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Original Article

Effect of Mobile Phone Radiation on Reproductive System and Behavior Using Male Albino Mice

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ABSTRACT

Background and objectives: This work was carried out to investigate the effect of mobile radiation on the behavior and reproductive system (histological study) in male albino mice. **Methods**: Twelve mice were divided into two groups. Group I: the control group was not exposed to mobile radiation (healthy mice); group II: mice were exposed to mobile radiation for one hour (active-ringing) per day for 90 days. At the end of the experiment, behavior scoring was carried out, followed by histological investigation of reproductive system. **Results:** In plus maze, anxiety measure, total lines crossed and total number of entries did not change compared to the control group. Also, in force swimming maze, the duration of immobility was not changed in mobile radiation exposed mice, compared to the control. Sperm morphology showed an increased in the percentage of abnormal sperm count in mice exposed to mobile radiation compared to the control mice group. Histological study showed that mice exposed to mobile phone radiation showed an increase of abnormal sperm shape compared to the control. A reduction of intraluminal spermatozoa, hypospermatogenic cells of seminiferous tubules was observed. Sperms also were very few inside the lumen of seminiferous tubules, and reduced number spermatogonia, spermatocytes, spermatid, and sertoli cells were observed. **Conclusion:** Exposure to mobile phone radiation has no effect on behavior; this indicates that it has no effect on the CNS for this duration of exposure. Histologically, this duration of exposure produced damage to testis structure and also in the morphology of sperms. Decrease in mobile phone usage each day keep the mind and body healthy.

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INTRODUCTION

Male infertility was increased during the recent years; infertility is affected by environmental hazards (Figure 1). Electromagnetic fields exposure may contribute to male infertility. Mobile phone has become one of the most common sources of non-ionizing radiations (NIR) [1].

Non ionizing radiation may produce heat, when the body is exposed to a large amount [2]. The body

regulate its temperature, but if exposures are too intense the body no longer copes [1].

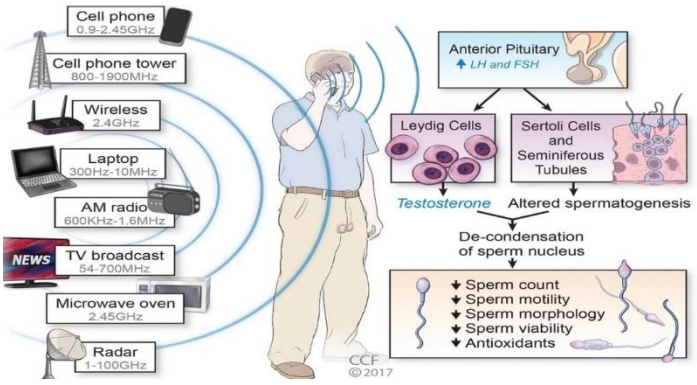

Figure 1: Environmental exposure to NIR [3]

Mobile phone user is exposed to radiation (900 Mhz - 2.8 Ghz) even if the person does not actually use the device for communication [4,5]. There is evidence that NIR produces free-radical damage in human, animal and plant [6]. Uncontrolled overproduction of reactive oxygen species (ROS) can lead to DNA damage such as single/double-strand breaks and crosslinks [7-10].

Human spermatozoa (male haploid gamete cell) subjected to radiofrequency radiation suffer from the formation of ROS, leading to decreased semen motility and vitality, and the increase of DNA adducts formation [11]. Exposure of semen to radiation led to the decrease of sperm motility and viability, and produced an increase in ROS level and a decrease in total antioxidant capacity [12]. Cleveland Clinic Foundation of Ohio (US) reports that the use of mobile phones decreases semen quality by reducing sperm count, motility, viability and normal morphology [3,13].

The reproductive system is usually exposed to mobile phone radiation. The mobile phone is usually carried out in waist belt or trouser pocket. Therefore, our work is carried out to investigate the effect of mobile radiation on the behavior, using plus maze and forced swimming maze, and reproductive system (histological study) in male albino mice.

METHODS

Design of the work

Male mice weighing 25-40gm bred in the animal house of Faculty of Pharmacy- University of Tripoli. Standard mice food pellet diet and water were free available. Mice were kept at room temperature (20-25˚C), and on 12h dark/light cycle in laboratory for at least 1 day before testing to acclimate with a new environment.

Twelve male albino mice were divided into two groups. Group 1 (n=6), was healthy mice without expose to mobile phone radiation; group 2 (n=6), exposed to mobile phone radiation for one hour (active-ringing) per day for ninety days. At the end of experiment, behaviour study were carried out using plus maze and forced swimming maze; this followed by histological examination of the testes (Fig 2).

