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2.45 GHz radio frequency radiation exposure induced changes on rat testes and protective effects of vitamins E and C

2.45 GHz radyo frekansı radyasyonunun rat testisinde oluşturduğu değişiklikler ve bu değişikliklere vitamin E ve vitamin C' nin koruyucu etkisi

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Öz

2.45 GHz radyo frekans elektromanyetik radyasyona (EMR) maruziyetin 12 haftalık sıçan testisleri üzerinde oluşturduğu değişiklikler ve bu değişikliklere vitamin E ve vitamin C'nin muhtemel koruyucu etkisini araştırmayı amaçladık. Bu çalışmada 28 adet Sprague-Dawley cinsi erkek sıçan kullanıldı. Sıçanlar 3 gruba ayrıldı: 1. grup: Kontrol grubu (3. gruba verilen miktarda oral gavaj ile su verildi), 2. grup: 2.45 GHz EMR' ye maruz bırakılan grup, 3. grup: 2.45 GHz EMR' ye maruziyet + Vitamin E ve C verilen grup (oral gavajla 50 mg vitamin E+ 200 mg vitamin C mg/kg ağırlık/gün). 2. ve 3. gruptaki sıçanlar 30 gün boyunca her gün aynı saatte 60 dakika 2.45 GHz EMR' ye maruz bırakıldı. 3. gruba vitamin E ve C, EMR uygulamasından 1 saat önce verildi. 30 günün sonunda sıçanlar anestezi altında servikal dislokasyon yöntemiyle sakrifiye edildi ve testisleri çıkarıldı. Rutin doku takibi yapıldı ve kesitler hematoksilen-eozin (H-E) ile boyandı. EMR' ye maruz kalan sıçanların testislerinin ağırlık ve hacimlerinde kısmen azalma tespit edildi. Histopatolojik incelemede ise seminifer tübül çapında ve Johnsen skorlama yönteminde EMR' ye maruz kalan sıçanlarda azalma tespit edildi. Ancak gruplar arası karşılaştırılmada istatistiksel olarak anlamlı fark gözlemlenmedi. Vitamin E ve C verilen grupta bu değişikliklerin grup 2' ye oranla daha az olduğu tespit edildi. Leydig hücre skorunda ise gruplar arasında fark gözlemlenmedi. Sonuç olarak EMR uygulamaları sıçan testisinde kısmen değişiklikler oluşturmaktadır ve bu değişikliklere karşı vitamin E ve vitamin C' nin bir miktar koruyucu etkisi bulunmaktadır.

Anahtar Kelimeler: Testis, sıçan, 2.45 GHz radyo frekansı, vitamin E, vitamin C

Abstract

The aim of this study was designed to determine the effects of 2.45 GHz radio frequency electromagnetic radiation on rat testes found in 12-week-old rats and the possible protective effects of vitamin E and vitamin C. Twenty-eight male Sprague-Dawley rats were used in this study. Rats were divided into three groups: Group I: Control group, (received oral gavages of water at an equal volume to that of vitamin E+ vitamin C used in group III), Group II: exposed to 2.45 GHz EMR, Group III: exposed to 2.45 GHz EMR + Vitamins E and C treated group (50 mg vitamin E + 200 mg vitamin C mg / kg body weight / day, oral gavages). Group II and group III were exposed to 2.45 GHz EMR, for 60 minutes every day for 30 days. Vitamin E and C, was given 1 hour before the EMR application to Group III. The rats were sacrificed by cervical dislocation under anesthesia at the end of 30 days period, and the testes were dissected. Routine tissue procedure was applied and the sections were stained with hematoxylin–eosin (H-E). The weights and volumes of the testes were slightly reduced in the EMR exposed groups. In histopathological examinations, diameter of tubulus seminiferus and Johnsen scores were slightly reduced in the EMR exposed groups. However, no statistically significant difference was observed between groups. It is determined that, the changes were lower in vitamin E and C administered groups when compared to group II. There was no statistically significant difference in Leydig cell scores. In conclusion, EMR applications causes slightly changes in rat testes and, vitamin E and C have some protective effects.

Keywords: Testis, rat, 2450 MHz radio frequency, vitamin E, vitamin C

Introduction

In recent years, the use of electronic devices is increasing with each passing day. Electronic devices which simplify our daily life, such as laptops computers, mobile phones or tablet devices, radiate electromagnetic waves due to wireless system.

Studies had already shown the effects of 2.45-GHz electromagnetic radiation on different body parts such as testis, kidney, hippocampus, brain, thyroid, immune system, spleen and liver (1-6). The testes are more sensitive to a variety of stresses, like inflammation, hyperthermia and radiation (7,8). Testes are also very susceptible to oxidative damage induced by reactive oxygen species (ROS) because they contain large amounts of peroxidation-susceptible polyunsaturated fatty acids. On the other hand, testes contain low amounts of antioxidants (9,10). A recent study reported that exposure to Wi-Fi EMR induced oxidative stress, and decreased the levels of antioxidants in the kidney and testes of rats (11).

