Original

The Effect of Extremely Low Frequency (ELF) Electromagnetic Waves on the Prevention of Diabetes Induced by Streptozotocin in Mice: Blood Indicators

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ABSTRACT

Background: Magnetic fields can affect physiological systems and some diseases, such as diabetes.

Objective: The current study aimed to investigate the effect of a low-power magnetic field on the blood parameters of mice, with streptozotocin-induced diabetes.

Material and Methods: In this experimental study, 48 adult male mice were divided into six groups of eight. Diabetic mice were exposed to magnetic fields of 0.0005 and 0.005 tesla for 5 and 10 days using Helmholtz coils. Blood samples were collected every other day to measure blood factors and assess the magnetic field's effects on diabetes-related parameters.

Results: In the diabetes groups, blood protein levels decreased without any effect from the magnetic intervention. However, in three out of the four groups, blood albumin increased under the influence of the magnetic field. The induction of a magnetic field led to a decrease in blood Gamma-Glutamyl Transferase (GGT) activity. Additionally, the magnetic field resulted in increasing blood magnesium levels.

Conclusion: The effects of the magnetic field and diabetes on the measured blood parameters, including GGT and magnesium were independent. However, the blood albumin level, which was reduced under the influence of induced diabetes, was improved by the magnetic fields, especially in the magnetic field of the 0.005 tesla.

Keywords

Diabetic; ELF; GGT; Proteins; Magnesium; Magnetics; STZ; Waves

Introduction

Diabetes is the most prevalent chronic disease according to the World Health Organization (WHO) and claims the lives of four million individuals annually. A joint report by WHO and the World Diabetes Federation predicts a significant increase in diabetic patients, exceeding 592 million by 2035. Currently, over 8 million Iranians are estimated to be living with diabetes, facing not only medical and psychological burdens but also significant financial costs for both patients and society [1-3]. One of the critical approaches for controlling and preventing metabolic diseases is magneto therapy. However, the harmful effects of electromagnetic fields on cells and physiological systems are also under discussion. Electromagnetic fields (EMFs) are

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invisible areas of energy created by the combined effect of electric and magnetic fields, presented everywhere in the environment with an influence on various cellular processes [2-3]. A study investigating individuals exposed to high-frequency EMFs found a 40% increase in the rate of neurological disorders, including suicide, compared to a control group [4]. However, it's important to note that this study does not necessarily prove causation. Further research is needed to explore the potential link between high-frequency EMFs and neurological conditions, considering factors like study design, potential confounding variables, and replication of findings [4-5]. Recent research has indicated that low-frequency electromagnetic fields are utilized for treating human diseases, including bone fracture repair, at an intensity of 3-5 microamperes/mm^2 and higher levels, leading to bone formation and destruction, respectively [4-5]. These high-frequency fields might influence cellular functions by affecting proteins, called membrane glycoproteins, on the cell surface [6].

Based on the frequency range, Extremely Low Frequency (ELF) fields include frequencies between 3 and 300 Hz, and Very Low Frequency (VLF) fields include frequencies between 3 and 300 kHz. Electromagnetic radiation, especially low-frequency radiation, has increased around us [3]. These waves spread in the environment with a specific wavelength and frequency at the speed of light, called electromagnetic radiation. The amount of energy absorption and penetration of these radiations depends on the frequency, radiation type, and the tissue [4].

Based on the impact of electromagnetic radiation on living organisms, they are categorized into two groups. The first group includes ionizing waves, which directly and indirectly have biological effects and cause DNA molecule destruction and damage to genetic materials, which are very dangerous for human health and living beings. Radiations with high frequency, short wavelength, and high penetrating power such as gamma, x, and cosmic rays are among these. The second group includes non-ionizing radiations with low frequency, long wavelength, and low penetration power, which do not have enough energy to break the chemical bonds of molecules and atoms [2]. The majority of the radiation in our environment falls within the low-frequency and very low-frequency electromagnetic spectrum, ranging from zero Hz to 300 kHz [5].

