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ORIGINAL ARTICLE

Effect of exposure to cell phone radiation on testicular function of male albino rats

Mohamed A. Alghannam[1], Randa S. Gomaa [1], Eman R. Abozaid [1], Yasmeen A. A. Diab[1] *

[1] Medical Physiology Department, Faculty of Medicine, Zagazig University, Sharqia, Egypt

***Corresponding Author:**
Yasmeen Akmal Ahmed Diab, Medical Physiology Department, Faculty of Medicine, Zagazig University, Sharqia, Egypt
E-mail: yasmeenakmal92@gmail.com

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ABSTRACT

Background: The possible health effects of radio frequency-modulated electromagnetic fields (RF-EMF) emitted by mobile phones, cordless phones, base stations, and Wi-Fi routers are a growing concern for the public around the world. This work aimed to study the possible effects of electromagnetic radiation from mobile phones on the oxidant and antioxidant status of testicular tissue and testicular function of male albino rats and its underlying mechanism.

Methods: Twenty male albino rats were divided into two groups: control group A (vehicle driving) and rats were exposed to the phone when the phone was turned off. Exposure to mobile radiation in group B (call mode). Group B was exposed to electromagnetic frequencies for 60 minutes every day for 6 weeks.

Results: Mobile radiation exposure in male rats resulted in a significant decrease in serum levels of FSH, LH, testosterone, epididymal sperm count, and sperm motility but weight gain or testicular weight changes were negligible, the testicular Malondialdehyde (MDA) significantly increased when compared with the control group, the testicular antioxidant Superoxide Dismutase (SOD) and Glutathione Peroxidase (GPx) activity showed a significant decrease in comparison to control group and there were structural changes in the form of basement membrane rupture, decreased s permatogenic cells, intertubular fibrosis, and interstitial cell proliferation.

Conclusions: We confirmed the harmful effect of mobile phone radiation on testicular function in male albino rats

Keywords: Electromagnetic radiation; testes; rats.

INTRODUCTION

Radiation can be divided into ionizing and non-ionizing radiation, the latter of which is divided into two forms: extremely low frequency (ELF) or power line (60 Hz) electromagnetic field (EMF) and radio frequency (RF). generated by radio wave/microwave products [1]. The modulated radio frequency electromagnetic field (RF-EMF) emitted by mobile phones, cordless phones, base stations, and Wi-Fi routers can have an impact on health, which has become an issue of increasing public concern around the world.

The production of reactive oxygen species (ROS) is related to increased exposure to EMR [2], and together with free radicals affects the reproductive system of humans and animals [3] Widespread concerns about exposure to EMRs have increased with declining fertility in the population [4].

In addition to reduced sperm production, disorders of sperm structure and motility, abnormal sperm function, or poor sperm delivery are generally associated with testicular oxidative stress caused by RFR exposure [5-7].

This work aimed to study the possible effects of electromagnetic radiation from mobile phones on the oxidant and antioxidant status of testicular tissue and testicular function of male albino rats and its underlying mechanism.

METHODS

Experimental animals:

A total of 20 adult male albino rats (local strains)

weighing 160-180 grams were obtained from the animal house of the Zagazig University School of Veterinary Medicine. Before the start of the experiment, the rats were acclimatized in the animal room for three weeks [8] The experimental protocol was approved by the Department of Physiology and the Committee of the Institutional Animal Care Unit of Zagazig University (ZU-IACUC; Sharkia; Egypt) Approval number: "ZU-IACUC / 3 / F / 117/2019.

All animal experiments comply with the ARRIVE guidelines and are carried out in accordance with the U.K. Animals.

Animals are kept in plastic cages (30 inches long, 18 inches wide, and 24 inches high). Under hygienic conditions, 10 rats per cage were kept in the henhouse of the Department of Physiology of the Faculty of Medicine of the University of Zagazig.

Experimental animals:

The subjects of the study were 20 healthy adult male albino rats (10-12 weeks). After 3 weeks of adaptation, the rats were divided into 2 groups (n=10). Group A: The shame control group was exposed to a closed cell phone for 6 weeks. Group B: They are exposed to mobile radiation for 1 hour every day for 6 weeks.

