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Pathological and immunohistochemical effects of electromagnetic fields on rat liver

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ABSTRACT

The aim of this study was to investigate the possible effects of 50 Hz electromagnetic fields (EMFs) generated by high power lines on the morphology of rat liver and apoptosis. For this purpose, 28 adult male rats weighing 200-250 g were used. The treatment groups were kept in a shelter that were 7.5 m far from the high power lines carrying 170 kV (50 Hz) energy. The control animals (Group 1) were kept in an animal house where no electromagnetic fields were exposed. The animals of the groups 2, 3 and 4 were exposed to EMF (170 kV, 50 Hz) continously for 1, 2 and 3 months, respectively. At the end of the study periods, the animals were sacrificed and their livers were collected. Histopathological evaluation indicated that inflammatory cell infiltration localized in the portal areas was seen in the liver of experimental groups rats. Bax and Bcl-2 positive staining was observed in the liver of control and experimental groups, but both Bax and Bcl-2 staining was intense in Group 3. In conclusion, long- term exposure to EMFs may cause inflammatory changes and apoptosis in liver of rats.

Key words: Bax, Bcl-2, Electromagnetic fields, Liver, Pathology, Rat.

INTRODUCTION

The term electromagnetic fields (EMFs) defines the effect that particles with electric charge create around them. EMFs are created by the generation, transmission, distribution, and end use of electrical energy, and so, EMFs are present everywhere in our environment though invisible (Aldrich and Easterly, 1987; Henshaw, 2002). Low-level electrical and magnetic fields are produced by home appliances, lighting fixtures, TV sets, etc. The dominant frequency is 60 Hz (Hertz, cycles per second), which is the frequency of household fields (Aldrich and Easterly, 1987). Power frequency fields, 60 Hz in the USA, Canada and South America, and 50 Hz in Europe and elsewhere are extremely low in electromagnetic frequency terms. Such fields are described as ELF-EMF. There is essentially no propagation of energy at power frequencies, yet there are induced currents in conducting objects nearby (Aldrich and Easterly, 1987). At 50 Hz, the wavelength of the electromagnetic field is 6000 km. At practical distances from power lines, within what is known as the near field, the electric and magnetic fields are essentially separate entities and should be treated as such. The terms electric and magnetic fields will be used to describe power line fields (WHO, 1987; Henshaw, 2002).

As such, the possible adverse effects of EMFs on healthy have been extensively studied in both experiments involving animals and humans over the past several decades (Ryan *et al.,* 1996; Berg, 1999; Brent, 1999; Lee *et al.*, 2004; Tarantino *et al.,* 2005). Some researchers have suggested there is relationship between exposure to EMFs and reproductive toxic effects (Aldrich and Easterly, 1987; Lin and Lee, 1994; Al-Akhras *et al.,* 2001; Aydin *et al.,* 2009). Due to increasing concern about the potential effects of magnetic fields on health, many studies have been directed toward understanding the effects of low magnetic fields. Epidemiological and laboratory reports suggest that children exposed to EMFs from power lines are at a greater risk of developing leukemia, and that adults exposed to EMFs at work run a higher risk of leukemia and brain cancer (Lin and Lee, 1994; Wartenberg, 2001; Henshaw, 2002).

The animal studies have attempted to determine the effects of exposure to low energy magnetic fields on health; however, the findings have proven to be rather contradictory. Investigations on the effects of the EMFs on the liver are limited (Erpek *et al.,* 2007; Holovska *et al*., 2014; Luo *et al*., 2016; Mortazavi *et al*., 2016). In these studies, some researchers report that EMF causes mild pathological

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changes in the liver such as inflammation, fatty cahanges and necrosis (Holovska *et al.,* 2014; Mortazavi *et al.,* 2016), while some researchers claim that there is no effect on biochemical parameters showing of liver damage (Luo *et al.,* 2016). The aim of present study is to investigate the possible effects of 50 Hz electromagnetic fields on the morphology of the liver and apoptosis.

