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Monitoring Of Rat's Blood Rheology after Exposure to 50-Hz Electric Fields (Occupational Study)

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Abstract: *The great use of electrical appliances in different life applications is one of the most obvious concerns because of its possible health drawbacks. The present paper is aimed to study the biological effects of exposure to extremely low frequency electric fields. Blood, as a main vital system to human life, was chosen to be the biomarker for the evaluation of the risk associating exposures to such fields. Six groups of animals are used; group A considered as sham group that housed at normal environmental conditions didn't receive any treatments. The other five groups B, C, D, E and F exposed to electric fields for different exposure periods 5, 10, 15, 20, 30 days respectively. Whole blood samples are collected immediately after sacrificing from each animal and then prepared for further measurements. Rheological measurements aremade and power low model is applied for different blood samples. Results indicated remarkable changes in viscosity coefficient and consistency index at different shear rates for exposed samples in comparison to sham animals. From the results it may be concluded that, electric field exposures may alter blood rheological properties in a manner that more concerns should be taken regarding such fields and new exposure limits have to be proposed in the future.*
Keywords: *Electric field, blood,viscosity coefficient, power law model, consistencyflow index*

I. Introduction

Until recently, the natural electromagnetic background was relatively constant, but the situation changed markedly and precipitously with the development of modern communications and electrical power systems. The environment is now heavily laden with man-made electromagnetic fields (EMFs) from radio; TV, microwave relay, and many similar sources. Static magnetic fields (SMFs) are time-independent fields of constant strength. Extremely Low Frequency electromagnetic Fields (ELF-EMF)are electromagnetic oscillating fields defined as having frequencies below 300 Hz. ELF-EMFs are very important from a public health standpoint because of the wide spread use of electrical power at 50 or 60 Hz in most countries. Electric and magnetic fields exist around electrical equipments and wiring throughout industry. Residential peoples and occupational workers who maintain transmission and distribution lines may be exposed to very high electric and magnetic fields [1]. *Purushothaman G., et al 2013* carried out an investigation was by exposing the adult male albino rats to the magnetic field of 202μT against the control group and both were analyzed for haematological and biochemical changes. The results showed that the magnetic field exposed (MF) animals showed significant increase in RBCs, WBCs, Hbs and platelet count as well as decrease in Red blood cell indices values of MCV, MCH and MCHC. In addition, the MF exposed group also showed significant increase of AST and ALT levels in plasma indicating the involvement of MF on liver cell membranes. The haematological parameters and liver enzymes were affected by the electromagnetic field exposure suggesting the possible induction of hazardous biological effects during the exposure to magnetic field[2].*DilekUlker., et al 2009* indicated that the applied ELF-EMF exposure may induce slight but statistically significant alterations in some hematological parameters of rats, within the physiological range. There was no significant difference in total leukocyte, neutrofil, lymphocyte, monocyte, eosinophil and basophil counts, or in erythrocyte, Hct, MCH, MCHC, RDW, PLT and PDW levels between the exposed and sham-exposed groups [3].*Touitou Y., et al 2013* studied male electrical workers chronically exposed to 50-Hz magnetic fields for a period of 1–20 years by examining the nocturnal profiles of their blood counts. The data was quite small; only 15 workers and the same number of controls. The exposure levels were also rather low. According to the writers, the results suggest that magnetic fields have no cumulative effects on the haematological or immune system functions [4].*MagdiYEl-Ashry et al 2008,* conducted a study to evaluate the influence of 50 Hz magnetic field on some liver enzymes tests; alanine aminolransferase (ALT), aspartate aminolransferase (AST), alkaline phosphatase (ALP), serum bilirubin, serum albumin and serum protein. The study was conducted on Sparague-Dawely male rats of an average weight of 140-160 gm. The exposure period was 7 days (2 hours/day). The results showed an increase ($p<0.05$) in all investigated liver enzymes [5].

