillare, B., Behari, J., 1998. Effect of amplitude-modulated radiofrequency radiation on reproduction pattern in rats. *Electromagn. Biol. Med.* 17, 43–55.
- Kim, Y.W., Kim, H.S., Lee, J.S., Kim, Y.J., Lee, S.K., Seo, J.N., Jung, K.C., Kim, N., Gimm, Y.M., 2009. Effects of 60 Hz 14 microT magnetic field on the apoptosis of testicular germ cell in mice. *Bioelectromagnetics* 30, 66–72.
- Koyu, A., Cesur, G., Ozguner, F., Akdogan, M., Mollaoglu, H., Ozen, S., 2005. Effects of 900 MHz electromagnetic field on TSH and thyroid hormones in rats. *Toxicol. Lett.* 157, 257–262.
- La Vignera, S., Condorelli, R.A., Vicari, E., D'Agata, R., Calogero, A.E., 2012. Effects of the exposure to mobile phones on male reproduction: a review of the literature. *J. Androl.* 33, 350–356.
- Lee, S.K., Son, I., Choi, H.J., Ahnn, J., 2013. Dicarbonyl/l-xylulose reductase (DCXR): the multifunctional pentosuria enzyme. *Int. J. Biochem. Cell Biol.* 45, 2563–2567.
- Liu, J., Tian, Z., Gao, B., Kunos, G., 2002. Dose-dependent activation of antiapoptotic and proapoptotic pathways by ethanol treatment in human vascular endothelial cells: differential involvement of adenosine. *J. Biol. Chem.* 277, 20927–20933.
- Lu, Y.S., Huang, B.T., Huang, Y.X., 2012. Reactive oxygen species formation and apoptosis in human peripheral blood mononuclear cell induced by 900MHz mobile phone radiation. *Oxid. Med. Cell. Longev.* 2012, 740280.
- Luo, Y., Wang, X., Chen, Y., Xu, S., Ding, G., Shi, C., 2013. Effects of electromagnetic radiation on morphology and TGF-β3 expression in mouse testicular tissue. *Toxicology* 310, 8–14.
- McCann, J., Dietrich, F., Rafferty, C., 1998. The genotoxic potential of electric and magnetic fields: an update. *Mutat. Res.* 411, 45–86.
- Meo, S.A., Al-Drees, A.M., Sufia, H., Khan, M.M., Imran, M.B., 2010. Effects of mobile phone radiation on serum testosterone in Wistar albino rats. *Saudi Med. J.* 31, 869–873.
- Mokarram, P., Sheikhi, M., Mortazavi, S.M.J., Saeb, S., Shokrpour, N., 2017. Effect of exposure to 900 mhz gsm mobile phone radiofrequency radiation on estrogen receptor methylation status in colon cells of male Sprague Dawley rats. *J. Biomed. Phys. Eng.* 7, 79–86.
- Narayanan, S.N., Kumar, R.S., Kedage, V., Nalini, K., Nayak, S., Bhat, P.G., 2014. Evaluation of oxidant stress and antioxidant defense in discrete brain regions of rats exposed to 900 MHz radiation. *Bratisl. Lek. Listy* 115, 260–266.
- Odaci, E., Bas, O., Kaplan, S., 2008. Effects of prenatal exposure to a 900 MHz electromagnetic field on the dentate gyrus of rats: a stereological and histopathological study. *Brain Res.* 1238, 224–229.
- Oh, J.J., Byun, S.S., Lee, S.E., Choe, G., Hong, S.K., 2018. Effect of electromagnetic waves from mobile phones on spermatogenesis in the era of 4G-LTE. *Biomed. Res. Int.* 2018, 1801798.
- Oyewopo, A.O., Olaniyi, S.K., Oyewopo, C.I., Jimoh, A.T., 2017. Radiofrequency electromagnetic radiation from cell phone causes defective testicular function in male Wistar rats. *Andrologia* 49, 1–6.
