

Effects of Chronic Exposure to Static Electromagnetic Field on Certain Histological Aspects of the Spleen and Some Haematological Parameters in Albino Rats

Mervat S. Zaghloul

Zoology Department, Faculty of Science, Benha University, Benha Egypt
mervat2009@hotmail.com

Abstract: Over the past few years, our environment has become seething electromagnetic smog that bombards our bodies every second of every day. Because electro-magnetic fields are invisible, we do not even realize they are there, although they are battering us mercilessly. Special attention has been given to the biological effects of magnetic fields. Thirty six male albino rats (*Rattus norvegicus*) were utilized in the present work to study the effects of static magnetic field (SMF) equaling 2 ml tesla on the spleen and some haematological parameters. Magnetic exposure was applied for 10 minutes for 3 days per week for two weeks. One day following magnetic exposure, the spleen showed congestion in the splenic sinusoids accompanied with thickening of the splenic capsule. A significant increase in the white blood cells and blood platelets was accompanied by enlargement of the white pulp were detected. Seven days following magnetic exposure, an increase of haemoglobin concentration; haematocrit and red blood cells was recorded accompanied with a highly significant decrease in blood iron. Later on, such increase was followed by a significant decrease in most haematological parameters after fifteen days of magnetic exposure. Hemosiderin granules were observed in the dilated splenic sinusoids at the areas of congestion. The splenic tissues and the haematological parameters appeared almost normal and manifested a tendency towards recovery after thirty days following magnetic exposure.

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Introduction:

During the past few decades, there was a growing concern about the increase in invisible environmental pollution due to the emission of electromagnetic waves (Tonini et al., 2001; Chakeres & de Vocht 2005). Numerous epidemiological studies have failed to find a correlation between magnetic field at different intensities and the appearance of any particular pathological changes (Reipert et al., 1997; Day, 1999; Schüz & Ahlbom, 2008; Calvente et al., 2010).

Previous studies showed that electromagnetic fields induced changes in haematological parameters in mice, rats and humans (High et al., 2000; Ali et al., 2003; Sihem et al., 2006; Hassan & Abdelkawi, 2010).

Considerable evidence has been accumulated regarding the biological effects of static magnetic field focused on sources of exposure and interaction mechanisms (Feychting, 2005; Rongen, 2005; Straume et al., 2008; Kundi et al., 2009). It was reported that paramagnetic properties of iron storing organs make these organs more likely to be affected by magnetic fields (Gorczyńska & Wegrzynowicz, 1991).

Other researchers demonstrated the interaction of static electromagnetic field (EMF) with the

immune system (Thun-Battersby et al., 1999 ; Marino et al., 2000 ; Attia & Yehya, 2002 ; Johansson, 2009)

The role of the spleen as a lymphatic organ storing iron in immune system and the increased application of magnetic field-generating equipment, stimulate the conduction of the present experimental study, targeting chronic exposure of the spleen to a moderate static magnetic field and some of haematological parameters in albino rats.

Material and Methods

Animals:

Thirty male albino rats (*Rattus norvegicus*), each weighs 120 ± 10 grams were utilized in the present study. The animals were housed in plastic cages to avoid any metallic interaction and were kept under similar normal laboratory conditions during the period of the experimental study.

The animals were divided into two groups:

Group I : This group included six rats used as control (unexposed animals).

Group II: This group included thirty rats exposed individually to constant electric magnetic field (direct current, DC) with flux density equal to 2 ml tesla.

EMF Exposure:

The exposure was applied for 10 minutes/ day, 3 days / week, for 2 weeks. The EMF was generated

by applying an electric current to the coil of artificial EMF apparatus constructed in the Department of Physics, Jubail Faculty of Education for Girls, Kingdom of Saudi Arabia. The animals were kept in a perforated plastic box, placed in the coil chamber of the apparatus. Then, a horizontal magnetic induction was applied to the whole animal body. The field strength was monitored with a gauss meter.

At the end of the experiment, all the animals were dissected and specimens of spleen were taken on 1, 7, 15, and 30 days post-irradiation. All the control animals and six animals of the second group were sacrificed after 1, 3, 6, 15 and 30 days following the exposure.

Histological methods:

Small spleen specimens were cut into small blocks, fixed in 10% neutral buffered formalin and processed up to paraffin pieces. Semi-serial sections of 6 μm were prepared and stained with Harris' hematoxylin and eosin (Drury & Wallington 1980) to illustrate the histological structure. Spleen specimens also stained for haemosiderin by **Perl's Prussian blue reaction technique (1867)** for detection of haemosiderin iron.

