ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Biochemical and Histological Effects of Mobile Phone Radiation on Enzymes and Tissues of Mice.

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ABSTRACT

The mobile phone has become one of the most successful inventions of the 20th Century. The effects of 900 MHz Radio frequency radiation (RF) from digital mobile may impose damaging health effects on human. The aim of this work was to study the effect of radiofrequency emitted from mobile phones on some biochemical and histopathological parameters of male white mice. In this study 40 mice were equally divided into control and exposed groups. Experimental groups were exposed to the phone calls per day for one month and 10 times, each time for 10 minutes. The control group received no radiation. Then, at the end of a month, changes in parameters were measured. The present result found significant decreases ($P<0.05$) in the levels of hemoglobin, hematocrit, red blood cells count, in addition to the platelet count after exposure to mobile phone. The most changes in the studied biochemical parameters were significant ($P<0.05$) with the exposure to an electromagnetic field. There was a different pathological damage in the heart, liver, or kidney to mobile phone exposure. The mobile radiation is harmful effects on enzyme activity and tissue. Exposure to electromagnetic fields is responsible for changes in enzyme and can effected on healthy.

Keywords: Wave mobile, Biochemistry enzymes, Histopathology, Mouse

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ISSN: 0975-8585

INTRODUCTION

The mobile phone has greatly changed people's lifestyle and is an integral part of everyday life. With the increased use of mobile phones, their potential effects on human health have become more important [1, 2]. The implications of this technology for human health are often overlooked. Innovations in cell phones may be associated with detrimental effects on the human brain, cardiovascular system, and more specifically male reproduction. Non-ionizing radiation (NIR) is widespread in human environment. The most frequent sources of NIR are mobile phones and cell towers which emit microwave radiation (MWR). They emit radiofrequency electromagnetic waves (RF-EMW), the effects of which have been unknown yet [3, 4].

Mobile phones and related telephony technologies transmit information that is encoded into electromagnetic waves in the microwave range around 900 MHz and 1800 MHz. In order to measure the impact of radio frequency electromagnetic waves on the human body a standardized unit called the SAR value (Specific Absorption Rate) was established. The SAR measures the rate of radiofrequency energy absorption in the body, expressed as watt/Kg. Device specific SAR tests are conducted at the highest power level of the device, in all four frequency bands [4, 5].

Our bodies act as antennas that absorb the radiation and convert it into alternating cross-current. When speaking into a cell phone, the sound wave from the speaker goes through a transmitter that converts the sound into a sine wave. Cell phone radiation may alter normal bodily functions. Many studies have looked at various body tissues reactions to the radiation exposure [6, 7].

In 2008, Baharara studied the effect of electromagnetic fields on mononuclear cells of the liver and spleen of mouse embryo and found significant effects on community and liver Kupffer cells and lymphocytes of the spleen are Megakariosit white polyps. Also, the total number of dividing cells in a significant increase has been observed [8]. However, many works showed that RF-EMR from a mobile phones, Wi-Fi, microwaves or other devices affected negatively, or general health, a number of studies in contrast did not note any abnormalities [7].

Studies showed that electromagnetic waves can increase the concentration of free radicals in chemical that the presences of free radicals that cause problems are uncontrollable directly. According to the mobile communication technology, a new technology, still not well understood long-term biological effects of electromagnetic fields [8].

The aim of this study was to investigate the possible effect of mobile phone microwave radiation on the serum of experimental animals and determination of enzyme activity for harmful effects of long-term exposure.

ANIMALS AND METHODOLOGY

In all of the research ethics of working with laboratory animals have been observed. A total of 40 mature and immature male mice were obtained from animal house of Jundishapur University, Ahvaz. They were divided in two groups, control (n=20) and exposure group (n=20) for the frequency. All animals in control and experimental groups were housed collectively in polycarbonate cages 30×40×40cm (W×L×H) and given ad libitum access to standard laboratory food and water. The housing room was maintained at 22 ± 2°C with 42±5% relative humidity and had 12-h light and 12-h dark cycle (light on 06:00–18:00 h). The experimental group was continually exposed to MWR from mobile phones. The microwave radiation was produced by two mobile test phone (model NOKIA 3110; NOKIA N73 Nokia Mobile Phones Ltd). The mobile telephone was situated in the center of the cage. Conversation mode was set to make waves in the mobile phone and to ensure the radiation on the aluminum sheet was placed over the cages. Experimental groups were exposed the phone calls per day for one month and 10 times, each time for 10 minutes.

