Effects of 50-Hz Magnetic Field on Some Biophysical Properties of Albino Rat's Blood

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ABATRACT

The effects of 50Hz magnetic field exposure on rat red blood cells (RBCs) structural properties were studied. Fifty male albino rats were equally divided into five main groups, namely groups A, B, C, D and E. Group A(10 animals) was used as the control group not subject to any treatment. Groups B, C, D and E (10 animals per group) were each continuously exposed to magnetic fields of (0.1, 0.2, 0.3 and 0.4 ± 0.01 mT-50Hz) respectively, for a period of 10 days. After which, all groups were immediately sacrificed and blood samples collected from each animal. The osmofragility and viscosity of RBCs were investigated for each collected blood sample. Results indicated obvious abnormality in the RBCs mechanical and rheological properties for the animals of groups B, C, D and E as compared to the control group A. It was concluded that further investigations are necessary, performed in cooperation with medical researchers, concerning regular medical examination of individuals exposed to such fields. Consequently, it becomes mandatory to revise dose limits recommended by the different commissions for exposure to such extremely low frequency magnetic fields below 0.5 mT.

Keywords: ELF, Electromagnetic Fields, RBCs fragility, Blood viscosity

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INTRODUCTION

Over the past years humans have created different intensities of electromagnetic fields through the various communication services and many electrical devices. Alongside the benefits

of such technologies, there is much concern regarding the influence of electric, magnetic and electromagnetic fields on the metabolism, biological processes and molecular mechanisms of cellular organisms [1, 2]. With the ever-increasing use of the technology, there has been increasing scientific evidence and public concern regarding the potential health risks from power-frequency or extremely low frequency (ELF) electromagnetic fields (EMFs) and from radiofrequency/microwave radiation emissions. The biological effects of ELF electric and magnetic fields have become a topic of considerable scientific scrutiny during the past two decades. The flurry of research in this area has contributed greatly to our understanding of the complex electromagnetic environment to which we are exposed. Yet, it has not abated the controversy associated with their harmful biological effects [3, 4].

The International Commission on Non Ionizing Radiation Protection (ICNIRP) - formally recognized as the nongovernmental organization in Non Ionizing Radiation (NIR) protection for the World Health Organization (WHO), the International Labour Organization (ILO) and the European Union (EU) - continuously monitors and periodically reviews critical scientific literature involving the physical characteristics and sources of NIR and their possible biological and adverse health effects. In doing so, the exposure guidelines developed by ICNIRP are intended to protect against the adverse health effects of NIR exposure. As adverse consequences of NIR exposure can vary tremendously from trivial to life threatening, a balanced judgment is thus required before deciding on acceptable exposure guidance [5].

The standard formulated guidelines establishing limits for occupational and residential EMF exposure are based on evaluation of biological effects that have been established to have health consequences. The main conclusion from the WHO reviews is that EMF exposures below the limits recommended in the ICNIRP international guidelines do not appear to have any known consequence on health. The guidelines show that acute biological effects have been established for exposure to ELF electric and magnetic fields in the frequency range up to 100 kHz that may have adverse consequences on health. On the other hand, consistent epidemiological evidence suggested that chronic low intensity ELF magnetic field (MF) exposure was associated with increased risk of childhood leukaemia. Consequently, establishing exposure limits through further examination of the low intensity ELF adverse health effects becomes imperative [6].

Saunders, 2003 [7]; found that the applied electric or magnetic field in the resonant frequency could have an impact on lymphocyte cells. In turn this may affect the immune system response in many diseases such as lymphatic anemia. As a result, natural ions, tissues, blood cells and body movements could be affected by EMF reducing the protective ability of white blood cells and impairing body function and control of such systems as the endocrine and nervous systems.

In the same regard, the influence of EMF exposure on biological systems, Jeong Jh, et al, 2005, [8]; found that exposure to continuous light and to ELF-EMF did not change significantly the rate of mitoses compared to sham-exposed rats. Whereas the amount of cell death was significantly increased in comparison to animal controls exposed to EMF in a 12-h dark-light cycle. In conclusion, long-term exposure to ELF-EMF, in animals housed under continuous light, may have reinforced cell alterations due to photic stress. Such findings suggested the synergistic

effect in vivo of stress and ELF-EMF exposure, advocating more rapid involution of the thymus possibly responsible for increased susceptibility to the hazardous effects of ELF-EMF.