Haematological and Blood chemical methods:

At the end of the experiment the blood samples were collected (0.5 ml approximately/sample) from the supra-orbital venous plexus of rats using heparinized syringes into two tubes. The first tube contained heparin as anticoagulant. The heparinized blood was used for RBC, haematocrit, haemoglobin, WBC and platelets analysis, using standard methods (Feldman et al., 2000).

The blood sample in the other tube was left for a short time to allow clotting. Clear serum samples were obtained by centrifugation at 3000 r.p.m. for 20 min and then kept at -20°C prior to biochemical analysis.

A serum level of iron was measured using Stanbio serum iron liquicolour commercial Kits, (Procedure No. 0370) according to the method by Weissman & Leggi (1974).

Statistical Analysis:

The data was present as the mean \pm SD for each correlation was calculated using Microsoft Excel.

Results

I. The control animals:

The spleen is surrounded by a fibrous connective tissue capsule interspersed with smooth muscle fibres. Irregular spaced trabeculae of smooth muscle and fibroelastic tissue emanate

from the splenic capsule into the splenic parenchyma containing blood and lymph vessels.

The parenchyma of the spleen is termed the pulp. Most of the pulp is soft red. It consists of large, irregular, thin-walled blood vessels, splenic sinusoids, interposed between sheets of thin connective tissues and splenic cords. The splenic cords are the masses of cells in between the sinusoids. They contain a lot of erythrocytes (RBCs) and some other cell types as macrophages and megakaryocytes.

Within the red pulp, small oval or/and rounded grayish blue stained areas represent the white pulps. They consist almost entirely of lymphocytes, in a peculiar association with the arterial blood supply (Fig. 1).

The splenic tissue reveals a scanty amount of blue stained haemosiderin granules that are diffused and distributed throughout the red pulp cords (Fig. 2).

II. The SMF-exposed animals:

1. One day post exposure to SMF:

The spleen had a thick connective tissue capsule, variable in its thickness at different areas. Numerous thick trabeculae extended from the splenic capsule inwards, branched and divided the spleen into numerous parts. There were multiple clear areas in the subcapsular spaces and few haemorrhagic areas as directed inwards. Within the red pulps, small foci of cellular necrosis were observed and the splenic sinusoids were slightly dilated and congested with blood cells (Fig. 3).

Most of the white pulps were large and irregular in shape revealing cellular proliferation of immunoplastic cells, and macrophages. Small foci of cellular lesions were observed scattering throughout the white pulps (Fig. 4).

2. Seven days post exposure to SMF:

Numerous subcapsular clear spaces were found between the capsule and trabeculae. Few other ones containing haemolysed RBCs were observed between the splenic parenchyma (Fig. 5).

The cells of the splenic cords revealed necrotic changes scattering throughout the red pulp. The splenic sinusoids were dilated contained some of erythrocytes or hemolysed blood. Numerous inflammatory cells and megakaryocytes were noted in the splenic parenchyma and sinusoids.

The white pulps were numerous, fragmented and appeared diffused in an irregular manner in the red pulp (Fig. 6).

3. Fifteen days post exposure to SMF:

The splenic vasculature revealed progressive changes represented by congestion of the splenic sinuses and diffusion of haemorrhagic and hemolysed areas throughout the red pulps. Numerous inflammatory cells, macrophage and megakaryocytes were observed in the splenic parenchyma and sinusoids (Fig.7).

Some splenic white pulps suffered from lymphoid depletion and the others became hyperplastic.

Heavy iron blue pigments of hemosiderin granules were observed in the dilated red pulp spaces which were filled with hemolysed red blood cells (Fig.8).

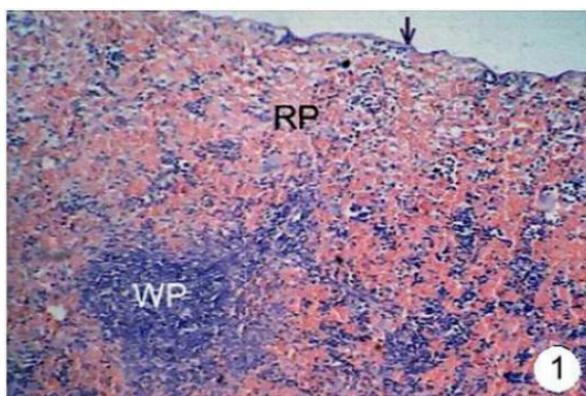


Fig. 1: A photomicrograph of a spleen section of control rat showing a fibrous connective tissue capsule surrounding the splenic pulp (arrow). Most of the pulp is a soft red pulp (RP) and some oval grayish blue-stained areas represent the white pulp (WP). (H&E stain X250)

4. Thirty days post the end of exposure to SMF:

The splenic tissues appeared almost normal and manifested a tendency towards recovery. Some significant signs towards complete vasculature and tissues recovery were observed in both red and white pulp as compared with the previous examined groups, where no areas of blood haemorrhage or haemolysis were observed.

