Melatonin Decreases the Morphological Changes in Liver Induced by Magnetic Field Exposure in Rats

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Abstract

Melatonin decreases the morphological changes in liver induced by magnetic field exposure in rats.

Aim: *The purpose of this study was to investigate the possible effect of melatonin (m) on morphological changes induced by magnetic fields exposure in rat liver.*

Methods: *Thirty young male wistar albino rats were used. Rats divided into 3 groups. First group (control group) received daily intra-peritoneal injections of saline for two weeks. Second group was magnetic field (MF) exposed to rats (n:10). Exposure was 27.12 MHz magnetic field energy, 2.5 hours/day for two weeks. Third group (MF+m) was exposed magnetic fields and had intra-peritoneal single dose of 4 mg/kg/ daily melatonin for two weeks.*

The liver tissue samples were taken under ether anesthesia at the end of the experiment and fixed in 10 % formalin. The liver samples blocked in paraffin after routin methods. Paraffin sections were dyed with hematoxylen-eosin and examined under 'Olympus BX50' light microscopy.

Results: *In histological examinations, mixed cell infiltrations noticed in the periportal area, a high enlargement in the portal area sinusoidal dilatations, granular and vacuolar degenerations in the cytoplasm of the liver parenchyma were observed in MF group. The severity of these lesions in the MF+m group was lower than that in the MF group.*

Conclusion: *Melatonin partially reduces the histo-pathologic effect of magnetic field on liver tissue in rats.*

Key words: *magnetic field, melatonin, liver, morphological changes.*

For many years biological effect of magnetic fields has been the subject of great interest. Based on the current body of knowledge, many specialists suggest that it may play some role in the process of embryogenesis and teratogenesis; disturbing the functioning of the central nervous and immunological systems; and effect into cellular growth and differentiation, particularly in terms of carcinogenesis. However, sufficient evidence collaborating the assumption of health hazard arising from general exposure to magnetic field has not been provided (25).

Over the past half- century, use of electromagnetic energy, especially telecommunication for commercial, military and medical fields have been increased dramatically. The vast body of research on the biological effect of some certain frequency motivated us to consantrate on them. For example 27.12 MHz band has been assigned for citizens band, as commercial communications and therapeutic applications. This assigment has been accepted all over the world. There is no enough result of biological effect of this frequency. Diathermy devices are being used commonly at hospitals. Desing power out-put of devices are about 500 watts. So, many patient may be under the possible treatment of 27.12 MHz radiofrequency electromagnetic radiation (RFR). Particularly, in rural areas, people use CB transceivers most commonly. Permitted out-put power of them is 4W, for hand-held portable and mobile communication. But it's very easy to use a linear RF amplifier of several- hundred watts. This means that there is a potential hazard for people.

Since there is a large number of industrial, scientific, and medical devices operatine at or near 27.12 MHz which are likely to cause hyperthermia in humans, our study was considered to be based on this amount of MF exposure. High-intensity 27.12 MHz radio-frequency radiation was determined to be teratogenic in rats during most of the gestation period (10).

Free radical mechanism for the effects of environmental MF on biological systems was discussed in different studies. Arguments are provided to suggest that the encounters of freely diffusing pairs of radicals are unlikely to produce significant effects in biology .The relevance of these effects in relations to cancer initiation, promotion and progression were discussed in these studies (2, 18, 19).

Melatonin which is known to function as an antioxidant in vitro and in vivo (14, 22, 23).

Melatonin exerts a protective effect against acute liver injuries induced by endo-toxic shock and ischemia re-perfusion in rats despide of its antioxidant action (21, 24).

We aimed to investigate the possible effect of melatonin on morphological changes in liver induced by magnetic fields exposure.

Materials and methods

Thirty albino young male (6 weeks old) Wistar Strain rats weighing 120-140 g were used ın the study. Animals were housed temperature (21± ^{O}C) and maintained on a 12: 12 h light-dark cycle with food and water ad libitum. They were divided into 3 groups.

Control group (C) (n:10)

The animals in control group received 0.1 ml/100g/day physiologic saline intra-peritoneal injections for two weeks.

Only magnetic field (MF) exposed group (n:10)

Only magnetic field exposed group had daily intraperitoneal injections of physiologic saline (0.1 ml / 100g) containing 1% ethanol for two weeks.