The cell has various ways to alleviate the effects of oxidative stress, either by repairing the damage or by directly diminishing the occurrence of oxidative damage through enzymatic and non-enzymatic antioxidants (12). Vitamin E and vitamin C are well known potent antioxidants. Vitamin E is the most important lipophilic antioxidant and mainly in the cell membranes (13). Vitamin C is the most important hydrophilic free-radical scavenger in extracellular fluids and protecting biomembranes from peroxidative damage (14). Some studies have revealed that a combination of vitamins E and C decrease the lipid peroxidation caused by oxidative stress (15). In a different study, 900 MHz electromagnetic radiation has made histopathological changes in the rat endometrium, and vitamin E and C reduced these changes (12). Therefore, vitamins E and C can have interactive effects (16).

The effect of vitamins E and C on rat testis tissue after 2,45 GHz EMR exposed animals has not been reported. Therefore, we aimed to investigate the effects of 2.45 GHz EMR exposure on testes, and possible protective effects of vitamins E and C on the testes injury, induced by EMR exposure.

Material and Methods

Animals

Twenty-eight, 12-week-old male Spraque-Dawley rats were used in the study. The rats were obtained from the Experimental Research Unit of Süleyman Demirel University. The study was approved by the Süleyman Demirel University Animal Ethics Committee (26 February 2013, No: 04). The rats were maintained under standard laboratory conditions (temperature 22 oC; light/dark cycle 12/12 h; ad libitium with standard rat chow and tap water).

The rats were randomly divided into three groups, as follows;

Group I (n=8): control group; received oral gavages of water at an equal volume to that of vitamin E+ vitamin C used in group III.

Group II (n=10): EMR exposed group

Group III (n=10): EMR exposed + Vitamins E and C treated group (50 mg vitamin E + 200 mg vitamin C mg / kg body weight / day, oral gavages).

Vitamins E (Evigen; Aksu Farma, Istanbul, Turkey) and C (Redoxon; Roche, Basel, Switzerland) were administered one hour before the EMR application for Group III. The rats in group II and III were exposed to 2.45 GHz EMR 1h/day for 30 days. The beginning exposure time was 10:00 am every day. The control group, group II and, group III were kept individually in stainless steel cages in the same room under standard laboratory conditions (temperature 22 oC; light/dark cycle 12/12 h; ad libitium with standard rat chow and tap water) during the out of EMR application time. The body weights of the rats were measured at the beginning and at the end of the experiment.

EMR Exposure Design

An electromagnetic energy generator (SET ELECO, Set Elektronik, Istanbul), and its monopole antenna was used for Wi-Fi like electromagnetic exposure application. 2.45 GHz experimental exposure, a 217 Hz pulsed radio frequency (RF) source and setup was tested in the Electromagnetic Laboratory of the Department of Electronics and Communication Engineering at Süleyman Demirel University. This RF generator is able to create electric field densities from 1 to 100 V/m on the exposure setup. Using this generator, 5 V/m electric field intensity can be obtained to get the realistic value as 0.1 W/kg of whole body specific absorption rate (SAR). Antenna power out was limited to 0.8 W (17). The electric field density was set 5 V/m in order to get a 100 mW/kg whole testes average SAR. Rats in the study group were exposed to 2.45 GHz EMR for 1 h/day, 7 days/week for 30 days. In order not to affect the control group, exposure process was conducted in an electromagnetically clean room. After the EMR exposure process the rats of group II, and III were kept under standard laboratory conditions in the same room with group I. The rats of each group were kept separate cages. The clean room is made by using stainless steel sheets for covering the walls of experiment room in the Experimental Animals Laboratory of Süleyman Demirel University. The average shielding effectiveness value of 100 dB on the frequency band is obtained. Electromagnetic measurements and instrumentations were conducted by the Department of Electronics and Communication Engineering.

During the exposure, each rat was placed in a special plastic holder (15x5 cm diameter). The quality of the transmitted signal was checked using spectrum analyzer (Promax AE-566) with its appropriate near-field probes. The power density measurements were made with an EMR survey meter (Holaday Industries, Inc., Eden Prairie, MN, USA). In order to provide the same whole body exposure to all 20 rats at the same time, the exposure setup was located in the same distance from the monopole antenna. Because the antenna is omni directional, and the rats have the full physical symmetry, the exposure setup can produce equal exposure to the rats. At 2.45 GHz, dielectric permittivity , conductivity , and specific weight   of rat tissues were found from the tables of scientific literature (18). By using the methodology mentioned above, whole body average SAR was found as the value of 0.1 W/kg.

