

24h Extremely Low Frequency Electromagnetic Field Exposure Modulates Immune Responses in Rats

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Abstract: - We examined the immune response induced by 24-hour continuous exposure to an ultra-low-frequency electromagnetic field (EMF) in white rats. We placed the experimental group wild-type albino rats in a special coil generating an electromagnetic field of 2 mT at a frequency of 50 Hz for 24 hours. The results of the study demonstrate that after 24 hours of continuous exposure to electromagnetic field, a significant increase in plasma potassium concentrations collected from the rats' tail veins was recorded (from 5.49 ± 0.46 to 13.07 ± 3.05 mmol/L). At the same time, the percentage of basophils in the blood increased significantly from 0.50 ± 0.09 to $1.03 \pm 0.10\%$, while the platelet concentration decreased from $617.8 \pm 15.2 \times 10^9/L$ to $323.5 \pm 114.5 \times 10^9/L$. The high standard deviation of immune system biomarkers allows us to conclude that the body responds through the involvement of individual mechanisms.

Key-Words: - Electromagnetic Field, Immune system, Catalase, Thrombocyte, IL-1, IL-10, Potassium.

Received: March 29, 2026. Revised: April 23, 2026. Accepted: May 29, 2026. Published: October 6, 2026.

1 Introduction

Modern humans live in an environment of electromagnetic fields. EMFs of varying frequencies and strengths are found in the home environment [1]. Mobile phones, television antennas, microwave ovens, medical equipment, and electric vehicles generate certain electronic signals and emit ionizing or non-ionizing radiation [2].

Such radiation affects the human body in different ways and causes reversible or irreversible biological changes [3]. Numerous studies are being conducted on the biological effects of radiation, so its proper use and benefits are becoming increasingly widespread, although incorrect knowledge or excessive exposure to radiation increases the risk of developing diseases [4].

Radiation has multiple characteristics, and its effects are also varied. For example, the same frequency of non-ionizing radiation can be characterized by different wavelengths, energies,

intensities, etc. [5]. The presence of these multiple variables means the presence of multiple biological effects, so although the basic mechanisms of radiation action are already well understood, certain combinations of variable parameters cause different changes in living organisms and alter their parameters in different ways [6], [7], [8].

It is known that radiation, including non-ionizing radiation, even in low doses, affects living organisms and can disrupt homeostasis [9].

Ionizing radiation, such as X-rays, gamma rays, α , β , and others, damages biological structures.

Free radicals, formed as a result of radiation-induced damage to proteins, lipids, and nucleic acids, in turn, have a destructive effect on biological systems. The toxic effects of non-ionizing radiation (UV, RF, MF, EMG, etc.) occur largely through changes in membrane potential and, unlike reactive oxygen species (ROS), cause less aggressive changes (e.g., thermal effects, etc.) [10], [11], [12].

In our study, we aimed to examine changes in the immune response caused by 24-hour continuous exposure to non-ionizing radiation, specifically ultra-low frequency electromagnetic fields (ULF-EMF).

In general, stress, if the body is able to cope with it, can be considered positive, as the body becomes more resilient and tolerant to it [13]. In the case of radiation and in toxicology in general, this type of stress and the resulting response are known as hormesis. A hormetic effect refers to a response to stress, such as radiation, by strengthening the body's antioxidant systems. In other words, hormesis refers to the "strengthening" of the body by stress [14], [15].

However, this concept is controversial among scientists [16], since when the balance between the oxidative and antioxidant systems is disturbed, there is a high risk that the immune system will no longer be able to cope with stress [17], which means that various pathological processes may develop in the body [18]. In addition, since the exact boundary between hormesis and the onset of a pathological reaction is unknown, it is difficult to say whether hormesis is one of the reactions of the immune system that lasts a relatively long time, or a transitional stage, or one of the types of immune responses followed by a standard immune response [19]. Thus, the differences in the body's reactions indicate that the immune system responds to stress with various specific and nonspecific reactions, including the action of ELF-EMF [20]. The answer depends on several factors, namely the state of the body (weight, age, stability of the immune system, state of the antioxidant system, concomitant diseases, etc.) [21], as well as the physical characteristics of the ELF-EMF, such as duration of action, energy, wavelength, etc. [22], [23].

