VITAMIN E REDUCES THE EFFECT OF MAGNETIC FIELD ON DEPRESSION IN THE BLOOD PARAMETERS AND IN THE INTEGRITY OF ERYTROCYTES IN ALBINO MICE

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Abstract. In this work the effect of sinusoidal 50 Hz, 2 mT magnetic field generated by electrical devices was evaluated on the blood parameters, erythrocyte counts (red blood cells/ RBCs), hemoglobin content (Hb), toxicity, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), white blood cells (WBCs), platelet counts (PLT), osmotic fragility test, and blood film test. Three groups, each of 10 mice were used, group one used as unexposed (control), group two exposed to the field for total period (45 day, 2 hours/day) and group three exposed to the same field and period after adding vitamin E (20 mg/kg) body weight (b.wt.) in the drinking water. The result showed a significant depression in the blood parameters of group two relative to control group, but for group three blood parameters are improved. In addition, the hemolytic percentages were (0.40 %, 0.30 %, 0.35 %) for the control (group one), exposed (group two), protected (group three), respectively. Blood film showed low stainable leucocytes and crenate damaged erythrocytes for group two but for group three erythrocytes nearly restored their integrity. The results recorded showed that the side effects of magnetic field on the blood of mice decreased after using antioxidant vitamin E.

Key words: Electromagnetic field, blood parameters, osmotic fragility, blood film, vitamin E.

INTRODUCTION

Wherever there are electric current, electric motors and electronic equipment are able to induce electromagnetic fields (EMFs) in our homes, at work and in school [28].

Prolonged exposure to 50 Hz, 2 mT magnetic fields is biologically toxic in rats. The main damaging role of the 50 Hz magnetic fields may be on the cellular membrane that plays an important role on the physiological functions of the cells

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and on the cell-to-cell communication. The changes in the mechanical properties of RBCs membrane as a result of the whole body exposure of the animal to the magnetic field for prolonged periods may damage the heart muscle and other critical organs [5].

Chromosomes aberration, micronuclei, sister chromatid exchanges and DNA strand-breakage, induced by extremely low frequency electromagnetic field (ELF-EMF) exposure levels ranging from 1 mT to 10 mT [8, 10, 26].

Abd El Ghany [1] and Liu. [21] indicated that the cell membrane properties were highly affected by the magnetic fields, which proved that it was biologically toxic and has anxiogenic effects. Elsayed [12] concluded that exposure to ELF-EMF for long time has hazardous effects on RBCs membrane, and is also affecting blood viscosity, therefore it may be hazardous to physiotherapists. Also, static magnetic field (SMF) have side effects on hematological, hepatic and renal systems [14].

Amara *et al.* [6] and Okano [27] concluded that SMF exposure has induced different metabolic and hematological effects, which appeared to be related to the duration of exposure. The change in biochemical parameters of SMF-exposed rats reflected probably hepatic damage.

Mohammad Nejad *et al.* [25] reported that actively lymphocytic proliferation is more sensitive to environmental factors including magnetic fields. Thus, exposure to SMF decreased the serum iron level.

Studies on the blood of the rates [18] might provide guidance for the assessment of the occupational toxicity and the public health significance after exposure to EMFs. The results showed that EMF produces pronounced changes in the molecular structure of hemoglobin and induced force acting on the charged particle, which may activate rouleaux formation of RBCs [8].

In this study we used vitamin E, which can be found naturally in some foods, added to others and available as dietary supplement. Vitamin E is the collective name for a group of fat-soluble compounds with distinctive antioxidant activities [34].

Antioxidants protect cells from the damage effect of free radicals, which are molecules that contain an unshared electron. The existence of damage cells caused by free radicals might contribute to the development of cardiovascular disease and cancer. Unshared electron is highly energetic and react rapidly with oxygen to form reactive oxygen species (ROS) [35]. Vitamin E refers to the plant-derived, lipid-soluble antioxidants: tocopherols and tocotrienols. The best-established bio-chemical function of vitamin E is its action as a lipid antioxidant [29].

Biochemical studies on the mechanism of antioxidant action of vitamin E were described by Packer [29] in a review that present status of vitamin E as an antioxidant. In this paper is underlined the antioxidant action of vitamin E on breaking of propagation chains of free radicals.

