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Original Article

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

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ABSTRACT

Objectives: To investigate histopathological effect on reproductive system and the behavioral effect in female albino mice due to exposure to mobile phone radiation. **Methods:** Twelve female albino mice were divided into two groups. Group 1, was healthy mice without expose to mobile phone radiation; group 2, exposed to mobile phone radiation for one hour (ringing) per day for ninety days. At the end of the experiment, behaviour study was carried out using plus maze and forced swimming maze; followed by histological examination of the ovary and uterus. **Results:** Mice exposed to mobile phone radiation did not show any changes in behavior. While, mice exposed to electromagnetic radiation from mobile phone showed ovarian cortex reduction in number of primordial and primary follicles, absence of oocyte inside the follicle, and large amount of vacuolated cytoplasm in Corpus luteum. In addition, mobile phone radiation exposed mice uterus showed abnormal histological features of the inner mucosa, or endometrium. Glands appears as focal inactive atrophic glands with hyperchromatic nuclei; atrophy of the uterine glands, and number of glands reduction in the stroma were observed. In myometrium, large blood vessels were observed. **Conclusion:** Exposure to mobile phone radiation has no effect either on behavior or any CNS function during exposure. Histologically, this duration of exposure produced damage in ovary and uterus. It is advised to limit the hours of mobile phone usage each day to keep the mind and body healthy.

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INTRODUCTION

People can be exposed to radio frequency radiation (RF), either naturally as from sunlight, and lightning strikes; or Man-made RF radiation as Broadcasting radio and television signals, mobile phones and cell phone towers [1, 2].

Mobile phones have become an integral part of human life; they make everything convenient and time saving. Electromagnetic radiation (EMR) emitted from mobile phones is non-ionizing radiation (NIR); it is considered one of the most dangerous forms of environmental pollution [3].

Biological effects of NIR could be physiological, biochemical or behavioral; it interacts with tissue through the generation of heat. The hazards depend on the ability of heat to penetrate the human body and on the absorption property of different tissues [1].

There is evidence that non-ionized electromagnetic fields (mobile phone radiation) produce free-radical damage in human, animals and plants [4]. Mobile phones are usually carried at waist level, either in front and back pocket of trousers or in hand bag; therefore, this research work was carried out to investigate the behavioral effect and the histopathological changes in reproductive system due to exposure to mobile phone radiation in female albino mice.

METHODS

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

Animals

Twelve female mice aged between 25 to 40gm were bred in the animal house of Faculty of Pharmacy, the University of Tripoli. Standard mice food pellet diet and water were free available. The animals were kept at room temperature (20-25°C), and on 12h dark/light cycle. Animals were kept in laboratory for at least 1 day before the experiment to acclimate with the new environment.

Design of the work

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

Elevated Plus- Maze

The maze is composed of two open arms (30*5cm) and two closed arms (30*5*15cm) that extended from a common central square area (5*5cm). The apparatus was elevated to a height of 45 cm above floor level [5]. Mice were gently handled by the right hand and placed on the central square area of the maze facing the close arm.

Parameters were scored to evaluate the anxiolytic effect and spontaneous motor activity in the elevated plus-maze. The scores include time spent by the mouse in each of the arms, lines crossed in closed or open arms, and the number of entries into closed or open arms. An arm entry was defined as the entry of all four paws into the arm [6]. The total lines crossed and the 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 minutes of the 6 minute testing period [9]. Immobility behavior is defined as the floating of the animal on water surface with front paws together and making hind limb movements necessary to keep float.

Histological study

At the end of radiation exposure, mice were sacrificed; ovaries and uteri of healthy and mobile phone radiation 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 concentration ranging from 70% to 100% (absolute). The dehydrated tissues were cleared in

xylene (twice), infiltrated and then were embedded in paraffin wax. Ovary and uterus 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 [10]. Sections from each study group were evaluated for structural changes; without the histologist knowing to which group the sample belongs. Light microscopy (Leica, Germany) was used for the evaluations.

Statistical analysis

Descriptive statistical analysis was performed using IBM SPSS (version 13), also verification whether the data were normally distributed was based on using Kolmogrove-Simirnove maximum deviation test for goodness of fit. If the parameters were parametric, treatments were compared by one-way ANOVA, Post-Hoc test (LSD test). If the parameters were nonparametric, treatments were compared by the Mann-Whitney *U* test for unmatched sample. The differences were considered significant at the *P* value ≤ 0.05. The values are expressed as the mean ± standard error.

