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Histological, Ultrastructural and Immunohistochemical Studies of the Low Frequency Electromagnetic Field Effect on Thymus, Spleen and Liver of Albino Swiss Mice

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Abstract: The effect of low frequency electromagnetic field (EMF) of 50 Hz at flux density of 100 μT on the immune system and liver tissues of mice was tested. The mice were exposed to short (10 and 20 days) and long (50 days) time of exposure. The long time exposure (50 days) of the magnetic field affected greatly all tissues more than those of short time (10 and 20 days). Histologically, during the exposure time of the experiment, spleen and liver tissues were more affected than thymus tissue. Destruction in spleen and liver sections were observed. Ultrastructural observations of the liver hepatocytes revealed highly irregular nuclei, autolyzed chromatin and most of the mitochondria were degenerated and had lost their cristae. Moreover, megakaryocytes were scattered in most spleen sections. Thymus tissue revealed a decrease in cell population of the medullary areas and showed the presence of certain pyknosis in most cortical regions. The immunohistochemical studies showed a gradual decrease in the enzymatic activity of glutathion-S- transferase (GST-p) of experimental mice group with the increase in the duration of the exposure time indicating the inability of spleen and liver tissues to protect themselves. However, thymus tissue showed a proportional increase in the enzymatic activity with the increase of the time of exposure to the EMF indicating its reactivity.

Key words: Electromagnetic field, glutathion S transferase, megakaryocytes, pyknosis, immunohisto-chemistry, thymus

Introduction

Recent experimental evidence suggests that human exposed to static magnetic fields (SMF) may face health hazards (Feinendegen and Muhlensiepen, 1988). Magnetic field is an important environmental factor and has become a topic of considerable interest in recent times. Several home/environmental sources generating electromagnetic field (EMF), such as 50-60 Hz high voltage transmission lines, video display terminals, electric blankets, clinical nuclear magnetic resonance (NMR) imaging procedures, etc., may interact with the human body (Benquet and Roux, 1998). Exposure may range between 0.01 and 1 μT in homes and offices and up to the mT range in steelworks and during welding. In addition, some doses of EMF are medically applied in the treatment of rheumatic diseases (Rusovan and Kanje, 1992).

Many reports have led to a growing awareness that even weak magnetic and EMFs might modify the biochemical and physiological processes (Liburdy, 1995). Also, Rosen and Lubowsky (1987) and Rudolph *et al.* (1988) discussed the potential hazards of imposed magnetic fields (MF) and the interactions of magnetic fields with cells, tissues and organs.

Since the physiological maturation and/or involution of the thymus may be accelerated by endogenous or exogenous factors, the thymus may be regarded as a possible target of 50 Hz electric and magnetic fields (Quaglino *et al.*, 2000).

Many studies have demonstrated the interactions of the low frequency EMF with the immune system (Cadossi *et al.*, 1992; Walleczek, 1992; Thun-Battersby *et al.*, 1999; Marino *et al.*, 2000). Benquet and Roux (1998) had examined *in vitro* the effects of SMF on the cellular immune parameters of C57B1/6 murine macrophages, spleen lymphocytes and thymic cells. Also, Urban and Schreiber (1988) had reported that any impairment of the immune response by MF exposure could reduce the body's protection against the development and progression of neoplasia. Moreover, Thun - Battersby *et al.* (1999) suggested that power - line (50/60Hz) magnetic fields may reduce immune function, which could lower resistance to infection or cancer.

Furthermore, the structural and functional changes in organelles of liver cells in rats exposed to MF were described and it was reported that paramagnetic properties of iron-storing organs such as liver, spleen and bone marrow make these organs more likely to be affected by the magnetic fields (Gorczynska and Wegrzynowicz, 1991).

The glutathion S-transferases (GST-p) are a group of multi functional enzymes of glutathion that occurs in all tissues of the body. They perform several roles in the detoxification of a broad spectrum of electrophilic reactive drugs (Mannervik, 1985).

In view of the above mentioned considerations, the investigation was conducted to evaluate the possible histological, ultrastructural and enzymatic changes that might occur in thymus, spleen and liver of mice when exposed to low frequency (50 Hz) electromagnetic field for short (10, 20 days) and long (50 days) exposure periods.

Materials and Methods

Two groups of male and female mice (control and experimental), each consists of 10 mice, aged 6 - 8 weeks and weighing 25-30 g. were used. The mice were housed in plastic cages and fed with a standard diet. The mice of the experimental group were exposed continuously to the magnetic field at a flux density of 100 μT for periods of 10, 20 and 50 days. The exposure system of the used MF in this experiment was constructed at Biophysics Department, Medical Research Institute, Alexandria University Egypt (Mohamed *et al.*, 2000).