Brown hemosiderin granules were seen in the cytoplasm of macrophages (Fig.9).

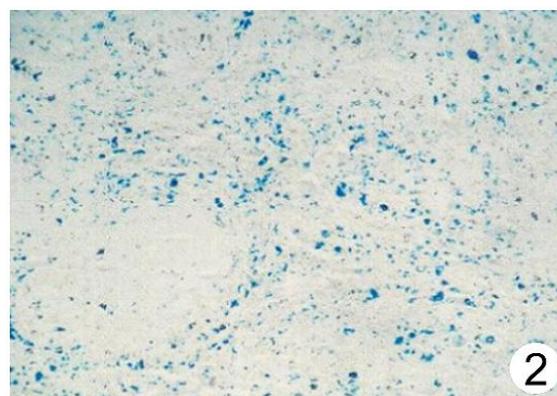


Fig. 2: A photomicrograph of a spleen section of control rat showing a scanty amount of blue-stained hemosiderin granules diffusely distributed throughout the red pulp cords. (Perl's Prussian blue stain X400)

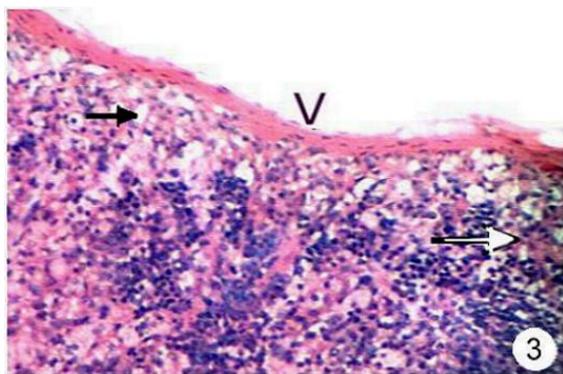


Fig. 3: A photomicrograph of a spleen section of rat after one day following the end of SMF exposure showing a thick connective tissue capsule (arrowhead), Multiple clear sinusoids in the subcapsular space (black arrow) and few haemorrhagic (white arrow). (H&E stain X400)

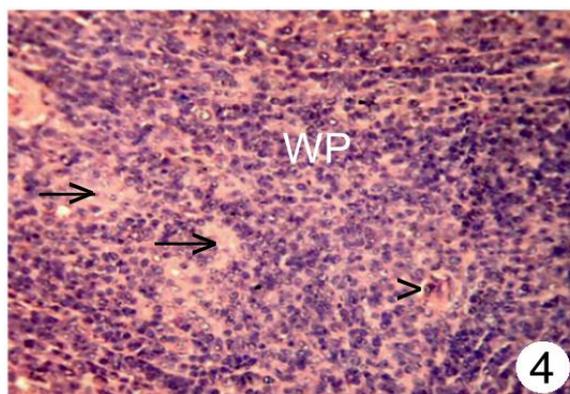


Fig. 4: A photomicrograph of a spleen section of rat after one day following the end of SMF exposure showing large and irregular white pulps (WP) revealing cellular proliferation of homogeneous population of immunoplastic cells, and macrophages (arrowhead) in addition to small foci of cellular lesions (arrow). (H&E stain X400)

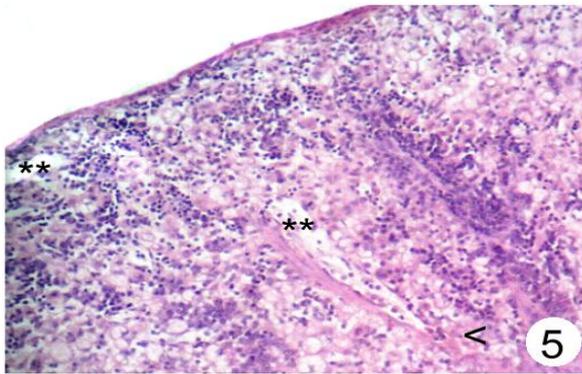


Fig. 5: A photomicrograph of a spleen section of rat after seven days following the end of SMF exposure showing numerous subcapsular clear spaces (***) and few other ones containing haemolysed RBCs (arrowhead) between splenic parenchyma. (H&E stain X250)