RESULTS

Behavior results

In plus maze (table 1), anxiety measure, total lines crossed and total number of entries were not changed in mice exposed to mobile phone radiation compared to the control group (p= 0.08, 0.965, 0.764 respectively).

Table 1: Effect of cell phone radiation on behavior using plus maze in female 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)	0.88±0.038	0.08	54.1±7.16	0.965	12.17±2.5	0.764
Radiation exposure (n=6)	0.68±0.112		53.5±15.0		11.0±2.61	

Using swimming maze (table 2), the duration of immobility was not changed in mobile phone radiation exposed mice compared to the control group (p = 0.683).

Table 2: Effect of mobile phone radiation on behavior using swimming maze in female albino mice

Treatments	Duration of immobility	
	Mean ± SE	P value
Control (n=6)	179.8 ± 11.05	0.683
Radiation exposure (n=6)	186.1 ± 10.26	

Histological Study

Ovary of control group

On examination of hematoxylin and eosin (H&E) stained sections of control mice ovary showed that the ovary was covered by a single layer of simple cuboidal (germinal) epithelium. The ovarian parenchyma formed of cortex and medulla (Fig.1). In the ovarian cortex, the primordial follicles were seen underneath the tunica albuginea (Fig.2). Growing follicles were seen in the ovarian cortex; includes primary, secondary and mature Graafian follicles.

The secondary follicle formed of large primary oocyte in the center and surrounded by zona pellucida and multilayers of follicular granulosa cells; the cystic spaces within the granulosa cells coalesce and form a large central cavity (the follicular antrum) (Fig.1,2). The mature Graffian follicle was larger and located near the surface. It is formed of primary oocyte surrounded by clear zona pellucida and few follicular cells, called corona radiata.

The whole follicle is surrounded by theca interna, and theca externa (Fig.1). The corpus luteum is formed of both granulosa and theca lutein cells; these were polyhedral cells containing large spherical nuclei and large amount of vacuolated cytoplasm (Fig.1,2).

Uterus of control group

Examination of hematoxylin and eosin stained sections of control mice uteri revealed that; the inner mucosa, or endometrium, consists of a surface columnar epithelium and lamina propria (stroma), the lamina propria containing numerous blood vessels and endometrial glands, which are simple or branched tubular glands (Fig. 3). Also, the lamina propria consists of loose connective rich with an abundance of fibers and smooth muscles.

The middle muscular layer, or myometrium, is composed of an inner circular and outer longitudinal smooth muscles. The outer layer, the perimetrium is the serosa of the uterus, composed of loose connective tissue with a large number of lymphatic vessels (Fig. 4).

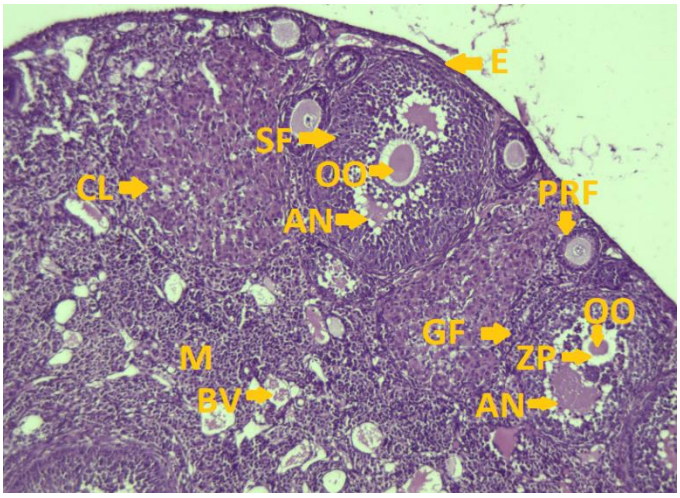

***Figure. 2**, A photomicrograph of control mouse ovary, showing: Primary follicle (PRF) with a single layer of follicular cells surrounding an oocyte. Secondary follicle (SF) with antrum (AN), central oocyte (OO). Graafian follicle (GF) with peripheral oocyte (OO), large antrum (AN) and zona pellucida (ZP). Corpus luteum (CL). Ovary was surrounded by a germinal epithelium (E), and middle medulla (M) with blood vessels (BV). (H&E. 20x).*