At the end of experimental time, the control and EMF exposed mice were killed by cervical dislocation. The thymus, spleen and liver were dissected out quickly and subjected to the following procedures:

1) For the histological studies, small pieces of the previous tissues were fixed in Bouin's fluid and processed to get 4 μ thick paraffin sections to be stained with haematoxylin and eosin (H&E).
2) 5 μm thick paraffin sections of thymus, spleen and liver were stained for immunohistochemical detection of the placental form of the enzyme glutathione S- transferase (GST-p) according to an indirect peroxidase staining technique (Hsu *et al.*, 1981).
3) For electron microscopic studies, small blocks of liver of both control and 50 days exposed mice to the EMF were prepared.

Ultrathin 50 nm thick sections were cut, double stained with uranyle acetate and lead citrate (Reynolds, 1963) and examined under JEOL 100s transmission electron microscope at 60 Kv accelerating voltage.

Results

Different sections of thymus of mice exposed to the low frequency EMF revealed slight histological changes comparing to sections of thymus of the control mice (Fig. 1). A moderate

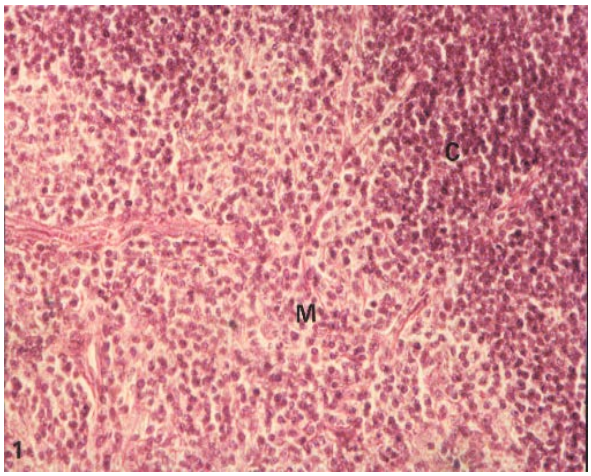


Fig. 1: Section of control thymus of mice showing a dark cortical region (C); a light medullary area (M). H& E, X 400.

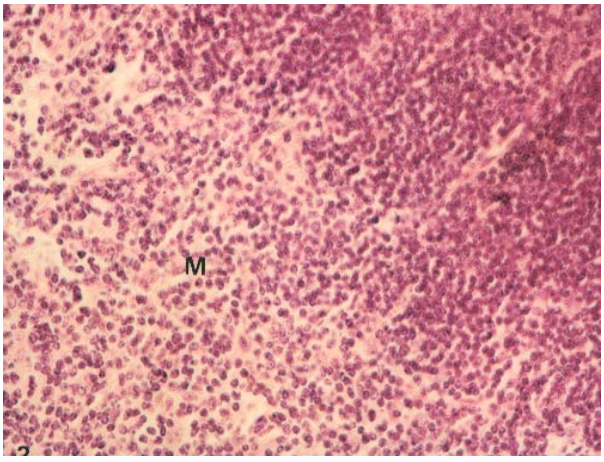


Fig. 2: Section of thymus of mice exposed to 20 days of low frequency EMF showing a moderate decrease in thymocyte population in the medullary area (M). H& E, X 400.

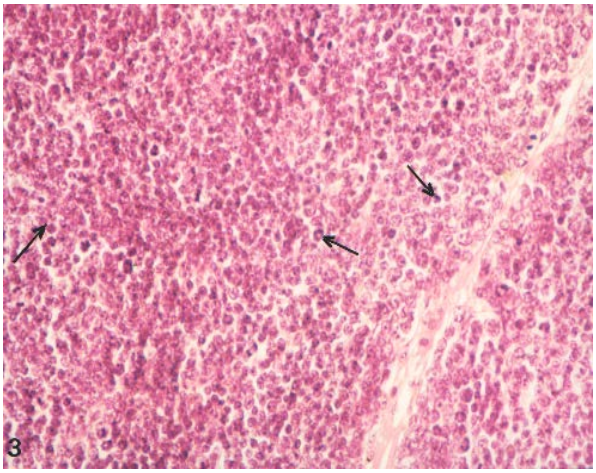


Fig. 3: Section of thymus of mice exposed to 50 days of low frequency EMF showing the presence of many pyknotic nuclei (arrows) in the cortical area. H& E, X 400.

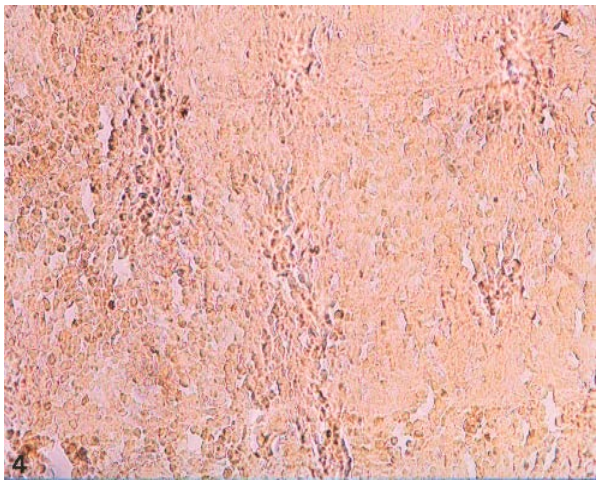


Fig. 4: Section of thymus of control mice showing the presence of brownish - red fine granules of GST-p enzyme reaction in most thymocytes. DAB - stain, X400.

