

Exposure Effects of 50 Hz, 1 Gauss Magnetic Field on the Histoarchitecture changes of Liver, Testis and Kidney of Mature Male Albino Rats

Ahmad Elbaz and Wael AM ghonimi*

Department of Histology and Cytology, Faculty of Veterinary Medicine, Zagazig University, Zagazig, Egypt

Abstract

The present investigation was carried out on twenty eight mature male albino rats from laboratory animal unite in the faculty of veterinary medicine, Zagazig University, Egypt. The rats were divided into four equally groups; I, II, III and IV of seven rats for each. Group I and II are control groups but the group I was kept in normal day condition and group II was kept in 12 hours Light / 12 hours dark cycle during the period of experiment without exposure to electromagnetic field (EMF), at constant temperature about 25°C. Group III was exposed to 50 Hz, 1 Gauss electromagnetic field (EMF) consciously for 21 days. Group IV was kept for 48 days post the end of the exposure period; 21 days and then complete to 48 days without exposure to MF for studying the delayed effects of MF exposure (reversibility or irreversibility). At the end of experiment, all groups were scarified by cervical dislocation and the liver, testis and kidneys were separated immediately and small pieces from them were taken, fixed in 10% neutral buffered formalin, processed till paraffin sections and stained histologically with Hematoxylin and Eosin (HandE) and Green Masson's Trichrome. Groups I, II showed normal histological structure of liver, testis and kidney without any abnormalities. Group III showed severe pathological lesions in the liver, testis and kidney. Regarding group IV, the histological examination of all organs showed the same normal histological structure as that in the control groups (I, II).

Keywords: Electromagnetic field; Histoarchitecture; Albino rat; Steatosis; Hyperplasia; Vacuolation; Congestion

Introduction

Humans beings are unavoidably exposed to ambient electromagnetic fields (EMF) generated from various electrical devices and from power transmission lines. All of the electronic equipment's that we use in our daily life without thinking how much we use or how often we use creates EMF [1].

With the ever-increasing use of the electric technology, electromagnetic fields especially the extremely low frequency electromagnetic fields (ELF-EMF) have become a part of the modern life. These fields are produced by all electric devices, including high energy sources like power lines and microwaves, but also found in low energy devices such as cell phones [2].

In exposed rat group to 50 Hz horizontal electric field for 8 hours/day for 8 weeks, many histological alterations such as focal tubular atrophy, necrosis and degeneration of the seminiferous epithelium were observed in the testes [3]. Moreover, Zare et al. [4] clarified that the exposed group of guinea pigs to 50 Hz electric field for 2 hours daily for 5 days cause cytoplasmic vacuolations of the hepatocytes. Furthermore, swelling in the epithelial cells of renal tubules and subsequently cell necrosis were observed with glomerular dilatation. And also, atrophy of the seminiferous tubules was detected [4].

It was reported that extremely low frequency EMF induced tissue damage in different organs of the experimental animals [4,5]. Also, exposure to MF adversely affects spermatogenesis, Sertoli and Leydig cells of experimental animals [6-8].

Recently, the histological and physiological studies have increased in the evaluation of the effects of electromagnetic fields on human health [4,9-14].

The aim of our work is to investigate the possible histological changes of the effects of the electromagnetic fields (EMFs) on the liver,

testis and kidney of male rats as well as clarifying if these effects could be reversible or irreversible.

Materials and Methods

Animals

Twenty eight mature male albino rats weighing between 292-329 gm and six months age were purchased from laboratory animal unite in the faculty of Veterinary Medicine, Zagazig University and transported to the laboratory of biophysics department, faculty of Science, Cairo University. The animals were acclimatized for one week in the laboratory before beginning of the experiment to avoid transportation stress. Animals were kept in a 12 hr light/12 hr dark cycle, at constant temperature of 25°C, food and water were available *ad libitum*.

