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Research Article

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Effect of 50 Hz, 0.85 mT Magnetic Fields on Total and Differential Leukocytic

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Abstract In current societies, people cannot avoid the exposure to MFs including the ELF-EMF generated by power lines because electricity is widely used and there are various types of electrical appliances. Scientific and public interests in potential health risks related to exposure to ELF-EMF have grown in the last twenty years. Consequently, many cell functions and biological systems may be affected by such risks. Furthermore, the findings of various kinds of human and animal studies which deal with the biological effect of exposure to low frequency EMFs have constantly been both positive and negative. A relationship between subtle bio-effects and exposure to power frequency MFs seems to be shown by few of these studies. To break macromolecular bonds, the extremely frequency electromagnetic field does not have sufficient photon energy. Health could be affected by exposure to ELF-EMFs with subtle bio-effects. Besides, the haematological and immune functions through these mechanisms may be affected by ELF-EMFs. The studies which have investigated the ELF-EMF effects on haematological systems have conflicting results. Therefore, the current study have been carried out in order to estimate the potential effects of in-vivo exposure to extremely frequency electromagnetic fields on the haematological parameters of rats' entire blood. In the current study, the strength of magnetic fields exists in occupational and public environments and is within the limits which comply with the guideline standards of magnetic field exposure in public and occupational environments. Moreover, their intensity is within the range of magnetic fields generated from different electrical appliances.

Keywords Magnetic field, Biological effect, Leukocytic, Differential leukocytic

Introduction

External atmospheric electrostatic field (ESF) appears between the positively charged upper atmospheric layer and a negatively charged Earth surface. In clear weather conditions, ESF in the atmosphere usually varies between 100 and 150 V/m [1]. Intensity of the external ESF is prone to significant fluctuations due to different reasons both natural and human-made. Naturally, as the cloud approaches, at a ground level the field may first increase and then reverse with the ground becoming positively charged. As a result, ESF tension up to 3 kV/m might be observed [2,3]. Man-made causes of the enhancement of ESF tension are different and might be divided into the following groups: air pollution, application of electric devices and synthetic clothing/utensils, body insulation from the Earth. Pollutant aerosols remove small ions from the air, which are very important to atmospheric electric conductivity. This decreases air conductivity and increases the potential gradient [4,5]. Electrical devices at home represent additional sources of ESF. In recent years' household appliances have dramatically changed our electrical environment. ESF of several hundreds of kilovolts per meter can be produced by handling and treating of plastics or by walking on non-conducting carpets. Body insulation from the Earth is a widespread unfavourable phenomenon in our reality, contributing to extra ESF generation. He said that when body potential was the same as the Earth's electric potential (was grounded), it became an extension of the Earth's gigantic electric system. The Earth's potential becomes a "working agent that cancels, reduces, or

pushes away electric fields from the body." Otherwise, changes in the ambient voltage induced on the body might be observed. Thus, insulation of the human body from the Earth contributes to ESF and probably is one of the main reasons for enhancement of body electric potential. This means that both ESF enhancement and body insulation from the Earth should result in similar outcomes. In particular, Marino et al. [6], and Harutyunyan and Artsruni [7], reported changes in serum protein fractions of rats exposed to vertical fields of 0.6–19.7 kV/m and 200 kV/m. These results were consistent with data provided by Sokal and Sokal [8], who showed increased content of alfa1- and alfa-2 globulin fractions in serum of humans grounded for 7 days. In our experiments, the mentioned fractions were diminished in ESF-exposed rats. Next, grounding the body substantially increased zeta potential and decreased red blood cell (RBC) aggregation [9]. Accordingly, opposite effects of the external ESF on physical parameters of RBC were reported [10,11]. Effects of an externally applied electric field on biological systems have been investigated over the past few decades using various methods and toward various targets. However, the mechanism of ESF effect on biological objects has not yet been fully understood. The reason may be that ESF induces various changes depending on exposure strength, duration, and other experimental conditions [12]. In some reports, microscopic views of structural changes in the cells were illustrated, including changes in cell orientation [13], shape [14], cell migration, and cell differentiation [15]. Very high voltage cell-to-cell fusion was registered [16]. Disbalance in pro-/antioxidant system of blood and alterations in plasma protein content in ESF-exposed rats were also demonstrated [17,18]. So, based on the above-provided data, one could expect blood cell alterations in animals exposed to ESF. In this regard, the report of Grandolfo [19], seems strange: "No significant differences were detected in blood cell counts, blood proteins or blood chemistry in mice exposed to ESF 340 kV/m." These contradictions regarding biological effects of ESF forced us to study hematology in female rats exposed to ESF. To solve this task, in our experiments the in vivo study was accompanied with in vitro measurements in ESF exposed blood samples. The choice of sex was based on earlier data of Leitgeb et al. [20], evidencing that women are generally more sensitive to electromagnetic field than men.

