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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^{o} 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^{0} C temperature and finally in paraffin wax for 24 hrs at 60^{0} 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].

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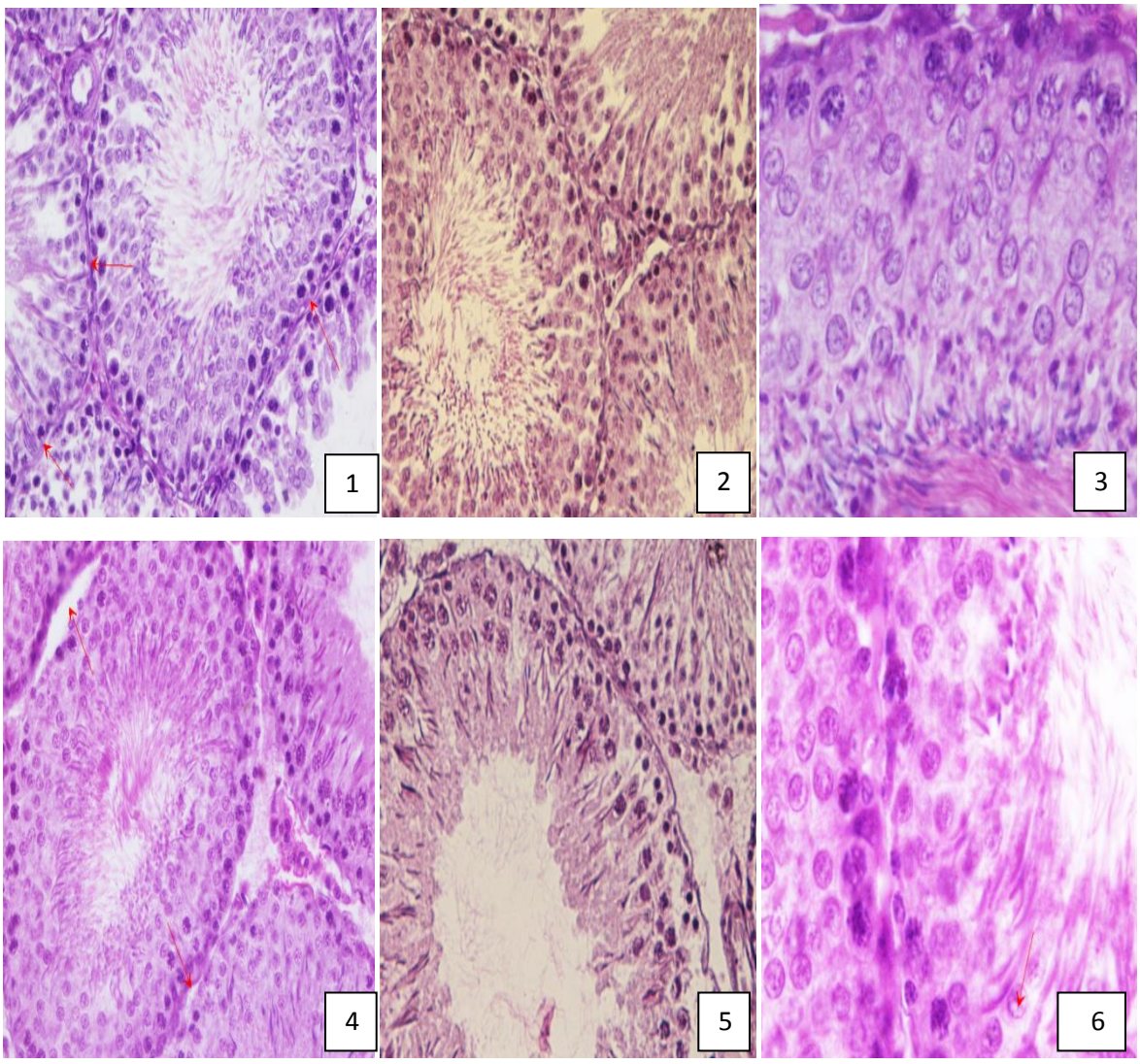

Fig. 1: Testicular section of rat as control showing normal spermatogenesis within seminiferous tubule. Intact germinal epithelial layers of adjacent seminiferous tubules are clearly visible.
(H & E staining, 400 X)

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].

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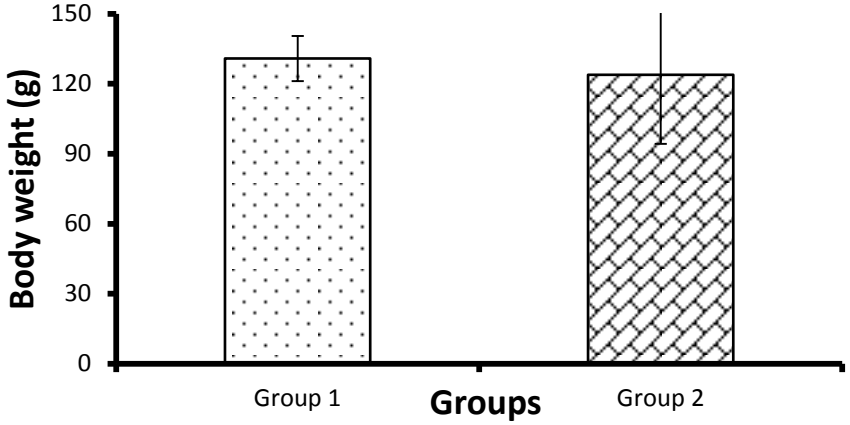

Fig. 7: Comparison of baseline rat body weight of control and exposure group

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.

