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The Influence of Magnetic Fields on Selected Physiological Parameters of Blood and Tissues in Mice

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Abstract

Background: Exposure to Magnetic fields (MFs)induces metabolic and haematological changes. **Aims**: This study aimed to highlight the influence of exposure to different applied magnetic fields on SOD, MDA and GSH levels in the liver, LDH and CPK activities in the muscle and GABA levels in the brain, as well as some haematological parameters. **Methods**: Adult male albino mice divided into 5 equal groups (n = 6), the control group and four exposure groups that exposed to MFs of 20, 40, 60 and 80 Gauss for 5 min/day for 5 days. **Results:** Exposure to MFs induced significant decreases in total GSH levels and SOD activity but induced a significant increase in MDA levels in the liver. By contrast, MF exposure significantly increased total LDH and total CPK activities in the muscle. The results revealed a significant increase in GABA levels in the brain, as well as decreases in haemoglobin (Hb), haematocrit (Hct), and RBCs counts, in addition to platelet (Plt) counts, after exposure to 20, 40, 60 and 80 Gauss MFs. After exposure to 40 Gauss MF, the mice showed pathological changes in RBCs, including a micronucleus and a serrated edge, with a mild incidence of echinocytes). In the group exposed to a 60 Gauss MF, examination of blood smears clearly showed changes in cell size, with the emergence of abnormal forms, including many areas with no RBCs (rouleaux formation). **Conclusion**: The changes in the biochemical parameters of SMF-exposed mice probably reflect hepatic damage and anaemia caused by kidney failure.

Keywords: Magnetic; Antioxidants; MDA; SOD; GABA and Echinocytes

Introduction

The planet is surrounded by magnetic fields (MFs) generated by the earth, solar storms, variations in the weather and everyday electrical events. Recently, scientists have discovered that external MFs can affect the body in both positive and negative ways, and such clinical observations have revealed new avenues of study **[1].** Exposure to SMFs induces metabolic and haematological changes that correlate with the length of exposure. Moreover, exposure to voltage reduces RBC function and metabolic activity; thus, it was proposed that increased toxicity in organs was an outcome of RBC failure **[2].**

Numerous environmental factors may interfere with this phenomenon, including long-term exposure to ELF-MF **[3]**. Many studies have revealed the effects of electromagnetic field (EMF) exposure on total antioxidant activity, including SOD, GPx, vitamin E and A concentrations, MDA and selenium concentrations in erythrocytes and the plasma **[4]**. The time of application of MFs influenced biomass and GSH concentrations in yeast **[5]. Huber et al. [6]** reported that there is a correlation between exposure to SMF and oxidative stress through a disturbed redox balance, which leads to physiological perturbances.

Abo-Neima et al., [7] indicated that after exposure rats to MF, there is a significant decrease in the hematological Constituents of blood such as Hb concentration, Hct percentage and RBCs as compared to control group. The viscosity of the blood was increased for animals of all groups as compared with control group. The differences in viscosity demonstrate the effects of RBCs aggregation and deformability respectively.

Exposure to SMF caused alteration in haematological and biological parameter due to proliferation of blood cells and enzymes release in blood related to duration of exposure. Lipid metabolism also altered because of membrane integrity. The decrease of body weight might be due to reduction in body fluid and protein content including hormonal changes and relatively loss in liver weight were also observed. In tissues, SMF exposure showed significant alteration in enzyme activities. The data showed that SMF effects on glucose and lipid metabolism and in addition, the conducted investigation on rat to examine the effect of SMF on loss in body weight. Therefore moderate intensity SMF seems to have anti-obese effect **[8].**Exposure to SMFs caused a significant increase in the plasma levels of nitric oxide (NO), malondialdehyde (MDA), and advanced oxidation protein products (AOPP), and a decrease in superoxide dismutase (SOD), glutathione (GSH), and glycation end products (AGEs) were observed in restraint stress model **[9].**

Materials and Methods

Experimental animals:

Thirty adult male albino Swiss mice (***Musmusculus)*** weighing 25-30 g were used in the present study. The animals were maintained under normal conditions and given food and water *ad libitum*. The mice were classified into 5 equal groups (n = 6) as follows:

I- Control group: The animals were left untreated as normal controls.
II- 20 Gauss group: The animals were exposed to 20 Gauss MFs.
III- 40 Gauss group: The animals were exposed to 40 Gauss MFs.
IV- 60 Gauss group: The animals were exposed to 60 Gauss MFs.
V- 80 Gauss group: The animals were exposed to 80 Gauss MFs.

