



Effect of 50 Hz, 0.85 mT Magnetic Fields on Hematological Parameters

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Abstract For long time, magnetic fields' positive and negative effects on human bodies have been investigated. The exposure to magnetic fields has a frequency which grew with a rapid improvement in several sciences and technologies, including systems of passenger transports, imaging diagnosis of magnetic resonance and nuclear magnetic resonance spectroscopy which depend on the magnetic levitations. Hence, the effect of magnetic fields on bodies has become important to be clarified. Static magnetic fields (SMFs) are "time independent fields whose intensity could be spatially dependent." In this study, there is no change in the time of magnetic fields which means it is static. But, several values are shown in the space which means it is spatially dependent. For the interaction with biological systems, there are four relevant parameters of SMF which are: target tissues, magnet support devices, dosing regimens, and magnet characteristics. Protecting SMFs' is so difficult by which biological tissues can be easily penetrated. Nevertheless, both the field's intensity and the field's gradient have a vital role in the SMF biological effects. Magnetic materials and moving charges, which exist in the tissue via different physical mechanisms, can be directly interacted with by SMFs.

Keywords Magnetic field, Biological effect, Hematological parameters

Introduction

SMFs have several common artificial sources, varying from normal refrigerator magnets to particular microwave ovens, audio-speaker components and battery-operated motors [1,2]. The availability of biological "windows" is suggested by several studies of *in-vitro* biological reactions to applied MFs [3,4]. Such "windows" represent amplitude' combinations and exposure' frequency and duration in which the ideal reaction is shown, and the reaction will be considerably less when it is outside this range. Accordingly, this shows that it will be to some extent more better. 45-50mT, 15-20mT and 0.5-2mT, are some windows for SMFs which were suggested. Regarding SMFs, numerous studies were conducted on behavioral and physiological reactions, genetic materials, cells and cellular components, and reproductions and developments [1,5]. Furthermore, they never propose any acute detrimental influence on physiological and behavioral parameters or on major development for the exposures of short terms when they are taken as a whole [2].

Yet, in *in-vitro* tests with hippocampal slides, few detrimental influences were observed, in which a long-term amplification of the excitatory postsynaptic potentials preceded by their small transient depression was resulted by the electromagnetic field of 2-3mT, while excitatory postsynaptic potentials were depressed by the electromagnetic field of 8-10mT. Taken together, the effects of sub-chronic and chronic exposures to SMFs are needed to be evaluated by long-lasting tests. There are many *in-vitro* and *in-vivo* studies regarding the biological influences of the 15-20mT SMF [1,5,6]. Nevertheless, on hematological samples, only *in-vitro* experiments evaluated the biological influences of 15mT SMF [6,7]. Besides, studies of SMFs exhibited that hematological parameters in mice and rats are altered by both very low frequency magnetic fields and SMFs. In addition, SMFs in mice also affect spleen B,T and total lymphocyte count [2].



According to these findings and the fact that biological systems are influenced by several influences of the downward and upward directed magnetic fields [1,5], the influences of sub-chronic long-term exposures to 16mT downward and upward oriented SMF on spleen cellularity and hematological parameters in mice was decided to be investigated in this study. Yet, all over the body of mice, the magnetic field that was used decreased vertically and ranged from 29.7mT to 5.8mT. Therefore, various mice body' parts were exposed to another field intensity. Potentially, 16mT was the mean of the magnetic field all over the tested volume. In experimental animals, no study was previously conducted to compare different biological influences of the SMF oriented downwards and upwards on hematological parameters.

There are growing interests on the potential health influences related to the exposures to very low frequency (ELF) electromagnetic fields (EMFs). ELF EMFs have the majority of human exposures, and are usually found under 200-300Hz, presented in work place and residential environments. The results indicated that the 50Hz magnetic fields (0.2-0.5mT) of rabbit red blood cells (RBCs) were exposed at the same time to the actions of oxygen radical generating systems, functioning as oxidizing agents, increased the cellular damage provoked *in vitro* by the oxidizing agents. Moreover, the decrease in the concentration of Mg in blood plasma and Fe and Mn in cerebrospinal fluid and the increase in the concentration of Ca and P were the results of constantly exposing cows to electric and magnetic fields (60Hz, 10kV/m, and 30mT) during 30 days. Besides, For the enhancement of neoplastic transformations *in vitro*, the potential for frequency magnetic fields' power by using promotion sensitive mice epidermal JB6 cells was evaluated. The data found did not strengthen the view that "environ environmental exposure to magnetic fields contribute to transformation" [8].