Thanaa E. et al, 2006; devoted a work to study the effect of exposing experimental male albino rats to a combined static and extremely low frequency (ELF) alternating magnetic fields of average intensity 3 mT, to evaluate the risk on human workers who occupationally exposed to similar fields. A total of thirty male albino rats were equally divided into three groups, A, B, and C. Group A served as a control group. Group B was exposed to static and extremely low frequency magnetic fields of 3mT, 8 hours/day for two successive weeks. Group C was exposed to the same condition as group B for four successive weeks. Osmotic fragility of RBCs, dielectric dispersion of hemoglobin (Hb) at frequency range of 12–105 Hz, Hb absorption, Hb electrophoresis, plasma alanine transaminase (ALT), aspirate transaminase (AST), malondialdehyde (MDA) levels and red blood cells (RBCs) catalase activity were carried out for all groups. The results indicated that exposure of animals to MFs of 3 mT results in an increase in RBCs osmotic fragility and decrease in its membrane elasticity, a partial change in Hb molecular structure without change in the electrophoretic band of Hb, increase in ALT and AST levels in plasma indicating some damage in liver cell membrane. The increase in MDA level and catalase activity indicates an increase in free radical level [6].*Nihal S. El-Bialy, et al; 2012*; aimed study to evaluate the possible effects of in vivo exposure to extremely low frequency magnetic fields (ELF-MF) on some hematological parameters, pathological variations and DNA structure in newborn rats. Six female pregnant Wistar rats were obtained from the National Research center in Egypt and gave birth to 30 rats at the animal house of Cairo University. The newborn rats were divided into two separate groups: one exposed group (50 Hz, 0.5 mT, 30 days, 24 h/day) and one control (sham). Red blood cells (RBCs), hemoglobin and hematocrit levels decreased significantly ($P< 0.02$) while white blood cells (WBCs) and platelet levels significantly increased ($P< 0.04$) in newborn rats that were exposed to ELF-MF. There was no significant difference in mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV) levels and DNA structure between the exposed and sham-exposed groups. ELF-MF induced a marked necro-degenerative change in kidney tissue and peri-portal fibrosis in liver tissues. The results indicated that the applied ELF-MF exposure may induce statistically significant alterations in some hematological parameters, kidney and liver tissues of newborn rats [7].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 hematological parameters in rat blood 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 [8-12].

After all these efforts of studies which mentioned above and the recommendations published by the authorized organizations, 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 work is devoted to study the hazard health effects accompanied with continuously and discretely exposure to ELF-MF 50-Hz on spleen of albino rats. Then after, the rheological parameters of whole blood are examined for all spleen tissues to reflect any deteriorationsattributed to the exposure.

II. Materials And Methods

Experimental Animals and Samples Collection:

In the presence work 65 male albino rats, each of average weight 200± 10 gm. divided into five main groups, namely group A, B, C, D, E and F. Animals of group A (15 animals) are used as a unexposed group and didn't receive any treatment and housed at normal environmental conditions (the temperature inside the lab varied between 240 and 270 C, lighting condition are natural light from large windows during the day and complete darkness during the night). Animals of group B, C, D, E and F(10 animals per each) were exposed to an electric field of 4 kV/m and 50 Hz, for periods of 5, 10, 15, 20 and 30 days to 8h/day respectively. At the end of the exposure period the animals were immediately sacrificed and blood samples from each animal was collected for experimental investigation.

Electric Field Exposure System:

The exposure cage consisted of Perspex chamber, with an exposure volume of dimension 100x30x35 cm^3 located between two parallel aluminum plates, which extended vertically along two parallel sides of the exposure cage as shown in Figure-1. In order to prevent any animal shock from direct contacts with the electrodes, the aluminum plates were covered by front fixed Perspex plates of similar measure. It is worthy to mention that, the Perspex material has a negligible effect on the field homogeneity. The two aluminum electrodes were connected to a step up transformer with an output voltage of 4 kV/m and 50Hz when connected to the main supply. For more precautions an electric timer was used to adjust the exposure times specially when mains fall. The electric inside the chamber was measured through the use of field meterand was found to be homogeneous and reads 4 kV/m.Food and water are 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.No temperature differences were observed between exposed and unexposed groups during the exposure.

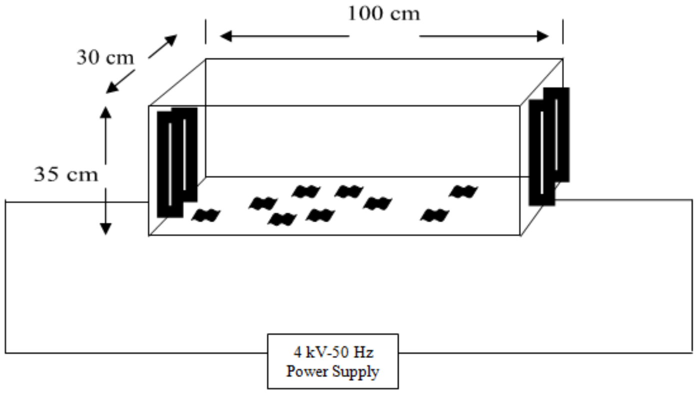

Figure-1: Schematic diagram ofelectric exposure system.

