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ABSTRACT

The possible adverse reproductive effects resulting from exposure to electromagnetic fields (EMF) are currently of great public concern. The objective of the present study is to reveal possible effects of electromagnetic fields emitted from a CDMA mobile phone on the ultrastructural aspects of the testicular cells. Approximately 6 weeks old Swiss albino rats were procured from LLRUVAS, Hisar, Haryana. Rats were acclimatized in plastic cages in a room maintained at 24 ± 1°C and 50 ± 5% humidity with an alternating 12 h light-darkness cycle. After one week, rats were exposed under electromagnetic radiation emitted from a CDMA mobile phone with 3 hrs exposure followed by 30 minutes rest and then again 3 hrs exposure per day for five months. One sham group was kept away from the source of radiation and was used as control for the experimental groups. Immediately after the last irradiation, rats were sacrificed and their testes were analyzed using Electron Microscopic techniques. Studies revealed pycnotic nuclei in germ cells, vacuolization in spermatogenic cells and detachment of spermatogonia and sertoli cells from basal lamina. Shrinkage was induced on the surface of the seminiferous epithelium due to exposure. Residual cytoplasm and debris of degenerating cells were also observed in the seminiferous tubules.

KEYWORDS: Radio Frequency Electromagnetic Fields (RF-EMF), Mobile Phone, Swiss Albino Rats, Testes, Seminiferous Tubules.

INTRODUCTION

Mobile phones and cell towers are amongst the most common sources of electromagnetic radiations. Increasing number of devices emitting such radiations raised the valid question concerning their safety and the potential risk of human exposure and its limits (Valberg, 1996). The possible adverse reproductive effects resulting from exposure to electromagnetic fields are currently of great public concern. Some investigations have suggested that one of the environmental factors potentially involved in the etiology of DNA damage in human spermatozoa is an increased exposure to radio frequency electromagnetic...
radiation (RF-EMR) emitted from mobile phones. Initial studies revealed negative correlation between mobile phone usage reproductive toxicity (Fejes et al., 2005). Large doses of radiofrequency (RF)-EMF have been shown in previous studies to be related to genetic defects, such as changes in the integrity of epididymal mitochondrial DNA (Aitken et al., 2005), increased micronuclei for mutations (Tice et al., 2002), increased chromosomal instability (Sykes et al., 2001; Mashevich et al., 2003), altered proto-oncogene c-fos (Goswami et al., 1999) and changes in morphology and gene expression (Pacini et al., 2002). RF-EMF of the commercially available cell phones may affect the fertilizing potential of spermatozoa and this can explain the RF-EMF related infertility cases observed in numerous studies (Wdowiak et al., 2007). Experimental studies specifically designed to evaluate testicular damage caused by low intensity RF show conflicting results (Saunders and Kowalczuk, 1981; Dasdag et al., 1999, 2003; O zguner et al., 2005; Ribeiro et al., 2007; Yan et al., 2007). Tissues with higher hydration as testes were more sensitive to magnetic fields (Arutunian et al., 1998), yet many controversies regarding the biological effects on the organs were encountered. Some investigators reported affection of testicular germ cells (Lee et al., 2004) while others denied any magnetic field exposure related histopathological alteration in testicular tissue (Forgaces et al., 2004).

In the light of such consideration, present study was conducted to analyze the ultrastructural consequences of chronic exposure of RF-EMR emitted from domestic mobile phones on rat testes. To avoid any secondary thermal effect, temperature of the room was kept at 24°C where the animals were placed throughout the experiment.

**MATERIALS AND METHOD**

I. Experimental animals

After the clearance from local Institutional Animal Ethical Committee (IAEC), approximately 6 weeks old male Swiss albino rats, weighing 50-60 g were kept in steady-state micro-environmental conditions (24 ± 1°C and 50 ± 5 % humidity), housed in plastic cases with 6 per cage with an alternating 12 h light-darkness cycle. The cages were built to provide proper ventilation to keep the animals aerated and dimensions prevent the free movement of the animals away from the mobile phone. All animals were maintained at an animal care facility according to the guidelines for the use and care of laboratory animals and food and water were available ad libitum. Cleaning, changing water and food was provided to all animals, daily.
II. Experimental Design

After one week of acclimatization and quarantine, 24 male rats were divided at random into two groups of 12 animals i.e. one experimental and other control group. Rats in the experimental group were exposed under electromagnetic radiation emitted from a Code Division Multiple Access (CDMA) mobile phone with 3 hrs exposure, followed by 30 minutes rest and again 3 hrs exposure per day for five months. The sham controls were handled in the same manner as the treated ones, but were not irradiated at any point.