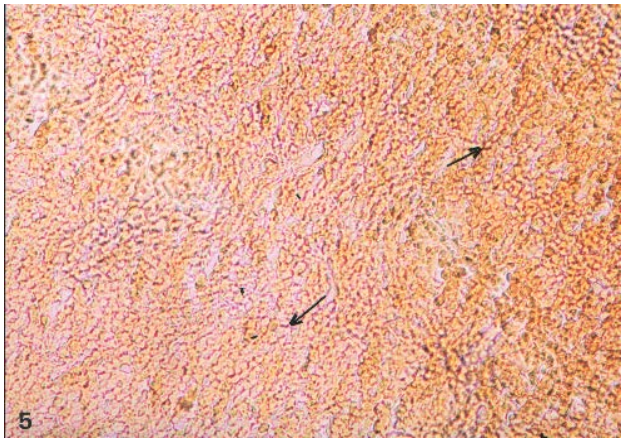


Fig. 5: Section of thymus of mice exposed to 50 days of low frequency EMF showing an obvious increase in GST-p enzyme activity in most thymocytes of both cortex and medullary regions (arrows). DAB - stain, X400.

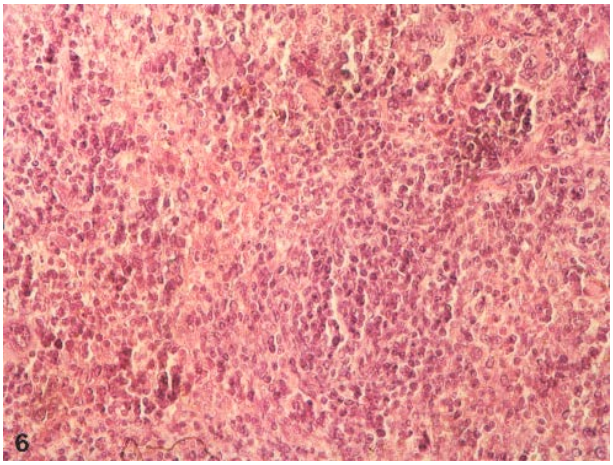


Fig. 6: Section of spleen of control mice showing the a less distinct appearance between red and white pulp areas. H& E, X 400.

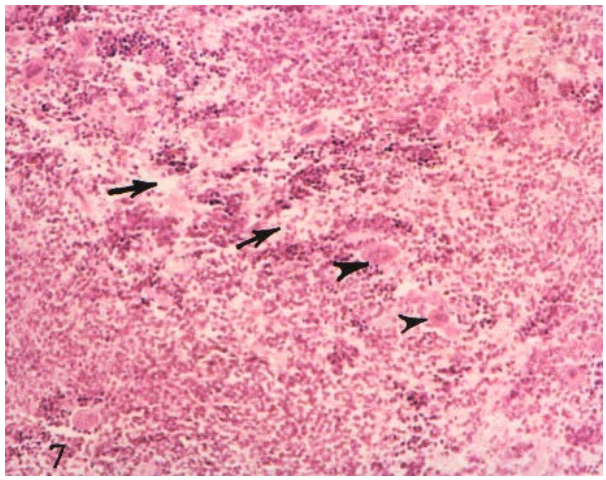


Fig. 7: Section of spleen of mice exposed to 20 days of low frequency EMF showing a decrease in the splenocyte population (arrows), note the presence of many megakaryocytes (arrowheads). H& E, X 400.

