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Original Article

Impact of Electrostatic Field on Select Enzymes, Oxidative Stress Markers and Haematological Parameters in Mice

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ABSTRACT

Background and Aim: With the rapid development of high-voltage direct current transmission, the possibility of health effects associated with electrostatic field has caused wide public concern. In this study, the effects of Electrostatic Field (ESF) exposure on haematological parameters and the activity of select antioxidant enzymes, including SOD, CPK, LDH, MDA, GSH and GABA, were investigated in mice. **Methods:** Adult Swiss male albino mice were divided into 4 equal groups (*n*=10). The mice were exposed for 5 consecutive days to SEFs with intensities of 0 kV/m (control, group I), 1 kV/m for 10 min/day (experimental group II), 5 kV/m for 10 min/day (experimental group III) or 10 kV/m for 10 min/day (experimental group IV). **Results:** The results revealed that SEFs significantly increased LDH and CPK activity in muscles; GABA concentrations in the brain, especially at higher intensities (5 and 10 kV/m); and MDA levels in the livers of exposed mice. On the other hand, mice exposed to different intensities of SEFs showed significant decreases in SOD activity and GSH levels in the liver. Exposure of mice to SEFs of 1, 5 and 10 kV/m for 10 min/day for 5 consecutive days induced pronounced declines in Hb, RBCs, WBCs, Hct, MCV, MCH and MCHC. The decreases in the average numbers of RBCs and WBCs and the levels of both Hb and Hct were significant. Additionally, when mice were exposed to 1 kV/m fields, RBCs in blood smears began to show poikilocytosis and marked hypochromia with anulocytes was observed. The blood smears of mice exposed to a higher SEF intensity (5 kV/m) clearly showed cell morphological changes and the emergence of abnormal forms, with many areas empty of RBCs (moderate incidence of echinocytes with moderate hypochromia). In mice exposed to SEFs of 10 kV/m, RBC morphology appeared completely different from normal morphology and the RBCs showed pathological changes; the outer membranes of the red corpuscles had changed and become serrated. **Conclusion:** Exposure of experimental animals to SEFs had negative effects on the brain, liver, muscles and blood, causing histological changes and disturbances in the functions of these tissues.

Keywords: Electrostatic field, Antioxidants, Echinocytes, Creatine phosphokinase, Lactate dehydrogenase.

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INTRODUCTION

Electrostatic field exist in the vicinity of high-voltage direct current transmission lines. The intensity of the SEF generated by a 750 kV power line can reach 12 kV/m at the cord minimal height (13 m).[1] SEFs can cause negative effects due to high electric tension and the nature and mechanisms of SEF-induced changes in biological tissues need further study. Recently, increases in the incidence of pathologies such as Alzheimer's disease, rheumatoid arthritis, diabetes, sepsis, chronic renal failure and respiratory distress syndrome have been observed. Oxidative stress, an imbalance in pro-/antioxidative processes that favours prooxidant reactions, is considered one of the aetiopathogenic factors of these.[2] There is increasing experimental evidence that externally applied SEFs exert various effects on oxidative processes and antioxidative defence systems. Strong SEFs cause transient inhibition of antioxidant enzyme activity in Red Blood Cells (RBCs) with subsequent adaptive stimulation of this activity after the end of the exposure cycle.[3]

The trans-membrane potential that appears in cells after exposure to electric fields increases the permeability of the cellular membrane, as in electroporation during gene transfection.[4] Thus, in addition to redox-sensitive intracellular dyes, we used standard Lactate Dehydrogenase (LDH) test to indirectly explore cellular membrane disruption along with general cytotoxicity, which is usually tested by these methods. Lu *et al.*[4] further stated that intracellular electroporation also occurs within organelle membranes, which we think might disturb the biochemical and

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redox balance within cells (especially with regards to organelles such as the mitochondria). Biochemical and redox balances might be disturbed to a greater extent if the intracellular membranes of the organelles are affected by SEFs, even at minute levels, than if only the plasma membranes were affected. Indirectly, the proposed methods (measurement of ROS fluorescent dyes, LDH, lipid peroxidation by Malondialdehyde (MDA) and antioxidative enzymes) will also indicate whether the changes are mainly cytoplasmic.[5]

MATERIALS AND METHODS

Experimental Animals

40 adult Swiss male albino mice (*Mus musculus)* weighing 25-30 g were used in the present study. The animals were kept under normal conditions and given access to food and water *ad libitum*. The mice were classified into 4 equal groups as follows:

Control group: The animals were left untreated to serve as normal controls.

1 kV/m group: The animals were exposed to a 1 kV/m electric field for 10 min/day for 5 days.

5 kV/m group: The animals were exposed to a 5 kV/m electric field for 10 min/day for 5 days.

10 kV/m group: The animals were exposed to a 10 kV/m electric field for 10 min/day for 5 days.

The animals were obtained from the Theoodor Bilharz Research Institute, Cairo, Egypt and all animal procedures were performed after approval from the Ethics Committee of the National Research Center (ECNRC), Egypt. The procedures were conducted in accordance with recommendations for the proper care and use of laboratory animals. The mice were sacrificed by cervical dislocation under light anaesthesia (ether).

Haematological Analysis

Haematological parameters were determined by using the haemocytometer method for RBC counts, WBC counts and platelet counts; the Wintrobe microhaematocrit method for PCV; and the Drabkin method for Hb determination, where Blood from caudal vein was drawn onto powdered potassium oxalate to a final concentration of approximately 0.2 per cent. After oxygenation by rotation in a stoppered flask the blood was sampled for dilutions suitable to spectrophotometric measurements and for oxygen content, especial care being observed in the mixing of cells and plasma at time of sampling.[6]

Lipid Peroxidation Estimation

Lipid peroxidation was estimated by measuring the level of thiobarbituric acid reactive substances in tissues by the method of Niehiu and Samuelsson.[7] The product was radiochemically pure as judged by thin layer chromatography and gas-liquid chromatography and had a specific activity of 5 x $l0^9$ disintegrations/min/micromole.

