JOURNAL OF CURRENT RESEARCH IN SCIENCE (ISSN 2322-5009)
CODEN (USA): JCRSDJ
2013, Vol. 1, No. 6, pp:601-604
Available at www.jcrs010.com

ORIGINAL ARTICLE

THE EFFECTS EMF ON LIVER OF RATS: A HISTOPATHOLOGICAL STUDY

Shahram Gharachorlou[1], Amirali Ebrahimneghazd[2], Hamid Mirzaie[3]
1- Assistant Professor, Department Of Pathobiology, Faculty Of Veterinary Medicine, Tabriz Branch, Islamic Azad University, Tabriz, Iran
2- Associate Professor, Department Of Anatomy, Faculty Of Medical Science, Tabriz Branch, Islamic Azad University, Tabriz, Iran
3- Associate Professor, Department Of Food Hygiene, Faculty Of Veterinary Medicine, Tabriz Branch, Islamic Azad University, Tabriz, Iran

ABSTRACT: Modern life conditions resulted in human permanent exposure to electromagnetic fields. Equipment such as printers, monitors, cell phone, television and so having an important role in human life are regarded as the generative sources of electromagnetic fields. Epidemiologic and laboratory studies on animals have demonstrated ill effects of electromagnetic fields on biologic systems. Previous studies have been done by means of light microscope in this regard; demonstrated ill effects of the field on morphologic changes in heart, vessels and prostate gland. Study Of effects of electromagnetic fields on liver tissue, from light microscope viewpoint is the purpose of this study. 30 female rats of wistar race with the weight of 150-200 gram were selected as laboratory model at this study and divided to two experiment and control group. The experiment group was exposed to electromagnetic field with 1mT intensity and frequently and of 50Hz eight hours each day for two month. After termination of this period, the experiment and control group animals were killed and samples of liver tissue were prepared to be studied by light microscope. Results of microscopic observations with Heamatoxylin-eosin staining in comparison with Trichrome staining made it clear that liver hepatocyte cells suffer from coagulation necrosis and nuclear and cytoplasm of hepatocyte suffer from vacuolization. Some cases of fatty liver were also observed. Even fibrosis of vessels was along with necrosis and apoptosis of vessels' endothelium cells and hepatocyte cells and kupffer cells and hemorrhage. Penetration of collagen fibers was observed in Trichrome staining in some cases. Dilatation of liver' central sinusoids and multiple presence of kupffer cells was seen as phagocytizer of necrosis hepatocyte. Also, penetration of mononuclear inflammation cells, increase of fibroblasts and wide necrosis of liver cells replaced by connective tissue were observed. Based on data obtained from our study it concludes that exposure to electromagnetic waves damage liver tissue which induce apoptosis in hepatocytes.

KEYWORDS: Electromagnetic field, liver, apoptosis, coagulation necrosis, fibrosis.

INTRODUCTION

Electromagnetic fields have been employed as useful tools in medical diagnosis. Recently, the use of electromagnetic fields has been expanded to therapeutic purposes because their interactions with living matter produce effects that initiate, accelerate or inhibit biological processes. Frequencies below 300 Hz are known as extremely low frequency electromagnetic fields (ELF-EMF) and do not have enough energy to break molecular bonds; for example, they do not cause direct damage to DNA (Repacholi and Greenebaum, 1999). Additionally, ELF-EMF are non-invasive and non-ionizing and even have non-thermal effects on cells and tissues. These properties have led to studies of the influence of ELF-EMF on the development of various diseases, including liver cell apoptosis. While some researchers associate ELF-EMF exposure with carcinogenesis (Girgert *et al.,* 2005; Chen *et al.,* 2000), other studies of experimental models and human cancers have shown that ELF-EMF do not increase the risk of several cancer types, including liver cancer, and that treatment with tumor-specific frequencies is feasible and well tolerated and may have biological efficacy in patients with advanced tumors (Barbault *et al.,* 2009; Galloni and Marino, 2000; Yasui *et al.,* 1997). Moreover, the exposure of female C3H/HeJ mice bearing mammary adenocarcinoma to a frequency of 120 Hz at intensities of 4 and 5 mT resulted in a significant reduction in the growth of the tumors, which is a phenomenon associated with angiogenesis

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Corresponding Author: Shahram Gharachorlou, Assistant Professor, Department Of Pathobiology, Faculty Of Veterinary Medicine, Tabriz Branch, Islamic Azad University, Tabriz, Iran. **E-mail:** dr.ali.gh1531@gmail.com

inhibition (Williams *et al.*, 2001). The exposure of female athymic nude mice with human breast cancer xenografts to a frequency of 120 Hz with an intensity of 15 mT, either alone or in combination with gamma radiation, resulted in decreased growth or reduced vascularization of the tumors (Cameron *et al.*, 2005). Similarly, the effect of 50 Hz at 0.5 μT and 0.5 mT on the development of chemically induced foci in rat livers showed a slight inhibition of their formation (Rannug *et al.*, 1993). This evidence that ELF-EMF inhibit carcinogenesis is not convincing, and the exact molecular mechanisms that account for its effects must be validated. The purpose of this study was to use the modified resistant hepatocyte model (MRHM), which induces a rapid proliferation of altered hepatocytes to form preneoplastic lesions in the rat liver (Carrasco-Legleu *et al.*, 2004), as a reliable model to seek information concerning to the effects of ELF-EMF on hepatocarcinogenesis. The aim of present study was to evaluate the effect of ELF-EMF on induction of apoptosis in liver of rats.