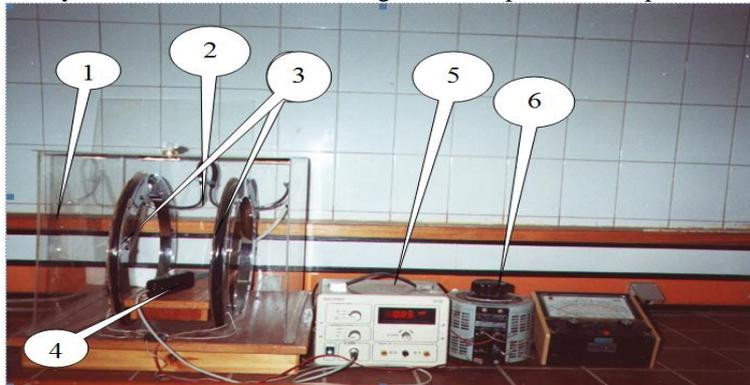
Thun-Battersby et al. [9] stated that "power line (50 or 60 Hz) MFs might reduce immune function, which could lower resistance to infection or cancer. However, EMFs could not induce detectable DNA fragmentation in either human peripheral blood leukocytes or polymorph nuclear cells". For more than 2hrs, cells were exposed to 50Hz pulsed magnetic fields of the type which would induce the maintenance of bone. These cells contain an important grouping on the intra-membrane protein distributions, in comparison with the control unexposed cells. The plasma membrane interferes and structures with the initiations of signal cascade pathways may be modified by the exposures to 50Hz, 2mT magnetic fields for 72hrs. According to the found data, an evidence exists when there is a biologically active exposure to 50Hz magnetic fields. A residential exposure to more than 0.2mT magnetic fields that exist within the permitted limit of constant exposures and are provided by IRPA may be caused by the houses which are formed adjacent to power lines and carry high currents [10]. A number of houses are formed extremely adjacent or beneath power lines in several countries.

Materials and Methods

1-Magnetic field exposure system

The source of magnetic field was Helmholtz two coils show in figure (1), the coils were distant by 15 cm, and each one was 30 cm in diameter and of 250 turns. The wire of the coil was 0.7 mm in diameter and its resistance was 13 ohm. The field was probed by a magnetic flux meter (ELWE 8533996, Cerligene, Germany).

In spite of the fact that the second method of direct measurements is a local method, i.e., it measures the local in space magnetic field; it was decided to use this method because of its low cost in money and efforts expenses. Actually, there are two classes of magnetic field probes: Hall probe and conductive probe.



- 1-Transparent plastic cover
- 2- Water circulating cooling system
- 3- Helmholtz coil
- 4- Magnetic probe
- 5- Flux meter
- 5- Flux meter
- 6- AC Variac

Figure 1: A view of the magnetic field generator system



Hall probe based on the Hall effect, has high sensitivity and wide frequency response but it needs a high power current stable source and will cause more perturbation of the medium owing to the introduction of driven current. Hence, the Hall probe is used for measuring steady state or very slowly varying magnetic field. When non steady magnetic field needs to be measured, it is preferred to use a conductive loop probe. This technique is useful for studying the field strength and its variations. Figure (2) is the schematic drawing of a typical probe system.