Various reports have discussed the impact of electromagnetic fields on genetic material and biological processes in living organisms [6]. It is important to note that the energy of electromagnetic fields is physically incapable of directly breaking the DNA of cells exposed to radiation [7]. However, electromagnetic fields can influence cell proliferation, and differentiation, disrupt the cell cycle, and communication between cells, increase DNA damage, produce free radicals, and alter antioxidant enzyme activities [8]. The extent of cellular and molecular changes induced by these waves depends on the duration of the radiation, its permeability in tissues, and the production of heat, which are influenced by the intensity and frequency of the waves [9]. Additionally, the response of cells varies according to the characteristics of the waves, such as waveform (sine or square), biological effects, and the type of cells exposed to radiation [10]. Magnetic fields, particularly those from mobile phones and hands-free devices, have a greater ability to penetrate living tissues, affecting enzyme activity [11], endocrine and metabolism [12], nerve conduction [13], growth cycle, fetal development [14], and the immune system [15]. The studies show that the electromagnetic field does not have enough energy to harm biological molecules. However, some researchers believe these fields can cause genetic damage to protective systems [16].

The absorption and penetration of radiation depend on its frequency and the tissue type [17-18]. Most environmental radiation falls within the low-frequency and very low-frequency electromagnetic range, with a frequency range between zero and 300 kHz [19-20]. Electromagnetic fields can affect cell proliferation, differentiation, cell cycle disruption, and communication in living systems [21]. The cellular and molecular changes induced by these waves depend on the duration of the radiation, its permeability in the tissues, and the production of heat, which are also dependent on the intensity and frequency of the waves [22]. The response of the cell varies according to the characteristics of the waves, such as waveform (sinusoidal or square), biological effects, and the type of exposed cells [23]. This research focuses on the impact of extremely low-frequency (ELF) electromagnetic waves generated by designed generators on preventing diabetes in a mouse model.

Material and Methods

Research plan

This experimental study (controlled intervention) was carried out at the Drug Applied Research Center (DARC) of Tabriz University of Medical Sciences, to study the effects of electromagnetic waves on streptozotocin-induced diabetes prevention in mice. This research was conducted by 48 male BALB/c mice weighing 30-35 grams. This research was conducted using 48 male BALB/c mice weighing 30-35 grams. To establish a biological balance and compatibility of the mice with the laboratory environment and to attain a stable state and relieve stress, they were kept in this center for two weeks (with a light regime of 12 hours of light and 12 hours of darkness; free access to water and food during the tests; an ambient temperature of 22±2 °C during the experimental period).

Diabetes induction method

To induce diabetes in the target groups, an intraperitoneal injection dose of 60 mg/kg of body weight of streptozotocin (STZ) dissolved in sterile normal saline was used to destroy beta cells and increase blood glucose levels to more than 180 mg/dl.

Design and construction of electromagnetic generator for induction of magnetic field

Two generators have been designed and built to create magnetic fields. These generators are wooden cylindrical structures without any metal parts. They have a height of 42 cm (distance between the two ring coils) and a diameter of 62 cm. Each generator consists of two coils (a pair of Helmholtz Coils) made of varnished grade 10 wire [24-25]. These coils are wound around the lower and upper cross-section surfaces of the cylinder, in a total of 160 coils, including 80 and 60 coils for the upper and the lower sections, respectively. The number of coils was determined using the formula for electromagnetic field intensity (B=μnl) (where B represents magnetic field intensity in Tesla, 4π×10μ represents a constant, n represents the number of turns per unit length, and I represent current intensity). To ensure the accuracy of the electromagnetic field output with intensities of 0.005 and 0.0005 tesla, the field intensity was measured using a tesla meter (Lutron EMF-828) after establishing the current in the circuit (Figure 1).

Animal grouping

The animals were randomly divided into 6 groups of 8 mice, as follows:

1- The control group: the animals were not subjected to any intervention (C).

2- The group, exposed to diabetes (without electromagnetic intervention) (D).

3- The group, exposed to diabetes and a 0.0005-tesla electromagnetic field for 5 days (DLF5).

4- The group, exposed to diabetes and a 0.0005-tesla electromagnetic field for 10 days (DLF10).

5- The group, exposed to diabetes and a 0.005-tesla electromagnetic field for 5 days (DHF5).

Figure 1: Different parts of electromagnetic generators

6- The group, exposed to diabetes and a 0.005-tesla electromagnetic field for 10 days (DHF10).

