BIOPHYSICAL AND BIOCHEMICAL CHANGES IN THE CHARACTERISTICS OF RAT BLOOD EXPOSED TO COMBINED ALTERNATING AND STATIC MAGNETIC FIELDS (*IN VIVO* STUDY)

THANAA E. SHALABY, M. M. SHAWKI

Biophysics Department, Medical Research Institute, Alexandria University, Egypt

Abstract. The present work is devoted to study the effect of exposing experimental male albino rats to a combined static and extremely low frequency (ELF) alternating magnetic fields of average intensity 3 mT, to evaluate the risk on human workers who occupationally exposed to similar fields. A total of thirty male albino rats were equally divided into three groups, A, B, and C. Group A served as control group. Group B was exposed to static and extremely low frequency magnetic fields of 3mT, 8 hours/day for two successive weeks. Group C was exposed to the same condition as group B for four successive weeks. Osmotic fragility of RBCs, dielectric dispersion of hemoglobin (Hb) at frequency range of $12–10^5$ Hz, Hb absorption, Hb electrophoresis, plasma alanine transaminase (ALT), aspirate transaminase (AST), malondialdehyde (MDA) levels and red blood cells (RBCs) catalase activity were carried out for all groups. The results indicated that exposure of animals to MFs of 3 mT results in increase in RBCs osmotic fragility and decrease in its membrane elasticity, a partial change in Hb molecular structure without change in electrophoretic band of Hb, increase in ALT and AST levels in plasma indicating some damage in liver cell membrane. The increase in MDA level and catalase activity indicates an increase in free radicals level.

Key words: static and ELF alternating magnetic fields, osmotic fragility, dielectric dispersion, hemoglobin, electrophoresis, MDA catalase, ALT, AST, free radicals.

INTRODUCTION

Most of theories addressing the mechanism of interaction between biological system and magnetic fields (MFs) suggest that, the plasma membrane, by virtue of its bioelectrical properties, is the site, where MFs exert their primary effect [14]. The flow of Ca^{+2} across the cell membrane in response to extra-cellular signals is an important means of transmitting signals from the outside to the interior of the cell [12].

Baureus *et al.* [2] studied the influence of ELF magnetic fields on the transport of Ca^{2+} in a biological system consisting of highly purified plasma membrane vesicles. The authors showed that suitable combinations of static and

Received May 2006;
in final form June 2006.

ROMANIAN J. BIOPHYS., Vol. 16, No. 3, P. 169–180, BUCHAREST, 2006

time varying magnetic fields directly interact with the Ca^{2+} channel protein in the cell membrane, and they also confirmed this model quantitatively.

Static and ELF MFs were hypothesized to selectively act on cell signaling, through their effects on charged matter motion, thus influencing cell survival mechanisms in transformed cells that are characterized by electrical disorders [21]. Yost and Liburdy [23], tested the hypothesis that ELF alternating magnetic fields acted in combination with static magnetic field to alter calcium signaling in the lymphocytes.

The aim of the present work is to study the effect of exposure of rats to a combined alternating and static magnetic fields of average intensity 3 mT on some biophysical and biochemical changes of rat blood, as a step forward, to evaluate the risk from occupational exposures of man to similar fields.

MATERIAL AND METHODS

EXPOSURE FACILITIES

The MF exposure was provided by a device that produces static and alternating MFs generated by two coils. This device was locally manufactured. Each coil has a mean diameter of 23.7 cm and 2100 copper wire turns. The resistance and the diameter of the wire used were 21.7 Ohm per 1000 meter and 1.1 mm respectively. The two coils separated by a distance of 11.5 cm in which animals were placed. One coil was connected to a variac fed from a main source of (220 V, 50 Hz) to supply AC current of 0.25 A, from which the alternating MF was obtained, the other coil was connected to a variac and high ampere rectifier bridge (NI-630) to supply DC current of 0.25 A, from which static MF was obtained. The magnetic field intensity of the alternating magnetic field was measured using a homemade search coil (0.1 mm in diameter and 100 turns). The field intensity of static magnetic field was measured using Teslameter model ("Laboratorio elettrofisco", Nervinao, Italy, model CTM2000 with a 3.5×12 mm^2 Hall probe), at different distances from the coil. The animal exposure unit is made of plexiglas with dimensions of 15 cm $\times$ 15 cm $\times$ 10 cm. This unit was placed between the two coils. The overall exposure of the combined fields AC (1.54 mT) and the static field (1.46 mT) was 3 mT.