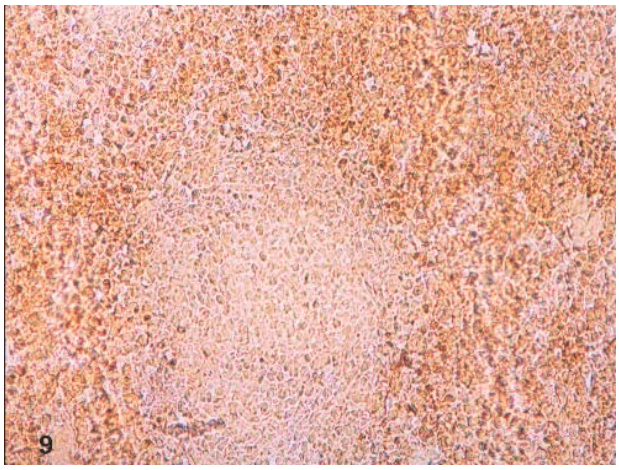
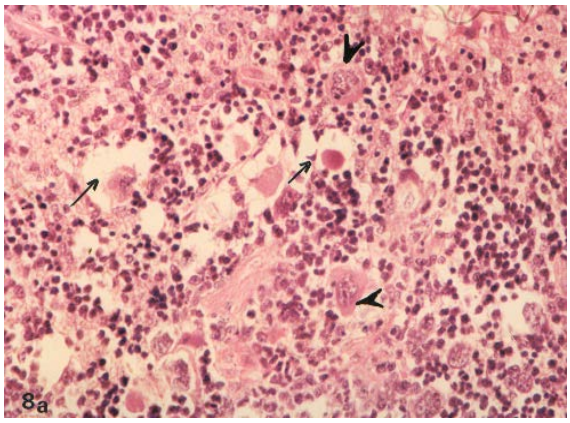


Fig. 9: Section of spleen of control mice showing the presence of brownish - red fine granules of GST-p enzyme activity in the nuclei of most splenocytes. DAB - stain, X400.

(A)



(B)

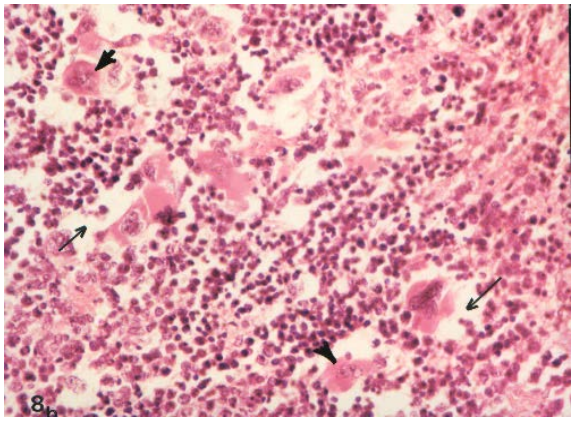


Fig. 8 a,b: Section of spleen of mice exposed to 50 days of low frequency EMF revealing many degenerated areas (arrows); arrowheads point at megakaryocytes. H& E, X 400.

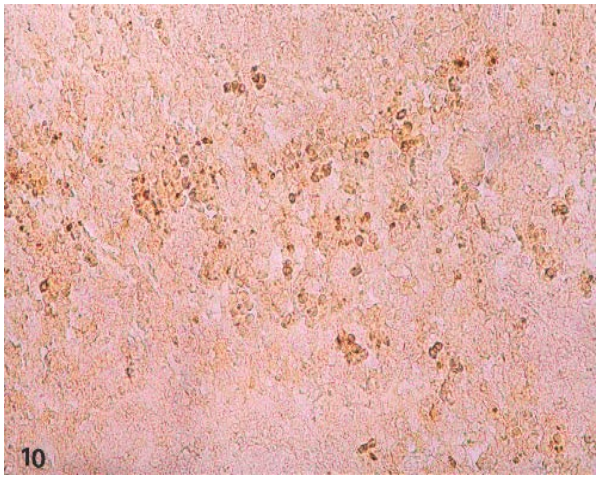


Fig. 10: Section of spleen of mice exposed to 20 days of low frequency EMF showing a slight decrease in the GST-p enzyme activity. DAB - stain, X400.

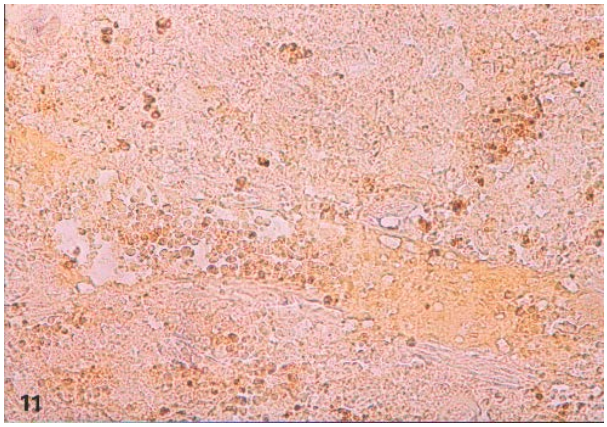


Fig. 11: Section of spleen of mice exposed to 50 days of low frequency EMF showing an obvious decrease in the GST-p enzyme activity of most splenocytes. DAB - stain, X400.

