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Original Article

2600 MHz Radiofrequency Radiation Exposure and Protective Role of Melatonin: Effects on Epididymis

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ABSTRACT

Objective: In this study, our aim is to investigate the possible protective effects of melatonin on potential damage caused by 2600 MHz radiofrequency (RF) radiation exposure on epididymis.

Materials and Methods: 36 Wistar Albino male rats were divided into six groups. Group 1: Control, Group 2: Sham Control (mirror of RF exposure), Group 3: Sham Melatonin (mirror of RF exposure and melatonin injection), Group 4: Melatonin (melatonin injection), Group 5: RF (2600 MHz RF exposure), Group 6: RF and Melatonin (2600 MHz RF exposure and melatonin injection). During the experiment, body weights were measured and at the end of the 30-day experimental period, epididymis tissues were collected and examined with Hematoxylin-Eosin.

Results: Epididymis was distinguished in normal histological appearance in the Control, Sham Control, Sham Melatonin and Melatonin groups. In the RF group, some structural degenerations were observed such as irregular tubule profile, deterioration and vacuolization in epithelium, loss of stereocilia, seperation in lateral and basal junctions of epithelium and immature sperm formation. In the RF and Melatonin group, the general histological appearance was found to be similar to the control groups except for some continuing degenerations.

Conclusion: Taken together, 2600 MHz RF radiation exposure caused some structural degenerations on epididymis and melatonin administration provided therapeutic effects on these degenerations.

Keywords: radiofrequency radiation, melatonin, epididymis

INTRODUCTION

Extremely low frequency microwaves from public transportation systems; high frequency microwaves from mobile phones, base stations, microwave ovens, radio/television/computers and strong frequency microwaves from magnetic resonance imaging are the most common sources of electromagnetic fields (EMFs) and the rate of exposure to EMFs has increased during the last century [1]. Considering uses of mobile phones, that the radiofrequency (RF) radiation range reaches to 2600 MHz with 4th generation, one of the most important sources of EMFs [1,2]. It has known that EMFs cause some neurological

disorders and increase the cancer risks. Additionally, especially its long term effects cause some detrimental effects on male fertility [3]. RF and microwave radiations emitted from mobile phones may lead to infertility for reasons such as single and double strand DNA breaks, decrease in sperm concentrations, seminiferous tubule diameters and serum testosterone levels, reduction in sperm vitality and motility, increased in reactive oxygen species (ROS) [4].

Harmful effects of EMFs exposure depends on the overproduction of ROS and changing of prooxidant/oxidant balance so antioxidant treatments like superoxide dismutase, catalase, glutathione, some vitamins such as A, C, E and also melatonin can be helpful in preventing or reducing some RF radiation damages [1,5]. In their study published in 2005, Hata et al. hypothesized that low RF radiation may reduce melatonin synthesis in the pineal gland [6] therefore, the use of melatonin as an antioxidant treatment in RF exposure may be an important key point. The chief secretory product of the pineal gland, melatonin (N-acetyl-5-methoxytryptamine) in RF exposure has been studied in several experimental studies including brain, kidney, heart, eye and as well as in many organs [1,7].

Melatonin has been reported to inhibit oxidative stress and early apoptosis in germ cells, improving sperm viability and count [8], to effect the synthesis and release of the hypothalamic gonadotropin-releasing hormones (GNRHs) [9]. It also directly controls the function of Leydig and Sertoli cells of testis and epithelial cells of epididymis, in addition to indirectly regulating spermatogenic cells. Additionally, melatonin membrane receptors MT_1 and MT_2 has been demonstrated in testis and epididymis of some species. Also it has been reported that the proliferation in epithelial cells of epididymis has induced and their function has regulated by melatonin [9].

It has known that epididymis provide an important microenvironment for sperm maturation and once this microenvironment is damaged, sperm maturation could be damaged and result in infertility. But at present, few studies have been reported to investigate the effects of RF radiation in epididymal microenviroenment. Taken together, the possible effects of 2600 MHz RF radiation exposure and protective role of melatonin, that is known to be so important for epidiymis because of the reasons described above, on these effects were examined in this study.