Materials and Methods

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).

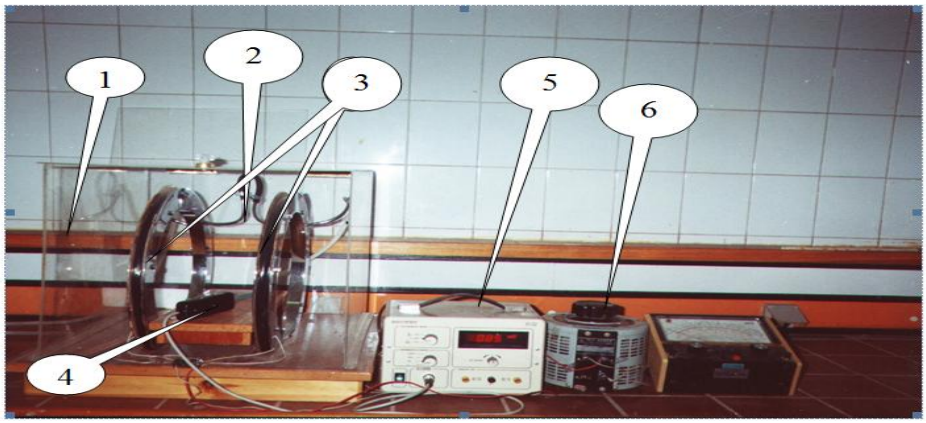

Figure 1: A view of the magnetic field generator system.
1-Transparent plastic cover 2- Water circulating cooling system 3- Helmholtz coil 4- Magnetic probe 5- Flux meter 6- AC Variac

The method for measuring magnetic fields can be divided into two classes:

a) Indirect measurements in which we measure the effect of the magnetic field and from a simple calculation, the magnetic field itself could be estimated. Spectral analysis of the effect of the magnetic field on the radiation medium (Zeeman Effect, Faraday Effect…etc) is a good example of the indirect measurements of the magnetic field.

b) Direct measurements in which a magnetic probe is directly trusted into the medium to measure the magnetic field at the point where the probe is located [14].

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.

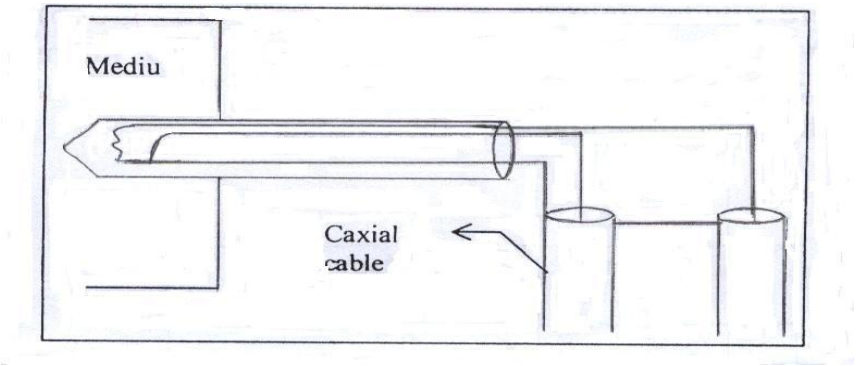

Figure 2: Schematic drawing of the magnetic probe

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.

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.

Methods

Blood samples were collected using orbital sinus technique (Sanaford method) [22]. The whole blood was used to determine White blood cells (WBCs), differential leukocytosis (Neutrophils, Lymphocyte, Monocytes, Eosinophils and Basophils) and platelets counts. This study was fulfilled by using complete blood counter (Selly, France).

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 [23]. The data are representing 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 mean ± standard errors of the electronic counts of total leukocytes in table (1) and (2) of the different groups of rats were investigation before exposure to magnetic field ranged between 11429±105 and 12231±247 while the leukocytic count of these groups of rats were 11420±15 count/mm3 after exposure to magnetic field for 2 hours only and 11507±122 after exposure for 14 hours / seven successive days. The result showed a small reduction but have no significance.

The reduction of neutrophils obtained after exposure is significant at the level of $p<0.05$ while these were significant increased level of the counts of the lymphocytes after exposure to magnetic field at the level of $p<0.05$. The mean ± S.E. of count of the neutrophils ranged between 3105±121 after exposure to 14 hours / 7 day with a percentage of reduction equals to 56% and 6229±37 after exposure for two hours only with a percentage of reduction equals to 13% . The percentage of increased count of lymphocytes after exposure to magnetic field ranged between 12% after exposure of two hours and 95% after exposure for 14 hours / seven successive days.

