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Induced Effects of Magnetic Field on The Retina of Rat's Eye

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HUMAN intervention and the application of electrical energy in everyday life have led to the fact that magnetic fields are considered one of the constituents of the environment. In fact, these fields have harmful effects on biological systems. Therefore, the aim of this study is to investigate the effects of magnetic fields on the retina of a rat's eye. At the same time, some biophysical properties of hemoglobin for a group of rats exposed to different magnetic fields were detected. Forty- eight mature male Wister rats weighing (150 – 180 g) were exposed to magnetic fields in the range from 120 Gauss to 160 Gauss. An electroretinogram (ERG) of all the retinas was recorded, and the retina was collected from a rat's eye and and tested through Deoxyribonucleic Acid (DNA) fragmentation. This was followed by testing effect of a magnetic field (MF) on hemoglobin by measuring viscosity and conductivity.

The data indicated that there is no change in all the measured parameters of the retina and blood after exposure to 120 and 130 Gauss. Furthermore, there was a decline in visual function and an increase in DNA fragmentation by increasing the exposure dose up to 160 Gauss. The results also showed a significant increase ($p \leq 0.001$) in both relative viscosity and electrical conductivity of blood after exposure to 130 Gauss and this increase continued until the exposure of rats to 160 Gauss. The present results suggested that the magnetic field exposure may induce ROS generation oxidative stress, which is an early step leading to cellular damage after exposure and consequently changes in ERG (retinal function). MF exposure caused apoptosis in retinal tissue and alterations in the blood characteristics.

Keywards: Magnetic field, retina, ERG, apoptosis, Blood viscosity and Conductivity

Introduction

Magnetic fields are produced by electrical appliances, power lines, electromagnets and everything that carries electric current. The level of magnetic fields to which humans may be exposed has considerably increased over the last century. The exposure level is normally limited for practical reasons. The magnetic field can affect biological processes in various aspects [1].

Magnetic field affects physiological processes such as increased cell activity, depolarization of cell membranes, changes in membrane potential, and increasing of inflammation. A magnetic field causes damage to the ocular surface between the eyelids and is associated with symptoms of ocular discomfort. The magnetic field also reduces the production of aqueous humor, which leads to dry eye syndrome and chronic conjunctivitis [2].

Magnetic field exposure leads to morphological alterations of the conjunctiva and reductions in the number of goblet cells. It also induces magnetophosphenes and electrical activity in the retina, which is recorded by ganglion cells. Magnetic fields also change the visually evoked potential (VEP) of the retina and also lead to cataract development [3].

In order to perform quantitative analysis of photoreceptors response to retinal pigment epithelium and neurosensory retinal destruction, ERG is done at different magnetic field exposure time. The obtained ERG data showed changes in the physical parameters of a-and b-waves. These changes depend on the intensity and duration of exposure [4]. Moreover, it was reported that the static magnetic field of significant induction inhibits DNA synthesis in mice, therefore suppressing tumor growth and even affecting normal tissues.

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On the other hand, the inhibition of apoptosis by the magnetic field can cause an increase in the risk of cancer development **[5],[6].** Some studies showed that one of the mechanisms by which magnetic fields interact with biological systems is that they cause changes in the spin states of reactive oxygen species (ROS) which in turn can lead to possible adverse effects on cell function [7].

Therefore, the present study was designed to investigate the effects of a magnetic field on the retina of a rat's eye. Moreover, some biophysical properties of hemoglobin for a group of rats exposed to different magnetic fields were detected.

Material and Methods

Experimental animals

Forty-eight mature male Wister rats weighing (150 – 180 g) were used in this study. The rats were selected from the animal house at the Research Institute of Ophthalmology, Giza, Egypt. All animals were housed in specially designed cages at room temperature (22 - 25^{o} C) and fed on a laboratory-balanced diet. The use of animals in the present study was in agreement with the guidelines from the Association of Research in Vision and Ophthalmology (ARVO) statement and approved by the Research Institute of Ophthalmology ethical committee (RIO-ethical committee). The animals were classified into 6 groups of 8 rats each, as follows:

Group (A): was used as the control group.

Group (B): was exposed to a magnetic field (MF) of 120 Gauss, for 6 hours.