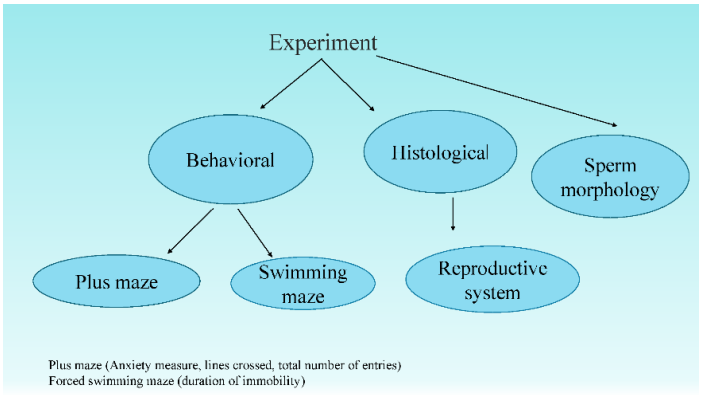

Figure 2, Design of the work

This experimental research on animals was conducted according to ethical rules of the Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmacy – University of Tripoli.

Behavioral study

Elevated Plus- Maze

The maze is composed of two open arms (30*5cm) and two close arms (30*5*15cm) that extended from a common central platform (5*5cm). The apparatus was elevated to height of 45 cm above floor level [5]. Mice

were gently handled by the right hand and placed on the center squire of the maze facing into the close arm.

Parameters scored to evaluate anxiolytic effect and spontaneous motor activity are time spent by the mouse in each of the arms, lines crossed in close or open arms, and the number of entries into close or open arms. An arm entry was defined as the entry of all four paws into the arm [6]. The total line crossed, total number of entries were calculated. The total line crossed and the total arm entries [7,8] express the spontaneous motor activity. Anxiety measures was calculated by the time spent in close arm by the total time of the test [8]. The duration of the test was 4 minutes.

Forced Swimming Maze

Mice were placed individually in glass cylinders (height 27 cm, diameter 15 cm) filled with water to a height of 16 cm (maintained at 23-25°C). Duration of the test was 6 minutes. The time of the two behavior parameters (duration of immobility and duration of climbing) was recorded during last 4 min of the 6 min testing period [9]. Immobility behavior is defined as the animal floated on the surface with front paws together and made only those movements with hind limb which were necessary to keep float.

Histological study

At the end of radiation exposure, mice were sacrificed; testes of healthy and exposed mice were removed and then were fixed in 10% formalin for 24 hours. The specimens were washed twice with 70% alcohol. The fixed tissues were dehydrated in an ascending series of alcohol ranging from 70% to 100% (absolute). The dehydrated tissues were cleared in xylene (twice), infiltrated and then were embedded in paraffin wax. Testes were sectioned on rotary microtome; sections were 5µm in thickness. The prepared sections were stained by routine methods using Hematoxylin-eosin method. The stained sections were examined under the microscope, and the different cell types were carefully studied and photographed [14]. Testis sections from each study group were evaluated for structural changes, blind by a histologist. Light microscopy (Leica, Germany) was used for the evaluations.

RESULTS

Behavioral study

In plus maze, anxiety measure, total lines crossed and total number of entries were not changed in mice exposed to mobile radiation compared to the control group (unexposed to mobile radiation) (Table 1).

Table 1, Effect of mobile radiation on behavior using plus maze in male albino mice

Treatments	Anxiety measure		Total lines crossed		Total number of entries	
	Mean ± SE	P value	Mean ± SE	P value	Mean ± SE	P value
Control (n=6)	0.903 ± 0.046	0.785	35.6 ± 3.65	0.711	8.4 ± 1.03	0.541
Radiation exposure (n=6)	0.920 ± 0.036		35.5 ± 4.92		7.25 ± 1.54	

The duration of immobility in force swimming maze were not changed in mice exposed to mobile radiation compared to the control group (Table 2).

Table 2, Effect of mobile radiation on behavior using swimming maze in male albino mice

Treatments	Duration of immobility	
	Mean ± SE	P compared to control
Control (n=6)	177.85 ± 5.942	0.231
Radiation exposure (n=6)	193.30 ± 10.571	

Sperm morphology

Sperm morphology examination showed that the percentage of abnormal sperm count in mice exposed to mobile radiation was significantly higher compared to the control group (p=0.000) (Fig 3, 4).

