

Effects of Whole Body Exposure to Extremely Low Frequency Magnetic Field (ELF-MF) on Physical and Biological Parameters in vivo Rats

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ABSTRACT

The use and convenience of electrical appliances is increasing in daily lives and they are the cause of harmful effects caused by magnetic fields (MF). The present study aimed to evaluate the physical and biological effects in vivo for the whole body irradiation in rats as well as its effect on the oxidative stress of brain tissues. **Methods:** Animals are divided into three groups, non-exposed control group (N) and whole body irradiated groups exposed to 0.5 and 1.5mT for 3hrs/day for 5 consecutive days. Animals in all groups are sacrificed by decapitation at the end of the exposure period. **Results:** Measurement of blood physical and biological parameters when exposed to 0.5mT shows a diminish of WBCs and lymphocytes counts, while platelets and segmented neutrophil counts are increased. Meanwhile exposures to 1.5mT don't alter these parameters. The mean value of the electrical conductivity of hemoglobin for rats exposed to both doses of MF compared to control group is significantly decreased. The slight increase in hemoglobin viscosity resulted in a less quantity of water absorbed by blood cells which lead to reduction in electrical conductivity. The electrophoretic patterns of serum proteins reveal significant reductions in α -1, α -2 and β -globulins in rats exposed to the MFs (0.5mT and 1.5mT). Moreover, albumin and α -2 globulin are significantly reduced in rats exposed to 1.5mT than those exposed to 0.5mT. The decrements may have resulted from disturbed protein synthesis in the liver. Both values of MFs(0.5&1.5mT) cause elevations of Malondialdehyde (MDA) in serum and brain tissue homogenate, while the total antioxidant capacity is reduced significantly. **Conclusion:** Electrical conductivity of rat's hemoglobin is significantly reduced. Enhancement of reactive oxygen species may give rise to functional and morphological disturbances in red blood cells through oxidative processes. The antioxidant defense of living system can be deteriorated by the effect of magnetic field which leads to oxidative stress.

Key words: Extremely Low Frequency Magnetic Field (ELF-MF), Malondialdehyde (MDA), Total Antioxidant Capacity (TAC)

Introduction

In modern society, humans are commonly exposed to magnetic field including extremely low frequency magnetic field (ELF-MF), which is generally generated by power lines and many kinds of electric appliances (Strasak and Smarad, 2002; Sabo *et al.*, 2002 and Xu, and Okano, 2002). The ELF-MF covers the frequency range of 3Hz to 3 kHz. The ELF-MF for many years is considered as an absolutely neutral form of radiation, but epidemiological data and results of experimental *in vitro* and *in vivo* studies have caused more attention to be paid to low frequency 50 or 60 Hz (Sobczak, 2002). These results of animal and human studies dealing with the biological effects of exposure to ELF-MF have consistently been positive or negative (Fiorani *et al.*, 1997).

Recent studies have shown that ELF-MF can change cell behavior and activation by affecting the biochemical and/or biophysical processes. Physical processes at the atomic level are the basis of reactions between biomolecules in ELF-MF, since the field can magnetically affect chemical bonds between adjacent atoms and alters energy levels and spin orientation of electrons. This contributes to an increase in the activity, concentration, and lifetime of free radicals (Simko and Mattsson, 2004 and Rollwitz *et al.*, 2004).

Results obtained in present study on the effects of ELF-MF on biological parameters are conflicting, and this leads to verify the present work. This work aimed to evaluate the possible effects of exposure to ELF-MF dose on whole body exposed rats.

Materials and methods

Experimental Animals

Thirty adult male albino rats (300-400 g body weight) are housed in especially designed cages (5rats/cage) and allowed free access to tap water and pellet diet. Animals are divided into 3 groups (10 rats each). Control

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group (N) non exposed normal rats and two whole body irradiated groups exposed to 0.5mT and 1.5mT for 3hrs/day for 5 consecutive days.