EXPERIMENTAL ANIMALS

Thirty male albino rats of 150 g average weight were used throughout the study. The animals were housed in plastic cages that permit normal ventilation and daylight, with diet and water available *ad libitum*. The animals were divided equally into the following groups:

Group A: served as control group.

Group B: was exposed to the magnetic fields of 3mT; 8 hours/day, 6 days/week. The total exposure period was 2 successive weeks.

Group C: was exposed to the magnetic fields of 3mT; 8 hours/day, 6 days/week. The total exposure period was 4 successive weeks.

At the end of the exposure time, all rats were sacrificed by cervical decapitation, and the blood was collected using heparinized syringe. The plasma and hemoglobin were obtained [16]. Heparinized blood was centrifuged at 1100 g for 10 minutes at 4 ºC using a temperature-controlled centrifuge. The packed RBCs were washed with five volumes saline-buffered solution and gently agitated for two minutes then centrifuged to separate the washed cells. The former step was repeated three times, part of the cleaned RBCs was lysed with five volumes of distilled deionized water. The following measurements were performed:

DIELECTRIC PROPERTIES OF HEMOGLOBIN

Dielectric measurements were run in the frequency range from 12 – 10^5 Hz using dielectric bridge (LCR meter istek-819 Japan). Hemoglobin solutions were placed in a homemade dielectric cell which contains two squared platinum electrodes with area of 1 cm^2, each with an inter electrode distance of 1 cm. The measurements were always done at constant temperature (20±0.5°C), the dielectric cell was electrostatically shielded using aluminum foil as to avoid any extraneous electromagnetic interference during the measuring process. The relative permittivity ε', the dielectric loss factor, ε'', and the conductivity, σ, were calculated [5]. The relative permittivity ε' for the sample was calculated at each frequency by the equation:

$$\varepsilon' = \text{Hemoglobin capacitance / Air capacitance} \tag{1}$$

$$\text{Air capacitance} = \frac{\varepsilon_0 d}{A} \tag{2}$$

where ε_0 is the vacuum permittivity which equals 8.85×10^{-12} F/m; A is the electrode surface area in meter square; d is the distance between the two electrodes in meter.

The dielectric loss factor, ε'', was calculated from the following equation:

$$\varepsilon'' = \frac{\varepsilon'}{2\pi fRC} \tag{3}$$

where f is the frequency in Hz; R is the resistance in Ω; C is the capacitance in Farad.

The conductivity, σ, was calculated using the following equation:

$$\sigma = \frac{d}{RA} \tag{4}$$

where R is the resistance in Ohm and σ the conductivity in Siemens/meter.

HEMOGLOBIN ABSORPTION SPECTRUM AND ELECTROPHORESIS

Hemoglobin absorption spectra of control and experimental rats were measured in the wavelengths range from 200–600 nm [17], using spectrophotometer (Shimadzu UV-model 1601 Spectrophotometer fitted with PC system). Hemoglobin bands were separated on cellulose acetate [20].

DETERMINATION OF THE OSMOTIC FRAGILITY OF RED BLOOD CELLS

Osmotic fragility test of RBCs was measured [22], the test was carried out within 1 hour of collection. The whole blood was added to varying concentrations of buffered NaCl, (pH = 7.4 and temperature kept at 25 ºC). The hemolysis percentage (% H) was calculated from the following equation:

$$\% H \text{ at certain concentration} = \frac{A \text{at this concentration}}{A \text{of } 0\% \text{ tube}} \times 100 \qquad (5)$$

where A is the supernatant absorption using spectrophotometer (Shimadzu UV-model 1601 Spectrophotometer) at 540 nm.