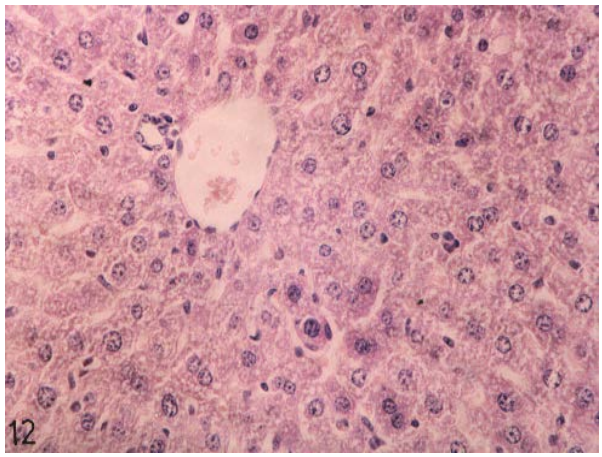


Fig. 12: Section of liver of control mice showing the regular hepatocyte strand with normal cytoplasmic density and nuclear morphology. H& E, X 400.

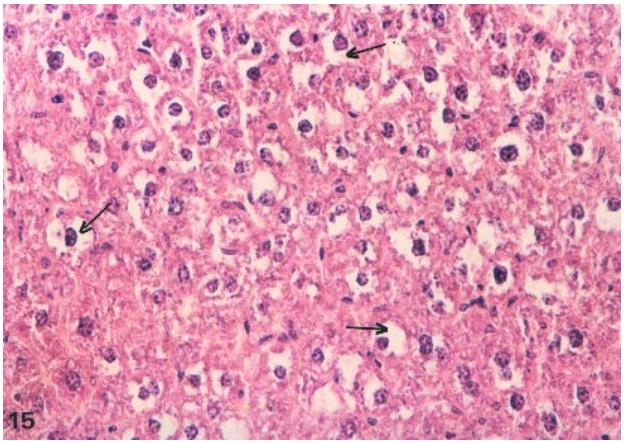


Fig. 15: Section of liver mice exposed to 50 days of low frequency EMF showing the highly cytoplasmic vacuolation of most hepatocytes (arrows). H& E, X 400.

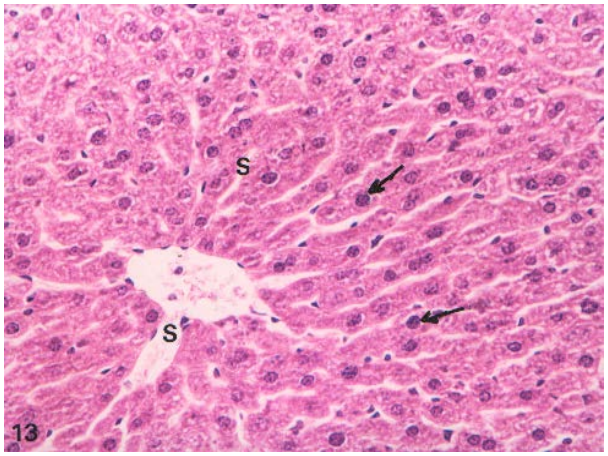


Fig. 13: Section of liver mice exposed to 10 days of low frequency EMF showing mild centrolobular sinusoidal (S) dilatation; many dense clumped nuclei (arrows). H&E, X 400.

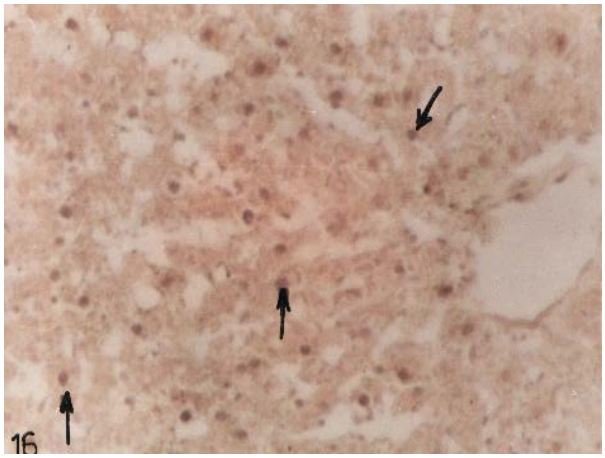


Fig. 16: Section of liver of control mice showing a moderate positive reaction of GST-p as brownish-red fine granules in the cytoplasm and nuclei (N) of most hepatocytes in the middle regions of the hepatic lobules. DAB - stain, X400.

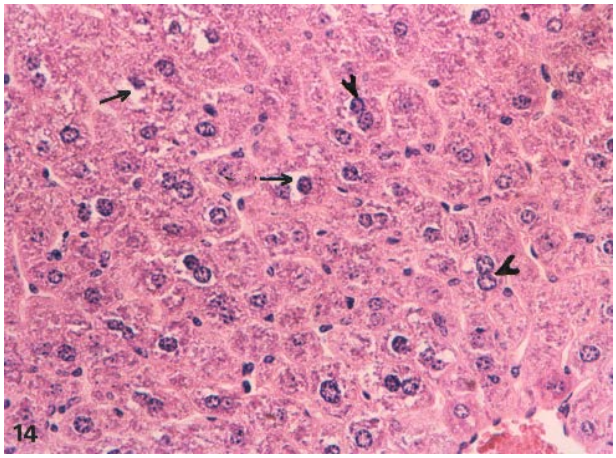


Fig. 14: Section of liver mice exposed to 20 days of low frequency EMF, showing mild vacuolation in some hepatocyte cytoplasm (arrows); arrowheads pointed at binucleated cells. H& E, X 400.