Blood Viscosity Measurements

Programmable rotating Viscometer model DV-II (Spindle SC4-18/Sample chamber SC4-13p) 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 ± 0.2C° and 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 (Sec^{-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. We plot relation between Viscosity (Cp) as Y axis and shear rate (Sec^{-1}) as X axis for each sample to get the viscosity curve.

Figure-2: TheViscometertestdevice(ViscometermodelDV-Ⅱ(SpindleSC4-18/SamplechamberSC4-13(p))manufacturedbyaBrookfieldcompanyintheUSA).

Statistical Analysis:

The statistical analysis was performed using the student's *t*-test with a minimal confidence level of 0.05 for statistical significance. Each experiment was performed at least three times with a minimum of three samples per termination point, resulting in a total number of a six samples for each experiment.

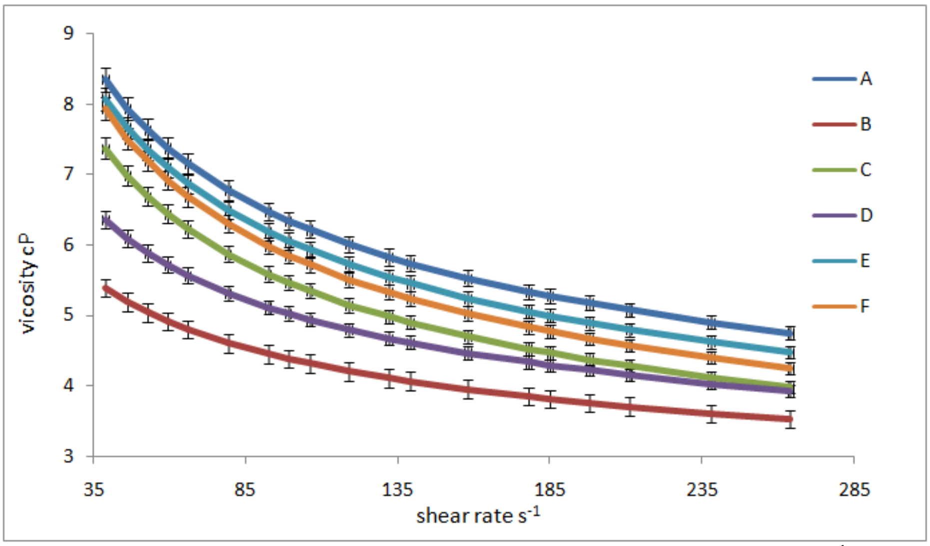

Figure-3: The variation of blood viscosity (cP) as a function of shear rate (s^{-1}) for the samples collected from the control group A and the groups B, C, D, E and F.

Table-1: 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 rate132 s^{-1})	Flow index (n)	Consistency index (k)
Control group A	6.481 ± 0.136*	5.83 ± 0.212*	0.702 ± 0.003*	36.13 ± 0.261*
Exposed group B	4.45 ± 0.235*	4.11± 0.361*	0.703± 0.002*	32.26 ± 0.142*
Exposed group C	5.59 ± 0.213*	4.98 ± 0.263*	0.828 ± 0.005*	15.17± 0.211*
Exposed group D	5.11 ± 0.354*	4.67 ± 0.242*	0.849 ± 0.003*	11.90 ± 0.334*
Exposed group E	6.19 ± 0.244*	5.55 ± 0.433*	0.852 ± 0.004*	9.83 ± 0.182*
Exposed group F	5.99 ± 0.18*	5.33 ± 0.183*	0.82 ± 0.005*	9.73 ± 0.122*

III. Results And Discussion

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 (3).

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 ,$\gamma^n$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 ηis 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, E and Fand tabulated in Table-1. 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.

IV. Conclusion

It is concluded from the present findings that exposure to such fields may cause significant physiological alterations on blood rheology and functionality. Also, one may conclude that exposure to 4-kV/m; 50-Hz electric field for occupational workers is risky and may cause health consequences. Further, the need for more concerns should be taken regarding such fields and new exposure limits have to be proposed in the future.

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