Magnetic fields exposed and melatonin (MF+m) treated group (n: 10)

Melatonin (Sigma Chemical Co.) was dissolved in ethanol with further dilution in physiological saline. Exposure was 2.5 hours/day, 6 days/week for two weeks. The magnetic fields exposed animals had intraperitoneal single dose of 4 mg/kg/day melatonin (0.1 ml / 100g) at 10:00 o'clock daily for two weeks following magnetic fields exposure.

Magnetic fields exposure

Commercial CB hand-held portable transceiver was used, (Midland, USA, Labelled of 4 Watts, 40 Channel). Each rat was irradiated seperately in a cage. MF energy at 27.12 MHz passes through the cage. The cage of animals has been made of only wood. No use any metal objects for undisturbed and homogenous field. The size of wooden cage was 40 x 40 x 30 cm.

First, we made by-pass the push-to talk switch of transceiver, in order to use as RF source in test set up. On first channel, we measured RF power on dummy load connected antenna connector. Dummy-load was 50 ohms for matched antennas impedance. This channel frequency had been measured 27.12 MHz with Frequency Counter.

To obtain pure antenna radiation pattern; a stainless steel ground plane was used under the cage. A standard plane size 1 x 0.5 m.

The ground plane's size was proper with respect to the irradition wavelength because the cage was near field area at such frequency so the MF must be homogeneus. Finally experiment test set-up has been prepared for power experiment test set up had been prepared for power and dosimetry calibration. A monopole magnet whip antenna had been used radiating element As known well; monopole antenna was exactly bi-directional. So we have got the homogenous MF with adjustable electrical parameters. We have taken some measures on the target points in the cage. We have used a powermeter and Standing Wave Ratio Meter (S.W.R), a spectrum analyzer whit loop probe antenna for calibration.

We have taken 38 measure within the cage and then made a statistical averaging (SPSS Statical Program) in order to obtain average power density for the cage. The average power density is obtained 14.47 Mw/cm^2. According to the "International Non-Ionizing Radiation Committee" exposure standards; for 27 MHz, for 6 min., exposure limit is 0.2 mW/cm^2. This value is for General Public. For occupational exposure limit was 1 mW/cm^2 (7) as considered general public exposure limit. We have to consider occupational exposure limit. Therefore our limit is 1 Mw/cm^2. In other words; in this study; our exposure is always over the recommended limit.

At the end of study all animals were sacrified under ether anesthesia, and sections of the liver were obtained and fixed in 10% formalin and dehydrated by alcohol, embedded in paraffin, and stained with hematoxylin-eosin. Histological sections were examined and photographed under light microscopy (Olympus BX50).

Diameter of portal area, sinusoidal wideness, degeneration percentage of hepatocytes were measured in 5 sections of liver tissue from each rat. The portal area diameter was calculated as a mean diameter [$(t_r + v_r) / 2$] from vertical (v_r) and tranvers (t_r) diameters measured by an ocular micrometer for 100 portal area under light microscope (x 40 magnification).

Sinusoidal wideness measured by an ocular micrometer under light microscope (x 40 magnification) was an average of 1000 sinusoidal wideness.

The method of Tsukamoto and Towner (24) was modified for the evaluation of hepatocytes degeneration as follows: 1+ = 0 to 25 % of hepatocytes with degeneration (little); 2 + = 25 to 50 (median); 3 + = 50 to % 75 (high); and 4 + > 75 % (very high) .

The mean with SD (standard deviation) of the data were compared for two groups and sex by SPSS 6.0 for Windows software (Student-t test for comparison of the control and experimental groups parameters, Chi-square (χ^2) test used to compare distribution percentage of hepatocyte degeneration in liver).

Results

The liver of control group had normal histological structures (Fig.1).

The significant differences in MF group was observed as compared to controls. In the MF exposed group, mixed cell infiltrations noticed in the periportal area and liver parenchyma. Cytoplasm of most hepatocytes could not be framed in a natural disposition. The cytoplasm had thin granular and vacuolar appearance with a great degree of degeneration and 'lipocyte-like' cells characterized by micro-vesicular fatty change. The degenerated hepatocytes with dark eosinophilic cytoplasm and pyknotic nucleus forming small groups were found around hepatocytes in periportal area. A moderate sinusoidal dilatations ($p<0.01$), a high enlargement and congestion in the portal area ($p<0.001$) were seen in liver samples compared to control group (fig. 2a, b).