Exposure facility

Animals were exposed to a homogenous magnetic field generated by 4 solenoids of 270 turns each of electrically insulated 2.2 mm copper wire, wound around a copper cylindrical chamber of 55 cm external diameter. Animals were kept in special plastic cages fixed on supports inside the irradiation chamber. Food and water were kept in special open containers fixed on the walls of the cages. Cleaning and changing water and food was done for all animals twice daily. Animals were exposed to the magnetic field in a free volume of about 2000 cm² at

*Corresponding author: Wael AM ghonimi, Department of Histology and Cytology, Faculty of Veterinary Medicine, Zagazig University, Zagazig, Egypt, Tel: 00201222498246; Fax: +2-055-2283683; E-mail: drwael_histology@yahoo.com; waghonimi@zu.edu.eg

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the center of the solenoids. The magnetic field was measured by means of a hand held Gauss (G)/Tesla (T) meter (Model 4048 with probe T-4048.001 by Bell Technologies, Inc., USA), and the magnetic flux density in the area where the animals were housed, was 1 ± 0.1 G. The coils were connected to a Variac fed from the mains (220 V and 50 Hz).

Experimental design

The male rats were divided into four equally groups, namely group I, group II, group III and group IV of seven rats for each. Group III and IV were exposed to electromagnetic field (EMF) consciously for 21 days. Group III was scarified at the end of 21 days of exposure by cervical dislocation. Group IV was kept for 48 days post the end of the exposure period for studying the delayed effects of MF exposure (reversibility or irreversibility). This group was kept away from the MF in similar conditions of control groups. The length of spermatogenesis in male rats is 48 days [15]. The control groups I and II were kept in normal condition but group I was kept in normal day condition and group II was kept in 12 hours Light/12 hours dark cycle during the period of experiment, at constant temperature about 25°C, food and water were available ad libitum. At the end of experiment, all groups were scarified by cervical dislocation and the liver, testis and kidney were separated immediately and small pieces from them were taken, fixed in 10% neutral buffered formalin, dehydrated, cleared and paraffin ionized for paraffin blocks and 5 micron sections were obtained and stained by different histological stains according to Bancroft and Gamble [16].

Results

Histological findings

Liver: The liver from control groups (I, II) showed normal structure of hepatic lobules and portal areas, where, there was no obvious degeneration or necrosis, no congestion, no proliferation of hepatic sinusoids as well as no formation of fibrous intervals (Figures 1A and 1B). The normal microscopic architecture of the liver is composed of hexagonal hepatic lobules. The later, are loosely separated from each other by a very thin connective tissue septa or trabeculae, so the demarcations in between the hepatic lobules are not clear (Figures 1A and 1B). Furthermore, each hepatic lobule is bounded centrally with central vein (Figures 1B and 1C). The major compartment of each hepatic lobule is the hepatocytes that represent about 80% of the hepatic lobule and appeared irregular polygonal or polyhedral shaped cells typically with single, central, large vesicular nucleus however, some binucleated cells are occasionally observed, occupying a central position of the hepatocytes. Hepatocytes are dorsally radiating from the central vein to the portal area forming the hepatic cords (Figures 1C-1E). On the other hand, liver from rats exposed to EMF (group III) showed severe steatosis, diffuse degeneration and necrosis of hepatic tissues (Figures 1F and 1G). Fibrous tissue proliferation with anti-inflammatory cells infiltration in the portal areas. Hexagonal lobules are centered on the central vein have a portal triad containing branches of the portal vein, hepatic artery and bile duct exhibited mild

