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Research Article

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Effect of 50 Hz , 0.85 mT Magnetic Fields on Antioxidant System

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Abstract Genetically, living organisms are adapted to the natural magnetic field (MF) of earth because they exist in it. The constant magnetic field of earth has flux density which ranges from 30 and 60 μT. Moreover, permanent magnets generate artificial magnetic fields that are highly used in devices like home appliances, speakers and microphones and in diagnostics and medical imaging. Furthermore, in medical treatment, MF has been effectively used, mostly in disorders of the gastrointestinal tracts, of the ocular, cardiovascular, respiratory, musculoskeletal and nervous systems, and in disorders of skins and soft tissues. MFs have biological effects like their anti-edema and anti-inflammatory properties and the enhancement of soft tissue repairs. These effects form scientific bases for MFs' clinical applications and experimentally have been established. Nonetheless, the MFs' applications may have the risks of functional disorders in biological systems, cells and tissues. In contrast, the lifetime and activity of free radicals can be increased by the exposure to MFs, from which oxidative stress is caused. Changes in the intracellular calcium releases, in the regulation of gene expressions and in the antioxidant enzyme activities can be promoted by the production of enhanced reactive oxygen species (ROS). The cell membrane properties are also modified by oxidative stress which affects a cell process like death, proliferation and growth. Besides, the pathogenesis of several disorders such as cancers as well as cardiovascular and skin disorders can be caused by increased oxidative stress. Against the immoderate productions of reactive nitrogen and oxygen species, bodies are protected by enzymatic and non-enzymatic antioxidant defense systems which have three levels. The first level prevents the production of free radicals by its enzymes including glutathione reductase, glutathione peroxidase, catalase and superoxide dismutase. The second level scavenges the active radicals by its lipid or water-soluble low molecular weight antioxidants including tocopherol, ascorbic acid, flavonoids and glutathione.

Keywords Magnetic field, Biological effect, Antioxidant system, Lipid peroxide, Glutathione

Introduction

Medical devices, residential power lines and household electrical wiring produces "extremely low-frequency electromagnetic field (ELF-EMF)" which is defined as "an electromagnetic wave of frequency 0–300 Hz that emits a non-thermal effect of non-ionizing radiation"[1]. ELF-EMFs can perform as risk factors of the oxidative stress occurrences in brain tissues as indicated by many reports [2]. Another study has proposed that the evidence of ELF-EMF-induced oxidative stress was done by: (1) reduction in activities of glutathione peroxidase (GSH-Px) and brain superoxide dismutase (SOD); (2) increase in activities of adenosine deaminase (ADA) and brain xanthine oxidase (XO); and (3) increase in levels of nitric oxide (NO) and malondialdehyde (MDA) in brain tissues [3]. Extensive attentions have globally been attracted by the influences of ELF-EMFs on human health, biological and central neurotransmitter systems with the growing uses of electromagnetic techniques. Oxidative stress acts as a serious imbalance between the anti-oxidants which cause potential tissues and organ damages and the productions of reactive oxygen species (ROS) [5], though the techniques of ELF-

EMFs increasing brain disorders look to be a significant factor [4]. The capacity of intrinsic cellular scavengers is noticeably exceeded by the ROS production in brains during the exposure of ELF-EMFs. Anti-oxidant enzymes "SOD, CAT and GSH-Px enzymes activities decreased" are activated by immoderate ROS [6] which, however, impairs mitochondrial functions, and damages cellular constituents such as lipids and proteins (MDA level increased). ELF-EMFs can cause oxidative stress injury which affects the brain tissue that can be protected by proposed natural antioxidants which prevent both the peroxidation as well as the reduce in the anti-oxidant enzyme levels. The exposure to magnetic field can also lead to the enhancement of somatic recombination which can be suppressed by supplementing of a non-specific antioxidant which is “vitamin E” as verified by another previous study [7-8].