H. E. Baieth; 2008 , [9]; studies on the blood of rats provided guidance for the assessment of occupational and public health significance of exposure to EMFs. Apparent changes in the viscosity of animal blood following exposure to EMFs (3,5 and 10 gauss) was examined. Results indicated elevated hematocrite (HCT) and decreased blood viscosity with increased EMF. Red blood cell permeability and deformability and the electrical properties of hemoglobin (conductivity and relaxation time) were also examined. The study revealed EMF exposure produced pronounced changes in hemoglobin molecular structure with induced force acting on the charged particle of charge q which may activate the Rouleau formation of red blood cells (RBCs).

Dilek Ulker ., et al; 2009, [10]; evaluated the possible effects of in vivo exposure to ELF-EMF (0.97 mT, 50 and 100 days, 3 h/day) on whole blood parameters (hematological parameters) in rats. Eosinophil, hemoglobin and MPV levels significantly decreased in rats that were exposed to EMF for 50 days. When the data for rats exposed for 50 days and 100 days were compared, it was found that MPV levels in rats exposed for 100 days were significantly lower.

Gabriele Gerardi., et al;2008 [11] A series of experiments on rats have been performed in this study to evaluate the effects of long time (50 days) exposure to ELF-EMFs and amplitude (non thermal), testing whether the metabolic processes would be affected. Several biochemical parameters have been evaluated by comparing their values during the periods of exposure and non exposure. The evidence that long term exposure to EMFs with a well defined frequency may have relevant effects on parameters such as body weight, blood glucose and fatty acid metabolism has been obtained.

Studies of childhood cancers were followed by studies of adult cancers in occupational as well as residential settings and by effects of EMFs on reproduction. Residential exposure was associated with miscarriages while occupational exposure was linked to various reproductive problems as well as adult cancers including primary brain tumors, leukemia, and breast cancer. Similarities between childhood and adult cancers raised concern [12]. This evaluation of the carcinogenicity of EMFs is of particular relevance to this environmental health criteria document. A number of relevant studies have been published following this assessment indicated that the increased risk of several cancers, such as childhood leukemia led to the classification of ELF-MF by the International Agency for Research on Cancer (IARC) as a “possible human carcinogen” in 2002 [13].

In spite of all these studies that have been carried out over the past years there is still no persuasive evidence that the fields pose any risks on all biophysical parameters at low doses. Moreover, the limited researches on the effect of ELF-MF on the biophysical parameters that can affect blood such as osmotic fragility, solubility, viscosity and the change in the morphological properties of the RBCs membrane which make some international organizations such as the (WHO), (ICNIRP) and (IARC) recommended doing more researches in all areas of exposure to the magnetic fields to measure it’s risk degree on health [12-14].

However, still the question can the exposure to ELF-MF promote cancer or initiate any other health hazards still has no clear answer and needs a lot of work to be handled, this gives us an enthusiasms to perform this work. This work is devoted for creating a technique of experimental application of magnetic field using coils that can interact with the biological object which resembles the general kind of exposure of humans near technical devices and used in research as well as in therapy [15].

Moreover to study the hazard health effects accompanied with the exposure to ELF-MF on RBCs of albino rats as a reasonable biomarker to reflect any deterioration attributed to the exposure on the circulatory system and the injury on the bone marrow. An investigation carried out immediately after 10-days continuous exposure (24 hours/day) for groups of animals that exposed to different low doses of extremely low frequency magnetic field and then examine the influence of the exposure (under our circumstances) on osmotic fragility of the RBCs membrane, solubility of cell membrane proteins, viscosity of blood and the morphological shape of RBCs in blood films. It is worthy to mention that, the effects of electric or the combined field is out of the scope of this study.