In our pilot study, we examined several immune system biomarkers in small groups of rats. Specifically, the primary objective of this study was to determine the relationship between the levels of catalase, monocytes, and interleukins, as well as platelet and potassium levels, in response to low-frequency electromagnetic fields (LFEMF). Since exposure duration is a variable that significantly influences the clinical picture, we chose to examine LFEMF-induced changes over a 24-hour period of continuous exposure. For other characteristics, we used a 50 Hz electromagnetic wave frequency for LFEMF.

2 Materials and Methods

2.1 Experiment Design

Adult male wild-type albino (length – 12 cm, 6 months old) were selected for the study and divided into experimental (n=8) and control (n=8) groups.

Experimental group rats were exposed to a continuous EMF for 24 hours. Immediately after EMF exposure, blood was collected from the tail vein of both the experimental and control group rats for analysis (Figure 1).

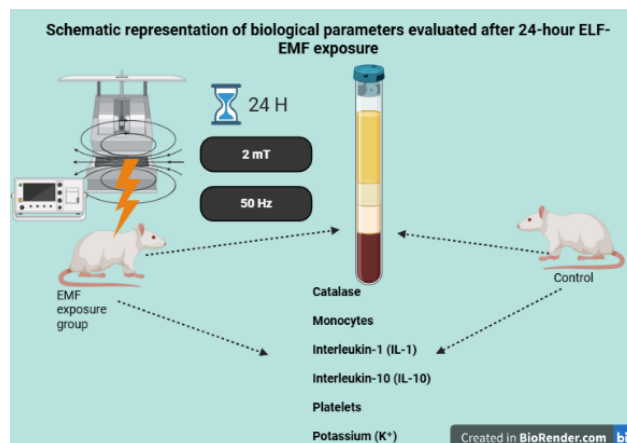


Fig. 1: 24-hour effect of 2 mT, frequency: 50 Hz EMF on the oxidative system (Catalase, IL-1, IL-10) in a rat model - experiment design.

Source: created by the authors

2.2 EMF Generation

The EMF generation was performed using a Helmholtz coil available in our laboratory. We placed the cage containing the animal within the coil. The animal was free to move around within the cage, and food and water were provided freely and voluntarily.

The following parameters were taken into account during the EMF generation:

The exposure lasted for 24 hours continuously, namely, the rats in the experimental group were placed in the coil for 24 hours (from 10:00 AM to 10:00 AM the next day). No additional lighting was used in the experimental laboratory room (only natural light).

The EMF intensity was monitored using a calibrated magnetometer at various points in the exposure chamber, where the parameters of the generated EMF were as follows: magnetic field amplitude: 2 mT, frequency: 50 Hz.

Taking these parameters into account, the EMF was uniformly distributed throughout the rat's body, and, in particular, the biological penetration depth was calculated using the standard formula [22]:

$$\delta = \sqrt{\frac{2}{\omega \mu \sigma}} \quad (1)$$

where $\omega=2\pi f$; $f=50$ Hz; $\mu \approx \mu_0=4\pi \times 10^{-7}$ H/m ;
 $\delta \gg 0.12$ S/m

2.3 Blood Analysis

Blood was collected from the tail vein of control rats. Blood was collected from the tail vein of experimental rats immediately after the end of exposure, at one-hour intervals.

The following parameters were determined in the collected blood and plasma to assess immune responses and cellular stress:

Potassium concentration (in plasma) [23]: This is an important indicator of electrolyte homeostasis, the concentration of which also changes as a result of stress, platelet activity, or immune responses.

Monocyte and platelet concentration: One of the important factors determining the immune and hematological status is the concentration of monocytes and platelets in the peripheral blood [24], as they actively participate in cellular stress reactions.

Catalase concentration in blood plasma: Catalase prevents the formation of free radicals and, accordingly, by assessing its concentration, it is possible to assess the activity of the body's antioxidant system [25], i.e., catalase is an indicator of the activity of the antioxidant system.

Specifically, it decomposes hydrogen peroxide (H_2O_2) into water and free oxygen (O_2), thereby reducing the risk of free radical formation. It is an indicator of oxidative stress.