CK Estimation

CK is also known as Creatine Phosphokinase (CPK). CK was estimated by the method of Olexová *et al.*[8] Test solutions consisted of 2 different mixtures, (A) and (B). One milliliter of (A) solution contained 2 µ Adenosine Triphosphate (ATP), tromethamine aminomethane (pH 9.0) and Mo nil. of serum. (B) solution contained creatine in addition to all the components of (A) solution. (A) and (B) were incubated at 38ºC and the reaction was started by adding ATP. After 30 min the reaction was stopped by adding 2 mL of 20% cold trichloroacetic acid per 3 mL. reaction mixture and the Difference between (A) and (B) was considered to be the transphosphorylated phosphate from ATP to creatine.

SOD Estimation

Superoxide Dismutase (SOD) was assayed using the method of Lima *et al.*[9] The assay mixture in a total volume of 1 mL consisted 0.1 mol/L sodium phosphate buffer (pH 7.8) and 0.08 mmol/L EDTA at a 1:1 proportion. The 0.1 mL of tissue sample (1:1000) after dilution added to 2.3 mL of distilled water, after which 1 mL of assay mixture with EDTA and sodium phosphate buffer. The increase in absorbance measured spectrophotometrically at 406 nm.

GSH Estimation

Glutathione (GSH) concentrations determined by the method of Niehius and Samuelsson.[7] Reduced glutathione was measured by reaction with 5,5'-Dithiobis (2-Nitrobenzoic acid) (DTNB) to give a compound that absorbs at 412 nm (Ellman's method). Reduced glutathione in the supernatant fractions was also assayed enzymatically using glutathione S-transferase and an excess of the other substrate.

GABA Estimation

Gamma-Aminobutyric Acid (GABA) concentrations were determined by the method of Serel *et al.*[10] In order to test the practicality of the ectrophoretic method, determinations in brains of rats with varying GABA concentrations were carried out. The same tissue samples also analyzed in duplicate using a Labotron aminoacid. analyzer (Liguimat 2).

Preparation of Tissue Homogenates

Small pieces of the brain, liver and muscle collected and rinsed in 10% buffered neutral formalin solution. The remainder of the tissue (0.5 g) was independently homogenized in 5 mL of cold phosphate-buffered saline (pH 7.4, 0.1 M) using a Universal Laboratory Aid homogenizer, filtered and centrifuged at 3000 rpm for 15 min at 4ºC. The supernatants containing cell suspensions collected and stored at 20ºC until further use in bioassays.[11,12]

Electrostatic Field Experimental Setup

The schematic diagram of the SEF setup is represented in Figure 1. The setup included a high-voltage power supply and a parallel plate capacitor was used to produce a homogeneous electric field. The distance between the plates could be adjusted to a maximum of 7 cm and measured with an accuracy of 0.1 cm.[13] Figure 1 was created by co-author Mohamed Elywa.

Statistical Analysis of Data

The results presented here are the mean±SE. The results were analysed using one-way Analysis of Variance (ANOVA) and the group means were compared using Duncan's Multiple Range Test (DMRT) using SPSS version 12 for Windows. The findings were considered statistically significant if $p<0.05$.

RESULTS

Table 1 shows GABA concentration in the brains, CPK and LDH activities in the muscles, MDA, GSH levels and SOD activities in the liver of the mice exposed to 1, 5 and 10 kV/m electric fields for 10 in/day for 5 days. Mice exposed to 1 kV/m EF showed a non-significant decrease ($p>0.05$) in GABA, GSH, LDH and CPK levels in comparison to the control group and with a percentage difference of -1.39%, -3.75%, -5.52% and -11,37% respectively. While, the levels of MDA and SOD are increased non-significantly ($p>0.05$) at the same group in comparison to the control group. Mice exposed to 5 kV/m and 10 kV/m EFs showed a significant increase ($p>0.05$) in GABA, MDA, LDH and CPK levels in comparison to control levels. While the levels of levels of SOD and GSH are decreased significantly ($p>0.05$) at the same doses in comparison to the control group.

Table 2 shows the effects of exposure to 1, 5 and 10 kV/m electric fields for 10 min/day for 5 days on haematological parameters of mice. The collective data on the effects of exposure to 1, 5 and 10 kV/m electric fields for 10 min/day for 5 days on haematological parameters in mice are shown in Table . There were significant differences in most of the haematological parameters (Hb, RBCs, Hct, Platelets (Plts) MCV, MCH, MCHC and WBCs) between the control and exposed groups of mice. Compared to no exposure, low-intensity electric field exposure (1 kV/m) significantly decreased Hb concentrations (15.10±1.30 vs. 11.35±0.80 g/dL), RBC counts (9.06±0.18 vs. 7.90±0.23×10^6/mm^3), Hct (51.04±2.6 vs. 42.15±2.2%), MCH (17.37±1.01 vs. 14.32±0.58) and MCHC (30.82±1.76 vs. 26.84±0.84). The maximal decrease was recorded for Hb concentrations, which were 24.86% lower in the 1 kV/m group than in the control group. In contrast, the decreases in WBCs and MCV were not significant ($p>0.05$). Compared to control mice, mice exposed to 5 kV/m electric fields showed very highly significant ($p<0.001$) decreases in Hb concentration (15.10±1.30 vs. 8.10±0.40 g/dL), Hct (51.04±2.6 vs. 30.15±0.30) and MCH (17.37±1.01 vs. 12.29±0.05). In addition, compared to control group mice, 5 kV/m group mice exhibited highly significant ($p<0.01$) decreases in RBC counts (9.06±0.18 vs. 6.60±0.50×10^6/mm^3), MCV (58.67±3.20 vs. 45.76±2.00) and WBC counts (2.19±0.05 vs. 1.70±0.08×10^3/mm^3) but significant decreases in MCHC (30.82±1.76 vs. 26.86±0.73). The maximal decreases of 40.93% and 46.37%, respectively, were recorded for Hct and Hb concentrations. Compared to control mice, mice exposed to high-intensity electric fields (10 kV/m) exhibited very highly significant ($p<0.01$) decreases in RBC counts (9.06±0.18 vs. 6.70±0.33×10^6/mm^3), Hct (51.04±2.6 vs. 37.45±0.40) and WBC counts (2.19±0.05 vs. 1.65±0.09×10^3/mm^3). Furthermore, high-intensity field exposure caused highly significant ($p<0.01$) decreases in Hb concentrations (15.10±1.30 vs. 11.25±0.13). On the other hand, the decreases in MCV, MCH and MCHC were not significant ($p>0.05$). The maximal decreases of 26.07% and 26.63% were recorded for RBC counts and Hct, respectively.

