Occupational studyof electric field on hematological parameters and biophysical blood properties for diagnosing anemia in albino rats

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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 study was performed to investigate the occupational study of electric field of strength 50Hz-3Kv/m on blood viscosity, hemolysis, osmotic fragility and hematological parameters. 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. Three groups of animals are used; group A considered as control group that housed at normal environmental conditions which didn't receive any treatments, the other two groups B and C exposed to electric fields for different exposure periods15, 30 days (8hours/day,5day/week)respectively. Animals of group B will divide into two subgroups B_1&B_2, and animals of group C will divide into two subgroups C_1& C_2. SubgroupsB_2&$C_2$are the recovery groups which didn't receive any treatment for a period 15 day post exposed.Experimental results revealed high significant changes in osmofragility, blood constituent and its viscosity which affect on a blood circulation because of many body problem alsoshowed that exposure to electric field originated different metabolic and hematological disruption. From the results it may be concluded that, electric field exposures may alter the blood mechanical and rheological properties. Also, it is suggested that more concerns should be taken regarding such fields and new exposure limits have to be proposed in the future.

Key words: electric field-osmotic fragility-blood hematology-viscosity-blood structure- red blood cell-white blood cells.

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INTRODUCTION

All living organisms are exposed to man-made sources of Electromagnetic Fields (EMFs) such as high voltage transmission lines, distribution lines, household electric wiring and appliances operated in 50-60Hz frequency which is in the range of Extremely Low Frequency (ELF) in various degrees**[1]**.Increased usage of EM principles for domestic and industrial purposes proves that EMF plays an important role in our daily life **[2]**. In modern society, the use of electricity is so widespread that it is impossible to avoid exposure to ELF-EMF produced by power lines and many kinds of electrical appliances **[3, 4, 5, 6]**. EM hazarded was considered one of the most dangerous types of pollution **[7,8]**due to EMFs which affected the functions of cells of the body **[9]**.The results of many different types of animal and human studies dealing with the biological effects of exposure to (ELF- EMF) have consistently been both positive and negative **[6,10]**.

Blood is the only tissue that flows throughout your body, this red liquid carries oxygen and nutrients to all parts of the body and waste products back to your lungs, kidneys and liver for disposal. It is also an essential part of your immune system, crucial to fluid and temperature balance, a hydraulic fluid for certain functions and a highway for hormonal messages **[11]**.The aim of the work was to study the variation of hematological

parameters and biophysical properties of whole blood in rats exposed to ELF-EMFs.EM waves occur imbalance in the cell power which disturbed their function. This disturbance induced according to the severity of the disorder and the damage induced **[12]**. Many studies reported that radiation from mobile phones absorbed by the human body and turn inside to heat. This biological effect leads to the continuation of the existence of many damage risks caused to human and their vital organs.

The effects of the interaction between EMFs and the haemodynamics of the arterial system have been studied by Sud and Sekhon**[13]**, they found that the method requires the derivation of an expression of the conductance of a single artery in the presence of a transverse magnetic field**[7]**.Blood is a complex fluid whose flow properties are significantly affected by the arrangement, orientation, and deformability of red blood cells **[14]**,blood viscosity is a measure of the resistance of blood to flow, which is being deformed by either shear stress or extensional stress.

Blood is a liquid which consists of plasma and particles such as red blood cells, white blood cells and platelets, however, the blood viscosity depend upon the plasma viscosity and hematocrit. Blood behave like a viscoelastic fluid; in addition several pathologies are accompanied by significant changes in the mechanical properties of blood which result in alteration in blood viscosity and viscoelastic properties. As RBCs form rouleaux tumbling disturbs the normal flow pattern and requires the consumption of energy resulting in an increase in blood viscosity at low shear rate.RBCs by themselves have been shown to exhibit viscoelastic properties, other factors contributing to the viscoelastic properties of blood are the plasma viscosity, plasma composition, temperature and the shear rate. Together, these factors make the streaming blood viscoelastic. The aim of this work was to study the effect of electric field produced from technological devices on blood viscosity, osmotic fragility and blood component of rats.

ELF-EMF also affects the immune and hematological systems through these mechanisms. Results obtained in studies on the effects of ELF-EMF on hematological parameters are conflicting; this led us to carry out the present study. The aim of the present work is to study the radiation hazard through measurements of possible changes on some biophysical properties of red blood cell membrane of albino rats in vivo studies through the measurements of the osmofragility of the RBCs membrane to evaluate any changes in the mechanical properties of the cellular membrane, also measurements whole blood viscosity to evaluate any changes in the membrane elasticity or in the permeability and shape which may cause change in the whole blood viscosity, and study the blood film as being magnified to 1000 times in order to follow surface changes in the RBCs morphology.