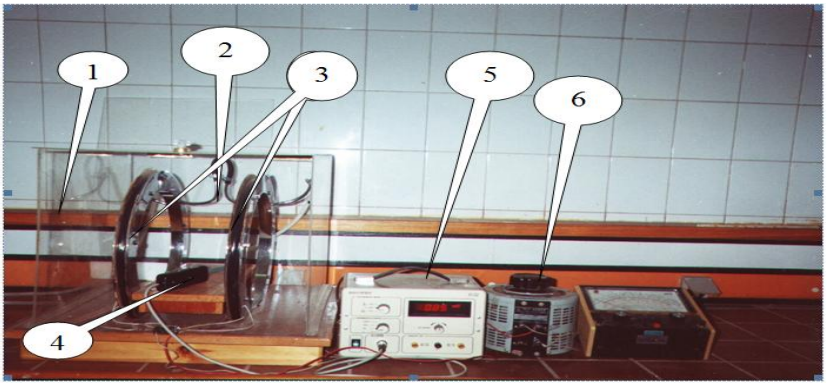
Epilepsy is “one of the most common neurologic disorder in which the patient experiences chronic abnormal bursts of electrical discharge in the brain”. Nearly 0.8% of people globally is suffering from epilepsy. Various symptoms like speech loss, full or incomplete consciousness loss, unusual sensory experiences and/or uncontrollable motor behavior in persons with epilepsy can be caused by severe and continuous seizure activities. Brain injuries or chemical imbalances cause epilepsy that is associated with continuous neuronal injuries. In *in-vivo* models, some kinds of epileptic seizures have been induced so far by using few physical or chemical agents [9].

The brain is extremely susceptible to oxidative stress Because of its limited antioxidant capacity, high lipid content as well as its higher energy requirements. It spends 20% of the metabolic oxygen; however, it is approximately considered as 2% of the body mass [10]. In healthy neuronal cells, ROS generations are under homeostatic control because there is a balance between ROS generations and the activities of antioxidants. Nonetheless, the levels of antioxidant may be exceeded causing oxidative damages and lead to death of necrotic cells after the exposure of the cells to physical and/or chemical agents like radiations [11]. The brain is being more vulnerable to oxidative stress by the reduced activity of antioxidants, which is exhibited as higher lipid peroxidation. In brains, glutathione (GSH) has a vital role in defending free radicals, and it is one of the main antioxidants. Moreover, oxidative neuronal death is related to GSH’s continuous depletion [12]. Oxidative damage has emerged as a technique which can have a key role in the epilepsy’s progressions and etiology. In new born pigs, the hydroxyl radicals and superoxide anions’ levels raise during seizures. Epileptic seizures might be induced by the free radicals’ immoderate levels through inactivating glutamine synthases or inhibiting glutamate decarboxylases [13].

Materials and Methods

1. Magnetic field exposure system

The source of magnetic field was Helmholtz two coils show in figure (1), the coils were distant by 15 cm, and each one was 30 cm in diameter and of 250 turns. The wire of the coil was 0.7 mm in diameter and its resistance was 13 ohm. The field was probed by a magnetic flux meter (ELWE 8533996, Cerligene, Germany).



1-Transparent plastic cover
2- Water circulating cooling system
3- Helmholtz coil
4- Magnetic probe
5- Flux meter
5- Flux meter
6- AC Variac

Figure 1: A view of the magnetic field generator system

In spite of the fact that the second method of direct measurements is a local method, i.e., it measures the local in space magnetic field; it was decided to use this method because of its low cost in money and efforts expenses. Actually, there are two classes of magnetic field probes: Hall probe and conductive probe. Hall probe based on

the Hall effect, has high sensitivity and wide frequency response but it needs a high power current stable source and will cause more perturbation of the medium owing to the introduction of driven current. Hence, the Hall probe is used for measuring steady state or very slowly varying magnetic field. When non steady magnetic field needs to measured, it is preferred to use conductive loop probe. This technique is useful for studying the field strength and its variations figure (2) is the schematic drawing of a typical probe system.