Table 1: GABA concentration in the brains, CPK and LDH activities in the muscles, MDA, GSH levels and SOD activities in the liver of the mice exposed to 1, 5 and 10 kV/m electric fields for 10 min/day for 5 days.

Groups	GABA (µg/g of tissue)	MDA (nmol/mg of protein)	SOD (U/mg of protein)	GSH (mmol/mg of protein)	LDH (µmol/min/mg of protein)	CPK (µmoles/min/mg protein)
Control	178.83±1.96	0.14±0.023	3.51±0.044	0.56±0.017	33.87±0.81	88.77±1.68
1 kV/m	176.33±5.45	0.15±0.012	3.53±0.024	0.54±0.030	32.00±2.08	78.67±5.92
P	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05
% diff.	-1.39	6.66	0.56	-3.75	-5.52	-11.37
5 kV/m	199.67±5.78	0.25±0.010	2.22±0.018	0.39±0.018	42.90±1.00	180.67±12.41
P	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05
% diff.	11.65	78.57	-36.75	-30.35	26.66	103.52
10 kV/m	235.00±2.88	0.35±0.029	1.98±0.060	0.25±0.023	57.33±4.91	236.60±18.55
P	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05
% diff.	31.40	150	-43.58	-55.35	69.26	166.53

Non-significant: $p>0.05$. Significant: $p<0.05$.

Histological Results

Figure 2 shows the representative image of Giemsa-stained cells. Blood smear from the control group showing normal biconcave disc-shaped erythrocytes (arrows) with central pale areas and anulocytes. Figure 3 shows the representative image of Giemsa-stained cells. Blood smear from the 1 kV/m group showing poikilocytosis and marked hypochromia. Figure 4 shows the representative image of Giemsa-stained cells. Blood smear from the 5 kV/m group showing a moderate incidence of echinocytes (black arrow), moderate hypochromia (red arrow) and target cells (yellow arrows). Figure 5 shows the representative image of Giemsa-stained cells. Blood smear from the 10 kV/m group showing a high incidence of echinocytes (crenated cells with spine-like projections).

DISCUSSION

The potential of SEFs adversely affect the health of the human population is an issue that continues to receive a great deal of attention in both public and scientific forums. There is growing evidence that the effects of SEFs are mediated by the formation of ROS and free radicals, which are highly reactive, removing hydrogen atoms from fatty acids, causing lipid peroxidation and ultimately resulting in cell death.[14] Toxic oxygen free radicals are extremely reactive and can cause considerable damage to biomolecules, such as RNA, enzymes, membranes, proteins and lipids, which may in turn lead to various pathological consequences. Normally, oxygen free radicals neutralized by highly efficient systems in the body. These systems include antioxidant enzymes such as SOD, LDH and CPK.

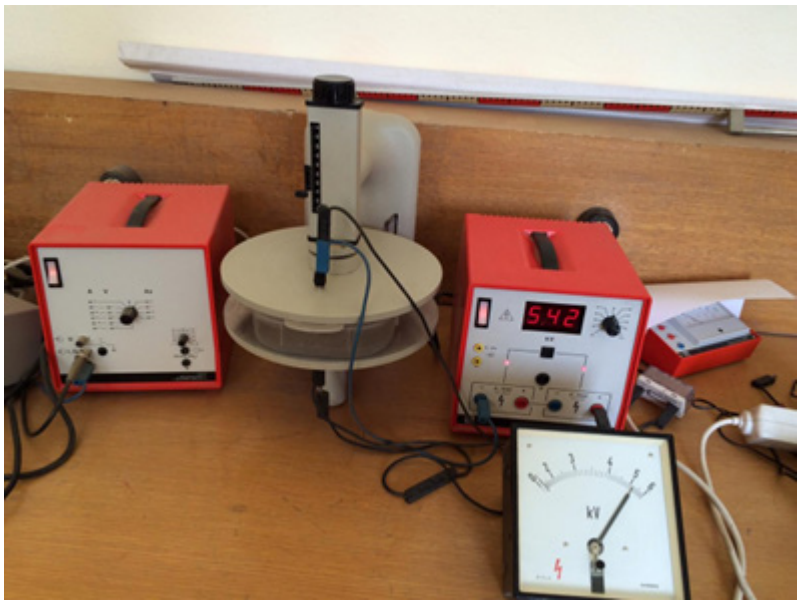

Figure 1: The external electrostatic field setup.

Table 2: Effects of exposure to 1, 5 and 10 kV/m electric fields for 10 min/day for 5 days on haematological parameters of mice.