MATERIALS AND METHODS

Twenty eight adult male rats, weighting 200-250g, were randomly divided into 4 groups of 7 animals of control (Group 1) and 21 animals of experiment group. The rats in the experiment group (n =21) were placed in an experiment cottage (2 x 2 x 1.85m), and 7.5 m away from a 170 kV energy transfer line as reported previously (Aydin *et al.,* 2007), and exposed continuously to 50 Hz field from the electromagnetic field (EMF) for 1 month (Group 2), 2 months (Group 3) and 3 months (Group 4), 7 animals each group. To determine the intensity of magnetic flow and the distribution character, measurements were taken in the cottage where the animals were located at the same height. The animals in the control group (n= 7) were kept under the same conditions without an electromagnetic field. The rats were kept in cages specially designed to minimize field fear (stress). The size of the cages was similar to that of commercially available rat cages, (33 x 13 x 15 cm). The walls of the cages consisted of perspex. Water bottles were mounted outside the cages. The rats had *ad libitum* access to maintenance feed and water. Commercially obtained cork flakes were used as bedding material. The cages were washed once a week. Animals were maintained under standard laboratory conditions on a 12-h light–12-h dark cycle in a temperature-controlled room at 21-22°C. All animals in this study was considered and approved by the Ethical Committee on Animal Experiments of the University of Firat.

Measurements of electric and electromagnetic fields: The ELF-EMFs values were measured in the experiment barn, which had 7.5 meters vertical distance to 170 kV (50 Hz) high frequency lines that were 10 m away from the transformer station (ELF 1.66± 0.01kV/m, EMF 48.21±1.58 mG). ELF-EMFs measurements were taken twice a day for four months, and the average values of power and frequency were calculated. The electromagnetic field was measured by a Gauss meter (hand type gauss meter / F.W.BEL Model / 4080 Frequency Range /25-1000 H Accuracy /<± 2% Measurement Type / True RMS). As the frequency of the present system and the transformer center is 50 Hz, the intensity of the magnetic flow was measured by this device in the 50 Hz caliber. The ELF-EMFs measurements were also taken in the laboratories housing the control group, and the average values were calculated. The average value of the electrical field (ELF) was 0.75±0.05 V/m whereas the value of the electromagnetic field (EMF) was calculated to be 0.48± 0.05 mG.

Histological analysis of the livers: At the end of the experiment, the rats were sacrificed by cervical dislocation under light ether anesthesia. The livers were fixed in 10% buffered formalin, embedded in paraffin, and then sectioned at 5 µm and stained with hematoxylin-eosin, and evaluated by light microscopy. In addition, immunohistochemical staining of all cross sections was performed using the avidin-biotin-complex (ABC; DAKO, Carpinteria, CA, USA) method with Bax and Bcl-2 antibodies.

RESULTS AND DISCUSSION

Livers of rats in the control group had normal histology; hepatic lobules with a central vein at its center and portal areas at its peripheral corners were located connective tissue. Hepatocytes formed trabecules by extending from central veins in the center of lobules to peripheral corners in irregular radial cords, each of which were separated by sinusoids containing Kupffer cells. In the experimental groups, only inflammation was seen in the liver as histopathological changes, and the severe of inflammation varied due to the exposed time. While, in Group 2 and 3, the inflammation was mild, in Group 4 was moderate. Inflammation including macrophages and lymphocytes was localized at portal areas of the liver. Immunohistochemical evaluation indicated that Bax and Bcl-2 positive staining was observed in the liver of experimental and control group rats, but the density of the staining was changed depending on exposed time and groups. Bax positive staining was intensive in Group 3 (Fig-1), followed by Groups 2 and 4. Bcl-2 overexpression was seen in Group 3 like Bax- positive staining (Fig-2), but followed by Groups 4 and 2. Immuon positive staining was localized in cytoplasm of the hepatocytes.

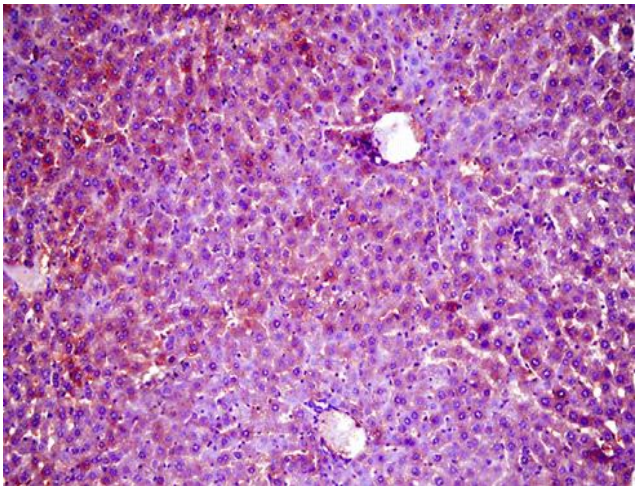

Fig-1: Photomicrograph, liver of a rat in Group 3 exposed to EMFs for 2 months. Bax – positive staining in cytoplasm of the hepatocytes (red). Immunohistochemical staining, ABC method, 3-amino-9-ethylcarbazole chromogen with Mayer hematoxylin counterstain X20.