All groups are exposed to MFs for 5 min/day for 5 consecutive days.

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

Experimental setup

The experimental setup illustrated in Fig. 1.A coil with a variable number of turns per unit length placed on a stand for coils and tubes and connected to a high-current power supply. The axial B-probe connected to the teslameter via the multicore cable, clamped with the stand rod from the probe apparatus, and aligned so that the Hall sensor (a) was located in the centre of the plastic body of the coil. **This is designed by Co-author: Mohammed Elywa.**

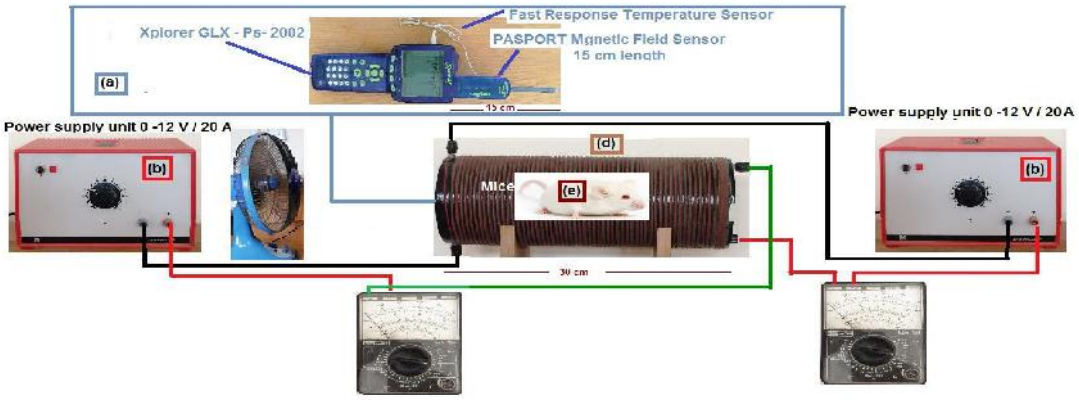

Figure 1: Schematic diagram of the SMF circuit. a) Xplorer GLX –PS-2002 with temperature and magnetic field probes used to measure the magnetic field intensity and the temperature simultaneously. b) a regulated d. c. voltage of low ripple. c) Multimeter to measure the d. c, current. d) double wrapped coil used to produce static magnetic field. e) a mice cage. And using a fan to cool the temperature rising. **This is designed by Co-author: Mohammed Elywa.**

Haematological analyses: Haematological parameters were determined by using the haemocytometer method for RBC counts, WBC counts and platelet counts; the Wintrobemicrohaematocrit 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 **[10].**

Lipid peroxidation estimation: Lipid peroxidation was estimated by measuring the level of thiobarbituric acid reactive substances in tissues by using the method described by Niehiu and Samuelsson in 1968**[11].**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. [12]** Test solutions consisted of 2 different mixtures, (A) and (B). One milliliter of (A) solution contained 2µ adenosine triphosphate (ATP), tromethamineaminomethane (pH 9.0), and half milliliter of serum. (B) Solution contained creatine in addition to all the components of (A) solution. (A) and (B) were incubated at 38 C, and the reaction was started by adding ATP. After 30 minutes 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: SOD was assayed using the method of **Lima et al. [13]** 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 was 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: GSH concentrations were determined by the method of **Niehius and Samuelsson [11].** Reduced glutathione (GSH) 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: GABA concentrations were determined by the method of **Serel et al. [14].** 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 were also analyzed in duplicate using a Labotronaminoacid. analyzer (Liguimat 2).