Magnetic field exposure device

The homogeneous magnetic field generator is consisted of coil placed on a wooden rack. The coil consists of 320 turns from electrically insulated 2 mm copper wire thickness is wound in a homogenous way around a copper cylinder of 2 mm thick, 50 cm diameter and 60cm length. The ends of the coil are connected to variac fed from the mains ($\approx 220V$ and 50 Hz) to produce different alternating magnetic fields. The magnetic field strength inside magnetic chamber (where the animal housed) is adjusted by changing the voltage across the coil to generate magnetic field of 0.5mT and 1.5mT in the area where the animals housed.

The magnetic field inside the chamber is measured at different locations by Gauss/tesla meter in order to find out the most homogeneous zone inside the cage.

Rats are kept in special plastic cages that permit normal ventilation. The effects of plastic on field uniformity are negligible as claimed by Kaune, (1979).

Methods

Animals in all groups are sacrificed by decapitation at the end of the experimental period. Each blood sample is divided into three tubes; one containing heparin for the physical measurement of hemoglobin and the second with EDTA for measuring the hematological indices and blood film, while the third tube is a plain without anticoagulant for separation of serum by centrifuging blood samples at 4000 rpm for 5min.

Hematology

Complete blood counting (CBC) is determined automatically by an electronic counter (Coulter Electronics). Blood smear film is stained with hematoxylin and eosin (H&E).

Measurements of physical properties of hemoglobin

Hemoglobin is extracted according to Trivelli method (Trivelli *et al.*, 1971). The packed RBCs are washed three times with 5 volumes saline then re-centrifuged and lysed with two volumes of deionized water. The hemolysate is centrifuged at 10,000 rpm for 20 min. at 4°C to remove erythrocyte ghosts.

Relative viscosity of hemoglobin is measured at constant temperature of $25\pm 1^\circ C$ by Ostwald viscometer. The viscosity of the solution is calculated by the following equation: $\eta_s / \eta_w = d_s t_s / d_w t_w$ Where η , d and t are viscosity, density and time of flow, respectively, for sample (s) and water (w). The relative viscosity of hemoglobin (Hb) is calculated by measuring the time of flow, using the same viscometer for constant volume of sample and water.

Electrical conductivity of hemoglobin is measured by using a conductivity meter (Hi 8633, Hanna instruments, USA).

Serum protein electrophoresis is performed by full automated system (miniLITE CELL, Italy). Malondialdehyde (MDA) and total antioxidant capacity (TAC) is determined in serum (Li and Chow, 1994) and whole tissue homogenate (5%) according to the method of and Koracevic *et al.* (2001).

Brain samples are fixed in 10% formol saline for 24 hrs. Then washed, cleared in xylene and embedded in paraffin at 56°C in hot air oven for 24 hrs. The tissue sections are stained by hematoxylin & eosin stain for routine examination through the light electric microscope (Banchroft *et al.*, 1996).

Statistical analysis

All results are expressed as the mean \pm SD. Statistical analysis is performed with statistical package for the social science for Windows (SPSS, version 11.0, Chicago, IL, USA). The data are analyzed by one-way analysis of variance (ANOVA). To compare the difference among the groups, post hoc testing is performed by the least significant difference (LSD) test. The *P*-value less than 0.05 are considered statistically significant.

Results

Data in table (1) reveal significant reduction ($p < 0.01$) in white blood cells (WBCs) and ($p < 0.006$) in lymphocyte, while platelets and segmented cells are significantly elevated ($p < 0.04$ & 0.002, respectively) in rats exposed to 0.5mT, compared to control rats. Rats exposed to 1.5mT show non-significant alteration in all parameters except the mean cell hemoglobin concentration (MCHC) which is significantly reduced ($p < 0.02$) as

compared to control rats. Multiple comparison analysis shows that exposure to 1.5mT cause significant reductions to near the control values in platelets count, Hb concentration and segmented cell % ($p < 0.01$, 0.04 and 0.02, respectively) as compared with group exposed to 0.5mT.