Interleukin 1 and 10 (in plasma) [26], [27]: Changes in concentration and ratio reflect immune and inflammatory responses caused by physical stress.

2.4 Statistical Analysis

The data were statistically processed by us using the GraphPrism program (standard statistical program). We determined the mean $\pm$ standard deviation (SD). The data of the control and electromagnetic field exposure groups were compared using an independent samples t-test, and one-way ANOVA. A p-value < 0.05 was considered statistically significant.

The study was conducted in accordance with international GLP standards. The protocol of all experiments on rats was approved by the Laboratory Animal Care and Use Committee. The principles of ethical treatment of animals were observed in

accordance with the Georgian legislation and international treaties.

3 Results

Statistically significant changes in several parameters were observed in blood collected from the tail vein of rats after 24-hour exposure to 50 Hz, 2 mT EMF.

3.1 Changes in Platelet Count

Analysis of peripheral blood collected from the tail vein of control rats revealed stable platelet counts (low standard deviation) with a mean value of $617.8 \pm 15.2 \times 10^9/L$. After 24 hours of continuous exposure to an electromagnetic field with a frequency of 50 Hz and an intensity of 2 mT, rats in the experimental group showed high platelet count variability: $323.5 \pm 114.5 \times 10^9/L$. The difference between the two groups was statistically significant ($p = 0.013$), indicating a significant decrease in platelet count after 24 hours of continuous exposure to an EMF with a frequency of 50 Hz and an intensity of 2 mT (Table 1).

Table 1. Changes in platelet count induced by 24 hours of continuous exposure to an electromagnetic field with a frequency of 50 Hz and an intensity of 2 mT

Parameter - Platelets	Value
Unit	$\times 10^9 /L$
Control (Mean $\pm$ SD)	617.8 ± 15.2
EMF (Mean $\pm$ SD)	323.5 ± 114.5
P-value (t-test)	<0.01
ANOVA F-value (df)	25.94 (1,6)
ANOVA p-value	<0.001

Source: created by the authors

3.2 Changes in Basophil Percentage

Analysis of blood collected from the tail vein of rats in the study groups revealed (Table 2) that 24-hour continuous exposure to an ultra-low frequency (ULF) electromagnetic field of 50 Hz and 2 mT intensity altered the percentage of basophils. Specifically, while the percentage of basophils in the control group was $0.50 \pm 0.09\%$, after 24-hour continuous exposure to an EM field of 50 Hz and 2 mT intensity, the percentage increased to $1.03 \pm 0.10\%$ ($p < 0.001$).

Table 2. Quantitative changes in the percentage of basophils in blood collected from the tail vein of rats in the control and experimental groups (24-hour exposure to an EM field of 50 Hz and 2 mT intensity) are shown

Parameter Basophil	Value
Unit	%
Control (Mean $\pm$ SD)	0.50 $\pm$ 0.09
EMF (Mean $\pm$ SD)	1.03 $\pm$ 0.10
P-value (t-test)	<0.001
ANOVA F-value (df)	71.20 (1,7)
t	8.48

Source: created by the authors

3.3 Changes in Potassium Concentration

The results of the study show (Table 3) that after 24 hours of continuous exposure to an electromagnetic field with a frequency of 50 Hz and an intensity of 2 mT, the potassium level changed by a statistically significant amount. Specifically, the calcium concentration in the blood taken from the tail vein of rats in the control group was 5.49 ± 0.46 mmol/L. However, after 24 hours of exposure to non-peak EMF (frequency 0 Hz and intensity 2 mT) in the experimental group of rats, this indicator doubled to 13.07 ± 3.05 mmol/L ($p < 0.01$), i.e., after 24 hours of continuous exposure to an electromagnetic field with a frequency of 50 Hz and an intensity of 2 mT, pronounced hyperkalemia is observed.