Group (C): was exposed to a magnetic field (MF) of 130 Gauss, for 6 hours.

Group (D): was exposed to a magnetic field MF) of 140 Gauss, for 6 hours.

Group (E): was exposed to a magnetic field (MF) of 150 Gauss, for 6 hours.

Group (F): was exposed to a magnetic field (MF) of 160 Gauss, for 6 hours.

Magnetic field exposure device

The homogeneous magnetic field generator in which animals were housed and exposed consists of a coil placed on a wooden rack. The coil consists of 320 turns of electrically insulated 2 mm-thick copper wire wound in a homogenous way around a copper cylinder of 2mm-thickness, 50 cm-diameter, and 60 cm-long. The cylinder wall was earthed to eliminate the effects of the electric field. The ends of the coil were connected to a variac fed from the mains (220 Vpp and 50 Hz) to produce different alternating electric magnetic field. The magnetic field strength inside the magnetic chamber (where the animals were housed) was adjusted by changing the voltage across the coil by the variac to produce a magnetic field of different strengths (120 to 160 Gauss) in the area where the animals were housed, as shown in Fig 1.

Fig. 1. The components of exposure device.

Electroretinogram (ERG)

The rats were dark-adapted for 3 hours before the electrophysiological recording. They were anesthetized intravenous with 35 mg/kg sodium thiopental, and after establishing the anesthesia, rats were placed on the pad of an operating table, where their body temperature was maintained at 37°C. Each rat was positioned with its head resting to one side and local anesthetizing eye drops were also applied. The pupil of the recorded eye was dilated with topical 1% mydriacyl. A white flash was used in this work with a fixed intensity of 4 lux and a duration of 0.2 seconds. ERG was recorded using the sensor PS-2111 and its electrodes (PASCO, Roseville, CA) which connect to the PASPORT interface directly to the computer. One electrode was placed at the corneal periphery as an active electrode. The other two electrodes were placed on the skin of the lower eyelid and on the ear as reference and earthed electrodes. The electrodes were placed on the skin the ear as reference. The resulted electrophysiological signals were collected and analyzed by Data Studio 1.9.8 software (PASCO, Roseville, CA).

Retina and blood sample collection

The rats were immediately euthanized at the end of a cardiac puncture. The eyes were enucleated and the retinas are carefully separated. The following measurements were carried out in retinas of control and treated rats.

The rats were anaesthetized with diethyl ether. Blood samples were taken from the animals by draining the blood from the eyes into a heparinized tube, using capillary tubes. The tubes sealed and gently checked ready for performing measurements. Hemoglobin was extracted by a modification of the method of [8].

Relative viscosity and Conductivity measurements:

Before Viscosity and conductivity measurements, hemoglobin concentration was adjusted by an appropriate dilution with deionized water at room temperature (25 ±1ºC) on the base of heme absorption band at 576 nm where the absorbance of Hb at 576 nm = 0.5. The relative viscosity was calculated at constant temperature by measuring the time of flow of a constant volume of samples and water by using Ostwald viscometer from the equation:

$$\eta s/ \eta w = dsts/dwtw$$

where η, d, and t are viscosity, density and time of flow respectively for the sample (s) and water (w). The electrical conductivity was determined by a conductivity meter type HI 8633, with a dip type cell which has fully protected electrodes made by Hanna instruments manufactured in Germany.

DNA fragmentation analysis:

The rat's retinas of both control group and all the treated ones were subjected to DNA fragmentation analysis. Agarose gel electrophoresis was applied to compare fragmented and intact DNA fractions of control and treated rat retinas as described previously by **[9].** Gel electrophoresis apparatus **(**Model Horizon 58, Gibco BRL) was used to run the electrophoresis by loading 20 µl of the DNA samples and DNA markers (Boehringer Mannheim) to the wells of 1% agarose gel containing ethidium bromide 0.5 mg/ml in TBE buffer (in 800 ml) of H2O dissolve 108 g Tris base (89 mM), 55 g boric acid (89 mM), 40 ml 0.5M EDTA, pH 8.0 (2mM); bring to 1 liter with H2O. Use diluted (1:10). Gel electrophoresis was viewed using Bio-Rad™ gel documentation system with the analysis software Quantity One®.