MATERIALS AND METHODS

50 male and 50 female mice were selected for the study. The generator apparatus of EMF was Helmholtz coil which generates frequency of 50Hz and severity of 1mT (Bancroft and Gamble, 2002). The voltage and amplitude were controlling by a voltmeter.

Mice were kept in cages by chance in order to mate as monogamous method. Detection of pregnancy was made by observing the vaginal plugging. Of 50 pregnant mice, 25 of them were selected by chance in order to exposure and 25 of them were selected as normal control (Bayazit, 2009; Borbely *et al.*, 1999).

Pregnancy period of animals was 3 weeks then 30 of neonates from each group were selected. In experimental group, animals were kept under EMF condition 5 weeks more (Bracken *et al.*, 1995). In the late of weeks 5, 15 of them from each group selected for sampling. The animals of different groups were sacrificed under light anesthesia (diethyl ether) 1 day after the end of the treatment. The liver samples fixed in a 10% neutral-buffered formalin solution were embedded in paraffin and were used for histopathological examination. Five micrometer-thick sections were cut, deparaffinized, hydrated, and stained with hematoxylin-eosin. A minimum of 10 fields for each slide were examined (Dana *et al.*, 2005). For study of apoptosis, TUNEL staining was made on samples (Freeman and Crapo, 1982; Hanafy *et al.*, 2010).

2.1. TUNEL staining

TUNEL staining was done based on following steps:

1- Preparing of sections with 4μ thickness.
2- Deparaffining of sections using pure xylenol
3- Putting the sections in the microwave with a power of 700W for 10 minutes.
4- Incubation of samples in the phosphate buffer containing H2O2 for 10 minutes.
5- Incubation at 37°C for 60 minutes.
6- Washing the sections 3 times in the PBS.
7- Incubation in Antiflourescein-pad for 30 minutes.
8- Washing the sections 3 times in the PBS.
9- Applying the sections with Diamin benzidine (DAB).
10- Differentiate staining with hematoxylin method.

RESULTS

Hepatocytes, bile ducts and Rymak strands are seen normal in control group (Figure 1).

Also, TUNEL staining showed a normal structure of hepatocytes, ducts and sinusoids the nucleus of cells are normal and there is a clear membrane between the cells and vessels have normal endothelial cells (Figure 2).

The slides which were stained with hematoxylin method showed wide pathological changes in the liver structure. Pyknotic nucleus with necrosis of endothelial cells and dissociation in along were obvious and it seen without membranous in association with nearby cells which indicates early apoptosis (Figure 3).

In TUNEL staining it has been seen that endothelial cells and endothelial membrane thinning and multiple dissociation along the endothelial cells lining blood vessels and sinusoids and the space below the cut-off membrane and endothelial lining that caused by endothelial cells and even atrophy of hepatocytes and necrosis in the liver is associated with extensive necrosis of hepatocytes in the liver (Figures 4, 5).

Apoptotic cells in the liver were observed in the control group and hepatocytes, Kupffer and endothelial cells are seen with brown nuclei.

In general, there was a wide necrosis in the liver which has been observed in TUNEL Slides and based on Fisher T-test results there is a significant difference statistically ($P<0.01$).

The presence of fibrous connective tissue and replacing the connective tissue with necrotic cells indicate irregular structure. Rymak strands, sinusoids and blood vessels are visible that are accompanied with hypoplasia associated with apoptosis and coagulation necrosis.

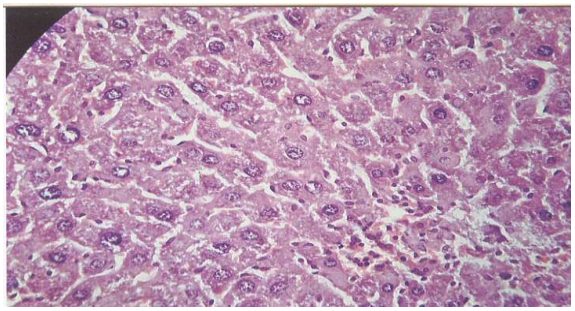

Figure 1: microscopic view of control group with normal hepatocytes, ducts and Kupffer cells (H&E, 400x).

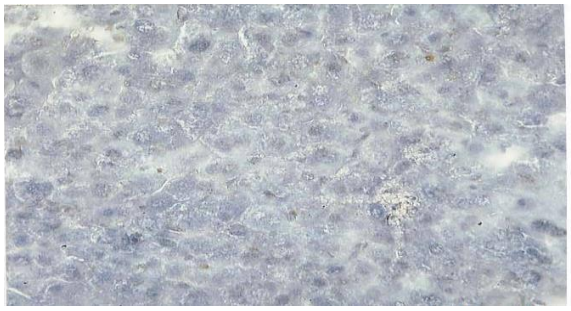

Figure 2: microscopic view of control group with normal hepatocytes, ducts and Kupffer cells (TUNEL, 400x).