Table 1. Blood cell counting and blood indices (expressed as mean±SD) in the different experimental groups.

Parameters	Groups	N	0.5 mT	1.5 mT
RBCs $\times 10^6$ (cell/ μ l)		7.05±0.87	7.56±0.77	7.29±1.16
WBCs $\times 10^3$ (cell/ μ l)		18.36±2.96	13.52±3.08*	15.92±2.05
Platelets $\times 10^3$ (cell/ μ l)		490.40±114.48	693.40±196.43*	421.20±102.35#
HCT (%)		36.40±3.20	40.48±4.11	39.30±5.35
Hb (g/dl)		13.42±0.75	14.90±1.24	13.02±1.83#
Staff (%)		0.80±0.45	1.00±0.44	1.20±0.55
Segmented (%)		20.20±0.84	34.60±8.26*	25.60±5.37#
Lymphocyte (%)		64.80±4.15	47.40±12.34*	57.20±6.34
Monocyt (%)		9.60±1.34	11.00±3.24	12.40±2.41
Basophil (%)		0	0	0
Eosinophil (%)		4.60±2.07	5.00±2.00	6.20±3.56
MCV (fl)		52.00±4.37	53.62±2.36	50.60±8.95
MHC (pg)		19.14±1.32	19.70±0.96	18.94±1.12
MCHC (g/dl)		36.92±1.77	36.82±0.78	35.08±0.61**

* Significance vs control group, # significance vs 0.5 mT.

The blood samples of groups exposed to magnetic field (MF) show that, red blood cells (RBCs) serrated with the edge, the cells called Echinocytes erythrocytes. In addition, RBCs were staked next to each other to form rouleaux as well as pale corpuscles can be noted (Figure 1).

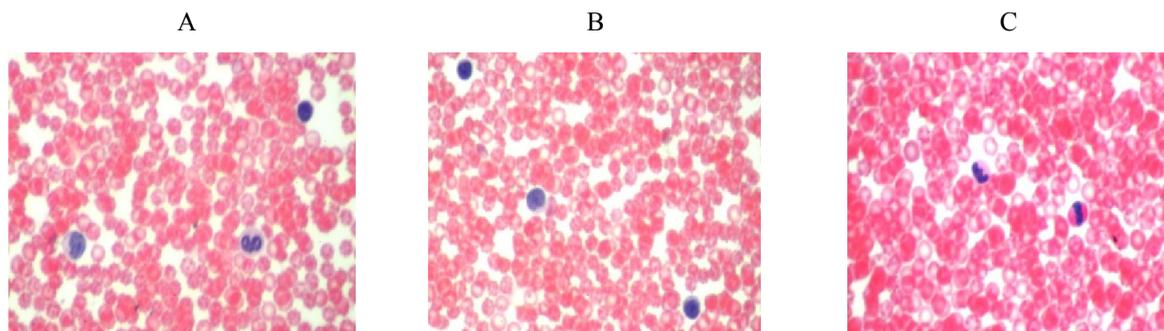
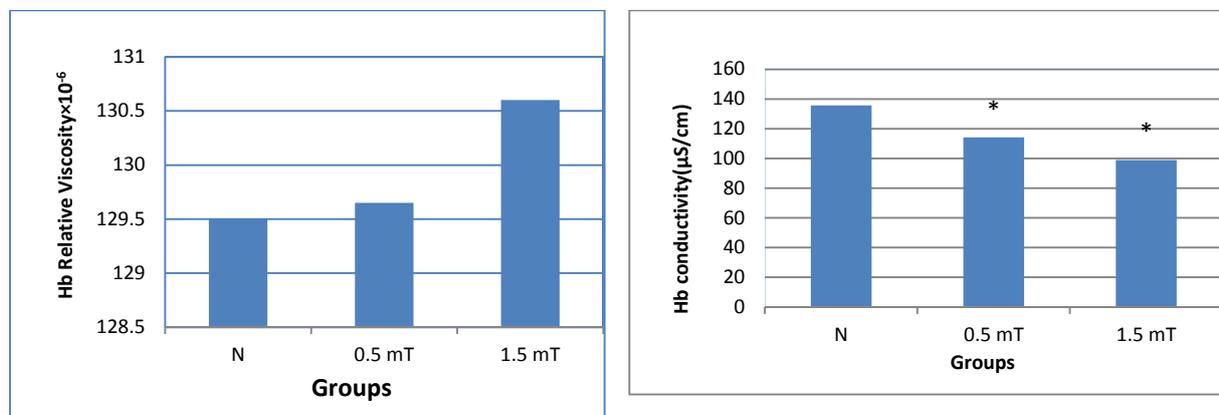