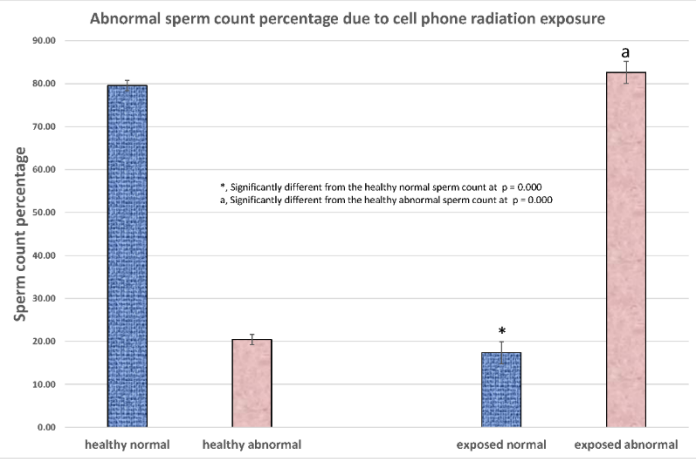

Figure 3. Normal and abnormal sperm count in healthy and mobile phone radiation exposure mice

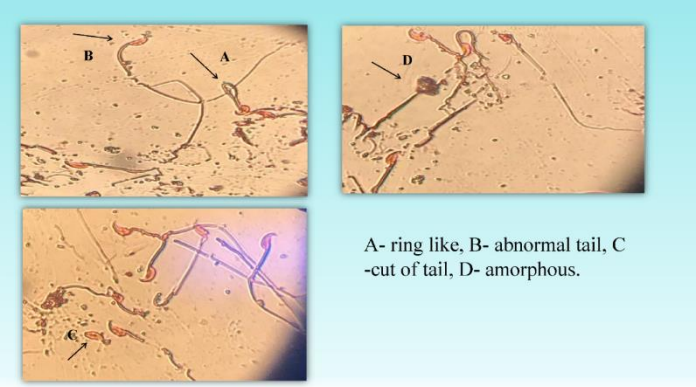

Figure 4. Abnormal sperm after mobile phone radiation exposure of male albino mice

Histological study

Healthy group

Sections of the testis of the control group showed the seminiferous tubule lumen full of mature spermatozoa and complete spermatogenesis, normal histological features of seminiferous tubules; seminiferous tubules, separated from each other by narrow interstitial spaces containing interstitial cell of Leydig, (Fig. 5). These seminiferous tubules containing spermatogenic cells and Sertoli cells; Sertoli cells have pale cytoplasm, which were observed among the spermatogenic cells. The spermatogenic cells were formed of spermatogonia, primary spermatocytes and spermatids. The spermatogonia appeared as small cell under basement membrane, smooth muscle (meyoid) cells at the membrane (Fig. 6).

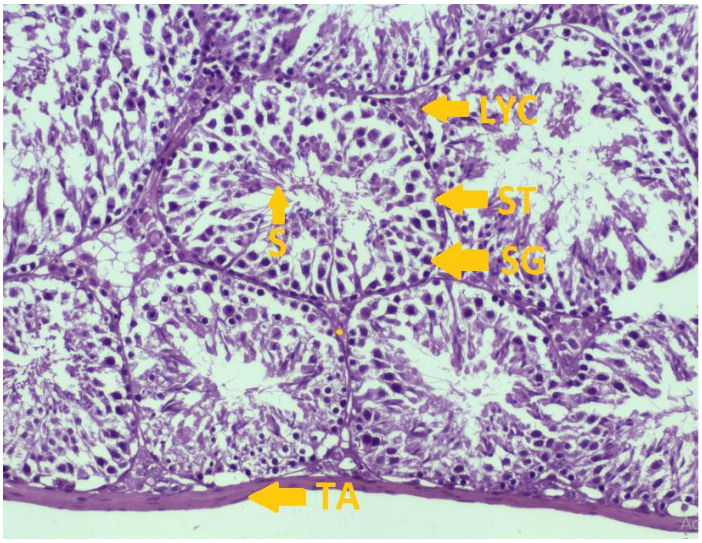

Figure 5, A photomicrograph of testis of group I (control group), showed:
a) Seminiferous tubules (ST) contain normal spermatogenic cell (SG).
b) Normal formation of sperms in the lumen of seminiferous tubules (S).
c) Normal interstitial space contains leydig cells (LYC).
d) Normal covering layer, tunica albuginea (TA). (H&E, 20x)

