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The Effect of Static Electric Field on Hematological Parameters of Albino Mice

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ABSTRACT : **The impact of static electric fields (SEF) on mice blood parameters is studied under normal temperature and pressure conditions and in the same location. The experiments were carried out using two static electrical field strengths of 67 kV/m and 133 kV/m, respectively. Experimental 35 mice have been subdivided into seven groups: a control group (T1), and three groups (T2, T3, and T3) that were exposed to 67 kV/m for three periods of 0.5 h , 1 h, and 1.5 h daily for 21 days, respectively. The other three groups (T4, T5, and T6) were exposed to 133 kV/m for the same periods of 0.5 h, 1 h, and 1.5 h daily for 21 days, respectively. The results indicate that there is a highly significant mean difference in blood parameters that are; white blood cells (WBC), red blood cells (RBC), and platelets (PLT) using an intensity of 133 kV/m rather than using an intensity of 67 kV/m. moreover, mice that exposed to 133 kV/m suffer aggressive behaviors and baldness. Indead, the results indicate that there is a significant difference ($p < 0.05$) when using animals exposed to 133 kV/m for 1.5 h, this means that increasing SEF strength and exposure time might affect not only on blood parameters but also on the animal behaviors.**

Key words: **static electric field, albino mice, blood tests**

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I. INTRODUCTION

Several studies were performed on the biological effects of static electric fields (SEF, simplified as E) at different levels. The importance is that the SEF is one of several environmental factors to which all-biological objects are exposed. There are natural and artificial (human-made) static electric fields (SEF), the natural electric field encountered above the surface of the earth that relies on the time and location at which it is measured. The main cause of human-made static electric fields in the environment is charge separation as a result of friction. For example, walking on non-conductive carpets causes 10-500 kV/m [1]. The electric field is defined as the applying electrostatic force on a moving charge (SEF=F/Q in Newton per Coulomb). Two parallel conducting plates, connected with a direct current DC power supply and separated by distance (d), produce SEF, defined as the applied voltage per separating distance (SEF = V/d in volts/m). In a previous study, the effect of SEF on the hair of humans and animals was examined. They were able to perceive the presence of hair movements caused by electrostatic forces of SEF at sufficiently high levels [2]. Exposure to SEF with an intensity of 56.3 kV/m caused a temporary oxidative stress response in the liver that is expressed. This biological response results in an increase in the mitochondrial membrane potential of hepatocytes [3]. The surface electrical charge of the red blood cells is negative and the repulsive force between them occurs in normal conditions and makes the cells separate from each other. While the presence of a carboxyl group of sialic acids in their cell membrane, the negative charges create a repulsive zeta potential between them [4]. The membrane protein bonds of RBCs cause their agglutination, which can be specific or nonspecific. In a nonspecific way called rouleaux, RBC agglutination occurs when RBC cells are connected strongly by their edges and slowly move in the plasma due to increased blood viscosity. Breaking the protein bonds between two RBCs needs a stronger force acting perpendicular to the membrane surface [5]. Rats

exposed to 200 kV/m indicate that blood haematology shows the damage of WBC and platelets in vitro and in vivo despite alterations in the count and RBC affected 14 days after the end of exposure [6]. The exposure to SEF with a strength of 56.3 1.4 kV/m for 49 days has done [7, 8]. Their study shows the limited effects on male reproductive capacity. Moreover, they showed that the only biological event observed was the loss of mitochondria cristae in spermatogenic cells. In previous studies, the exposure duration of EF impacts varied from a few hours to several months and mostly ranged from 5 to 20 days [9]. Thus, three weeks were chosen as the exposure duration of SEF during this study. In a dose-dependent manner, plasma glucocorticoid (GC) levels could be suppressed by exposure to EF in BALB/c mice, and an experimental model developed to assess the effect of EFs in vivo shows that 50 Hz EFs can control the endocrine system in stressed mice [10].

In a review, the study discusses [11], electric field stimulation means to control cell orientation, migration, and phenotype in vitro and in vivo. SEF up to 10 V/cm is favoured among investigators, as such signals are primarily encountered in the extracellular space of plants and animals.