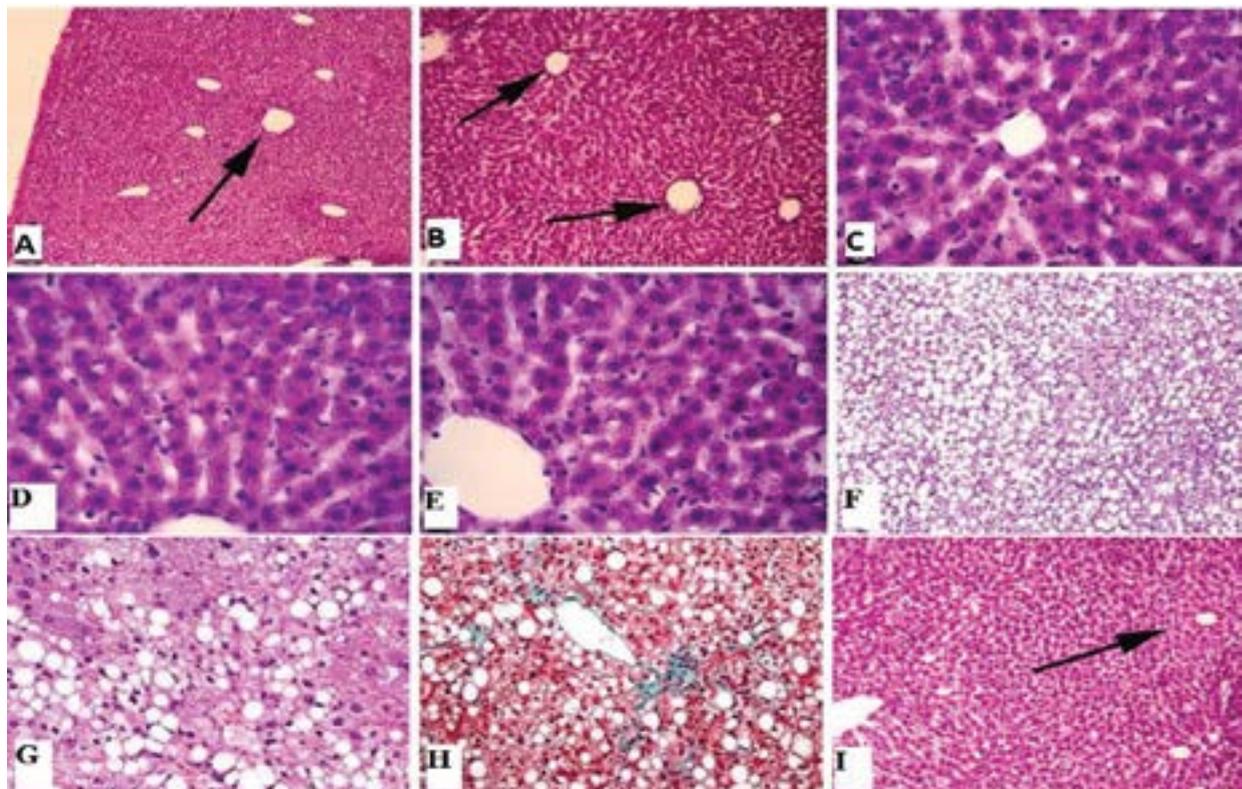


Figure 1: (A, B): Section of mature albino rats' liver of control group (I & II) showing normal, intact hepatic parenchyma; hepatic lobules, portal areas and normal central vein (arrow). A, B) H&E; A) Obj.4X : Oc.10X; B) Obj.10X : Oc.10X. (C-E): Section of mature albino rats' liver of control group showing normal hepatocytes of irregular polygonal shaped cells with single, central, large vesicular nucleus. Normal and regular hepatic cords that were dorsally radiating from the central vein. C, D, E) H&E; C, D, E) Obj.40X : Oc.10X. (F-H): Section of mature albino rats' liver of exposed group to EMF (III) showing severe steatosis, diffuse degeneration, necrosis of hepatic tissues and fibrous tissue proliferation with anti-inflammatory cells infiltration in the portal areas and with moderate disorganization of hepatic cords. F, G) H&E; H) Green Masson's Trichrome; F) Obj.10X : Oc.10X; G) Obj.40X : Oc.10X; H) Obj.40X : Oc.10X. (I): Section of mature albino rats' liver of group (IV) that kept for 48 days post the end of exposure to EMF without exposure showing the normal, intact liver parenchyma (arrow). H&E; I) Obj.10X: Oc.10X.

to moderate congestion of the hepatic artery, sinusoids and Portal vein (Figure 1H). Furthermore, dilatation of the Portal vein and moderate disorganization of hepatic cords were observed. In addition, fusions of the portal triad were recognized (Figure 1H). Regarding group IV, that kept for 48 days post the end of exposure to EMF without exposure, the histological examination of the liver showed normal, intact parenchyma (Figure 1I) as that in the control groups (I and II).