Results

The electroretinogram ERG

The electroretinogram (ERG) responses of the dark-adapted eyes for all groups were illustrated in Figure (2). The mean and standard deviation of the amplitude of both a-and b-waves for all groups are shown in Table (1). For control, the amplitude of a-wave has mean values of 0.958 ± 0.029 µV while the b-wave is 1.769 ± 0.053 µV. It is noticed that there was a notable change in the ERG of the group which was exposed to 140 Gauss in the form of significant decrease ($p<0.05$) in a- and b- wave amplitudes in comparison with control. Furthermore, there was a decline in visual function when increasing the exposure dose up to 160 Gauss. The percentage difference for a- wave was 19% in its amplitude whereas the percentage difference in b- wave were 14%, i.e. a-wave is more affected than b-wave (table 1). All parameters of groups which exposed to 120 & 130 Gauss concerning ERG were had no significant changes compared to control values. Figure (3) and Figure (4) showed the histogram of a-wave and b-wave respectively for experimental groups compared to control groups.

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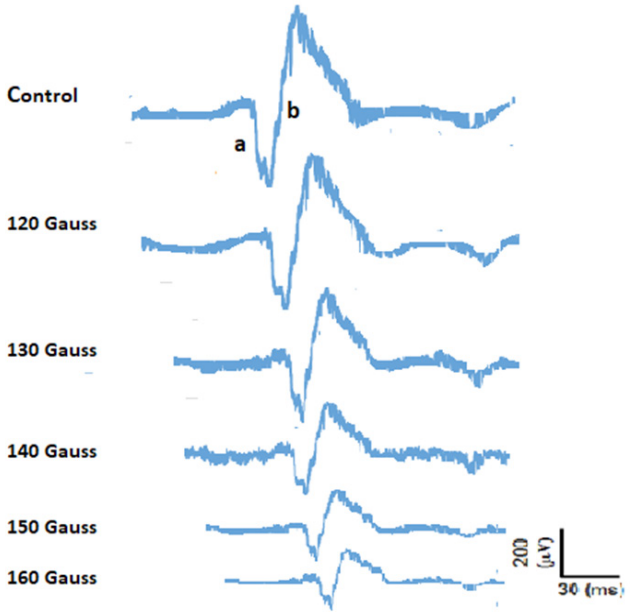

Fig. 2. ERG responses of the dark-adapted eyes for all the studied groups.

TABLE 1. Electrophysiological measurements of control and all the studied groups.

Groups	a-wave	b-wave
A	0.958 ± 0.029	1.769 ± 0.053
B	0.946 ± 0.023	1.757 ± 0.051
C	0.921±0.018	1.724 ± 0.052
D	0.901 ± 0.017	1.696 ± 0.034
E	0.859 ± 0.0 31	1.619 ± 0.024
F	0.800±0.029	1.523 ± 0.030

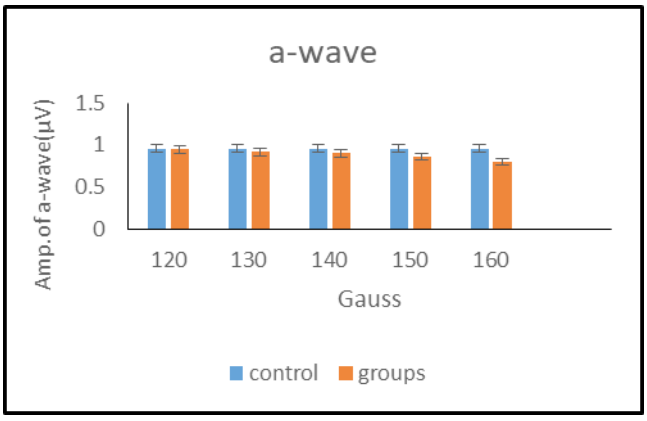

Fig. 3. The mean value of the electrophysiological measurements of a-wave (μv) ± S.D for control and all the studied groups.