Group	Control	1 kV/m			5 kV/m			10 kV/m		
Haem. Param.	Mean±SE	Mean±SE	*p*-value	% Diff.	Mean±SE	*p*-value	% Diff.	Mean±SE	*p*-value	% Diff.
Hb (g/dL)	15.10±1.30	11.35±0.80	<0.05	-24.86	8.10±0.40	<0.001	-46.37	11.25±0.13	<0.01	-25.52
RBCs (10^6/mm^3)	9.06±0.18	7.90±0.23	<0.05	-12.83	6.60±0.50	<0.01	-27.17	6.70±0.33	<0.001	-26.07
Hct (%)	51.04±2.6	42.15±2.2	<0.05	-17.42	30.15±0.30	<0.001	-40.93	37.45±0.40	<0.001	-26.63
MCV (fl)	58.67±3.20	53.28±2.76	>0.05	-9.19	45.76±2.00	<0.01	-22.01	55.90±3.45	>0.05	-4.72
MCH (pg)	17.37±1.01	14.32±0.58	<0.05	-17.57	12.29±0.05	<0.001	-29.25	16.79±0.09	>0.05	-3.31
MCHC (g/dl)	30.82±1.76	26.84±0.84	<0.05	-12.92	26.86±0.73	<0.05	-12.87	30.01±1.06	>0.05	-2.64
WBCs (10^3/mm^3)	2.19±0.05	2.25±0.02	>0.05	2.86	1.70±0.08	<0.01	-22.29	1.65±0.09	<0.001	-24.57

The *p*-values refer to the significance of the difference between the indicated value and the control value.Non-significant: $p>0.05$; Significant: $p<0.05$; Highly significant: $p<0.01$. Very highly significant: $p<0.001$.

The present data showed that hepatic GSH content was markedly decreased in exposed mice and this decrease was highly significant at the highest strength (10 kV/m) of exposure. Our results seem to be consistent with those of Rifat *et al.*[15] who reported that GSH levels in the intestine, liver, testes and spleen of mice exposed to 10 GHz microwave radiation for 30 days were very significantly lower than those in the same tissues of control mice.

In the present study, the observed decrease in the activity of the antioxidant enzyme GSH in the liver following SEF exposure may have been due to the damaging effects of free radicals produced following SEF exposure or, alternatively, may have been due to a direct effect of formaldehyde formed from free radical oxidation on this enzyme. GSH acts as a free radical scavenger and regenerator of alpha tocopherol and plays a significant role in sustaining protein sulfhydryl groups.[16]

Decreases in hepatic GSH levels result in increased susceptibility to hepatic injury via induction of lipid peroxidation.[17] GSH is the main antioxidant found in liver cells and plays a protective role in the metabolism of a large number of toxic agents, including oxidative stress-causing agents. SEF toxicity has been associated with decreased hepatic GSH, which may reflect depletion of GSH due to overproduction of ROS and subsequent oxidative stress caused by SEF. Mailankot *et al.*[18] reported that compared to control conditions, exposure to continuous microwave radiation at 2450 MHz and 0.25 mW/cm^2 induced significant decreases in the activity of the antioxidant enzymes GSH-Px, SOD and Catalase (CAT). Decreased Lipid Peroxidase (LPO) and GSH content in the testes and epididymis of rats exposed to 0.9/1.8 GHz has also been reported.[19]

SOD is an antioxidant enzyme that functions with GSH-PX and GST to protect organisms from toxic free radicals, mainly superoxide anion free radicals.[20] The liver is the most important detoxification organ in the body and contains an abundance of mitochondria, the main organelles that generate superoxide anion free radicals.[21] Thus, estimates of the hepatic activity of SOD reflect oxidative stress status to some extent. In this study, statistically significant decreases in hepatic SOD activity were observed in mice exposed to the highest field intensities (5 and 10 kV/m) after an exposure time of 5 days compared to control mice. These decreases in SOD activity may indicate increased superoxide anion free radical production after the 5-day exposure time. Güler *et al.*[22] hypothesized that molecular $O2^{\bullet-}$ is transformed into superoxide anion free radicals as a result of energy transfer in tissues in electric fields. Thus, in our study, the enhancement of superoxide anion free radical production in mice upon exposure to the low-intensity (1 kV/m) electric field for 5 days may have increased the synthesis of SOD, which can eliminate superoxide anion free radicals by forming H_2O_2. This may represent a self-regulatory mechanism that protects cells from injury caused by increased ROS. This hypothesis is supported by the similar activity of SOD between the 1 kV/m group and the control group ($p>0.05$). However, SOD activity was again significantly decreased in both higher-intensity exposure groups (5 and 10 kV/m). This may have been because the adverse effects of SEF exposure were cumulative and the mice were not able to maintain homeostasis when the adverse effects exceeded a certain threshold. Cieslar *et al.*[23] exposed male Wistar rats to an SEF with an intensity of 32 kV/m for 10 days (4 hr/day) and found no significant differences in erythrocytes or serum SOD activity. However, significant differences ($p<0.05$) were observed in the serum when the exposure level was increased to 48 kV/m, indicating that the effect was dose-dependent. Further support for our result was obtained by Wu *et al.*[20] who found that the activity of the antioxidant enzyme SOD was not significantly increased ($p>0.05$) in the liver homogenate of mice exposed for 7 days to an environmental SEF of 2.3 kV/m; on the other hand, an electric field intensity of 9.2 kV/m significantly decreased the activity of SOD after 35 days of exposure.