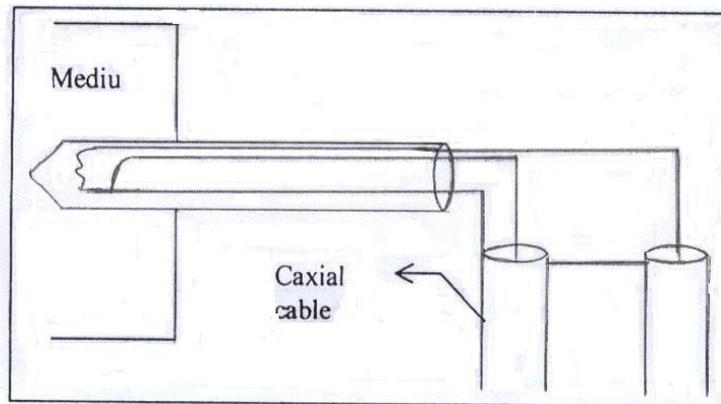


Figure 2: Schematic drawing of the magnetic probe

The sensor is a small coil made of several windings of small-wire diameter. The coil is fixed on a support insulator piece and then connected with a coaxial cable. When the coil is placed into a varying magnetic field, an inductive electromotive potential (ϵ) will be produced across the two ends of the coil. If the size of the coil is so small the magnetic field within the coil can be regarded as uniform so the output voltage and the value of the required measured magnetic field is directly shown in the pre-calibrated scales of the magnetic probe. The magnetic field was first well mapped to investigate the best area between the two coils at X, Y and Z direction at which the used field intensity is approximately constant. A flux meter EL WE 8533996 was used to map the magnetic field in the area between the Helmholtz coils. The area of constant magnetic field was chosen to be the exposure area in which the cage was located. The investigated rats, except the control ones, were all exposed to a low frequency 50 Hz magnetic field of intensity of about 0.85 mT (8.5 G). The control group was exposed to a sham (not energized) field.

2-Experimental Animals

The experiments were carried out on 56 male Sprague-Dawley rats, of about 150 gm mean weight. They were obtained from the breeding unit of National Research Center, Dokki, Giza. The rats were housed eight per cage in a well-ventilated room ($25 \pm 2^\circ\text{C}$), while the relative humidity was $(43 \pm 3)\%$ and 12 hours light and dark cycle at the animal house of the Zoology Department, Suez Canal University. They were kept at the Biophysics laboratory, where they have been exposed to the magnetic field, for at least one week before exposure. The rats were regularly fed on a standard diet ad libitum.

Rats were divided into seven main groups (8 rats each).

- Group 1: Rats were exposed to the magnetic field for 1 day (2 hours/day) and the blood samples were collected before and after exposure.

- Group 2: Rats were exposed to the magnetic field for 2 days (2 hours/day) and the blood samples were collected before and after exposure in both days.

- Group 3: Rats were exposed to the magnetic field for 3 days (2 hours/day) and the blood samples were collected before and after exposure at the first and last day.

- Group 4: Rats were exposed to the magnetic field for 4 days (2 hours/day) and the blood samples were collected before and after exposure at the first and last day.

Group 5: Rats were exposed to the magnetic field for 5 days (2 hours/day) and the blood samples were collected before and after exposure at the first and last day.

- Group 6: Rats were exposed to the magnetic field for 6 days (2 hours/day) and the blood samples were collected before and after exposure at the first and last day.

- Group 7: Rats were exposed to the magnetic field for 7 days (2 hours/day) and the blood samples were collected before and after exposure at the first and last day.



3. Methods

Blood samples were collected using orbital sinus technique (Sanford method) [12]. The whole blood was used to determine hematological indices. Hematological indices hemoglobin content (Hb), hematocrit values (Ht %), red blood cells count (RBCs) and red blood cell indices [Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin concentration (MCHC)]. This study was fulfilled by using complete blood counter (Selly, France).

The effect of magnetic field was determined by comparing the values of before and after in each group using Student's unpaired t-test used Senecor method [13]. The data are represents by the mean values \pm standard error from 8 rats/group and the differences are considered statistically significant at the level of $P < 0.05$.

Results & Discussion

Fifty six male Spargue-Dawely rats used in this study divided in to seven groups eight rats each. All rats were exposed for two hours daily to extremely low frequency (0.85 mT) magnetic field figure (1). Blood samples were collected using orbital sinus technique (Sanford method) before and after exposure for 2 hours for the first day, 4 hours/2 days, 6 hours/3 days, 8 hours/4 days, 10 hours/5 days, 12 hours/6 days and 14 hours/7 days respectively.

The results in table (1) and (2) demonstrate the hematological indices of rats before and after exposure to magnetic field. The total hemoglobin of rats before exposure to magnetic field was ranged between 11.5 ± 0.18 to 12.1 ± 0.14 gm/dl. After exposure the total hemoglobin was ranged between 11.9 ± 0.13 after exposure to two hours only and 8.2 ± 0.08 after 14 hours /seven days exposure. The reduction of hemoglobin levels after exposure was in reflection on the hematocrit values and red blood cell count.

The data for hematological indices showed a remarkable reduction especially after exposure for 8,10,12 and 14 hours respectively, but had no change in 1st, 2nd and 3rd exposure to magnetic field.

The values of hematocrit without exposure ranged between $35 \pm 0.65\%$ and $37 \pm 0.92\%$. The values have changed between 37 ± 0.42 and 27 ± 0.37 after exposure to two hours only and 14 hours / seven days respectively.