In an effort to control and monitor the produced RF energy; background noise and other possible unwanted noises were measured by using specific tools and equipments.

In this study, Finite Difference Time Domain (FDTD) method was used in order to evaluate absorbed RF energy in the biological tissues (19,20). For SAR calculation, firstly average electric field was noted. Then the distance between certain tissue and the antenna was determined. Finally, electrical parameters of tissue on the certain frequency band were found from scientific literature. SAR values were calculated through the FDTD method with MATLAB software (19,21).

Histopathological examinations

At the end of the experimental period, the rats were sacrificed by cervical dislocation under anesthesia via intra-peritoneal injection of ketamine hydrochloride (90 mg/kg) and xylazine (5mg/kg). The testes were removed for histopathological examination, and were bisected and fixed in 10% formaldehyde solution. The testes tissue samples were embedded in paraffin, cross sections were cut in 5 μm thickness and stained with hematoxylin-eosin. Histologic sections were assessed and photographed by Olympus BX51 photomicroscope. The mean diameter of the seminiferous tubules and Johnsen score was evaluated in the microscopic examination. The stage of the seminiferous tubules of germ cells in the testis of serial sections (spermatogenesis) with Johnsen testicular biopsy score and the number of Leydig cells in the interstitial space were evaluated.

Statistical analyses for Johnsen score, Leydig cells, diameter of the seminiferous tubules, and weight and volume of the testes were performed using SPSS 17.00 statistical package (SPSS Inc, Chicago, IL, USA). The significance level was set at 0.05. Data were expressed as mean ± Standard deviation. The non-parametric Mann-Whitney's U-test was used for comparisons of groups and, P and x^2 values were presented in relevant tables.

Results

There were no statistically significant differences in the means of the body weights among the groups at the end of the experimental period (Table 1).

Table 1. Mean Body Weight of rats (means±SD)(g)

Groups	Beginning of the experiment	End of the experiment
I	273.62±23.06	**309.75±26.35**
II	262.60±24.85	**294.20±22.51**
III	257.40±39.13	**281.30±38.91**

It was observed that weights and volumes of the rat testes were decreased in the EMR exposed groups when compared to the control group. However, the decrease was lower in the Vitamins E and C administered group. Statistically significant difference was not observed between groups ($p > 0.05$), (Table 2).

Table 2: Comparison of morphological and histopathological parameters between the control (group I), EMR exposed (group II), EMR exposed + Vitamins E and C administered (group III) groups (means±SD).

Group	Weight[a] (g)	Volume[b] (ml)	Diameter of[c] Tubulus Seminiferus	Johnsen[d] Scores	Leydig[e] Cell Scores
I	6.96±1.28	7.03±1.38	258.74±12.37	9.98±0.54	3±0.0
II	6.65±0.89	6.77±0.88	255.45±10.53	9.91±0.14	3±0.0
III	6.94±0.35	7.30±0.40	260.32±8.09	9.96±0.08	3±0.0
a,b,c,d,e P>0.05 No significant differences					

In histopathological examinations, the control group testes tissues were observed as normal (Figure 1).

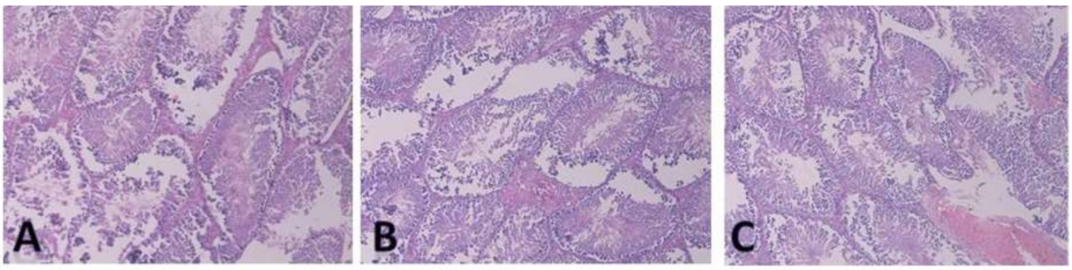

Figure 1: Histological section of the testes (X40, hematoxylin-eosin [H&E]). A; group I : control group, Normal appearance of leydig cells and seminiferous tubules of the testes tissue. B; group II : EMR exposed group, C; group III: EMR exposed + Vitamins E and C treated group.

Diameter of tubulus seminiferus and Johnsen scores were decreased in the EMR exposed groups, but the decreases were lower in the Vitamins E and C administered group. However, statistically significant difference was not observed between groups ($p > 0.05$), (Table 2) (Figure 1).

There was no difference between the groups in Leydig cell scores (Table 1).

Discussion

Wireless devices have a widespread use in today's modern world. These wireless devices use higher frequency and usually have a longer exposure time and wider area of exposure (22). Like laptop computers which contain wireless technology, generally used near reproductive organs and may have harmful effects on testes (23). Oxidative stress plays some role in the EMR exposure effects on body tissues (24,12).