The small behavior was obtained in number of total platelets where they reduced form 988±17 to 546±12 at the level of $p<0.05$ when compared with those obtained before exposure to magnetic field. The effect of magnetic field (0.85 mT) on the total and differential leukocytic counts is show in table (1) and (2). The total leukocytic count after exposure to different intervals time ranged between 12180±140 and 11420±15 which indicated that a certain level of reduction was obtained without any significance when compared to those levels obtained before exposure to magnetic field. The results in table (2) showed also a significance elevation in the counts of lymphocytes at the level of $p<0.05$ and a significance reduction in neutrophils after exposure to low frequency magnetic field. The small behavior was obtained in the number of total platelets showed in figure (3) where they reduced from 988±17 to 546±12 at the level of $p<0.05$ when compared with those obtained before exposure to magnetic field.

The most significant changes were observed in WBCs. The increase was registered after short-term electrostatic field (ESF) exposure. After long-term ESF exposure, a moderate decrease of these cells was shown. In the in vitro experiments, we observed diminished number of WBC in blood samples exposed to ESF. Both the increase of WBC count after short-term exposure of animals to ESF and the decrease after long-term exposure can be explained by WBC damage in peripheral blood and their compensatory mobilization from the bone marrow. This assumption is supported by the DNA comet test. Using this test, we showed enhanced DNA

strand-brakes after ESF exposure both in vivo and in vitro. Moreover, both regimens of in vivo ESF applications resulted with DNA damage in WBCs of peripheral blood. The genotoxisity of external ESF was shown in an earlier study of McConn et al. [24]. They used DNA comet test to characterize ESF (200 kV/m) effect on DNA strand in blood leukocytes in rats for the first time. We did not find any report on a similar effect of ESF. However, Lai and Singh [25] reported increase of DNA double and single strand breaks in brain cells at in vivo exposure to magnetic field. These authors showed that magnetic field-induced DNA strand breaks were caused by free radicals, since treatment of the animals with free radical scavengers blocked these effects. DNA damage observed in our work may result from free radical action since activation of prooxidant processes was shown for this physical factor [26,27]. The fact that alterations of WBC count were mainly stipulated by changes in MON and PMN content also confirms a free radical-dependent mechanism of ESF-induced genomic DNA damage. It is well known that phagocytes (MON and PMN) possesses strong free radical-generating mechanisms, and their damage might induce oxidative burst [28]. Kindzelskii and Petty [29] reported enhanced reactive oxygen production by neutrophils and cellular DNA damage after exposure of electric field. RBC count altered significantly neither after short-term ESF exposure in vivo nor after ESF exposure to blood in vitro. Nevertheless, we observed enhanced RBC count after long-term exposure of rats to ESF. This was accompanied by accordant alterations in HGB, HCT, MCV, and MCH. It is noteworthy that RBC size (MCV) was diminished both after long- and short-term ESF exposures in vivo. Appropriate changes were also observed on RBC histogram. Although the RBC count and hemoglobin parameters were minimally altered for ESF-exposed rats, the shape of RBCs was significantly affected. ESF caused RBC deformation both in vivo and in vitro. This finding is supported by earlier results reported by Artsruni et al. [30]. A decrease in the strength of peripheral proteins binding to erythrocyte membranes and an increase in microviscosity of the lipid bilayer was shown. Such alterations in physical parameters of RBC membranes might be responsible for above-described deformations. Similar results were shown by Gass et al. [31] upon application of high frequency electric field. Authors described changes in RBC shape and production of various membrane structures: long filopodia-like processes, retraction fibers, and lamella-like structures.

Table 1: Effect of Low Frequency Magnetic Field (0.85 mT) on Total Leukocytic Counts

Time of exposure (hours)	Leukocytic count /mm^3		Neutrophils Count /mm^3		Lymphocytes count /mm^3	
	before	After	before	After	before	After
2	11429 ±105	11420±15 (-0.08)	7132 ± 129	6229 ± 37* (-13)	3617 ± 134	4575 ± 116* (+12)
4	11708±144	11699±194 (-0.08)	7061 ± 110	6228±158* (-11)	4008 ± 142	4856 ±143* (+26)
6	11644 ±192	11604±122 (-0.3)	7154 ± 175	5603±158* (-21.7)	3836 ± 126	5386 ± 151* (+40)
8	12231±247	12180±140 (-0.4)	7515 ± 334	5014±144* (-33.3)	4021 ± 142	6209 ± 1688* (+54)
10	11626±108	11546±132 (-0.7)	7178 ±119	4721±112* (-34.2)	3820 ± 96	6726 ± 161* (+76)
12	11765 ±244	11663 ±128 (-0.9)	7316 ± 182	3605 ± 86* (-50.7)	3811 ± 115	7404 ± 169* (+94)
14	11660±201	11507 ±122 (-1.3)	7108 ± 122	3105±121* (-56)	3984 ± 167	7786 ± 227* (+95)

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 $p<0.05$. Percentages of changes level before and after are in parenthesis.