Testes: Sections of the testis of control groups I and II stained by H and E showed a thick layer of fibrous tissue, capsule or (tunica albuginea) which enclosed a number of nearest seminiferous tubules. The latter, are separated by interstitial spaces which comprised interstitial tissue and interstitial Leydig cells (Figure 2C). The seminiferous tubules appeared rounded or oval in shape (Figures 2A and 2B) and are surrounded by a thin basal Lamina (Figure 2E). The stratified germinal epithelium that

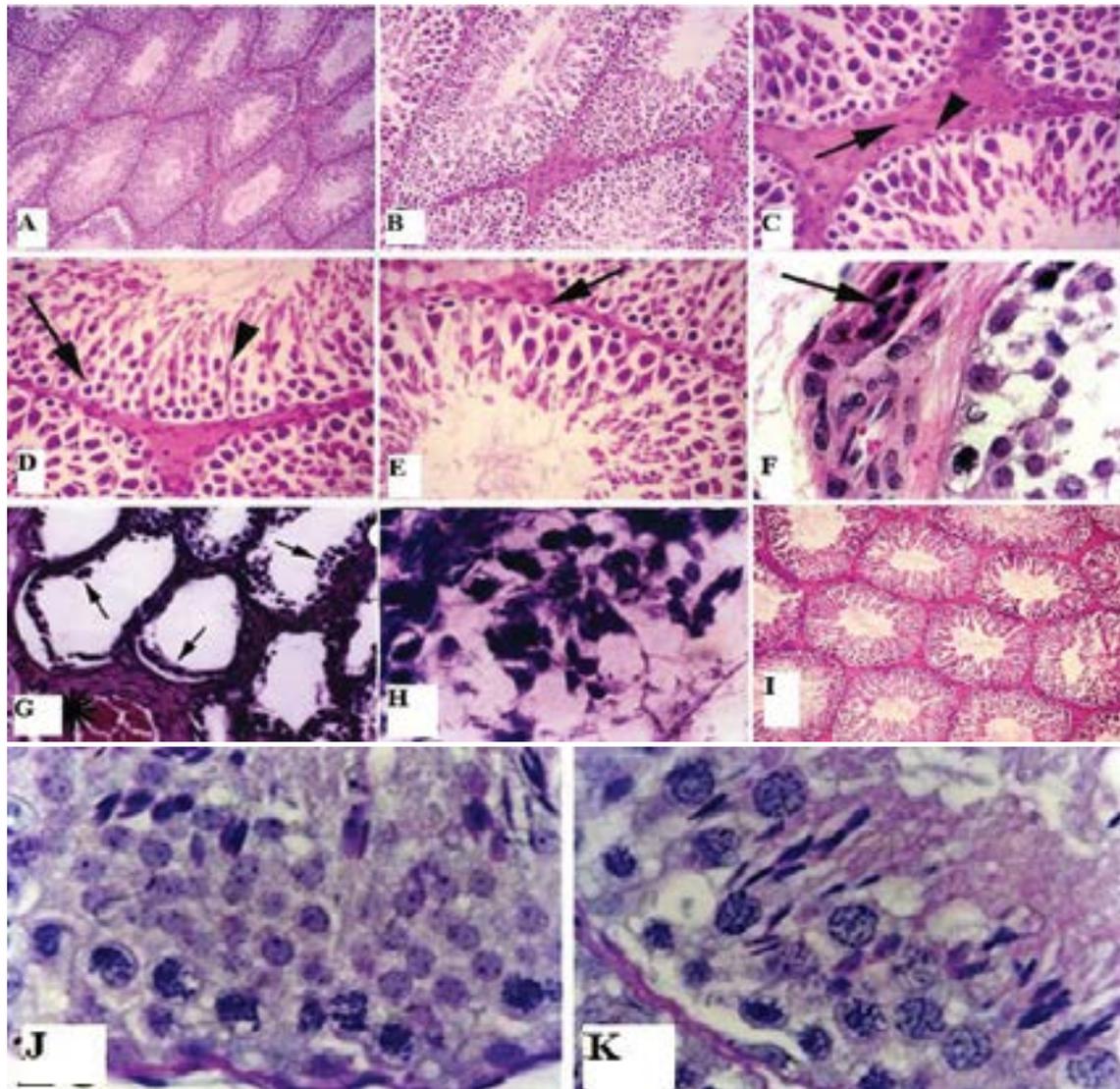


Figure 2: (A): Section of mature albino rats' testis of control group (I and II) showing normal, intact rounded or oval seminiferous tubules. **H&E (Obj.4X : Oc.10X)**. (B): Section of mature albino rats' testis of control group showing that the seminiferous tubules were laterally separated from each other by a considerable amount of interstitial connective tissue. **H&E (Obj.10X: Oc.10X)**. (C): Section of mature albino rats' testis of control group showing normal interstitial connective tissue with ovoid or polygonal leydig cells (arrow) and flat myoid cells (arrow head). **H&E (Obj.40X: Oc.10X)**. (D): Section of mature albino rats' testis of control group showing the normal seminiferous tubules that was lined with stratified seminiferous epithelium; proliferating highly divided numerous spermatogenic cells with non-divided elongated sertoli cells. **H&E (Obj.40X: Oc.10X)**. (E): Section of mature albino rats' testis of control group showing that the stratified seminiferous epithelium rested on a thin basal lamina and is formed of different types of spermatogenic cells which represented the different stages of spermatogenesis. **H&E (Obj.40X: Oc.10X)**. (F): Section of mature albino rats' testis of exposed group to EMF (III) showing proliferation and hyperplasia of the interstitial connective tissue and leydig cells (arrow). **H&E (Obj.40X: Oc.10X)**. (G): Section of mature albino rats' testis of exposed group to EMF (III) showing atrophy in the seminiferous tubules and disorganization of the germinal epithelium with loss of the spermatogenic cells specially spermatocytes and spermatids (arrow). Dilatation of the interstitial blood vessels with marked congestion and engorgement with large number of blood cells (star). **H&E (Obj.10X: Oc.10X)**. (H): Section of mature albino rats' testis of exposed group to EMF (III) showing degeneration and necrosis of the spermatogenic cells. **H&E (Obj.100X: Oc.10X)**. (I-K): Section of mature albino rats' testis of group (IV) that kept for 48 days post the end of exposure to EMF without exposure showing the normal, intact seminiferous tubules **Figure 2I**. And the normal stratified seminiferous lining epithelium **Figure 2 J, K**. (I, J, K) **H&E**; (I) **Obj.10X: Oc.10X**; (J) **Obj.100X: Oc.10X**; (K) **Obj.100X: Oc.10X**.