BIOCHEMICAL ASSESSMENT

The biochemical parameters determined were: alanine aminotransferase (ALT) [10], asparate aminotransferase (AST) activities [18], malondialdehyde (MDA) concentration of blood plasma [4] and catalase activity in RBCs [7].

STATISTICAL ANALYSIS

Statistical analysis was performed to obtain the mean and standard deviation. The data analysis was performed using the SPSS-10 package (release 3, SPSS Inc., Chicago III) running on MCROVAX 3500. ANOVA test was used to compare between the means of the different parameters of the three studied groups. A difference was considered significant at probability $p < 0.05$.

RESULTS

Figs. 1a, 1b and 1c illustrate the variations of log ε', log ε'' and the conductivity σ as a function of log frequency f in the range of 12–10^5 Hz, for control Hb and that extracted from mice exposed to MF of average intensity 3 mT for either 2 weeks (group B) or 4 weeks (group C). It is clear from figures that, there is an increase in the relative permittivity of group B as compared to that of

group A, with larger increase of group C than group B. On the other hand, no change occurred in the dielectric loss between group B and group A, while a small increase between group C and group B may be seen in this range of frequency. In case of conductivity curve, there is an increase in the conductivity of group B than group A, and larger increase between group C and group B.

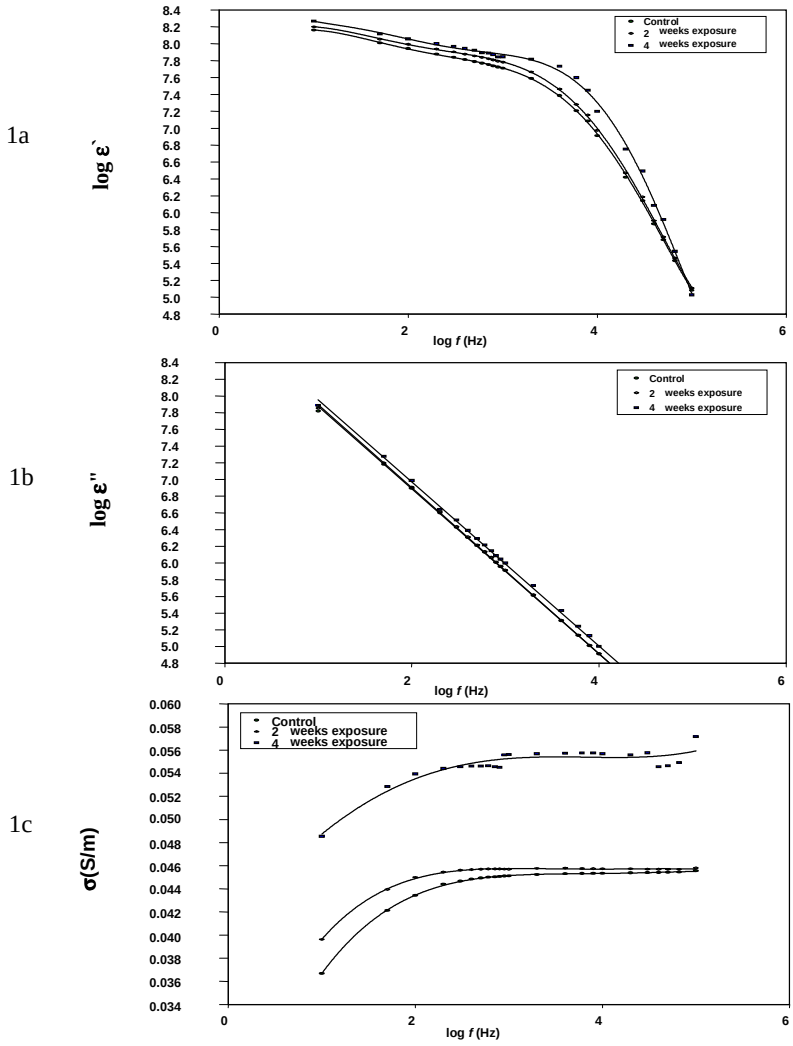

Fig. 1. The variations of log ε' (1a), log ε" (1b) and the conductivity σ (1c) as a function of log frequency *f* for Hb extracted from blood of all studied groups.