Biological activity of vitamin E is different from its antioxidant activity, and there is a preference for alpha-tocopherol. This preference is achieved through the selective degradation and excretion of other vitamin E forms and the selective retention of alpha-tocopherol, mediated by the hepatic alpha-tocopherol transfer protein [22].

The aim of the present study was to throw light on the changes of RBCs membrane structure in blood of mice exposed to ELF-EMF and also to study the action of antioxidant vitamin E on reducing the side effects of ELF-EMF. First, there were measured several hematological parameters in all three groups of mice, such as erythrocyte counts, hemoglobin content, toxicity, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, white blood cells and platelet counts. Secondly, the mechanical properties of cell membrane were studied by the osmotic fragility test. Third, the changes in the morphological shape of blood cells were studied by blood film test.

MATERIALS AND METHODS

EXPERIMENTAL ANIMALS

In the present study, 30 healthy Swiss albino mice males (3 months old) with an average weight 30–45 gram were used [17]. The mice have been obtained from the breeding farm, Faculty of Veterinary Medicine, Zagazig University. Animals were kept under standard hygienic measures against infection diseases in plastic cages. Animals were classified into 3 groups, each of 10 mice.

Group one (control)

Mice were maintained in the same experimental condition but without the magnetic field.

Group two

Mice were exposed to EMF at 2 mT strength, 50 Hz frequency. The plastic cage with mice were placed in the middle of the magnetic field device.

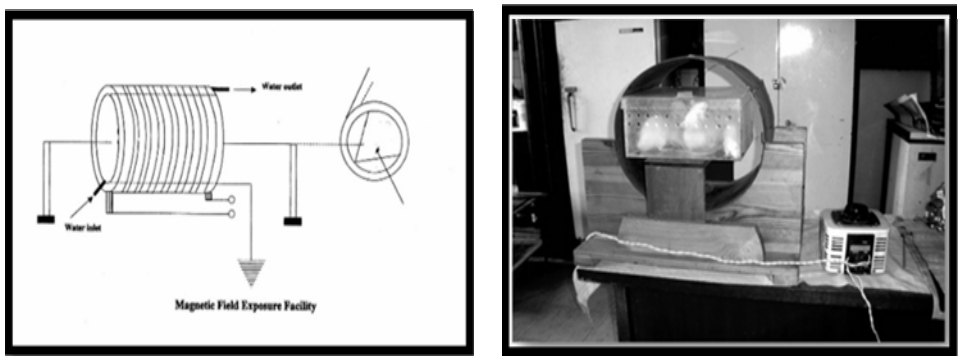
Group three

Mice were exposed to EMF at 2 mT strength, 50 Hz, after adding 20 mg/kg b.wt. vitamin E as antioxidant reagent according to Sharma and Giupta [32].

Animals of group two and three were exposed to EMF daily for a period of 45 days at a rate of 2 hours/day. The total exposure period was 90 hours at the end of experimental period. It started from 8 a.m. to 10 a.m. every day.

THE EXPOSURE APPARATUS

Homogenous magnetic field generator (Fig. 1) was designed and constructed in Cairo University in the electronic center and represents a solenoid, consisting of coil 320 turns from electrically insulated 2 mm thick cupper wire, twisted in a homogenous way around a cupper cylinder of 2 mm thick, 40 cm diameter and 40 cm in length [3, 4, 13, 30]. The cylinder wall was earthed. The ends of the coil connected to an autotransformer (Variac, U.S.) fed from the mains (220 V and 50 Hz). The field strength was adjusted by changing the voltage across the coil using the autotransformer as shown in Figure 1. Mice from group two and three were maintained in plastic cages that permit normal ventilation and the cages were placed in the middle of the cylinder coil to get homogenous and higher magnetic field exposure. Group one was kept in similar plastic cages that permits normal ventilation and housed in similar condition (shame expose), but without magnetic field [17].



(A) (B)

Fig. 1. Images of the electrical system used in experiments to generate EMF: (A) diagram of the magnetic exposed system (B) the image shows the animal which were exposed continuously as a group in plastic cage on the shelf within the solenoid [3, 4, 13, 30].

HEMATOLOGICAL STUDIES

Mice were anesthetized with ketamine (Sigma, Madrid, Spain). Two blood samples were obtained from the retro-orbital venous plexus of mice at 45 days from the beginning of the experiment. The samples were collected either on 0.2 % EDTA anticoagulant for evaluation of erythrocyte (RBCs count, Hb content, HCT, MCV, MCH and MCHC) and WBC (total and differential leukocytes count or on

ammonium oxalate anticoagulant for platelets count, using standard techniques, according to Lawis Mitchell [20].