lined the seminiferous tubules, consists of two distinct populations of cells; the proliferating, highly dividing numerous spermatogenic cells [Figures 2D and 2E] and non-dividing fewer Sertoli cells (Figure 2D). Sertoli cells appeared as elongated cells, with irregular poorly defined outline and oval basal nuclei (Figure 2D). Different types of spermatogenic cells represented the different stages of spermatogenesis (Figures 2D and 2E). The spermatogonia resting on a thin connective tissue, basal lamina and having small and relatively dark nuclei. Spermatocytes appeared as large cells with large oval nuclei. Inner to primary spermatocytes, there were the secondary spermatocytes with their relatively smaller size followed by spermatids and Spermatozoa (Figures 2C-2E). The spermatids were detected at their different steps of spermatogenesis. The cells first appeared rounded with central rounded nuclei (round spermatids) and gradually, they become elongating spermatids that form the spermatozoa with their characteristic shape (Figures 2C-2E). In between the tubules, the interstitial tissue has blood vessels with clusters of cells of ovoid or polygonal shape and spherical nuclei representing the Leydig cell are present (Figure 2C).

Microscopic examination of the testes exposed to EMF (group III) revealed some alterations in both the interstitial tissue and seminiferous tubules that are at variance with those obtained in the control. There was disorganization of the normal appearance of the testes with overall different degrees of atrophy in the seminiferous tubules. At the same time a disorganization of the germinal epithelium, with loss of the spermatogenic cells specially spermatocytes and spermatids and exfoliation of the germ cells are also observed (Figure 2G). In the seminiferous tubular lumen and almost all tubules showed severe atrophy as they were devoid of epithelium, with only Sertoli cells and spermatogonia present within the depleted tubules. Sperms were hardly seen (Figure 2G). The spermatogenic cells showed degeneration and/or necrosis (Figure 2H). Moreover, atrophied seminiferous tubules decrease in the germ cell population and fusion between some seminiferous tubules are observed (Figure 2G).

