

## HISTOPATHOLOGICAL STUDY ON ELECTROMAGNETIC LOW FREQUENCY (50HZ) EFFECTS ON INDUCTION OF APOPTOSIS IN LIVER OF RAT

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**ABSTRACT:** Humans are continuously exposed to harmful environmental factors, including Electromagnetic Fields (EMF) produced by home appliances, diagnostic tools and industrial instruments. Study Of effects of electromagnetic fields on liver tissue, from light microscope viewpoint is the purpose of this study. Material and Methods: 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 (50.Hz) 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: 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.

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

### INTRODUCTION

It is known that Electromagnetic Field (EMF), as an environmental factor, can produce functional and structural disorders. Progressive dependency of modern life to electrical devices has increased public concern about the potential effects of EMF on health. Humans are, indeed, continuously exposed to such harmful environmental factors produced by home appliances, diagnostic tools and industrial instruments with widely varying frequencies ([Torregrossa, 2005](#)). It has been suggested that electric and magnetic fields at environmental levels may extend the lifetime of free radicals and result in DNA damage ([Michael et al., 1999](#)). EMFs could have a harmful effect on cellular metabolism by affecting biochemical reactions, behavior of charged molecules and ion channels, synthesis of macromolecules ([Levin, 2003](#)). 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

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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 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  $\mu$ T 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

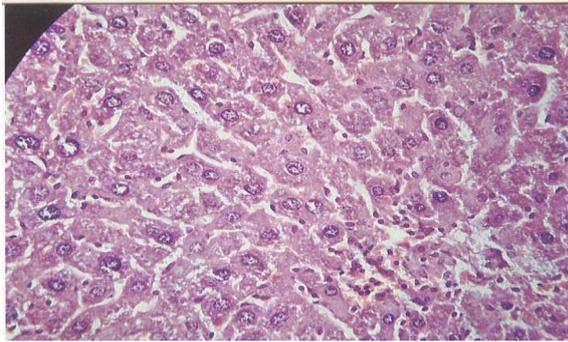
TUNEL staining was done based on following steps:

- 1- Preparing of sections with 4 $\mu$  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 H<sub>2</sub>O<sub>2</sub> for 10 minutes.
- 5- Incubation at 37°C for 60 minutes.
- 6- Washing the sections 3 times in the PBS.
- 7- Incubation in Antifluorescein-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.

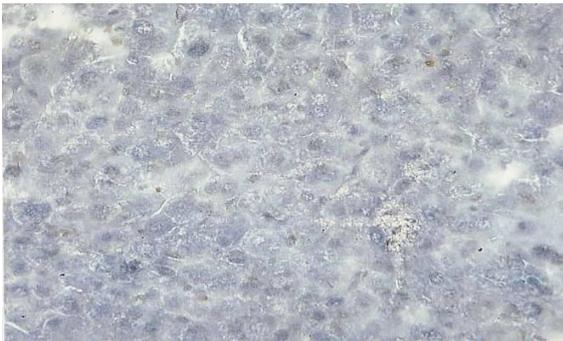
#### RESULTS

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 (Figures 3, 4). 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 5, 6). 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

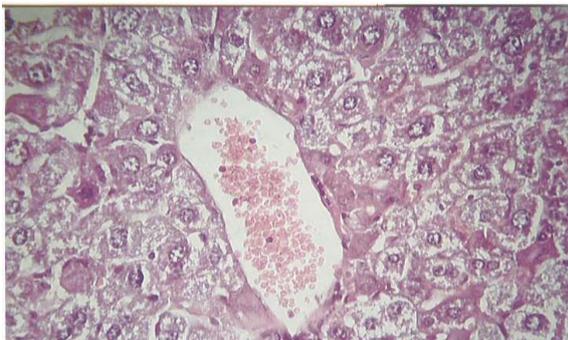
Fisher T-test results there is a significant ( $P < 0.01$ ) difference statistically (figures 7,8). The presence of fibrous connective tissue and replacing the connective tissue with necrotic cells indicate irregular structure. Rymak strands, sinusoids and blood vessels are visible that are accompanied with hypoplasia associated with apoptosis and coagulation necrosis.



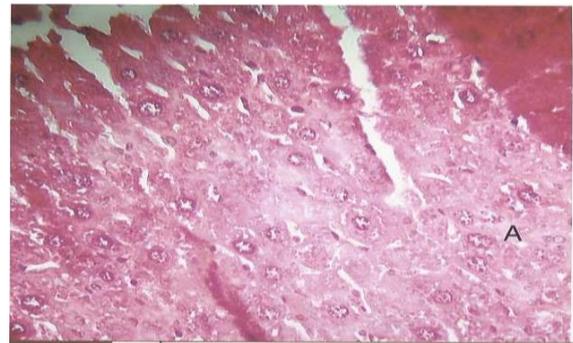
**Figure 1:** microscopic view of control group with normal hepatocytes, ducts and Kupffer cells (H&E, 400x).