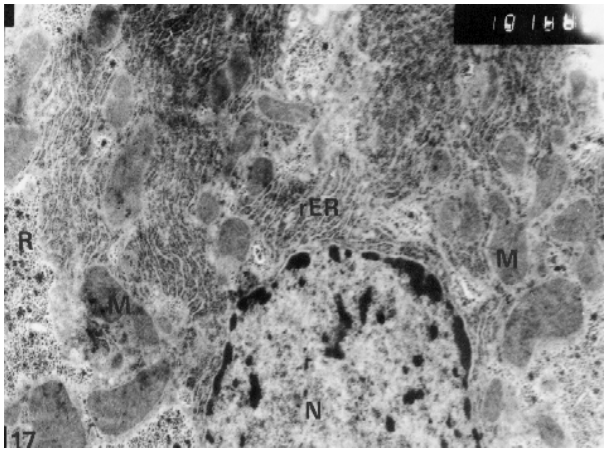


Fig. 17: Electron micrograph of liver of control mice showing a part of the hepatocyte nucleus (N); dense mitochondria (M); rough endoplasmic reticulum (rER); ribosomes (R). (Glutaraldehyde fixed, uranyle acetate and lead citrate - stained preparation, X8000).

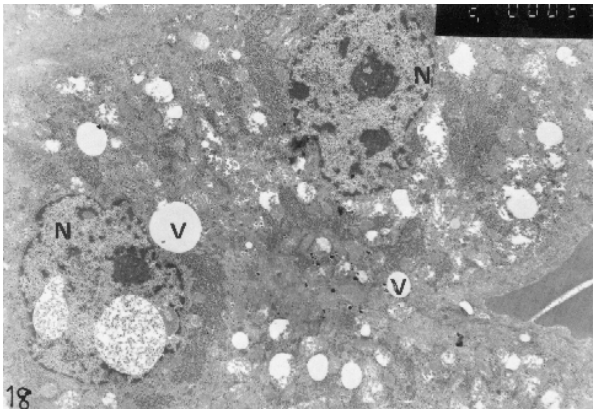


Fig. 18: Electron micrograph of liver mice exposed to 50 days of low frequency EMF showing irregularly-shaped hepatocyte nuclei (N); general vacuolation in the hepatocyte cytoplasm (V). (Glutaraldehyde fixed, uranyle acetate and lead citrate - stained preparation,X 2700).

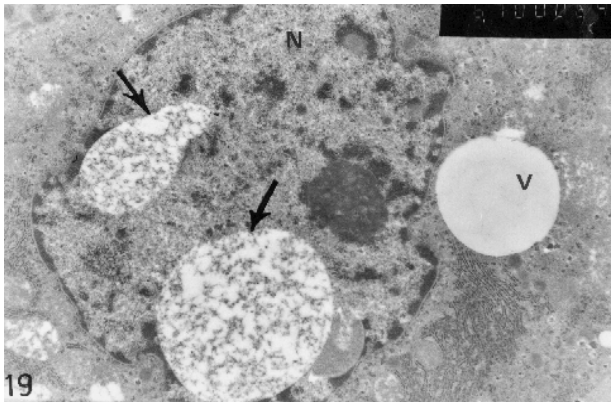


Fig. 19: Enlarged part of the previous figure showing an irregular shaped hepatocyte nucleus (N) with an obvious autolyzed nucleoplasm (arrows); large vacuole (V). (Glutaraldehyde fixed, uranyle acetate and lead citrate - stained preparation, X 6700).

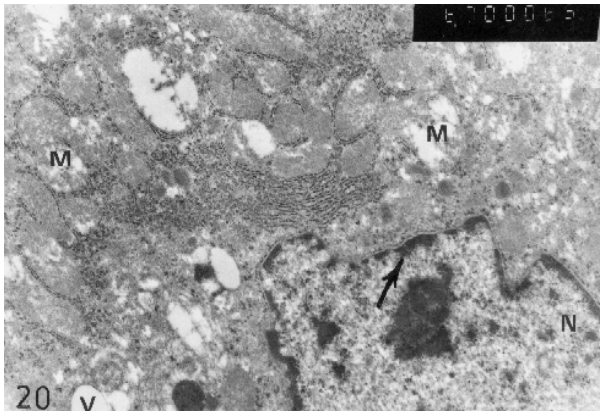


Fig. 20: Electron micrograph of liver mice exposed to 50 days of low frequency EMF showing a part of an irregular-shaped hepatocyte nucleus (N) with thin peripherally placed heterochromatin (arrow); vacuolized cytoplasm (V); destructed mitochondria (M). (Glutaraldehyde fixed, uranyle acetate and lead citrate - stained preparation, X 6700).