MATERIALS AND METHODS

Chemicals

Melatonin (N-acetyl-5-methoxytryptamine) (> 98.0 %) was purchased from Tokyo Chemical Industry (TCI) (Lot: 4O2HH-RH) and dissolved in 10% pure ethanol that contained a phosphate buffer saline (PBS) (pH=7.4) and stored at -20^{0}C. All other chemicals were obtained from standard commercial suppliers.

Radiation Exposure

A vector signal generator (Rohde & Schwartz, SMBV100A, Germany) was used to create 2600 MHz radio-frequency (RF) radiation in all of the experimental sessions Vector signal generator was used to stimulate the emission of mobile phones. The frequency, modulation and power exposed to animals were similar with the radiation from mobile phones. Administrations were made using horn antenna (ETS Lindgren, Model 3164-03, Frequency Range: 400 MHz-6 GHz, USA). EMA measurements were performed using the pre-study EMA probe (Narda – EMR 300, type 8.3 probe, Germany) while the generator was in on-off state. The rats were housed in a plastic cage (34x24x13 cm) placed symmetrically along the perpendicular axis, 11 cm below the mid-line of the horn antenna. Exposure performed approximately 11 cm away from antenna and because of this exposure could be defined as far field exposure and we could say that situation is similar with our exposure from mobile phones. Administrations started after one week adjustment period. Far field applications were made according to the formula R>2D2/λ by considering Rayleigh approximation. E field values were measured in the middle and corners of the box where the administration was made. Power density value was calculated as 0.14 mW/cm^2 (**Figure 1**).

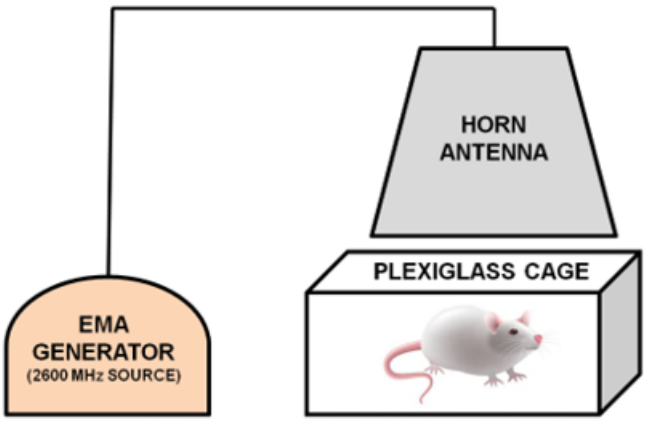


Figure 1. RF exposure system

Table 1. Experimental design

Groups	Number of Subjects	Administrations
Control	N=6	No administration
Sham Control	N=6	Mirror of RF exposure procedure in all aspects except the delivery of RF
Sham Melatonin	N=6	Mirror of RF exposure procedure in all aspects except the delivery of RF and received 10 mg/kg subcutaneous Melatonin injection
Melatonin	N=6	10 mg/kg subcutaneous Melatonin injection
RF	N=6	2600 MHz RF exposure (30 minutes/day)
RF + Melatonin	N=6	2600 MHz RF exposure (30 minutes/day) and 10 mg/kg subcutaneous Melatonin injection

Animals

Thirty six 200-250 gr weight Wistar Albino male rats (Gazi Universty Medical School Experimental Animal Breeding and Experimental Research Center, Ankara, Turkey) were used in this research. They were housed in clean, sterile, polypropylene cages under standard vivarium conditions (12-hour light/dark cycle) with access to water and standard rat chow (Korkutelim Yem Ltd., Antalya, Turkey). The animals were housed four per cage in an air-conditioned animal room at 22 ± 3°C and 55 ± 10% humidity. The animals were acclimatized to laboratory conditions for four weeks prior to the start of the experiments.

Experimental Design

Thirty six male Wistar albino rats were divided into six equal groups (See **Table 1**).

RF exposure administrations were applied between 10.00 am-3.00 pm and subcutaneous melatonin injection was applied at 4.00 pm. The dose and application time of melatonin was performed in accordance with the study by Akbulut et al. [10]. During the experiment, body weights were measured each day for each group and each animal. At the end of the 30-day experimental period, epididymis samples were collected under ketamine (45 mg/ kg) and xylazine (5 mg/kg) anesthesia.