MATERIALS and METHODS

Experimental Animals

In the present work 50 male albino rats, each of average weight 160 ± 20 grams divided into five main groups, namely group A,B,C,D and E . Animals of group A (10 animals) are used as a control group and didn't receive any treatment and housed in normal environmental conditions (the temperature inside the lab varied between 22° and 25° C, lighting condition are natural light from large windows during the day and complete darkness during the night). Animals of group B, C, D and E (10 animals per each) were continuously exposed to a magnetic field of (0.1, 0.2, 0.3 and 0.4 ± 0.01 mT-50Hz) respectively, for a period of 10 days. Animals of All groups were immediately sacrificed and blood samples from each animal were collected for experimental investigations directly after the end of the exposure.

Magnetic Field Exposure Facility

The magnetic field exposure system was locally manufactured in the Radiation Physics Laboratory at Alexandria University in Egypt. The magnetic field exposure unit generates a homogeneous magnetic field by 4 solenoids of 48 turns each of electrically insulated 2 mm copper wire, wound around a copper cylindrical chamber of 60 cm external diameter as shown in Figure (1).

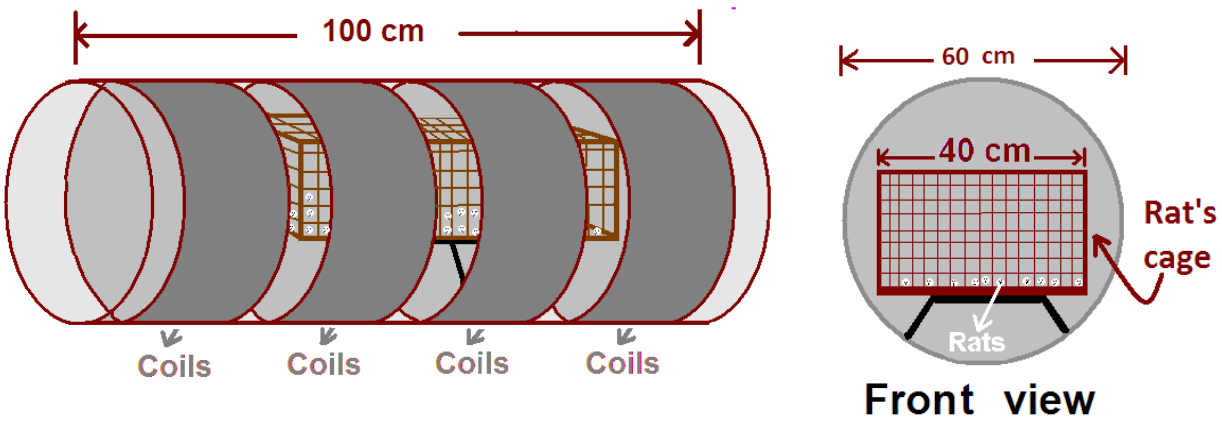

Figure (1): A diagram of the magnetic exposure system. It is composed of 4 solenoids producing homogeneous magnetic field at their central axis using a sinusoidal current of 50 Hz. Animals were exposed continuously as a group in a plastic cage on the shelf within the solenoid.

Animals were kept in special plastic cages fixed on supports inside the irradiation chamber with an exposure volume of dimension 40x30x35 cm^3 located inside the coil. Food and water were kept in special open containers fixed on the walls of the cages. Cleaning and changing water and food were done for all animals twice daily. Animals were exposed to the magnetic field in a free volume at the center axis of the solenoids. A 220 V and 50 Hz sinusoidal power frequency current were fed through the solenoid in the exposure system. The prepared circuit was able to generate an effective magnetic field with sinusoidal wave of frequency of 50 Hz. The magnetic flux density was measured by using a digital Teslameter (Phywe, 209101074, Göttingen, Germany) to ensure homogeneity of the field. No temperature differences were observed between exposed and sham groups during the exposure. Magnetic field measurements showed that, under the conditions of the experiment, the magnetic field exposure system produced a homogenous flux density in all readings and stable frequency of 50 Hz with negligible harmonics and no transients. The magnetic flux density in the area where the animals were housed was 0.04 ± 0.01 mT as measured by Teslameter. The coils were connected to a variac fed from the mains (220 V and 50 Hz). For more precautions an electric timer was used to adjust the exposure times especially when mains fall.