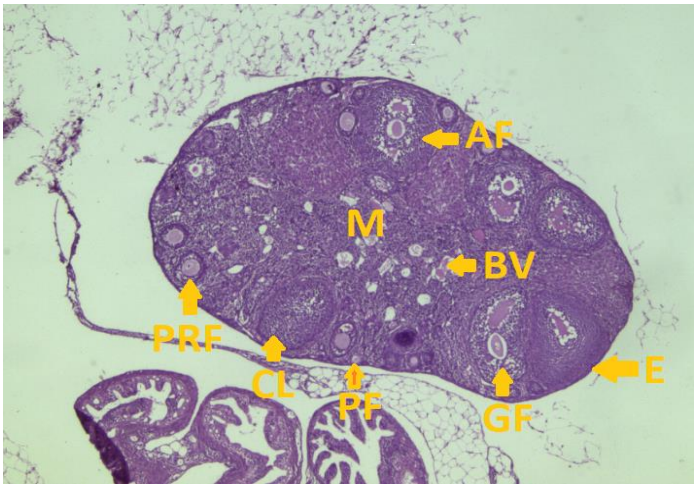

***Figure. 1**, A photomicrograph of control mouse ovary (l power), showing: Cortex containing different ovarian follicles; primordial follicle (PF), primary follicle (PRF), antral follicle (AF), Graafian follicle (GF) and corpus luteum (CL). Medulla (M) containing blood vessels (BV). Ovary was covered with a layer of germinal epithelium (E). (H&E. 10x).*

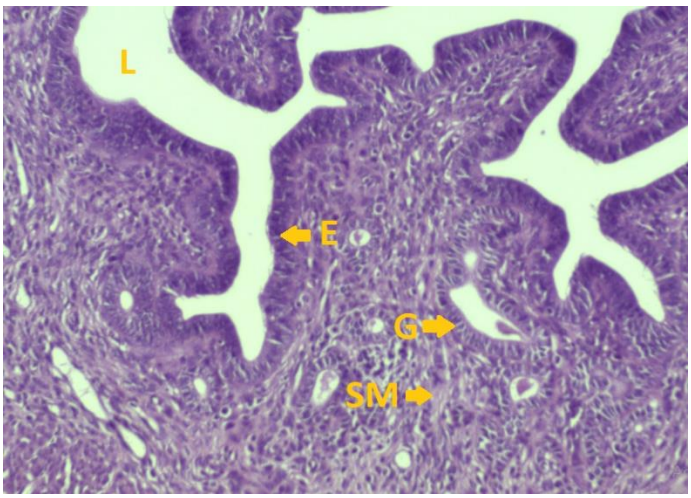

***Figure. 3**, A photomicrograph of control mouse uterus, showing: Endometrium, consists of a surface simple columnar epithelium (E), Stroma containing many endometrial glands (G), smooth muscles (SM). Lumen of the uterus (L). (H&E, 40x).*

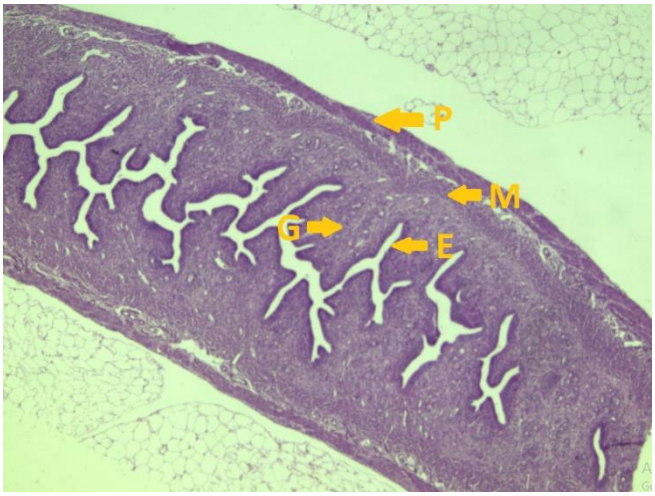

***Figure. 4**, A photomicrograph of control mouse uterus (low power), showing: Endometrium lining with epithelium layer (E), and many uterine glands (G). Myometrium (M). Perimetrium (P), the serosa of the uterus. (H&E, 4x).*

Ovary of mobile phone radiation exposed group

Sections of the mice ovary that exposed to mobile radiation showed normal appearance of germinal epithelium covering the ovary. The ovarian cortex, showed significantly reduction in number of primordial and primary follicles were seen underneath the tunica albuginea and absence of oocyte inside the follicle (Fig.5,6). Normal antral and mature Graafian follicles, and the secondary follicle show normal appearance.