III. Histopathological examination

A. Extraction of the testes

Immediately after the last irradiation, the rats were sacrificed by overdose of ether. Testes were dissected out and decapsulated, put in buffered glutaraldehyde 2.5% for one hour and then cubes of 1mm in dimension were cut for transmission electron microscopy and cubes of 1cm for scanning electron microscopy by a sharp razor from the outer layer of the testes with careful manipulation. All the samples were then transferred in a fixative (2.5% glutaraldehyde and 2% paraformaldehyde in 0.1M phosphate buffer of ph 7.4) for 12 hr at 4° C (Aisha et al., 2006).

B. Processing for transmission electron microscopy

Washing was given to the samples by phosphate buffer (ph 7.4) three times for 10 minutes each. The slices were then fixed in 1% osmium tetroxide for 1 hr followed by another washing. Dehydration of the samples was performed by gradually increasing concentration of ethyl alcohol for 30 minutes each and then in absolute alcohol for 1 hr. After treating with propylene oxide, samples were embedded in spur resin to form gelatin blocks. Blocks were trimmed and ultra thin sections (300 Å) were cut and picked up on copper grids. Uranyl acetate and lead citrate were used to stain the sections which were examined and photographed by transmission electron microscopy.

C. Processing for scanning electron microscopy

Samples were fixed at room temperature and rinsed three times using the same buffer used for the fixative for five minutes each rinse. After passing through 1% osmium tetroxide for 1 hr, samples were again rinsed three times using the same buffer for 5 minutes each rinse. Then samples were dehydrated using gradually increasing concentration of ethyl alcohol for 10 minutes each and then in absolute alcohol for 1 hr.
Finally samples were passed through the step of critical point dry followed by mounting and coating with conductive material.

RESULTS

1. Transmission electron microscopy

Electron microscopic examination of the testes of the control adult rats illustrated the normal secondary spermatocytes and few early spermatids with acrosome cap formation (fig. 1). Seminiferous tubules were consisting of spermatogenic cells at different stages of differentiation together with supporting sertoli cells. Sertoli cells were present in close proximity to the basement membrane with adjacent spermatogonia situated towards the lumen of the seminiferous tubules. Primary young spermatids were present as large rounded cells with oval nucleus, containing different cell organelles. Mitochondria were present with ill defined cristae. Acrosome formation at earlier stage was noticed (fig. 2). Myoid cells were present outside the basal lamina which encircled the seminiferous tubules.

The exposed testes to Radio Frequency Electromagnetic Fields (RF-EMF) showed variable degenerative changes in the spermatogenic cells. Some cells showed electron dense areas and vacuolation within the cytoplasm, (fig. 3). Irregular shaped and multiple vacuolated mitochondria were also observed. (fig. 4).

2. Scanning electron microscopy

Scanning electron micrograph of control testicular tissue illustrates the cross section of seminiferous tubules. Furrow like depression was observed running longitudinally on the outer surface of it. Cells of varied shapes and sizes were clearly visible in it. Outside the seminiferous tubule, interstitial tissue was made up of connective tissue having Leydig cells, myoid cells and blood vessel (fig. 5). Various dividing germ cells were arranged in the specific manner in the seminiferous epithelium. Larger cells occupied the basal part and smaller cells were situated towards the lumen and the surface of spermatogonia, spermatocytes and spermatids was smooth (fig. 6).