Histochemical Analyses

Epididymis tissue samples were fixed in 10% neutral formaldehyde for seventy-two hours. After washing in tap water, the samples were dehydrated through ascending alcohols and cleared in xylene, then embedded in paraffin. Hematoxylin-Eosin was performed on 4 µm thick sections. The histomorphological changes were evaluated in epididymis using a photographic light microscope (DM4000B Image Analyze System; Leica, Wetzlar, Germany), a Leica DFC280 plus camera and an LAS software program (Leica).

Statistical Analyses

The body weights of the animals were analyzed using the statistic program. All data was presented as Mean ± Standard Deviation (SD). The differences between the groups for body weights were analysed by Mann Whitney U test and $p<0.05$ was considered as statistically significant.

RESULTS

The cross sections of epididymis were distinguished in normal histological appearance in the Control, Sham Control, Sham Melatonin and Melatonin groups. Epididymal tubules were lined by pseudostratified columnar epithelium with stereocilia and all of the tubules were surrounded by smooth muscle and connective tissue. Intertubular region also was distinguished in normal histologic structure with connective tissue rich in collagen fibers and blood vessels. Basal cells with heterochromatic round shaped nucleus and principal cells with relatively pale stained oval nucleus were observed in the epithelium. Mature sperms were present in the lumen of each epididymal tubules (**Figures 2**-**5**).

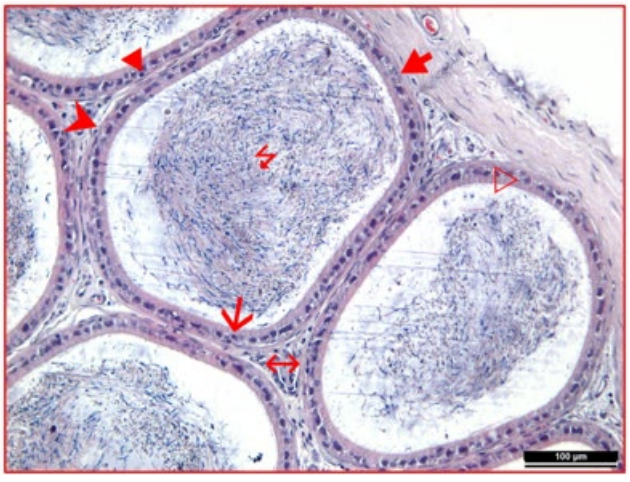


Figure 2. Epididymal cross sections for the Control group showed epididymal tubules: ➔, pseudostratified columnar epithelium with stereocilia: ↘, connective tissue around the tubules: ➤, intertubular connective tissue: ↔, basal cells: ▶, principal cells: ▷, sperms: ↯(Hematoxylin-Eosin x200)

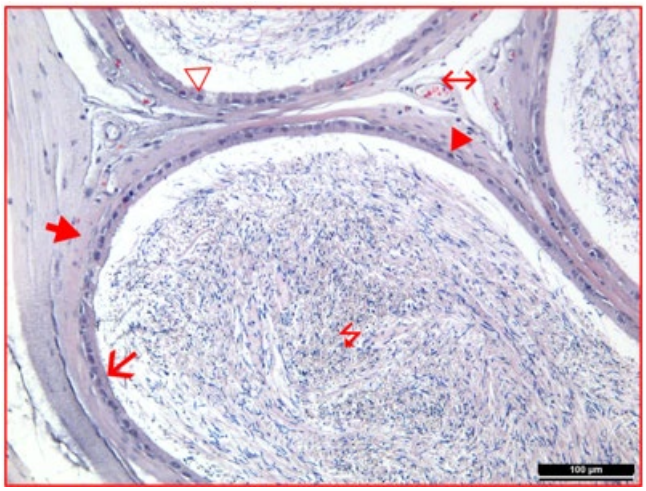


Figure 3. Epididymal cross sections for the Sham Control group showed epididymal tubules: ➔, pseudostratified columnar epithelium with stereocilia: ↘, intertubular connective tissue: ↔, basal cells: ▶, principal cells: ▷, sperms: ↯ (Hematoxylin-Eosin x200)