Table 2: Effect of Low Frequency Magnetic Field (0.85 mT) on Differential Leukocytic Counts

Time of exposure (hours)	Monocytes count / mm^3		Eosinophils count / mm^3		Basophils count /mm^3	
	before	After	before	After	before	After
2	329 ± 29	307 ± 21 (-6)	249 ± 27	206 ± 20 (-17)	102 ± 14	103 ± 14 (+0.9)
4	333 ± 31	281 ± 22 (-15)	234 ± 2	247 ± 12 (+5)	72 ± 21	87 ± 19 (+20)
6	334 ± 24	304 ± 27 (-8)	219 ± 15	247 ± 12 (+12)	101 ± 26	74 ± 22 (-26)
8	341 ± 42	267 ± 20 (-21)	252 ± 15	239 ± 4 (-5)	102 ± 14	87 ± 19 (-14)
10	292 ± 24	276 ± 21 (-5)	249 ± 27	247 ± 27 (-8)	87 ± 19	72 ± 21 (-17)
12	295 ± 24	279 ± 21 (-5)	236 ± 4	248 ± 13 (+5)	107 ± 21	75 ± 22 (-26)
14	276 ± 18	292 ± 22 (+5)	204 ± 18	234 ± 4 (+14)	88 ± 19	87 ± 19 (-1)

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 $p<0.05$.
Percentages of changes level before and after are in parenthesis.

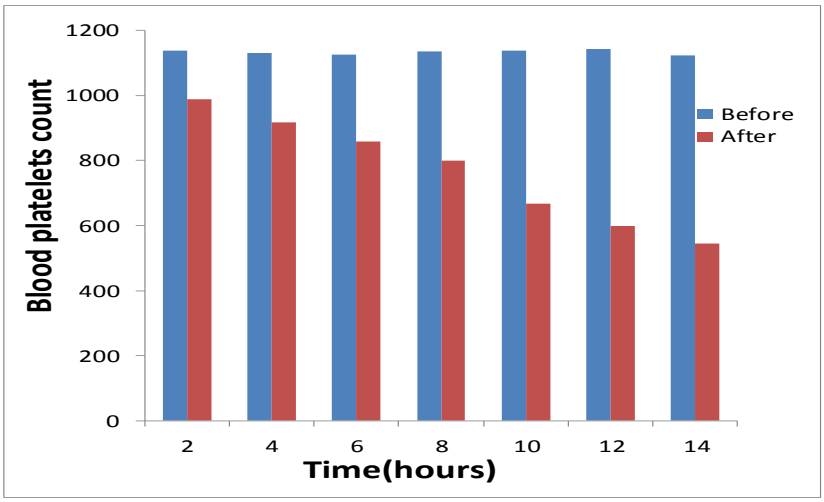

Figure 3: Effect of low frequency magnetic field (0.85mT) on the blood platelets count (103/mm^3) of rat

Serrated RBC was also observed in mice exposed to mobile phone-induced electromagnetic field [32]. Thus, it seems that both electrostatic fields and time-varying electromagnetic fields might exert similar biological effects. PLT count significantly increased in the blood of rats after short-term ESF exposure and remained unaffected after long-term ESF exposure. Simultaneously, the moderate decrease of PLT count was observed in blood exposed to ESF in vitro. The situation was similar to that of WBC. Namely, an increase of PLT count might be a secondary outcome of PLT initial damage/decrease. Alterations of PLT-dependent processes are expected in such a situation. Indeed, Cassiano et al. [33] and Harutyunyan and Artsruni [34] have shown acceleration of blood coagulation in ESF-exposed animals. Also, as was shown by Zhang et al. [35], intense electric fields (nanosecond pulses) cause human platelet aggregation, increase of intracellular-free Ca+2 ion concentration, and the release of plateletderived growth factor. All of these are generally in compliance with our results. A detailed study of hemostasis in rats exposed to external ESF is a matter for our future investigations. The above-discussed damage of blood cells and release from the bone marrow may stipulate a wavelike character of ESF post-effects. This aspect is very poorly covered in the literature. Earlier, Artsruni et al. [36]

achieved a similar result for protein synthesis and degradation in the liver of ESF-exposed rats. Explanations of the physical mechanisms of ESF biological effects are beyond the scope of this article; however, it has been shown that the application of electric fields to biological systems can lead to changes in ionic and molecular currents, lifetimes of free radicals, and orientation of molecules [37,38,39]. All mentioned processes have obviously contributed to biological consequence of ESF exposure described in the present work.

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