Sampling, blood parameters assay, and statistical analysis

During the experiment, blood samples were taken from mice every 48 hours (on the second, fourth, sixth, eighth, tenth, and twelfth days), by scratching the tail of the mice to measure blood glucose using a glucometer [24]. An hour after the last exposure to the magnetic field, all mice were anesthetized with ketamine (100 mg/kg) and xylazine (5 mg/kg) via intraperitoneal injection, and blood was drawn from the heart. The blood samples were then centrifuged at 4 °C with 3500 rpm for 10 minutes using a Sigma 3000 model centrifuge to measure serum indicators, including Iron, Urea, Creatinine, Lipoprotein-Low Density (LDL), Triglycerides, Cholesterol, Protein, activity of Alkaline Phosphatase (ALP), Albumin, Calcium, CK, g-GT, High Density (HDL), Alanine Aminotransferase (ALT), Lactate Dehydrogenase (LDH), Aspartate Aminotransferase (AST), magnesium, phosphorus, and uric acid. The data analysis was conducted using SPSS software (version 22), and the results were presented as mean ± standard deviation. The differences between the mean values of blood glucose and other parameters among different groups were assessed using one-way ANOVA followed by Tukey's test, with a significance level of P-value<0.05 (Figure 2).

Results

Blood Glucose

The blood glucose levels in the treatment groups, as depicted in Figure 3, fluctuated throughout the experiment. By the twelfth day, the glucose levels in all induced diabetes groups fell within the diabetic range. Furthermore, the blood glucose levels of the irradiated groups did not show a significant difference from the diabetic group without electromagnetic intervention after becoming diabetic.

Gamma glutamyl transferase (GGT)

Figure 4 displays the blood GGT activity in the studied groups. In all four groups exposed to electromagnetic radiation, there was a significant decrease in blood GGT activity after developing diabetes compared to the diabetic group without radiation (D) and the control