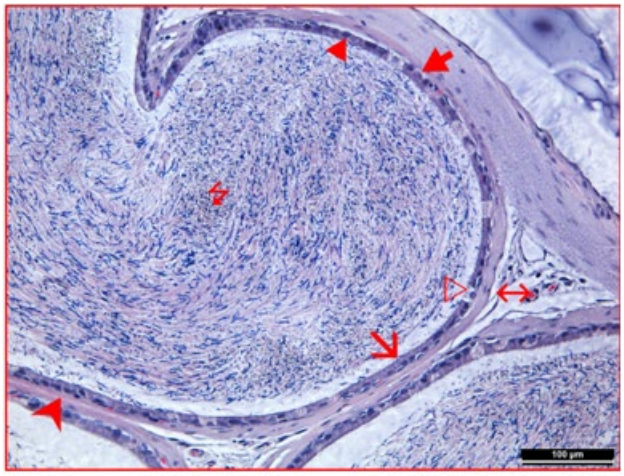


Figure 4. Epididymal cross sections for the Sham Melatonin group showed epididymal tubules: ➔, pseudostratified columnar epithelium with stereocilia: ↘, connective tissue around the tubules: ➤, intertubular connective tissue: ↔, basal cells: ▶, principal cells: ▷, sperms: ↯(Hematoxylin-Eosin x200)

In the 2600 MHz RF radiation exposure group irregular tubule profile was seen, in these tubules the lumen was closed and did not allow the sperm passage. It was distinguished that tubules were not lined by its normal pseudostratified columnar epithelium with stereocilia. Instead, cell proliferations like cell stacks were seen in some regions and also single cuboidal or columnar epithelium were seen in some parts. Especially in regions that epithelium proliferated, stereocilia structures could not be distinguished in anyway. Additionally, stereocilia damage were also seen in some other regions. Seperation and distortion were detected in the lateral and basal (membrane) connections of epithelial cells lining the tubules. It was observed that sperms were seen in the lumen

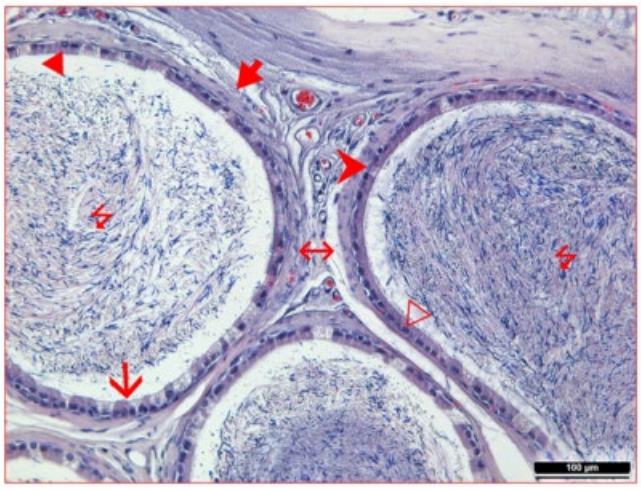


Figure 5. Epididymal cross sections for the Melatonin group showed epididymal tubules: ➔, pseudostratified columnar epithelium with stereocilia: ↘, connective tissue around the tubules: ➤, intertubular connective tissue: ↔, basal cells: ▶, principal cells: ▷, sperms: ↯(Hematoxylin-Eosin x200)

of tubules decreased relatively compared to other groups, and almost no mature sperms were observed in some tubules. Hyalinized structures with eosinophilic staining properties, immature sperms, some waste products and agglutinated sperms were distinguished in the lumen besides mature sperms. In the epithelial cells it was found that transparent cells containing endostatic vesicles in their apical cytoplasm became dominant, that is thought to be associated with increased cell residues in the lumen. Additionally, large-transparent and dense vacuolated cells were distinguished in the epithelium, which increased incidence especially in pathological cases at corpus-cauda junction. It was found that the connective tissue and smooth muscles surrounding the tubules became extremely thick in this group, especially with increased collagen fiber density. In contrast, it was observed that there was an extensive loss of connective tissue elements in the intertubular area, especially in some regions (**Figures 6** and **7**).