Biochemical analysis

At the end of the exposure time of two months, in both control and exposed groups, animal were anesthesia directly into the heart, blood samples were taken in plastic heparinized tubes and serum was separate. Hematology Analyzer (Sysmex, KX-21) for evaluation of Cell blood counter (CBC), included white

blood cell (WBC), red blood cell(RBC), hemoglobin (Hb), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and Platelet count (Plt). The outdoor activities of enzymes and variables, including, Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Alkaline phosphatase (ALP), High density lipoprotein (HDL), creatine phosphokinase (CPK), albumin (Alb), total protein (pro.T), creatinine (Cr), and blood urea nitrogen (BUN) were measured by auto analyzer (Hitachi 912). To measure, acid phosphatase (ACP) and α-Amylase were measured in handling procedure by pars azmoon kit and to measure β-galactosidase (β-Gal) substrate was prepared and was done in a signed protocol.

Histopathological sample preparation

At the end of experiment, liver, heart, kidney and testis tissue were collected for histopathological examination. All tissues were fixed in formalin solution (10%) and dehydration, cleaning paraffin embedding, section and cut with hematoxylin and eosin (H&E) and were studied by light microscope.

Statistical analysis

Data were analyzed using a commercially available statistical software package (SPSS v. 22.0). Results were presented as means ±SD. Statistical significance was determined by the level of $p<0.05$ using the Student's t-test. For nonparametric data Mann- Whitney test was used.

RESULTS

Biochemical analysis

The results of the study changes in biochemical parameter of the mouse upon exposure to electromagnetic radiations (EMR) from live cell phones are presented in **Table 1**.

Table 1: Biochemical parameters and effects of mobile phone MWR in the mice in both groups: Experimental and Control

variable	Exposed mature group	Exposed immature group	Control mature group	Control immature group	P-value Exposed& Control immature	P-value Exposed& Control mature
WBC(×10^9 L)	1.9±0.12	2.1±0.13	1.57±0.09	1.45±0.08	P<0.001	P<0.001
RBC(×10 ^{12}L)	8.2± 0.09	8.2±0.09	8.56±0.13	8.24±0.04	P<0.001	P<0.001
Hb(g/dl)	12.29±0.20	11.29±0.17	12.29±0.17	11.41±0.12	P=0.095	P=0.207
HCT (%)	37.67±0.14	41.54±0.58	39.78±0.15	43.39±0.27	P<0.001	P<0.001
MCV(fl)	45.6±0.17	48.17±0.14	50.16±0.09	52.41±0.11	P<0.001	P<0.001
MCH(pg)	15.12±0.10	12.52±0.32	16.18±0.13	13.80±0.10	P<0.001	P<0.001
MCHC(g/dl)	32.76±0.13	26.62±0.10	33.10±0.08	26.51±0.09	P<0.05	P<0.001
PLT(×10 ^{9}L)	542.7±0.94	921.2±1.13	659.30±2.11	767.9±0.99	P<0.001	P<0.001
ALT(U/l)	52±0.81	60.80±0.78	47.60±1.17	51.00±0.81	P<0.001	P<0.001
AST(U/l)	286.6±1.07	294.20±0.78	265.90±0.73	275.90±0.73	P<0.001	P<0.001
ALP(U/l)	348.8±0.78	296.1±0.7	314.3±0.94	263.5±1.5	P<0.001	P<0.001
ACP(U/l)	9.34±0.60	8.24±0.66	2.7±0.35	2.38±0.26	P<0.001	P<0.001
α-Amylase (U/l)	61.60±0.78	53.59±0.79	83.19±0.85	92.3±0.69	P<0.001	P<0.001
β.Gal(U/l)	19.57±0.89	22.26±1.16	21.83±1.03	24.35±1.14	P<0.001	P<0.001
HDL(mg/dl))	41.14±0.11	43.14±0.11	47.15±0.08	51.13±0.10	P<0.001	P<0.001
CPK(U/l)	2622±0.81	1642±0.84	891.90±284.92	832.3±0.94	P<0.001	P<0.001
Alb(g/dl)	2.7±0.08	2.6±0.09	3.15±0.07	3.07±0.08	P<0.001	P<0.001
Pro.T(g/dl)	4.8±0.07	4.9±0.06	5.54±0.01	5.68±0.0	P<0.001	P<0.001
Cr (mg/dl)	0.18±0.08	0.11±0.07	0.14±0.00	0.11±0.0	P<0.05	P<0.001
BUN(mg/dl)	27±0.08	34.11±0.99	18.11±0.08	26.14±0.10	P<0.001	P<0.001