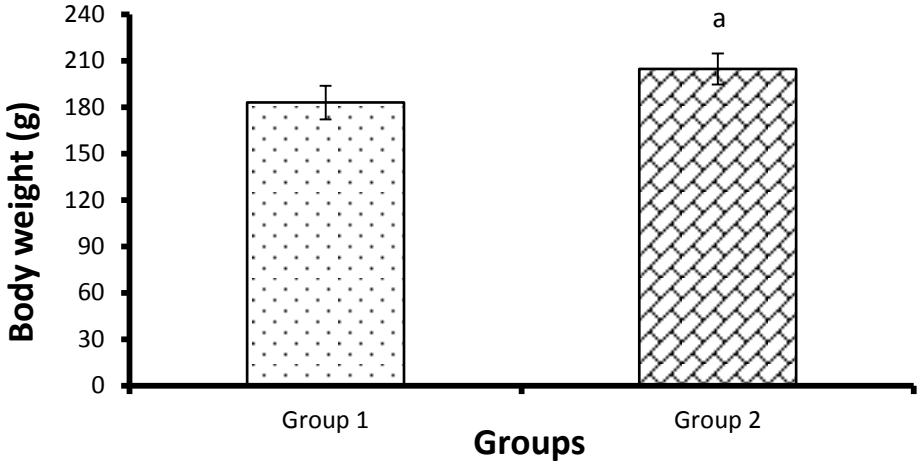

Fig. 8: Changes in body weight of control and exposure groups after 6 months

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.

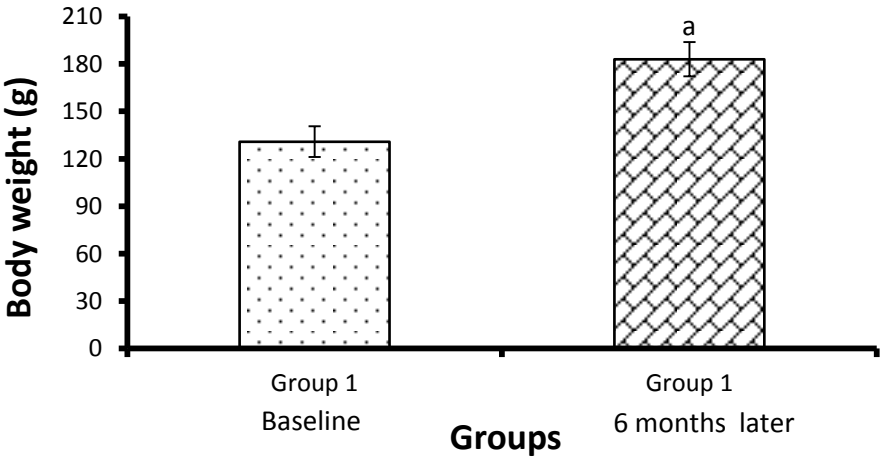

Fig. 9: Changes in baseline body weight of control group after 6 months

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.

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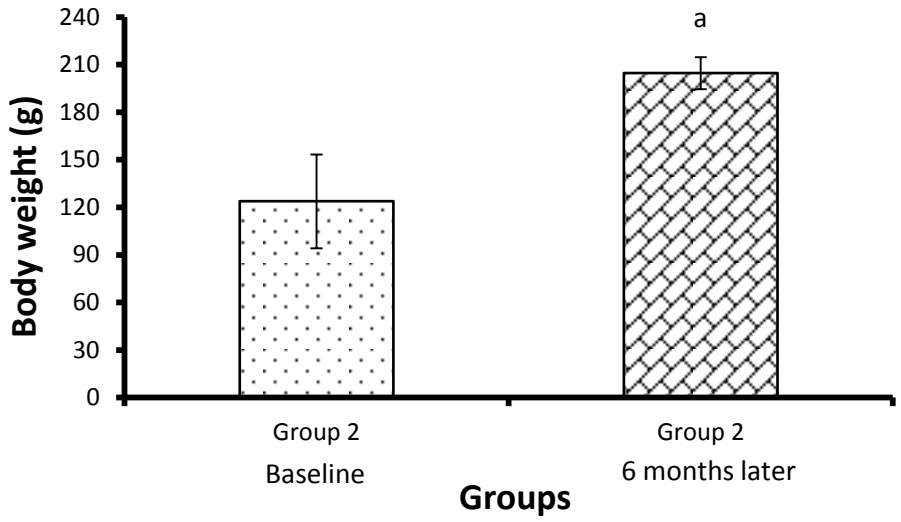

Fig. 10: Changes in baseline body weight of exposure group after 6 months

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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