II. Materials and Methods

2.1. Experimental animals:

Thirty-five healthy adult male Wistar albino mice aged 4 weeks old and weighing 27 ± 3 g were obtained and maintained at the Breeding Animal House of the Faculty of Medicine, Zagazig University, Egypt. Animals were kept for acclimatization (1 week) in plastic cages with stainless steel wire-bar lids at a controlled temperature (25 ± 1 °C) and humidity (55 ± 5%) in a 12:12 h light-dark cycle in an artificially illuminated room, completely free from chemical contamination. They were fed and allowed to access it and drink water freely. The reporting in the manuscript follows the recommendations in the ARRIVE guidelines. The mice were divided into seven groups, each group having five mice as shown in Table 1. Whereas T1 is the control (sham) group (SEF = 0 V/m), and three groups (T2, T3, and T3) were exposed to 67 kV/m for three periods (0.5, 1, and 1.5 hours) daily for 21 days, respectively. And another three groups (T4, T5, and T6) were exposed to 133 kV/m for periods (0.5, 1, and 1.5 hours) respectively, daily for 21 days.

Ethics statement

All animal protocols were approved by the Ethical Committee of Zagazig University (ZU-IACUC committee), approval number (ZU-IACUC/1/F/257/2022)

Table 1. Treatment group, applied static electric field (SEF), daily exposured time and total experimental time

Treatment Groups	E (kV/m)	Exposure time (h)	Total time (day)
T1	0	0	21 days
T2	67	0.5	
T3		1	
T4		1.5	
T5	133	0.5	
T6		1	
T7		1.5	

2.2. SEF System Setup

Figure 1 shows the SEF system components [12]. (1) Parallel plate capacitor, (2) indicates the DC power supply, (3) I-Measuring amplifier D to measure the charge, current and voltage, (4) two-way switch, (5) Multimeter LDanalog, and (6) the mice cage.

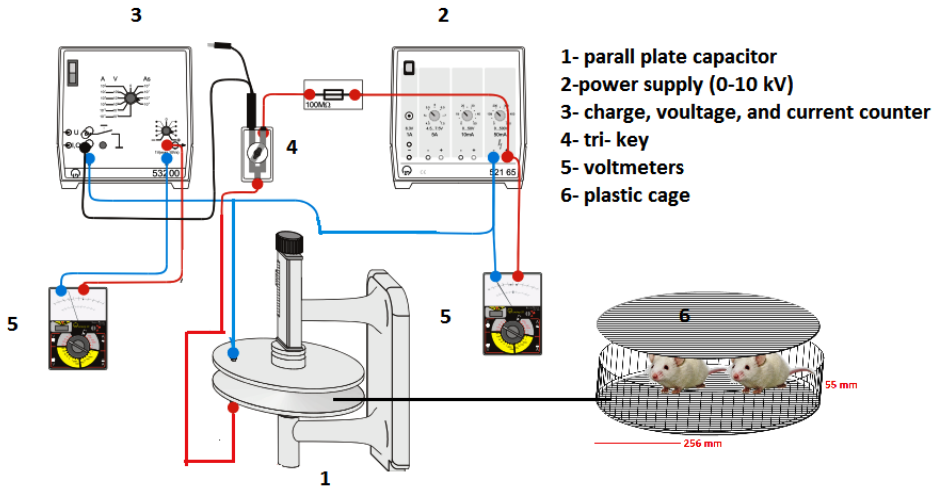

Figure 1. Measuring of the Static Electric Field by measuring the distance between charged plate capacitor of 70 mm apart, plate area= 51492.57 mm^2 and Plate thickness: 7 mm, high power supply 0-10 kV and voltmeter connect to volt sensor12.the plastic cage has height 55 mm and surface area is 51492.57 mm^2

The input AC voltage is 220 V and the output DC voltage is 0-10 kV where the SEF strength produced DC voltage is up to 160 kV, which is generated between two parallel electrodes capacitors. The device consists of two plates separated by a distance of 0 to 7 cm that is continuously adjustable; fine adjustment of the plate spacing at 1/10 mm intervals over a length of 2 mm; plate diameter: 256 mm; and plate thickness: 7 mm.

The movable plate is connected to the earth socket of the power supply and the I-Measuring amplifier the isolated plate connected via the resistor 100 MΩ to the positive socket of the power supply.

For measuring the charge on the capacitor the I-Measuring Amplifier D is switched to the range 10^{-8} As. The 3 V or 10 V DC range may be selected at multimeter. Then, for example, an output voltage of 3 V corresponds to $3x10^{-8}$ As.

A handmade cage is designed to be suitable for the above device in Fig. 1. Its diameter is 256 mm and its thickness is 55 mm. The cage is made of local plastic strips of thickness of 0.5 cm and the separated distance is 0.5 cm as shown in figure 1 (6).