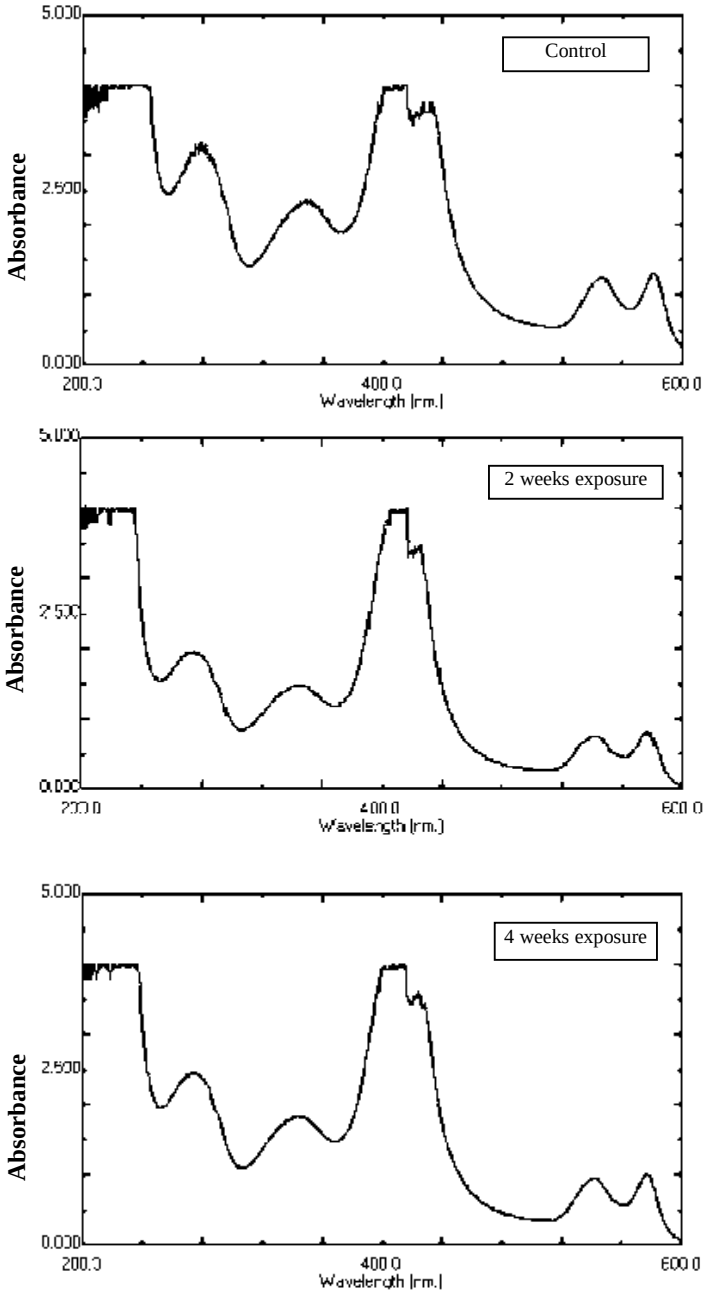

Fig. 2. Hemoglobin absorption spectra.

Hemoglobin absorption spectra (Fig. 2) indicate the appearance of the well-known hemoglobin characteristic bands at 220, 280, 340, 410, 540, and 578 nm. These bands correspond to aliphatic amino acids, aromatic amino acids, globin-heme interaction, soret band, nitrogen ion bonds in porphyrine rings and heme-heme interaction bands respectively. The figure indicates no obvious change in the absorbance of the main characteristic bands between the three groups. Figure 3 represents the Hb bands of the three studied groups.