The interstitial connective tissue and Leydig cells of group (III) showed proliferation (Figure 2F). The blood vessels in such tissue exhibited marked congestion as indicated by their dilation and their engorgement with a large number of blood cells. In addition, haemorrhagic areas in the intertubular tissue are observed (Figure 2G). The Leydig cells and intertubular connective tissue showed signs of hyperplasia and the intertubular space became wider (Figure 2F). But in other groups, the interstitial tissues resembled that of control one.

Regarding group IV, that kept for 48 days post the end of exposure to EMF without exposure, the histological examination of the testes showed normal, intact seminiferous tubules and interstitial connective tissue (Figure 2I), with normal stratified seminiferous lining epithelium; spermatogenic cells (Figures 2J and 2K) as that in the control groups (I and II)

Kidney: The kidney from control groups (I, II) showed normal structure of the renal tissues without any histological abnormalities. Kidney is covered by a thin connective tissue capsule (Figure 3A). The parenchyma of the kidney consists of large number of uriniferous tubules that are consisted of nephron and the collecting tubules. Two regions are found in rat kidney; the outer cortex and the inner medulla (Figure 3A). The renal cortex was observed consisting of numerous renal corpuscles; a thin walled expansion of the proximal end of nephron and deeply invaginated to form double-walled cup-shaped structure; Bowman's capsules. The concavity of the cup is occupied by a tuft of capillaries; glomerulus. Each renal corpuscle has a vascular pole,

where the afferent and efferent arterioles enter and leave, and a urinary pole where the proximal convoluted tubule begins (Figures 3A and 3B).

Bowman's capsules, is a double-walled cup, the internal layer; visceral layer that is made of modified epithelial cells, stellate in shape with several radiating processes that embrace the underlying glomerular capillaries special cells; podocytes that are unique to the kidney. Moreover, the visceral layer envelops the capillaries of the glomerulus while, the external layer; parietal layer is formed of simple squamous epithelium and is surrounded with the proximal and distal convoluted tubules and loop of henle (Figure 3B).

In cross sections, the renal corpuscles were observed surrounding with groups of tubes like structures. Some of them were lined with 3-5 simple cuboidal cells with single central spherical nucleus and acidophilic granular cytoplasm; proximal convoluted tubules. Others were lined with 5-8 simple cuboidal cells with single spherical nucleus located near the lumen and less acidophilic cytoplasm; distal convoluted tubules (Figure 3B). Some of them were lined with simple squamous epithelium; descending limb of henle loop. Others have wide lumen and lined with simple cuboidal cells; connecting tubules (Figure 3C).

Regarding the histological changes in the kidney sections of group (III), we found variable histological changes in glomeruli and some parts of the renal tubules. Such changes exhibited an existence of hyperemia with swelling in the lining epithelium of the glomeruli associated with a peri-glomerular focal area (Figure 3D). Also, they were represented by dramatic renal injury with tubular cell swelling, loss of brush border of proximal convoluted tubules as well as necrotic lesions (Figure 3E). Dilatation and hyperemia in the intertubular cortical blood vessels are seen clearly (Figures 3F and 3G) and the presence of leucocytes infiltration, mainly composed of lymphocytes in interstitial tissues (Figure 3H). In group IV the kidney sections revealed a normal histological structure as in a control group (Figure 3I).

Discussion

The potential of EMF adversely affecting the health of the human population is an issue which continues to receive a great deal of attention in both public and scientific forums. The harmful biological effects of EMF ionizing radiations has been demonstrated on the liver [4,5], gonads [1,6,7], skin and brain [1].

The extent of liver changes has been shown to depend on the duration of the exposure to EMF radiation, where our results clarified that, liver from rats exposed to EMF, group III showed severe steatosis, diffuse degeneration and necrosis of hepatic tissues. We suggest that these changes could be considered as sign of metabolic alterations under the influence of the exposure to the EMF. Repeated exposure to the electromagnetic radiation (EMF) is able to induce hepatic tissue damage. The degree of damage increased with time of exposure to EMF. Previously similar tissue changes have been described using lower frequency EMR [17] in rat. Also liver sections from group IV were the same of that in group I and II and this indicates that the effect of EMF was reversible.