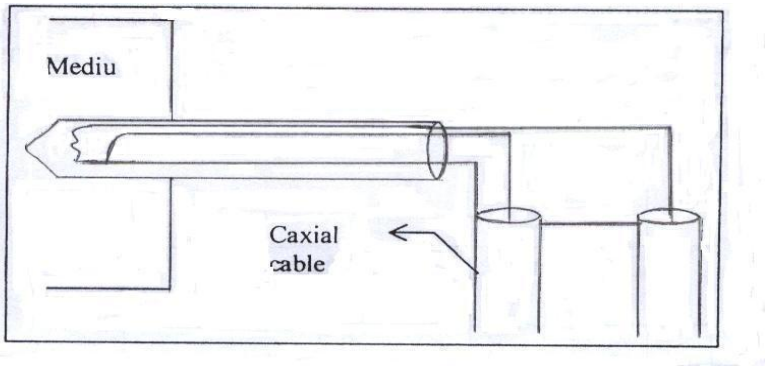


Figure 2: Schematic drawing of the magnetic probe

The sensor is a small coil made of several winding small-wire diameter. The coil is fixed on a support insulator piece and then connected with coaxial cable. When the coil is placed into a varying magnetic field, an inductive electromotive potential (ε) well produced across the two ends of the coil. If the size of the coil is so small the magnetic field within the coil can be regarded as uniform so the output voltage and the value of the required measured magnetic field is directly shown in the pre-calibrated scales of the magnetic probe. The magnetic field was firstly well mapped to investigate the best area between the two coils at X, Y and Z direction at which the used field intensity is approximately constant. A flux meter EL WE 8533996 was used to map the magnetic field in the area between the Helmholtz coils. The area of constant magnetic field was chosen to be the exposure area in which the cage was located. The investigated rats, except the control ones, were all exposed to a low frequency 50 Hz magnetic field of intensity of about 0.85 mT (8.5 G). The control group was exposed to a sham (not energized) field.

2. Experimental Animals

The experiments were carried out on 56 rats male Sprague-Dawely rats, of about 150 gm mean weight. They were obtained from the breeding unit of National Research Center, Dokki, Giza. The rats were housed eight per cage in a well-ventilated room (25±2ºC), while the relative humidity was (43±3) % and 12 hours light and dark cycle at the animal house of the Zoology Department, Suez Canal University. They were kept at the Biophysics laboratory, where they have been exposed to the magnetic field, for at least one week before exposure. The rats were regularly fed on a standard diet ad libitum.

Rats were divided into seven main groups (8 rats each).

- Group 1: Rats were exposed to the magnetic field for 1 day (2 hours/day) and the blood samples were collected before and after exposure.
- Group 2: Rats were exposed to the magnetic field for 2 days(2 hours/day) and the blood samples were collected before and after exposure in both days.
- Group 3: Rats were exposed to the magnetic field for 3 days(2 hours/day) and the blood samples were collected before and after exposure at the first and last day.
- Group 4: Rats were exposed to the magnetic field for 4 days(2 hours/day) and the blood samples were collected before and after exposure at the first and last day.

Group 5: Rats were exposed to the magnetic field for 5 days(2 hours/day) and the blood samples were collected before and after exposure at the first and last day.

- Group 6: Rats were exposed to the magnetic field for 6 days(2 hours/day) and the blood samples were collected before and after exposure at the first and last day.
- Group 7: Rats were exposed to the magnetic field for 7 days(2 hours/day) and the blood samples were collected before and after exposure at the first and last day.

3. Methods

Blood samples were collected using orbital sinus technique (Sanaford method) [15]. The whole blood was used to determine reduced glutathione (GSH) content. To obtain serum, the collected blood was incubated at 37ºC for one hour in clean dry test tubes, then centrifuged at 3000 rpm for 20 min. Sera were aspirated and stored at-20 ºC until used. Serum was used in the determination of the levels of lipid peroxide.

Heparinized blood was used in the determination of blood reduced glutathione according to the Beutler method [16].

Reagents:

(1) Precipitating solution 1.67 gm glacial metaphosphoric acid, 0.2 gm ethylene di-amine tetra acetic acid (EDTA) and 30 g NaCl in 100 ml of distilled water.