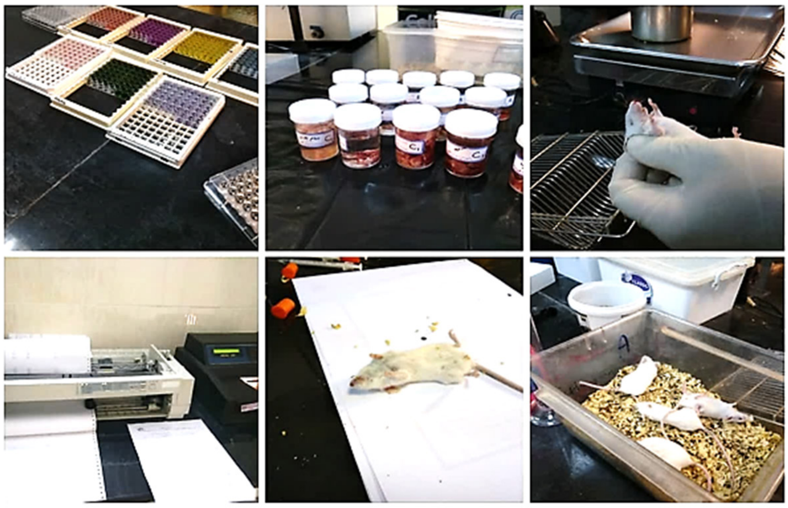

Figure 2: Sampling steps and laboratory tests

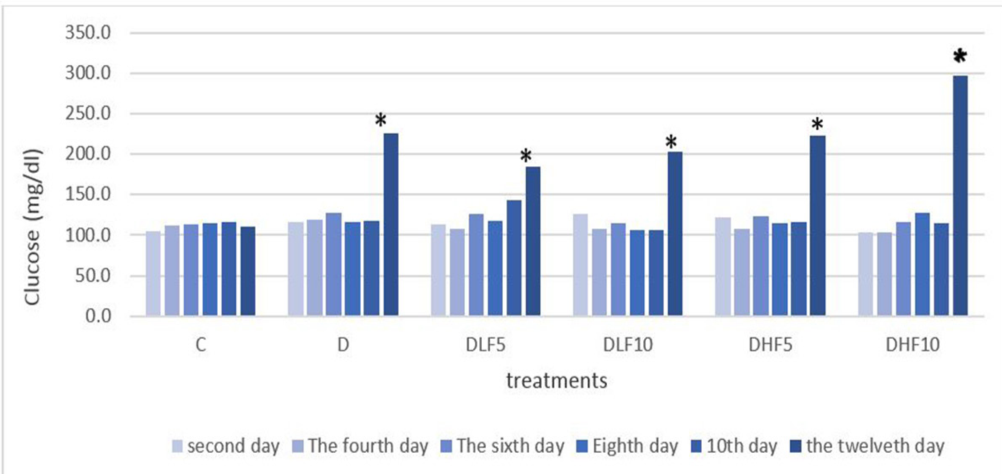

Figure 3: Comparison of blood glucose levels in the experimental groups and trial even days (from the second to the twelfth)
*Indicates a significant difference compared to group C and other test days in all groups (P<0.01). Control (c), exposed to diabetes (D), exposed to diabetes and a 0.0005 tesla electromagnetic field for 5 days (DLF5), exposed to diabetes and a 0.0005 tesla electromagnetic field for 10 days (DLF10), exposed to diabetes and a 0.005 tesla electromagnetic field for 5 days (DHF5), exposed to diabetes and a 0.005 tesla electromagnetic field for 10 days (DHF10).

group (C) (P-value<0.01).

Blood Magnesium

Figure 5 displays the blood magnesium levels in the experimental groups. The DLF10, DHF10, and DHF5 groups exhibited a significant increase in magnesium levels after developing diabetes, compared to the control (C) and diabetic (D) groups (P-value<0.01).

Blood Protein

The total blood protein levels in the studied groups are illustrated in Figure 6. All diabetic groups exhibited a significant decrease

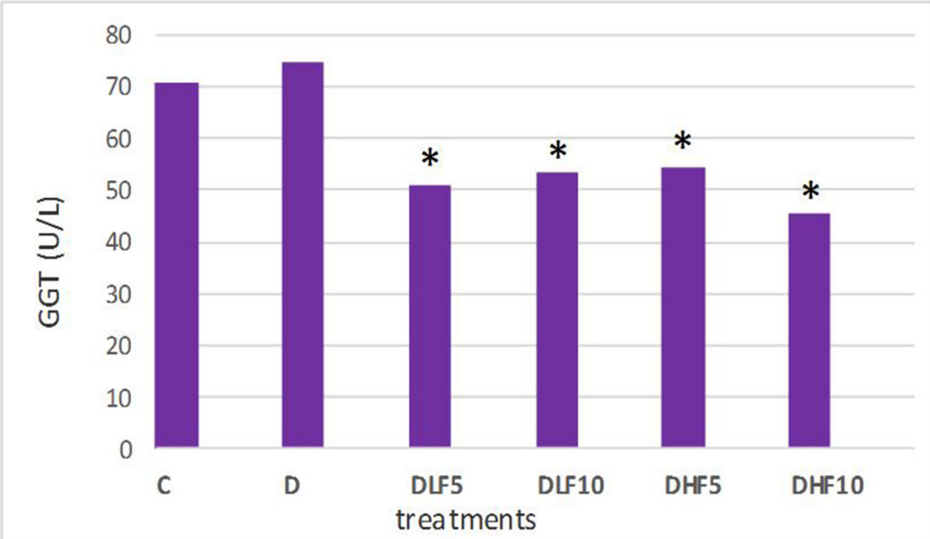

Figure 4: Comparison of blood Gamma-glutamyl transferase (GGT) activity in the experimental groups.
*Indicates a significant difference compared to other groups (*P*-value<0.01). Control (c), exposed to diabetes (D), exposed to diabetes and a 0.0005-tesla electromagnetic field for 5 days (DLF5), exposed to diabetes and a 0.0005-tesla electromagnetic field for 10 days (DLF10), exposed to diabetes and a 0.005-tesla electromagnetic field for 5 days (DHF5), exposed to diabetes and a 0.005-tesla electromagnetic field for 10 days (DHF10).