OSMOTIC FRAGILITY

Osmotic fragility test of the whole blood was performed for control and experimental groups according to Dacie and Lewis [11]. The amount of hemolysis was then determined by reading the supernatants absorbance using a UV/visible spectrophotometer (Jasco V-530, made in Japan) [9].

PREPARATION OF BLOOD FILM

By the wedge method, a drop of well mixed blood without any EDTA (minimum of 10 gentle inversions) is placed on the base of a slide close to one end (about 1 cm from the edge) with a pipette/capillary tube. Then, a smear is made with the spreader inclined at an angle of about 30 to 45 degrees to the blood [33].

STATISTICAL ANALYSIS

Data collected throughout, laboratory investigations and outcome measures coded, entered, and analyzed using Microsoft Excel software. Data were then imported into Statistical Package for the Social Sciences (SPSS version 20.0) software for analysis. According to the type of data, quantitative continues group represented by mean ± SD, the following tests were used to test differences for significance. Differences between parametric quantitative multiple by ANOVA probability value p value was set at $p < 0.05$ for significant results and at 0.001 for a high significant result.

RESULTS

BLOOD PARAMETERS LIST

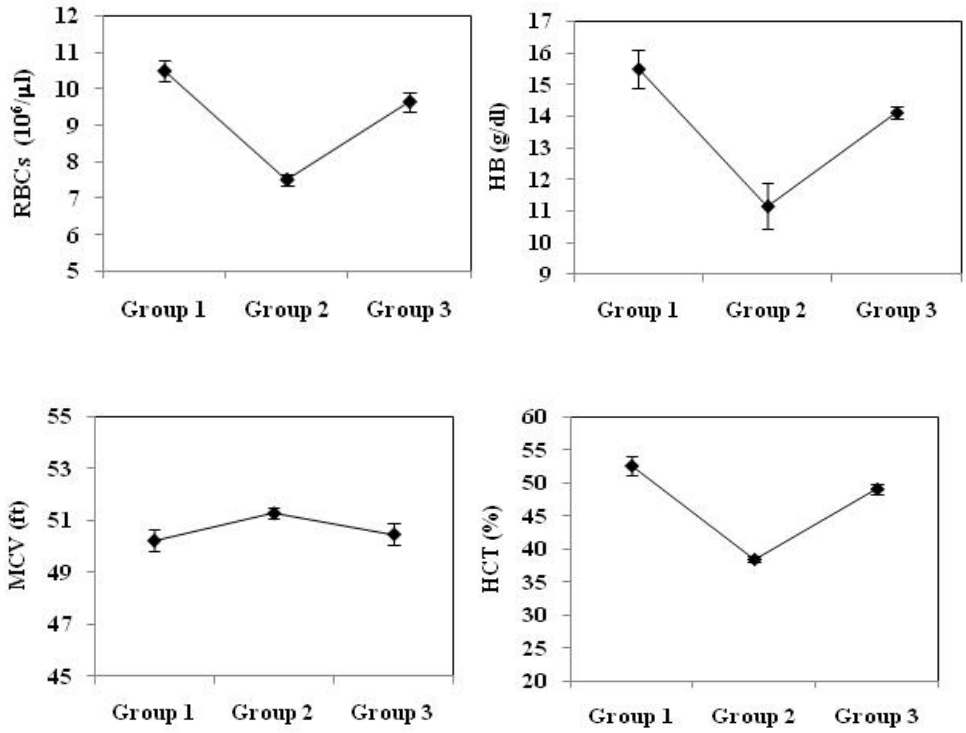
The mean values of blood parameters (RBCs, HB, HCT, MCV, MCH, MCHC, PLT, LCT) are shown in Table 1 and Figure 2 for the three groups at $p < 0.05$. It is clear that there is a significant decrease in the blood parameters of group 2 relative to the control group. Also, it is clear that for the parameters of the protected group with vitamin E (group three) the results indicated an improvement in all the parameters due to the fact that the mice drank water with antioxidant vitamin E. Anemia, one of more common blood disorders, occurs when number of

red blood cells or protein in takes are inadequate [24]. The results in Table 1 explain that the rate of number of RBCs for control group was 11.16±0.29 × 10^6 µL and for group two was 7.5±0.15 × 10^6 µL, but for protected group (group three) it was improved and began to increase similar to the control group 9.65±0.26 × 10^6 µL.