The experiment lasted 6 weeks using the same brand and the same model of mobile phones, the performance of mobile phones is as follows: -The highest SAR value is 0.96 W / kg, and the carrier frequency of each mobile phone is 890- 915 MHz frequency band Modulation frequency of 217 Hz, maximum mean power of 250 mW and 2 W The maximum peak power [9].

After making sure that all mobile phones are turned on and the answering mode (answer mode) activated, call another group of mobile phones from a group of GSM (Global System for Mobile Communications) phones. Then put the mobile phone in a plastic experiment box without metal accessories, put the mobile phone in a special area in the middle of the experiment cage to avoid possible damage from mice, and place the phone mobile in the experiment cage. The center of the cage provides nearly equal electromagnetic radiation for the entire body of the animal [10].

During the exposure period, the animals can move freely in the cage. The electromagnetic field radiation generated by the mobile phone is approximately equal to the average downlink frequency of the Orange network in Egypt (935-945) MHz electromagnetic field = 935 + 945 = 1880/2 = 940MHz Group A: control group. (Sham operation group): Let the rats stay in a cage of the same size in a separate similar room, turn off the mobile phone at the same time and touch the mobile phone for 6 weeks [7].

Blood sampling and biochemical analysis

All rats were sacrificed by intraperitoneal injection of ketamine (90 mg/kg body weight). Blood samples were taken directly from the cardiac puncture immediately and then centrifuged at approximately 3000 rpm for 10 minutes to collect serum.

Gonadal extraction and testicular tissue preparation

Through a vertical midline abdominal incision, carefully remove and dissect the testicle and epididymis. Use an electronic scale to measure the weight of the testicles. After removing adhered connective tissue, testicular tissue was washed with ice-cold saline and one testis from each animal was fixed in 10% formalin for histopathological and immunohistochemical evaluation. The contralateral testis was immediately immersed in liquid nitrogen and stored at -80 ° C until used for biochemical enzymatic determination and Western blot analysis.

Sperm analysis

The right epididymis of each rat was dissected, extracted, and ground in 2 ml Hank buffered saline (HBSS) at 37°C [11]. After 5 minutes of incubation at 37°C, the sperm in the tail of the epididymis was measured using a standard blood count method. Extract epididymal fluid from the WBC pipette (white blood cell pipette) to the 0.5 mark, and extract the semen diluent (sodium bicarbonate 5g, formalin 1ml, distilled water 99.0ml) to the "11" mark, and then mix well. Add one drop to the chamber of the hemocytometer and allow the sperm to settle by keeping the hemocytometer in a humid place (moist chamber) for 1 hour.

After incubation, count the number of sperm in the appropriate large squares of the hemocytometer under an optical microscope. Sperm concentration refers to the number of sperm in each milliliter of liquid and is calculated using the following

formula. Sperm count = the number of sperm counted x dilution factor x volume factor/number of areas counted [12]. The percentage of sperm motility is calculated using the percentage of live sperm to the total number of sperm (active and inactive). Sperm that does not move at all is considered to be immobile, while other sperm that show a certain movement are considered to be motile [13].

Evaluation of serum testosterone, FSH, and LH levels.

Free testosterone levels were measured using a testosterone enzyme immunoassay kit (catalog number: BC1115, BioCheck, Inc. 323 Vintage Park Dr. Foster City, CA 94404). FSH used rat enzyme immunoassay kit (catalog number: BC-1029, BioCheck, Inc 323 Vintage Park Dr. Foster City, CA 94404. LH Using Rat Enzyme Immunoassay Test Kit (catalog number: BC-1031, BioCheck, Inc 323) Vintage Park) Dr. Foster City, CA 94404)-According to the manufacturer's agreement.

Evaluation of testicular tissue oxidative stress markers.

Testicular tissue is homogenized in 50 mM potassium phosphate (pH 7.4). The homogenate was used to evaluate the oxidative stress parameters; the rat ELISA kit (SAE, Egypt) was used to measure SOD activity, and the rat ELISA kit (SAE) was used. , De Ober, Cairo, Egypt) to measure GPx activity. And MDA content used rat ELISA kit (Egyptian Biotechnology Company (SAE), Obur City, Cairo, Egypt) according to the manufacturer's agreement.

Histopathological examination.