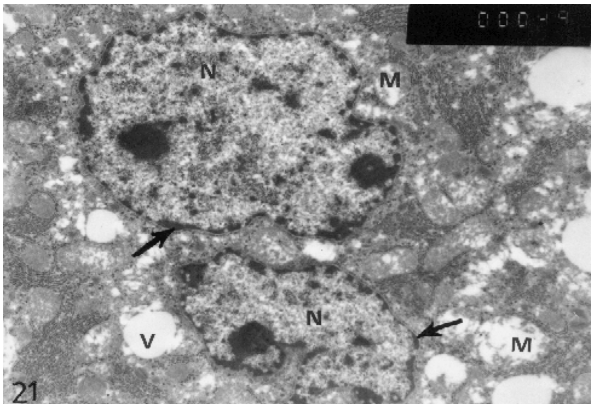


Fig. 21: Electron micrograph of liver mice exposed to 50 days of low frequency EMF showing irregularly-shaped hepatocyte nuclei (N) with thin peripherally placed heterochromatin (arrow); vacuolized cytoplasm (V); the mitochondria (M) had loss their matrix and cristae. (Glutaraldehyde fixed, uranyle acetate and lead citrate - stained preparation, X 8000).

decrease in thymocyte population was observed in the medullary areas in thymus section of mice exposed to 20 days of the low frequency EMF (Fig. 2). Also, an observable pyknosis was appeared in the cortical areas in sections of mice exposed to 50 days (Fig. 3).

The immunohistochemical reaction for glutathion transferase enzyme activities (GST-p) in thymus tissue which were detected by the immunoperoxidase-DAB stain, showed the presence of brownish-red fine granules distributed in the nuclei of thymocytes of both cortex and medulla, while, it was negative in the cytoplasm. The results revealed a proportional increase in the enzyme activity in thymus tissue of all experimental mice groups with the increase of the exposure time to the EMF (10, 20 and 50 days respectively) (compare Fig. 4 of control and Fig. 5 of treated).

The light microscopic examination showed that comparing with the control spleen sections of mice (Fig. 6), certain decrease in splenocyte population with many vacuolated areas in the white pulp areas were observed in spleen mice exposed to 20 and 50 days of low frequency EMF, indicating partial spleen atrophy (Figs. 7, 8 a,b). Many megakaryocytes were observed in most spleen sections of these mice (Figs. 7, 8 a,b). These cells are characterized by numerous clustered nuclei and barely identified cytoplasm.

The immunohistochemical reactions of mice spleen sections which exposed to the low frequency EMF showed a gradual decrease in the glutathion transferase (GST-p) enzyme activity with the increase in the time of exposure (10, 20 and 50 days respectively) (compare Fig. 9 of control and Figs. 10 and 11 of treated).

The histological examination of liver tissue of mice exposed to the low frequency EMF for 10 days showed that the hepatic cords at certain regions became separated from each other by mild centrolobular sinusoidal spaces. In addition, large dense clumped nuclei were observed in many hepatocytes (Fig. 12, control and Fig. 13, treated). However, after 20 and 50 days of exposed mice to the EMF, vacuolation in most hepatocyte cytoplasm was noticed (Figs. 14 and 15) with an obvious increase in the number of the binucleate cells (Fig. 14).

The immunohistochemical enzyme activity of GST-p in the hepatocyte nuclei showed very weak reactions in the different sections of all experimental mice groups comparing with the control (Fig. 16), indicating the damaging effect of the low frequency EMF on liver tissue and its inability to protect itself.

In addition, the electron microscopic observations revealed a strong influence of the EMF on the nuclei and mitochondria of most hepatocytes of mice exposed to 50 days, comparing to the

hepatocytes of the control mice (Fig. 17, 18). The results showed that some of the hepatocyte nuclei were irregular in shape, containing very thin layer of peripherally placed heterochromatin with an obvious autolyzed nucleoplasm (Fig. 19). In addition, many of the mitochondria were disintegrated, exhibiting a considerable loss of their matrix and their cristae (Fig. 20 and 21).

Discussion

Various mechanisms can explain the effect of EMF on organ function and induce cellular changes that the electromagnetic field might amplify electric currents in tissues and cells or affect these currents through resonance with local field focus (Sagan, 1992). The present results are in consisting with the results of many authors who had reported that the effect of the EMF is critically depend on the duration of exposure and the strength of the field (Mevissen *et al.*, 1998). Only a few studies have dealt with the *in vitro* effect of EMFs on the structural changes of immune system.