Table 1

Blood parameters (mean ± S.E.M) under the effect of EMF (2 mT) compared with the control male albino mice

Blood parameters	**Control**	**Treated**	**Protected**	***p***
RBCs (×10^6/µL)	11.16±0.29	7.50±0.15	9.65±0.26	0.0001
Hb (g/dL)	15.70±0.61	11.16±0.74	14.12±0.1	0.001
HCT (%)	52.64±1.48	38.50±0.35	49.10±0.78	0.0001
MCV (fL)	50.24±0.5	51.28±0.19	50.48±0.51	0.244
MCH (pg)	15.14±0.29	14.60±0.15	14.66±0.11	0.002
MCHC (g/dL)	30.60±1.14	28.48±0.35	29.20±0.83	0.006
PLT (×10^3/µL)	865.40±4.21	649.40±15.5	787.80±26.5	0.00001
LCT (×10^3/µL)	5.54±0.23	3.98±0.24	4.40±0.22	0.0001



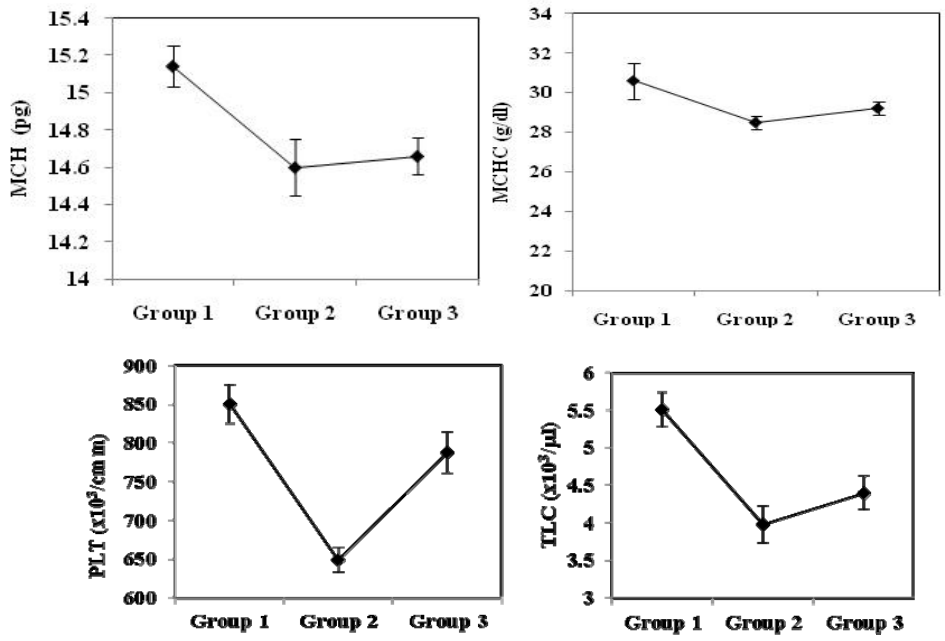


Fig. 2. Detection of the changes in blood parametars for the three groups.

From the above results, it is noticed that there is a decrease in the amount of hemoglobin (from 15.70±0.61 to 11.16±0.74 g/dL) due to decreasing of RBCs number after exposing the rats to ELF-EMF (group 2) compared with control group. For protected group, hemoglobin level was improved and began to increase similar to the control group.

OSMOTIC FRAGILITY TEST

The osmotic fragility test is employed to help diagnosis different types of anemia the physical properties of the red blood cell are altered. The main factor affecting the fragility test is the shape of the RBCs, which in turn is dependent on the volume, surface and functional state of the red blood cell membrane, as shown in Figure 3.

It is clear from Table 2, that the hemolysis percentages were 0.400±0.014 %, 0.300±0.014 %, and 0.350±0.017 % for the three groups, respectively. For mice of group two, we noticed that the hemolysis % was faster (0.3 %) and rupture will take place much more quickly than control group (0.4 %) and protected group (0.35 %) at 5.5 % concentration of NaCl as shown in Figure 3. Also, Table 2 explains that the changes are highly significant at ($p < 0.05$).