The testes were immersed in Bouin's solution, then immersed in paraffin, sectioned to a thickness of 5 µm, and stained with hematoxylin and eosin. Examine the sections under a light microscope and assess the general histological appearance. The histopathological analysis is performed blindly by expert pathologists.

Statistical analysis

The data obtained in this study are expressed as the mean ± SD of the quantitative variables and the statistical analysis was performed using the SPSS program (SPSS Inc. Chicago, IL, USA). Analysis of variance with the [post hoc (LSD)] test was used to compare the mean between all study groups, with a p-value <0.05. 0.05 is considered statistically significant.

RESULTS

Effect of mobile radiation exposure on body weight gain and testicular weights

The present study showed that mobile radiation exposure didn't significantly affect body weight gain or testicular weight. (Table 1).

Effect of mobile radiation exposure on sperm characteristics

Mobile radiation exposure showed a significant decrease in sperm count and motility compared to control rats. (Table 1).

Effect of mobile radiation exposure on serum testosterone, FSH, and LH levels.

Mobile radiation exposure showed a significant decrease in serum testosterone levels in addition to a decrease in serum FSH and LH levels compared to control rats. (Table 1).

Effect of mobile radiation exposure on testicular tissue oxidative stress and lipid peroxidation parameter

The testicular MDA level as a marker of lipid peroxidation was elevated significantly in exposed rats, in association with significant reductions of testicular GPx and SOD activity compared to control rats. (Table 2).

Histopathologic results

Light photomicroscopic picture of H & E stains x 400 magnification. Group A showing: Normal testis formed of uniform seminiferous tubules lined by normal layers of spermatogenic cells up to mature sperm formation, basement membrane of normal thickness, and normal Leydig cells (Figure1A). Group B showed rupture of the basement membrane, dropped off spermatogenic cells, intertubular fibrosis, and Leydig cell hyperplasia (Figure 1B).

Table 1: Effect of mobile radiation exposure on body wt gain, testicular weights, sperm count, sperm motility, serum testosterone, FSH, and LH level in all studied groups.

Parameters		Group A	Group B
Body weight gain (gm)	Mean ± SD	69.5 ± 14.8	67.4 ±10.6
	P-value of LSD		P > 0.05
Right testicular wt (gm)	Mean ± SD	1.68 ±0.081	1.69±0.088
	P-value of LSD		P > 0.05
Left testicular wt (gm)	Mean ± SD	1.67 ±0.083	1.7 ± 0.09
	P-value of LSD		P > 0.05
Sperm count ($x10^6$ spermatozoa /ml)	Mean ± SD	73.5± 5.87	38.9 ± 5.15
	P-value of LSD		P < 0.001
Sperm motility rate (%)	Mean ± SD	69.5 ± 5.52	61.1 ± 4.72
	P-value of LSD		P < 0.01
serum Testosterone (ng/ml)	Mean ± SD	6.27 ± 1.18	4.37 ± 0.8
	P-value of LSD		P < 0.01
Serum FSH levels (mIU/mL)	Mean ± SD	3 ± 0.61	2.12 ± 0.55
	P-value of LSD		P < 0.01
Serum LH levels (mIU/mL)	Mean ± SD	4.13 ± 0.65	3.18 ±0.45
	P-value of LSD		P < 0.01

Table 2: Effect of mobile radiation exposure on the testicular levels of MDA, SOD activity, and GPx activity in studied groups.

Parameters	Group A		Group B
MDA (nmol/gm protein)	Mean ± SD	119.6 ± 7.5	231.7 ± 8.38 7.5
	P-value of LSD		P < 0.001
SOD activity (U/gm protein)	Mean ± SD	80 ± 8.42	59.9 ± 11.35
	P-value of LSD		P < 0.001
GPX activity (U/gm) protein)	Mean ± SD	30.4 ±5.85	22.7 ± 4.22
	P-value of LSD		P < 0.001