Scanning electron micrograph of testicular tissue exposed under electromagnetic radiations showing sharp edge craters and shrinkage induced on the surface of degenerating cells in the seminiferous epithelium due to exposure(fig. 7). The residual cytoplasm and debris of degenerating cells in the epithelium were clearly visible. Ruptured sperm head and distorted tail were also observed (fig. 8).
Plate I: Transmission electron micrographs of sections of testes of control adult rat showing:

Fig. (1): Part of a seminiferous tubule. Spermatocyte (SC) with large central nucleus (N) and copious cytoplasm containing cell organelles. (x 3700)

Fig. (2): A spermatid with oval nucleus developing the acrosome body (AB). Golgi zone was present in the cytoplasm (→). (x 5000)

Plate II: Transmission electron micrographs of sections of testes of exposed adult rat showing:

Fig. (3): Irregular shape of mitochondria having initial vacuolation (→). Lysosomes are normal. Lipid droplets (L) are numerous in the cytoplasm. (x4500)

Fig. (4): The cytoplasm has been occupied by excessive vacuoles (→) and lipid droplets (L) disturbing the normal distribution of other cell organelles. (x4500)
Plate III Scanning electron micrographs of sections of testes of control adult rat showing:

Fig. (5): Intact germinal epithelium layer surrounding the seminiferous tubule. Various spermatogenic cells of varied shapes and sizes were clearly visible in it. Outside the seminiferous tubule, interstitial tissue was made up of connective tissue having Leydig cells, myoid cells and blood vessel. (x1100)

Fig. (6): Various dividing germ cells were arranged in the specific manner in the seminiferous epithelium and larger cells occupied the basal part and smaller cells were situated towards the lumen in the seminiferous epithelium. Heads of mature spermatids invaded in the apical portions of sertoli cells and the tail was coiled and protruding out from the surface towards the lumen of seminiferous tubule. (x2940)

Plate IV: Scanning electron micrographs of sections of testes of exposed adult rat showing:

Fig. (7): The spermatogonia, spermatocytes, sertoli cells and spermatids were degenerated after the radiation exposure (→) (x2560)

Fig. (8): Abnormal sperm head (→) showing ruptured cytoplasm and debris of degenerated germ cells (D). (x2660)
DISCUSSION

Testis is the site of an intense proliferation and differentiation of the germinal cells that will become the sperm cells. Mammalian spermatozoa leaving the testis have to undergo distinct morphological and biochemical changes during the epididymal transit before being capable to fertilize the ovum. Standby communication signals did not significantly affect the sperm parameters but prolonged daily usage of mobile phone might have negative effect on testicular tissue. Mailankot et al., (2009) showed that difference in total sperm count was not significant, but the percent of motile sperm in the non thermal level of RF exposed animals was reduced to 40%. Oxidative stress has been known for some time to limit the fertilizing potential of spermatozoa through the induction of peroxidative damage to the sperm plasma membrane(Aitken et al., 1989 and Jones et al., 1979). Oxidative stress is also known to be associated with DNA damage in human spermatozoa (Shen et al., 2000). The source of the free radicals responsible for generating such stress appears to be the mitochondria(Coppers et al., 2008). Some investigations described that one of the key environmental factors involved in the stimulation of sperm mitochondria to produce high level of reactive oxygen species (ROS), might be excess exposure to RF-EMF from sources such as mobile phones (Geoffry et al., 2009). It has been found that exposure of mice to EMF caused atrophy in the seminiferous tubule (Khayyat et al., 2011). Rajaei et al., (2009) reported that exposure to EMF for long periods could decrease the diameter of reproductive ducts.

In the present study some alterations in testicular tissue were observed when exposed to RF-EMF emitted from domestic mobile phones. The degenerative changes in seminiferous tubules are in accordance to Sert et al., (2002) and Lee et al., (2004) who reported that exposure to EMF resulted in reduction in the number of well organized seminiferous tubules and increased germ cell death. Sert et al. (2002) added that in EMF exposed rats abnormal shapes of the sperms with abnormal heads were observed. This was in accordance with the present study. In addition, Large number of fat droplets and vacuoles in the cytoplasm were found in exposed groups as compared to the control group animals. Irregular shaped and vacuolated mitochondria were also visible in exposed groups studies.