In the 2600 MHz RF radiation exposure and Melatonin group, the general histological appearance was found to be similar to the control groups. It was noted that the irregular tubule formation and cell vacuolization were completely eliminated, the epithelium surrounding the tubules was viewed as a normal pseudostratified columnar epithelium with stereocilia and the intertubular connective tissue returned to its normal appearance. On the other hand, it was determined that the connective tissue surrounding some tubules still displayed a thick appearance, but not as thick as in the RF group and seperation continued in the basal membrane connections of epithelial cells lining the tubules in some regions (**Figure 8**).

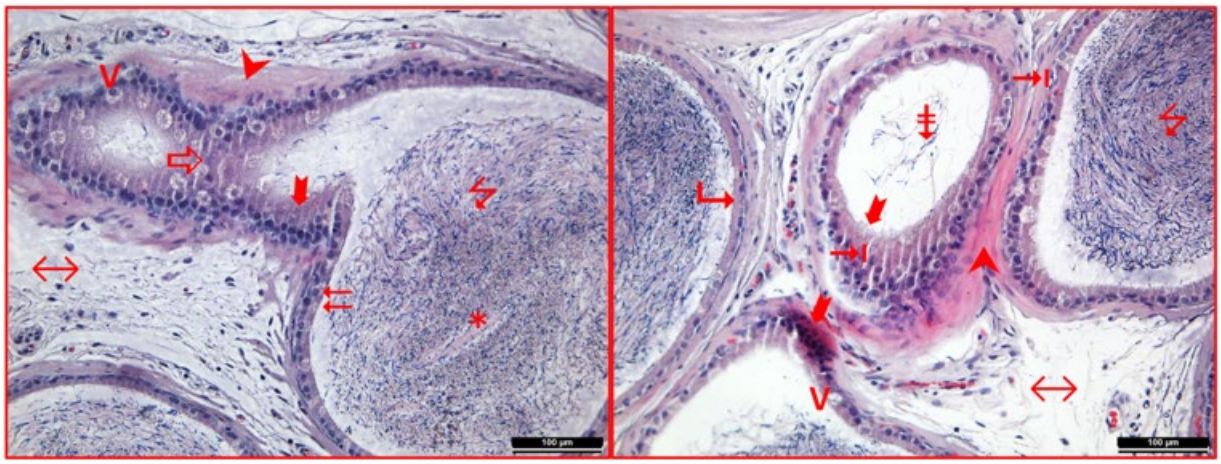


Figure 6. Epididymal cross sections for the RF group showed irregular tubule: ⇨, proliferated epithelium: ➸, cuboidal/columnar epithelium: ↳, loss of stereocilia: ⇉, seperation of lateral and basal junctions: ↦, hyalinization:*, immature sperms and waste products: ǂ, vacuolization: V, sperms: ↯, thick connective tissue around the tubules: ➤, an extensive loss of connective tissue elements in the intertubular area: ↔ (Hematoxylin-Eosin x200)