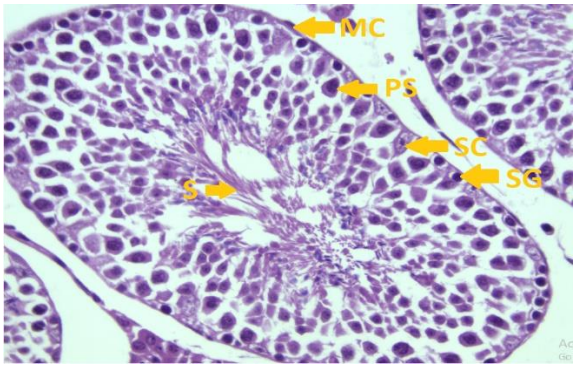

Figure 6. Photomicrograph of seminiferous tubule of testis of group I (control), showed:
a) Normal spermatogonia (SG), primary spermatocytes (PS) and sertoli cells (SC).
b) Meyoid, smooth muscle cells (MC) at a basement membrane.
c) Normal formation of sperms (S) in the lumen of seminiferous tubules. (H&E, 40x)

Mobile phone radiation exposure group

Sections of testis of the mobile radiation exposed group showed that the testicular effect was predominant, reduction of intraluminal spermatozoa and hypospermatogenic cells of seminiferous tubules (Fig. 7). Cells of spermatogenesis including spermatogonia, spermatocytes, spermatid, and sertoli cells numbers were significantly reduced. Sperms were also very few inside the lumen of seminiferous tubules in comparison with the control groups (Fig. 8).

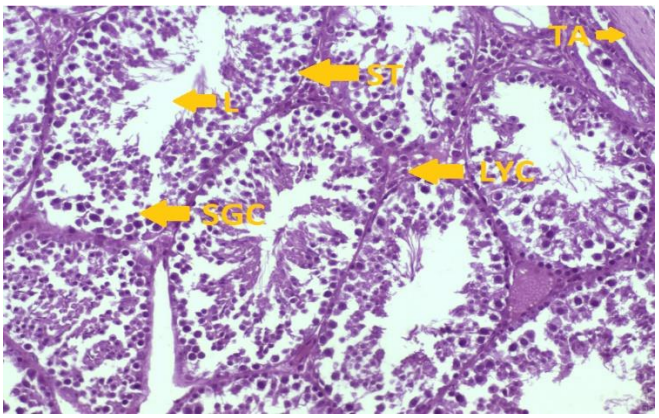

Figure 7. A photomicrograph of testis of group II (the mobile radiation exposed), showed:

- ***a)*** *Seminiferous tubules (ST) contain fewer spermatogenic cells (SGC).*
- ***b)*** *Reduction in formation of sperms (S) in the lumen of seminiferous tubules (L).*
- ***c)*** *Normal testicular wall with normal tunica albuginea (TA).*
- ***d)*** *Normal interstitial space contains leydig cells (LYC). (H&E, 20x)*

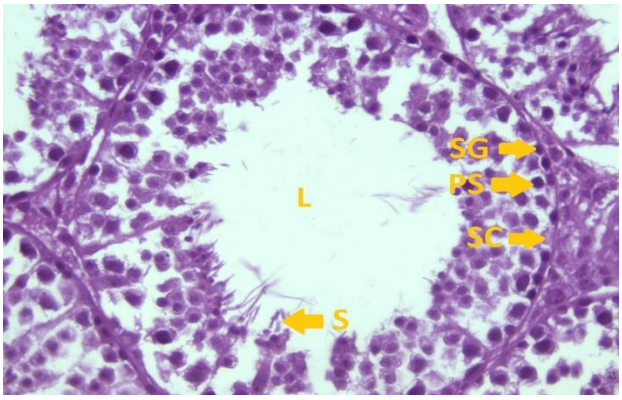

Figure 8. Photomicrograph of seminiferous tubule of group II (the mobile radiation exposed) showed:

- ***a)*** *Reduction in number of spermatogonia (SG), primary spermatocytes (PS) and sertoli cells (SC).*
- ***b)*** *Reduction in formation of sperms (S) in the lumen of seminiferous tubules (L). (H&E, 40x)*

DISCUSSION AND CONCLUSION

The Behavior of mice exposed to mobile radiation, using plus maze and forced swimming maze, was not changed compared to the control. Although it was found that animals exposed to mobile radiation showed a decrease in locomotor activity [15]; this difference in observation could be due to the difference in the design of the experiment.