In the MF+m group; mixed cell infiltrations decreased in periportal area and disappeared from the liver parenchyma, even they were almost disappeared in periportal area. Hepatocytes with dense eosinophilic cytoplasm and 'lipocyte-like' cells were still seen in the liver samples. A thin granular and vacuolar degeneration ($p<0.05$) were observed, compared to MF group. A small enlargement ($p<0.05$) and disappeared of congestion in portal area were seen and sinusoidal dilatations were still lasting ($p<0.01$) in liver tissues, compared with MF group (fig. 3a, b).

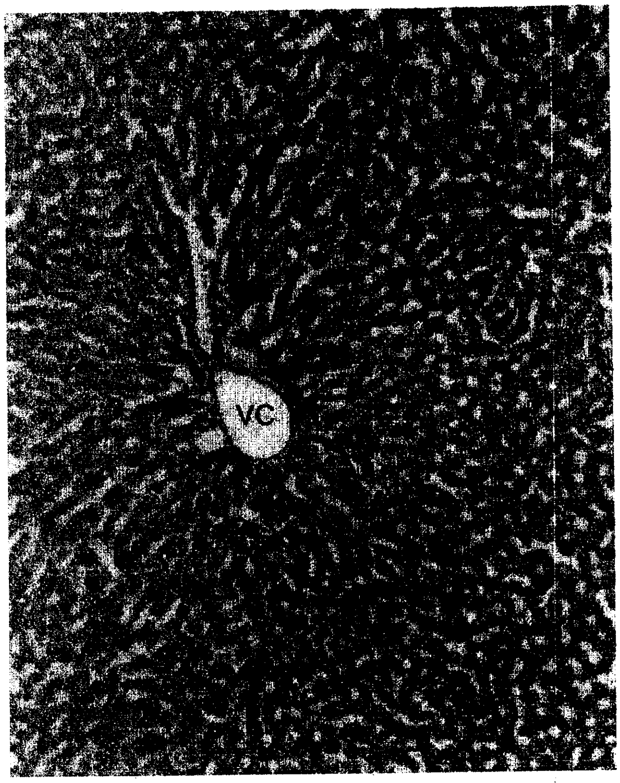

Figure 1: Histological apperance of rat liver tissue. Normal lobular structure is seen (a sample taken from the middle lobe of the liver). VC: vena centralis (Hematoxylin-eosin, x120).

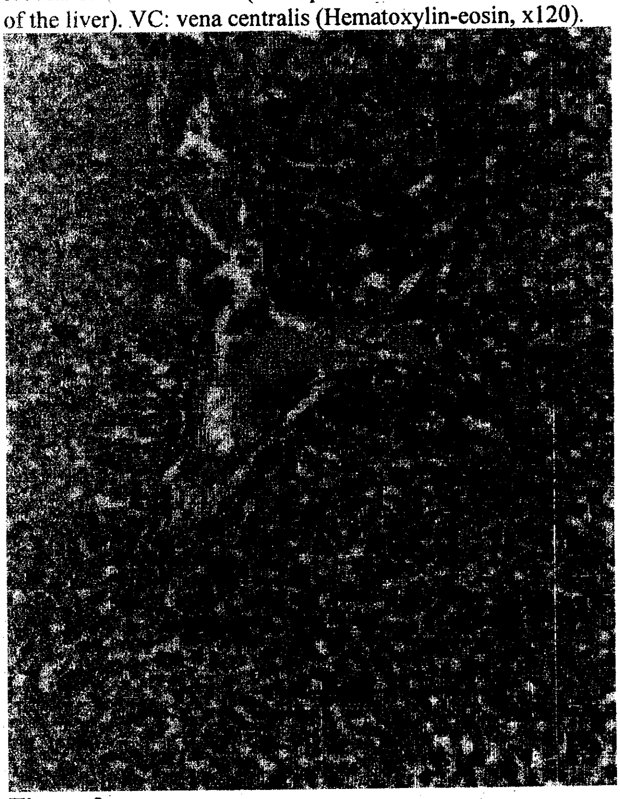

Figure 2 a.