Preparation of tissue homogenates: Small pieces of the brain, liver, and muscle were 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 4 ºC. The supernatants containing cell suspensions were collected and stored at -20 ºC until further use in bioassays **[15].**

Statistical analysis: The results presented here are the mean ± SEM of 8 mice in each group. 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 $P<0.05$ **[16].**

Results

Table (1): Brain GABA levels , CPK and LDH activities in muscles**,** MDA, GSH levels and SOD activities in liver of the mice exposed to20, 40, 60 and 80 Gauss MFs for 5 min/day for 5 days.

Groups	GABA (µg/g of tissue)	CPK (µmol/min/mg of protein)	MDA (nmol/mg of protein)	SOD (U/mg of protein)	GSH (mmol/mg of protein)	LDH (µmol/min/mg of protein)
Control	178.83±1.96	88.77±1.68	0.14±0.023	3.51±0.044	0.56±0.017	33.87±0.81
20 Gauss	184.67 ± 1.85	81.33±2.59	0.18±0.018	3.50±0.061	0.55±0.030	33.66±2.96
P	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05
% diff.	3.26	-8.38	28.57	-0.28	-1.78	-0.62
40 Gauss	207.00± 4.72	240.00±7.75	0.41±0.034	1.85±0.031	0.19±0.021	59.66±5.48
P	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
% diff.	15.75	170.36	192.85	-47.29	-66.07	76.14
60 Gauss	238.67 ± 6.35	290.00±20.81	0.63±0.020	1.55±0.026	0.09±0.005	75.66±1.76
P	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
% diff.	33.46	226.68	350.00	-55.84	-83.92	123.38
80 Gauss	272.67 ± 6.39	326.30±18.44	0.81±0.040	1.24±0.029	0.04±0.006	87.33±3.84
P	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
% diff.	52.47	267.57	478.57	-64.67	-92.85	157.83

Mice exposed to a 20 Gauss MF showed a non-significant increase (P>0.05) in GABA and MDA levels in comparison to the control group and with a percentage difference of 3.26% and 28.57% respectively. While, the levels of CPK, SOD, GSH and LDH are decreased non-significantly (P<0.05)at the same group in comparison to the control group. Mice exposed to 40, 60 and 80 Gauss MFs showed a significant increase (P<0.05) in GABA, MDA, CPK, and LDH 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): Haematological parameters of the mice exposed to20, 40, 60 and 80 Gauss MFs for 5 min/day for 5 days.

	Control	20 Gauss			40 Gauss			60 Gauss			80 Gauss		
	Mean±SE	**Mean±SE**	**P value**	**% diff.**	**Mean±SE**	**P value**	**% diff.**	**Mean±SE**	**P value**	**% diff.**	**Mean±SE**	**P- value**	**% diff.**
Hb	15.10±0.33	10.60±0.19	<0.001	-29.82	7.55±0.08	<0.001	-50.01	12.00±0.22	*<0.05*	-20.55	12.40±0.30	*<0.05*	-17.93
RBCs	9.06±0.19	6.40±0.07	<0.001	-29.38	5.05±0.02	<0.001	-44.28	7.55±0.11	*<0.05*	-16.69	8.44±0.24	*>0.05*	-6.90
Hct	51.04±3.02	38.20±2.1	<0.001	-25.16	26.07±1.03	<0.001	-48.92	38.95±2.8	*<0.05*	-23.69	43.75±2.49	*<0.05*	-14.29
MCV	58.67±3.87	59.79±4.09	*>0.05*	1.91	52.58±3.25	*>0.05*	-10.38	51.18±2.12	*<0.05*	-12.77	54.01±3.02	*>0.05*	-7.94
Plt	675±39.6	388±22.4	<0.001	-42.52	235.50±18.4	<0.001	-65.11	302.00±20.1	<0.001	-55.26	664.58±4.6	*>0.05*	-1.54
WBCs	2.19±0.26	3.30±0.34	*<0.05*	50.86	2.05±1.08	*>0.05*	-6.29	1.40±0.03	<0.001	-36.00	3.23±0.27	*<0.05*	47.62

P values refer to significant differences compared to the control group.
Non-significant (N.S.): p>0.05. Significant: p<0.05.
Highly significant: p<0.01. More highly significant: p<0.001