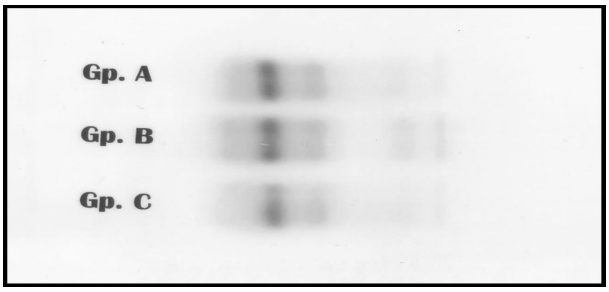

Fig. 3. Hemoglobin electrophoresis.

Figure 4 shows the results of osmotic fragility measurements for RBCs collected from animals of the different groups, where the percentage of hemolysed cells is plotted as a function of the concentration percentage of NaCl. For analysis of these results, the curves were differentiated and plotted as a function of NaCl concentration percentage as shown in Fig. 5. From Figures 4 and 5 it is possible to calculate median corpuscular fragility (MCF); (the NaCl concentration at which 50% of RBCs are hemolyzed) and the maximum half width ($W_{\text{h max}}$) as given in Table 1.

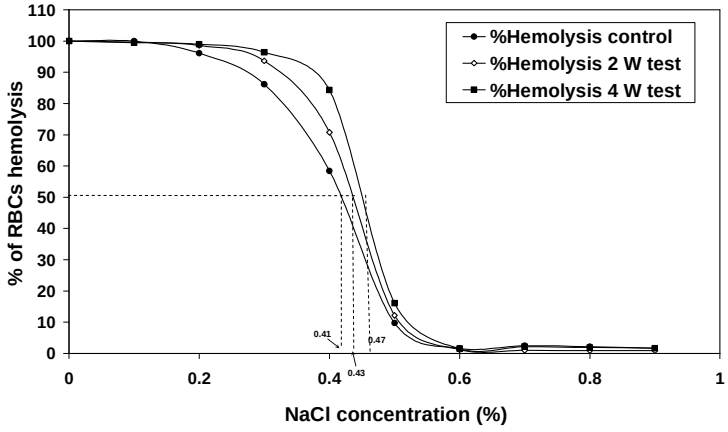

Fig. 4. Osmotic fragility curves.

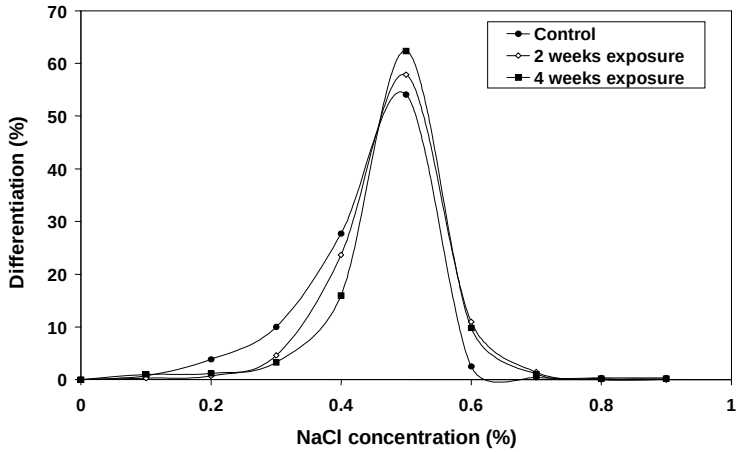

Fig. 5. Osmotic fragility curve differentiation.

Table 1

Red blood cells mean corpuscular fragility (MCF) calculated in g% NaCl, and the corresponding maximum half width ($W_{h\,max}$) as calculated from Fig. 5.

Group	MCF (g% NaCl)	W_{max} (arbitrary units)
A	0.41	1.2
B	0.43	1.1
C	0.47	0.9

Table 2 illustrates the measured biochemical parameters (ALT, AST, malondialdehyde and catalase) of the blood collected from control and exposed groups. It is clear from the table, the plasma ALT, AST levels were significantly elevated from 42.9±7.06 IU/L and 146.1±17.34 IU/L (plasma control) up to 53.6 ±4.2 IU/L and 165.8±13.78 U/L ($p < 0.001$) in group B, and up to 90.2±10.7 IU/L and 200.3±15.62 IU/L ($p < 0.001$) in group C. Malondialdehyde level in the plasma showed no significant increase in group B as compared to group A, the elevation was only 5.14%, but a significant increase in group C as compared to group A (23.4%) and group B (17.36%). Catalase activity in RBCs of blood of the three studied groups indicates a significant increase from 9.46±0.63 (10^3 IU/g Hb) in control group up to 10.25±0.41(10^3 IU/g Hb) in group B, and 10.57±0.42 (10^3 IU/g Hb) in group C ($p < 0.001$), this represents a percentage change of 8.42% and 11.83%, respectively.