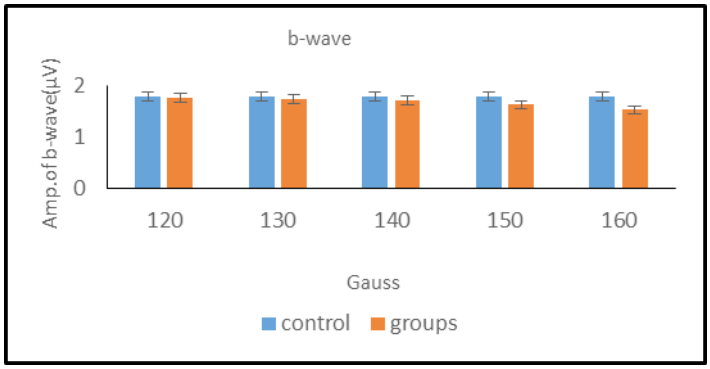

Fig. 4. The mean value of the electrophysiological measurements of b-wave(µv) ± S.D for control and all the studied groups.

DNA fragmentation measurements

Agarose gel electrophoresis of retinal DNA for control and exposed groups to a magnetic field showed a time-dependent appearance of the typical ladder pattern of internucleosomal fragmentation, a characteristic of apoptosis. A faint ladder pattern was barely noticeable in the exposed group (C), but maximum band intensity was noted in the group (F) exposed to 160G. Results also showed no DNA ladder was present in the control retina and group (B). Table (2) illustrates the number of bands, base pairs (bp) and percentage of bp in each band for the ladder and all groups. In the table, the ladder reflects 3 bands with different percentages for 140G in contrast to the control lake of the DNA ladder, and the bands increase by increasing the intensity of the magnetic field to 160G. The fragmentations increased by 9 bp with increasing the intensity of the magnetic field to 160G.

Relative Viscosity measurements

Table (3) shows how the average relative viscosity of hemoglobin changed in rats that were exposed to an alternating magnetic field at different doses and for fixed amounts of time. This was compared to the rats that were not exposed to the field. Also, they are presented by the histogram shown in figure (6). The results show that there was a non-significant change in relative viscosity for rats exposed to 120G (B groups) compared to the control group. The results also showed a significant increase ($p \leq 0.001$) in relative viscosity after exposure to 130 Gauss and this increase

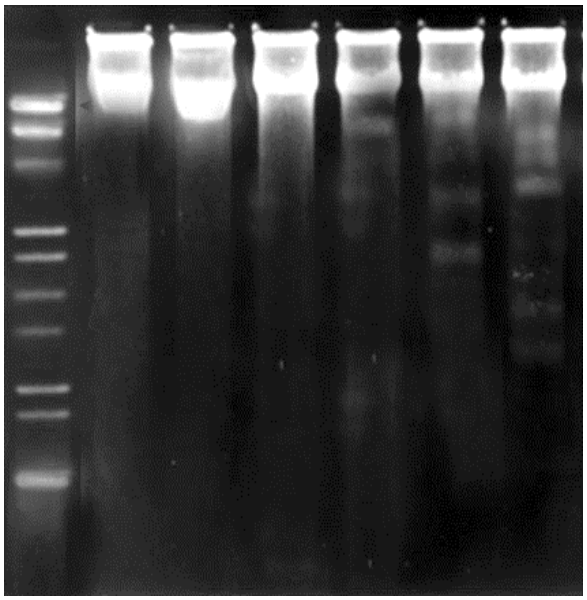

Fig. 5. DNA fragmentation analysis by gel electrophoresis. The retinal samples from control and all the studied groups. The first column is the marker (100 bp).

TABLE 2. Bands and percentage of bp fragmentation for ladder and different groups exposed to magnetic field compared to control.

Band	Marker		A		B		C		D		E		F	
	BP	%	BP	%	BP	%	BP	%	BP	%	BP	%	BP	%
1			1613.11	100	1613.11	100	1613.11	32.75	1613.11	30.37	1613.11	26.6	1613.11	24.93
2							1145.68	62.75	1156.24	44.79	1145.68	48.93	1156.24	42.79
3	1000	21.92												
4									934.53	10.96	953.03	8.92		
5	900	16.16											888.79	9.28
6									827.45	4.21	837.04	3.61	827.45	7.26
7	800	7.41												
8							762.98	4.5	758.1	3.93	748.62	4.69	762.98	6.18
9	700	8.06												
10													667.51	1.85
11											623.96	5.1		
12	600	6.26												
13											546.04	2.15	541.73	2.39
14	500	7.39												
15													459.36	2.86
16	400	5.85												
17													368.41	2.46
18	300	5.52												
19									274.37	5.73				
20	200	6.12												
21	100	15.31												
Number of bands							3		6		7		9	

TABLE 3. Hemoglobin relative viscosity of blood from control rats and all the studied groups.