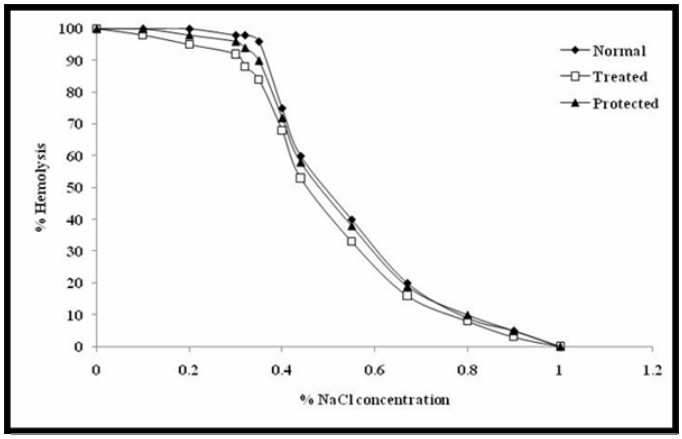


Fig. 3. Hemolysis % of blood samples from group 1, 2, and 3.

Table 2

Hemolysis % (mean ± SD) under the effect of EMF (2 mT) compared with the control male albino mice

Groups	**Hemolysis %**	**SD**	***p***
Control (group 1)	0.400	±0.01414	0.0001
Treated (group 2)	0.300	±0.01414	0.0001
Protected (group 3)	0.350	±0.01710	0.0001

BLOOD FILM

Blood film of control mice (group one)

Figure 4 represents the morphological shape of normal RBCs for direct blood smear of control group which showing intact leucocyte (curved arrow) and erythrocytes (straight arrow).

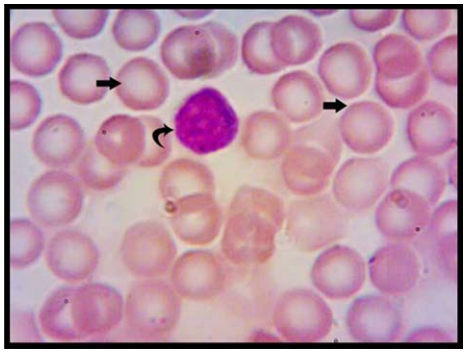


Fig. 4. Blood film of normal mice showing intact leucocyte (curved arrow) and erythrocytes (straight arrow).

Blood of EMF-exposed mice (group two)

Figures 5 and 6 represent fragmented leucocytes (curved arrow), crenated RBC_s (straight arrows) and other erythrocytes in rouleaux formation (arrow head) also indicates the presence of abnormal shape and irregularity in the cell membrane of the blood of group two and the absence of electrostatic positive charge on the surface of RBCs cellular.

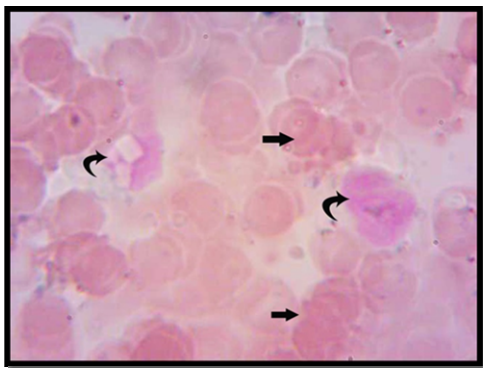


Fig. 5. Blood film of EMF-exposed mice showing damaged and low stainable leucocytes (curved arrows) and crenated and damaged erythrocytes (straight arrows).

The results showing the membrane dysfunctions, which lead to sticking cells together and forming one common membrane.

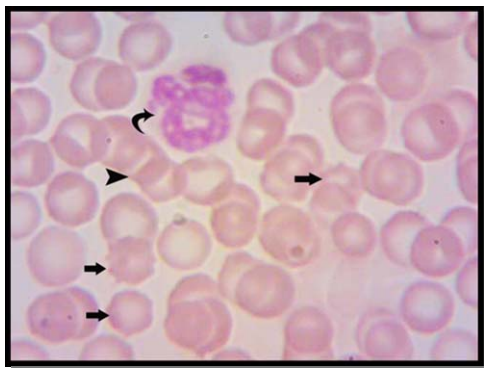


Fig. 6. Blood film of EMF-exposed mice exhibiting fragmented leucocytes (curved arrow), crenate RBCs (straight arrows) and other erythrocytes in rouleaux formation (arrow head).