(2) Phosphate solution 0.3 M Na_2 HPO_4.

(3) DTNB: reagent-40 mg 5, dithiobis-2-nitrobenzoic acid in 100 ml 1% sodium citrate.

Assay procedure:

(1) 0.2 ml of blood was added to 1.8 ml distilled water.

(2) 3.0 ml of precipitating solution was added and the samples were immediately vortexes.

(3) The mixture was centrifuged at 2200 rpm for 15 minutes at room temperature.

(4) 1.0 ml of supernatant was added to 4 ml of the phosphate solution.

(5) One half ml of DTNB reagent was added and the absorbance was measured at 412 nm.

Glutathione concentration is calculated from the following equation:

$$\text{Glutathione concentration} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \text{standard concentration}$$

Determination of the extend of lipid peroxide of serum was based on the measurement of thiobarbituric acid (TBA) reactants according to the Sharma method [17].

Lipid peroxide assay:

(1) 0.5 ml of serum was mixed with 3 ml of 1.0% phosphoric acid (pH 2.0) and 1 ml of 0.6% TBA in air-light tubes and were put in boiling water for 45 min.

(2) The samples were cooled in ice then 5 ml butanol were added along with thorough stirring of the mixture.

(3) The butanol phase was separated by centrifugation at 10000 rpm for 10 minutes and transferred to glass cuvettes.

(4) The color of the TBA chromogen was measured at 520 nm and 532 nm in UV visible spectrophotometer.

(5) The difference between absorbance at 520 nm and at 532 gives rise to the TBA value, which primarily represents the malondialdehyde concentration and was taken as a measure of lipid peroxide.

The effect of magnetic field was determined by comparing the values of before and after in each group using Student's unpaired t-test used Senecor method [18]. The data are represents by the mean values ± standard error from 8 rats/group and the differences are considered statistically significant at the level of $P < 0.05$.

Results & Discussion

Fifty six male Spargue-Dawely rats used in this study divided in to seven groups eight rats each. All rats were exposed for two hours daily to extremely low frequency (0.85 mT) magnetic field figure (1). Blood samples were collected using orbital sinus technique (Sanford method) before and after exposure for 2 hours for the first day, 4 hours/2 days, 6 hours/3 days, 8 hours/4 days, 10 hours/5 days, 12 hours/6 days and 14 hours/7 days respectively.

The results in table (1) showed that the level of total lipid peroxide ranged between 0.38±0.009 µmol/l after exposure for 2 hours only and 0.62±0.012 µmol/l after exposure for 14 hours/7 days. These level of serum total lipid peroxide after exposure to magnetic field were significantly increased than those obtained before exposure at the level of $p<0.05$. The percentage of difference between the levels obtained before and those obtained after exposure to magnetic field was ranged between 5.6% after exposure for two hours only and 71.4% after exposure for 14 hours/ seven days.

In contrast, the level obtained for GSH was reduced form 3.0±0.05 mg/ml after exposure for two hours only and 1.4±0.08 mg/ml after exposure for 14 hours / 7 days. This reduction is due to the effect of exposure to magnetic field were ranged between 2.9% after two hours only and 57.6% after exposure for 14 hours / seven days.

The reduced form of glutathione (GSH) is a tripeptide of cystine, glutamic acid and glycine. It is the principle nonprotein-sulfhydryl compound in the tissue. In addition to being a major cofactor for glutathioneperoxidase enzyme system which is responsible to detoxi by the highly oxidative moieties produced as a byproduct for the metalolion in the cell (free radical), GSH is necessary for the stability of sulfhydryl-containing enzymes, and protects hemoglobin cell lipid membranes of the cell and in organelles and other cofactors from oxidation [19].