In our study, we choose testes because testes are extremely susceptible to oxidative damage induced by ROS, and the testes are more sensitive to a variety of stresses, such as inflammation, hyperthermia, and radiation (7-10).

The effect of the EMR on testicular tissue at different frequencies on rats investigated in several studies. Lots of studies have indicated that, EMR effected male reproductive tissues (32) but some other studies have been conflicting (2).

Previous studies reported no significant difference between testicular weights of the rats exposed to EMR (23,26,27). On the other hand, Imai et al. and results of our studies are reported EMR reduced the weight of the rat's testes (25).

Table 3 shows the effects of EMR at different frequencies (MHz) and different exposure time period on Johnsen score of rat's testes. While Ozguner et al. (900 MHz) and

Kim et al. (2450 MHz) did not observe any significant effect (27,28). However, Saygın et al. (29) and results of our studies are reported.

Table 3: Effects of different frequencies (MHz) on rat's Johnsen score.

Reference	Frequency (MHz)	Time (daily/weekly/ total)	Effects
Ozguner et al. 2005	900	30 minutes per day, 5 days a week for 4 weeks	No
Kim et al. 2007	2450	for 1 hour or 2 hours a day for 8 weeks	No
Saygın et al. 2011	2450	60 minutes/day for 28 days	Yes (significant)
Present Study	2450	60 minutes per day, 7 days a week for 30 days	Yes

Leydig cells are another histopathological parameter of testis. Lots of studies show that EMR effects Leydig cells in rats as shown in Table 4 (27-29). However, Viera Almášiová et al. and we did not observe any marked morphological changes in Leydig cells (2,30).

Studies on the effects of EMR at different frequencies and different exposure time periods on rats seminiferous tubular diameters are presented in Table 5. Previous studies and our results are reported that EMR decreased the seminiferous tubular diameters of testes (27,28,31,32).

Table 4: Effects of different frequencies (MHz) on rat's Leydig cells

Reference	Frequency (MHz)	Time (daily/weekly/total)	Effects
Saygın et al. 2011	2450	60 minutes/day for 28 days	decrease
Ozguner et al. 2005	900	30 minutes per day, 5 days a week for 4 weeks	statistical difference
Kim et al. 2007	2450	for 1 hour or 2 hours a day for 8 weeks	statistical difference
Viera Almášiová et al. 2014	2450	3h daily applications for 3 weeks	No
Viera Almášiová et al. 2013	2450	3h daily applications for 3 weeks	No
Present Study	2450	60 minutes per day, 7 days a week for 4 weeks	No

Table 5: Effects of different frequencies (MHz) on rat's seminiferous tubular diameters.

Reference	Frequency (MHz)	Time (daily/weekly/total)	Effects
Dasdağ et al. 1999	900	2 hours per day, 7 days a week for 4 weeks	decrease
Saygın et al. 2011	2450	60 minutes/day for 28 days	No
Ozguner et al. 2005	900	30 minutes per day, 5 days a week for 4 weeks	decrease
Kim et al. 2007	2450	for 1 hour or 2 hours a day for 8 weeks	decrease
S. Shahin et al. 2014	2450	2 hours per day, 7 days a week for 4 weeks	decrease
Present Study	2450	60 minutes per day, 7 days a week for 30 days	decrease

Vitamin E and vitamin C are well known potent antioxidants. In an experimental study, it has been shown that 900 MHz radiofrequency has some histopathologic changes in rat endometrium, and vitamins E and C reduced these changes (12). In previous studies, vitamin E plus vitamin C administration was found to reduce the oxidative stress in rat testes (33). The results of present study reported that vitamin E plus vitamin C administration reduced the morphological and histopathological damages in rat testes.

The body weights of the rats were measured at the beginning and end of the experiment. Our results showed that there is no statistically significant difference in weight gain between the control and exposure groups. These results were consistent with previously reported studies (34-35), except Salah et al. (36) who reported a loss in body weight in rats exposed to 2.45 GHz EMR. In our knowledge, there is no current data on effects of 2.45 GHz EMR on rat's testes, and protective effects of vitamins E and C.

According to literature and our results, 2.45 GHz EMR exposure has negative effects on testes. However, these effects depends on frequencies and length of exposure time. Therefore, we believe that there is a need for further studies with different frequencies and exposure periods to prove the protective effect of vitamins E and C on oxidative stress and histopathologic damage in the testes caused by EMR.

Conclusion

In conclusion, the results demonstrated that wireless (2.45 GHz) have negative morphological and histopathological effects on testes. However, the results also indicated that Vitamin E and C treatment has a protective effect on testes tissue damaged through 2.45-GHz EMR harmful effects.

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