Groups	Hemoglobin relative viscosity
A	2.57 ± 0.007
B	2.58 ± 0.012
C	3.015 ± 0.021**
D	3.048 ± 0.022*
E	3.128 ±0.019*
F	3.205 ±0.027*

Values expressed as (mean ± S.D).*:P≤0.05(Significant). **: P≤0.001(High significant).

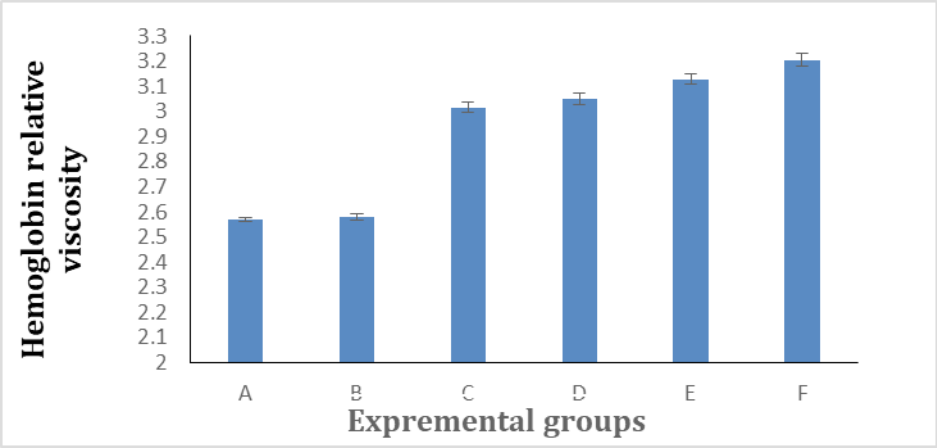

Fig. 6. The mean values of the relative viscosity measurements ± SD of blood from control rats and all the studied groups.

continued until rats were exposed to 160 Gauss.

Electrical conductivity measurements

The average readings of hemoglobin electrical conductivity for the control and rats of all the studied groups are tabulated in table (4). The obtained data revealed a significant increase increase (P≤0.001) in electrical conductivity of hemoglobin for the exposed group (c). The measured electrical conductivity increases by increasing the exposure dose to 160G compared to to the control group (figure 7). Results also showed that hemoglobin electrical conductivity had no significant increase in the group exposed to 120 Gauss.

TABLE 4. Hemoglobin electrical conductivity of blood from control rats and all the studied groups.

Groups	Hemoglobin relative conductivity µs/cm
A	403.75 ± 7.395
B	408.75 ± 10.825
C	507 ± 7.714**
D	616.25 ± 9.601**
E	910 ± 7.906**
F	983 ± 9.601*
Values expressed as (mean ± S.D). *:P≤0.05(Significant). **:P≤0.001 (High significant).	

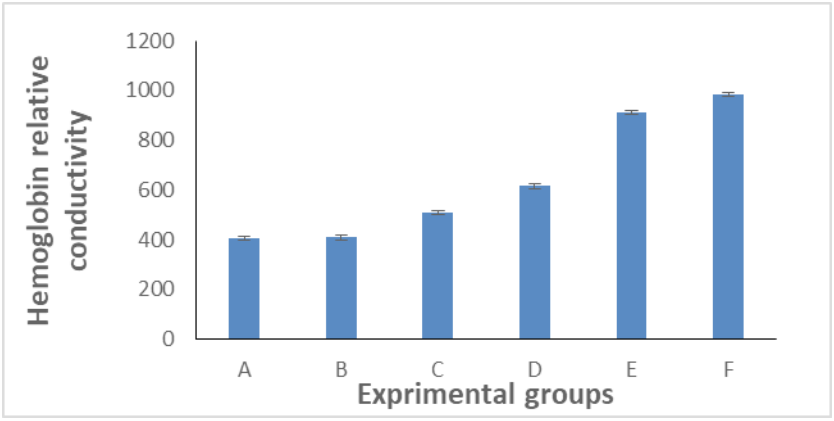

Fig. 7. The mean values of the electrical conductivity measurements (µs/cm± SD) of blood from control rats and all the studied groups.