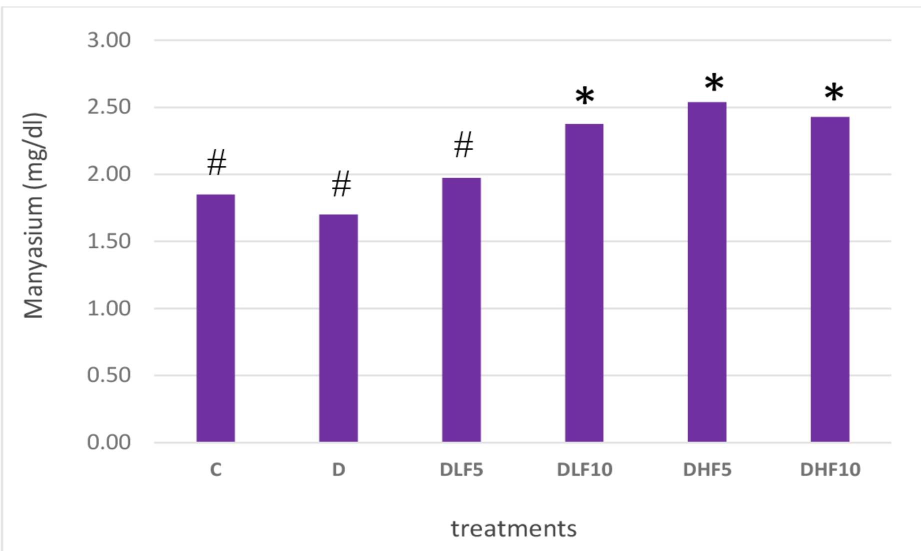

Figure 5: Comparison of blood magnesium levels in the experimental groups.
*Indicates a significant difference compared to other groups (*P*-value<0.01).
#indicates a significant difference compared to other groups (*P*-value<0.01).
Control (c), exposed to diabetes (D), exposed to diabetes and a 0.0005 tesla electromagnetic field for 5 days (DLF5), exposed to diabetes and a 0.0005-tesla electromagnetic field for 10 days (DLF10), exposed to diabetes and a 0.005-tesla electromagnetic field for 5 days (DHF5), exposed to diabetes and a 0.005-tesla electromagnetic field for 10 days (DHF10).

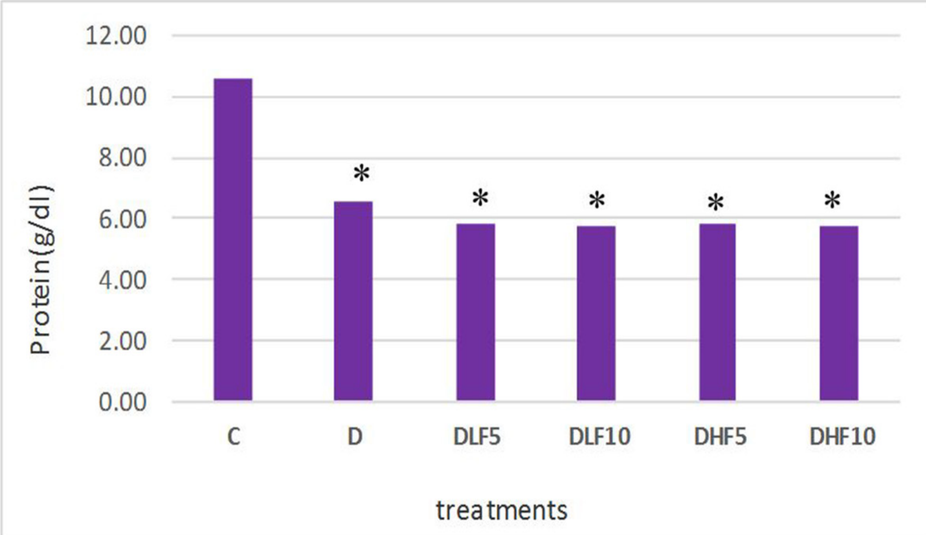

Figure 6: Comparison of the amount of total blood protein in the experimental groups. *Indicating a significant difference from group C (*P*-value<0.05).
Control (c), exposed to diabetes (D), exposed to diabetes and a 0.0005 tesla electromagnetic field for 5 days (DLF5), exposed to diabetes and a 0.0005-tesla electromagnetic field for 10 days (DLF10), exposed to diabetes and a 0.005- tesla electromagnetic field for 5 days (DHF5), exposed to diabetes and a 0.005-tesla electromagnetic field for 10 days (DHF10).

in blood total protein compared to the control group (*P*-value<0.05), and magnetic fields did not impact this decrease.

Blood Albumin

The blood albumin levels in the experimental groups are presented in Figure 7. In the presence of diabetes, there was a significant decrease in blood albumin levels compared to the control group (*P*-value<0.01). Among the diabetic groups treated with DHF5, FLF10, and DHF10, there was a significant increase in albumin levels compared to the diabetic group (*P*-value<0.01), similar to the control group. However, the albumin levels in the DLF5 group did not differ from those in the diabetic group.

Other Blood Parameters

This research found no significant differences (*P*-value>0.05) in therapeutic interventions for the ALP, ALT, AST, LDH, creatine phosphokinase (CPK), and LDL and HDL, as well as uric acid, urea, iron, creatinine, phosphorus, calcium, triglycerides, and cholesterol in the test groups.