The mature Graafian follicle was large and located near the surface. It is formed of primary oocyte surrounded by a clear zona pellucida and few follicular cells, called corona radiata. The whole follicle is surrounded by theca interna, and theca externa. Corpus luteum is formed of both granulosa and theca lutein cells, these were polyhedral cells containing large spherical nuclei and large amount of vacuolated cytoplasm (Fig.6).

Uterus of mobile phone radiation exposed group

Sections of the exposed mice uterus showed in some areas abnormal histological features of the inner mucosa, or endometrium, that the lining simple columnar epithelial cells of endometrium and some glands extends to the underlying lamina propria (stroma); glands appears as focal inactive atrophic glands with hyperchromatic nuclei appeared darkly stained. Also histopathology observations showed atrophy of the uterine glands that reduces number of glands in the stroma (Fig.7,8).

The middle muscular layer, or myometrium is composed of an inner circular and outer longitudinal smooth muscle layer; the region in between the two layers of smooth muscle contains large blood vessels. The outer layer, the perimetrium, is the serosa of the uterus, composed of loose connective tissue with a large number of lymphatic vessels (Fig. 9).

The histological structures of the uterus in the mobile phone radiation exposed group was different in some endometrial glands to that of the control group (Fig.7,8). Glands in the exposed group appears as focal inactive atrophic glands, with hyperchromatic nuclei appeared darkly stained and reduces number of glands in the stroma.

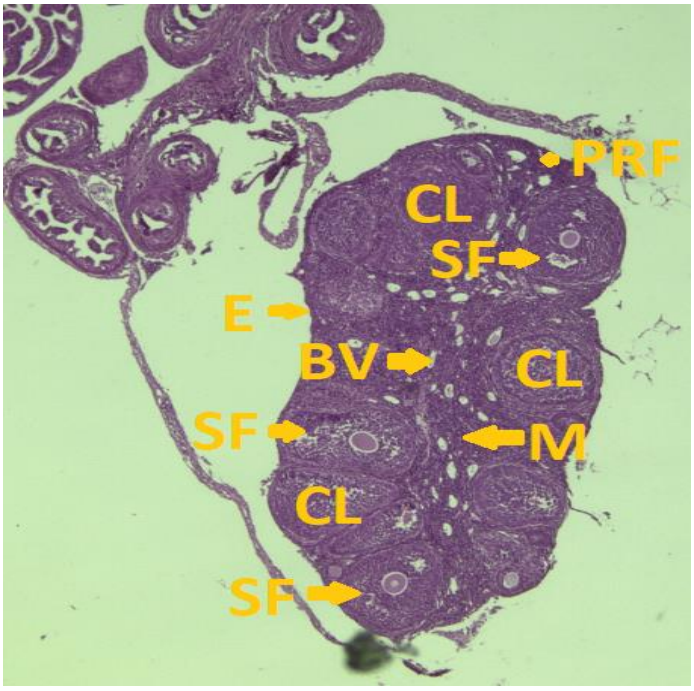

***Figure. 5,** A photomicrograph of exposed mouse ovary (low power), showing histological features of: Cortex containing remnants of primary follicles (PRF) that lack oocytes. Many antral secondary follicles (SF) and corpus leutum (CL). Medulla (M) containing blood vessels (BV). Covered with a layer of germinal epithelium (E). (H&E. 4x).*