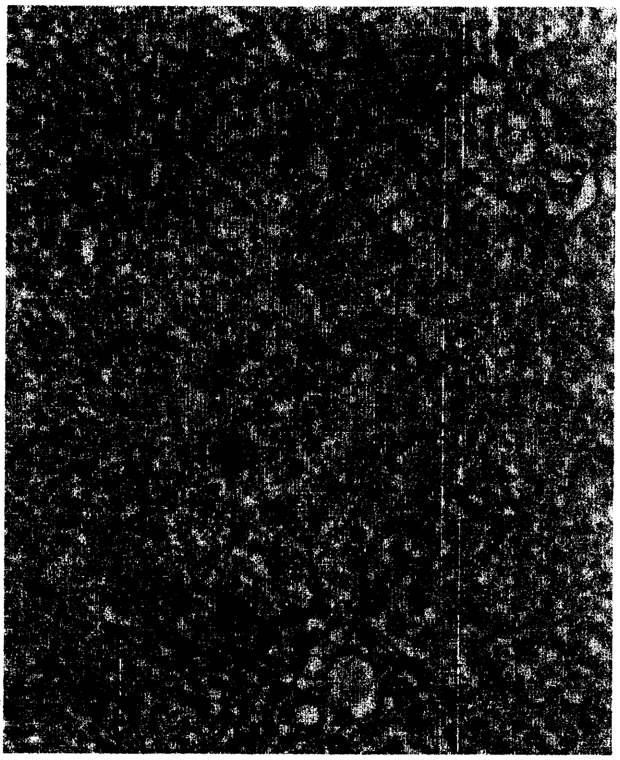

Figure 2b: MF treated group. In (a) and (b) mixed cell infiltrations (asterisks) are seen. Hepatocytes with dense eosinophilic cytoplasm (thin arrows) and ' lipocyte-like' cells (thick arrows) characterized by microvesicular fatty change and hydropic degeneration are observed (HE., x120).

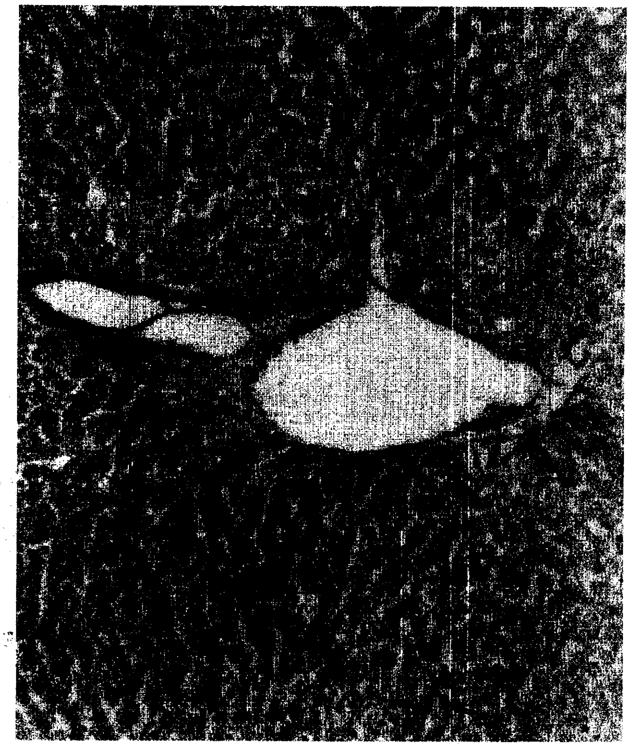

Figure 3 a.

Figure 3 b. MF+m treated group in periportal area (a) and parenchyma (b) mixed cell infiltrations are disappeared. However, hepatocytes with dense eosinophilic cytoplasm (thin arrows) and ' lipocyte-like' cells (thick arrows) are seen in the liver samples (HE, x120).

Discussion

The effects of very small magnetic fields on chemical reactions involving free radicals are well established and reproducible. They are fully understood and form an unique basis for rationalizing why such fields might affect biological systems. Free radicals occur widely in the body, both intrinsically in normal biochemical reactions and extrinsically in their creation by exposure to UV light and high- energy radiation (2).