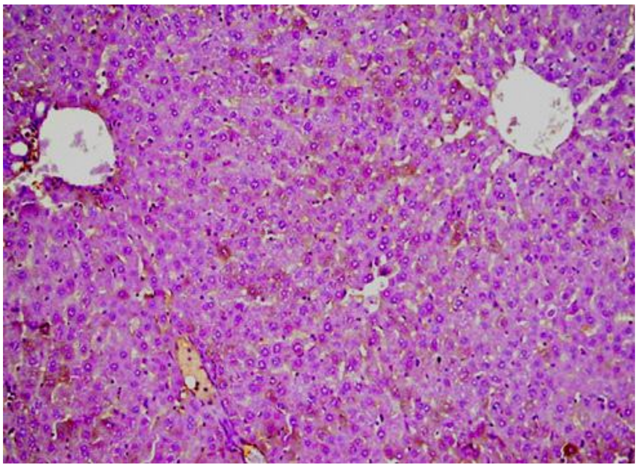

Fig-2: Photomicrograph, liver of a rat in Group 3 exposed to EMFs for 2 months. Bcl-2 – positive staining in cytoplasm of the hepatocytes (red). Immunohistochemical staining, ABC method, 3-amino-9-ethylcarbazole chromogen with Mayer hematoxylin counterstain X20.

The effects of EMA on human and laboratory animals are of interest to many researchers. The studies investigating effects of electromagnetic fields on health have been conducted by using experimentally induced electromagnetic field (Lee *et al.,* 2006; Aydin *et al.,* 2007). But it is well known that high voltage lines surrounding us like a network have hazardous effects on health and these effects are known to occur in a long time (Lin and Lee, 1994; Lee *et al.,* 2006; Aydin *et al.,* 2009). Increased incidences of leukemia in children and brain cancer in adults living under the influence of electromagnetic fields have been evaluated (Lin and Lee, 1994; Henshaw, 2002). However, difference of frequency, amplitude and exposure time of EMFs results in distinct outcomes in repeated investigations. So it is difficult to compare researches with each other. Thus, different results have been reported regarding changes in the liver of laboratory animals exposed to EMF. Some researchers reported that EMF generated by 50 Hz electric current did not cause significant microscopic findings in rat liver (Margonato *et al.*, 1995; Boorman *et al.,* 1997; Zecca *et al.,* 1998; Mortazavi *et al.,* 2016), while others reported that histopathological changes such as sinusoidal dilation, vacuolar degeneration, necrosis and inflammatory cell infiltration were seen in the liver (Gocimen *et al.,* 2002; Yeniterzi *et al*., 2002; Erpek *et al.,* 2007; Luo *et al.,* 2017). In this study, only mononuclear cell infiltrations were observed in the liver of EMFs-exposed rats, and inflammatory cell infiltration was observed to increase with the duration of exposure.

The mechanism by which EMFs cause damage to tissues and cells is unknown. However, it has been reported that EMFs cause damage by altering the electrical structure of cell membrane proteins and by increasing free radicals (Tsong, 1992; Seyhan and Canseven, 2006). Besides, in another study, researchers reported that free oxygen radicals induced by EMF in liver caused hepatic injury, and lipid peroxidation of plasma and organelle membranes by cytochrome P-450 enzyme system (Koyu *et al.*, 2009). In the present study, Bax and Bcl-2 overexpression was seen in the experimental groups compared to control group. Bax is a pro-apoptotic marker, and acts apoptotic inducer (Oltavi *et al.,* 1993). Bcl-2 is an anti-apoptotic marker, and involved in the manipulation of cell survival outcomes after cytotoxic stress (Oltavi *et al.,* 1993; Basu and Haldar, 1998). Cell loss and cell division processes are homeostatically balanced to allow adaptation to changing conditions, and also to generate and maintain the complex dynamic architecture of tissues (Oltavi *et al.,* 1993; Basu and Haldar, 1998). It has been reported that overexpression of the pro- and anti-apoptotic markers can be an important factor for regulation of apoptosis (Basu and Haldar, 1998).

In conclusion, the findings of this study indicate that continuous and long- term exposure to 50 Hz EMFs may cause inflammatory cell infiltration in the liver of rats. In addition, EMFs may give rise to initiate of apoptosis in hepatocytes.

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