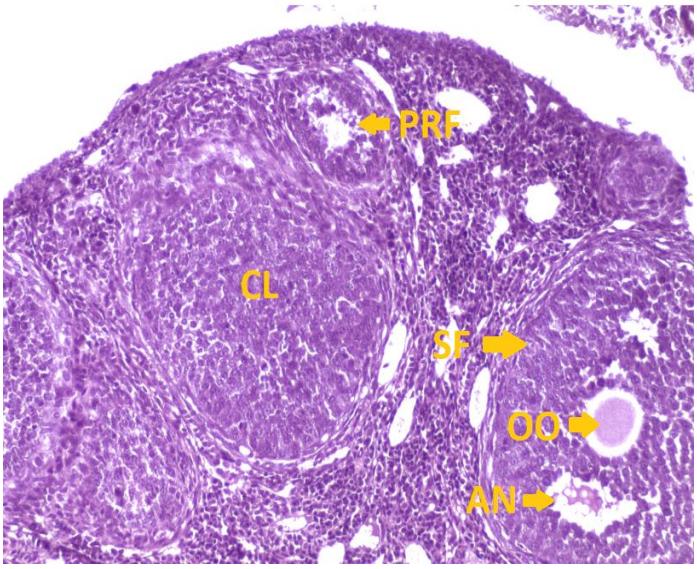

***Figure. 6**, A photomicrograph of exposed mouse ovary, showing histological features of: Remnants of primary follicles (PRF) that lack oocyte inside follicle. Secondary follicle (SF) with antrum (AN) and a central oocyte (OO). Corpus leutium (CL). (H&E. 20x).*

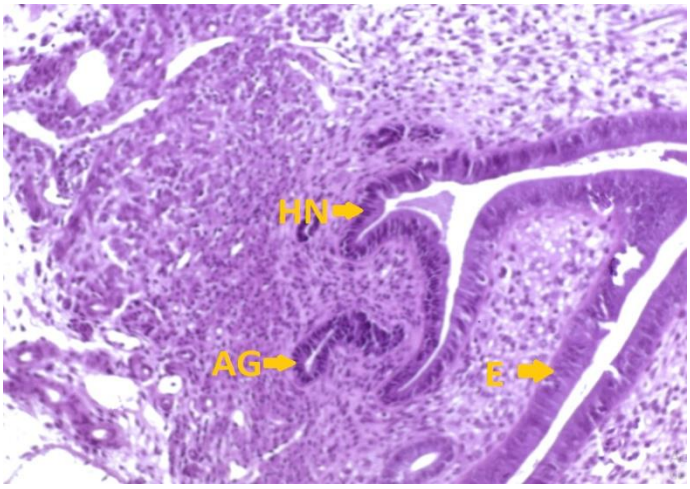

***Figure 7.** A photomicrograph of exposed mouse uterus, showing: Endometrium lining with columnar epithelial cells (E), some are with hyperchromatic nuclei appeared darkly stained (HN). Decrease of number of uterine glands and some glands are atrophic (AG) with hyperchromatic nuclei appeared darkly stained. (H&E, 20x).*

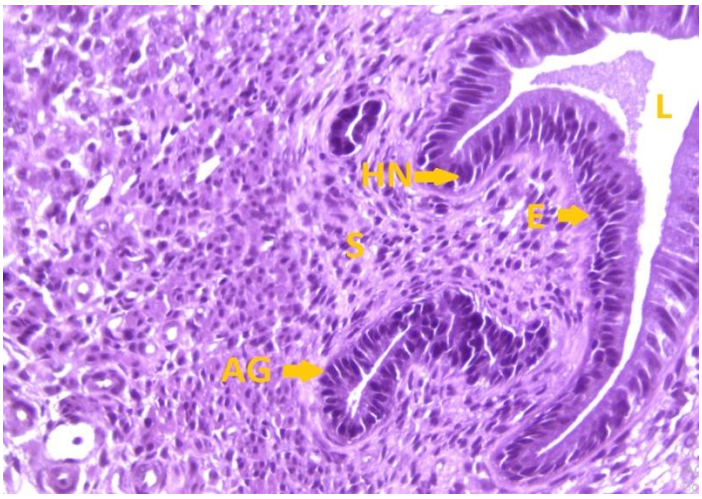

***Figure 8.** A photomicrograph of exposed mouse uterus, showing: Endometrium containing some columnar epithelial cells (E) are with hyperchromatic nuclei appeared darkly stained (HN). Decrease of number of uterine glands, some atrophic uterine glands (AG) with hyperchromatic nuclei appeared darkly stained in the stroma (S). Lumen of the endometrium (L). (H&E, 40x).*