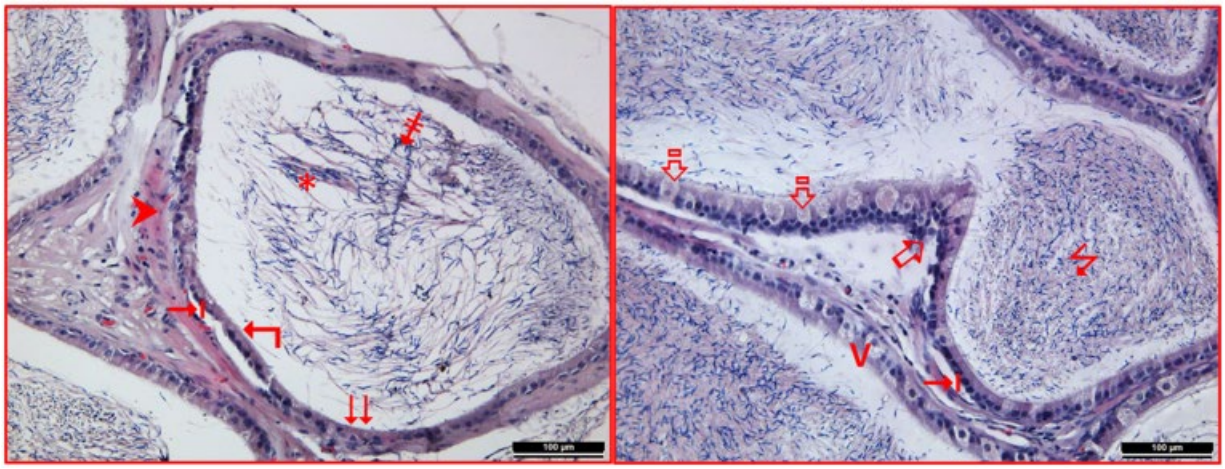


Figure 7. Epididymal cross sections for the RF group showed irregular tubule: ⇨, cuboidal/columnar epithelium: ↳, loss of stereocilia: ⇉, seperation of lateral and basal junctions: ↦, hyalinization:*, immature sperms and waste products: ǂ, vacuolization: V, sperms: ↯, transparent cells containing endostatic vesicles: ⇪, thick connective tissue around the tubules: ➤ (Hematoxylin-Eosin x200)

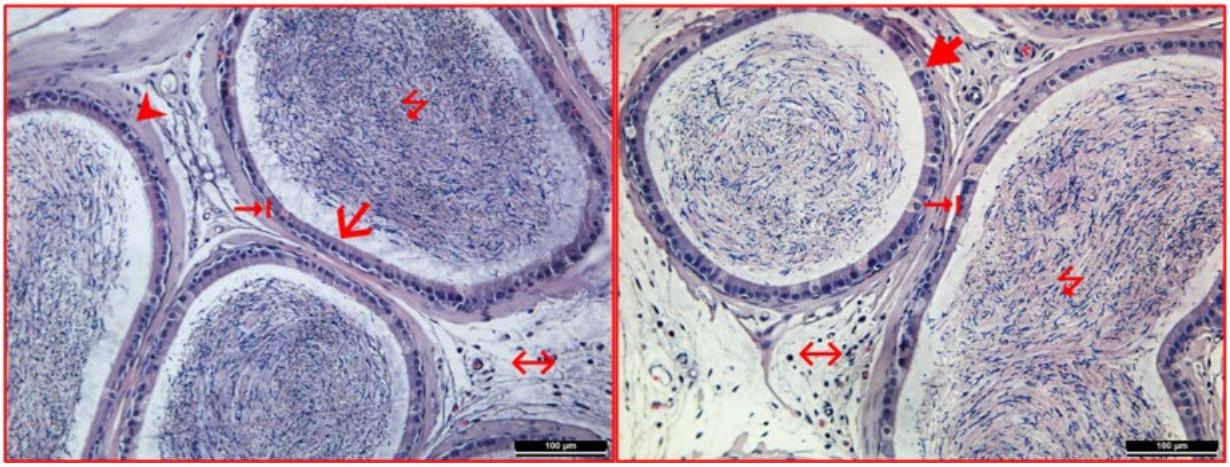
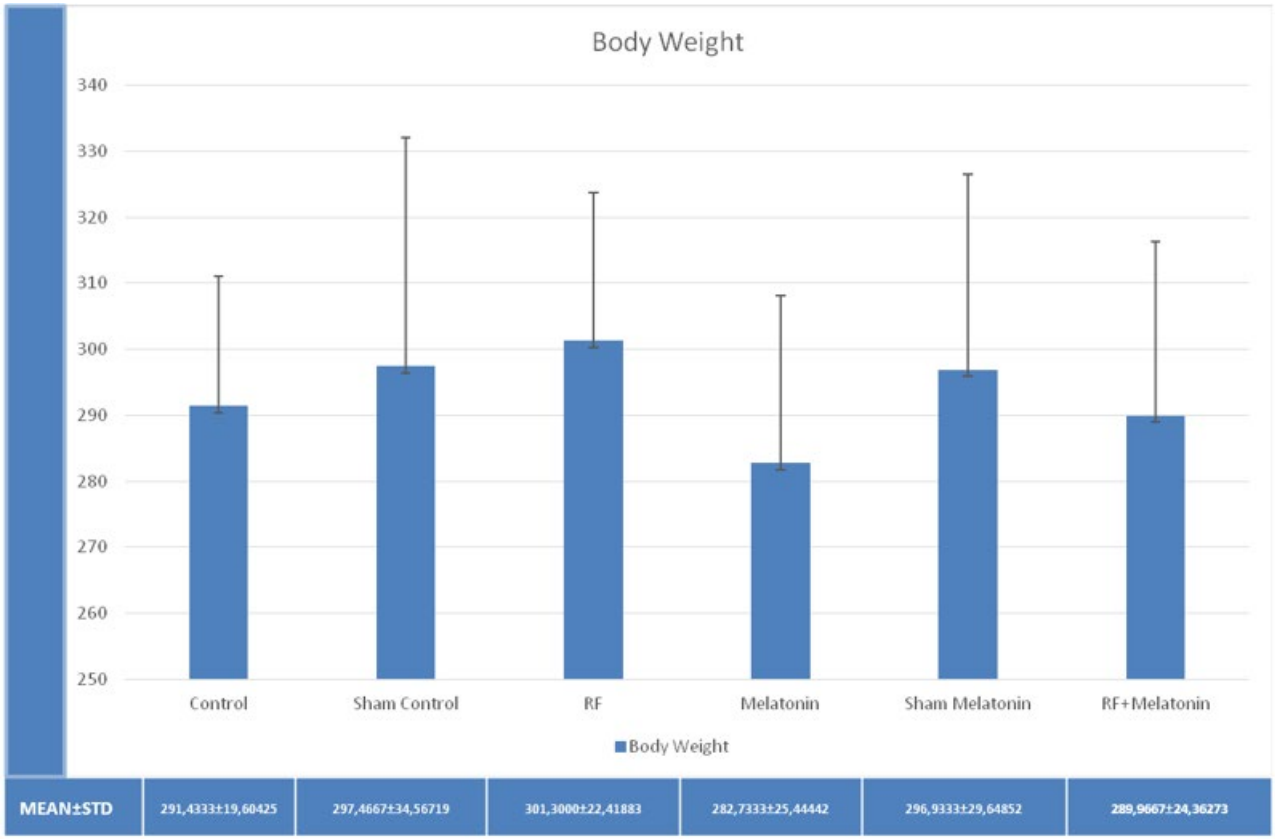