Table 3. Quantitative changes in potassium concentration in blood taken from the tail vein of rats caused by 24-hour exposure to EMF with a frequency of 50 Hz and an intensity of 2 mW

Parameter Potassium	Value
Unit	mmol/L
Control (Mean $\pm$ SD)	5.49 $\pm$ 0.46
EMF (Mean $\pm$ SD)	13.07 $\pm$ 3.05
t-value	4.92
df	6
P-value	<0.01

Source: created by the authors

3.4 Monocytes, catalase, Interleukins (1, 10)

In our study, 24-hour exposure of rats to 50 Hz, 2 mT EMFs did not have a statistically significant effect on the percentage of monocytes in peripheral blood, or the concentrations of catalase, IL-1, and IL-10. Considering that the number of rats in the experimental and control groups was 8, we may have obtained different rates and changes in some parameters in the larger experimental group (Table 4).

A high standard deviation was recorded in the parameter of changes in the percentage of monocytes in the blood sample taken from the tail vein after 24 hours of continuous exposure to a 50 Hz, 2 mT EMF, therefore, this data cannot be considered statistically reliable.

A similar change (statistically unreliable, due to high SD) was also obtained in the case of catalase concentration, i.e., despite the fact that the concentration of catalase increased during 24 hours of continuous exposure to a 50 Hz frequency and 2 mT intensity EMF, high variability in the SD indicator does not allow us to consider the change statistically significant ($p=0.23$).

The interleukin levels (IL-1 and L-10) is also characterized by high variability (high standard deviation), as a result of which no changes in these indices were confirmed in the EMF group after 24 hours of exposure to an EMF with a frequency of 50 Hz and an intensity of 2 mT.

Table 4. Quantitative indices (percentage) of monocytes and changes in catalase concentration after 24 hours of exposure to an EMF with a frequency of 50 Hz and an intensity of 2 mT

Parameter	Unit	Control (Mean $\pm$ SD)	EMF (Mean $\pm$ SD)	P-value
Monocytes	%	1.50 $\pm$ 0.29	2.88 $\pm$ 2.48	0.26
Catalase	IU/ml	460.7 $\pm$ 6.34	600.2 $\pm$ 222.9	0.23
Interleukin 1 (IL-1)	pg/ml	3.25 $\pm$ 0.87	3.80 $\pm$ 2.57	0.72
Interleukin 10 (IL-10)	pg/ml	4.05 $\pm$ 0.96	6.43 $\pm$ 4.24	0.29

Source: created by the authors

4 Discussion

In our study, we examined the immune response of rats to 24-hour continuous exposure to an EMF with a frequency of 50 Hz and an intensity of 2 mT.

The results of the study demonstrate that extremely low-frequency EMFs (50 Hz) selectively affect the body and its biological and biochemical processes, as evidenced by the changes in several blood parameters observed in our study.

The results of the study indicate that during 24-hour continuous exposure to an electromagnetic field with a frequency of 50 Hz and an intensity of 2 mT, some parameters change consistently, while others show high variability (high standard deviation). For example, in the control group of rats, platelet counts are relatively stable and characterized by less variability and a low standard

deviation, while in the experimental rats in the EMF-exposed group, platelet count variability is quite high, which may indicate individual sensitivity to electromagnetic fields. Despite this, a statistically significant decrease in platelet concentration was observed after 24-hour continuous exposure to an electromagnetic field with a frequency of 50 Hz and an intensity of 2 mT.

The thrombocytopenia in this case may be caused by both direct and indirect effects of EMF, namely, EMF can shorten the lifespan of platelets and simultaneously suppress the formation of new platelets [28], [29]. This can be explained by the fact that platelets are formed from hematopoietic stem cells (HSCs) in 4–7 days [30], and their lifespan in the blood averages approximately 7–10 days [31]. In our study, EMF exposure lasted only 24 hours, so platelet production was likely not significantly affected. Theoretically, if platelet production were completely suppressed by 24 hours of EMF exposure, the reduction in platelet count in the blood would be a maximum of 15–25%. According to our data, the platelet count in the EMF-exposed group decreased by approximately 48% (we calculated this parameter as follows: $\text{change in thrombocytopenia} = (\text{Control} - \text{EMF}) / \text{Control} \times 100$), which excludes the existence of only one mechanism caused by EMF exposure—a decrease or cessation of the functional activity of megakaryocytes. Accordingly, along with a delay in platelet formation, accelerated platelet destruction or other mechanisms that could be considered the cause of thrombocytopenia may occur [32].