The SEF values in Table 1 refer to the theoretical calculation depending on the law E=V/d. Indeed, we make a comparison between both electric fields; one measured using measured charge (experimental) and the other using where Q is the charge on the capacitor plates separated by a distance of 6 cm and the area of the plate. And the other uses applied voltage divided by the distance between plates. Table 2 indicates that the theoretical calculation is about twice the experimental estimation.

Table 2 A comparison between measured electric field strength by measure the charge on the capacitor plates and the theoretical calculation using E= V/d

$\frac{V}{volt}$	$\frac{Q}{AS}$	$\frac{C}{farad}$	$\frac{E(practical)}{V/m}$	$\frac{E\ (theoretical)}{V/m}$
0.E+00	0.E+00	0.E+00	0.E+00	0.E+00
1.E+03	4.E-08	4.E-11	8.E+03	2.E+04
2.E+03	7.E-08	3.E-11	2.E+04	3.E+04
3.E+03	1.E-07	3.E-11	2.E+04	5.E+04
4.E+03	1.E-07	3.E-11	3.E+04	7.E+04
5.E+03	2.E-07	3.E-11	3.E+04	8.E+04
6.E+03	2.E-07	3.E-11	4.E+04	1.E+05

2.3. Physiological Analysis

The hematological parameters were determined by using the hemocytometer method for RBCs count, WBCs counts, MCV, MCH, MCHC and HCT, Wintrobemacrohematocrite method for PCV, and Drabkin method for Hb determination [13,14].

2.4. Statistical Analysis:

The results presented here are the means ± SE of 5 mice in each group. The results were analyzed using a one-way analysis of variance ANOVA. if $P < 0.05$ indicated that there is a statistically significant difference.

III. Results

3.1 Unusual symptoms

At the end of the experiment, i.e., after 21 days of SEF exposure, we observed that there were changes in the mice's behaviors; hair loss and weight loss occurred; and aggressive behavior occurred that made the mice bite themselves, as shown in figure 2.

Figure 2. Effect of SEF of 157 kV/m on mice. (A) Disappear clearly the hair loss when the animals exposed to SEF for 0.5 h daily (10 days from starting point). (B) Shows the hair loss and the aggressive behaviors when animals exposed to SEF for 1.5 h (at the end of experiment).

3.2 Blood indicators levels changing

Red blood cells are one of three types of cells that circulate in the plasma. Hematocrit (Hct) is a measurement of the quantity of RBC counts associated with whole blood cell counts. Hemoglobin (Hb) is a protein located inside RBCs and contains an iron molecule; Hb carries oxygen from the lung to whole body cells and returns some carbon dioxide from those cells to the lung again (John P. Cunha, D. O.). Hct and Hb may be used to diagnose anemia diseases.

The results in Table 3 indicate that by applying high SEF (133 kV/m) to mice, the symptoms of anemia occurred. This high SEF of 133 kV/m reduces RBC counts by 40.2% and 31.0% when exposed for 1 hour and 1.5 hours, respectively. Moreover, Hct decreased by 37.2% and 34.8% for 1 hour and 1.5 hours, respectively. Hemoglobin levels also decrease by 39.3% and 30.6 when exposed for 1 hour and 1.5 hours, respectively.

Table 3. Pairwise Comparisons, mean ±SD and p-value of quantities of RBC, Hb and Hct of the seven treatment groups

RBC					Hb					Hct				
PC	Mean ± SD (106 /µL)		p-value	change %	PC	Mean ± SD (g/dL)		p-value	change %	PC	Mean ± SD (%)		p-value	change %
T1:T2	T1	7.44±0.20	0 .989	-4.2	T1:T2	T1	12.61±0.21	0 .998	-1.8	T1:T2	T1	33.75±0.27	0 .995	2.5
	T2	7.13±0.14				T2	12.38±0.08				T2	34.6±0.25		
T1:T3	T1	7.44±0.20	0 .535	-14.1	T1:T3	T1	12.61±0.21	0 .442	-10.9	T1:T3	T1	33.75±0.27	0 .830	-7.0
	T3	6.39±0.12				T3	11.23±0.27				T3	31.38±0.83		
T1:T4	T1	7.44±0.20	0 .874	-8.5	T1:T4	T1	12.61±0.21	0 .495	-10.4	T1:T4	T1	33.75±0.27	0 .874	-6.4
	T4	6.81±0.24				T4	11.3±0.43				T4	31.6±1.22		
T1:T5	T1	7.44±0.20	0 .0001	0.4	T1:T5	T1	12.61±0.21	0 .115	-16.3	T1:T5	T1	33.75±0.27	0 .304	-13.4
	T5	7.47±0.58				T5	10.55±0.60				T5	29.23±1.75		
T1:T6	T1	7.44±0.20	0 .005	-40.2	T1:T6	T1	12.61±0.21	0 .007	-39.3	T1:T6	T1	33.75±0.27	0 .007	-37.2
	T6	4.45±0.56				T6	7.65±0.91				T6	21.18±2.58		
T1:T7	T1	7.44±0.20	0.027	-31.0	T1:T7	T1	12.61±0.21	0 .032	-30.6	T1:T7	T1	33.75±0.27	0 .012	-34.8
	T7	5.13±0.25				T7	8.75±0.48				T7	22±1.40		