Blood of EMF-exposed mice and presented with vitamin E (group three)

In Figure 7, the blood film from ELF-EMF-exposed mice, protected with vitamin E, is presented, showing leucocyte (curved arrow) and erythrocytes (straight arrows) nearly restoring their integrity.

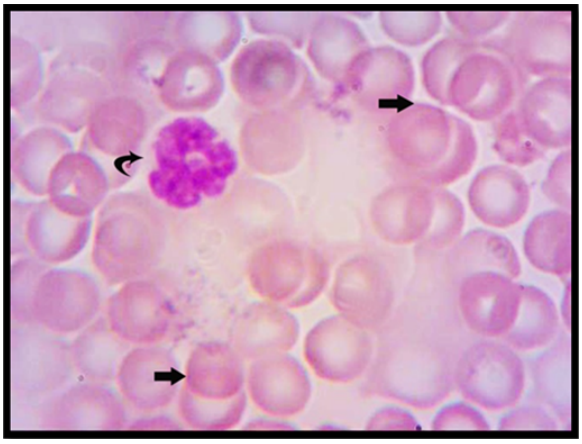


Fig. 7. Blood film of EMF-exposed mice protected with vitamin E showing leucocyte (curved arrow) and erythrocytes (straight arrows) nearly restoring their integrity.

DISCUSSION

The presented work was directed to study the biological changes on the structure properties of blood cells collected from mice exposed to magnetic field 2 mT especially focused on the changes of the mechanical properties of RBCs membrane.

On the other hand, during the last years, the background effects of ELF-EMF, generated from power lines and electric appliance, have both got the interests of scientists and the matter of public concern. Several recent published works found that even small magnetic field strengths at 50 Hz are harming to biological systems [5, 19].

Protection of cells from the damaging effects of free radicals, which are molecules that contain an unshared electron are the aim of scientists. Have been reported elevated levels of free radicals from the cells exposed to magnetic field [15]. Free radicals damage cells might contribute to the development of cardiovascular disease and cancer [14, 31, 36].

The present study indicated a significant decrease in all the parameters of the exposed mice (group two) relative to the control mice, which indicate appearance of anemia. The 50 Hz ELF-EMF increased micronucleated polychromatic erythrocytes (MNPCEs) in bone marrow from mice [2]. Also, Fraham [16] reported that all tested flux densities significantly stimulated formation and release of free radicals.

Changes of the mechanical properties will cause changes in the permeability of the cellular membrane and cause the remarkable changes in the membrane morphology as can be noted from the blood film up to certain extent [5].

Ali *et al.* [5] reported that the exposure of animals to 50 Hz, 2 mT magnetic field resulted in decrease of RBC membrane elasticity and permeability. There were noticed changes in the structure of hemoglobin, no sign of repair in the structure of newly generated RBCs after removing the animal from the magnetic field, which indicates that the blood generating system was severely injured in exposed mice.

Also, it was noticed from the analysis of the blood films the irregularity in the shape of RBCs membrane and sticking of several cells together forming common cellular membrane in case of group two. This phenomenon was not noticed for healthy blood. Normal blood cells do not stick together because of Coulomb repulsive forces between the positive electrostatic charges on the outer surface of the cell membrane [5]. These electrostatic charges are formed on the surface of the normal healthy RBCs as result of the K^+ ion pump which forms the resting potential across cellular membranes. Normal and healthy cell membranes are in the liquid crystalline state at which only K^+ and Na^+ can be pumped through the membranes [5].

Osmotic fragility study of the RBCs membrane indicated a significant change of hemolysis values for group two as compared with control group. The change in the values of hemolysis indicated a modification of the elasticity of the cell membrane, which is mainly due to changes in the intermolecular forces between macromolecules [5].

The changes in the amount of hemoglobin give us indication that there are some diseases [23, 37], which have abnormal types of hemoglobin. According to this study, the using of vitamin E reduces the side effects of forming free radicals due to exposing to ELF-EMF [7, 14].

CONCLUSIONS

Exposure to 2 mT, 50 Hz magnetic field is risky and deteriorates the physiological functions of the red blood cells, so health care consideration should be followed. Vitamin E can be used to decrease the side effects of the magnetic field.