Fig. 1: Photomicrograph of blood smears from control (A), 0.5mT (B) and 1.5mT (C) groups by hematoxylin-eosin staining (original magnification X 40).

Although haemoglobin viscosity shows slight elevations in rats exposed to 0.5 and 1.5mT, these elevations are statistically non-significant ($p > 0.05$), compared to normal group. As regard to electrical conductivity of haemoglobin, significant reductions are observed in rats exposed to 0.5 and 1.5mT as compared to normal group (Fig.2).



* Significance vs control group.

Fig. 2: Mean values of hemoglobin relative viscosity and conductivity for control and different experimental groups.

Rats exposed to MF revealed no alteration in serum total protein, γ -globulin and A/G ratio as illustrated in table (2) and Fig. (3). Rats exposed to 0.5mT revealed significant reduction in both α -1 and β -globulins ($p < 0.01$ & 0.03 , respectively), while rats exposed to 1.5mT showed significant reductions in both α -1 and α -2 globulins ($p < 0.03$ & 0.001 , respectively), compared to control rats. Moreover, exposure to 1.5mT causes significant reduction in serum albumin and α -2 globulin ($p < 0.02$ & 0.01 , respectively) as compared to rats exposed to 0.5mT.

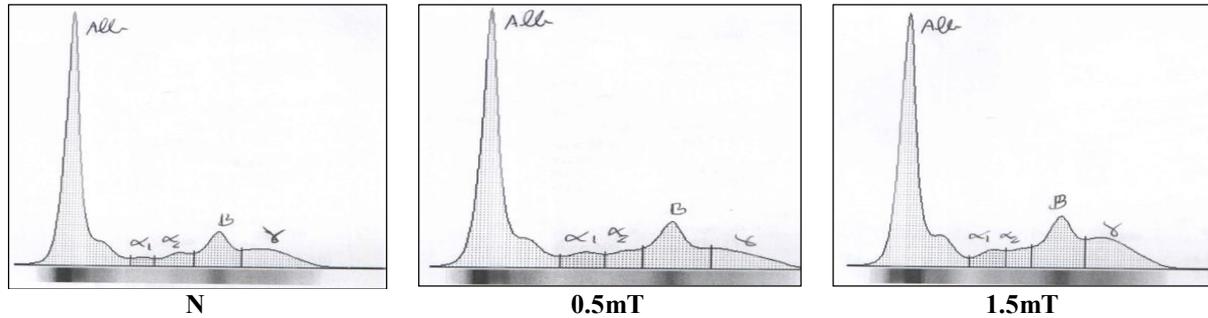


Fig. 3: Electrophoretic patterns of serum proteins for various groups

Table 2: Serum levels of total protein, albumin and globulins as well as albumin to globulin ratio (expressed as mean \pm SD) for various experimental groups.