However, present findings were found to be contradictory to some earlier published work. Cairnie and Harding (1981) found no significant differences in histological evaluations of testes and sperm count between controls and animals exposed to 2.45 GHz for 16h/day for 1-30 days. Hae-June et al.(2010) who exposed the male rats to RF 90 min/day, 5 days per week for 12 weeks, reported no alterations in caudal epididymis sperm count, frequency of

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spermatogenesis stages. Ribeiro et al. (2007) also found that RF exposure for 1 hr/day for 11 weeks did not induce any differences in histological examinations of testes and sperm count when compared to the unexposed control group. Here, it is worth to notice that present exposure duration was quite higher than that of above cited examples as the rats were exposed to RF six hours/day, seven days/week for 20 weeks.

CONCLUSION
The present findings demonstrated that chronic cell phone exposure up to five months adversely affected the testicular tissue of rats. Vacuolated irregular shaped mitochondria and numerous lipid droplets were observed in the cytoplasm. Germ cells at different developmental stages were degenerated after the radiation exposure. Ruptured sperm heads were observed along with the debris of degenerated germ cells.

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REFERENCES
Objective and Scope

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Histological Changes in Rat Testes after Exposure to Mobile Phone Radiations

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Abstract: In the recent times, observing the decreasing trend of male reproductive health in men and animals, the present study was aimed to find out histological changes in rat testes exposed to radio frequency electromagnetic radiation (RF-EMF) emitted from commercially available mobile phones. After clearance from Institutional Animal Ethical Committee (IAEC), approximately 5 weeks old male Swiss albino rats, weighing 40-50 g were kept in steady-state micro-environmental conditions (24 ± 1° C and 50 ± 5 % humidity). Rats were given radiation exposure using Code Division Multiple Access (CDMA) mobile phone with 3 hrs exposure, followed by 30 minutes rest and again 3 hrs exposure per day for 6 months. The control group was handled in the same manner as the treated ones, but was not irradiated at any point. Histological examination of testicular tissue exposed to electromagnetic radiation revealed the detachment of adjacent seminiferous tubules and decreased number of Leydig cells at interstitial space. Spermatozoa density in seminiferous tubules was found decreased. Cells with vacuolated cytoplasm and condensed nuclei were also observed.

Keywords: RF-EMF, mobile, albino rats, testes, seminiferous tubules.

I. INTRODUCTION

Many electronic types of equipment create electromagnetic fields [1], but mobile phones have become most popular devices worldwide since last decade. It has raised the public concern regarding human safety related to radio frequency electromagnetic radiation (RF-EMF) emitted from commercially available mobile phones. Recently, several studies related to potential adverse effects of RF-EMF on various body parts of human and animals have been conducted. Histological and physiological studies have evaluated the effects of electromagnetic radiations on human health [2], [3], [4], [5], [6], [7], [8]. It has been shown that prolonged exposure to electromagnetic fields (EMF) without any protection may produce some adverse effects on human body [9]. Penafiel et al. [10] showed that the radiation from TDMA (time division multiple access) digital cellular phones can cause significant changes in ornithine decarboxylase activity, which is essential for cell growth and DNA synthesis. Roschke and Mann [11] did not observe any change in electroencephalogram (EEG) of subjects exposed to radiation emitted by cellular phones. Cain et al. [12] reported that tumor formation in vitro was not induced after repeated exposure to radiofrequency fields. Jensh [13] reported that animals exposed to radiations of 915 MHz did not cause any significant histological alterations. Andrea et al. [14] did not observe any significant differences between brain, heart and liver tissue of irradiated and control animals. Adverse effects of microwave radiation have also been established on brain and eyes of rats [15], [16]. Fertilizing capacity of sperm was reported to be reduced after exposure to RF radiation [17]. Mc Ree et al. [18] suggested that sperm count and reproductive capacity decreased after microwave exposure during embryonic development. An increased exposure to RF-EMR emitted from mobile phones is also believed to be one of the environmental factors potentially involved in the DNA damage in human spermatozoa. Large doses of radiofrequency (RF)-EMF have been shown in previous studies to be related to genetic defects, such as changes in the integrity of epididymal mitochondrial DNA [19], increased micronuclei for mutations [20], increased chromosomal instability [21], [22], altered proto-oncogene c-fos [23] and changes in morphology and gene expression [24]. Experimental studies specifically designed to evaluate testicular damage caused by
low intensity RF show conflicting results [25], [26], [27], [28], [29], [30]. The purpose of this study is to investigate the histological changes originating from EMR emitted by cellular phones.