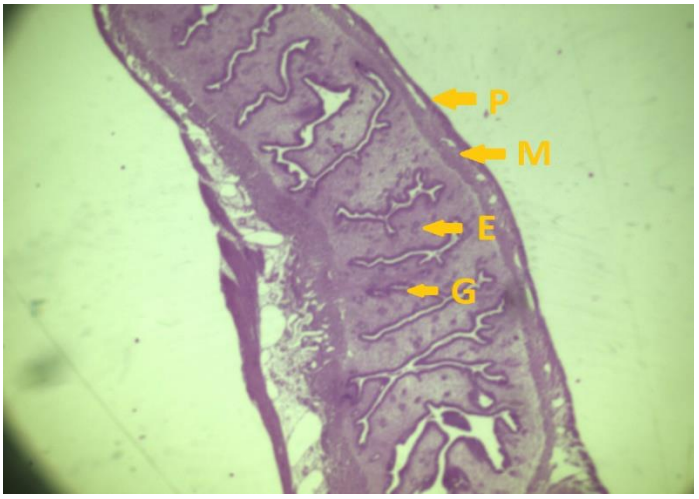

***Figure 9.** A photomicrograph of exposed mouse uterus (low power), showing: Endometrium containing epithelium layer (E), with decrease of number of uterine glands (G), Myometrium (M) and Perimetrium (P), the serosa of the uterus. (H&E, 4x).*

DISSCUSION AND CONCLUSION

Mice exposed to mobile phone radiation for one hour per day (ringing) for ninety days did not produce any changes in behavior. This duration of exposure has no effect on the CNS. It was found that mice exposed to electromagnetic radiation (EMR) showed suppression in motor component. A study on rats suggested that

"it is possible that the rats were under stress; the motivation to interact with the environment decreases, leading to an excessive self-accentuation of the animal" [11]. In our work the behavior parameters in plus maze and forced swimming maze were not changed, mice may need more exposure time to observe significant changes in behavior. Mice were used in our work, which may be less sensitive to EMR emitted from mobile phone than rats.

In the investigation of ovary in mice exposed to EMR from mobile phone, we observed the ovarian cortex with a reduction in number of primordial and primary follicles and absence of oocyte inside the follicle. Large amount of vacuolated cytoplasm was observed in Corpus luteum.

In mobile phone radiation exposed mice, uteri showed in some area's abnormal histological features of the inner mucosa, or endometrium. Glands appear as focal inactive atrophic glands with hyperchromatic nuclei. Observations showed atrophy of the uterine glands that reduces number of glands in the stroma. In myometrium, large blood vessels were observed.

Invisible mobile phone radiation causes significant damage to reproductive organs in human beings and may lead to infertility [3]. Exposure of people to EMR may produce harmful effects through the stimulation of oxidative stress, lower antioxidant levels and increase the level of lipid peroxidation; this indicates the increase in the free radicals [12, 13], leading to infertility [13].

It was concluded that EMR exposure might induce DNA damage due to increased oxidative stress [14]. Although EMR energy is not enough to damage DNA directly, indirect mechanisms, such as the free radical have been proposed to explain EMR-induced DNA damage [15 - 17].

It was reported that EMR is responsible for the decrease of melatonin levels in the brain pineal gland [18, 19]; the decrease in melatonin concentrations increased the oxidative stress [20]. Melatonin acts as potent antioxidant to detoxify ROS and stimulates antioxidative enzymes [20, 21]. Melatonin reduces oxidative stress and protects membrane lipids, cytosolic proteins, nuclear and mitochondrial DNA from oxidative damage [22].

Khaki et al., [23] concluded that long term exposure to EMR could produce an irreversible damage that lead to infertility. They found that the electromagnetic field has harmful effects on oocyte and the ovarian follicles; also produce deleterious effect on gonadal tissue and gametogenesis.

The obtained results from the present study reported that exposure to mobile phone radiation for one hour/day for three months did not show any changes in the behavior; this indicates that there is no effect on CNS after mobile phone radiation exposure for this duration of time. In the histological study, the ovarian cortex showed significant reduction in number of primordial and primary follicles seen underneath the tunica albuginea; also, there was absence of oocyte inside the follicle. Corpus luteum is formed of both granulosa and theca lutein cells, these were polyhedral cells containing large spherical nuclei and large amount of vacuolated cytoplasm.

Sections of the exposed mice uteri showed abnormal histological features of the inner mucosa, and endometrium; abnormality appeared in the lining simple columnar epithelial cells of endometrium; some glands extends to the underlying lamina propria (stroma). Glands appeared as focal inactive atrophic glands with darkly stained hyperchromatic nuclei. Also, histopathology observations showed atrophy of the uterine glands and the reduction of the number of glands in the stroma. The middle muscular layer, or myometrium is composed of an inner circular and outer longitudinal smooth muscle layer; the region in between the two layers of smooth muscle contains large blood vessels. The uteri in the mobile phone radiation exposed group was different in some endometrial glands to that of the control group.

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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