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ULTRASTRUCTURAL CHANGES IN RAT TESTICULAR TISSUE AFTER WHOLE BODY EXPOSURE TO ELECTROMAGNETIC RADIATION EMITTED FROM MOBILE PHONES

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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^{o} 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; Ozguner et al., 2005; Ribeiro et al., 2007; Yan et al., 2007). Tissues with higher hydration as testes were more sensitive to magnetic fields (Arutiunian 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 form domestic mobile phone on rat testes . To avoid any secondary thermal effect, temperature of the room was kept at 24^0 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^o 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 4^0 C (Aisha et al., 2006).

B. Processing for transmission electron microscopy

Washing was given to the samples by phosphate buffer (ph 7.4) three timesfor 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 A^0) 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

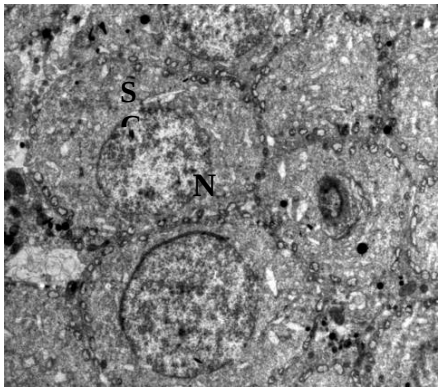
Electron microscopic examination of the testes of the control adult rats illustrated the normal secondary spermatocytes and few early spermatids with acrosome cap formation (fig. 1). Seminiferous tubules were consisting of spermatogenic cells at different stages of differentiation together with supporting 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.

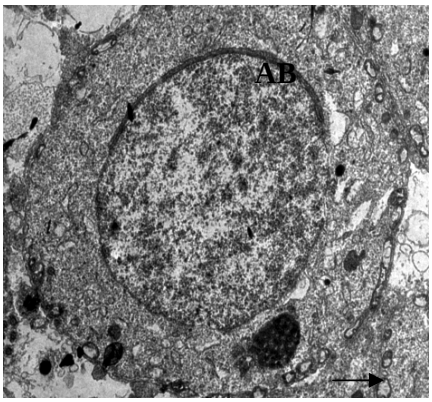
The exposed testes to Radio Frequency Electromagnetic Fields (RF-EMF) showed variable degenerative changes in the spermatogenic cells. Some cells showed electron dense areas and vacuolation within the cytoplasm. (fig. 3). Irregular shaped and multiple vacuolated mitochondria were also observed. (fig.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



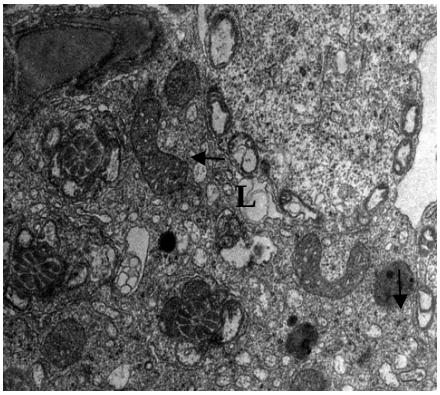


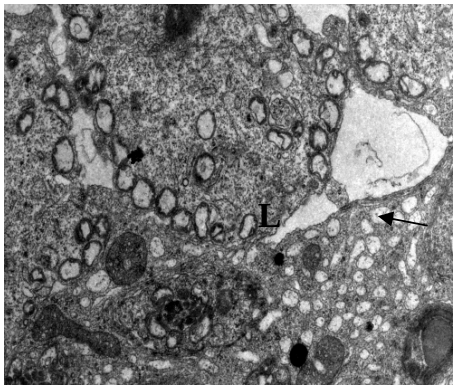
1 **2**

Plate 1: 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)





3 **4**

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)

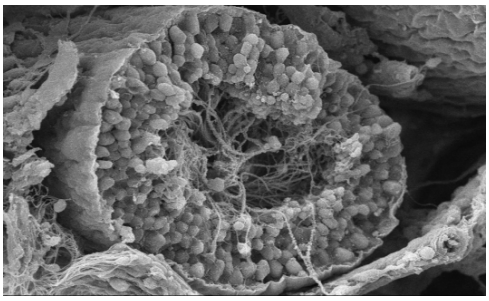
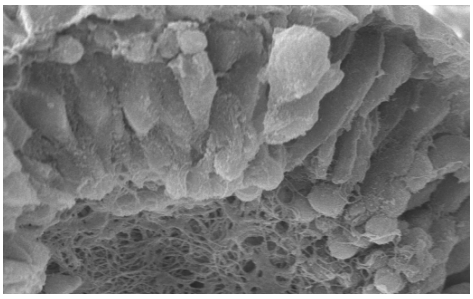



Plate III Scanning electron micrographs of sections of testes of control adult rat showing:

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)

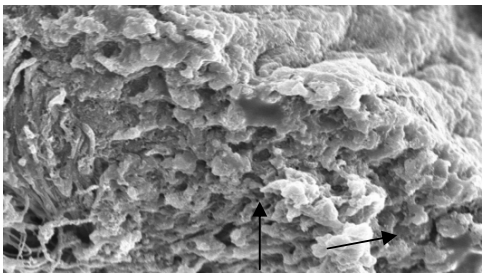
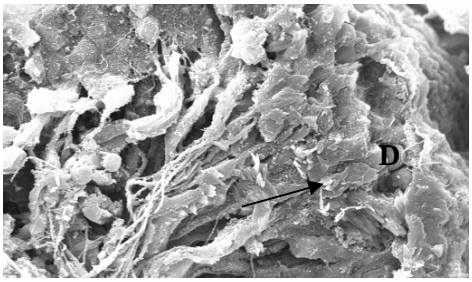



Plate IV: Scanning electron micrographs of sections of testes of exposed adult rat showing:

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 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

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 upto 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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