RBCs Membrane Osmotic Fragility

In the osmotic fragility test, 0.05 ml of blood sample was added to varying concentrations [0%-100%] of a buffered sodium chloride solution (normal saline 0.9% NaCl) and allowed to

incubate at room temperature for a period of 30 min, then remix the test tubes gently and centrifuge at 3000 r.p.m (centrifugal model 800- 4000/min, made in China). Spectrophotometer (UV/visible spectrophotometer LKB-Novaspec, made in England) was used to measure the hemolysis percentage for all blood samples collected from each group. [hemolysis is the liberation of hemoglobin from RBCs] [16].

Blood Viscosity Test

Programmable rotating Viscometer model DV-II (Spindle SC4-18 / Sample chamber SC4-13 (p)) manufactured by a Brookfield company in the USA was used for measuring the whole blood viscosity at different shear rates. The Viscometer is provided with a circulating water bath to control the temperature of the sample. All viscosity measurements of the samples were controlled to be at 37 ± 0.2 ^{o}C; 6.7ml of heparinized blood collected from each individual were put in the Viscometer chamber for the run of the experiment. The viscosity of each sample was measured in centipoises (cP) at different shear rate ($\mathbf{s^{-1}}$). At each shear rate, the samples were left in dynamic motion in the Viscometer in order to get a stable reading of the viscosity. Then plot relation between viscosity (cP) as Y axis and shear rate (s^{-1}) as X axis for each sample to get the viscosity curve.[17]

RESULTS AND DISCUSSION

Osmotic Fragility

The osmotic fragility test for all blood samples was carried out as mentioned before and different parameters were examined and calculated. These parameters are as follows:
(C_s%) is the percentage of NaCl concentration at which hemolysis starts to occur which characterizes the transport of water molecules through the RBCs membrane and hence its permeability, (C_{max}%) the maximum rate of hemolysis, ($H_{50\%}$) the concentration percentage of NaCl that leads to 50% hemolysis, and (W_{hmax}%) the width at half maximum which represents the relative elastic range of the RBCs membrane. Figure (2) shows the variation of the hemolysis percentage as a function of percentage of NaCl concentration in buffer solution for RBCs collected from animals of group A. The data in the figure were differentiated and plotted as a function of NaCl concentration percentage as presented in figure (3). The average values of the C_s, C_{max}, W_{hmax} and $H_{50\%}$ percentages were calculated for all animals from each group A, B,C,D and E and tabulated in the table (1). The shape of the hemolysis differential curve is the ideal one for the healthy RBCs as mentioned in literatures [18, 19].

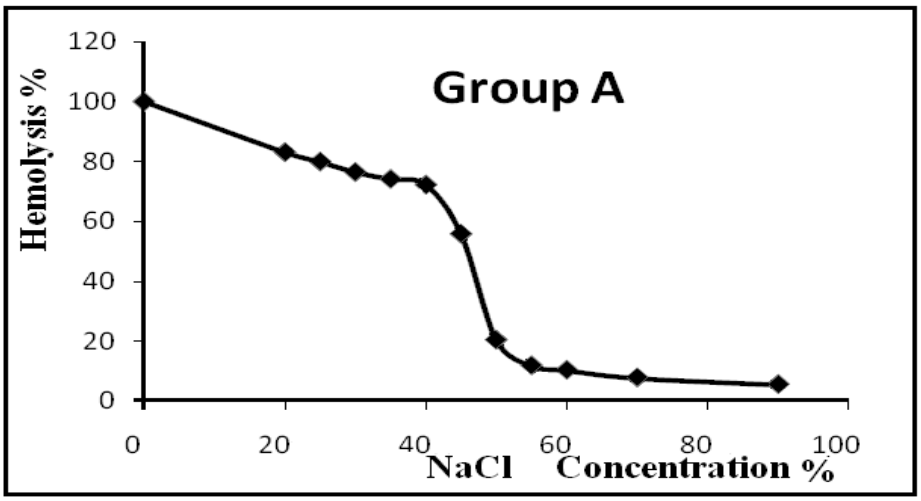

Figure. (2): The variation of the hemolysis percentage as a function of NaCl concentration percentage in buffer solution for red blood cells collected from animals of control group A.

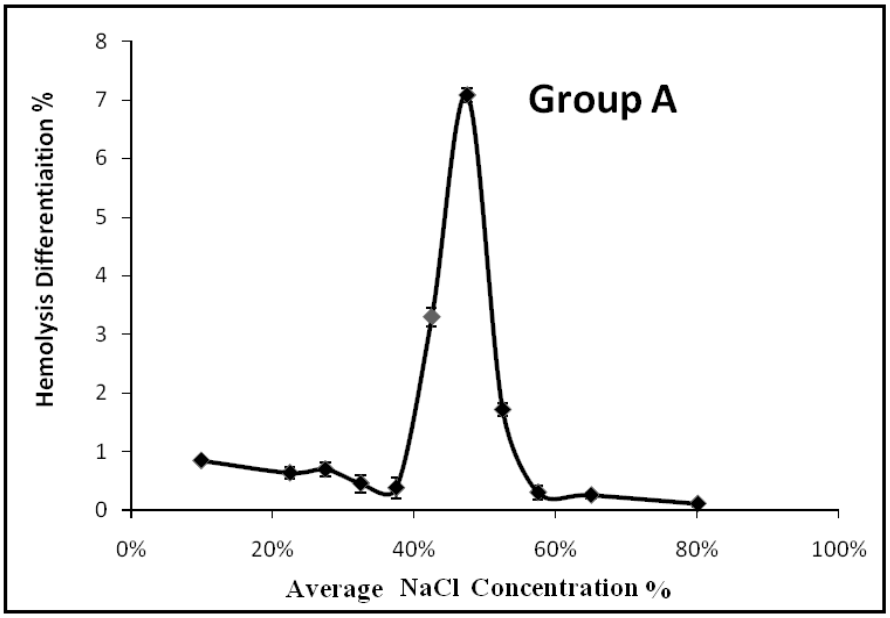

Figure.(3): The differential data plotted as a function of the average NaCl concentration percentage of samples collected from group A.

Figure (4) illustrates the osmotic fragility curves for the RBCs collected from exposed animals of groups B,C,D and E as compared to the control group A. The differential plots for the curves are presented in Figure (5). It's clear from the osmotic fragility characteristic curves in the figures that the percentage concentration of NaCl at which maximum hemolysis and maximum rate of hemolysis occurred were shifted to lower values as compared with the control ones.

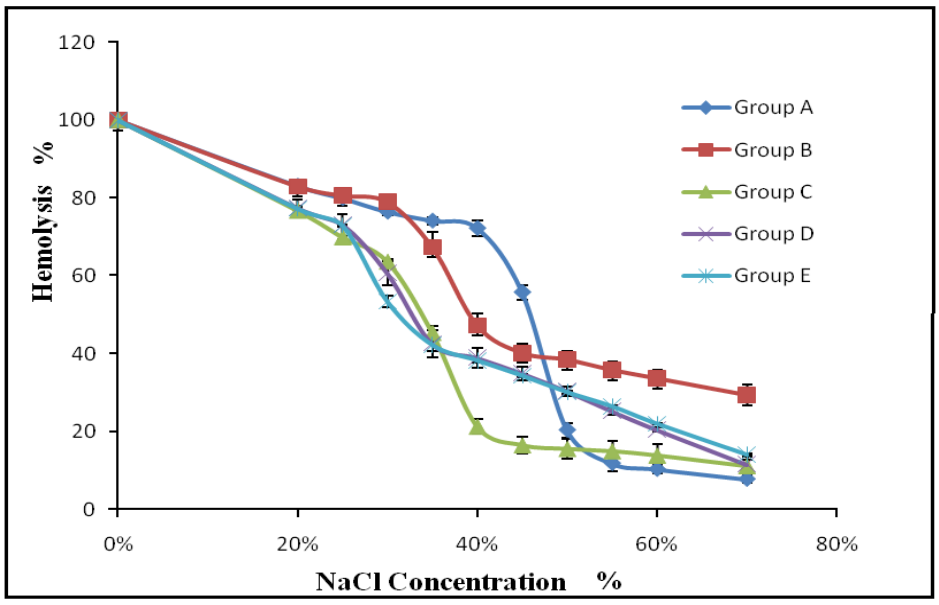

Figure.(4): The variation of the hemolysis percentage of animals RBCs as a function of the NaCl concentration % for samples collected from all groups.

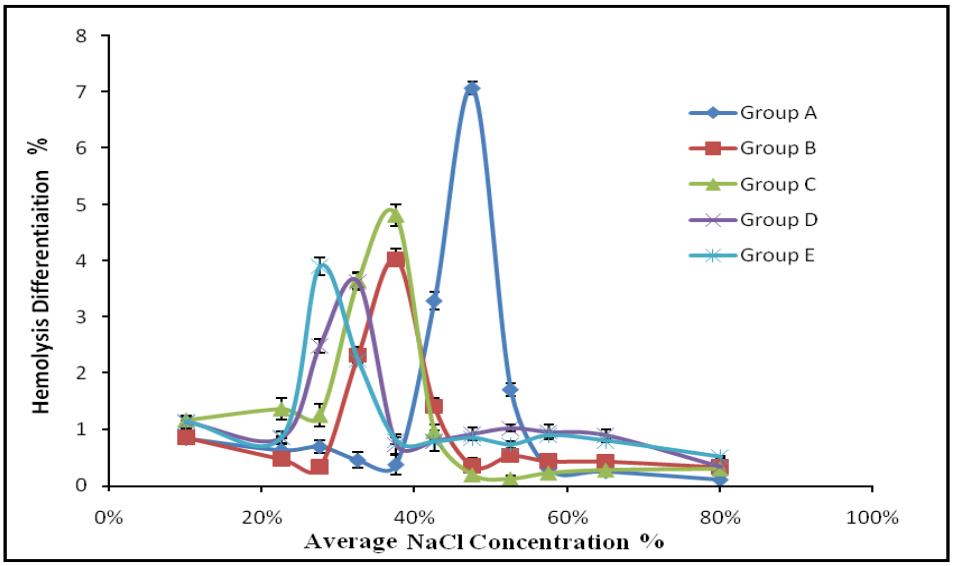

Figure.(5): The differential data plotted as a function of the average NaCl concentration percentage of samples collected from the control group A and the groups B, C, D and E which exposed to different low magnetic field doses.

The results of the osmotic fragility curve can give a good information about the changes that may occurred in the elasticity and ionic permeability of the RBCs membrane, which play the major role in the metabolic activities of RBCs. The shift of the main peak and Cs to the lower value of NaCl concentration after exposure to ELF MF indicates changes in the membrane permeability to water molecules. The changes in the RBCs permeability will cause disturbance in their function and mutual interactions with neighboring cells.

Table.(1): The average values for each parameter calculated after the osmotic fragility test from all animals from each group

Parameters / Group	C_S%	C_{max}%	H_{50}%	W_{hmax}
Control group A	37.5 ± 0.32*	47.7 ±0.41*	47 ±0.31*	8.17 ± 0.02*
Exposed group B	28.4 ± 0.21*	36.9 ± 0.32*	39.8 ± 0.14*	9.71 ± 0.02*
Exposed group C	30.5 ± 0.25*	35.9 ± 0.34*	34.4 ± 0.19*	10.57 ± 0.01*
Exposed group D	25.5 ± 0.36*	30.4 ± 0.26*	31.4 ± 0.27*	9.31 ± 0.02*
Exposed group E	22.6 ± 0.22*	27.8 ± 0.23*	30.4 ± 0.25*	8.57 ± 0.01*

* The standard error values.

Viscosity

Rheological measurements of whole blood and red blood cell suspensions demonstrate unique non-Newtonian behavior i.e. yield stress; shear thinning, thixotropy and viscoelasticity [20]. Blood behaves like a non-Newtonian fluid whose viscosity varies with shear rate. The non-Newtonian characteristics of blood come from the presence of various cells in the blood (typically making up 45% of the blood's volume), which make blood a suspension of particles. As the blood begins to move, these particles (or cells) interact with plasma and among themselves and hence hemorheologic parameters of blood include whole blood viscosity, plasma viscosity, red cell aggregation, and red cell deformability (or rigidity) are varied [21]. The effect of stirring rate can be determined with the rotary Viscometer; by rotating its spindle at different speeds. For each of these speeds, the shear rate is calculated (related to the rotation speed of the

spindle) and shear stress is measured (related to the torque needed to rotate the spindle). Then a plot of the shear stress and shear rate is obtained, as shown in Figure (8).

Of note that the fluid shear thinning is the decrease of viscosity with increasing shear rate and blood behaves non-Newtonian fluid at low shear rate and change its behavior to Newtonian fluid for high shear rate flow, i.e. the case of flow through larger arteries. It has been pointed out that in some diseased conditions, blood exhibits remarkable non-Newtonian properties.

The rheological properties of blood can be described by one of the most widely used forms of the general non-Newtonian constitutive relation is a power-law model,

$$\tau = k\,\gamma^{n} \qquad (1)$$

Where τ is the shear stress ,γ^{n} is the shear rate, the constant **k** (consistency index) is a measure of the consistency of the fluid and its viscous nature where the higher the k is, the more viscous the fluid is. **n** (rheological flow index) is a measure of the degree of non-Newtonian behavior: the greater the departure from the unity, the more pronounced the non-Newtonian properties of the fluid are. Rheological flow index values are almost unaffected by the erythrocytes concentration. The dependencies of rheological parameters k and n on the hematocrite values are also found. The viscosity of the power-law fluid can be expressed as

$$\eta = k\,\gamma^{n-1} \qquad (2)$$

Where η is non-Newtonian viscosity. If $(n<1)$ a shear-thinning fluid is obtained and if $(n>1)$ a shear-thickening fluid is obtained; But if $(n=1)$ a Newtonian fluid is obtained.

The viscosity test with different shear rates for all blood samples was carried out as mentioned before and different parameters were calculated by applying the power-law model. Figures (6) and (7) show the viscosity (cP) as a function of shear rate (s^{-1}) of blood collected from animals of group A and animals of groups B,C,D and E with compare to the control one. The average values for the viscosity of the whole blood and the flow index (n), the consistency index (k) were calculated for all animals from each group A,B,C,D and E and tabulated in the Table (2). The average values of the whole blood viscosity indicated a pronounced decrease for the exposed groups as compared to the control ones. The data obtained from the power law model for the exposed samples indicate a significant increase in the flow index and significant decrease in the consistency index. The correlation between shear stress and shear rate defining the flow behavior of a liquid is graphically displayed as shown in Figure (8). This diagram is called a flow curve. These results illuminate remarkable changes in the rheological properties of the exposed blood samples which may be due to the changes of the cellular blood contents as a result of structural changes and uncontrollable blood membrane uptake.

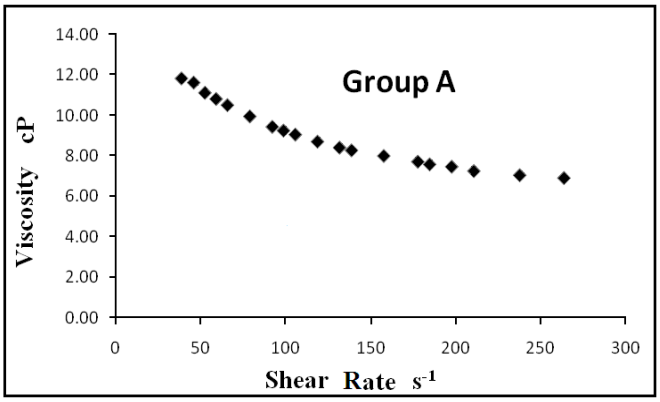

Figure (6): The variation of blood viscosity (cP) as a function of shear rate (s^{-1}) for the samples collected from the control group A.

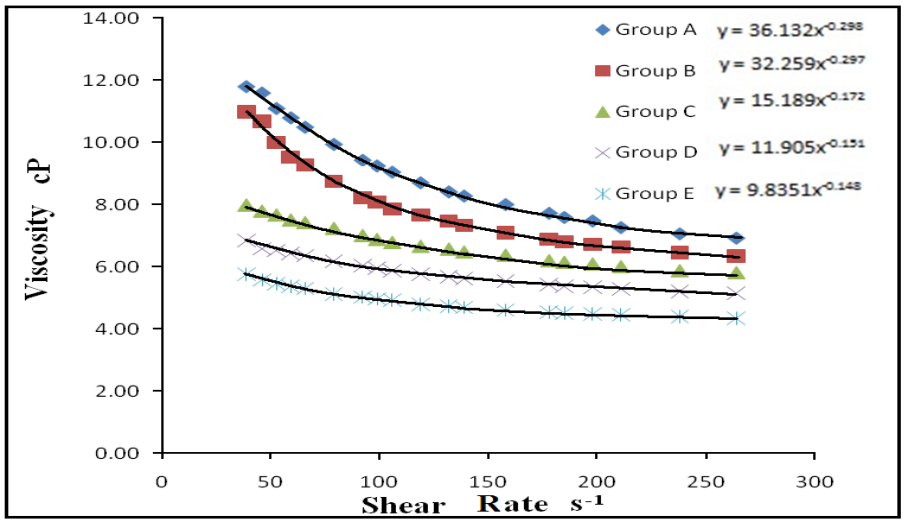

Figure (7): The variation of blood viscosity (cP) as a function of shear rate (s^{-1}) for the samples collected from the control group A and the groups B, C, D and E which exposed to different low magnetic field doses.

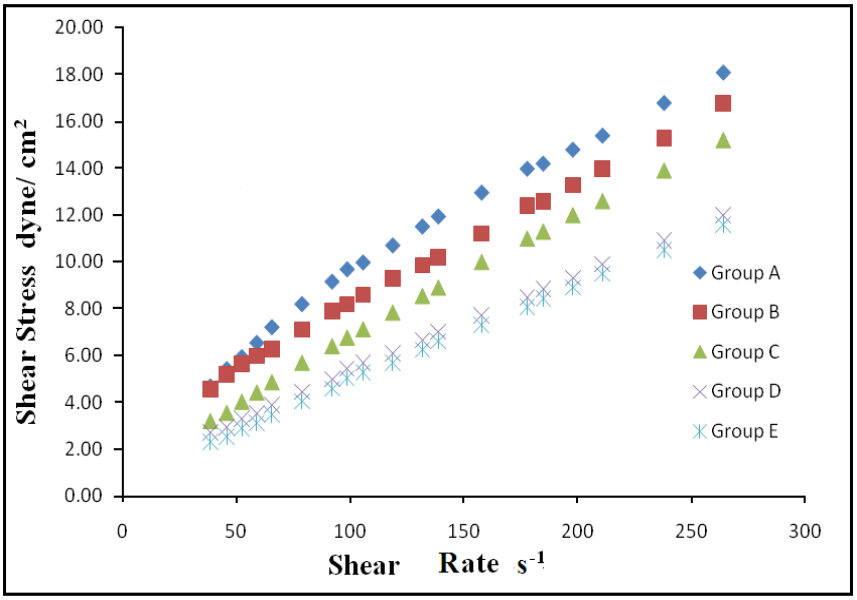

Figure (8): The relation between shear rate and shear stress of blood at 37.2 ^{o}C

Table.(2): The rheological parameters of the average values for each parameter from all animals from each group

Parameters / Group	Viscosity cP (shear rate 92.4 s^{-1})	Viscosity cP (shear rate 132 s^{-1})	Flow index (n)	Consistency index (k)
Control group A	9.431 ± 0.136*	8.420 ± 0.212*	0.702 ± 0.003*	36.13 ± 0.261*
Exposed group B	8.242 ± 0.235*	7.481 ± 0.361*	0.703± 0.002*	32.26 ± 0.142*
Exposed group C	7.024 ± 0.213*	6.582 ± 0.263*	0.828 ± 0.005*	15.17± 0.211*
Exposed group D	6.031 ± 0.354*	5.673 ± 0.242*	0.849 ± 0.003*	11.90 ± 0.334*
Exposed group E	5.033 ± 0.244*	4.741 ± 0.433*	0.852 ± 0.004*	9.83 ± 0.182*

* The standard error values.

Conclusion

There are remarkable changes in the RBCs mechanical and rheological properties after exposure to that fields in a manner that prolonged exposures to it are biological toxic. It is recommended not to allow buildings close or down to power-lines and a special protocol for buildings permission should be done in a way that exposures to such fields are omitted. More investigations must be carried out in cooperation with medical researchers to do frequent medical examination for people who exposed to such fields. According to the vicinity of the present findings, it's necessary to revise the dose limits recommended by different commissions for exposure to extremely low frequency magnetic fields below 0.5 mT.

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