The mean \pm S.E. of red blood cell count for the different experimental groups ranged between 4.0 ± 0.6 and 3.8 ± 0.07 while after exposure of two hours was 4.0 ± 0.08 and 3.0 ± 0.03 for those after exposure for 14 hours.

The hematological indices of groups of rats after exposure to low frequency magnetic field (0.85 mT) showed that there is no remarkable changes in the values MCV, MCH and MCHC. While the hemoglobin levels were ranged between 11.9 ± 0.13 and 8.2 ± 0.08 which significant at the level at $p < 0.05$ when compared with those levels before exposure. These values have same reflection on the levels of hematocrit and erythrocytes counts which demonstrated a significant reduction in their values at the level at $p < 0.05$ when compared with those before exposure to magnetic field.

The results in table (1) and (2) through which a significant reduction was obtained in the hemoglobin, hematocrit, erythrocytes and neutrophils came in agreement with those obtained by Bonhomme et al., 1998 [14]. As far as platelets count are concerned Gorczynska and Wegrzynowicz [15], reported that Exposure of guinea pigs to homogeneous magnetic fields as low as 0.005 T for 1 hour, 7 days a week, for 6 weeks led to a decreased platelet count, increased platelet aggregation, increased prothrombin and partial thromboplastin times, decreased fibrinogen and increased fibrinolysis. Those effects were reversible within 2 months of discontinuation of exposure to the magnetic field [15], which agreed with those obtained from our studies.

Table 1: Effect of Low Frequency Magnetic Field (0.85 mT) on Hematological Indices.

Time of exposure (hours)	Hemoglobin content (gm /dl)		Hematocrit value (%)		Erythrocytes count ($10^6/\text{mm}^3$)	
	before	After	before	After	before	After
2	12.1 ± 0.14	$11.9 \pm 0.13(-1.7)$	37 ± 0.15	$37 \pm 0.42 (0)$	4.0 ± 0.05	$4.0 \pm 0.05 (0)$
4	12.0 ± 0.14	$11.0 \pm 0.12*(-8.3)$	37 ± 0.35	$35 \pm 0.51*(-5.4)$	4.0 ± 0.05	$3.8 \pm 0.05 (-5)$
6	11.9 ± 0.15	$9.9 \pm 0.14*(-16.8)$	37 ± 0.15	$32 \pm 0.19*(-13.5)$	4.6 ± 0.05	$3.5 \pm 0.02* (-12.5)$
8	11.8 ± 0.23	$9.4 \pm 0.09*(-20.3)$	36 ± 0.35	$31 \pm 0.26*(-13.9)$	3.9 ± 0.05	$3.4 \pm 0.03* (-12.8)$
10	11.7 ± 0.13	$9.1 \pm 0.128*(-22.2)$	36 ± 0.59	$30 \pm 0.37*(-16.7)$	3.9 ± 0.05	$3.3 \pm 0.04 * (-15.4)$
12	11.5 ± 0.21	$8.8 \pm 0.08*(-23.5)$	36 ± 0.80	$29 \pm 0.30*(-19.4)$	3.9 ± 0.08	$3.2 \pm 0.03* (-17.9)$
14	11.2 ± 0.18	$8.2 \pm 0.08*(-26.8)$	35 ± 0.65	$27 \pm 0.37*(-22.9)$	3.8 ± 0.07	$3.0 \pm 0.03*(-21.1)$

The data represented as mean values \pm standard error for 8 rats.

*Significant difference between before and after exposure of groups to the magnetic field at the level of $p < 0.05$.

Percentages of changes level before and after are in parenthesis.