Parameters		Groups	N	0.5mT	1.5mT
Total protein (g/dl)			5.76 \pm 0.43	5.76 \pm 0.51	5.02 \pm 1.05
Albumin (g/dl)			3.41 \pm 0.24	3.61 \pm 0.43	2.90 \pm 0.55 [#]
Globulin (g/dl)	α -1		0.33 \pm 0.03	0.21 \pm 0.08 [*]	0.23 \pm 0.07 [*]
	α -2		0.33 \pm 0.03	0.28 \pm 0.03	0.23 \pm 0.04 ^{*#}
	β		1.05 \pm 0.03	0.89 \pm 0.15 [*]	0.97 \pm 0.11
	γ		0.61 \pm 0.05	0.63 \pm 0.05	0.68 \pm 0.25
A/G Albumin to Globulin ratio			1.65 \pm 0.39	1.79 \pm 0.11	1.46 \pm 0.15

* Significance vs control group, # significance vs 0.5mT.

Serum level of MDA is significantly elevated ($p < 0.01$) in rats exposed only to 1.5mT, while both values of MF (0.5 & 1.5mT) cause significant elevation ($p < 0.002$ & 0.0001 , respectively) in brain MDA, compared to control. As regard to serum TAC, rats exposed to 1.5mT reveals pronounced drop, compared to both control and 0.5mT groups ($p < 0.001$ & 0.03 , respectively). The total antioxidant capacity in brain is significantly reduced ($p < 0.001$ & 0.0001 , respectively) due to effect of MF (0.5 & 1.5mT) compared to normal group (table 3).

Table 3. Concentration of MDA and TAC in g/dl in Serum (s) and brain tissue (b), (expressed as mean \pm SD) for different groups.

Groups	Parameters	sMDA	sTAC	bMDA	bTAC
N		5.33 \pm 2.88	2.24 \pm 0.15	9.90 \pm 4.87	1.18 \pm 0.33
0.5mT		7.16 \pm 1.99	1.90 \pm 0.29	30.17 \pm 11.14 [*]	0.56 \pm 0.15 [*]
1.5mT		10.12 \pm 2.93 [*]	1.46 \pm 0.38 ^{*#}	40.58 \pm 6.13 [*]	0.39 \pm 0.08 [*]

* Significance vs control group, # significance vs 0.5mT.

The morphology of neurons in the meninges (A), hippocampus (B) and striatum (C) are normal in H&E-stained sections taken from the control group, Fig. (4). As regard to rats exposed to 0.5mT, focal haemorrhage in the meninges, nuclear pyknosis and degeneration in the neuronal cells of the subiculum of the hippocampus and diffuse gliosis with vacuolization in the matrix of striatum are detected.

The meninges as well as brain fissures in rats exposed to 1.5mT show severe congestion in the blood vessels with oedema. There is nuclear pyknosis in the degenerated neuronal cells of fascia dentate of the hippocampus, in addition to vacuolization in the matrix of the striatum.