II. MATERIAL AND METHOD

Animals:

Ethical clearance was sought from local Institutional Animal Ethical Committee (IAEC), approximately 5 weeks old male Swiss albino rats, weighing 40-50 g were kept in steady-state micro-environmental conditions (24 ± 1° C and 50 ± 5 % humidity), housed in plastic cases with 6 per cage with an alternating 12 hrs light-darkness cycle. Proper ventilation was ensured to keep the animals aerated and dimensions of the cases prevented the free movement of the animals away from the mobile phone. All animals were maintained at an animal care facility according to the guidelines for the use and care of laboratory animals. Standard laboratory animal feed and water were given ad libitum Along with daily cleaning and changing of water, the food was provided to all animals.

Experimental Design:

Animals were acclimatized to experimental conditions prior to the onset of exposure for 1 week. 12 male rats were divided at random into two groups of 6 animals as experimental and control group. Experimental group was exposed under electromagnetic radiation emitted from a commercially available Code Division Multiple Access (CDMA) mobile phone with 3 hrs exposure, followed by 30 minutes rest and again 3 hrs exposure per day for six months. The sham control was handled in the same manner as the treated ones, but was not irradiated at any point.

Histology:

The rats were sacrificed by overdose of ether after the last exposure of radiation. Testes were dissected out and decapsulated tissue was fixed in Bouins fixative for 72 hrs. Washing was given to the samples under running tap water for 2 hrs followed by dehydration through 70% alcohol for 12 hrs, 90% alcohol for 10 minutes, absolute alcohol for 10 minutes, absolute alcohol + xylene for 8 minutes, xylene for 6 minutes, xylene + paraffin wax for overnight at 60° C temperature and finally in paraffin wax for 24 hrs at 60° C. Then small cubical blocks were made of the testes sample to be embedded in the paraffin wax. Next day, section cutting was done using microtome and fine ribbons (7 μm thick) were put on precoated microscopic glass slides with egg albumin and glycerine. After complete drying, slides were stained in Haematoxylin & Eosin stains according to the standardized procedure and finally slides were examined under microscope.

Body weight:

The rat body weight of control and experimental group was recorded at two time points i.e. at the initiation of experiment (baseline data) and after six months.

Statistical analysis:

Data was analyzed by Student’s t-test. P<0.05 was considered significant.

III. RESULTS

Histological examination of testes:

The testes of rats of control group contain a number of seminiferous tubules with connecting tissue separating them, boundary tissue consists of outer layer of collagen fiber, normal sized seminiferous tubules which were full of spermatogenic cells with scanty interstitial tissue and few Leydig cells and intact germinal epithelial layers of adjacent seminiferous tubules were present [Fig. 1], [Fig. 2], [Fig. 3]. The light microscopic examination of the testes sections in exposed group revealed some alterations in both the interstitial tissue and seminiferous tubules. The detachment between the adjacent seminiferous tubules was observed at several places and number of Leydig’s cells at interstitial space was observed as decreased [Fig. 4], [Fig. 5]. Leydig cells secrete male reproductive hormone i.e. testosterone which is required in very high quantities for maintenance of reproductive tract. Large vacuoles, condensed nuclei and free floating cells were present in many spermatogenic cells at different developmental stages [Fig. 6].
Fig. 1: Testicular section of rat as control showing normal spermatogenesis within seminiferous tubule. Intact germinal epithelial layers of adjacent seminiferous tubules are clearly visible. (H & E staining, 400 X)

Fig. 2: Testicular section of control group showing normal spermatogenesis and proper arrangement of cells in the seminiferous tubule and the interstitium. (H & E staining, 400 X)

Fig. 3: Testicular section of control group showing healthy arrangement of spermatogenic cells. (H & E staining, 1000 X)

Fig. 4: Testicular tissue exposed to electromagnetic radiation (3hrs/day up to 6 months) revealing detached adjacent seminiferous tubules (arrow) and decreased number of Leydig’s cells at interstitial space. (H & E staining, 400 X)