PC= Pairwise Comparisons, M=the mean and p=the significant p-value ≤ 0.5

The complete blood count (CBC) is a group of tests that estimate the white blood cells (WBC), red blood cells (RBC), and platelets (PLT) that circulate in our blood. The cells that improve the immune systems of our bodies are the white blood cells.

White blood cells are considered the first line of defense of the body and act as an army inside our bodies to protect us from infectious diseases, foreign invaders, and pathogens. Two types of WBC are lymphocytes (produced mainly by the lymphomatous organs) and phagocytes that are produced by the bone marrow. Phagocytes have a multi-loop nucleus within the cytoplasm, and lymphocytes include a huge nucleus where each WBC has a single process in the immune system.

Table 4 indicates the pair wise comparisons, mean, p-value, and percentage change that relied on the control treatment group T1 and each of the six treated groups (T2—T7). The results show that when applying a high static electric field such as 133 kV/m, there is a significant difference between groups where the p-value reaches 0.00001 (very high significance). WBC counts show a high significance difference when using 133 kV/m (Table 1), this high SEF causes decreasing in WBC counts in about 31.3%, 45.1%, and 52.1% by using exposure times of 0.5 hour, 1 hour, and 1.5 hours, respectively. Moreover, lymphocyte counts decreased by 33.6%, 52.9%, and 51.5 for 0.5 hour, 1 hour, and 1.5 hours, respectively. As shown, add to this granulate cell decrease.

Table 4. Pairwise Comparisons, mean and p-value of WBC, LYM and GRA of the seven treatment groups

	WBC				LYM					GRA				
PC	Mean ± SD (103 /µL)		p-value	change %	PC	Mean ± SD (103 /µL)		p-value	change %	PC	Mean ± SD (103 /µL)		p-value	change %
T1:T2	T1	7.47±0.27	0.995	-3.9	T1:T2	T1	7.47±0.30	0.995	-3.9	T1:T2	T1	0.15±0.01	0 .999	6.7
	T2	7.18±0.24				T2	7.18±0.25				T2	0.16±0.02		
T1:T3	T1	7.47±0.27	0 .988	5.6	T1:T3	T1	7.47±0.30	0.988	5.6	T1:T3	T1	0.15±0.01	0 .275	-53.3
	T3	7.89±0.85				T3	7.89±0.83				T3	0.07±0.01		
T1:T4	T1	7.47±0.27	0.950	9.2	T1:T4	T1	7.47±0.30	0 .950	9.2	T1:T4	T1	0.15±0.01	0 .910	26.7
	T4	8.16±0.86				T4	8.16±0.81				T4	0.19±0.03		

T1:T5	T1	7.47±0.27	0.002	-31.3	T1:T5	T1	7.47±0.30	0 .008	-33.6	T1:T5	T1	0.15±0.01	0 .128	-66.7
	T5	5.13±0.14				T5	4.96±0.15				T5	0.05±0.01		
T1:T6	T1	7.47±0.27	0 .0001	-45.0	T1:T6	T1	7.47±0.30	0 .0001	-52.9	T1:T6	T1	0.15±0.01	0 .001	-66.7
	T6	4.11±0.43				T6	3.52±0.34				T6	0.05±0.01		
T1:T7	T1	7.47±0.27	0.0001	-52.1	T1:T7	T1	7.47±0.30	0 .0001	-51.5	T1:T7	T1	0.15±0.01	0 .003	-60.0
	T7	3.58±0.22				T7	3.62±0.26				T7	0.06±0.01		

PC= Pairwise Comparisons, M=the mean and p=the significant p-value ≤ 0.5

Table 5 indicates the noticed effect of a high static electric field of 133 kV/m with the exposure times of 1 hour and 1.5 hours on the PLT, where the reduction of PLT counts is 41.2% and 66.8%, respectively. On the other hand, MPV and PDW counts were reduced by 20.4% and 6.8%, respectively.