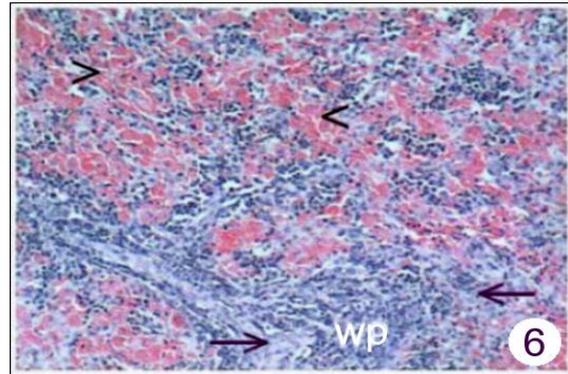


Fig. 6: A photomicrograph of a spleen section of rat after seven days following the end of SMF exposure showing dilated splenic sinusoids containing some of blood cells (arrowhead), or hemolysed blood. The white pulps (WP) (WP) are numerous, fragmented and appear diffused in an irregular manner throughout the red pulp. (H&E Stain X400)

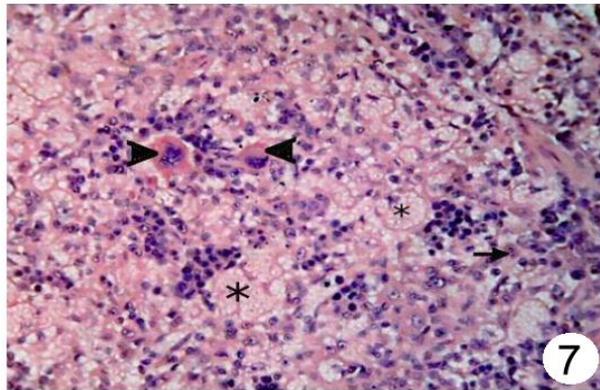


Fig. 7: A photomicrograph of a spleen section of rat after fifteen days following the end of SMF exposure showing haemorrhagic and haemolysed sinusoids diffused throughout the red pulps (*), some inflammatory cells, macrophage (arrow) and megakaryocytes (arrowheads) are observed in the splenic parenchyma. (H&E Stain X400)

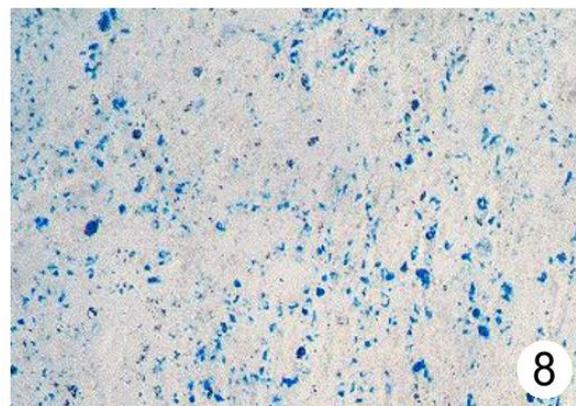


Fig. 8: A photomicrograph of a spleen section of rat after fifteen days following the end of SMF exposure showing heavy iron blue pigments of haemosiderin granules in the dilated red pulp spaces. (Perl's Prussian blue method X400)

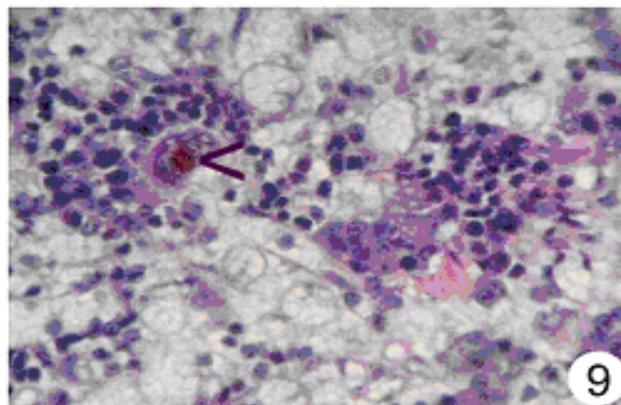


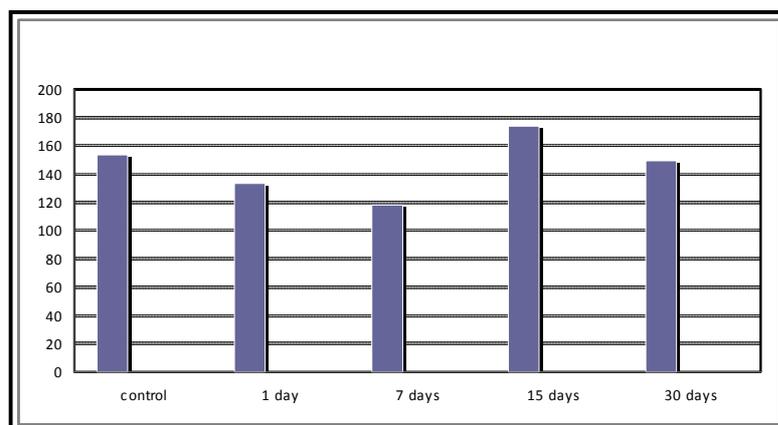
Fig. 9: A photomicrograph of a spleen section of rat after thirty days following the end of SMF exposure showing brown haemosiderin granules (arrowhead) in the cytoplasm of macrophages. (H&E Stain X600)