REFERENCES

1. ABD EL GHANY, W.S.M., *Effects of Electromagnetic Fields on Some Biophysical-Biochemical Properties of Rat's Blood* In Vivo *Exposure*, PhD Thesis, Biophysics Department, Faculty of Science, Cairo University, Giza, Egypt, 2000.
2. ALCARAZ, M., E. OLMOS, M. ALCARAZ-SAURA, D.G. ACHEL, J. CASTILLO, Effect of long-term 50 Hz magnetic field exposure on the micronucleated polychromatic erythrocytes of mice, *Electromagnetic Biology and Medicine*, 2014, **33**(1), 51–57.
3. ALI, F.M., R.H. EL GEBALY, M.A. EL HAG, A.M. ROHAIM, Solid Ehrlich tumor growth treatment by magnetic waves, *Technology and Health Care*, 2011, **19**(6), 455–467.

4. ALI, F.M., R.H. EL-GEBALY, A.M. HAMAD, Combination of bacteriolytic therapy with magnetic field for Ehrlich tumour treatment, *General Physiology and Biophysics*, 2017, **36**(3), 259–271.
5. ALI, F.M., W.S. MOHAMED, M.R. MOHAMED, Effect of 50 Hz, 0.2 mT magnetic fields on RBC properties and heart functions of albino rats, *Bioelectromagnetics*, 2003, **24**(8), 535–545.
6. AMARA, S., H. ABDELMELEK, M.B. SALEM, R. ABIDI, M. SAKLY, Effects of static magnetic field exposure on hematological and biochemical parameters in rats, *Brazilian Archives of Biology and Technology*, 2006, **49**(6), 889–895.
7. AZIZ, I.A., H.J. EL-KHOZONDAR, M. SHABAT, K. ELWASIFE, A. MOHAMED-OSMAN, Effect of electromagnetic field on body weight and blood indices in albino rats and the therapeutic action of vitamin C or E, *Romanian J. Biophys*., 2010, **20**(3), 235–244.
8. BAIETH, H.A., Physical parameters of blood as a non-Newtonian fluid, *International Journal of Biomedical Science, IJBS*, **4**(4), 2008, 323–329.
9. BROWN, B.A., *Hematology: Principles and Procedures*, Lea & Febiger, Philadelphia, PA, US, 1993, pp. 458–462.
10. CHUNG, M.K., J.C. KIM, S.H. MYUNG, D.I. LEE, Developmental toxicity evaluation of ELF magnetic fields in Sprague-Dawley rats, *Bioelectromagnetics,* 2003, **24**(4), 231–240.
11. DACIE, J.V., S.M. LEWIS, *Practical Haematology*, 7th ed., Charchill and Livingston, London, 1991, pp. 196–200.
12. EL SAYED, W.H.A., *Effect of Extremely Low Frequency Magnetic Field on Mechanical Properties of Red Blood Cells in Rats*, PhD Thesis, Department of Basic Science, Faculty of Physical Therapy, Cairo University, 2005.
13. EL-GEBALY, R.H., N.S. EL-BIALY, M.M. RAGEH, A 50 Hz 0.5 mT magnetic field induces cytogenetic effects and biological alterations in Wistar rat, *Life Sci. J.*, 2012, **9**(4), 1875–1881.
14. EMRE, M., S. CETINER, S. ZENCIR, I. UNLUKURT, I. KAHRAMAN, Z. TOPCU, Oxidative stress and apoptosis in relation to exposure to magnetic field, *Cell Biochemistry and Biophysics*, 2011, **59**(2), 71–77.
15. FIORANI, M., B. BIAGIARELLI, F. VETRANO, G. GUIDI, M. DACHÀ, V. STOCCHI, *In vitro* effects of 50 Hz magnetic fields on oxidatively damaged rabbit red blood cells, *Bioelectromagnetics*, 1997, **18**(2), 125–131.
16. FRAHM, J., M. LANTOW, M. LUPKE, D.G. WEISS, M. SIMKÓ, Alteration in cellular functions in mouse macrophages after exposure to 50 Hz magnetic fields, *Journal of Cellular Biochemistry*, 2006, **99**(1), 168–177.
17. HANAFY, M.S., N.H. EL-SHAER, A. EL ATTAR, M.H ARAFA, A study on the effect of vitamin E on histomorphological and immunohistochemical changes induced by electromagnetic field, *Egyptian Academic Journal of Biological Sciences*, 2019, **11**(1), 85–99.
18. IBRAHIIM, M.H., M.S. HANAFY, S.A NASSARA, A. SHAHWAN, Effects of radiofrequency radiation emitted from mobile phone on hematological parameters in albino mice, *Arab J. Nucl. Sci. Appl.*, 2020, **53**(3),189–196.
19. LAI, H., N.P. SINGH, Magnetic-field-induced DNA strand breaks in brain cells of the rat, *Environmental Health Perspectives*, 2004, **112**(6), 687–694.
20. LEWIS, S.M., B.J. BAIN, I. BATES, J.V. DACIE, *Dacie and Lewis Practical Haematology*, Churchill Livingstone, 2006.
21. LIU, T., S. WANG, L. HE, K. YE, Anxiogenic effect of chronic exposure to extremely low frequency magnetic field in adult rats, *Neuroscience Letters*, 2008, **434**(1), 12–17.
22. MANOR, D., S. MORLEY, The α-tocopherol transfer protein, *Vitamins & Hormones*, 2007, **76**, 45–65.
23. MARIAM, S.A., A.E.G. NAWAL, Effects of exposure to electromagnetic field on of some hematological parameters in mice, *Open Journal of Medicinal Chemistry*, 2012, **2**, 30–42