GSH plays a critical role in many cellular processes, including the metabolism and detoxification of oxidants, metals and other reactive electrophilic compounds of both endogenous and exogenous origin [20]. The results of this study also showed a significant decrease in blood GSH content, at the level of $p<0.05$ accompanied by an increase in lipid peroxide, after 2,4,6,8,10,12 and 14 hours of exposure to low frequency magnetic field. GSH depletion could be related to its involvement in the detoxification of the deleterious effects of increased free radical reduced within the cell, the reason of in which Lipid peroxide was highly produce after the exposure to the magnetic field.

This might be due to increased level of oxidized form of GSSG which inhibit the glucose meonophosphate pathway which is responsible for the continues supply of GSH within the cell [21]. This also came agreement with those obtained by Fiorani, et al., 1997 [22].

Lipid peroxides increase in many diseases and in tissues poisoned by a Variety of toxins [23]. It was established many years ago that disrupted tissues undergo lipid peroxide more quickly than healthy ones. This means that lipid peroxides accumulate in a brain homogenate much more quickly than they do in an isolated intact brain [24].

Reasons for this increased peroxidizability of damaged tissues include inactivation of some antioxidants, leakage of antioxidants from the cell, and the release of metal ions (especially iron and copper) from storage sites and from metalloproteins hydrolyzed by enzymes released from damaged lysosmes. Hence the series of events:

Diseases or toxins ⟶ cell death or damage

increased lipid peroxide ⟶ can explain many of the reports of increased lipid peroxide in disease or toxic state [25].

In reality, the elimination of hydrogen peroxide in the cytosol of an any cell especially the red blood cells pout a pressure on the damaged cell membrane producing total lipid peroxide leading to the hemolysis of red blood cell i.e. reducing the number of red blood cell and oxidizing hemoglobin (natural) to oxidized hemoglobin (Met-Hb); (denatured). This means that increased level of free radicals will stimulate lipid peroxidation through the inhibition of glutathione reductise /peroxide system. The result of which accumulation of total lipid peroxide and oxidized form of glutathione (GSSG). In addition, free radicals will inhibit Met-Hbreductase leading to accumulation of met-hemoglobin [26,27]. This finding is also in agreement with what was previously postulate that the exposure of erythrocytes to the oxidant system promoted a depletion of GSH, a change in energy charge, and hemoglobin oxidation with production of Met-Hb [28,29].

Table1: Effect of Low Frequency Magnetic Field (0.85 mT) on Antioxidant system

Time of exposure (hours)	Serum total lipid peroxide (µmol/l)			Level of blood reduced glutathione (mgm /ml)		
	Before exposure	After exposure	% of change	Before exposure	After exposure	% of change
2	0.36 ± 0.005	0.38 ± 0.009*	+ 5.6	3.4 ± 0.08	3.0 ± 0.05*	- 2.9
4	0.34 ± 0.007	0.45 ± 0.006*	+ 32.4	3.3± 0.08	2.8± 0.13*	- 6.1
6	0.35 ± 0.006	0.50 ± 0.008*	+ 42.9	3.3 ±0.07	2.5 ±0.09*	- 24.2
8	0.35 ± 0.009	0.53 ± 0.007*	+ 51.4	3.4± 0.08	2.1± 0.06*	- 38.2
10	0.36 ± 0.009	0.57 ± 0.011*	+ 58.3	3.3± 0.o6	2.0± 0.13*	- 39.4
12	0.35 ± 0.010	0.60 ± 0.009*	+ 71.4	3.4 ±0.08	1.6 ± 0.07*	- 52.9
14	0.35 ± 0.008	0.62 ± 0.012*	+ 71.4	3.3 ±0.04	1.4 ± 0.08*	- 57.6