Figure 8. Epididymal cross sections for the RF and Melatonin group showed epididymal tubules: ➔, pseudostratified columnar epithelium with stereocilia: ⇲, thick connective tissue around the tubules: ➤, intertubular connective tissue: ↔, sperms: ↯, seperation of lateral and basal junctions: ↦ (Hematoxylin-Eosin x200)



MEAN±STD	291,4333±19,60425	297,4667±34,56719	301,3000±22,41883	282,7333±25,44442	296,9333±29,64852	289,9667±24,36273

Graphic 1. Comparative statistical graphs showing body weights

Body weights that were measured during the experiment showed no statistically significant difference ($p>0.05$) (**Graphic 1**).

DISCUSSION

RF radiation exposure emitted from mobile phones cause several hazardous effects on the reproductive pattern such as testicular cancer and reproductive outcomes along biological effects such as enzyme induction, toxicological effects like genotoxicity and carcinogenicity [11,12]. Because of these strong relationships between RF radiation exposure and reproductive pattern, RF exposure studies with animals and RF exposure-related reproductive outcomes have become an important subject area of recent studies.

Khillare and Behari exposed the adult male rats to only 200 MHz RF fields for 35 days and after that they mated this with normal female rats in their study. They found significant decrease in fertility through exposed rats that they thought were linked with ultrastructural changes in seminiferous tubules, Leydig cells and spermatids [13]. In another similar study, 220 MHz RF exposure for 1 month effected sperm quality in rats, disrupted the secreting function of Leydig cells and increased the apoptosis of testicular tissue [14].

According to study of Alkis et al., the testis is the most sensitive organ to EMF pollution, and they researched the effects of 900, 1800 and 2100 MHz RF exposure on testicular tissue for 6 months. They found that RF exposure caused to increasing total oxidant status, oxidative stress index, malondialdehyde and DNA single-strand breaks in the testicular tissue especially at 1800 and 2100 MHz frequencies [4].