Table 2

Change in ALT, AST (IU/L), malondialdehyde (nmol/mL) and catalase (10^3 IU/g Hb) levels in the blood collected from control rats (group A), and that exposed to a combined MFs of an average intensity of 3 mT (8 h/day, 6 day/week) for either 2 weeks (group B) or 4 weeks (group C).

Parameter		Group A	Group B	Group C
ALT (IU/L)	Mean ±SD	42.9±7.06	53.6 ±4.2	90.2±10.7
	F-value		101.479	
	p		0.001	
	% change	24.94		110.26
AST (IU/L)	Mean ±SD	146.1±17.34	165.8 ±13.78	200.3±15.62
	F-value		30.741	
	p		0.001	
	% change	13.48		37.1
MDA (nmol/mL)	Mean ±SD	2.04±0.09	2.15±0.09	2.52±0.17
	F-value		40.466	
	p		0.001	
	% change	5.14		23.4
Catalase (10^3 IU/gHb)	Mean ±SD	9.46±0.63	10.25±0.41	10.57±0.42
	F-value		13.458	
	p		0.001	
	% change	8.42		11.83

F is the statistical value of ANOVA test.

DISCUSSION AND CONCLUSION

The change in the RBCs osmotic fragility of exposed and unexposed animals may be due to magnetically induced changes in the properties of RBCs membranes. Modification in the physical condition of the proteins on the cell membrane may lead to change in the permeability of the RBCs membrane [8]. Some proteins on the cell membrane (ion channels) act as pores through which the liquid (water) bound to ions carried inside the cell. The applied MF may cause variation on the electrical charge distributed on the RBCs membrane. The redistribution of the electrical charges may affect the ion channels proteins as a cause of unbalance in the ionic concentration, which may lead to change in osmotic fragility. Electromagnetic force (e.m.f.) produced by MFs can increase the amount of intracellular cations, leading to an increase of the influx of water into the cell. The increase of water into the cell leads to an increase in the hydrostatic pressure on the inner cell membrane that usually ends with hemolysis [13]. The increase of water into the cell may be due to a cationic accumulation provoked by a reduction Na^+ channel activity, caused by inhibition of ATP synthesis or even by an enzymatic (Na^+-K^+-ATPase) inactivation [11]. The Na^+-K^+-ATPase, an integral protein of outer cell membrane, supports the ionic hemostasis of the cell which is under control of Na^+, K^+, Mg^+ and ATP [3].

The width at half maximum of the differential plot ($W_{h\,max}$) as shown in Fig. 5 (Table 1), represents the relative decrease in the elastic limit of RBCs' membrane. The increase in $W_{h\,max}$ will represent the increase in cellular membrane elasticity, so the elasticity of RBCs membrane decreased as a result of MFs exposure.

It is well known [1] that, RBCs have to be squeezed and deformed to pass in blood capillary vessels of diameter smaller than that of RBCs itself, the degree of squeezing of RBCs depend mainly on their membrane elasticity. Therefore, the decrease of the RBCs membrane elasticity will lead to the increase of the blood capillary resistance for RBCs passage to the body cells for carrying normal metabolism, and hence toxicity in some organs may occur [1]. It is clear from the Table that, the change in the mechanical properties of RBCs membrane proved to be time dependent.

The electrophoretic pattern of Hb molecule in all groups remains unchanged in the Hb absorption band at wavelengths 220, 400 and 540 nm for rats exposed to a combined static and alternating MFs for either two or four weeks with respect to that of the control group. But a partial increase of the Hb absorption bands at wavelengths 280, 340 and 578 nm that represent aromatic amino acids, globin-heme, heme-heme interactions respectively, was noticed in group C against group B against group A, indicating partial loss of Hb molecule stability.