Table 5. Pairwise Comparisons, mean ± SD and p-value of PLT, MPV and PDW of the seven treatment groups

PLT					MPV					PDW				
PC	Mean ± SD (103/µL)		p-value	change %	PC	Mean ± SD (fL)		p-value	change %	PC	Mean ± SD (%)		p-value	change %
T1:T2	T1	504.5±11.752	0 .943	19.9	T1:T2	T1	6.19±0.204	0 .831	5.5	T1:T2	T1	14.59±0.24	0 .999	0.3
	T2	605±63.771				T2	6.53±0.109				T2	14.63±0.024		
T1:T3	T1	504.5±11.752	0 .999	-3.0	T1:T3	T1	6.19±0.204	0 .525	8.2	T1:T3	T1	14.59±0.24	0 .721	2.8
	T3	489.25±89.792				T3	6.7±0.096				T3	15±0.147		
T1:T4	T1	504.5±11.752	0 .971	16.5	T1:T4	T1	6.19±0.204	0 .795	5.8	T1:T4	T1	14.59±0.24	0 .804	2.5
	T4	587.5±29.485				T4	6.55±0.105				T4	14.95±0.111		
T1:T5	T1	504.5±11.752	0 .999	2.1	T1:T5	T1	6.19±0.204	0 .867	5.0	T1:T5	T1	14.59±0.24	0 .839	2.3
	T5	515±77.284				T5	6.5±0.188				T5	14.93±0.191		
T1:T6	T1	504.5±11.752	0.001	-41.2	T1:T6	T1	6.19±0.204	0 .724	4.2	T1:T6	T1	14.59±0.24	0 .867	1.1
	T6	296.75±30.047				T6	6.45±0.048				T6	14.75±0.083		
T1:T7	T1	504.5±11.752	0 .0001	-66.8	T1:T7	T1	6.19±0.204	0 .007	20.4	T1:T7	T1	14.59±0.24	0 .022	6.8
	T7	167.5±17.304				T7	7.45±0.165				T7	15.58±0.120		

PC= Pairwise Comparisons, M=the mean and p=the significant p-value ≤ 0.5

3.3 Photomicrograph of blood smear

The results in Figure 3 show a photomicrograph of a blood smear. Fig.3 (a) indicates the control group (T1) that shows normal disc biconcave erythrocytes (red arrow) with central pallor area and peripheral HGB and a few hyperchromic cells (black arrow). Fig. 3 (b) indicates the treated group (T2) that shows some irregular-shaped erythrocytes (red arrow) with central pallor area and peripheral Hb, and some hypochromic cells (black arrow). Fig. 3 (c) indicates the treated group (T3) that displays many attached erythrocytes (red arrow) forming rouleaux and the appearance of numerous hypochromic cells (black arrow). Fig. 3 (d) indicates the treated group (T4) that shows a large number of attached erythrocytes (red arrow) forming rouleaux and the appearance of numerous hypochromic cells (black arrow). Fig. 3 (e) indicates the treated group (T5) that shows many attached erythrocytes (red arrow) forming rouleaux and the presence of echinocytes (blue arrow) and other moderate hypochromasia cells (black arrow). Fig. 3 (f) indicates the treated group (T6) that shows a column of erythrocytes (red arrow) forming rouleaux and the presence of echinocytes (blue arrow) and other moderate hypochromasia cells (black arrow). Fig. 3 (g) shows the treated group (T7) that exhibits rouleaux of erythrocytes (red arrow) forming and a high incidence of echinocytes (blue arrow) with marked Hypochromasia cells (black arrow).