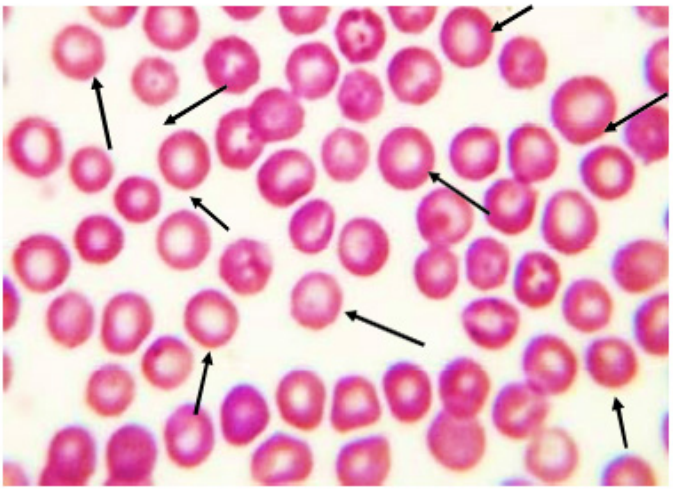

Figure 2: Representative image of Giemsa-stained cells. Blood smear from the control group showing normal biconcave disc-shaped erythrocytes (arrows) with central pale areas and anulocytes.

MDA concentrations in hepatocytes were also determined in this study. MDA is a specific and chemically stable product of lipid peroxidation, during which free radicals attack polyunsaturated fatty acids in cell membranes to ultimately cause cell damage. Thus, MDA is also regarded as a reliable biological marker of oxidative stress.[22] Güler *et al.*[22] found significantly elevated MDA levels and significantly decreased antioxidant enzyme activity in guinea pigs that were exposed to an ELF electric field. The authors also found that N-acetyl-L-cysteine application had protective effects against ELF electric field-induced oxidative stress. The results of a study by Wu *et al.*[20] revealed that MDA concentrations in mice exposed to 9.2 kV/m for 14 and 21 days were significantly higher than those in control mice. Additionally, Balci *et al.*[24] detected clear increases in MDA levels due to oxidative stress in the cornea, in accordance with previous studies. In corneas exposed to PC monitor radiation, the levels of MDA, an indicator of lipid peroxidation, were significantly

elevated. The results of this study agree with those of previous studies showing significant increases in MDA concentrations after exposure to SEFs of different intensities (1, 5 and 10 kV/m). These data indicate that SEFs of certain intensities increase lipid peroxidation in biological membranes in liver tissues.

The enzyme LDH is an important enzyme and has been studied as a general marker of cellular health. The findings of our study suggested that LDH levels were increased in exposure groups (5 and 10 kV/m) compared to the control group. These observations are consistent with those of Yuan *et al.*[25] who also observed increased levels of LDH in the serum of volunteers occupationally exposed to very high-frequency radiation. Peppes *et al.*[26] showed significant positive correlations between angiographic findings and peak serum levels of myocardial enzymes (CPK, Aspartate aminotransferase (AST) and LDH) and inflammatory biomarkers were identified. Therefore, the finding in this study that SEF increased muscle enzyme levels in mice may suggest that muscular damage occurred in the exposed groups. Miliša *et al.*[5] mentioned that increases in metabolic (or mitochondrial) activity can be confirmed by increases in LDH levels; therefore, the 5 kV/m field increased metabolic activity. Mitochondria are sensitive to electromagnetic radiation.[27] Since LDH was measured in the medium, the observed increases could also mean that the cell membranes were disrupted, causing LDH leakage to the surrounding environment.[5] Concerning the significant elevation in muscle CPK activity in mice exposed to 5 and 10 kV/m SEFs, our results seem to be consistent with those of Ali *et al.*[28] who found that CPK levels in rat testes were highly significantly increased in all animals exposed to a 50 Hz and 0.05 mT EMF. Moreover, the highly significant increases in CPK levels in muscle were mainly attributable to damage to the cellular membrane in cells of different organs.

The present study investigated the effects of exposure to SEFs with different intensities of 1, 5 and 10 kV/m for 5 days (10 min/day) on the levels of the amino acid neurotransmitter GABA in the mouse brain. GABA levels decreased non-significantly ($p>0.05$) in mice exposed to lower-intensity SEFs (1 kV/m), but mice exposed to higher intensities (5 and 10 kV/m) exhibited significant increases ($p<0.05$) in GABA levels. A similar situation was reported by Xu *et al.*[29] who reported that full-body exposure of mice to an SEF with a certain intensity (2.30 kV/m) for 7, 21 and 35 days (24 hr/day) did not cause significant changes in glutamic acid and GABA levels in the hippocampus, but exposure to an SEF with a high intensity (9.20 kV/m) for 21 and 35 days clearly increased GABA levels.

In the central nervous system, GABA is the primary inhibitory neurotransmitter.[30] GABA can act on its receptor and then regulate appropriate levels of inhibitory signals essential for synapse plasticity, which is the basis for learning and memory.

In our study, SEF exposure decreased WBC and RBC counts. The decreases in Hb concentrations in this study may be attributable to the interaction between the iron in haem and the electric field, as the electric field enters the body and acts on ions in all vital organs, including the spleen, bone marrow, kidneys and liver. We observed that the exposed groups exhibited lower Hb concentrations and RBC counts than the control group, suggesting an anaemia-like stage. Our results are consistent with those previously reported by Bonhomme-Faivre *et al.*[31] Changes in the composition of Hb inside the RBC membrane were observed; such changes affect the physiological function of RBCs and their capacity to transport oxygen, as observed previously with reduced Hb concentrations after exposure to electromagnetic waves.[32] Destruction in splenic tissue has also been reported,[33] confirming the harmful effects of SEFs on haemopoietic tissue. The spleen is a lymphatic organ that stores blood corpuscles. Spleen hyperfunction increases the rate of destruction of RBCs, WBCs and Plts, which can ultimately lead to a decrease in Hb