Data are presented as mean ± SD

White blood cell (WBC), red blood cell(RBC), hemoglobin (Hb), hematocrit (HCT), mean corpuscular volume(MCV), mean corpuscular hemoglobin(MCH), mean corpuscular hemoglobin concentration (MCHC), Platelet count(Plt),Aspartate aminotransferase(AST), Alanine aminotransferase(ALT), Alkaline phosphatase(ALP), acid phosphatase(ACP), β-galactosidase(β-Gal), High-density lipoprotein(HDL),creatine phosphokinase(CPK),albumin(Alb), total protein(pro.T),creatinine(Cr), blood urea nitrogen(BUN).

CBC

The parameters of cell blood counter (CBC) were measured under affection of electromagnets of mobiles. In both groups of mature and immature mice with mobile radiation compared to the control group, the RBC, HCT, MCV, MCH and MCHC were significantly declined (P<0.001). In group with mobile radiation compared to the control group the WBC and PLT were significantly increased (P<0.001). But our result in the Hb had not significantly changes and failed.

Heart indicator

Creatine phosphokinase (CPK) and High-density lipoprotein (HDL) reflects cardiac function were measured in this experiment and significantly were changed (P<0.05). The results showed that HDL and CPK was increased in the group exposed mobile ray compared to control group.

Kidney indicator

The trial also assessed kidney function. Results showed significantly a decrease in Albumin and total protein in exposed of radiation group, but BUN and creatinine increased in this group compared to control group.

Liver enzyme and other parameters

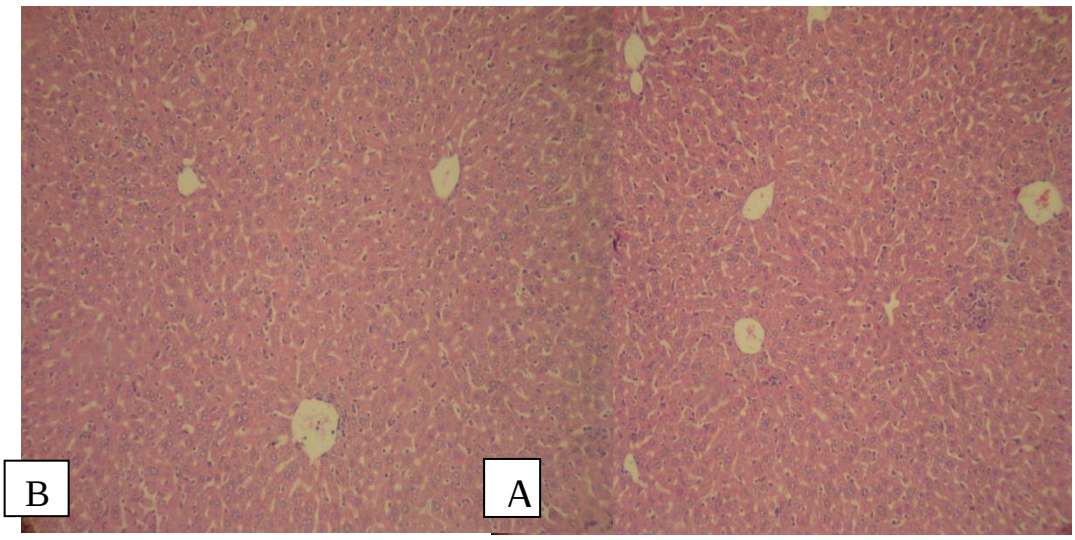
The results of measurements of liver enzyme in the experimental groups changed significantly compared to controls (P < 0.05).

The results show that AST, ALT, ALP and ACP group increased in mobile radiation exposure group compared to control group. While our results show that α –Amylase and β.Gal decreases in the experimental group compared to control group.

Histopathological changes

Liver

The structure of the liver in the control group was normal and histopathological changes were not observed. But, in exposure group we observe structural changes such as: condensation nuclei in some cells, irregular cell arrangement, infiltration of inflammatory cells, swelling and fatty changes of hepatocytes, and granulation of cytoplasm (**Figure1**).



ISSN: 0975-8585

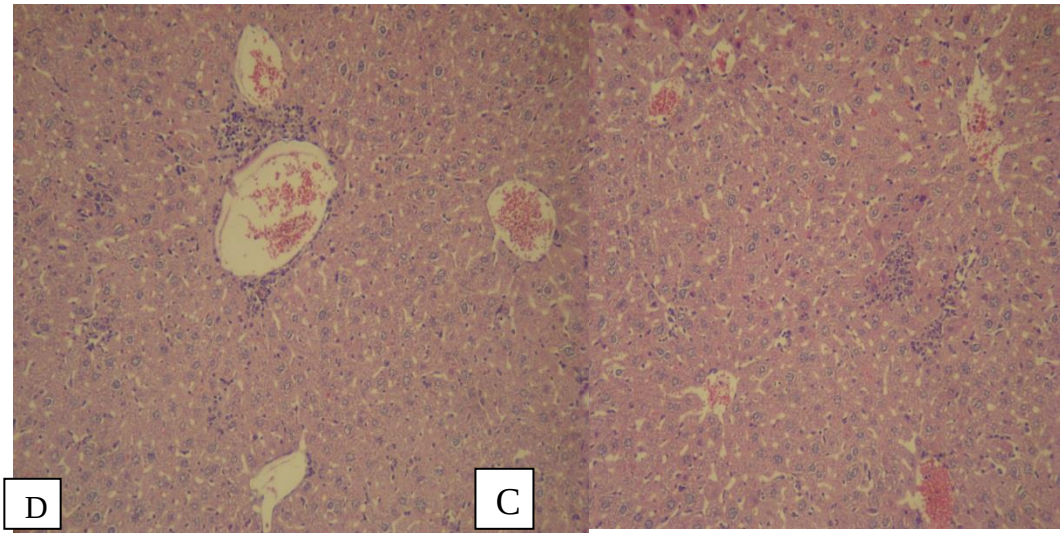
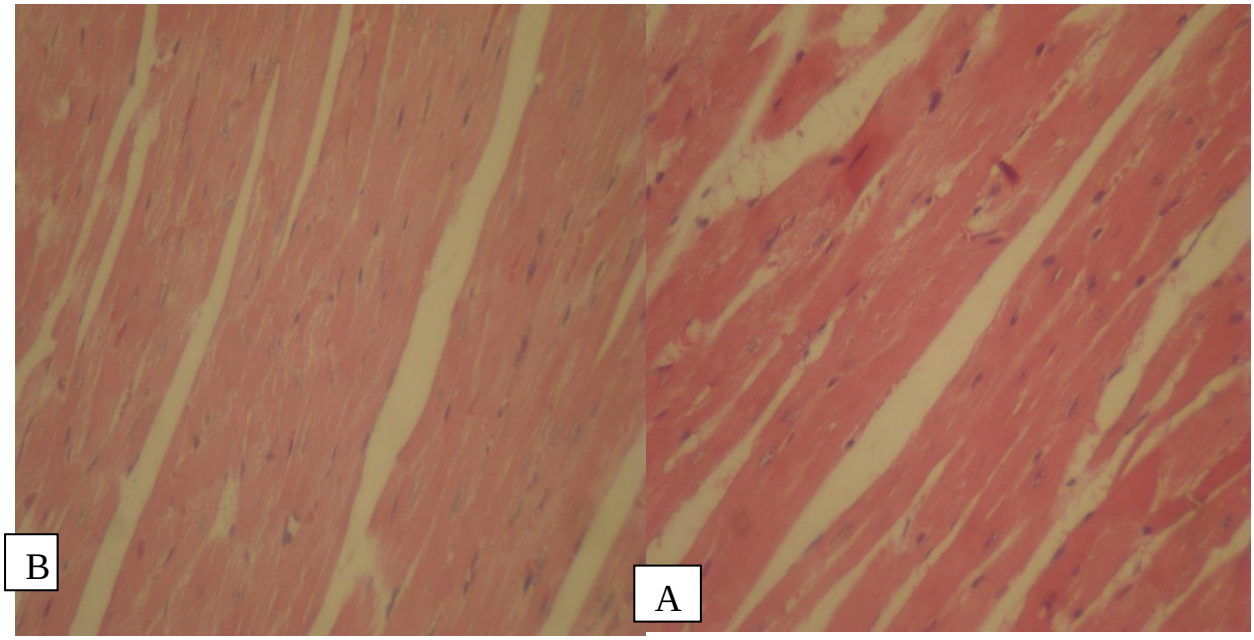


Figure 1: Photomicrograph light of the liver; A) control immature; B) control mature; C) exposed immature; D) and exposed mature (H & E staining)

Heart

Light microscopic examination of cardiac muscle in the control group showed normal muscle fibers with centrally located nuclei on the myositis. The cardiac muscle in the group exposed demonstrated congestion of blood vessels and extravasations of RBCs. In addition, disruption of few fibers and hypertrophy of many of the cardiac myositis with condense nuclei was detected, accompanied by infiltration of inflammatory cells (**Figure2**).



ISSN: 0975-8585

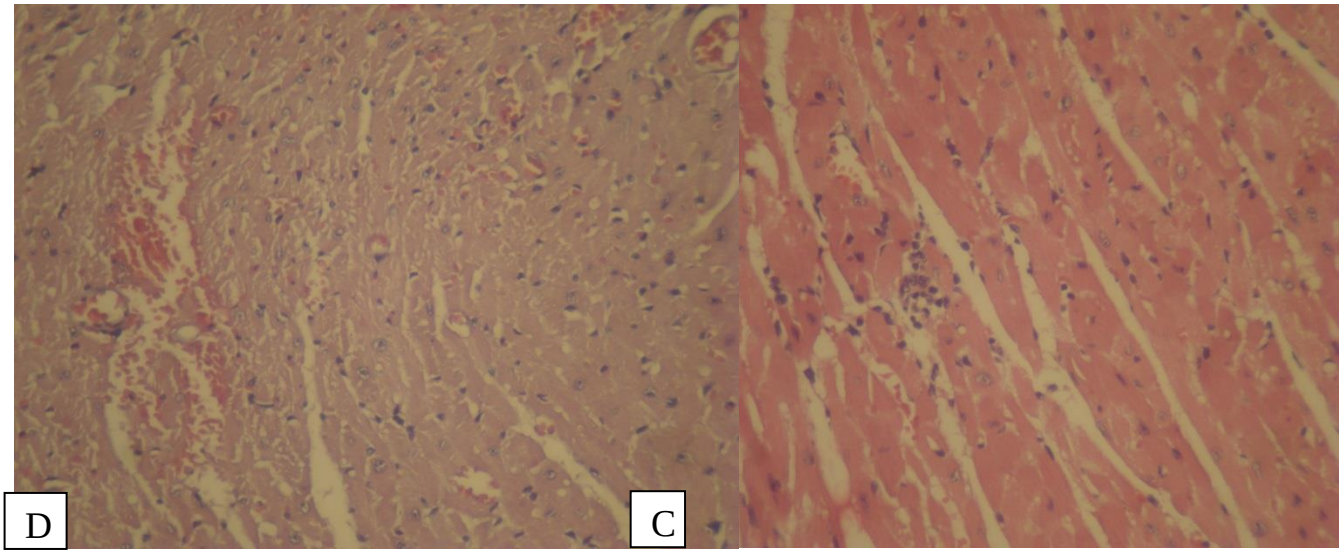
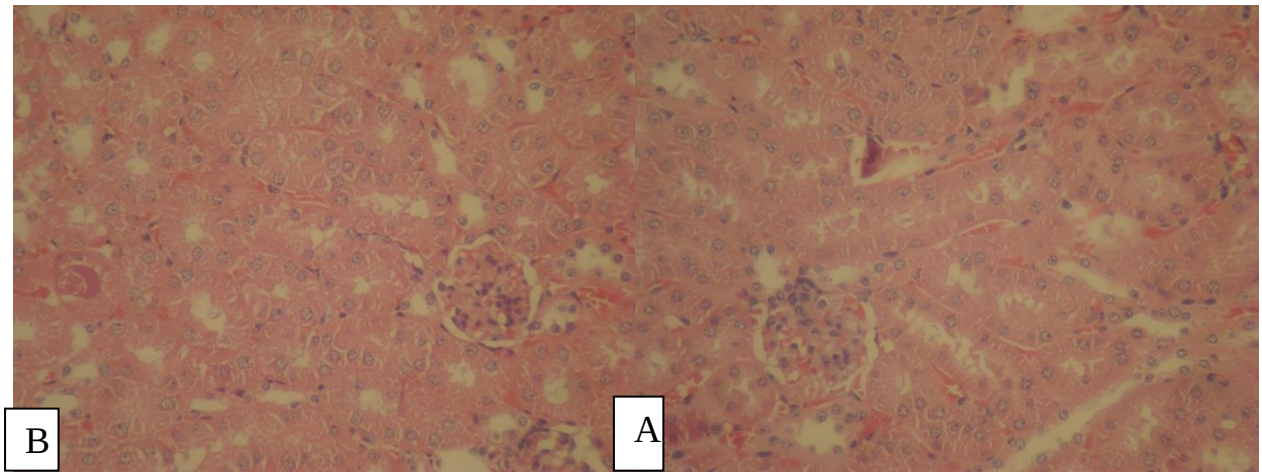


Figure 2: Photomicrograph light of the heart; A) control immature; B) control mature; C) exposed immature; and D) exposed mature (H & E staining)

Kidney

Control group was observed to have a regular morphology of renal parenchyma. But, the kidney tissues showed glomerular damage, dilatation of Bowman's capsule, formation of large spaces between the tubules, interstitial inflammation, hemorrhage, and congestion in the vessels in exposure group. The medullary region comprised of medullary collecting ducts, straight portions of the proximal as well as distal tubules and thin limbs of the loop of Henle with changed histological structure **(Figure3**).



ISSN: 0975-8585

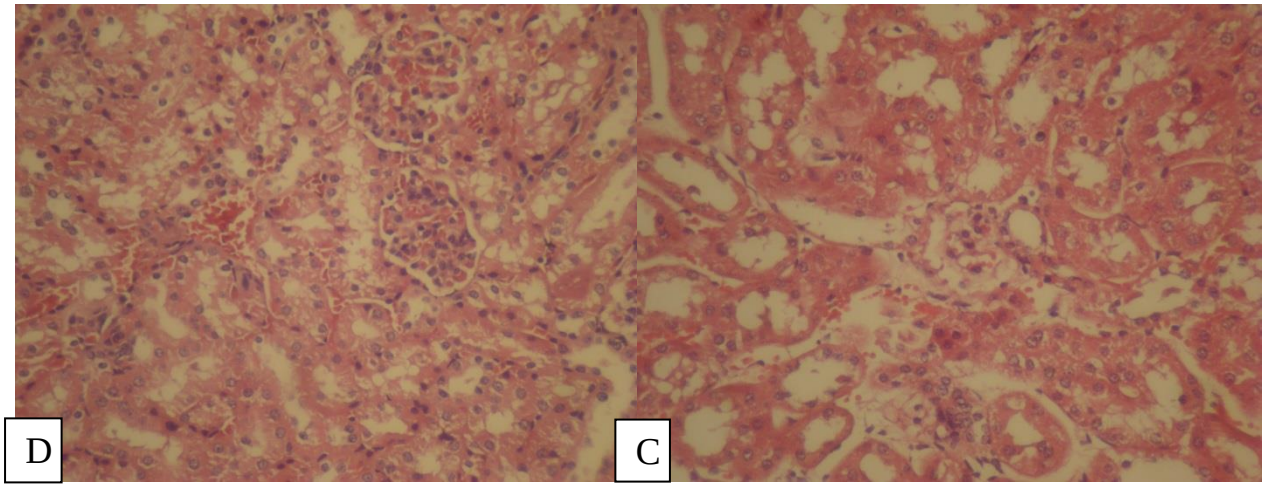


Figure 3: Photomicrograph light of the kidney; A) control immature; B) control mature; C) exposed immature; and D) exposed mature (H & E staining)

Testis

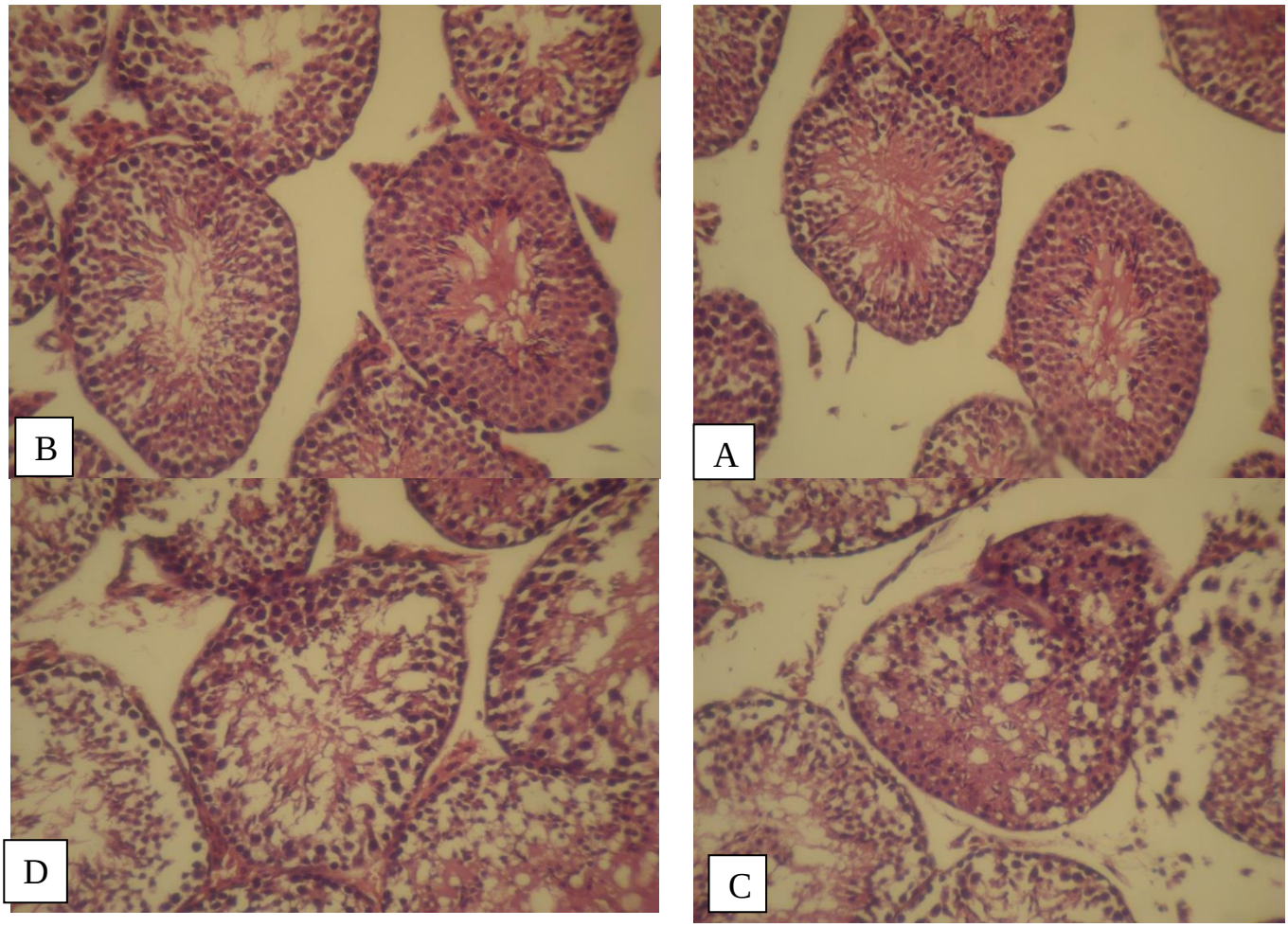


Figure 4: The photo micrograph light of the Testis; A) control immature; B) control mature; C) exposed immature; and D) exposed mature (H & E staining)

The testicular tissue in control group was normal and didn't showed any structural changes. Observation of the testicular parenchyma in exposure group indicated various degenerative changes when compared to control group. The seminiferous epithelium contained many empty spaces that probably indicate

edema in germinal epithelium. Also, thickness of germinal epithelium was decreased due to dislodged cells. The spermatocytes were mostly normal, but some possessed vacuolar cytoplasm (**Figure4**).

DISCUSSION

Assessments of blood parameters are the most important means by which to determine the health situation of experimental animals. In our study there was a change in red blood cells and their contents when exposed to electro- magnetic waves generated by mobile phones. These evaluations are diagnostic for certain diseases such as anemia, leukemia and detect the presence of the inflammation. The decrease in numbers of red blood cells was shown in some diseases as anemia. Mean Corpuscular Hemoglobin (MCH) has medical significance in the diagnosis of some types of anemia. The electromagnetic waves of exposure to human from mobile phones lead to damage and a clear influence on the cell walls, especially the walls of red blood cells and cause an imbalance in blood enzymes [9, 10]. MWR increases cell apoptosis and induces functional disorders in many cell types, which even after time could be approached in cancer treatment [11].

Serum activities of AST, ALT, ALP, and ACP change significantly after the exposure to MWR. It has become clear that NIR similar these from mobile phones are mighty of applying direct effect at cellular and subcellular levels by destabilizing cell membranes and affecting signal transduction pathways. The direct effect of this process is leaking of cytosolic enzymes and energy metabolism disturbances in hepatocytes. Microwave radiation altered the function of the cell membrane with increasing penetrance and ion flux. Significant increase in ALT activity indicates the cytotoxic effect of non-ionizing radiation on hepatocytes inducing apoptosis and necrosis and cell damages [3, 12]. So the changes and increased liver enzymes in the experimental groups are representative of damage to the liver tissue and also can produce free radicals. The waves are also environmental factors that even low intensities it is also stressful due to the side effects can affect the health of organisms [13]. The main effects of electromagnetic fields have increased body temperature. High temperatures and cause damage to internal body organs, vessels, internal bleeding causes. In fact, one of the mechanisms that cause cell damage, which is why hypothermia [13, 14]. Studies have shown that mammalian and human cells that electromagnetic radiation generated free radicals cause oxidative stress and impaired antioxidant system and genotoxic effect [15].

The Enzyme LDH is a present enzyme and has been studied as a general marker of cellular health. In our study, the findings suggest that LDH are increased in exposure group. These observations are in agreement with the reports by Yuan *et al*., who have also seen increased levels of LDH in the serum of volunteers occupationally exposed to very high frequency radiations [16]. These observations indicate that exposure to the radiofrequency radiation increases cell death and changes the homeostasis of the tissue [17]. Peppese*t al*. showed the significant positive correlations between angiographic findings, and peak serum myocardial enzyme levels (CPK, AST, and LDH), and inflammatory biomarkers were identified. They also showed the association between HDL levels and CAD correlation [18]. Therefore, due to our result in this study the effect of radiation increased cardiac enzymes in the exposed group and can be suggested that cardiovascular disease increased in this group.

The present data showed an elevation in the concentration of urea and creatinine of mouse's sera in the exposed group. The mobile phones emitting 900-MHz electromagnetic radiation may be mainly absorbed by kidneys because they are often carried in belts, according to which, may influence the serum creatinine level and urea [19].

In the present study, exposure to EMR led to a marked reduction in total protein and albumin in the exposed group. The decreased level of total protein in mouse to exposure group is also in agreement with the results of Kula *et al*. [20]. The decrease in the level of protein, can be explained due to the impaired synthesis of proteins, mainly albumin in liver cells or malfunction of the absorption process as a result to the exposure of the mouse to the electromagnetic field [21].

Radio frequency electromagnetic radiation from mobile phones induces oxidative stress in rats. The damage to such tissues may have a part in increasing of serum levels of creatinine. There is an association between cell phone radiation and cellular damage, which in turn, may result in increased serum levels of creatinine [22, 23].

In case of function mechanism of electromagnetic fields, it is believed that EMF with high energy waves cause the rise of local temperature where these waves contact together and like ionizing rays lead to the formation of free radicals and create their destructive effects. Free radicals attack lipids, changing in their nature and breaking protein bounds cause cell damage [24, 25].

In the present study, pathological effects of EMF radiation emitted from a mobile phone were evaluated by determining the existence of pathological damages in the heart, liver, and kidney. Our results showed that some pathological damages include inflammation, hemorrhage, and congestion that is in agreement with Sepehrimanesh *et al*.'s study [26]. Some of studied have reported that mobile phone radiation can cause a decrease in serum testosterone concentration and this could be due to the effects of radiation on Leydig cells, the pituitary, or the hypothalamus and an alteration in gonadotropin secretion [24, 27]. In biological systems, the effect of RF-radiation in generation of free radicals, increased lipid peroxidation and tissue damage with vascular congestion that aggregate of blood vessels and extravasation of RBCs in the myocardium, together with the disruption of few cardiac fibers [28]. These findings were suggested to be due to free radical generation with EMF and lead to damage of large cellular molecules such as inflammation, atherosclerosis, and carcinogenesis [29].

CONCLUSION

Our results show that the mobile radiation is harmful effects on enzyme activity and tissue. The negative effect on human health is due to hypothermia and free radical that increase oxidative stress and cause damage to internal body organs.

ACKNOWLEDGMENTS

This research has been conducted with the support of student research committee, with number of 93S25 in Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran. We appreciate the Animal Laboratory and Department of Clinical Biochemistry helped us to conduct the present study.

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