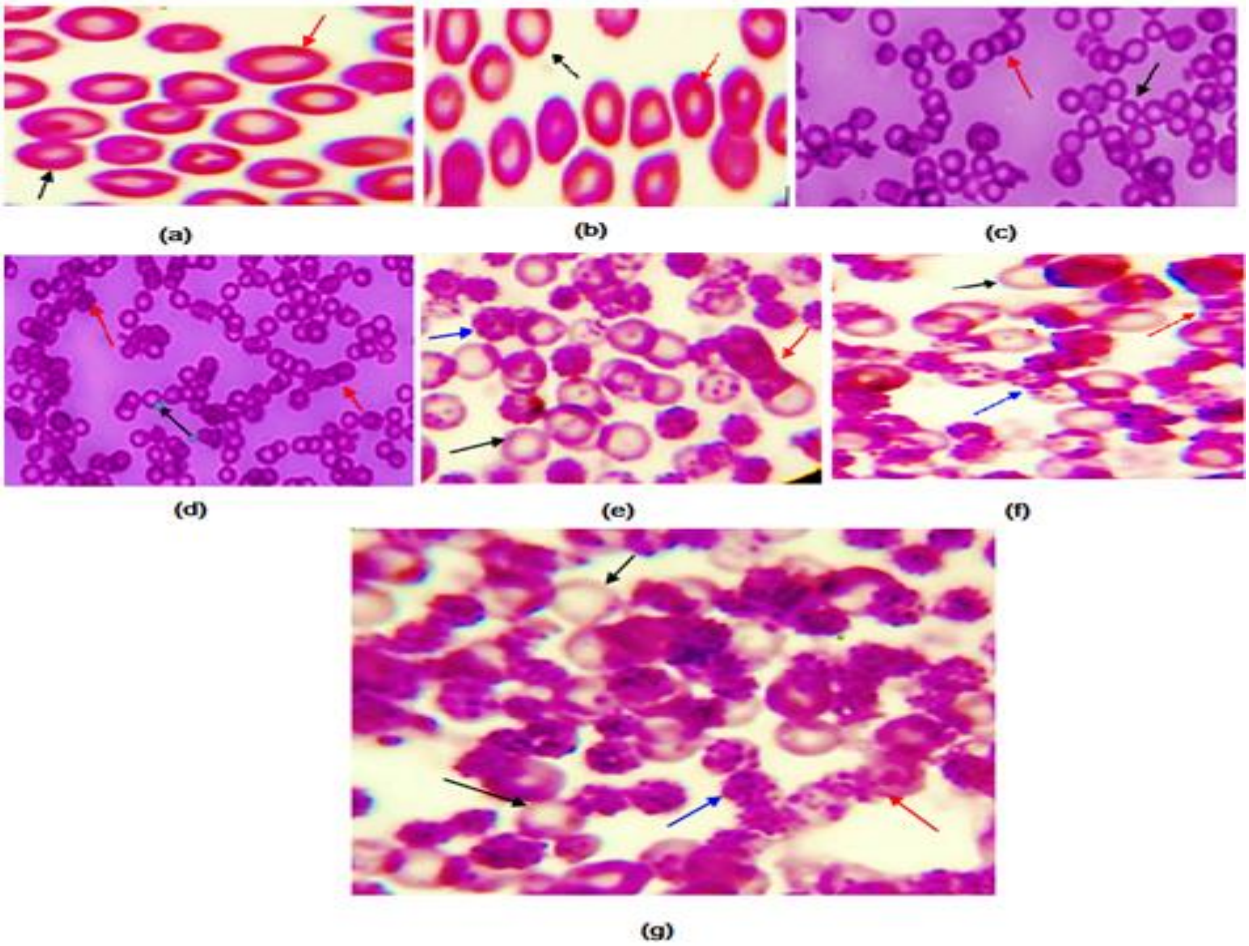

Figure 3. A photomicrograph of blood smear. (a) Control group (T1). (b) treatment group (T2) using SEF intensity of 67 kV/m and exposed time 0.5 h. (c) treatment group (T3) using SEF intensity of 67 kV/m and exposed time 1 h. (d) treatment group (T4) using SEF intensity of 67 kV/m and exposed time 1.5 h. (e) treatment group (T5) using SEF intensity of 133 kV/m and exposed time 0.5 h. (f) treatment group (T6) using SEF intensity of 133 kV/m and exposed time 1 h. (g) treatment group (T7) using SEF intensity of 133 kV/m and exposed time 1.5 h.

The photomicrograph of the blood smear shows a large number of attached erythrocytes forming rouleaux and the appearance of numerous hypochromic cells in mice exposed to 67 kV/m for 1 hour and 1 hour and 1.5 hours. While mice exposed to 133 kV/m for 0.5 hour, 1 hour & 1.5 hours exhibit rouleaux of erythrocytes forming and a high incidence of echinocytes with marked hypochromic cells.