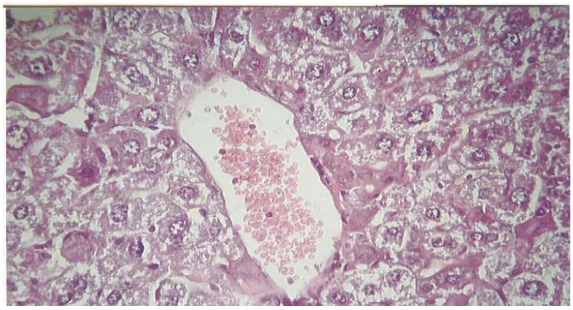

Figure 3: microscopic view of experimental group. Pyknotic nucleus with necrosis of endothelial cells and dissociation in along were obvious and it seen without membranous in association with nearby cells which indicates early apoptosis (H&E, 400x).

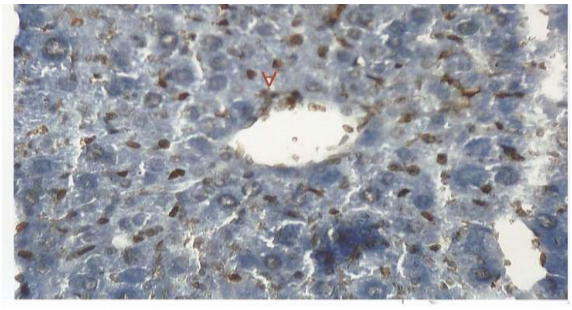

Figure 4: microscopic view of experimental group. Pyknotic nucleus with necrosis of endothelial cells and apoptosis are seen (TUNEL, 400x).

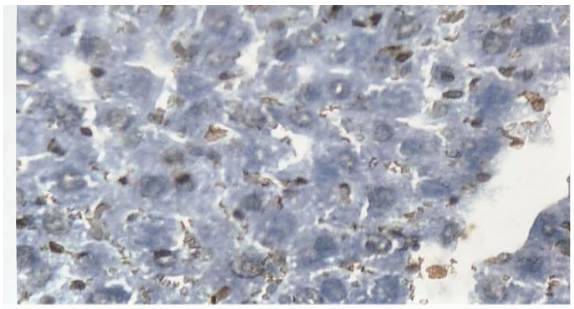

Figure 5: Microscopic view of experimental group. Apoptotic cells in the liver are seen, Kupffer and endothelial cells are seen with brown nuclei (TUNEL, 400x).

DISCUSSION AND CONCLUSION

Our data showed that EMF not only cause coagulation necrosis and apoptosis in hepatocytes also results in mild inductor signs in the cells that may cause affect the cell cycle and cessation of cell cycles and death finally (Borbely *et al.*, 1999).

Oxidize fatty acids and lipids through the formation of free radicals attack cell membranes and yield damage, including rupture of proteins with sulfide bonds. By considering crucial role of cell membrane damage caused by electromagnetic waves and apoptosis, programmed cell death can be justified (Hanafy *et al.*, 2010).

Mitochondria in the cytoplasm of the hepatocytes as well as are the first cytoplasmic organelles that affected by the electromagnetic field thus, by considering the frequent number of mitochondria and their dilation, could not involve in cell oxidation process and play an important role in cellular energy production activities, thereby, apoptosis starts (Bayazit, 2009).

Other changes that have seen in the cell include heterochromatin appearance of nucleus and neutrocells that are generally believed to have been started under the influence of electromagnetic waves through the induction of apoptosis is necrotic cells.

The mechanism of induction the apoptosis in using ELF is that when these waves contract with intracellular fluid they make a heat and increase in the cell temperature which form a shock and microvacuoles in cells which carry the energy and temperature and by penetration of these into the DNA of cells induce apoptosis (Hanafy *et al.*, 2010).

The second mechanism of apoptosis using ELF is to formation of H_2O_2 and free radicals especially O radical which penetrate into the cell DNA and induce P53 gene which is in charge of apoptosis (Hanafy *et al.*, 2010; Freeman and Crapo, 1982).

Some authors have suggested the therapeutic use of ELF-EMF for cancer treatment because, in different experimental models, ELF-EMF have been able to inhibit the growth of cancer cell lines and tumors; however, few *in vivo* experiments have been performed to investigate the molecular mechanisms of ELF-EMF in cancer development (Cameron *et al.*, 2005). The anti-carcinogenic effect of ELF-EMF could result from the inhibition of cell proliferation and/or apoptosis induction. *In vitro* studies have reported the pro-apoptotic action of ELF-EMF and that this effect is associated with an increase in the number of annexin-V- and TUNEL-positive cells and caspase 3 activities (Mi *et al.*, 2007).

In conclusion and by comparison of above mentioned references it comes that the exposure to ELF yields to damage hepatocytes by induction of apoptosis and necrosis.

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