Haematological results;**Table (1): Effect of EMF on serum iron levels ($\mu\text{g}/\text{dl}$) of adult male rats**

GROUP	Serum iron $\mu\text{g}/\text{dl}$ ($M \pm SE$)
Control	153.44 \pm 9.56
One day	133.49 \pm 7.46*
7 days	118.49 \pm 11.56**
15 days	173.54 \pm 17.46*
30 days	149.34 \pm 5.37

All data are Mean value \pm Standard error.

N= 6 animals of each group.

* Significant at ($p < 0.05$).** Highly significant at (< 0.01).**Table (2): Effect of EMF on red blood cells (RBCs) count ($10^6/\text{mm}^3$) of adult male rats**

GROUP	Red blood cells (RBCs) $10^6/\text{mm}^3$ ($M \pm SE$)
Control	7.25 \pm 0.13
One day	7.16 \pm 0.42
7 days	7.89 \pm 0.29*
15 days	6.67 \pm 0.36*
30 days	7.88 \pm 0.51

All data are Mean value \pm Standard error.

N= 6 animals of each group.

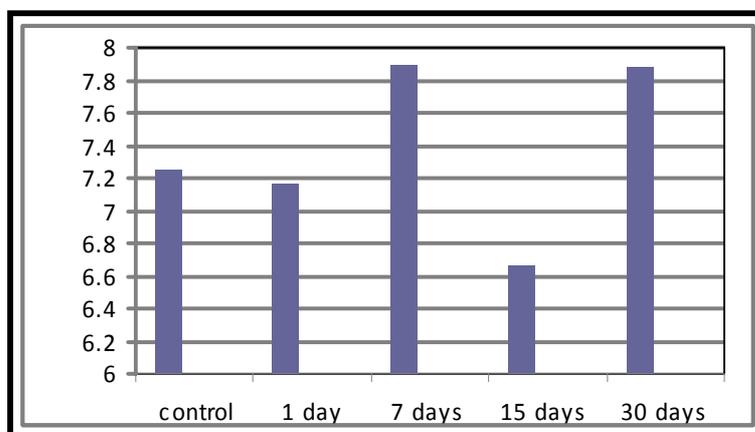
* Significant at ($p < 0.05$).** Highly significant at (< 0.01).

Table (3): Effect of EMF on haemoglobin levels (g/dl) of adult male rats

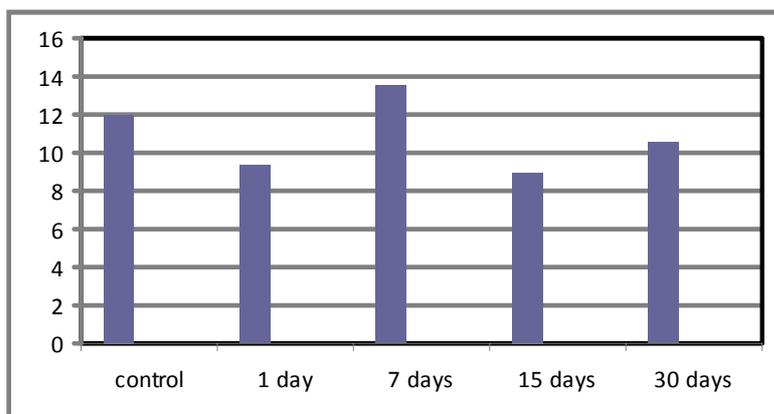
GROUP	Haemoglobin levels (g/dl) (M [±] SE)
Control	11.98 ± 0.25
One day	9.34 ± 0.21*
7 days	13.54 ± 0.46*
15 days	8.95 ± 0.39*
30 days	10.55 ± 0.43

All data are Mean value[±] Standard error.

* Significant at (p < 0.05).

N= 6 animals of each group.

** Highly significant at (< 0.01).

**Table (4): Effect of EMF on haematocrit value % of adult male rats**

GROUP	Ht (%)
Control	36.21 ± 0.45
One day	31.19 ± 0.26*
7 days	41.54 ± 0.76*
15 days	29.54 ± 0.46**
30 days	37.34 ± 0.76

All data are Mean value[±] Standard error

* Significant at (p < 0.05).