The microscopic examination of the testis in the groups I, II and IV showed that there are no any histological changes and the effect of 1 G EMF on the testis was reversible. Our results revealed that, sections from group III that are subjected to Mf for 21 days, showed some alterations in both the interstitial tissue and seminiferous tubules that are at variance with those changes obtained in the control and other groups. There was disorganization of the normal appearance of the testis with overall different degrees of atrophy in the seminiferous

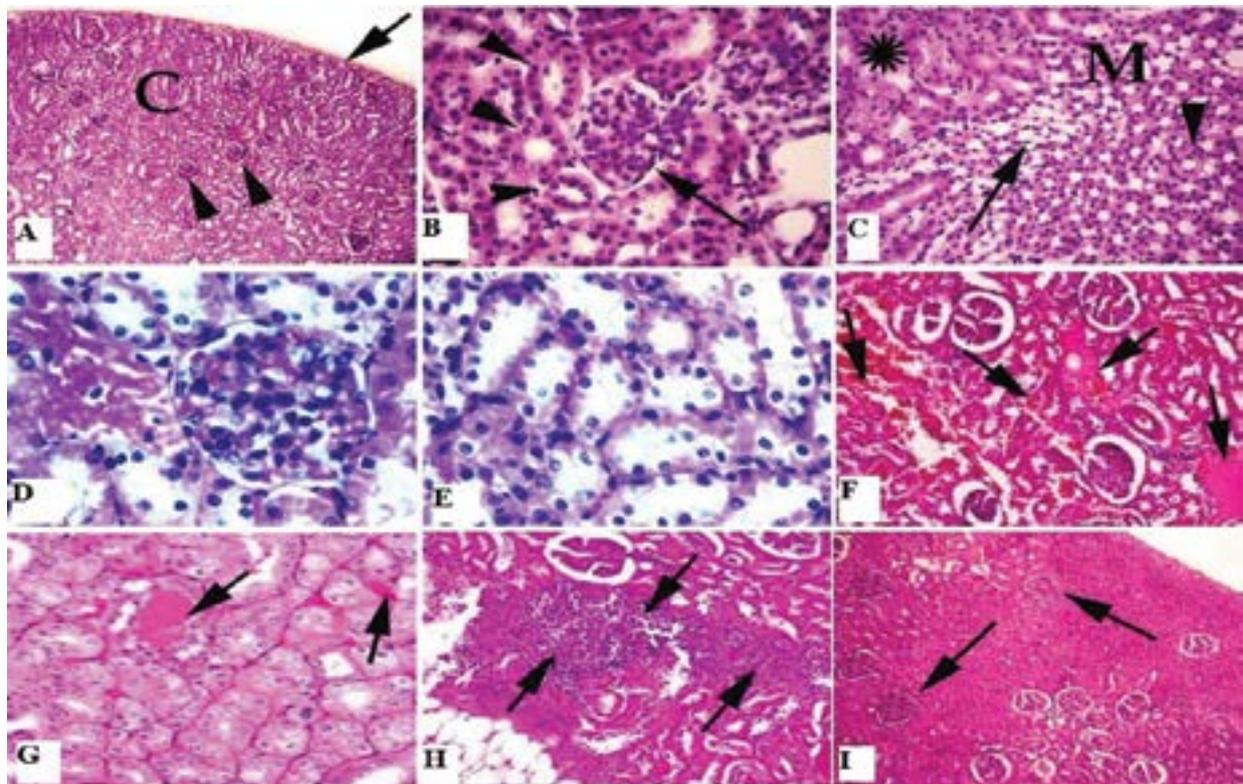


Figure 3: (A): Section of mature albino rats' kidney of control group (I & II) showing normal, intact renal cortex (C), Bowman's capsule (arrow head) and thin connective tissue capsule (arrow). H&E; (Obj.4X: Oc.10X). (B): Section of mature albino rats' kidney of control group showing normal, intact renal corpuscle of glomerulus and bowman's capsule (arrow), and are surrounded with proximal and distal convoluted tubules (arrow head). H&E (Obj.40X: Oc.10X). (C): Section of mature albino rats' kidney of control group showing normal, intact medulla (M) of distal convoluted tubules (star), loop of henel (arrow) and connecting tubule (arrow head). H&E; (Obj.x 10: Oc.10X). (D): Section of mature albino rats' kidney of exposed group to EMF (III) showing hyperemia with swelling in the lining epithelium of the glomeruli associated with a peri-glomerular focal area. H&E; (Obj.40 X: Oc.10X). (E): Section of mature albino rats' kidney of exposed group to EMF (III) showing dramatic renal injury with tubular cell swelling with loss of brush border of convoluted tubules as well as necrosis. H&E; (Obj.40 X: Oc.10X). (F, G): Section of mature albino rats' kidney of exposed group to EMF (III) showing dilatation and hyperemia in the intertubular cortical blood vessels (arrow). 6, 7) H&E (Obj.10X: Oc.10X). (H): Section of mature albino rats' kidney of exposed group to EMF (III) showing leucocytic infiltration that are mainly of lymphocytes (arrow). H&E; Obj.10X: Oc.10X. (I): Section of mature albino rats' kidney of group (IV) that kept for 48 days post the end of exposure to EMF without exposure showing the normal, intact renal corpuscles (arrow). H&E I) Obj.4X: Oc.10X.

tubules, disorganization of the germinal epithelium, loss or exfoliations of the germ cells specially spermatocytes with only sertoli cells and spermatogonia within the depleted tubules. The interstitial connective tissue and Leydig cells of group III showed proliferation. The blood vessels in such tissue exhibited marked congestion as indicated by their dilation and their engorgement with a large number of blood cells. In addition, the Leydig cells and intertubular connective tissue showed signs of hyperplasia and the intertubular space became