Discussion

The study found that STZ caused hyperglycemia, an experimental model of type 1 diabetes, in mice by damaging β-cells. According to reports, STZ enters pancreatic β-cells through glucose transporter-2 (GLUT2) and damages the cell membrane within 1-2 days, leading to DNA strand breaks. This process reduces the cellular level of NAD+ and causes β-cells necrosis. Since GLUT2 is also present in rodent kidneys, STZ uptake by kidney cells may expose them to similar necrosis as β-cells. Other studies have shown that STZ induces diabetes and severe insulin deficiency in mice and other rodents [15-17].

Considering that, the debate over the effects of electromagnetic fields with different intensities on living beings, particularly humans, remains a challenging issue in the

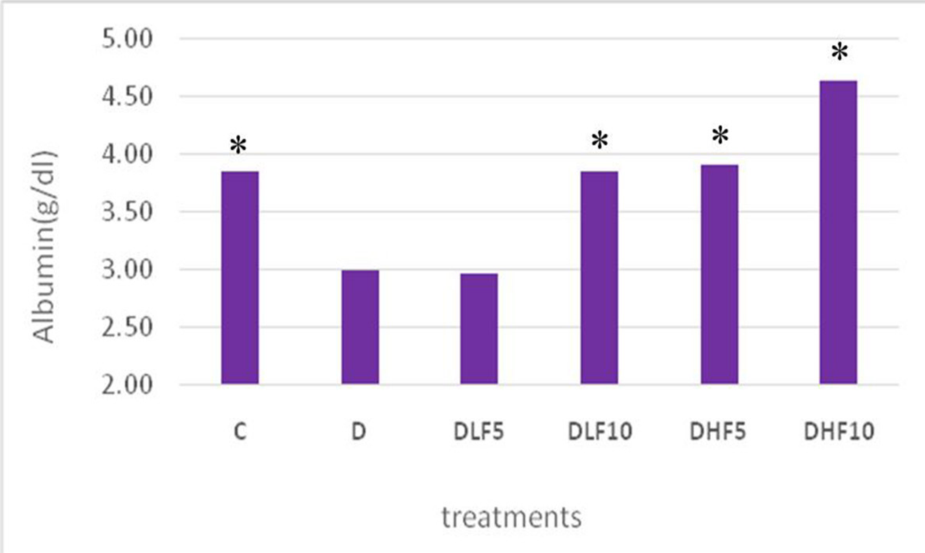

Figure 7: Comparison of blood albumin levels in the experimental groups.
*Indicates a significant difference compared to D and FLF5 groups (*P*-value<0.01).
Control (c), exposed to diabetes (D), exposed to diabetes and a 0.0005 tesla electromagnetic field for 5 days (DLF5), exposed to diabetes and a 0.0005-tesla electromagnetic field for 10 days (DLF10), exposed to diabetes and a 0.005-tesla electromagnetic field for 5 days (DHF5), exposed to diabetes and a 0.005-tesla electromagnetic field for 10 days (DHF10).

scientific community [17]. The physical nature of electric fields affects static charges and is governed by Coulomb's law, while magnetic fields are based on Lorentz's law and act on moving charges. Due to the electrical nature and the presence of moving charges as transmitters of nerve signals, magnetic fields can influence these signals. Therefore, it is anticipated that the primary impact of magnetic fields from a physical standpoint will occur through the disruption of nerve impulses and their effect on systems reliant on these signals elsewhere in the body [15].

In this study, BAlb/c mice were exposed to a magnetic field with an intensity of 0.005 Tesla and a frequency of 0.0005 Tesla. The research demonstrated that the intensity and duration, of the magnetic field did not affect the blood glucose level. However, it is important to note that the potential effects of the field could vary based on factors such as pulse types, field intensity, and frequency. For instance, different studies have yielded varying results. In one study, male mice treated with streptozotocin were divided into six groups: healthy controls, insulin recipients, metformin recipients, and groups exposed to electromagnetic fields of 50, 25, and 100 Hz with an intensity of 250 microteslas for 45 minutes daily.

The results indicated that the healthy control group had a blood glucose level of 130±4.7, while the average serum glucose levels for the other groups before treatment were 181±10. After treatment, the serum glucose levels for groups 2 to 6 were 162±3.14, 165±3.7, 141±13.6, 165±14.2, and 169±9.5, respectively. The statistical analysis revealed that a magnetic field with a frequency of 25 Hz and an intensity of 250 microteslas reduced the blood glucose of diabetic mice [18].