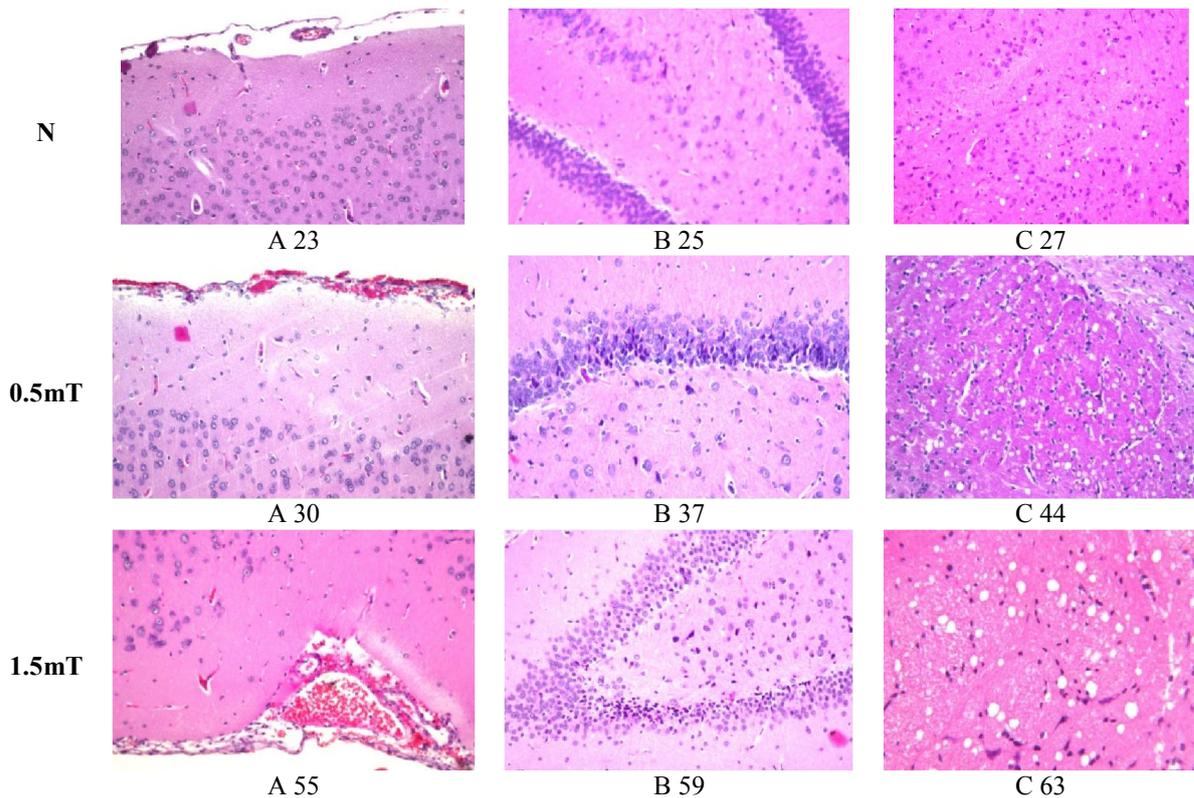


Fig. 4: Light microphotographs showing the morphology of the different brain regions [meninges (A), hippocampus (B) and striatum (C)] in normal (N) and exposure to MF of (0.5mT & 1.5mT) groups using hematoxylin-eosin staining (original magnification X 40).

Discussion

Measurements of blood physical and biological parameters are most important indicator for knowing health status of living organisms (Soud, 2004). Also these parameters are diagnostic for certain diseases such as anemia, leukemia and detection of inflammation (Fatayer, 2006).

The major findings of the present study are: that exposure to 0.5mT MF leads to a diminish of WBCs count and lymphocyte, while platelets count and segmented neutrophil are increased. However, exposures to 1.5 mT don't alter these parameters. Fiorani *et al.*, (1997) indicated that 0.5 mT magnetic field had no effect on intact RBC. Similar findings are observed in present study: peripheral blood erythrocytes count is not changed for exposed rats.

Neutrophils are a type of phagocyte and are normally found in the bloodstream. Neutrophils are one of the first-sensors for inflammatory cells which rapidly migrate towards the site of inflammation (Jacobs *et al.*, 2010). In addition platelets are rapidly deployed to sites of inflammation by interacting with leukocytes and by secreting cytokines and other inflammatory mediators (Wagner and Burger, 2003).

The observed tendency of a decrease in leukocyte count and the elevation of neutrophils and platelets support the idea of inflammation induction by the MF.

Ali *et al.*, (2003) and Singh *et al.*, (2013), studied the effect of 50 Hz, 0.2 mT magnetic fields on red blood cells properties in albino rats. Authors reported drop in RBCs membrane elasticity, permeability, and changes in molecular structure of hemoglobin. These results confirm the present findings which are shown in blood smears photomicrograph of the groups exposed to MF reveal distorted shape and staked RBCs forming rouleaux. The red blood cells have increased ability to stick together may be due to change in size and charge on the surface as a result of exposure to magnetic field.