N= 6 animals of each group.

** Highly significant at (p < 0.01).

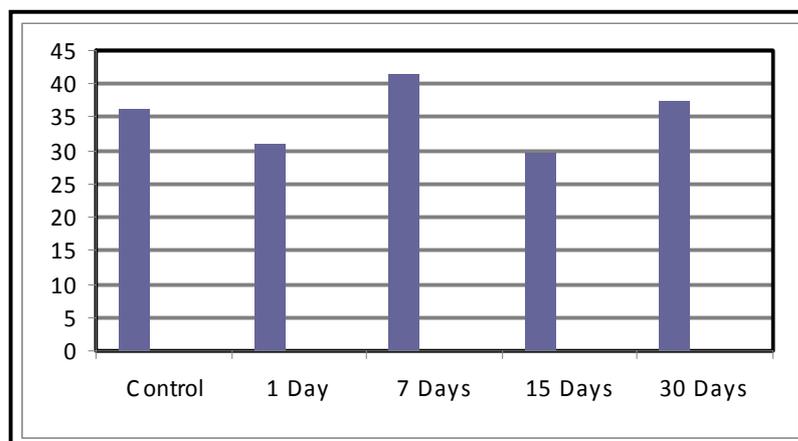


Table (5): Effect of EMF on white blood cells (WBCs) count ($10^3/\text{mm}^3$) of adult male rats

GROUP	Blood platelets $10^3/\text{mm}^3$ (M+SE)
Control	536.23 + 14.8
One day	628.17 +15.45*
7 days	694.45 + 35.65**
15 days	634.16 +12.73*
30 days	602.33+14.04

All data are Mean value± Standard error

N= 6 animals of each group.

* Significant at ($p < 0.05$).

** Highly significant at ($p < 0.01$).

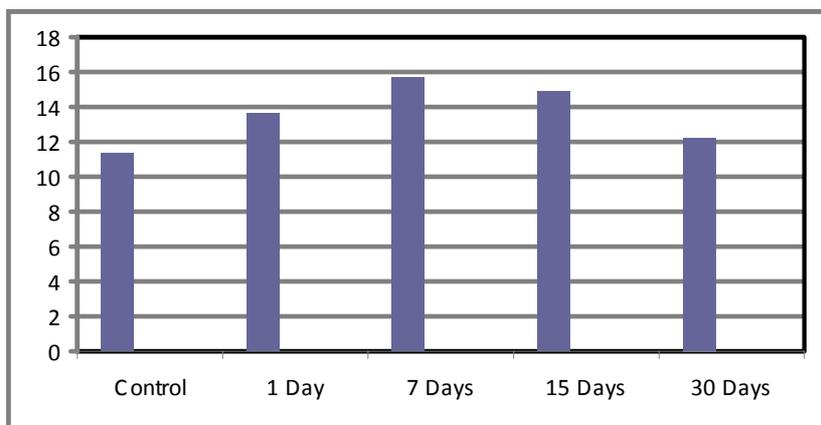


Table (6): Effect of EMF on blood platelets count ($10^3/\text{mm}^3$) of adult male rats blood platelets count ($10^3/\text{mm}^3$)

GROUP	White blood cells (WBCs) $10^3/\text{mm}^3$ (M±SE)
Control	11.35 ± 0.13
One day	13.65 ± 0.27*
7 days	15.68 ± 1.27**
15 days	14.89 ± 0.76**
30 days	12.23 ± 0.39

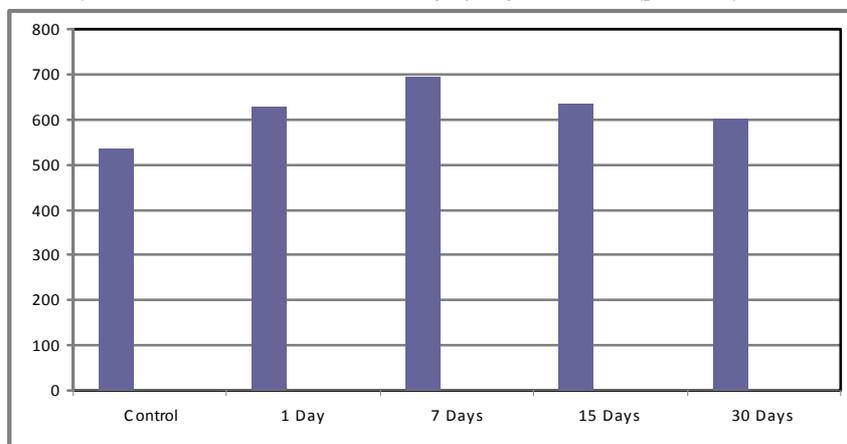
All data are value± Standard error

N= 6 animals of each group.