Similarly, Yu et al. investigated the long-term exposure to 4G smartphone RF radiation on the testis and they found that Spock3 and MMP14-Spock3 complexes overexpressed, MMP2 and MMP14-MMP2 activities suppressed in the testis. As a result of these expression changes, sperm quality decline improved and testicular injury rised [3].

Gautam et al. researched the effects of 3G mobile phone radiation exposure for 45 days on the male reproductive system. They found that significant increase of reactive oxygen species and lipid peroxidation levels caused to decrease the sperm count, alternate the sperm tail morphology so they concluded that the male fertility was affected [15].

According to our study, 2600 MHz RF radiation exposure caused serious structural degenerations on epididymis which is very important for the production of mature sperms

to gain the capacity for fertilizating the oocyte. Therefore, we believe that this damage will negatively affect the male fertility.

According to study of Chauhan et al., 2.45 GHz microwave radiation exposure for 35 days increased the lipid peroxide levels in several organs like liver, brain, kidney and caused histopathology in the testis and the caudal epididymis. Degeneration was seen in the epididymis epithelium, alterations of epithelium lining and reduced sizes of cell population were observed. For the researchers, these effects caused to decrease in sperm count, motility and daily sperm production because of the testicular and epididymal histopathological changes [16]. Similarly, An et al. investigated the sperm maturation in epididymis micro-environment in the case of 1.84 GHz RF radiation exposure but they did not find any effect in sperm maturation and epididymal microenvironment [17]. It would be appropriate to consider that the reason for this findings linked with the duration because the experiment took only 5 days. This finding also reveals the importance of long-term practice in this area.

Arık et al. studied the possible effects of 2100 MHz RF exposure on distribution of androgen receptor (AR) and histology in epididymis through hypertensive and normotensive rats. Accordingly, they found some degenerative changes in the histology of epididymis such as deletion in stereocilia, reduction in the amount of mature sperms, separation and distortion between the cell junctions of epithelial cells lining tubules and strong AR immunoreactivity in the hypertensive rats by 2100 MHz RF exposure [18].

Similarly, to the studies described above, in this study we found some structural degenerations in the epididymis such as irregular tubule profile, impairment and vacuolization of epithelium, loss of stereocilia, separation in lateral and basal junctions of epithelium and immature sperm formation. Also, it was distinguished that tubules were not lined by its normal pseudostratified columnar epithelium with stereocilia. Instead, cell proliferations like cell stacks were seen in some regions and also single cuboidal or columnar epithelium were seen in some parts.

Because of the reactive oxygen species formation background in the case of RF radiation exposure, antioxidant treatments can be helpful in preventing or reducing some radiation damages. Besides its therapeutic effects of detoxifying free radicals, melatonin has been shown to modulate apoptosis caused by EMF-induced oxidative stress [1].

Pandey and Giri applied 900 MHz RF exposure to Swiss albino mice for 35 days and gave 5 mg/kg melatonin for observing the possible protective effects of melatonin in their study. Accordingly, they found sperm head abnormalities, reduced level of total sperm count, lipid peroxides, glutathione, superoxide dismutase activities and caused to degeneration in testis histology by RF exposure. Additionally, they observed that melatonin administration inhibited spermatogenesis arrest, reduced oxidative stress, DNA damage and caused to improvement in testicular histology [8]. According to several studies, melatonin employs a multi-dimensional approach to deliver protection against RF exposure and its possible harmful effects [19].

By the 10 mg/kg melatonin administration, the general histological appearance was found to be similar to the control groups except for some continual degenerations like the connective tissue surrounding some tubules still displayed a thick appearance and separation continued in the basal membrane connections of epithelial cells lining the tubules in our study.

CONCLUSION

Taken together, it was concluded that 2600 MHz RF radiation exposure caused some serious structural degenerations on epididymis which is very important for the production of mature sperms to gain the capacity for fertilizing the oocyte. Additionally, melatonin administration provided therapeutic effects on these degenerations. We believe that this study is an important preliminary research that closes the gap in the literature to determining the damage of epididymis in the case of RF radiation exposure and leads the way for further investigations in this area.

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