24. MOHAMED, F.A., A.A. AHMED, B.M. EL-KAFOURY, N.N. LASHEEN, Study of the cardiovascular effects of exposure to electromagnetic field, *Life Science Journal*, 2011, **8**(1), 260–274.
25. MOHAMMADNEJAD, D., J.S. RAD, A. AZAMI, B. KHOJASTEH, F. RAJAEI, H.T.N. ABADEL, A. HEMATEI, M. VALILOU, A. LOTFI, Protective effect of vitamin E supplement in electromagnetic field induced damages in spleen: an ultrastructural and light microscopic studies, *Global Veterinaria*, 2010, **4**(4), 416–421.
26. OBE, G.V, Controversial cytogenetic observations in mammalian somatic cells exposed to extremely low frequency electromagnetic radiation: a review and future research recommendations, *Bioelectromagnetics,* 2005, **26**(5), 412–430.
27. OKANO, H., Effects of static magnetic fields in biology: role of free radicals. *Front. Biosci.*, 2008, **13**(1), 610–625.
28. ONGEL, K., N. GUMRAL, F. OZGUNER, The potential effects of electromagnetic field: a review, *Cell Membranes and Free Radical Research*, 2009, **1**(3), 85–89.
29. PACKER, L., Vitamin E is nature's master antioxidant, *Sci. Am. Sci. Med.*, 1994, **1**(1), 54–63.
30. RAGEH, M.M., R.H. EL-GEBALY, N.S. EL-BIALY, Assessment of genotoxic and cytotoxic hazards in brain and bone marrow cells of newborn rats exposed to extremely low-frequency magnetic field, *Journal of Biomedicine and Biotechnology*, 2012, **2012**, 1–7.
31. SALEM, A., A. HAFEDH, A. RACHED, S. MOHSEN, B.R KHEMAIS, Zinc prevents hematological and biochemical alterations induced by static magnetic field in rats, *Pharmacological Reports*, 2005, **57**(5), 616–622.
32. SHARMA, M., Y.K. GUPTA, Effect of alpha lipoic acid on intracerebro-ventricular streptozotocin model of cognitive impairment in rats, *Eur. Neuropsychophar.*, 2003, **13**, 241–247.
33. TKACHUK, D.C., J.V. HIRSCHMANN, M.M. WINTROBE, (Eds.), *Wintrobe's Atlas of Clinical Hematology*, Lippincott Williams & Wilkins, 2007.
34. TRABER, M.G., L. PACKER, Vitamin E beyond antioxidant function, *Am. J. Clin. Nutr.*, 1995, **62**(6),1501–1509.
35. TRABER, M.G., J. ATKINSON, Vitamin E, antioxidant and nothing more, *Free Radical Biology and Medicine*, 2007, **43**(1), 4–15.
36. VARSHNEY, G., V. KATIYAR, S. KUMAR, Effect of magnetic field on the blood flow in artery having multiple stenosis: a numerical study, *International Journal of Engineering, Science and Technology*, 2010, **2**(2), 967–982.
37. VERHAGEN, H., B. BUIJSSE, E. JANSEN, B. BUENO-DE-MESQUITA, The state of antioxidant affairs, *Nutrition Today*, 2006, **41**(6), 244–250.