Table (2): Significant differences were observed in most of the haematological blood parameters (Hb, RBCs, Hct, Plt, MCV and WBCs) between the control and the exposed groups of mice after exposure to20, 40, 60 and 80 Gauss MFs. MF exposure produced a more significant decrease *(p<0.001*) in Hb, RBCs, Hct and Plt after 20 and 40 Gauss MF exposure and a significant decrease (p <0.05) in Hb, RBCs and Hct after 60 Gauss MF exposure, but the decrease in Plt was more highly significant. In contrast, mice exposed to 80 Gauss MF showed significant decreases in both Hb and Hct and non-significant decreases in both RBCs and Plt in comparison to those of the control group. While the WBC count was significantly increased after exposure to 20 and 80 Gauss MFs, with percentage differences of 50.86% and 47.62%, respectively, this value decreased non-significantly after exposure to a 40 Gauss MF and was more significant after exposure to a 60 Gauss MF, with percentage differences of -6.29% and -36.00%, respectively, in comparison to the control group. On the other hand, MCV showed a non-significant increase after exposure to a 20 Gauss MF and a decrease after exposure to both 40 and 80 Gauss MFs, whereas a significant decrease was observed after exposure to a 60 Gauss MF, with percentage differences of -12.77%.

Histopathological results:

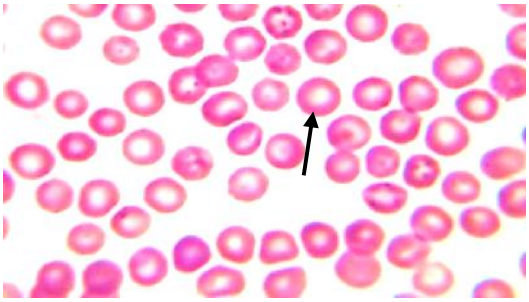

Figure 2

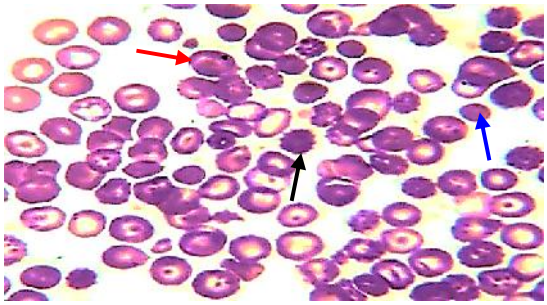

Figure 3

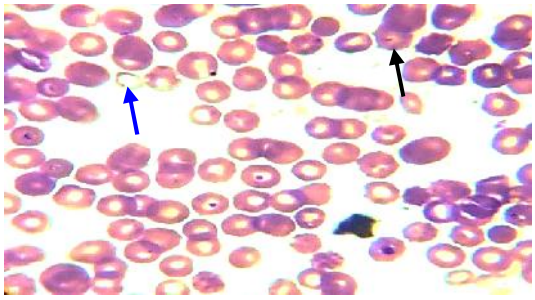

Figure 4

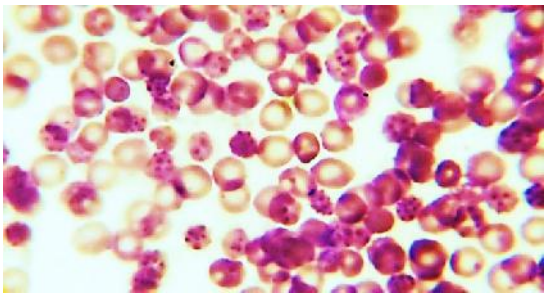

Figure 5

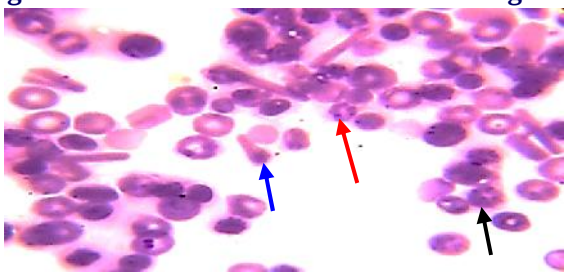

Figure 6

Figure. (2): Representative photo of a Giemsa-stained blood smear of control group showing normal disc-shaped biconcave erythrocytes (arrows) with a central pale area; Figure. (3): Representative photo of a Giemsa-stained blood smear of 20 Gauss group showing a moderate incidence of echinocytes (black arrow), marked hypochromasia (red arrow), target cells (yellow arrows) and microcytes (blue arrows); Figure. (4): Representative photo of a Giemsa-stained blood smear of 40 Gauss group showing a mild incidence of echinocytes (black arrow) and micronuclei (blue arrows); Figure. (5): Representative photo of a Giemsa-stained blood smear of 60 Gauss group showing a moderate incidence of echinocytes and the formation of a rouleaux that may lead to a clot; Figure. (6): Representative photo of a Giemsa-stained blood smear of 80 Gauss group showing a mild incidence of echinocytes (black arrow), bi-micronucleated erythrocytes (red arrow), and ovalocytes (blue arrows).

Discussion

Our studies showed a substantial increase in GABA levels in the brains of mice exposed to 40, 60 and 80 Gauss MFs for 5 min/day for 5 days. This observation is consistent with **Obata [17],** which reported that the exposure of adult rats to electromagnetic radiation (EMR) for 1 h daily for four months induced substantial increases in glutamate, aspartate, GABA and glycine levels but a strong decrease in glutamine levels. An increase in cerebral metabolism was observed immediately after electromagnetic irradiation over some exposed regions of the scalp, and this effect was interpreted as a consequence of altered activity induced by EMF exposure **[18].