- Sadik, N.A., 2008. Effects of diallyl sulfide and zinc on testicular steroidogenesis in cadmium-treated male rats. *J. Biochem. Mol. Toxicol.* 22, 345–353.

- Sepehrmanesh, M., Saeb, M., Nazifi, S., Kazemipour, N., Jelodar, G., Saeb, S., 2014. Impact of 900 MHz electromagnetic field exposure on main male reproductive hormone levels: a *Rattus norvegicus* model. *Int. J. Biometeorol.* 58, 1657–1663.
- Sharma, P., Parmar, J., Verma, P., Goyal, P., 2015. Radiation induced oxidative stress and its toxicity in testes of mice and their prevention by *Tinospora cordifolia* extract. *J. Reprod. Health Med.* 1, 64–75.
- Sirav, B., Seyhan, N., 2011. Effects of radiofrequency radiation exposure on the blood brain barrier permeability in male and female rats. *Electromag. Biol. Med.* 30, 253–260.
- Solek, P., Majchrowicz, L., Koziorowski, M., 2018. Aloe arborescens juice prevents EMF-induced oxidative stress and thus protects from pathophysiology in the male reproductive system in vitro. *Environ. Res.* 166, 141–149.
- Sonmez, O.F., Odaci, E., Bas, O., Kaplan, S., 2010. Purkinje cell number decreases in the adult female rat cerebellum following exposure to 900 MHz electromagnetic field. *Brain Res.* 1356, 95–101.
- Svedenstal, B.M., Johanson, K.J., Mild, K.H., 1999. DNA damage induced in brain cells of CBA mice exposed to magnetic fields. *In vivo.* 13, 551–552.
- Tabrah, F.L., Ross, P., Hoffmeier, M., Gilbert, F.Jr., 1998. Clinical report on long-term bone density after short-term EMF application. *Bioelectromagnetics.* 19, 75–78.
- Tang, J., Zhang, Y., Yang, L., Chen, Q., Tan, L., Zuo, S., Feng, H., Chen, Z., Zhu, G., 2015. Exposure to 900MHz electromagnetic fields activates the mkp-1/ERK pathway and causes blood-brain barrier damage and cognitive impairment in rats. *Brain Res.* 160, 92–101.
- Tenorio, B.M., Jimenez, G.C., Morais, R.N., Torres, S.M., Nogueira, R.A., Junior, V.A.S., 2011. Testicular development evaluation in rats exposed to 60 Hz and 1 mT electromagnetic field. *J. Appl. Toxicol.* 31, 223–230.
- Ulubay, M., Yahyazadeh, A., Deniz, Ö.G., Kivrak, E.G., Altunkaynak, B.Z., Erdem, G., Kaplan, S., 2015. Effects of prenatal 900 MHz electromagnetic field exposures on the histology of rat kidney. *Int. J. Radiat. Biol.* 91, 1–25.
- Vesselinova, L., 2015. Body mass index as a risk prediction and prevention factor for professional mixed low-intensity EMF burden. *Electromagn. Biol. Med.* 34, 238–243.
- Yahyazadeh, A., Deniz, Ö.G., Kaplan, A.A., Altun, G., Yurt, K.K., Davis, D., 2018. The genomic effects of cell phone exposure on the reproductive system. *Environ. Res.* 167, 684–693.
- Yahyazadeh, A., Altunkaynak, B.Z., 2019a. Protective effects of luteolin on rat testis following exposure to 900 MHz electromagnetic field. *Biotech. Histochem.* 1–10.
- Yahyazadeh, A., Altunkaynak, B.Z., 2019b. Investigation of the neuroprotective effects of thymoquinone on rat spinal cord exposed to 900 MHz electromagnetic field. *J. Chem. Neuroanat.*, 101657.
- Zalata, A., El-Samanoudy, A.Z., Shaalan, D., El-Baiomy, Y., Mostafa, T., 2015. In vitro effect of cell phone radiation on motility, DNA fragmentation and clusterin gene expression in human sperm. *Int. J. Fertil. Steril.* 9, 129–136.