MATERIALS AND METHODS

Experimental animals and exposure facility system

Experiments were performed on adult Albino rats at physics Department, Faculty of Science, Damanhour University, Egypt, under conventional laboratory conditions. The experimental animals kept in the same conditions for 2 weeks for adaptation. In the present work fifty male albino rats were used, each of average weight 170±10gm .The animals were housed in the same environmental conditions in plastic cages, and feed with constant balanced diet and tap water, which were equally divided into three groups namely A, B and C. Animals of group A are used as a control group and didn't receive any treatment and housed at 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 was divided into two subgroups namely B_1 and B_2 which were exposed to 50Hz, 3KV/m electric field for a period of 15 day (8 hours/day, 5day/week). Group B_2 animals were left to survive and housed at normal environmental conditions similar to control group A_1 for a period of 15 day post exposed. Animals of group C are divided into two subgroups namely C_1 and C_2 were exposed to the electric field for a period of 30 day (8 hours/day, 5day/week). Group C_2 animals were left to survive and housed at normal environmental conditions similar to control group A_1 for a period of 15 day post exposed. At the end of this period, all animals in control and experimental group were housed collectively in Perspex chamber, with an exposure volume of dimension 100x30x35 cm^3 located between two parallel cupper plates as shown in Fig .1, which extended vertically along two parallel sides of the exposure cage. In order to prevent any animal shock from direct contacts with the electrodes, the cupper plates were covered by a sheet of Polymethyl methacrylate. It is worthy to mention that, the Perspex material has a negligible effect on the field homogeneity**[15].** The two electrodes were connected to a step up transformer with an output voltage of 3Kv 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 field inside the chamber was measured through the use of field meter and was found to be homogeneous and reads 3Kv/m.

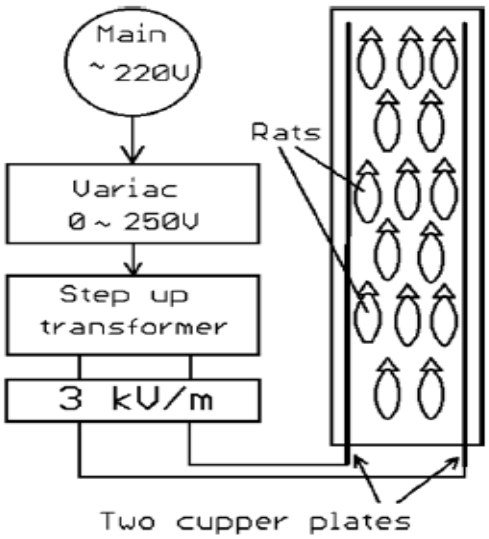

Fig.1. Schematic diagram for exposure facility system

Rats were anesthetized with ketamine HCL (50mg/kg), before killing animals, the blood from aorta was collected in plastic heparinized tubes for hematological and biophysical analysis.Determination of hematological parameterssuch as RBCs, WBCs counts, and hemoglobin concentration by an automatd hematologic analyzer using whole blood sample.

Hematological Studies

Collection of blood samples

The rats were slaughtered after the exposure periods, blood samples were taken at the end of exposure and collected on anti-clotting EDTA bottles**[18]**, and these samples were used in assessment of blood profile **[6].**

Hematological parameters Determination

In complete blood count, Dilekincluded the red blood cells (RBC) and white blood cells (WBC) counts were determined by the improved Neubauerhaemocytometer method. The haemoglobin (Hb) concentration was determined according to Jain (1986), using the cyanomethaemoglobin method and hematocrit (HCT). Measurements RBC indices are packed cell volume (PCV) was determined by the microhaematocrit method according to Dacie and Lewis (1991). Schilling method of differential lecukocyte count was used to determine the distribution of the various white blood cells **[19]**. Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were computed according to Jain (1986).white blood cell count (WBC).), Lymphocytes (Lym), Monocytes (Mono), Segmental neutrophils (S), andEosinophil granulocyte (Eos) were also determined.

Hemoglobin Determination

HC1 was taken into an ordinary pipette and was poured in the graduated dilution tube up to 20% mark; the heparinized blood was filled into the hemoglobin pipette up to 0.02 ml and transferred it into the dilution tube. The blood and HCl were stirred in the dilution tube with the stirrer. Distilled water was added until the color of the dilution and standard tubes matched with each other. The reading was noted which gave hemoglobin as g/dl of blood **[17]**.