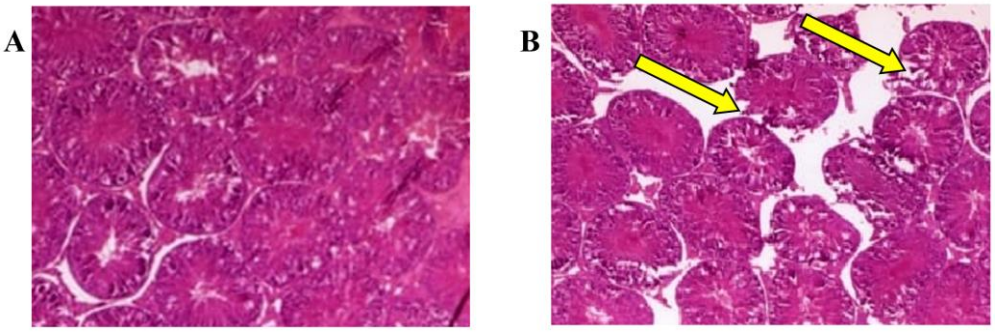

Figure 1: light photomicroscopic picture of H & E stain x 400 magnification (A) (group A) showing: Normal testis formed of uniform seminiferous tubules lined by normal layers of spermatogenic cells up to mature sperm formation, basement membrane of normal thickness and normal Leydig cells. (B) (group B) showing: rupture of the basement membrane, dropped off spermatogenic cells, intertubular fibrosis, and Leydig cells hyperplasia.

DISCUSSION

The rapid growth in the use of mobile phones has been accompanied by a parallel increase in electromagnetic field (EMF) density [14 and 15]. The direct biological effect of EMF is the thermal effect of absorbing EMF energy through induced

current or the thermal effect of long-term exposure [16].

This study showed that compared with the control group, the mobile radiation exposure of male rats resulted in a significant reduction in serum levels of FSH, LH, testosterone, epididymal sperm count, and sperm motility, and weight gain or changes in testicular weight were negligible.

Kumar et al. [17] reported a decrease in testosterone levels in male rats after exposure to microwaves at 10 GHz. On the other hand, Hajioun [18] and Salama et al. [19] found that mobile phones (800 MHz) did not change the serum testosterone level of rabbits, while Nisbet et al. [20] and Kim et al. [21] reported that electromagnetic fields (2.45 GHz) increased testosterone levels in rats, but did not change FSH/LH levels.

Our results are consistent with those of Meo et al. [22] Who reported that EMFs with a frequency of 84 MHz did not significantly affect body weight and testes. In the report of rabbits, 50 Hz ultra-low frequency (DES)-EMF caused a decrease in sperm motility and motility [23]. On the other hand, DES-EMF does not affect the motility and morphology of boar sperm [24]. However, Kesari et al. [25] reported a significant reduction in the total number of sperm after wireless exposure (2 hours/day for 35 days).

In this study, optical microscopy imaging of testicular tissue isolated from mobile rats exposed to radiation showed structural changes in the form of basement membrane rupture, decreased spermatogenic cells, intertubular fibrosis, and Leydig cell proliferation.

Interestingly, the recurrence of 50 Hz radiation at 8 hours/day within 8 months resulted in histological changes in the testis, including weight loss and a decrease in the normal diameter of the seminiferous tubules [26].

Regarding oxidative stress markers, this study showed that testicular malondialdehyde increased significantly in rats exposed to mobile radiation, and testis SOD and GPx antioxidant activities were significantly reduced.

La vigneira and others. [27] revealed that the damage caused by electromagnetic radiation promotes increased ROS production. Regarding the consumption of antioxidants, electromagnetic radiation can overwhelm the defense system and cause cell damage and apoptosis [1].

Furthermore, Gautam et al. [28] Lipid peroxidation and neurodegeneration in rat testes were found to be enlarged and GPx antioxidant enzymes were reduced.

Contrary to published results, some studies have found the beneficial effects of ELF-EMF on male reproductive potential. Hori and colleagues [29]. In addition, Darbandi et al. [30] It is assumed that exposure to 50 Hz ELF-EMF for several hours may have a beneficial effect on sperm morphology and movement, while longer exposure or ELF-EMF with different characteristics will negatively affect sperm quality or no effect.

CONCLUSION

Based on the current research results, we have confirmed the harmful effects of mobile phone radiation on the testicular function of male albino rats. This radiation exposure causes oxidative stress, which leads to changes in the structure and function of the testicular tissue, which manifests in the form of changes in testicular tissue parameters, sperm, and sex hormones.

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