The study of the dielectric properties of hemoglobin of rats exposed to combined static and alternating MFs for either two or four weeks showed an increase in the Hb conductivity against control (Fig. 1c). This change indicates a partial charging of the hemoglobin (Hb) molecule. The change in conductivity indicates that, exposure of the animals to the MFs demonstrated caused partial structural changes in their Hb molecules, which may affect their properties and hence the RBCs physiological function [1].

According to the radical pair mechanism [9] the free radicals lifetime in the animal's body increase due to exposure to magnetic fields. This mechanism explains the increase in the RBCs catalase activity in the 4 weeks exposed as compared to the control one. In fact, the increase in catalase activity is considered a defense mechanism to protect the body against hazards due to an increased level of free radicals. The same mechanism may explain the increase in malondialdhyde concentration in the 4 weeks exposed group by a noticeable percent as compared to the 2 weeks exposed group and the control group (32.40% and 5.14%, respectively). The increase in the stability of lipid peroxidation intermediates increases the chain reaction of unsaturated fatty acids leading to accumulation of its end product, which is malondialdehyde. The increase in malondialdehyde is in turn accompanied by increase in other free radicals as peroxide radical leading to an increase in the antioxidants concentration such as catalase which metabolizes H_2O_2 to water. There is wide acceptance among researchers that exposure to the magnetic field causes an increase in the free radicals leading to an increase in antioxidants concentration [24].

In rats exposed to MFs, a marked increase in AST and ALT activities in plasma was observed, indicating a form of hepatic injury as a direct effect of MFs.

ALT and AST concentrations increased in the 2 weeks exposed group as compared to the control by 24.94% and 13.48%, respectively. When the stress is further increased in the 4 weeks exposed group, more cell membranes were damaged resulting in an increase of ALT and AST compared to control by 110.26% and 37.10%. Our data are in agreement with those obtained by Taha and Mohamed [19]. This elevation initiates the process of lipid peroxidation in the plasma membrane adding to disturbance of membrane permeability and subsequent electrolyte imbalance [6]. Progress in magneto-chemistry refers the effects of MFs to the chemical reactions involving free radicals. Magnetic fields may increase the average radical concentration, lengthen their lifetime and enhance the probability of radical reaction with cellular components [15].

Conclusively, magnetic fields possess the potentials to induce hazardous biological effects in rats. The main damaging role of magnetic fields may be on the cellular membrane. The change in the mechanical properties of RBCs membrane as a result of whole body exposure of the animal to MFs is time dependant. And the change in RBCs physiological function may damage other organs such as liver and other critical organs.