In our work, mice exposed to mobile phone radiation one hour (active – ringing) per day for 90 days produced an increase of abnormal sperm shape compared to control. Reductions of intraluminal spermatozoa, hypospermatogenic cells of seminiferous tubules, were observed. Sperms were also very few inside the lumen of seminiferous tubules. The number of spermatogonia, Spermatocytes, spermatid, and sertoli cells was reduced.

Exposure to mobile phone radiation produces thermal effects; the body will regulate its temperature, but if exposures are too intense the body no longer copes [1]. The reproductive organs, particular the testes are at risk when exposed to mobile phone radiation; mobile phones are usually carried at waist level [4, 16, 17].

A study showed a growth of the thickness of membrane propria and an amount of collagen fiber, and also an increased number of electron-dense mitochondria and cellular structures; this indicates that the testes react on electromagnetic radiation exposure through morphological re-organization [18].

Mobile phone radiation interferes with the oxidative repair mechanisms leading to oxidative stress, causing damage of the cellular components including DNA [6]. Exposure to mobile radiation produces an increase in mitochondrial ROS, leading to DNA fragmentation,

and decreased sperm motility and viability [11,19-23]. It was reported that oxidative stress might be the main factor that causes sperm chromatin/ DNA damage [11]. The effect of radiation on DNA produces distortion of the acrosome, possibly leading to an inability to penetrate oocytes, causing in infertility [23].

It was found that electromagnetic radiation stimulates the release of mitochondrial ROS [11], and consequent activation of Akt signaling pathway, which is essential for anti-apoptotic action [4]. Mobile phone radiation exposure induced oxidative damage in mitochondria of sperm tail; this limited endogenous antioxidant defenses, which may produce oxidative damage leading to peroxidation of the sperm acrosomal membrane and diminished acrosin activity, which indicate the sperm function [24 - 26].

Mitochondria in spermatozoa supply the energy for sperm motility; any metabolic disruption in the electrons transport chain, due to exposure to mobile radiation, can produce mitochondrial ROS, thus affecting sperm motility [12, 27, 28]. Also Free radicals oxidize membrane phospholipids extracellularly, thus causing reduction in membrane fluidity with impaired motility [3]. Sperm motility is directly associated with mitochondrial dysfunction; defects in sperm mitochondrial ultrastructure may be associated with decreased sperm motility in humans [29, 30].

Studies reported that after mobile phone radiation exposure, there is a decrease in glutathione and superoxide production, in seminal fluid. These enzymes protect spermatozoa against the assault of ROS. The decreased glutathione level during sperm production correlated with disruption in the membrane integrity of spermatozoa [23,31].

In seminal plasma, there are high levels of endogenous antioxidants to protect spermatozoa from oxidative damage [32, 33]. Mobile phone exposure leads to the induction of oxidative stress by the generation of ROS in the sperm plasma membrane through activation of NADH oxidase [3]. Increase in ROS can stimulate endothelial growth factor (EGF) receptor, which in turn activates extra cellular signal regulated kinase (ERK) pathway. In ERK pathway, there is an activation of mitogen-activated protein kinase (MAPK), which has tumor promoting role [34].

Chronic exposure to ROS can activate various stress kinase (p38 MAP kinase); this activation can stimulate extra cellular signal regulated kinase (ERK) pathway, leading to phosphorylation of heat shock proteins (Hsp), which inhibits apoptosis. Inhibition of apoptosis might promote carcinogenesis by prolonging survival of cell with damaged DNA [34]. Heat shock proteins stabilizes endothelial stress fiber and enhance secretion of bFGF [35], this can lead to increase in permeability of blood testis barrier and causes infertility [34].

Exposure to mobile phone radiation is responsible for the decrease of melatonin levels in the brain pineal gland [31, 36]. Melatonin regulates LH and FSH secretion in the hypothalamus; this can alter the production of gonadal sex steroids, resulting in changes in the reproductive cycle [37, 38].

Our finding conclude that the histological investigation showed that mice exposed to mobile phone radiation, one hour per day for 90 days, produces an increase of abnormal sperm shape compared to the control. A reduction of intraluminal spermatozoa, hypospermatogenic cells of seminiferous tubules was observed. Sperms also were very few inside the lumen of seminiferous tubules, and reduced numbers of spermatogonia, spermatocytes, spermatid, and sertoli cells were observed.

Disclaimer

The article has been previously presented in the LCMPS2020 conference, Nov 14, 2020, Tripoli – Libya. This article has not been previously published, and is not part of a thesis project.

Conflict of Interest

There are no financial, personal, or professional conflicts of interest to declare.

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