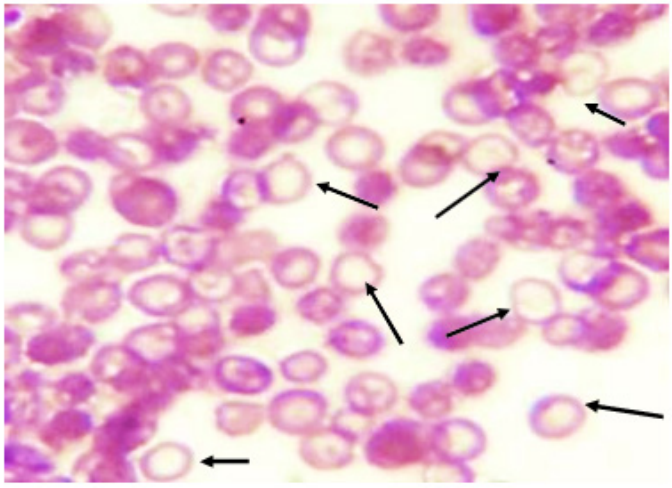

Figure 3: Representative image of Giemsa-stained cells. Blood smear from the 1 kV/m group showing poikilocytosis and marked hypochromia.

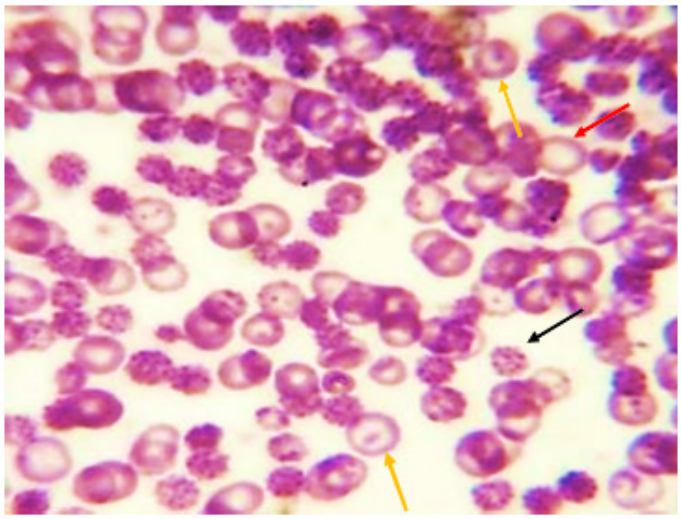

Figure 4: Representative image of Giemsa-stained cells. Blood smear from the 5 kV/m group showing a moderate incidence of echinocytes (black arrow), moderate hypochromia (red arrow) and target cells (yellow arrows).

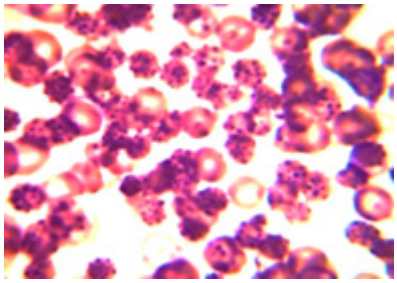

Figure 5: Representative image of Giemsa-stained cells. Blood smear from the 10 kV/m group showing a high incidence of echinocytes (crenated cells with spine-like projections).

concentration.[34] Any abnormality in the spleen as a result of an EMF can lead to haemolysis of RBCs within the spleen.[33] It has been proven by the production of haemosiderin granules in macrophages that EMF exposure results in increased phagocytic activity against RBCs.[35] The observed alterations in various haematological parameters clearly indicate that the haemopoietic system was adversely affected by the SEFs.

To identify RBC deformation, we analysed blood smears. Deformed RBCs, mainly echinocytes with multiple spine-like projections, were detected in samples from mice exposed to 1, 5 and 10 kV/m SEFs. The deformation of RBCs was most pronounced in mice exposed to the highest intensities (5 and 10 kV/m) of SEFs. The blood smears of the exposed groups exhibited RBCs with distorted shapes that were stacked together to form rouleaux (Figures 2-4). SEF exposure increased the ability of RBCs to stick together by increasing the viscosity of the blood. Most of the RBCs appeared pale in colour (hypochromic) because of a lack of Hb and the membrane structure was altered. Haemolysis of RBCs was also observed as the strength of the SEF increased. Our results are consistent with those of Singh *et al*. who reported that exposure of Swiss albino mice to radiation emitted from a video display unit 20 cm away at a power density of 0.295 W/cm2 altered the morphology of RBCs in blood smears and scanning electron micrographs. RBC counts and Hb concentrations were reduced until day 42 of irradiation. The effects were manifested as damage to neighbouring RBCs, likely a result of increased generation of ROS.[36] Thus, oxidative damage of membranes leads to various forms of abnormal RBCs, including elliptocytes, echinocytes, knizocytes and codocytes.[37]

CONCLUSION

SEF exposure caused different metabolic and haematologic effects that appeared to be related to the intensity of the SEF. The changes in biochemical parameters in SEF-exposed mice probably reflected hepatic damage and anaemia caused by kidney failure. Further studies are needed to better understand the effects of SEFs on biological systems.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

ESF: Electrostatic Field; **EMF:** Electromagnetic Field; **SOD:** Superoxide Dismutase; **GSH:** Glutathione; **MDA:** Malondialdehyde; **GABA:** Gamma-Aminobutyric Acid; **CPK:** Creatine Phosphokinase; **LDH:** Lactate Dehydrogenase; **RBC:** Red Blood Cell; **Hb:** Haemoglobin; **MCV:** Mean Corpuscular Volume; **WBC:** White Blood Cell; **Plt:** Platelet.