The applied static magnetic fields decline in a vertical way and were not uniform. Besides their vertical decline, the variations of fields in horizontal planes are intense and have local minima and maxima. For the test classifications, the averaged magnetic fields of 16mT in a spatial way function as the closest approximations by the windows criteria of the magnetic fields. In the mouse exposed to 16mT SMFs of all orientations, several investigations exhibited a raise in the cellularity of total spleen which additionally was less in the upward oriented SMFs than in the downward oriented SMFs or in the case of unexposed animals. An obvious raise in the down group and a statistically observed raise in SMFs exposed mice are shown by the spleen lymphocyte count. Furthermore, SMFs influence biological systems causing pro-inflammatory alterations, and a raise in producing species of responsive oxygen are shown in earlier studies [2,16]. In spleen granulocyte count, a statistically important decline was found with a trend of blood granulocyte count to be raised in mice and rats exposed to SMFs. Such results can be compared to others in different reports demonstrating granulocyte count raise in mice blood exposed to SMF [2]. The death of granulocytes and phagocytosis, which are related to producing free radicals, are raised in exposed animals' splenic tissues to all orientations of SMFs. This could be the elaboration beyond the pronounced alterations in this research. Between exposed and unexposed groups, there was not any change in red blood cells count or in spleen. Accordingly, there was also no change in red blood cell count shown by the exposed rats to very low frequency magnetic fields for 50 and 100 days, and also by the SMFs exposed mice for 30 days [2,17]. It was concluded that in spleen and blood of tested animals, red blood cells count are not influenced by different SMFs intensities and various exposures' times, which may be because of the rapid recovery of the red blood cells once they were exposed to the SMFs. Iron is "an absolute requirement for most forms of life because of its unusual flexibility to serve both as an electron donor and an acceptor". It could be properly toxic because the conversions of hydrogen peroxides to free radicals in the cell can be catalyzed by free iron. For preventing this, iron is bind to proteins by any life form which uses that iron allowing cells to get benefits out of the iron though restricting its capability to harm. In hemoglobin molecules of RBCs, the iron's majority is placed, and the remaining is stored in ferritin forms in bone marrow, liver and spleen. Accordingly, the major physiologic sources of iron's reservation in bodies are these stores. Moreover, iron is also stored by macrophages as a processing role to destroy and process hemoglobin. When red blood cells are damaged, iron is recycled by macrophages through placing it on transferrin molecules in which iron is carried in the blood. Furthermore, iron is required by macrophages' anti-bacterial activities. In mice, brain, heart, and liver are the major storage organs of iron [18].

In the up and down groups liver, in comparison with control animals, indicated an obvious decline of iron content, as shown in the current study. Additionally, in brain and spleen, the iron amount is also determined in the current study. Comparing to the control, there were differences in iron's dynamics in animals' spleens and brains exposed to the downward or upward directed fields whereas the iron's dynamics in animals' livers in the exposed groups was the same. Specifically, there was no change in the iron's amount in the animals' brains exposed to the upward magnetic field; there was a decrease in livers and a raise in spleens. On contrary, there was a decline in the iron's amount in animals' brains and livers exposed to the downward magnetic fields whereas there was still unaltered in their spleens. In addition, iron's relocations from storages in livers to spleens for potential lymphopoiesismay be suggested by the findings found in the animals exposed to the upward fields. Nevertheless, the downward magnetic fields influences on iron are more difficult to be elaborated. The removal of iron is not desired and it is firmly structured in brains. Although energy inadequacy can be resulted by too little iron, oxidative stress can be caused by too much iron. In animals exposed to the downward magnetic fields, it was not clear where iron of brains and livers transfers. In this process, macro-phages are considered to be potential destinations. For this hypothesis's examination, serum iron and transferrin were measured in all animals.

"Transferrin's are iron-binding blood plasma proteins that control the level of free iron in biological fluids" [19]. For iron, the transferrin's affinity is too high; however, it gradually declines with pH decreasing under neutrality. Livers are main sources for producing transferrin which, yet, is produced by other organs like brains. The transferrin vital function is in iron's transference from the duodenum's centres of absorption or from recycling centres of RBCs in reticuloendothelial macrophages to tissues. Moreover, transferrin is significant for the division of cells and erythropoiesis [20], it also, in the innate immunity, has a vital function. Furthermore, the survival of bacteria is impeded by the withholding of iron which is the responsible of transferrin. In inflammation, the transferrin's levels are therefore decreased [21]. Yet, throughout the mice acute phase responses, the transferrin's levels are increased in inflammation [22,23].

The main results were that a reduction in the levels of Hb, MPV, and eosinophil is induced by the exposures of ELF EMF. There was a statistically significant that in EMF exposed rats for 50 days, hemoglobin limitations obviously reduced. Lino [24], stated that "Hb is sensitive to EMF". Moreover, the applied MFs are oriented with erythrocytes as shown in other investigations [25]. There was a hypothesis that these findings are possibly



related to the alterations in the Hb conformations below the actions of MFs [26]. It was reported that levels of neutrophil, Hb and hematocrit are significantly reduced in comparison with the control groups following the exposures of ELF EMF (60Hz, 0.11mT, 6 months) [27]. In rats, hyperfunction of spleens is elaborated to be the cause for the pronounced hematological variability in responses to the exposures of ELF EMF [28]. Leukocytes, platelets, and the RBCs destruction rates are increased by the hyperfunction of spleens [29].