IV- Discussion

This work aims to continue to discover the influence of SEF on animals that currently use high DC power that produces 133 kV/m. According to other studies, low power SEF indicated that there is no significant influence on animals that long-lasting, full-body exposure to SEF with different intensities 15. Cell deformations and membrane structural changes occur when the cell is exposed to a static electric field 16. The present study demonstrates that the change in SEF intensity causes a change in leucocytes and their indices. Fig. 4(a, b, c) and Table 3 show the change in RBC counts as well as the Hb and Hct indices. Fig. 4(d, e, f) and table 4 also show changes in the WBC, LYM, and GAR indices. Fig. 4(g, h, i) and Table 4 show the change in PLT, MPV, and PDW indices for platelets. In all types of blood tests either at T1 or T2, there is no statistical significance because the mean values are the same as the control ones where $p > 0.05$ as shown in figure 4. This study demonstrates that the SEF influence not only relies on the exposure time but also depends on SEF intensities 17. The results show that by using the conditions (SEF=133 kV/m and 1.5 hours), we can obtain significant changes as shown in Fig. 4.

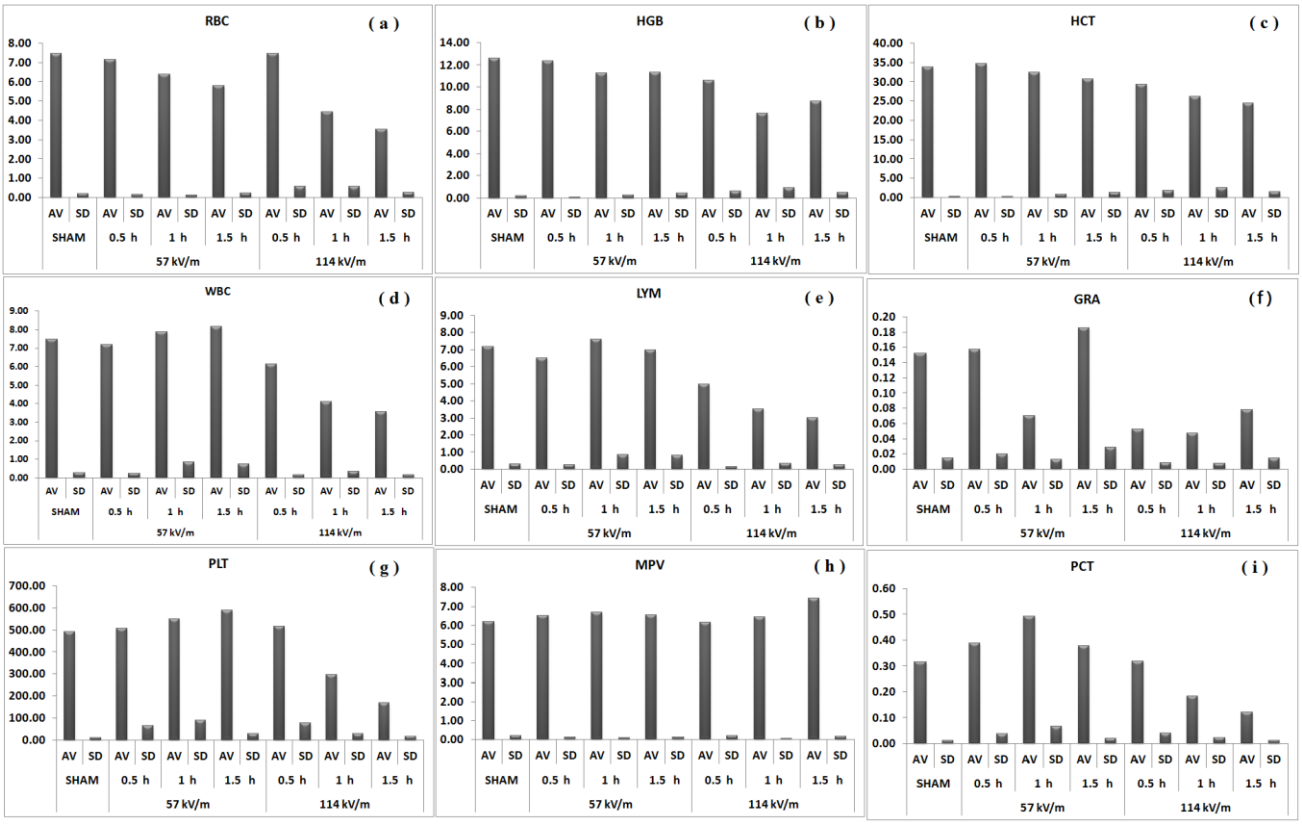

Figure 4. shows the change in some blood parameters that indicate the effect of SEF on whole-body mice where the mice exposure to two intensities 67kV/m and 133 kV/m for 0.5 h, 1 h and 1.5 h. (a,b,c) indicate the change in RBC counts, HGB levels and HCT levels respectively. (d,e,f) indicate the SEF effect on WB Ccounts and LYM and GRA levels. (g,h,i) shows the change in PLT counts and MPV, PCT levels.