* Significant at ($p < 0.05$).

** Highly significant at ($p < 0.01$).

data Mean



Discussion

The present experimental study showed that the chronic exposure to SMF induced different splenic histological and haematological disruption in albino rats. The earliest haematological responses reported one day after the end of exposure to SMF revealed a significant decrease in haemoglobin, serum iron and hematocrit values. However, no significant change in RBCs was detected.

This decrease could be attributed to the interaction between heme (iron) and SMF where the magnetic field penetrates the body and acts on ions in all organs, altering the cell membrane potential and distribution of ions (Kula, 1996; Berg, 1993). Regular exposure to SMF leads to an increase of plasma volume. Therefore, haemoglobin concentration was slightly below normal values in the presence of low serum iron levels (Amara et al., 2006 ; Chater et al., 2006). In addition, the static magnetic field may cause cardiovascular stress accompanied with a slow development of mild cardiac decompensation during the exposure period, hence developing heart failure with subsequent passive congestion and stagnant hypoxia (Walter & Israel 1987; Snover 1989 ; Grawford 1994). Thus, it can be concluded that as the body adapts to the higher oxygen needs, more fluid would be in the blood. In turn, measured haemoglobin in such cases would be apparently low. This is because it was diluted out by a larger plasma volume. Moreover, the red blood cells looked normal on the blood smear although haemoglobin, serum iron and haematocrit values were significantly decreased, hence resulted in anemia. Usually, this type of anemia is mild and appears like the pseudo-anemia caused by sports. In this respect, regular physical activity, especially extensive running and exercises increase iron loss causing mild iron deficiency (Bärtsch et al., 1998). True iron deficiency can even occur especially when nutritional iron intake is insufficient and iron demand is increased.

From the histopathological perspectives, the spleen which showed congestion or storage of RBCs in the splenic sinusoids was accompanied with a conspicuous thickening in the splenic capsule and trabeculae. In this context Cesta (2006) described that the splenic capsule is composed of dense fibrous tissue, elastic fibres, and smooth muscles.

However, animals that depend on running for their survival tend to have rather muscular splenic capsules; they can store erythrocytes and release them into the general circulation when needed for extra oxygen carrying capacity (Bacha & Linda, 2000).

The capsule and trabeculae of dogs, horses and cats contained more smooth muscle than that of mice and rats. So, the spleen of rodents do not

contracts as rapidly and tends to be varying in their gross appearance (Valli et al., 2002).

Thus, the thickness of the capsule, trabeculae and concentration of smooth muscles are very important agents to make strong contraction when the body needs the blood and the smooth muscle concentration may play a role in the immune reactions. Indeed, the thickened splenic capsule and trabeculae after chronic exposure to SMF allow the spleen to contract and eject stored extra erythrocytes from the splenic sinusoids when needed for extra oxygen. This is due to the resultant stagnant hypoxia like status. This view supports the opinion of Bacha and Linda (2000), who reported that animals evolved to live at low altitudes often have oxygen loading curves that depend on a high partial pressure of atmospheric oxygen. When moved to high elevations, they often generate extra erythrocytes to compensate for the thin air. These extra erythrocytes are stored in the spleen. Then they would be released when needed to deal with exertion. This was also supported by the work of Pinkus et al. (1986) in their study on human spleen.

A significant increase in white blood cells and blood platelets count that were accompanied by an enlargement of the white pulp masses was detected one day after the end of exposure to SMF.

It is clear that a splenic white pulp represents an active site of lymphocytic cell proliferation. This is supported by the most recent studies by Kaszuba et al.(2008) and Mohammadnejad et al. (2010),who reported that actively lymphocytic proliferaton are more sensitive to environmental factors including magnetic fields.

The increase in haemoglobin and haematocrit seven days after sub-acute exposure to SMF may be explained by the installation of hypoxia-like status which is probably resulting from the oxygen binding impairment of haemoglobin or iron metabolism disruption. Thus, exposure to SMF decreased the serum iron level. This is in accordance with previous studies showing that exposure to electromagnetic field induced a decrease in blood iron (Stashkov & Gorokhov, 1998; Nourmohammadi et al., 2001). Similarly, Hachulla(2000) reported that iron was decreased in plasma of French population living near riverside high-voltage transmission lines.

It is well documented that transferrin controlled transit of iron since intestinal enterocytes increase medullar erythroblasts and allowed recovery of iron after destruction of erythrocytes by macrophagic system (Wagner, 2000).