REFERENCES

1. Ali, F. M., Ahmed, W. N. A., Abdel Hamid, S., Abd El-Fattah, E. S., & Ahmed, M. (2011). Effects of exposures to a max of fast neurons and 50Hz 0.05mT magnetic field on rat testes (*in vivo* study). Journal of International Dental and Medical Research, 4(2), 94.
2. Balci, M., Namuslu, M., Devrim, E., & Durak, I. (2009). Effects of computer monitor-emitted radiation on oxidant/antioxidant balance in cornea and lens from rats. Molecular Vision, 15, 2521–2525.
3. Bonhomme-Faivre, L., Marion, S., Bezie, Y., Auclair, H., & Fredj, G. (1998). Study of human neurovegetative and hematologic effects of environmental low frequency (50Hz) electromagnetic fields produced by transformers. Archives of Environmental Health, 53, 92–97.
4. Burlaka, A., Selyuk, M., Gafurov, M., Lukin, S., Potaskalova, V., & Sidorik, E. (2014). Changes in mitochondrial functioning with electromagnetic radiation of ultrahigh frequency as revealed by electron paramagnetic resonance methods. International Journal of Radiation Biology, 90(5), 357–362. https://doi.org/10.3109/09553002.2014.899448
5. Cabrales, L. B., Ciria, H. C., Bruzón, R. P., Quevedo, M. S., Céspedes, M. C., & Salas, M. F. (2001). ELF magnetic field effects on some hematological and biochemical parameters of peripheral blood in mice. Electro- and Magnetobiology, 20(2), 185–191. https://doi.org/10.1081/JBC-100104142
6. Ciccoli, L., De Felice, C., Paccagnini, E., Leoncini, S., Pecorelli, A., Signorini, C., Belmonte, G., Guerranti, R., Cortelazzo, A., Gentile, M., Zollo, G., Durand, T., Valacchi, G., Rossi, M., & Hayek, J. (2013). Erythrocyte shape abnormalities, membrane oxidative damage and β-actin alterations: An unrecognized triad in classical autism. Mediators of Inflammation, 2013, 432616. https://doi.org/10.1155/2013/432616
7. Cieslar, G., Malyszek-Tumidajewicz, J., Sowa, P., & Sieron, A. (2012). Impact of static electric field on prooxidant-antioxidant balance in rats. COMPEL – The International Journal for Computation and Mathematics in Electrical and Electronic Engineering, 31(4), 1212–1223. https://doi.org/10.1108/03321641211227483
8. Cieslar, G., Sowa, P., & Sieron, A. (2003). Influence of high voltage static electric field on antioxidant enzymes activity in male rats. In Proceedings of the 25th Annual International Conference of the IEEE Engineering in Medicine and Biology Society. Mexico (pp. 3257–3260). https://doi.org/10.1109/IEMBS.2003.1280838
9. Dalle-Donne, I., Rossi, R., Giustarini, D., Milzani, A., & Colombo, R. (2003). Protein carbonyl groups as biomarkers of oxidative stress. Clinica Chimica Acta; International Journal of Clinical Chemistry, 329 (1–2), 23–38. https://doi.org/10.1016/s0009-8981(03)00003-2
10. Elywa, M. (2017). Biological effects of water treated using static electric field as revealed by rat blood tests. Romanian J. Biophys., 27 (3–4), 89–98.
11. Eraslan, G., Bilgili, A., Akdogan, M., Yarsan, E., Essiz, D., & Altintas, L. (2007). Studies on antioxidant enzymes in mice exposed to pulsed electromagnetic fields. Ecotoxicology and Environmental Safety, 66(2), 287–289. https://doi.org/10.1016/j.ecoenv.2006.01.001
12. Glantzounis, G. K., Tsimaris, I., Tselepis, A. D., Thomas, C., Galaris, D. A., & Tsimoyiannis, E. C. (2005). Alterations in plasma oxidative stress markers after laparoscopic operations of the upper and lower abdomen. Angiology, 56(4), 459–465. https://doi.org/10.1177/000331970505600414