** This increase in cerebral metabolism may result in an increase in the rate of cerebral glucose utilization, which represents the main energy source in the brain **[19].** Glucose is metabolized into amino acid neurotransmitters in neurons and glia **[20].** Thus, the rapid increase in brain GABA levels after exposure of the mice to 40, 60 and 80 Gauss MFs in the present study may be due to an increase in brain metabolism induced by MFs.

Concerning the significant elevation of muscle CPK and LDH activities in mice exposed to 40, 60 and 80 Gauss MFs. **Razavi1 et al. [21]** reported that hypoxia provoked a rapid loss of cellular ATP, followed by tissue functional and structural alterations, as revealed by an increase in LDH release. Thus, the increase in LDH activity following exposure to SMFs could indicate an adaptation that promotes anaerobic production of ATP in SMF-exposed rats **[22].** Thus, the increase in LDH activity in the plasma following SMF application could be associated with severe muscle damage.

The present results showed that mice exposed to 40, 60 and 80 Gauss MFs showed a significant increase in hepatic MDA levels with percentage differences of 192.85%, 350.00% and 478.57%, respectively. The present data showed a progressive increase in MDA levels with an increasing intensity of exposure. This relationship was in accordance with **Armitage et al. [23],** who showed an elevation in MDA levels in the liver and excretory organs, indicating oxidative stress in response to SMFs (128 mT, one h/day for thirty consecutive days).

Regarding SOD and GSH, the results of this study revealed that mice exposed to 40, 60 and 80 Gauss MFs showed significant decreases in hepatic SOD and GSH activities. GPx is an antioxidant enzyme that uses glutathione to reduce lipid hydroperoxides and hydrogen peroxide to reduce oxidative damage **[24].** The SOD activity and GSH, which metabolizes O_2^- and accelerates its conversion to H_2O_2, which is subsequently reduced to water by the GSH cycle, might also have contributed to damage reduction. Likewise, the depletion of H_2O_2 has an inhibitory effect on OH− formation and decreases the lipid peroxidation values **[9]**. A decrease in GPx activity suggests the excess use of glutathione and reflects increasing levels of tissue MDA and oxidative damage. The decreased GPx activity and increased MDA levels increase in the tissues of the groups exposed to MFs in this study reveal an increase in lipid peroxidation. SOD is also an important antioxidant system enzyme that decomposes superoxide anion radicals to H_2O_2. In this way, the toxicity of superoxide is eliminated, and free radicals are not generated by superoxide **[25].** These alterations may affect many processes within the cell, as free radical formation induces changes in enzyme activity, gene expression and membrane structure **[26].**