The increase of haemoglobin and red blood, seven days after exposure to SMF may be explained by the hypoxia-like status. However, the precise way

in which SMF induced hypoxia-like status has not yet been fully clarified. The hypothesis of an action of SMF on the geometrical conformation of haemoglobin was reinforced by the fact that SMF induced a prominent effect on the haemoglobin structure as previously demonstrated by **Amara et al. (2006)**.

Recent studies by **Hassan & Abdelkawi (2010)** showed that exposure of the animals to moderate and strong static magnetic fields induced change in the absorption spectra and conductivity measurements of hemoglobin molecules. Furthermore, they found different degrees of globin unfolding. They regarded them as a sign of molecular destabilization. This reflects the function of haemoglobin which would be converted from oxy-haemoglobin to non functional met haemoglobin with decreasing oxygen affinity.

After seven days following the end of exposure to SMF, the histopathology of splenic tissues revealed numerous dilated sinusoids containing some of erythrocytes and/or haemolysed blood. **Faine et al. (1999)** reported large zones of haemorrhage and some features of vascular congestion in the red pulps of infected spleens. In such cases, damage of the vascular walls may be a reason for altering the permeability of sinusoidal capillaries, allowing the leakage of red blood cells, their progressing to haemorrhagic areas and scattering throughout the red pulp. Thus congestion is very likely resulted from disruption of splenic vasculature.

In addition, the white pulps were numerous, fragmented and appeared diffused in an irregular manner throughout the red pulp, such pathologic consequences were confirmed haematologically by the significant increase of the number of white blood cells. Thus, it is clear that magnetic field affects the population of lymphatic cells and it has a suppressive effect on immune system.

These findings are in accordance with those of **Attia & Yehia (2002)** who reported a progressive depletion of splenocytes in the white pulp areas in addition to the fragmentation of the tissues. Numerous inflammatory cells, macrophages and megakaryocytes were also noted in the splenic parenchyma and sinusoids, seven and fifteen days after the end of exposure to SMF.

Regarding the increase in the number of macrophages, demonstrated in this study, it was clear that they are defensive and resistant cells. There were several reports showing that under stimulatory conditions and tissue damage, macrophages become more active (**Zidek et al., 1998; Cui & Benowitz 2009**) and their number increase (**Lissbrant et al., 2000**). On the other

hand **Simko et al. (2001)** have shown that magnetic field results in a significant increase of the phagocytic activity of macrophages.

Brown haemosiderin granules were seen in the cytoplasm of macrophages after thirty days following the end of exposure to SMF. These granules represent the result of haemolysis of red blood cells.

Haemosiderin was found in all cases that showed large zones of haemorrhage which resulted from disruption of splenic vasculature. Haemolysis of red blood cells results in the release of haemoglobin, which is then phagocytosed by macrophages and stored in the cytoplasm in the form of hemosiderin (**Damjanov, 1996; Faine et al., 1999**). Moreover, the ultrastructural appearance of haemosiderin pigments within siderosomes in splenic macrophages suggests that erythrocytes degeneration is contributed to be pigment production (**Ward & Reznik-Schüller, 1980**).

A significant decrease in most hematological parameters was reported after fifteen days following the end of exposure to SMF. Our data demonstrated that SMF exposure was associated with significant low levels of RBCs numbers and the haemoglobin was associated with a significant increase in serum iron level. These findings were further confirmed histopathologically in the spleen which showed splenic sinusoids with hemorrhagic or/and hemolysed blood and an increase of hemosiderin granules.

Based on Perl's Prussian blue reaction of iron, the haemosiderin granules observed in the dilated splenic sinusoids which were filled with haemolysed red blood cells. The accumulation of haemosiderin pigments in the spleen as iron storage organ is mainly due to the rapid and continuous destruction of erythrocytes with erythrophagia and breakdown of haemoglobin and its conversation to haemosiderin.

Histopathological features of the white pulps that revealed lymphoid depletion and others which were hyperplastic with proliferation of megakaryocytes. These features observed in this context were confirmed haematologically with significant increase in the blood platelets and WBCs after 15 days following the end of exposure to SMF.

Henrykowska et al. (2009) indicated that exposure to magnetic field induced oxidative stress and free radicals generation in human blood platelets, producing a number of adverse effects and thus may lead to systemic disturbances in the human body.

Thirty days following magnetic exposure, the splenic tissues appeared almost normal and manifested a tendency towards recovery.

Conclusion,

Several experiments are still necessary to elucidate which frequency, intensity, exposure time and other parameters involved with SMF in order to be safe, especially, these concurrent with the environmental pollutants. In turn, we should protect ourselves against this pollutant.

Corresponding author

Mervat S. Zaghloul
Zoology Department, Faculty of Science, Benha University, Benha Egypt
mervat2009@hotmail.com

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