The results showed that thymus of mice exposed to 10 days of exposure did not demonstrate any recognizable differences than that of the control. However, 20 days of exposure revealed a moderate loss in the lymphocyte populations in the medullary areas. Moreover, the thymus of mice exposed to 50 days of the low frequency EMF revealed an observable increase in the number of pyknotic cells in the cortical areas which is a type of apoptosis. An explanation may be direct put forward that EMF may affect thymic cell physiology. Worth to mention that apoptosis is the main process by which organs maintain cell mass and at the same time eliminate excess and aged cells that have lost their importance (Huppertz *et al.*, 1999).Benquet and Roux (1998) concluded that *in vitro* exposure to relatively low 0.02 - 0.15 T static MF intensities could alter functional parameters of murine macrophages, thymocytes and splenocytes. They demonstrated that static MF exposure decreased mitogenic responses in lymphocytes as well as it produced markedly increased apoptosis of thymic cells. Also, Quaglino *et al.* (2000) had suggested that, in the rat a prolonged exposure to 50 Hz electric and magnetic fields, independently from field strength, seems to affect thymic cell death and possibly thymic physiology. Since alterations in the balance of cell death and other parameters such as mitosis (Capri *et al.*, 1999) might interfere with the positive and negative selection of thymocytes and the immunosurveillance properties of the thymus (Urban and Schreiber, 1988). The results also revealed a gradual increase in the enzyme activity in thymus tissues of experimental mice groups indicating their reactivity .

Spleen pattern of the EMF exposed mice showed that spleen lesions became more prominent as the exposure time was increased. Therefore, 10 days of exposure mice to the EMF revealed nearly normal spleen pattern. However, progressive depletion of splenoctes in the white pulp areas in addition to the fragmentation of the tissue were the most prominent features of the effect of the EMF on sleep tissues of mice exposed continuously to 20 and 50 days. In addition, the results showed the presence of many megakaryocytes (polykaryocytes). Kamel *et al.* (1992) had reported that the term polykaryocyte for a type of multinucleated giant cells found in lymphoid tissues in association with a variety of reactive and neoplastic disorders and they display a T- cell phenotype. In addition, the present results demonstrated a gradual decrease in the GST-p enzyme activity in spleen tissues of experimental mice groups due to the increase in the time of exposure, indicating the inability of spleen to protect itself. Thun-Battersby *et al.* (1999) found that a prolonged (13 weeks) 50 Hz MF exposure of female rats at flux densities of 50 or 100 μT significantly suppressed the proliferative capacity of spleen T lymphocytes *in vivo* (Mevissen *et al.*, 1996, 1998). Also, Trembly *et al.* (1996) had reported that 6-weeks exposure of rats to a 60 Hz MF at a flux densities ranging from 2 to 2000 μT induced a significant decrease in the number of spleen CD4+ , CD8+ and CD5+ lymphocytes.

Furthermore, the present results involved a serious disturbance and a cellular response in the structure of hepatocyte organelles (nucleus and mitochondria) in mice exposed to the low frequency EMF as described in rats by Gorczynska and Wegrzynowicz (1991). The extent of these changes has been shown to depend on the duration of the exposure and strength of the applied fields. Vacuolation of the cytoplasm, multi nucleation and nuclear pleomorphism were also reported in this work. We suggest that these changes could be considered as sign of metabolic alterations under the influence of the exposure to the EMF. The characteristic feature of nuclear autolysis that appeared in the electron micrographs of most hepatocytes of mice exposed to 50 days of low frequency EMF showed the disintegration of the chromatin with loss of the nucleus altogether (karyolysis) and hence revealing the appearance of dead cells and dying cells in living tissues (Wheater *et al.*, 1993).

Moreover, Gorczynska and Wegrzynowicz (1991) found an increased number of cytoplasmic vacuoles, mitochondrial swelling and irregular distribution of nuclear chromatin in rat hepatocytes exposed to 10 mT static magnetic field. Also, they had reported that mitochondria usually seem to be the most sensitive, exhibiting a rapid structural and physiological response to the different kind of stress-generating factors. In addition, Voyedovodskaya *et al.* (1981) have been suggested that paramagnetic particles naturally located in the mitochondria of liver cells could make the liver particularly sensitive to imposed magnetic fields leading to modification of haemostasis either directly through origin or indirectly through changes in mitochondrial respiration of liver cells.

Furthermore, Mannervik (1985) have reported that glutathione plays a major role in protecting the liver against oxidative metabolites of drugs and many other xenobiotics and any reduction in the tissue concentration of glutathione or in the rate of biosynthesis can therefore lead to serious liver damage. Hence suggest that absence of the GST-p enzyme activity in the liver sections of experimental groups indicated the destructive effect of the EMF on the nuclei of the liver organ, so it had lost its ability to protect itself or detoxifying the broad spectrum of EMF.

Recommended that the human beings should be kept away from exposure to devices emitting EMF or at least their time of exposure must be shortened enough to diminish the hazards of exposure.

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