REFERENCES

1. ALI, F. M., S. MOHAMED, M. MOHAMED, Effect of 50 Hz, 0.2 mT magnetic field on RBC properties and heart function of albino rats, *Bioelectomag*., 2003, **24**, 535–545.
2. BAUREUS, K., M. SOMMARIAN, B. PERSSON, L. SALFORD, J. EBERHARDT, Interaction between weak low frequency magnetic fields and cell membrane, *Biolectromag*., 2003, **24**, 395–402.
3. BOLDYREV, A.A., Functional-activity of Na+-K+-pump in normal and pathological tissues, *Molecular and Chemical Neuropathology*, 1993, **19**, 83–93.
4. DRAPER, H., M. HADLEY, Malondialdehyde determination as an index of lipid peroxidation, *Met. Enzymo*., 1984, **105**, 114–118.
5. GABRIEL, C., E. CORTHOUT, The dielectric properties of biological tissues, *Phys. Med. Biol*., 1996, **41**, 2231–2249.
6. GULCH, K.W., O. LUTZ, Influence of strong static magnetic fields on heart muscle contraction, *Phys. Med. Biol*., 1986, **31**, 763–769.
7. JOHANSSON, L., L. BORG, A spectroscopic method for determination of catalase activity in small tissue samples, *Anal. Biochem*., 1988, **17**, 331–336.
8. KUCHEL, P.W., A. COY, P. STIBS, NMR "diffusion-diffraction" of water revealing alignment of erythrocytes in a magnetic field and their dimensions and membrane transport characteristics, *Magnetic Resonance in Medicine*., 1997, **37**, 637–643.
9. KULA, B., A. SOBCZAK, R. KUSKA, Effects of static and ELF. Magnetic fields on free radical processes in rat liver and kidney, *Electro Magneto Biol*., 2000, **19**, 99–105.
10. MIGUEL, L., J. BEDINI, A. ORDINAS, Study the serum alanine aminotransferase in blood donors in Spain, *Hematologica*, 1998, **83**, 237–239.
11. NAGINI, S., S. SELVAM, Biochemical indicators of membrane damage in the plasma and erythrocytes of rats fed the peroxisome proliferator di(2-ethylhexyl)phthalate, *Medical Science Research*, 1997, **25**, 119–121.
12. POLLARD, T., W. EARNSHAW, Membrane channels, In: *Cell Biology*, 1st ed., USA, Sounders, 2004, pp.125–148.
13. RABINI, R.A., E. PETRUZZI, R. STAFFOLANI, M. TESEI, P. FUMELLI, M. PAZZAGLI, L. MAZZANTI, Diabetes mellitus and subjects agening: A study on the ATP content and ATP-

related enzyme activities in human erythrocytes, *European Journal of Clinical Investigation*, 1997, **27**, 327–332.

14. ROSEN, A.D., Mechanism of action of moderate-intensity static magnetic fields on biological systems, *Cellular Biochemistry Biophysics*., 2003, **39**, 163–173.
15. SCAIANO, J.C., F.L. COZENS, J. MCLEAN, Model for rationalization of magnetic field effects in vivo. Application of the radical pair mechanism to biological systems, *Photochem. Photobiol*., 1994, **59**, 585–589.
16. SCHMIDT, R., Laboratory diagnosis of hemoglobinopathies. In: *Hematology. Clinical and Laboratory Practice*, Brick L. (ed.), 1st ed. USA, Mosby, 1993, pp. 327–371.
17. SHEEHAN, D., Spectroscopic techniques, In: *Physical Biochemistry. Principles and Applications*, 3rd ed., England, J. Wiley Ltd, 2000, pp. 61–78.
18. SPOTO, G., M. FIORONI, N. CORRADO, M. TIRIPODI., D. ADRIANO, Aspartate aminotransferase activity in human health and inflamed dental pulps, *J. Endodent*., 2001, **27**, 394–395.
19. TAHA, A., H. MOHAMED, The effect of magnetic field on the biochemical parameters of mice blood, *Pak. J. Med. Sci*., 2003, **19**, 36–40.
20. TAREK, M., R. FREDERIC, Erythrocytic disorders. In: *Clinical Diagnosis and Management by Laboratory Methods*, 18th ed., USA, Bernard M, Saunders W Company, 1996, pp. 617–663.
21. TOFANI, S., M. CINTORINO, D. BARONE, A. FERRARA, P. OSSOLA, K. ROLFO, F. ROLFO, F. RONCHETTO, S.A. TRIPODI, F. ARCURI, F. PEROGLIO, Tumor growth inhibition, apaptosis and loss of p53 expression induced in vitro and in vivo by magnetic fields, *Proc. Amer. Assoc. Cancer Res*., 1999, **40**, 488–496.
22. TURGEON, M., Manual procedures in hematology and coagulation. In: *Clinical Hematology. Theory and Procedures*, 2nd ed., England, Brown L Company, 1993, pp. 336–367.
23. YOST, M., R. LIBURDY, Time varying and static magnetic fields act in combination to alter calcium signal transduction in the lymphocytes, *FEBS Lett*., 1992, **296**, 117–122.
24. ZHADIN, M, F. BAMES, Frequency and amplitude windows in the combined action of DC and low frequency AC magnetic fields on ion thermal motion in a macromolecule; a theoretical analysis, *Bioelectromag*., 2005, **26**, 323–330.