Fig. 5: Testicular tissue of rat exposed to electromagnetic radiation (3hrs/day up to 6 months) showing decreased number of spermatozoa (arrow) and free floating cells. (H & E staining, 400 X)

Fig. 6: Testicular tissue of rat exposed to electromagnetic radiation (3hrs/day up to 6 months) showing large vacuoles (arrow), condensed nuclei and free floating cells. (H & E staining, 1000 X)

Body weight:

At the initiation of experiment, there was no significant (p>0.05) difference between control and experimental group [Fig. 7] whereas after 6 months, significant increase (p<0.05) was observed in the body weight of experimental group [Fig. 8]. Control group also showed gradual significant increase in body weight with time period [Fig. 9] like experimental group [Fig. 10].
Fig. 7: Comparison of baseline rat body weight of control and exposure group

n=6; Values are Mean ± SEM (unpaired Student's t-test). Group 1: Control; Group 2: Experimental group. The t-value was 0.224 at 5 degree of freedom and p>0.05.

Fig. 8: Changes in body weight of control and exposure groups after 6 months

n = 6; Values are Mean ± SEM (unpaired Student's t-test). a. p<0.05 vs group 1. Group 1: Control; Group 2: Experimental, exposure was 90 min + half an hour rest + 90 min per day. The t-value was 1.46 at 5 degree of freedom.

Fig. 9: Changes in baseline body weight of control group after 6 months

n= 6; Values are Mean ± SEM (paired Student's t-test). Group 1: Control. a. p<0.001 vs group 1 at Baseline. The t-value was 13.72 at 5 degree of freedom.
Fig. 10: Changes in baseline body weight of exposure group after 6 months

n= 6; Values are Mean ± SEM (paired Student’s t-test). Group 2: Experimental, exposure 90 min + half an hour rest + 90 min per day. a. p<0.001 vs group 2 at baseline. The t-value was 26.07 at 5 degree of freedom.

IV. DISCUSSION

The mobile phone users get exposed to different frequencies in different countries and continents. Exposure of radiofrequency energy depends upon the frequency of the cellular phone [31]. Male reproductive functions can possibly be affected by the electromagnetic field radiation generated by mobile phones via three mechanisms: an EMF-specific effect, a thermal molecular effect or a combination of both effects [32]. Animal model studies show that electromagnetic field radiations generated by mobile phones have a wide range of damaging effects on the male reproductive system sperm parameters [32]. A decrease in seminiferous tubule diameter was observed after 3 minutes exposure daily during 30 days using a conventional cellular telephone (890 to 915 MHz) [26], [27]. Similar results were reported by Ozguner et al. [28] with decrease in seminiferous epithelium thickness. The current study was done to verify whether radiation emitted by mobile cellular telephones may impair testicular function in adult rats. The results of the present study revealed that exposure of mobile phone radiation caused the detachment of neighboring seminiferous tubules. Among the healthy spermatogenic cells, some cells were also observed with vacuolated cytoplasm and condensed nuclei. The rats exposed to (RF-EMF) are showing faster body weight gain as compared to the non exposed control group. In agreement with the present study, Aydin et al. [33] found that exposure of rats to EMF caused deceleration of spermatogenesis and degeneration of germ cells. The exposure of mice to EMF for 12 days also induced an increase in maturation arrest in spermatogenesis, disorder in germinal cell distribution and a decrease in germ cell population [34]. These observations agree with that of Rajaei et al. [35] who mentioned that exposure to EMF for long periods could decrease the diameter of reproductive ducts and the length of the epithelial cells.

V. CONCLUSION

In the exposed animal group, testicular tissue revealed the detached adjacent seminiferous tubules and decreased number of Leydig’s cells at interstitial space. The number of spermatozoa was found to be decreased in experimental group as compared to respective control group. Large vacuoles and condensed nuclei were also noticed in experimental group. These histological changes are directly associated with the declined reproductive health of the animal. Present findings establish the role of radiation exposure in declined fertility in animals. Further studies can elaborate the molecular mechanism behind the cellular and tissue level damage in seminiferous tubule after exposure to electromagnetic radiation.

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