The data represented as mean values ± standard error for 8 rats.
*Significant difference between before and after exposure of groups to the magnetic field at the level of $p<0.05$.
On mankind and cells of mammalians, particular genes' expression can be influenced by static MF, thus these influences may be associated with both the magnetic flux density and the exposure period. Laramee et al. resulted in showing that when fibroblasts are exposed to MF of 1 to 440 mT, the heat shock protein expression of primary rats raised [30]. A heat shock protein is "a group of functionally related molecular chaperones that can be used as markers for cellular stress" [31]. Defense mechanisms of cell stress have several genes like proteins engaged in xenobiotic metabolisms, anti-oxidant and pro-oxidant enzymes as well as genes encoding molecular chaperones which might also be induced under oxidative stress of free radicals [32]. According to the existing data, Chekhun et al. found that free radical metabolisms can be disrupted and their concentrations can be elevated because cells are exposed to SMF [33]. The appropriate mechanisms of antioxidant defense, in return, can control damages of free radicals. Nevertheless, in previous research, SMFs with several magnetic levels of flux density (0.4, 0.55, and 0.7 T) have got no or insignificant influences on the expression of genes encoding enzymes engaged in systems of antioxidant defenses which are "SOD1, SOD2, GPX1, MGST1, GSR, and CAT" comparing to the control culture. Furthermore, there were no observable changes in the activity of antioxidant enzymes in fibroblasts of humans after their exposure to SMFs [34]. However, results associated with the effects of SMFs on the activity of cell antioxidants are inconsistent. In murine fibroblasts, absences of all effects of SMF is shown [35], but in myelomonocytic leukemia cells, oxidative stress is induced by the exposure to 6-mT SMF [36]. Besides, in-vitro on murine fibroblasts was done in a previous study which proposed that cells' tolerances to oxidative stress induced by fluoride ions are improved by exposure to fluorides and SMFs [37]. Likely, it has been reported that lipid peroxidation is elevated and antioxidant levels are reduced in brain tissues of many animals because of the exposure to radio frequency radiation (RFR).
The impacts of 890–915 MHz RFR, which were produced from cellular phones with 217 Hz modulation frequency on different oxidant and antioxidant levels inside the brain tissues of guinea boars, were examined by Meral et al. who exposed the boars to RFR during 12 hours per day (11 hours and 45 minutes for stand-by and 15 minutes for speaking mode) for 30 days. Finally, they concluded that the level of MDA is elevated and the enzyme activity of CAT and level of GSH are reduced in the brain tissue of those animals [38]. Lipid peroxidation in brain tissues of rats is raised when they are exposed to RFR according to Dasdag et al. [39] and Sokolovic et al. [40]. Additionally, Ilhan et al. reported that antioxidants such as Ginkgo Biloba may inhibit oxidative damage induced by RFR in brains [9].
Several neurodegenerative diseases like Parkinson and Alzheimer can be induced by prolonged exposure to ELF-EMF [41]. The neurotoxicity of ELF-EMF has pathogenesis in which oxidative damage made up by free radicals is shown [42]. ROS induces oxidative damage, that is unusually created from cellular metabolisms after the systems of antioxidants lose their ability for digestion and then structural and functional changes are induced in organs.Yet, the antioxidant systems are made up of antioxidant enzymes as well as different compounds of endogenous and dietary antioxidants which have an interaction with inactivate ROS. Bodies can be protected from oxidative damage by several antioxidants; the most significant ones are: SOD, CAT, GSH-Px [43]. The antioxidant capacity of body is indirectly reflected by these antioxidants' vitality.
O_2 is "one of the principal ROS produced in aerobic organisms, and is a highly cytotoxic ROS that is converted to hydrogen peroxide (H_2O_2) by SOD". H_2O_2 is converted to water and molecular oxygen by CAT or GPx. Therefore, relative reduces of CAT, SOD and GPx, which make the main enzymes of the antioxidant defense systems, may induce oxidative damage. This study indicates that the activity of SOD, CAT and GSH-Px of cerebral cortex as well as serum in the ELF-EMF group is significantly reduced, in comparison with the control. Thus, these findings show that mice exposed to ELF-EMF might not have sufficient antioxidant enzymes to completely remove ROS excesses. The tissue damage's level can be indirectly reflected by MDA which can measure lipid peroxidation. It was confirmed that [8] when mice are exposed to ELF-EMF, their levels of MDA in cerebral cortex and serum are significantly elevated. Therefore, ELF-EMF is confirmed to have the ability of promoting the lipid peroxides' formations [44] and causing oxidative stress in tissues [45].

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