In present work the mean values of electrical conductivity of haemoglobin for rats exposed to MF as compared to control group is significantly reduced due to the folding of molecules and decrease in the surface charge. The slight increase in haemoglobin viscosity resulted in a less quantity of water absorbed by the blood cells.

The electrophoretic patterns of serum proteins reveal significant reductions in α -1, α -2 and β -globulins in rats exposed to the MF (0.5mT and 1.5mT). Moreover, albumin and α -2 globulin are significantly reduced in rats exposed to 1.5mT than those exposed to 0.5mT. The decrements may have resulted from disturbed protein synthesis in the liver.

The magnetic field penetrates the body with little or no attenuation because the permeability of biological tissue is approximately as air. Consequently all tissues and cells of the body are exposed to the same magnetic field level (Arcos *et al.*, 1995).

The ELF-MF doesn't have enough energy to break macromolecular bonds (Seto *et al.*, 1986). One of the interaction mechanisms of magnetic field with biological systems is that it alters the spin states of reactive oxygen species (ROS) which, in turn, change the relative probabilities of recombination and other interactions (Fiorani *et al.*, 1997). If this is the case, the proportion of radicals reacting with macromolecules will increase, leading to possible adverse effects on cell function (Marino *et al.*, 2001). Moreover, the enhancement in the concentration of ROS may give rise to functional and morphological disturbances in the cell through oxidative stress, leading to reversible or irreversible tissue injury (Yokus *et al.*, 2008, Harakawa *et al.*, 2005, Yokus *et al.*, 2005).

The present study illustrates that both intensities of MF cause elevations of MDA in serum and brain tissue homogenate, while the total antioxidant capacity was reduced significantly. The present results are in line with (Goraca *et al.*, 2010, and Ciejka *et al.*, 2011).

A biomarker for lipid peroxidation is MDA, it is a highly toxic molecule and it has been implicated in a range of disease pathologies by producing oxidative damage in tissues (Del Rio *et al.*, 2005). Many studies reveal the effect of ELF-MF on lipid peroxidation (Kabuto *et al.*, 2001; Jelenkovic *et al.*, 2006). Excess oxygen free radicals induce lipid peroxidation, especially in brain, which is very vulnerable to free radical insults because it contains high concentrations of easily peroxidizable fatty acids (Halliwell, 2001). Therefore, overproduction of ROS can be an important mediator of damage to cell structures, including lipids and membranes, proteins, and DNA (Valko *et al.*, 2007). The living organism has defensive systems against free radicals. These antioxidant defense systems can be deteriorated by the effect of magnetic field which leads to oxidative stress (Lee *et al.*, 2004).

Magnetic field exposure results in deleterious morphological changes in the different parts of the brain. A focal hemorrhage in the meninges, nuclear pyknosis and degeneration of the neuronal cells of the subiculum are signs of brain tissue injury that leads to gliosis. Gliosis is the universal response of the CNS to tissue injury and occurs as a result of many acute conditions such as trauma, ischemia, and stroke. Gliosis in any form entails an alteration in cellular activity that has the potential to create widespread effects on neurons as well as other non-neural cells, causing either a loss of normal functions or a gain of detrimental ones (Sofroniew & Vinters, 2010 and Hamby & Sofroniew, 2010).

Conclusion

Exposure to extremely low frequency magnetic field (0.5&1.5mT) has led to the following:

- Drops in RBCs, membrane elasticity, permeability, and characteristics of hemoglobin.
- Electrical conductivity of rat's hemoglobin is significantly reduced.
- Enhancement of reactive oxygen species may give rise to functional and morphological disturbances in red blood cells through oxidative processes.
- The electrophoretic patterns of serum proteins reveal significant reductions in α -1, α -2, and β -globulins.
- The antioxidant defense of living system can be deteriorated by the effect of magnetic field which leads to oxidative stress.
- Degeneration of neuronal cells of the subiculum is a sign of brain tissue injury that leads to glioma.

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