Discussion

The present study is an attempt to investigate the effect of magnetic fields with different intensities (120-160 Gauss) on the rat retina by detecting its function by measuring ERG. It provides guidance for the assessment of the occupational and public health significance of magnetic fields and indicates areas that may be hazardous. At the same time, some physical blood parameters for these rats were measured as an important indicator for knowing the health status of living organisms.

The present study showed that all ERG parameters and many blood constituents were affected by the exposure to a magnetic field, which suggests functional changes in both. The ERG serves to register the potential of retinal cells in response to light. The assessment of retinal electrical activity was conducted with a- and b-wave amplitude of the electroretinogram: The a-wave is a negative wave reflecting the functional activity of photoreceptors, and b-wave is a positive wave reflecting the electrical activity of bipolar and Muller cells, with the possible involvement of the horizontal and amacrine cells [10],[11]. The b-wave is generated within the inner retinal layer, during which the blood supply is provided mainly by the retinal circulation. Therefore, the b-wave is supposed to be a good indicator for the inner retina and retinal circulation [12].

The obtained ERG data showed changes in the physical parameters of a-and b-waves. These changes beginning with exposure to 130 gauss and increasing with increasing intensity of exposure. The detected results seem to confirm the hypothesis that magnetic fields affect oxidative processes that occur in living biological cells and that this effect can be explained by the radical pair mechanism [13]. Oxidative stress is one of the most significant mechanisms concerned with magnetic fields. Under oxidative stress conditions, excessive reactive oxygen species (ROS) can injure cellular proteins, lipids and DNA, resulting in lesions in cells. [14] reported that exposure to oxidative stress will upset regular biological functions.

The retina is suitable for the generation of reactive ROS and resultant oxidative damage. The retina is one of the highest oxygen-consuming tissues in the human body. The highest oxygen level is found in photoreceptors that contain high levels of polyunsaturated fatty acids. The existence of this micro-environment, combined with abundant light exposure and a high energy demand, supports a highly oxidative milieu [15],[16].

Cleavage of DNA into oligonucleosomal-sized fragments are a biochemical hallmark of apoptosis or programmed cell death [17]. In the present study, agarose gel electrophoresis of retinal DNA obtained from control rats and different study study groups showed a dose-dependent appearance of the typical ladder pattern of internucleosomal fragmentation, a characteristic of apoptosis. So, the obtained results indicated that magnetic fields affect the function of the retina, accompanied by apoptosis. These changes can also be attributed to the ability of magnetic fields to produce ROS. Our result agreed with a previous study by [6] who explained the correlation between magnetic field exposure characteristics and their influence on apoptosis and ROS concentration. [6], [18] explained the role of ROS in apoptosis and damaging the DNA of the retina. It triggers cell death moreover DNA damage.

Excessive ROS could attack membrane phospholipids, impair mitochondrial function, and damage proteins, lipids, DNA and RNA to disrupt normal cellular processes [19],[20]. However, excessive ROS formation has been linked to vascular endothelial dysfunction, neuron degeneration, and inflammation in the retina. ROS can directly modify cellular molecules and impair their function [21].

The hematological parameters showed that exposure of hemoglobin molecules to magnetic fields induced great changes in their physical properties. When the whole body is exposed to a moderate and strong static magnetic field, there are different degrees of globin unfolding, which is termed a sign of molecular destabilization. The magnitude of destabilization increases with the increasing intensity of the magnetic field, which leads to the suggestion that hemoglobin exists in a new conformational state with low stabilization. These results are in agreement with the study of [22] who indicated that exposure of animals to magnetic fields These results are in the absorption spectrum of hemoglobin molecules.

The present results showed a significant increase in the relative viscosity of hemoglobin in rats exposed to magnetic fields (MF). This elevation may be due to the fact that MF caused

the aggregation and unfolding of hemoglobin molecules on protein parts, which in turn led to an increase in the viscosity of hemoglobin. This finding is in agreement with [23] who detected a change in the viscosity of hemoglobin molecules after exposure to a magnetic field.