wider. Such spermatogenesis arrested and disorders in the germinal cells shape are in agreement with the study of Ozguner et al. [10] who showed a decrease in germ cell population in the testis of rats exposed to EMF for 2 hr every day for ten days and Lee et al. [18], Khaki et al. [7] and Aydin et al. [8] in mice who claimed that exposure of male mice to nonionizing radiation for 12 days caused severe histopathological changes in the testis. Ongel et al. [1] and Rajaei et al. [19] in rats clarified that exposure to EMF for long periods induced the same results that were in agreement with our result. It might be suggested that the EMF affect the spermatogonia either directly by induction of apoptosis Lee et al. [18] and/or indirectly by induction of reactive oxygen species (Ros) and free radicals. Our result showed that the Leydig cells were affected by EMF and increased in both number and activities that are

parallel to the elevated testosterone level in the study of McDonald [20] in rat but disagree with result obtained by AL-Akhras et al. [21] in rats.

Regarding the kidney, our investigation clarified that the tissue sections from the control group (I, II) appeared as a normal structure without any abnormalities. On the other hand, sections obtained from group III after its exposure to 1 G EMF for 21 days showed that, histological changes in glomeruli and some parts of the renal tubules. Such changes as an existence of hyperemia, swelling in the lining epithelium of the glomeruli, a focal area of atrophied glomeruli, congested blood vessels, vacuolation, necrosis of the epithelium of the renal tubules and dilatation in the intertubular cortical blood vessels with leukocytes infiltration, of lymphocytes in interstitial tissues. Similar observation were recorded by Ozguner et al. [22], Al-Glaib et al. [11], Ongel et al. [1], Bayazit [23] who mentioned that the exposure to electromagnetic radiation (EMR) induced some atrophied glomeruli, leukocytes infiltration between the kidney tubules, and the vacuolation of some tubules. Furthermore, Oktem et al. [24] and Ozguner et al. [22] reported that renal impairment in animals exposed to mobile phone radiations are due to oxidative stress induced by EMFs and using of melatonin (as an antioxidant) may exhibit a protective effect against this impairment.

Conclusion

The present investigation was concluded that the exposed rat group to 1G electromagnetic field (EMF) consciously for 21 days showed severe histological changes; pathological lesions in liver, testis and kidney. But regarding the group that kept for 48 days post the end of exposure to EMF without exposure, there were no any pathological effects were recognized and the histological examinations were as that in the control groups.

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