Lai et al. (8) stated that an increase in DNA single- and double-strand breaks in brain cells of rats after acute exposure (two hours) to a sinusoidal 60-Hz MF. And in another study; it was reported that free radicals were involved in radio-frequency electromagnetic radiation-induced DNA damage in the brain cells of rats (18). According to the reports cumulated DNA breaks in brain cells can lead to neuro-degenerative diseases and cancer and an excess of free radicals in cells has been suggested to be cause of various human diseases (2, 19).

Boorman et al.(1) reported that no histological changes in linearly polarized 60 Hz MF for 8 weeks. Margonata et al. (11) informed no morphologic significant differences in control and experiment animals, to provide possible laboratory support to health risk evaluation associated with long-term, low-intensity magnetic field exposure, animal were exposed 22 h / day to a 50 Hz magnetic flux density of micro T for 32 weeks (a total of about 5000 h). Morphology and histology of liver were assessed at the end of exposure period. The another study, the chronic exposure of guinea pigs to the magnetic field of induction 0.005 T and 0.3 T were caused the morphological changes of spleen and functional disturbances of liver (5).

We determined histolo-morphological changes in liver tissue. Our study was based on intensity of 27.12 MHz MF exposure. Moreover, several epidemiologic studies have suggested that there may be an association between exposure to 60 Hz MF and the increasing incidence of cancer (6, 17). Also, Lary et al. (10) reported that 27.12 Hz MF was determined to be teratogenic in rats during most of the gestation period. Reiter et all. (16) explained that nocturnal pineal melatonin synthesis and serum melatonin levels had been decreased in rats exposed to pulsed DC MF.

Melatonin is a direct free radical scavenger, and recently many in vitro studies have shown that melatonin, a pineal hormone, functions effectively as an antioxidant. The drop in serum melatonin could theoretically be explained by an increased uptake of melatonin by tissues that were experiencing augmented levels of free radicals as a consequence of MF exposure. Since, melatonin levels were decreased, free radicals might cause oxidative effect which include DNA of cell proteins, membran lipids.

These data suggest that free radicals may play a role in the MF-induced DNA single-and double-strand breaks observed in liver cells of the rat. Consistent with this hypothesis is the fact that free radicals can cause damage to DNA and another macromolecules in cells. Particularly, oxygen free radicals have been shown to cause DNA strand breaks (12). In addition, a study has implicated free radicals as the cause of some of the biological effects observed after exposure to MF (9). Consequently, the encounters of freely diffusing pairs (F-pairs) of radicals are unlikely to produce significant effects in biology (2).

In the present study; we observed that morphological changes (mixed cell infiltration, vacuolar and granular degeneration, hepatocytes with dense eosinophilic, 'lipocyte-like' cells, enlargement periportal area and sinusoidal dilatation) in liver induced by MF. These findings may be explained MF effects indicated as the previous studies above.

Recently, many in vitro studies have shown that melatonin functions effectively as an antioxidant, i.e., a hydroxyl radical and a peroxyl radical scavenger (14, 22, 23).

It has also been shown that when animals and tissues were subjected to lipid peroxidation, melatonin causes a substantial protection against the oxidative destruction of lipids. It has also been reported that melatonin exerts a protective effect against acute liver injures induced by endo-toxic shock and ischemia-reperfusion in rats through its antioxidant action (20, 21). The effects of melatonin on the progression of liver injury and on changes in liver lipid peroxide (LPO) and reduced glutathione (GSH) contents with injury progression were reported by different investigators. It has been shown that LPO produced by oxidative stress damages cells and tissues. Hepatic tissues possess a high concentration of GSH. GSH is well known to protect liver cells against oxidative damage through chemical or enzymatic reactions (3, 4, 13, 15).

In conclusion; parenchyma and stromal structures were prevented partially from effects of magnetic fields in melatonin treated group. Also, mixed cell infiltrations almost disappearad in the periportal area and from parenchyma. Granular and vacuolar degeneration regression was observed. And congestion was disappeared in portal area. It can be suggested that melatonin has a minimal protective effect on 27.12 MHz EMF induced changes in liver tissue.

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