Moreover, [24], [25] found that exposure to a constant magnetic field of different intensities could promote the autoxidation rate of oxyhemoglobin to methemoglobin. These leads to the breaking hydrogen bonds between hydrophobic non-polar groups, leading to the unfolding of globular proteins. Intermolecular charge charge repulsion is a driving force for unfolding. exposure to a moderate and high-intensity constant magnetic field in the range of 130G–160G, which may cause the unfolding of globular proteins with the formation of new groups exposed to the surface besides the polar hydrophilic groups, leading to an increase in electrical conductivity. So, the obtained results revealed the hematotoxicological effects of magnetic fields.

Conclusion

This work done on 48 male rats exposed to magnetic fields in the range from 120 Gauss to 160 Gauss then, the retina collected from rat's eye (ERG) and (DNA) fragmentation measurements. This followed by testing the effect of magnetic field on (Hb) by measuring viscosity, conductivity and of hemoglobin. From the previous results, it has been shown that the first dose of the magnetic field effect starts from the value of 130 Gauss, and the effect increases with increasing dose, compared to the control group.

The present results suggest that magnetic field exposure induces ROS generation and oxidative stress, leading to cellular damage after exposure. The free radicals generated due to MF exposure attack polyunsaturated fatty acids, which results in lipid peroxidation that breaks down membranous structure, especially in the photoreceptor region, and consequently changes in ERG (retinal function). The magnetic field exposure also induces apoptosis in retinal tissue. Moreover, the exposure to MF causes an alteration in the blood characteristics.

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التأثيرات المستحثة للمجال المغناطيسي على شبكية عين الفأر

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إن التدخل البشري واستخدام الطاقة الكهربائية في الحياة اليومية أدى إلى اعتبار المجالات المغناطيسية أحد مكونات البيئة. في الواقع، هذه المجالات لها آثار ضارة على النظام البيولوجي. ولذلك فإن الهدف من هذه الدراسة هو دراسة تأثير المجال المغناطيسي على شبكية عين الفأر. وفي الوقت نفسه تم الكشف عن بعض الخصائص البيو فيزيائية للهيموجلوبين في مجموعة من الفئران المعرضة لمجالات مغناطيسية مختلفة. تم تعريض ثمانية وأربعين ذكرًا ناضجًا من فئران ويستر ألبينو بوزن (١٥٠ - ١٨٠ جم) لمجالات مغناطيسية تتراوح من١٢٠ جاوس إلى ١٦٠ جاوس. تم تسجيل مخطط كهربية الشبكية (ERG) لجميع شبكية العين وتم جمع الشبكية من عين الفأرواختبارها من خلال تجزئة حمض الديوكسي ريبونوكلييك (DNA). تلا ذلك اختبار تأثير المجال المغناطيسي على الهيموجلوبين من خلال قياس اللزوجة والموصلية. تشير البيانات إلى عدم وجود أي تغيير في جميع المعلمات المقاسة للشبكية والدم بعد التعرض لـ ١٢٠ و١٣٠جاوس. علاوة على ذلك، كان هناك انخفاض في الوظيفة البصرية وزيادة في تجزئة الحمض النووي عن طريق زيادة جرعة التعرض حتى ١٦٠ جاوس. كما أظهرت النتائج زيادة معنوية ($P\leq$٠,٠٠١) في كل من اللزوجة النسبية والتوصيل الكهربائي للدم بعد التعرض لـ ١٣٠ جاوس ويستمر هذا الارتفاع حتى تعرض الفئران لـ ١٦٠ جاوس. تشير النتائج الحالية إلى أن التعرض للمجال المغناطيسي قد يحفز الإجهاد التأكسدي الناتج عن ROS وهو خطوة مبكرة تؤدي إلى تلف الخلايا بعد التعرض وبالتالي تغييرات في ERG (وظيفة الشبكية) وأيضا يؤدي التعرض للـ MF إلى موت الخلايا المبرمج في أنسجة الشبكية. ويؤدي التعرض للـ MF إلى تغير في خصائص الدم.