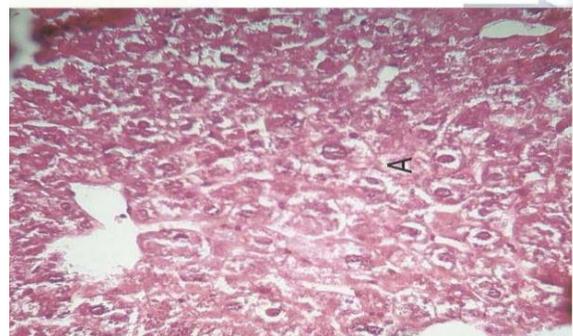
**Figure 2:** microscopic view of control group with normal hepatocytes, ducts and Kupffer cells (TUNEL, 400x).



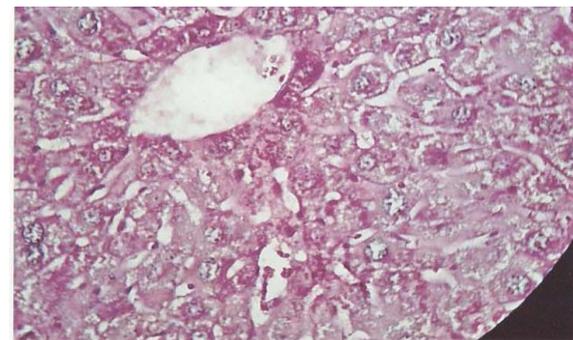
**Figure 3:** microscopic view of experimental group. Pyknotic nucleus with necrosis of endothelial cells and dissociation in along were obvious and it seen without membranous in association with nearby cells which indicates early apoptosis (H&E, 400x).



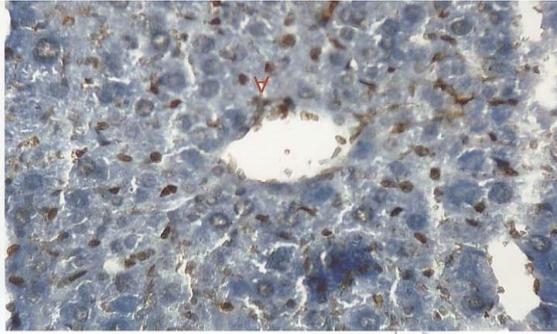
**Figure 4:** microscopic view of experimental group. Pyknotic nucleus with necrosis of endothelial cells and apoptosis are seen (H&E, 400x).



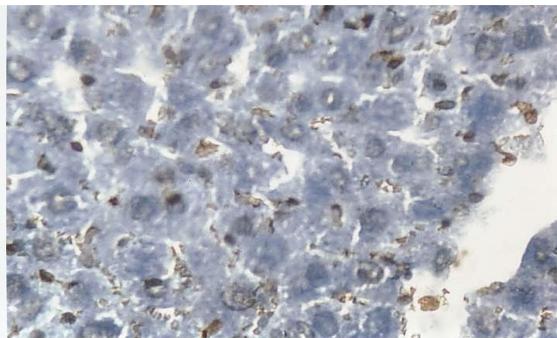
**Figure 5:** Microscopic view of experimental group. Apoptotic cells in the liver are seen, Kupffer and endothelial cells are seen with brown nuclei (H&E, 400x).



**Figure 6:** Microscopic view of experimental group. Apoptotic cells in the liver are seen, Kupffer and endothelial cells are seen with brown nuclei, dissociation of endothelial and free spaces are obvious (H&E, 400x).



**Figure 7:** Apoptotic nucleus of hepatocytes, endothelium cells and Kupffer are seen (TUNEL 400x).



**Figure 8:** Apoptotic nucleuses are seen with brown appearance and hepatocytes and Kupffer cells are seen in apoptotic form (TUNEL 400x).

#### DISCUSSION AND CONCLUSION

In support of this hypothesis; it is shown that EMF has a pro-oxidant effect ([Regoli et al., 2005](#)) and it is proposed that the effect of EMF is mediated by production of free radicals ([Bredt and Snyder, 1994](#)). According to [Peluso et al. \(1977\)](#), granulosa cells in atretic follicles undergo nuclear condensation and cytoplasmic blebbing, these type of changes occurred in EMF-exposed granulosa cells and followed by appearance of apoptotic bodies, a characteristic of late apoptosis. Present findings well agrees with pro-oxidant effect of EMF ([Regoli et al., 2005](#)) and that, oxidants are well known apoptosis-inducing factors ([Chandra et al., 2000](#)). 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 neutrocytes 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 activity ([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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