Along with thrombocytopenia, one of the most pronounced findings of the study is a sharp increase in the concentration of potassium in the peripheral plasma of rats in the EMF group, i.e., pronounced hyperkalemia. A significant ($p < 0.01$) increase in potassium levels in the EMF group compared to the control group indicates an electrolyte imbalance [33]. This may be due to EMF-induced changes in cell membrane permeability, leading to a significant shift of potassium from the intracellular space into the plasma [34], [35].

The increase in plasma potassium concentration in rats of the EMF group ($5.49 \pm 0.46 \rightarrow 13.07 \pm 3.05$ mmol/L) can also be explained by platelet lysis. It is known that the potassium concentration in platelets is significantly higher than in plasma (intracellular concentration of $K^+ \sim 100\text{--}150$ mmol/L versus plasma concentration of $K^+ \sim 4\text{--}5$ mmol/L) [36], therefore, during platelet lysis, a large amount of potassium is transferred into the plasma.

After exposure to EMF, a statistically significant increase in the percentage of basophils was observed. The percentage of basophils almost doubled under EMF exposure, which indicates a stress or proinflammatory response [37] and may indicate activation of the immune system caused by exposure to an electromagnetic field [38]. In our case, the obtained data support the hypothesis that EMF can influence the regulation of immune homeostasis, particularly through components of innate immunity [39].

No statistically significant changes were found in the percentage of monocytes, catalase activity, or interleukin (IL-1 and IL-10) concentrations. Although a certain upward trend was observed in the group exposed to EMF, the high variability significantly limits the interpretation of the results.

A selective increase in catalase activity may indicate the involvement of individual mechanisms of the oxidative stress response, although the obtained data do not allow us to consider this change reliable. Similarly, the high standard deviation of cytokines indicates differences in individual responses, and a longer exposure period or a larger number of animals may be required to increase statistical power.

It is important to note that this is a pilot study, and one limitation in the interpretation of the results is the relatively small number of animals in each group ($n = 8$), which reduces the sensitivity of the statistical analysis to some parameters. In future studies, we plan to examine larger cohorts and identify additional molecular biomarkers to more accurately assess the mechanisms of action of EMF.

5 Conclusion

Our results show that 24-hour exposure to an electromagnetic field with a frequency of 50 Hz and an intensity of 2 mT has significant effects on the blood of rats. In particular, a sharp (~48%) decrease in platelet count was observed in the EMF-exposed group, which exceeds the theoretically expected level. The decrease in platelet count may be due not only to a delay in their formation but also to accelerated lysis or other cellular mechanisms. 24-hour continuous exposure to an EMF with a frequency of 50 Hz and an intensity of 2 mT caused a significant increase in plasma potassium concentration (from 5.49 ± 0.46 to 13.07 ± 3.05 mmol/L). These changes can be explained by platelet analysis results [40], [41]. Specifically, the study shows that thrombocytopenia occurs during EMF exposure and that platelet lysis releases large amounts of potassium.

Exposure to an EMF with a frequency of 50 Hz and an intensity of 2 mT significantly increased the percentage of basophils in the peripheral blood of rats, suggesting that this reaction is the result of an adaptive immune response.

Since 24-hour continuous exposure to 50 Hz, 2 mT EMFs did not statistically significantly change the concentrations of monocytes, catalase, and interleukins (IL-1, IL-10) due to the high standard deviation, we suspect that a study conducted on a larger animal population may yield different results. These findings on changes in hematopoietic and electrolyte parameters confirm that 24-hour continuous exposure to 50 Hz, 2 mT EMFs, as well as even extremely low doses of non-ionizing radiation, pose a risk in medical and environmental contexts.

Declaration of Generative AI and AI-assisted Technologies in the Writing Process

The authors wrote, reviewed and edited the content as needed and they have not utilised artificial intelligence (AI) tools. The authors take full responsibility for the content of the publication.

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Contribution of Individual Authors to the Creation of a Scientific Article (Ghostwriting Policy)

The authors equally contributed in the present research, at all stages from the formulation of the problem to the final findings and solution.

Sources of Funding for Research Presented in a Scientific Article or Scientific Article Itself

No funding was received for conducting this study.

Conflict of Interest

The authors have no conflicts of interest to declare that are relevant to the content of this article.

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