The present results showed a significant reduction in the values of haemoglobin (Hb), RBCs, haematocrit (Hct) and platelets (Plt) in most mice exposed to 20, 40, 60 and 80 Gauss MFs in comparison to those of the control groups, while the WBC count was increased in the 20 and 80 Gauss groups but decreased in the 40 and 60 Gauss groups in comparison to that in the control group. This finding is consistent with research that demonstrated that there are substantial decreases in the amounts of several factors in the blood, including most Hb, the Hct, RBCs, MCV, MCH, and MCHC **[27].** Additionally, those authors observed a significant increase in the average WBC count, as well as the proportion of lymphocytes, and this increase was accompanied by cases of anaemia, such as macrocytic anaemia. This increase is also evidence of bleeding, which occurs as a protective mechanism after exposure to radiation and increased temperature **[28].** In the current study, mouse blood samples were collected after exposure to 20, 40, 60 and 80 Gauss SMFs separately for 5 min/day for 5 days and then stained with Giemsa stain. The RBCs of mice exposed to 20 Gauss MFs showed a moderate incidence of echinocytes with a central pale area, marked hypochromasia and microcytic cells. These results are in agreement with **Fairbrother [29],** who found that these different abnormal forms of RBCs were a pale colour with an increased rate of colour fading in the swabs from mice that were exposed to electromagnetic waves; this observation has been attributed to a reduced amount of haemoglobin inside RBCs.

Increased exposure to 40 Gauss MFs appears to have a clear influence on the blood swabs, with a mild incidence of echinocytes and micronuclei in the centre. Additionally, the sizes of RBCs differed from normal size, with clear areas between RBCs **(Fig. 4),** which suggests that the lysis of many cells leads to a substantial reduction in the number of RBCs, as shown in **"Table 2",** which reported a percentage difference of -44.28%. The present result showed that SMFs have a stronger effect on the blood, where a reduced intensity of colour inside the RBCs appears due to a reduced concentration of haemoglobin (hypochromic); this finding was also confirmed in **"Table 2",** where a decrease in the average concentration of haemoglobin inside RBCs was observed after exposure to 40 Gauss MFs, with a percentage difference of -50.01%. It was noted that the mice exposed to 60 Gauss exhibited varying RBC sizes (anisocytosis). Microscopic observation revealed that the aggregation of RBCs and rouleaux formation occurred in the plasma, and rouleaux were oriented in the direction in which their long axes were perpendicular to the magnetic field **[30].**

In this part of the study, many pathological changes in RBCs were observed in mice exposed to 80 Gauss MFs. RBCs with serrated walls were observed, with different numbers of humps, and other cells showed a structure surrounding the RBCs from the outside. These RBCs appear with a serrated edge and are called burr cells or called echinocyte erythrocytes (mild incidence of echinocytes), bi-micronucleated erythrocytes and ovalocyte cells were also observed. **Mckenzie et al. [31]**, confirming that these abnormal forms of RBCs were due to the presence of anaemia or anaemia caused by kidney failure. In the current study, changes and a clear imbalance in the forms and sizes of RBCs and their contents occurred after exposure to SMFs; this effect may be attributed to a malfunction and a clear change in the form of the membrane that surrounds the newly formed RBCs, which leads to an imbalance in the function of the membrane due to magnetic waves.

Conclusion: MF exposure caused different metabolic and haematological effects, which appeared to be related to the intensity of SMF exposure. The changes in the biochemical parameters of SMF-exposed mice probably reflected hepatic damage and anaemia caused by kidney failure. Further studies are needed to better understand the effects of MFs strength on biological systems.

Abbreviations: **MF**: Magnetic field, **SMF**: Static magnetic 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**: Platelets, **Hct**: Haematocrit.

Ethical approval and consent to participate: The animals were obtained from the Theodor Bilharz Research Institute in Cairo, Egypt, and all animal procedures were performed after approval from the Ethics Committee of the National Research Centre (ECNRC). I agree to provide related documentation.

Availability of data materials: The data are publicly available in the Supplementary File.

All data of this article are available on mendeley data website. Url: https://data.mendeley.com/drafts/psnw8ddgw8

Author contributions: HA carried out the physiological and histological studies, participated in the sequence alignment and drafted the manuscript. ME participated in the experiments presented in the article. **All authors read and approved the final version of the manuscript.**

Conflict of interest: none

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