13. Güler, G., Seyhan, N., & Aricioğlu, A. (2006). Effects of static and 50 Hz alternating electric fields on superoxide dismutase activity and TBARS levels in guinea pigs. General Physiology and Biophysics, 25(2), 177–193.
14. Güler, G., Turkozer, Z., Tomruk, A., & Seyhan, N. (2008). The protective effects of N-acetyl-L-cysteine and epigallocatechin-3-gallate on electric field-induced hepatic oxidative stress. International Journal of Radiation Biology, 84(8), 669–680. https://doi.org/10.1080/09553000802241747
15. Hayk, A. H. (2015). Carbonylation of plasma proteins and blood hemolysis induced by the action of external electrostatic field *in vitro*. Medicinal Chemistry, 5(5), 226–230.
16. Irmak, M. K., Fadillioğlu, E., Güleç, M., Erdoğan, H., Yağmurca, M., & Akyol, O. (2002). Effects of electromagnetic radiation from a cellular telephone on the oxidant and antioxidant levels in rabbits. Cell Biochemistry and Function, 20(4), 279–283. https://doi.org/10.1002/cbf.976
17. Megha, K., Deshmukh, P. S., Banerjee, B. D., Tripathi, A. K., & Abegaonkar, M. P. (2012). Microwave radiation induced oxidative stress, cognitive impairment and inflammation in brain of Fischer rats. Indian Journal of Experimental Biology, 50(12), 889–896.
18. Karabulut, A. B., Gül, M., Karabulut, E., Kiran, T. R., Ocak, S. G., & Otlu, O. (2010). Oxidant and antioxidant activity in rabbit livers treated with zoledronic acid. Transplantation Proceedings, 42(9), 3820–3822. https://doi.org/10.1016/j.transproceed.2010.06.017
19. Lima, P. L., Benassi, J. C., Pedrosa, R. C., Dal Magro, J., Oliveira, T. B., & Wilhelm Filho, D. (2006). Time-course variations of DNA damage and biomarkers of oxidative stress in tilapia (Oreochromis niloticus) exposed to effluents from a swine industry. Archives of Environmental Contamination and Toxicology, 50(1), 23–30. https://doi.org/10.1007/s00244-004-0178-x
20. Lu, W., Wu, K., Hu, X., Xie, X., Ning, J., Wang, C., Zhou, H., & Yang, G. (2017). Theoretical analysis of transmembrane potential of cells exposed to nanosecond pulsed electric field. International Journal of Radiation Biology, 93(2), 231–239. https://doi.org/10.1080/09553002.2017.1230244
21. Mailankot, M., Kunnath, A. P., Jayalekshmi, H., Koduru, B., & Valsalan, R. (2009). Radio frequency electromagnetic radiation (RF-EMR) from GSM (0.9/1.8GHz) mobile phones induces oxidative stress and reduces sperm motility in rats. Clinics, 64(6), 561–565. https://doi.org/10.1590/s1807-59322009000600011
22. Martínez-Sámano, J., Torres-Durán, P. V., Juárez-Oropeza, M. A., & Verdugo-Díaz, L. (2012). Effect of acute extremely low frequency electromagnetic field exposure on the antioxidant status and lipid levels in rat brain. Archives of Medical Research, 43(3), 183–189. https://doi.org/10.1016/j.arcmed.2012.04.003
23. Miliša, M., Đikić, D., Mandić, T., Grozić, D., Čolić, I., & Ostojić, A. (2017). Response of aquatic protists to electric field exposure. International Journal of Radiation Biology, 93(8), 818–830. https://doi.org/10.1080/09553002.2017.1321809
24. Mohammad, A., Esrafil, M., Fatemeh, P., Maryam, M., & Zahra, A. (2016). Biochemical and histological effects of mobile phone radiation on enzymes and tissues of mice. Research Journal of Pharmaceutical, Biological and Chemical Sciences, 7(5), 1962.
25. Moron, M. S., Depierre, J. W., & Mannervik, B. (1979). Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver. Biochimica et Biophysica Acta, 582(1), 67–78. https://doi.org/10.1016/0304-4165(79)90289-7
26. Niehaus, W. G., & Samuelsson, B. (1968). Formation of malondialdehyde from phospholipid arachidonate during microsomal lipidperoxidation. European Journal of Biochemistry, 6(1), 126–130. https://doi.org/10.1111/j.1432-1033.1968.tb00428.x
27. Olexová, L., Štefánik, P., & Kršková, L. (2016). Increased anxiety-like behaviour and altered GABAergic system in the amygdala and cerebellum of VPA rats-An animal model of autism. Neuroscience Letters, 629, 9–14. https://doi.org/10.1016/j.neulet.2016.06.035
28. Peppes, V., Rammos, G., Manios, E., Koroboki, E., Rokas, S., & Zakopoulos, N. (2008). Correlation between myocardial enzyme serum levels and markers of inflammation with severity of coronary artery disease and Gensini score: A hospital-based prospective study in Greek patients. Clinical Interventions in Aging, 3(4), 699–710. https://doi.org/10.2147/cia.s3551
29. Rifat, F., & Sisodia, R. (2017). Modulation of 10 GHz microwaves induced biochemical changes in different organs of Swiss albino mice by Prunus domestica fruit extract. International Journal of Pharmaceutical Sciences and Research, 8(1), 136–144.
30. Serel, T. A., Ozguner, F., & Soyupek, S. (2004). Prevention of shock wave-induced renal oxidative stress by melatonin: An experimental study. Urological Research, 32(1), 69–71. https://doi.org/10.1007/s00240-003-0397-z
31. Simkó, M., Droste, S., Kriehuber, R., & Weiss, D. G. (2001). Stimulation of phagocytosis and free radical production in murine macrophages by 50 Hz electromagnetic fields. European Journal of Cell Biology, 80(8), 562–566. https://doi.org/10.1078/0171-9335-00187
32. Wu, S. X., Xu, Y. Q., Di, G. Q., Jiang, J. H., Xin, L., & Wu, T. Y. (2016). Influence of environmental static electric field on antioxidant enzymes activities in hepatocytes of mice. Genetics and Molecular Research, 15(3), 150–388. https://doi.org/10.4238/gmr.15038800
33. Xu, Y., Wu, S., Di, G. Q., Ling, P., Jiang, J., & Bao, H. (2016). Influence of static electric field on cognition in mice. Bioengineered, 7(4), 241–245. https://doi.org/10.1080/21655979.2016.1197632
34. Yao, C., Li, X., & Xiong, Y. (2005). Instant effects of radio frequency electromagnetic wave on hemoglobin in single living intact red blood cell. Chinese Chemical Letters, 16(8), 1121–1124.
35. Yuan, Z.-Q., Li, F., Wang, D.-G., Wang, Y., & Zhang, P. (2004). Effect of low intensity and very high frequency electromagnetic radiation on occupationally exposed personnel. Zhonghua Lao Dong Wei Sheng Zhi Ye Bing Za Zhi [Chinese Journal of Industrial Hygiene and Occupational Diseases], 22(4), 267–269.
36. Zaghloul, M. S. (2011). Effects of chronic exposure to static electromagnetic field on certain histological aspects of the spleen and some hematological parameters in albino rats. Journal of American Science, 7(8), 383–394.
37. Zhao, L., Peng, R. Y., Wang, S. M., Wang, L. F., Gao, Y. B., Dong, J., Li, X., & Su, Z. T. (2012). Relationship between cognition function and hippocampus structure after long-term microwave exposure. Biomedical and Environmental Sciences, 25(2), 182–188. https://doi.org/10.3967/0895-3988.2012.02.009

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