Red Blood Cell Count

For RBC counting blood with an anticoagulant was used. Blood was drawn into the RBC diluting pipette exactly to the 0.5 mark, using gentle suction on the mouth piece. The lip of the pipette was wiped free of blood before inserting it in to the diluting fluid (Toission Solution). The diluting fluid was drawn up to the mark 101 above the bulb. The tube was rotated in a horizontal position to ensure uniform dispersion of the blood cells in the pipette **[17]**calculated by using the following formula:

RBC (million/mm) = (Cells counted / 5) x 10x 200

Packed Cell Volume (PCV)

Packed cell volume was measured using the heparinized blood in the plain capillary tubes (75mm x I mm). Tubes were filled approximately 1cm from the end. Holding it in the flame sealed the vacant ends of the tubes. Care was taken not to heat the blood. Capillary tubes were fixed in the hematocrit centrifuge machine. Then centrifugation was done at 13000 rpm for 5 minutes **[17]**.

Red Blood Cell Indices

From the values of PCV, Hb and RBC count following useful erythrocyte indices were empirically calculated **[17]**.

Mean Corpuscular Volume (MCV)

MCV expresses the average volume of the individual RBC and is calculated from the formula as given by Wintrobe**[17]**and Diem **[20]**.

MCV= Hematocrit x 10/RBC

MCV is expressed in femtoliter.

Mean Corpuscular Hemoglobin (MCH)

MCH is the amount of hemoglobin by weight in average red blood cell count and is calculated by the formula as given by Wintrobe**[17]** and Diem and Clenter**[17, 18]**.

MCH= Hemoglobin x 10/RBC

It is expressed in pictogram.

Mean Corpuscular Hemoglobin Concentration (MCHC)

MCHC is the concentration of hemoglobin in the average red blood cells or the ratio of weight of hemoglobin to the volume in which it is contained and is calculated from the formula as given by Wintrobe andZahra **[17, 18]**.

MCHC = Hemoglobin x 100/Hematocrit

Data presentation and statistical analysis

Data are reported as the mean± standard error of mean SEM. Statistical significance of the differences between means was assessed by Student's t-test. The level of significance was set at $p<0.05$.

RESULTS AND DISCUSSION

Anemia, one of the more common blood disorders, occurs when the number of healthy red blood cells decreased in the body. The nutritional or hypochromic anemia is common and in chronic blood loss where iron or protein intakes are in adequate**[17,18]**. It was observed from the results of the reading recorded in Table 1 that the rate of hemoglobin for control group was 14.18±0.27g/dl and for group B_1 which exposed to EF -50Hz-3Kv/m for a period 15day (8h/day-5day/week) began to decline to 12.02±0.57 g/dl and was 10.12±0.26 g/dl for group B_2 which exposed to the EF for the same period of group B_1 but kept for two weeks away from EF (recovery group)and then showed its lowest value for group C_1 at 30 day after exposure time with rate reached 9.94±1.18 g/dl and for recovery group C_2 the rate of haemoglobin was 8.81±0.07. From the above result it is noticed that there is a decrease in the rate of haemoglobin after prolonged time periods when exposed to 30 day compared with the average rate in the control group.

Table 1. RBCs, WBCs and Hemoglobin before and after exposure to electric fields

Parameters	RBCs(10^6/µl)	WBCs (10^3/µl)	Hemoglobin(g/dl)
A	5.00±0.07	7.87±0.51	14.18±0.27*
B_1	4.39±0.142*	6.78±0.92*	12.02±0.57*
B_2	3.80±0.06*	6.02±1.31*	10.12±0.26*
C_1	3.86±0.31*	5.23±0.38*	9.94±1.18*
C_2	3.55±1.04*	5.15±1.66*	8.81±0.07*

Mean ± SE of blood rats structure in each group.* p <0.05

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 that it consists of various types of cells such as RBCs and WBCs. Table.1; Fig.2Show hematological parameters for different groups of animalswhich indicates that there is a significant effect in blood rat structure due to exposure to electric field. Values of RBCs, WBCs are decreased with increasing exposure time and there is no improvement in hematological parameters for recovery groups.Also after exposed rats to electric field, there is a significant effect in hemoglobin molecule structure due to damage/or broken of it as seen in Table.1. This means that these components of blood are broken due to irradiated by electric fields from technological devices which used daily.