The dielectric constant:

To discuss the above results and interoperate the effect of the static electrical field when the mice bodies are considered insulators and do not conduct electricity, we believe that as shown in Fig. 5 (A) shows the charge distribution only on plates because the dielectric material between capacitor plates is air. (B) shows the charge distribution only on the dielectric material between the capacitor plates. (C) shows the charge distribution on both dielectric materials between capacitor plates is air and material, which reduces the total electric field strength. (D),

the induced static electric field that is created on the mouse surface area corresponding to the case (C). When a dielectric material (mice's whole bodies) is placed in an electric field, there is an induced electric charge on the up and down surfaces. We try to explain the effect of the electric field on the mice, but we do not have the ability to measure it in the mice. Therefore, we aim to make observations. One of the observations we noticed was that the mice exposed to SEF suffer hair loss, as shown in Fig. 2, and this may be due to the repulsion force between hair negative charge and the induced negative charge on the skin surface. The produced static electric field between the two opposite sides of the mice's whole bodies influences the charges of the cell membrane. Due to the mice moving all times of exposure, the location of hair loss differs from one mouse to the other, as shown in Fig. 2. The distribution of charges is either positive or negative in the cell. For example, the negative charges Q that are located around the RBC surface and affected by the electrostatic force resulting from the applied electric field strength can also affect the motion of ions through the cell membrane.

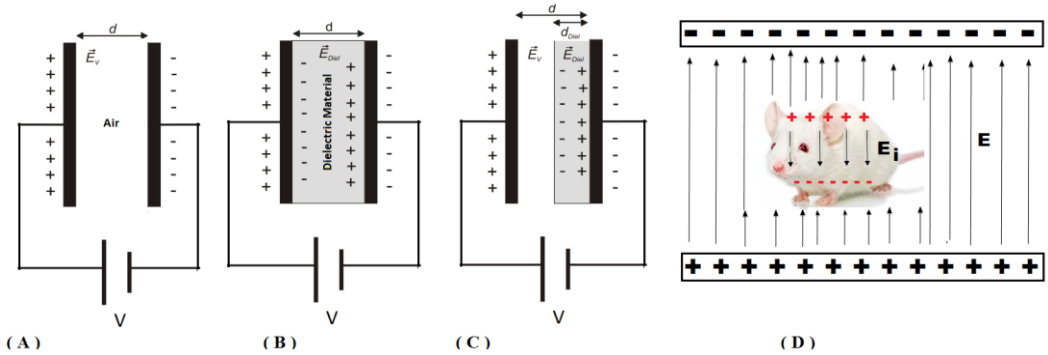

Figure 5. (A) shows E using dielectric material between capacitor plates is air (B) shows E using dielectric material between capacitor plates. (C) shows E using dielectric material between capacitor plates is air and material. (D) the induce static electric field that created on the mouse surface area corresponding like the case (C).

Finally, we did all the procedures to avoid the effect of cage material. We made it of very thin plastic material and constructed strips in it to allow air in the medium that surrounds the mice and between the capacitor plates. Therefore, there is no noticeable change in the charge or capacitance measuring values.

Conclusion

Depending on the results of this study and from the microscope results that show the change in blood parameters and/or indicators, RBC, WBC, PLT, and their related, finally, there is an observed difference between sham (control group) and the other treated groups that is shown in the photomicrograph of blood smear and levels of blood parameters. The impact of mice exposed to the static electric field was observed at the condition of E = 133 kV/m and exposure time of 1.5 hours over 21 days. More research is needed to determine whether these changes are linked to any organ disorder.

CONFLICT OF INTEREST

Consent for publication: Not applicable.

Competing interests: The authors declare that there are no conflicts of interest.

Availability of data materials: The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate: All animal procedures were carried out with the approval of the Ethics Committee of the National Research Center, Egypt, and following recommendations of the institutional animal care and use committee of the Zagazig University

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