On the other hand, it was proposed that after the exposure of subacute to SMFs, levels of Hb are increased [30]. In the group exposed for 50 days, the mean thrombocytes (MPV) volume is reduced by continuous exposures to ELF-EMF as explained by a number of investigations. In the peripheral blood, states of microthrombocyte can be caused by decreased MPV which, in thrombocytopenia of bone marrow insufficiency, is decreased. the bone marrow insufficiency idea is supported by the noticed trend of a reduction in leukocyte index and count. Though, in rats exposed to EMFs for 100 days, there is no reduction in MPV. Additionally, in white blood cells of the exposed rats, a trend exists for a reduction in MPV; however, this was not statistically significant. Contrarily, only in the 4th week, a raise in leukocyte count is induced by the exposure of rats to a magnetic field for 1, 2 and 4 weeks [31].

Table 2: Effect of Low Frequency Magnetic Field (0.85 mT) on Red blood cells Indices

Time of exposure (hours)	MCV (μ^3)		MCH (p gm)		MCHC (%)	
	before	After	before	After	before	After
2	92.5±0.34	92.5±0.31(0)	30.25±0.03	29.75±0.03(-1.7)	32.7±0.45	32.16±0.49(-1.7)
4	92.5±0.70	92.11±0.61(-0.4)	30.0±0.06	28.95±0.05(-3.5)	32.43±0.44	31.43±0.43(-3.1)
6	92.5±0.24	91.92±0.45(-0.6)	29.75±0.07	28.29±0.08(-4.9)	32.16±0.57	30.94±0.53(-3.8)
8	92.31±0.56	91.18±0.54(-1.2)	30.26±0.27	27.65±0.17*(-8.6)	32.78±0.52	30.32±0.49*(-7.5)
10	92.31±0.58	90.51±0.64(-1.9)	30.0±0.39	27.58±0.40*(-8.1)	32.50±0.62	30.35±0.41*(-6.6)
12	92.31±0.50	90.63±0.49(-1.8)	29.47±0.08	27.57±0.08*(-6.4)	31.94±0.53	30.34±0.57*(-5)
14	92.11±0.50	90.00±0.75(-2.3)	29.47±0.06	27.35±0.11*(-7.2)	32.00±0.55	30.57±0.76*(-3.9)

The data represented as mean values \pm standard error for 8 rats.

*Significant difference between before and after exposure of groups to the magnetic field at the level of $p < 0.05$. Percentages of changes level before and after are in parenthesis.

It was shown that there were no influences on the counts of white blood cells and hematocrit of mice exposed to a 1.89T DC magnetic field [29]. In the form of microcytes, the existence of hypochromic RBCs are shown by the MCV reduction at 100 days in the current study. Thus, it does not agree with another report indication that a raise in the level of MCV is induced by the exposure of SMFs to 128mT for 1 hour daily throughout five days [32]. Moreover, no change is observed in MCV levels in another study conducted by Mukewar and Baile [33]. It was suggested that the welders immunological and hematological parameters are not affected by ELF electromagnetic fields do not affect [34]. As a whole, some aforementioned reports and investigations showed that, in hematological parameters, there are a modification is caused by EMF, while in other studies, no modification is caused. Beyond such contradiction in the findings, there are several reasons which are possibly because of various setups of exposures, times of recoveries, goals of investigations and ways of assays and finally experimental conditions including alternative or static MFs, durations of MF times, various intensities and the frequency [35].

In the findings attained (MCV, eosinophil, and Hb), the reasons for the progressions of non-linear times can be related to the physiological defenses or to the adaptations of hemopoetic systems. The results of the current study indicated that body weights are not influenced by ELF-EMFs. Similar conclusions and findings were shown in previous studies. After 3 months, no change was observed in the body weight of control and experimental groups of rats exposed to EMFs at an intensity of 6.25T for 8hrs daily [36]. Likewise, after 32 weeks of exposing rats to EMF (50Hz, 5T), no change was found in their body weights as indicated by Margonato et al. [37]. Also, another investigation showed that after 10 weeks of exposing rats to EMF at 9.4T for 6hrs daily indicated no difference in their body weights [38]. Moreover, another study indicated that there was no change in body weights of exposed and control mice during the study, and also no change in the hematological parameters. It also showed that although there were essential differences, no biological effects were indicated and their magnitudes were too small. Any changes in hematological parameters such as body weights are caused by anatomic changes in livers which are hematopoietic organs [39].

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