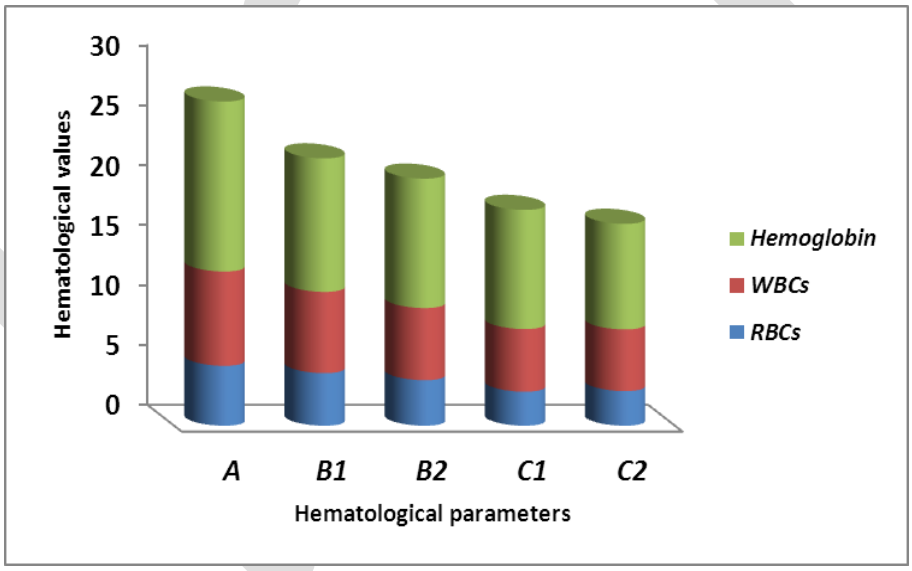

Fig.2. Hematological parameters for different groups of animals.

Data concerning blood hematological parameter levels for control and exposed groups which are given in Table.2. Results of the analysis are given as mean± standard deviation; also statistical differences were determined according to exposure period and groups. The results showed high statistically significant at level($p<0.001$) for PCV, MCV, MCHandMCHCfor all different groups of rats B_1,B_2,C_1, C_2as compared with control group A.

Mean corpuscular hemoglobin has medical importance in the diagnosis of some types of anemia, the results indicates as shown in Table.2.significant decreased in the value of MCH this decreased show disease of anemia such as iron deficiency **[21]**.The results showed that exposure to EF originated different metabolic and hematological disruption, which appeared to be related to the duration of exposure, the treatment to EF for a

period 30 days caused a decreased of hemoglobin concentration, white blood cells, red blood cells count as compared to control rats.

EF exposure induced a decreased in Eos, Lym and increased in Mon, and S. Our work agreement with the work performed by ***Seto et al., 1986*** demonstrated that a high intensity (80kV/m) 60Hz EF decreased Eosinophile, white cell counts and red cell parameters.

Table .2Hematological parameters for control and exposed rats.

Parameters	*Group (A)*	*Group (B_1)*	*Group (B_2)*	*Group (C_1)*	*Group (C_2)*
PCV (%)	48.25±1.01	40.18±1.93^{HS}	32.89±1.54^{S}	35.80±1.04^{HS}	31.84±1.16^{Hs}
MCV*(FL)*	96.00±0.78	91.52± 1.82^{Hs}	86.87±2.27^{VHS}	92.77±1.28^{VHS}	89.7±4.68^{VHS}
MCH *(Pg)*	28.36±0.16	27.4±0.53^{S}	26.65±0.62^{S}	25.75±0.41^{S}	24.78±0.74^{S}
MCHC*(g/dl)*	29.54±0.36	29.91±0.16^{S}	24.23±1.11 ^{VHS}	24.54±0.55 ^{VHS}	23.06±0.92 ^{VHS}
LYM (%)	42.1±4.33	32.63±2.12 ^{VHS}	30.2±1.02 ^{VHS}	36±2.34 ^{VHS}	35.25±2.55 ^{HS}
MON (%)	3.81±0.31	4.00±0.44 ^{NS}	3.4±0.67 ^{NS}	4.00±1.35 ^{NS}	3.5±0.64 ^{NS}
S (%)	40.6±2.11	49.9±2.18 ^{VHS}	60±0.70 ^{VHS}	53.25±1.93 ^{VHS}	56±3.24 ^{VHS}
Eos (%)	1.6±0.33	0.90±0.211^{HS}	0.80±0.63 ^{HS}	0.5±0.28 ^{HS}	0.70± 0.4 ^{HS}

Values represented Mean ± standard error
NS difference is not statistically significant between groups at level ($p>0.05$)
S difference is statistically significant between groups at level ($p<0.01$)
HS difference is highly statistically significant between groups at level ($p<0.001$)
VHS difference is very highly statistically significant between groups at level ($p<0.0001$)

From the results in Table.3 it is clear that the viscosity of the blood was increased for animals of group $B_{1\&}$ $B_2$and decreased for animals of group, C_1& C_2 as compared with control group A. The differences in viscosity which decreased and increased as compared with control groups demonstrate the effects of RBCs aggregation and deformability respectively.

Table .3.The calculated values of the relative viscosity for different groups of animals.

Groups	Relative viscosity (Mean ± SD)
A	353.8±17.29
B_1	382.36±13.93*VHS
B_2	393.6±7.50*VHS
C_1	340.00±14.74*VHS
C_2	343.75±26.46*VHS

* The values of the standard deviation

VHS difference is very highly statistically significant between groups at level ($p<0.0001$)

Osmotic fragility is a test to detect whether red blood cells are more likely to break down. In hematological studies, the osmotic fragility test provides an indication of the ratio of surface area/volume of the erythrocytes. In the osmotic fragility test whole blood was added to varying concentration of buffered sodium chloride solution and allowed to incubate at room temperature(25 ± 1^0C) **[14]**.The amount of hemolysis is measured through the spectrophotometer (UV/Vis type SPECTRO SC, made in USA). Carefully transfer the supernatants to cuvettes and read on a spectrophotometer at a wavelength of 550nm.Set the optical density at 0, using the supernatant in test tube No 1, which represents the blank, or 0% hemolysis. Test tube No.14 represents 100% hemolysis. The percent hemolysis was calculated for each supernatant as follow:

Percent Hemolysis = (O.D of supernatant/ O.D of supernatant in tube No.14) ×100

Where O.D= optical density. The percent hemolysis (H%) was then plotted as a function of the percentage of sodium chloride concentration (NaCl %) as seen in Fig.3.The percentage hemolysis (H%) is decreased with exposure to electric field and there is no improvement in the blood state for recovery group post exposed.

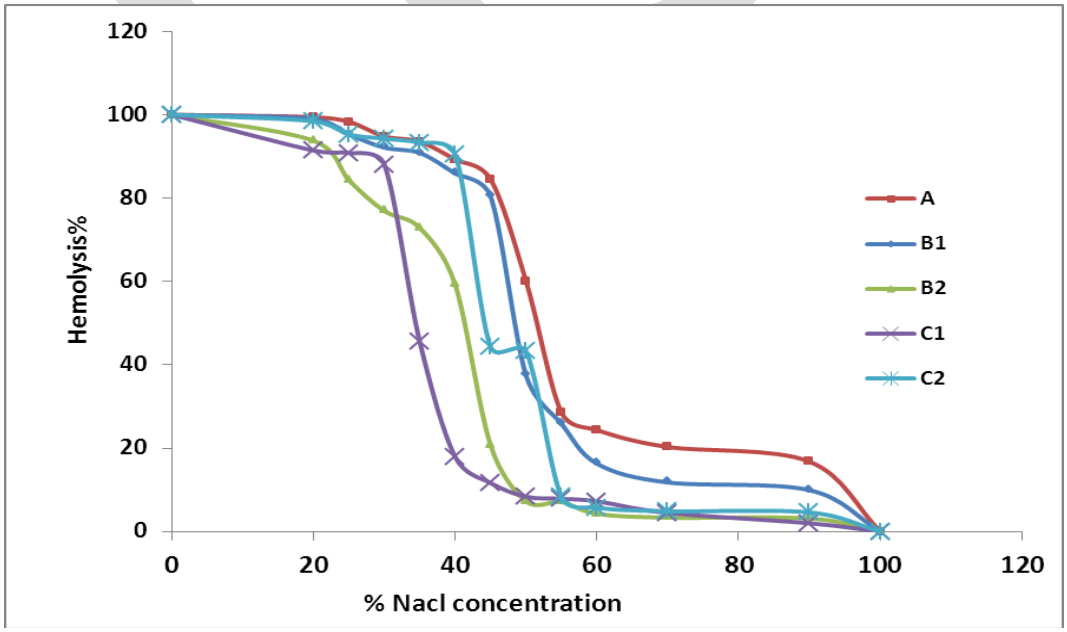

Fig.3 Percent hemolysis (H%) versus the percentage of sodium chloride concentration (NaCl %) before and after exposure to electric field.

Fig 4. Represent a photograph of a blood film for the control group as magnified to 1000 times. The figure indicates normal shape of the blood cells and the presence of electrostatic positive charges on the surface of RBCs cellular membrane; these positive electrostatic charges cause coulomb repulsive forces between adjacent cells that result in pressure on the cell membrane surface. This pressure could cause charges in blood morphology for adjacent cells (shown by arrows) and prevent cellular sticking.

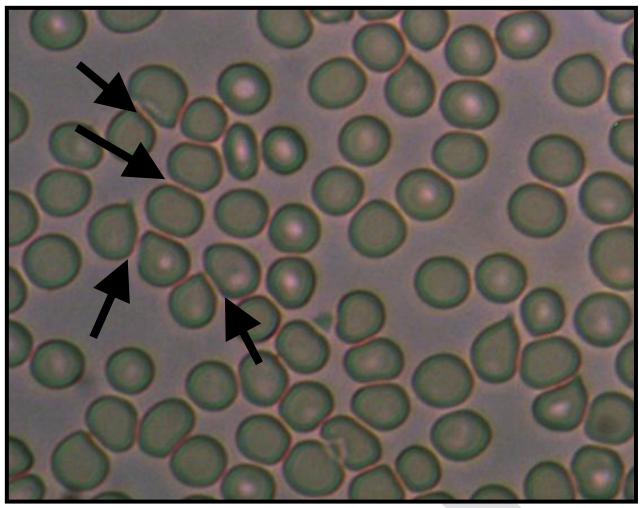

Fig.4. Morphological shape of normal RBCs for control group A(×1000), and the coulomb repulsive forces between adjacent cells that prevent cells from sticking together, which lead to a mechanical stress cause a momentary change on its shape as signed by arrows.

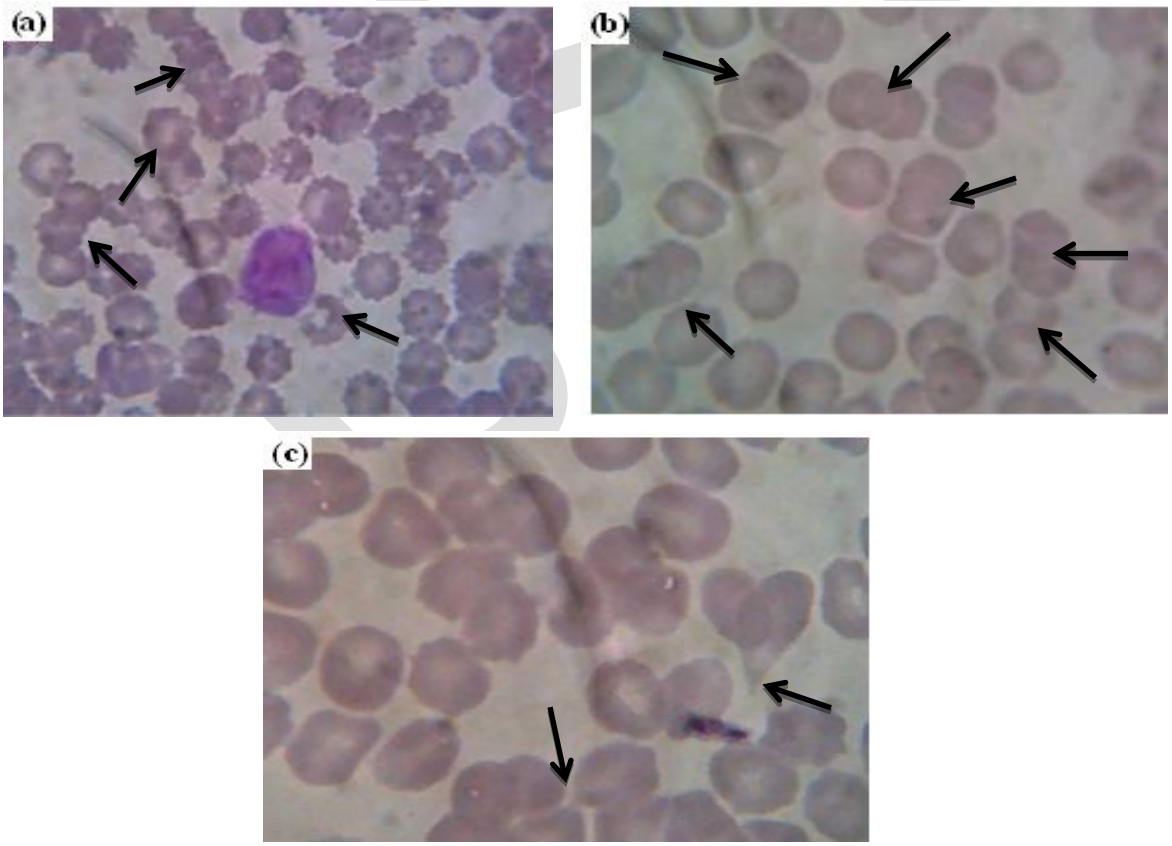

Fig.5 Morphological abnormal blood film for the 15-day exposed group B_1 (×1000) indicate the irregularity in the cell membrane the stuck of cells together in close approximate and deformed shapes with degradation surfaces (a) appears cells with basophilic stripping and the infusion of adjacent cells with the formation of a common membrane for more than cell (b)appears compact cells of erythrocytes(c) also appear sickle shape of RBCs as in Color Atlas of hematology, 2005 Marshall et al.2005.

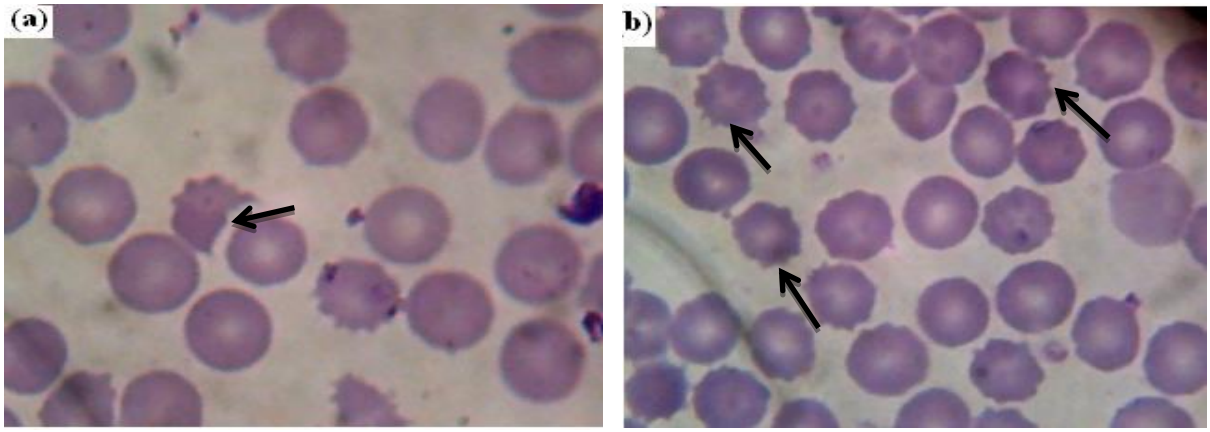

Fig.6.represents a photograph of a blood film for the recovery of 15-day exposed group B_2 as magnified to 1000 times. (a) Helmet or fragmented cells RBCs which known in microangiopathic hemolytic anemia and others as heart-valve hemolysis. (b) Irregular projections with abnormal volume and shapes as in color atlas.

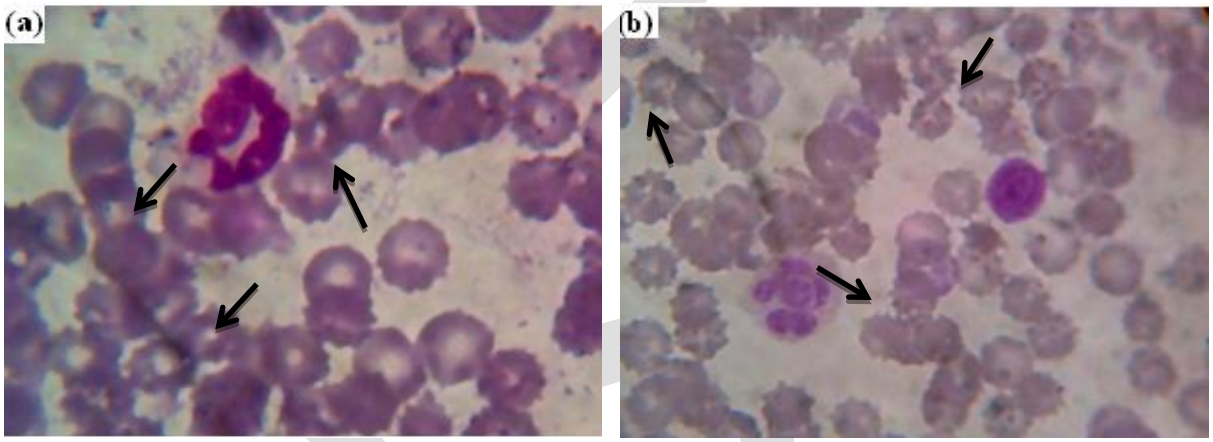

Fig.7.represents a photograph of a blood film for 30-day exposed group C_1 as magnified to 1000 times. (a) & (b) show irregular projections with abnormal volume and shapes, the RBCs stacked together in long chains as happened in "rouleaux formation" as in color atlas.

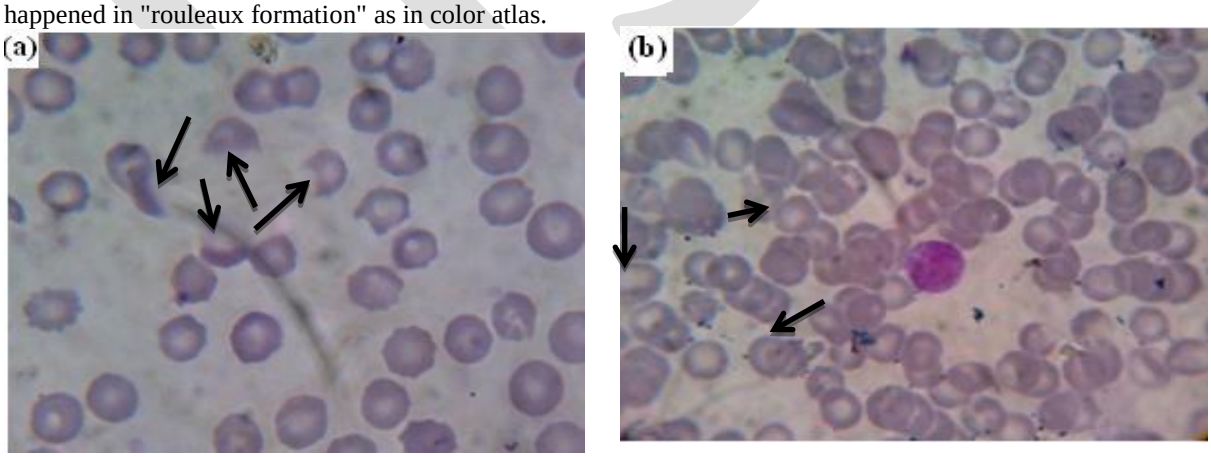

Fig.8.represents a photograph of a blood film for recovery of 30-day exposed group C_2 as magnified to 1000 times. (a) Show fragmented cells. (b)Show irregular projections with abnormal volume and shapes and the RBCs stacked together in long chains as happened in "rouleaux formation" as in color atlas.

Results of the morphological shape of blood samples from rats after exposed to electric field at different periods of time 15, 30 days and 15 day recovery for two periods of exposure time,blood films were stained with leishman stain. Fig.4.represent the morphological shape of normal RBCs for control group A (×1000), and the coulomb repulsive forces between adjacent cells that prevent cells from sticking together, which lead to a mechanical stress cause a momentary change on its shape as signed by arrows.Fig.5 depicts a photograph of abnormal blood film for the 15-day exposed group B_1 (×1000) this figure indicates the presence of abnormal shape and irregularity in the cell membrane of the blood cells and the absence of electrostatic positive charges on the surface of RBCs cellular membrane. This leads to loss of coulomb repulsive forces between adjacent neighboring cells. However, some cells stick together forming one common membrane (signed by arrow) the results are compared with the well-known blood diseases as given from Williams's hematology as color atlas of hematology. Fig.6.represents a photograph of a blood film for the recovery of 15-day exposed group B_2 as magnified to 1000 times. (a) Red blood cells were retained in their natural form to a large extent, close to the structure present in the control group also existed some Helmet or fragmented cells RBCs which known in microangiopathic hemolytic anemia and others as heart-valve hemolysis. (b) Irregular projections with abnormal volume and shapes as in color atlas.Fig.7. represents a photograph of a blood film for 30-day exposed group C_1 as magnified to 1000 times. (a) & (b) show irregular projections with abnormal volume and shapes and the RBCs stacked together in long chains as happened in "rouleaux formation" as in color atlas. Fig.8. represents a photograph of a blood film for recovery of 30-day exposed group C_2 as magnified to 1000 times. (a) Show fragmented cells. (b) Show irregular projections with abnormal volume and shapes and the RBCs stacked together in long chains as happened in "rouleaux formation" as in color atlas.

Measurements of blood parameters are most important means by which to determine the health status of experimental animals**[23, 7]**. Fatayer**[24]**noted that these measurements are diagnostic for certain diseases such as anemia, leukemia and detect the presence of the inflammation. The decreased in numbers of red blood cells was showed in some disease as anemia. The evidenced of the increase in the number of red blood cells indicated that the presence of disease which increases the production of red blood cells as polycythaemia due to disorder in the bone marrow **[24]**.

Indices of red blood cells give us a clear image of performance and efficiency of functional red blood cells, the concentration of hemoglobin, and describe the increase and decreased in the volume of red blood cells**[7]**.The most important of these indices that the average size of the red cells (mean cell volume), which helps in the diagnosis of some diseases, decreased in the size of the red cells showed in anemia. Fatayer**[24]** reported that the mean corpuscular hemoglobin concentration (MCHC) index helps in the diagnosis of different types of anemia. The results showed a significant reduction in the measurements of the blood, hemoglobin and hematocrit (PCV), in addition to the number of indices of red blood cells are RBC,MCV,MCH,MCHC, this decline is an indication of different types of anemia, as well as leukemia(***Mariam et al., 2012***).While it was observed a significant decreased in the average number of white blood cells WBC as well as the proportion of lymphocytes LY and this decreased is accompanied with different cases of anemia, it is also evidence of bleeding which arises under the effects of radiation and increased the temperature and resistance of the body**[7]**.The increase in the percentage of lymphocytes associated with lymphatic leukemia, or inflammation of the lymph gland.

EF exposure induced a decreased in Eosinophile, Hb ,PCV and MCV levels . Our work agreement with the work performed by ***Seto et al., 1986*** demonstrated that a high intensity (80kV/m) 60-Hz EF decreased eosinophile counts. They also recorded decreased white cell counts, although red cell parameters did not differ significantly.

Conclusion

From the results it may be concluded that, electric field exposures may alter the blood mechanical and rheological properties. Also, it is suggested that more concerns should be taken regarding such fields and new exposure limits have to be proposed in the future. EF exposure originated different metabolic and hematological effects, which appeared to be related to the duration of exposure.

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