In a separate study, the main difference from the current study was the frequency and type of the magnetic field. The test group, with 22 young BALB/C mice, was placed in a constant uniform magnetic field with a magnetic induction of 0.05 tesla created by a permanent magnet for 10 days, for at least 10 hours daily. The control group was subjected to similar conditions but without a magnetic field. Both groups were monitored 10 days before, during, and 10 days after the experiment, and were weighed daily. Subsequently, the test group was placed in the magnetic field for 15 days. On the fifteenth day, blood samples were taken from both groups. The blood glucose levels for the test group were 114.36±8.18 mg/dl, and for the control group, they were 113.81±4.46 mg/dl. Similar to the results of this research, no significant difference was observed [24-25]. During this period, the magnetic field and time led to significant changes in the blood GGT index in the irradiated and diabetic groups compared to the non-irradiated diabetic group, resulting in a decrease. Additionally, an increase in blood magnesium levels was observed in the irradiated and diabetic control groups. MRI imaging devices' magnetic field impacted the blood glucose in rats, causing a decrease compared to the control group [26-27]. Similar findings were reported in another study [28]. Conversely, a study with photo evidence showed blood glucose elevation after exposure to a weak magnetic field [29].

The current research findings indicate that cortisol concentration in the blood varies throughout the day, with the highest levels observed in the morning. Various factors, including stress, can alter its levels by up to 17 times. The reasons for the fluctuations in blood glucose levels due to magnetic fields have been diverse. Some studies have attributed the decrease in blood glucose to changes in cortisol and melatonin, as well as long-term effects on the pituitary-hypothalamus axis or the impact on the pituitary gland, which has been considered the primary cause of increased cortisol and its effect on blood glucose. This situation does not lend itself to a straightforward interpretation. However, the reaction to the stimulus depends on many factors, including field parameters, frequency, induction, polarization, radiation, pulse shape, irradiation time, and the physical and physiological conditions of the body during irradiation. Different studies based on the conditions, have obtained variable results. However, all studies show that magnetic fields have an effect similar to applying stress on the body and can change the level of cortisol based on this dependence. Some other studies have reported a decrease in the level of these hormones after applying a magnetic field [30].

Although the results of the present study did not show a significant effects on blood glucose levels, other studies conducted on the effect of magnetic fields on glucose levels have shown changes in the structure of beta cells in the pancreas and the amount of insulin synthesis and secretion. Also, indicated an increase in hypoglycemia, these waves affect the absorption of glucose in the tissue by facilitating the passage of glucose through the cell wall, and it probably affects the activity of insulin in insulin-dependent tissues; Therefore, these waves not only increase the absorption of glucose due to the increase in insulin secretion, but also enhance the secretion of insulin receptors and signals entering the target cells [31-32]. In this regard, another study has shown that short-term exposure to RF-EMF generated by mobile phone jammers can reduce fasting blood glucose levels in adult male rats [33].

Conclusion

As an achievement, the intensity and duration of the electromagnetic field used in this research did not affect the process of inducing diabetes and blood glucose levels. Although the total protein decreased under the influence of diabetes, albumin levels increased due to magnetic radiation. Furthermore, electromagnetic induction led to increased magnesium levels and reduced blood Gamma-Glutamyl Transferase. Various factors such as frequency, intensity, duration of radiation, sampling type, test timing, and physiological conditions can influence the magnetic effects on cells and body systems. Therefore, further research with emphasis on longer period and stronger electromagnetic intensity is suggested to complete the conclusion.

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Authors' Contribution

A. Dehghan as a master's student, carried out the implementation stages, especially the design and construction of electromagnetic generators. T. Vahdatpour as the supervisor professor, presented the idea of research, guided the implementation steps and wrote the article. M. Mesgari A. as the consultant professor, controlled the implementation steps in the research center and performed hematology tests. All the authors approved the final version of the article.

Ethical Approval

The animal procedures were approved (2021-01-13) in accordance with the related guidelines by the animal research ethics committee of Islamic Azad University (approval number: 162330414) and it was implemented in Tabriz University of Medical Sciences.

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This research was carried out at the personal expense of the master's student (Ali Dehghan).

Conflict of Interest

None

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