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EVALUATION OF THE BIOLOGICAL EFFECT OF ALTERNATING MAGNETIC FIELD ON RED BLOOD CELLS (RBCs) AND HEMOGLOBIN (Hb) ELECTROPHORETIC PROFILE OF SWISS ALBINO MICE.

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ABSTRACT

Recent development of modern industry, research and diagnostic equipment make it sometimes necessary for organisms to be exposed to a magnetic field. In the present study an attempt has been made to demonstrate the changes induced to the red blood cells (RBCs) and hemoglobin (Hb) protein pattern of male swiss albino mice by daily exposure to 2 milli Tesla, 50 Hertz Alternating Magnetic Field (8 hrs/day)for 5,10,15 and 20 days as direct effect groups and after periods of 30 and 45 days post exposure to MF as late effect groups. Hb electrophoretic analysis was made using (SDS-PAGE). The results exhibited major changes in the protein pattern which were observed all over the exposed groups of direct as well as the late effect groups. These changes involved changes in relative mobility and the total number of protein bands which was reflected as appearance of new bands and disappearance of others. The changes were also indicated in the percentages of similarity of Hb protein patterns between control groups and exposed groups. