The Effects of Static Magnetic Field on Rat Brain, Lungs, Liver, Pancreas and Blood Electrolytes

Mohammed Farahna*,‡, Mohammed A. Ali Omer*,†, Mohamed E. M. Garalnabi†, Adel Abdella Al-Ganim*, Saida Abdelkareem†, and Yahia M. Bushara*,‡

ABSTRACT

The effects of a static magnetic field of 1.5 Tesla during an exposure time of 0-3 hours was characterized in four groups (E_0, E_1, E_2, and E_3) of rat tissue (brain, lungs, liver, and pancreas) and blood electrolytes (sodium, potassium, and calcium). Before exposure, the average levels for electrolytes were 116.81± 3.67, 5.16 ± 0.28 mmol/l, and 0.23 ± 0.07 mg/dl respectively. Significant reductions (R^2 =0.98, P = 0.05) in sodium and calcium were seen following exposure in a time linear correlation; the reduction was 31.55% and 15.59% respectively. Potassium increased following the exposure time in a linear form; the increase was 47.76% at 3 hours of exposure. While the observed effects in tissues were primarily due to edema (vacuolations) and degeneration in brain tissue, alveolar congestion, emphysema, and hemorrhage were also seen in lung tissue. Atrophy was observed in the liver, and necrosis seen in the pancreas..

Key Words: static magnetic field, brain, lungs, liver, pancreas, blood electrolytes

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1. Introduction

Recently, the exposure to electromagnetic fields (EMF) has increased rapidly as the sources and the applications of such technologies increases. The sources of such EMF include microwave cookers, stations of electricity, electric motors and electronic equipment (Domenico and Sergio, 2004). The excessive exposure to EMF from these sources may be accompanied with considerable neurological degeneration and heart disease (Knave, 2001), although the components of MRI systems (magnetic field, gradient pulsed magnetic field, RF pulses, and electrodes) could induce reversible effects in patients under examination. However, a lack of vigilance or the ignorance of certain basic safety requirements could lead to serious adverse effects, including death (Kerviler *et al.*, 2005).

Also, some studies have reported that high-frequency EMF damages sleep quality (Borbely *et al.*, 1999), may lead to low birth weights (Mortazavi *et al.*, 2013), and to behavioral disorders in children (Divan *et al.*,2008) and young people (Divan *et al.*, 2012), and can cause migraines and headache-related symptoms in children (Sudan *et al.*, 2012).

Corresponding author: Dr. Mohammed Farahna
Address: *Radiologic Technology Department, and Department of Basic Health Sciences, College of Applied Medical Science, Qassim University, Buraidah-KSA. †College of Medical Radiologic Science, Sudan University of Science and Technology, Khartoum-Sudan. ‡Faculty of Medicine and Health sciences, Department of Anatomy, Al-Neelain University, Khartoum-Sudan.
e-mail ✉ farahnamohammed@hotmail.com
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In the study carried out by Odaci *et al.* (2013), they investigated the effect of a 900 megahertz (MHz) electromagnetic field (1 hour daily between days 13 and 21 of pregnancy) applied in the prenatal period on the spinal cord and motor behavior of female rat pups. The histological study on day 32 revealed pathological changes in the spinal cord and a significant increase in the EMF group rat pups' motor functions (p=0.037), which could explain the increase in rat pups' motor activities.

Human lymphocytes were simultaneously exposed to 4.75 T for static component and 0.7 mT for the pulsed component at 500 MHz generated by an NMR apparatus for 1 hour. This exposure increased the Ca^{2+} influx without any proliferative or pro-inflammatory effect on either unstimulated or PHA-stimulated lymphocytes (Aldinucci *et al.,* 2003; Junji, 2006). Also, human skin fibroblast cell morphology was modified with a concomitant decrease in the expression of some sugar residues of glycol-conjugates after a 1 hour exposure to a 0.2 T static magnetic field (Panci *et al.* 2003).

The worst but limited reports attributed to MR scanning, were seven death incidents: one death during examination for cerebral infarction, one involving a ferromagnetic cerebral aneurysm clip, and five related to inadvertent scanning of patients with cardiac pacemakers (Schenck, 2000).

For the sake of avoiding hazards of SMF, the Center for Devices and Radiological Health (CDRH, 1982) suggested that in case of diagnostic magnetic resonance applications exposure to SMF must not exceed 2 T. And the National Radiological Protection Board (NRPB, 1984) recommends that the exposure to SMF (*for MRI patients or volunteers*) should not exceed 2.5 T for the whole or a substantial portion of the body. While the Federal Health Office (FHO, 1984) recommends that patients imaged in MRI should not be exposed to SMF exceeding 2 T, and if the patient is re-exposed to fields higher than 2 T, there should be monitoring for cardiac and circulatory functions.

The current situation of EMF applications is accompanied with potential hazards, especially when the field strength exceeds 2-2.5 T or even within 1.5 T with considerable duration of exposure. Therefore the current study presents results for biological effects due to EMF exposure with 1.5 T and time variation up to 3 T.

2. Experimental section

2.1 Methods

Forty healthy Swiss Albino rats with average weight 187.2 g were kept at a temperature of 20°C and 50% humidity; this was maintained by using a thermometer and hygrometer respectively. Before starting the experiment, the rats were fed on wheat, meat extract, soya extract, folic acid and vitamin A and well water supply by water tap. The rats were divided into four groups: control group and E1, E2 and E3 exposed to 1.5 T for 1, 2 and 3 hours respectively using a MRI system (Philips - super conductive magnet). The serum electrolytes as Na^+, K^+ were measured before and after exposure using an electrolyte analyzer (Roche 9180) and Cromatest Calcium-Methylthymol Blue (Biosystem calcium kit) was used for Ca^{+2} analyses. The tissues of the brain, lungs, spleen and liver were dissected and preserved in formalin after the rats were sacrificed. The dissected tissues were prepared for light microscopy and the histology images obtained were diagnosed by three different veterinary histopathologists. Their results of diagnosing the specimens have been considered with the relative exposure times to SMF, while the diagnoses that disagreed were excluded.

2.2 Sample processed for histology

The dissected tissues were placed in fixative (formalin 10%) immediately after the removal from the rat's body to prevent post mortem changes such as putrefaction and autolysis, to enhance visual differentiation of structure by applications of biological dyes and chemicals, and to preserve cell constituents in a life-like manner with protection from hardening the naturally soft tissue. Then the tissues were dehydrated using isopropyl alcohol, immersed in xylene to remove the alcohol, then saturated with paraffin at melting points of 58°C in a 3 step paraffin bath. Then the saturated solidified tissue was oriented in a block form and set up in the microtome, ensuring that the temperature of the block and microtome's knife were constant. The sections obtained were attached to glass slides using gelatin (5%) as adhesive in the floatation bath. Then the samples were stained using hematoxylin and eosin (H&E stain) and characterized using a microscope with 40X magnification. Then images at areas

of interest were taken and diagnosed by 3 different veterinary histopathologists.

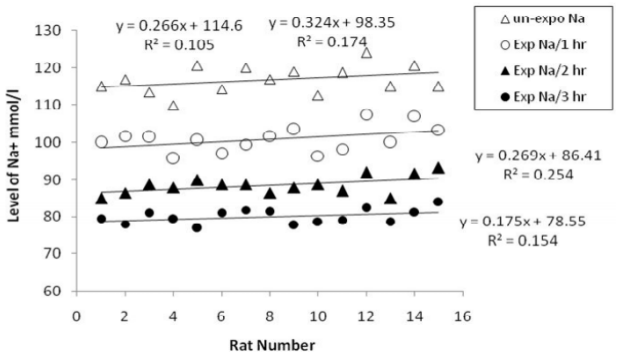

Figure 1. The correlation between rat number and their serum level of Na^{+} before and after exposure to 1.5 T of SMF for 0 – 3 hours.

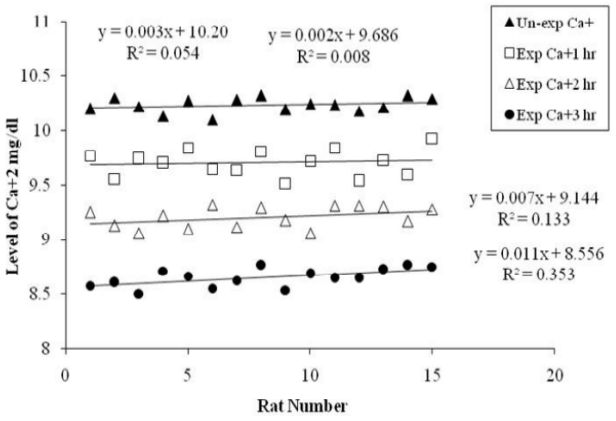

Figure 2. The correlation between rat number and their serum level of Ca^{+2} before and after exposure to 1.5 T of SMF for 0 – 3 hours.

3. Result and discussion

3.1. The results

The correlation between the Na^{+}, Ca^{+2}, and K^{+} electrolyte level and the rat number per experimental group are: ($y = 0.27x + 112.6$, $y = 0.32x + 98.35$, $y = 0.27 + 86.41$ and $y = 0.17x + 78.55$), where x refers to rat number and y refers to level of Na^{+} electrolyte, with a coefficient $R^2 = 0.1$ in average (for Na^{+} - Figure 1). ($y = 0.003x + 10.2$, $y = 0.002x + 9.69$, $y = 0.007x + 9.14$ and $y = 0.01x + 8.56$), where x refers to rat number and y refers to level of Ca^{+2} electrolyte, with a coefficient $R^2 = 0.1$ in average (for Ca^{+2} – Figure 2). ($y = 0.03x + 7.41$, $y = 0.02x + 6.7$, $y = 0.01x + 6.1$ and $y = 0.02x + 4.96$) where x refers to rat number and y refers to level of K^{+} electrolyte, with a coefficient $R^2 = 0.21$ in average (for K^{+} - Figure 3). For the correlation with the time of exposure to SMF of 1.5T for a duration of 0-3 hours, the Na^{+} and Ca^{+2} electrolytes showed a linear reduction following the time of exposure with an equation fitted in $y = -12.29x + 115.01$ (Figure 4) and $y = 0.52x + 10.24$ (Figure 5) where x refers to time of exposure in hours and y refers to Na^{+} and Ca^{+2} electrolytes level in mmol/l and mg/l respectively and the average significant coefficient $R^2 = 1$, $P = 0.05$. However, the K^{+} electrolytes showed an increasing rate based on the equation: $y = 0.81x + 5.25$ (Figure 6) where x refers to time of exposure in hours and y refers to K^{+} electrolytes level in mmol/l with an average significant coefficient $R^2 = 1$, $P = 0.05$.

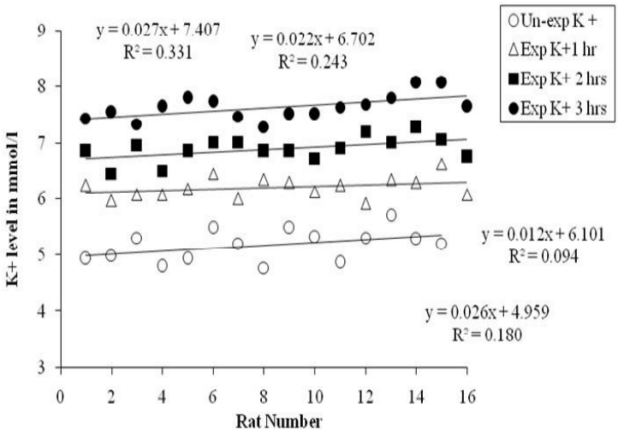

Figure 3. The correlation between rat number and their serum level of K^{+} before and after exposure to 1.5 T of SMF for 0 – 3 hours.

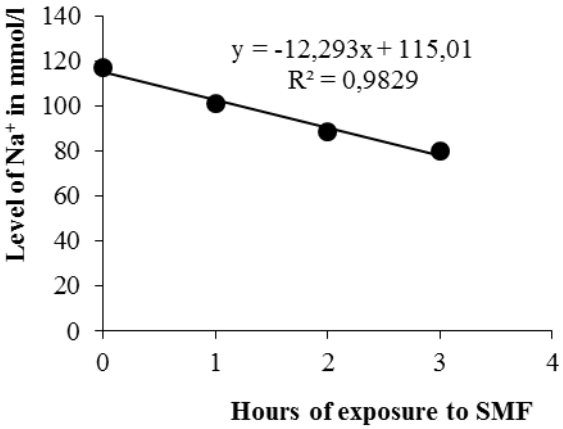

Figure 4. The correlation between the times of exposure to 1.5T SMF and serum level of Na^{+}

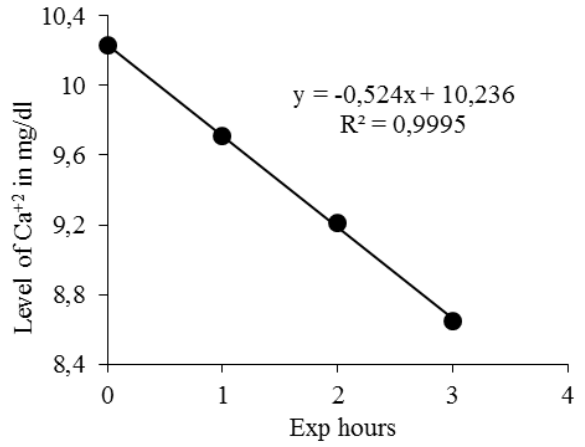

Figure 5. The correlation between the time of exposure to 1.5T SMF and serum level of Ca^{+2}.

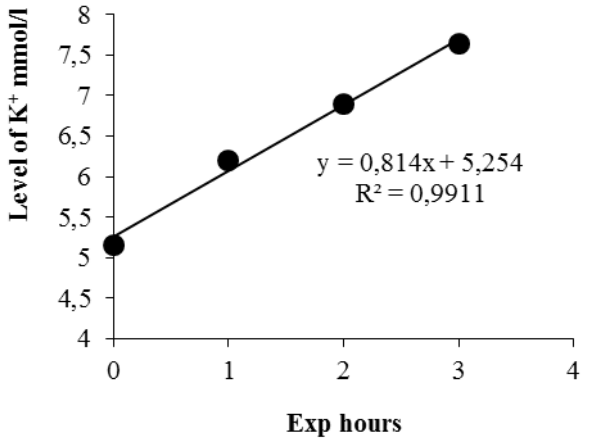

Figure 6. The correlation between the times of exposure to 1.5T SMF and serum level of K^{+}.

Figures 7 and 8 show histological changes induced: in lungs, this appeared as alveolar congestion and emphysema (Figure 7-a), excessive hemorrhage and blood clots in the lung (Figure 7-b), and degenerative brain tissues (cerebrum) with edema (vacuolations) (Figure 7-c). Atrophied hepatocytes (Figure 8-a) and necrotic cells in pancreatic tissues (Figure 8-b) were observed at 3 hours exposure to 1.5 as well.

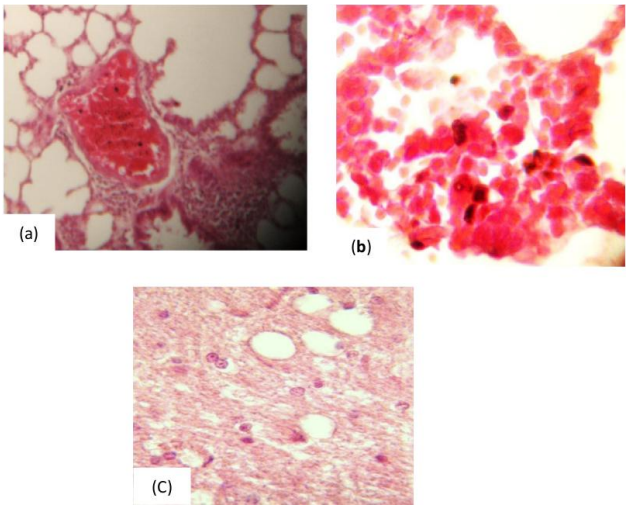

Figure 7. (a) Lung alveolar congestion and emphysema (b) excessive hemorrhage and blood clots in the lung (c) degenerative brain tissues (cerebrum) with edema (vacuolations) observed at 3 hours exposure to 1.5 T.

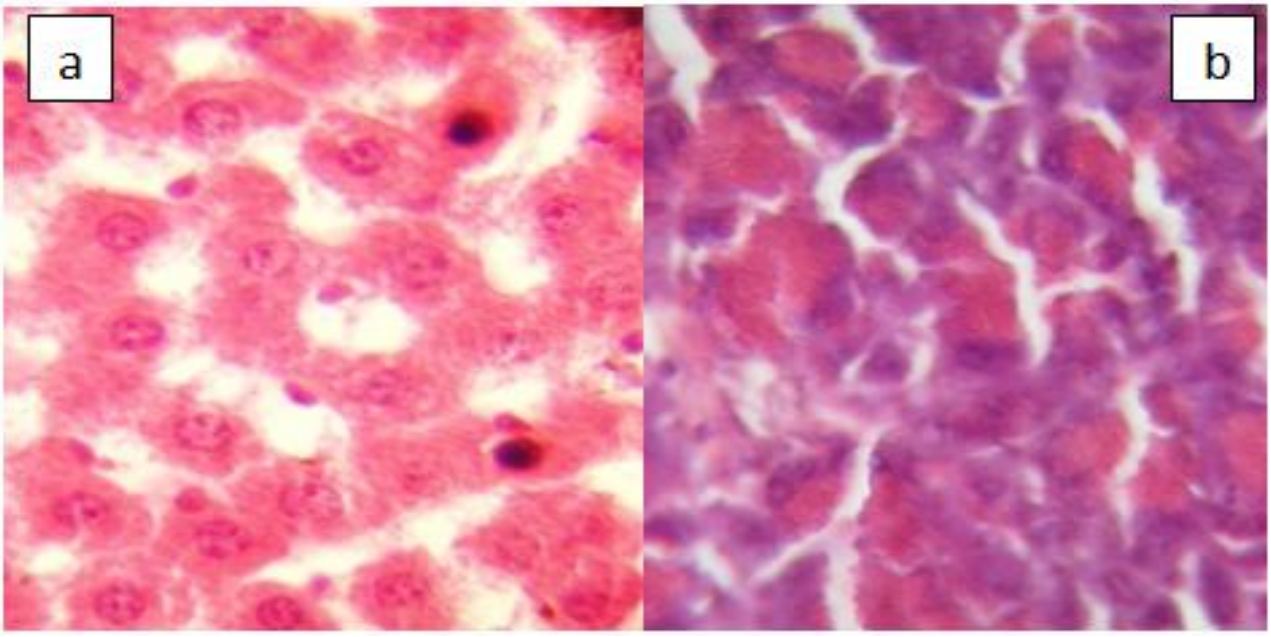

Figure 8. (a) Atrophied hepatocytes and (b) necrotic cells in pancreatic tissues observed at 3 hours exposure to 1.5 T.

3.2 Discussion

The effects of exposure to EMF have reported by several studies in this realm. Leo and Rio (2013) showed desirable effects on okra plants after exposure to EMF such as faster growth, increase in height, weight, sizes and number of fruits per plant as well as less insects and pests, while negative effects were seen in tomato plants. In the medical field, Markov (1994; 2007) stated that EMF generated by power lines and electrical appliances induces the risk of cancer; as an example, the risk of childhood leukemia has been noticed to be associated with exposure to extremely low frequency (ELF). Other confirmed significant effects associated with exposure to SMF with strengths of 1.5 -4 T was shown by Domenico and Sergio (2004); these were the sensations of nausea, vertigo, and metallic taste. The effects of static magnetic fields also have been described by Pacini *et al.,* (1999), in which the exposed human neural cell culture developed branched dendrites with changes in physiological function after 15 minutes of exposure; such branched dendrites increased with magnetic field strength in stimulating media collagen gel (Kangarlu *et al.,* 2004). This result could be utilized to improve repair of transected peripheral nerves by directing and stimulating axonal growth through a tube filled with magnetically aligned collagen gel. The decrease in apoptosis and cell proliferation with an increase in cell necrosis was observed in Vero cells exposed to 0.5 mT SMF, although, in the same conditions, rat astrocytes showed a significant increase in these three parameters (Buemi *et al.,* 2001; Gamboa *et al.,* 2007). Damage to lymphocytes by SMF (7 mT) increased up to 20% when they were treated with ferrous chloride ($FeCl_2$) (Zmyslony *et al.,* 2000; Junji, 2006). Moreover, exposure to SMF could increase the activity, concentration and lifetime of paramagnetic free radicals, leading to oxidative stress, genetic mutation and apoptosis (Zhao, 2011; Dini, 2010), and exposure to SMF initiates an iron-mediated process that increases free radical formation in brain cells, leading to the breaking of DNA strands and cell death (Soumaya *et al.*, 2013).

Although a recent study focused on exposure from mobile radiofrequency RF (Feychting *et al.,* 2005), the trend of this study was the study of static magnetic field effects of MRI in rats' tissues (brain, liver, spleen, pancreas and lung) and the blood electrolytes serum (Na^+, K^+ and Ca^{+2}). It is a specific consideration that the human electrolytes as Na^+, K^+ and Ca^{+2} levels are 145 mmol/l, 4.5 mmol/l and 5 mmol/l respectively, while for rats the Na^+ level was 149 (145-154) mEq/l (male) and 149 (143-154) mEq/l (female), the K^+ level was 6.8 (5.9-7.8) mEq/l (male) and 6.4 (5.5-7.4) mEq/l (female) and Ca^{+2} level was 11.7(10.8-12.7) mg/dl (male) and 11.6 (10.7-12.6) mg/dl (female) (Chatterjea and Rana, 2005). The observed biological effects of SMF could be ascribed to the interaction of magnetic fields with biological tissues which are characterized as electrodynamic effects in electrolyte flows or magneto-mechanical, leading to induction of electrical potential and currents (WHO, 1987). The strength of the static field in MRI has increased from 0.015 to 12 T during the last 25 years, which is about an 800 fold increase. In addition to low and high field systems 1.5 - 4 T, ultra-high field systems with field strengths above 4 T are now available for human MRI (Kangarlu *et al.,* 2004); therefore researchers sense the inevitability of safety procedures to be considered with the introduction of superconducting magnets in MRI which lead to human exposure to SMF up to several Tesla (T) (Silva *et al.,* 2005).

The effects of electromagnetic fields have been a matter of argument for a long time, although some scholars have shown some biological and morphological effects. Consistent with this trend, our experiments proved that exposure to EMF, specifically to 1.5 T for a duration up to 3 hours lead to significant reductions in the level of electrolytes (Na^+ and Ca^{+2}) as hyponatremia and hypocalcaemia respectively. Such decreases in the serum level of Na^+ and Ca^{+2} could be ascribed to alterations in ion-binding to membrane macromolecules (Saunder, 2005; Schenck, 2005), and due to emptying of intracellular Ca^{2+} stores and to Ca^{2+} influx from the extracellular medium (Bian *et al.,* 1997; Ki-Taek *et al.,* 2009), which is a general phenomenon, independent of apoptogenic stimulus.

The decrease of Na^+ and Ca^{+2} in serum after exposure to SMF has been shown by Gerasimova and Nakhil'nitskaia, (1977). They observed an increase in K^+ concentration (hyperkalemia) during an hour exposure and a decrease in Na^+ concentration during a three hour exposure to 4500 Oersted constant magnetic field CMF in rats.

The effect of EMF of K^+ in serum was a significant increasing linear form following the

increment of the exposure hours; such increase could be ascribed to the induced electric fields and currents circulating in the extracellular medium which in turn alters ion-binding to membrane macromolecules, influences ion transport across the membrane, and modifies ligand–receptor interactions at the cell membrane surface (Wilfried and Hannes, 2007). These results are in agreement with the study carried out by Gerasimova and Nakhil'nitskaia (1977).

The surprising histological effects due to EMF exposure, were observed as lung alveolar congestion and emphysema (Figure 7-a), excessive hemorrhage and blood clots in the lung (Figure 7-b, degenerative brain tissues (cerebrum) with edema (vacuolations) (Figure 7-c), atrophied hepatocytes (Figure 8-a) and necrotic cells in pancreatic tissues (Figure (8-b), which were observed at 3 hours exposure to 1.5 T. Such effects in tissue could be ascribed to induced electrical potential and currents by electrodynamics or magneto-mechanics (EHC 1987). Similar effects have been reported by others (Buemi *et al.,* 2001; Gamboa *et al.,* 2007). While Celikozlu *et al.* (2012) observed significant increases in the level of blood glucose, serum protein level, and weekly weight gain decreased, decreased pyramidal neuron numbers and increased ischemic neuron numbers (73%) at the cortex region of brain. And in the same realm, Odaci *et al.* (2013) showed a significant increase in rat pups' motor functions (p=0.037), which was ascribed to pathological changes in the spinal cord; while in a similar study, İkinci *et al.* (2013) showed that the application of a 900 MHz EMF in the prenatal period adversely affected female pups' learning behavior and also resulted in histopathologic changes appearing in the hippocampus. The destructive phenomena in tissues may be ascribed to the creation of free-radicals by EMF exposure, which in turn attacks membrane lipids and changes its nature by breaking protein bonds (Zare *et al.* 2007).

4. Conclusion and outlook

The effects of EMF in living tissues is confirmed without doubt, and the severity of damage is significantly related to the magnetic field strength and the duration of exposure as well as to the type of tissue exposed. In contrast with the current assumptions about EMF effects, this study on biological effects proved the hazards arises due to EMF exposure up specific limits, and since the absorbed energy from EMF sources follows the concept of accumulated dose, further studies related to the dose limits as well as fractionation related to frequency of exposure would be valuable. The results of those kinds of experiments could be better confirmed by Raman spectroscopy and/or stereological studies to avoid subjective characterization that may affect the significance of induced effects. Moreover and since there is biological changes to living tissue, the treatment of certain tumors, behaviors and improving the genetic criteria in plants, vegetables... etc. can be contemplated.

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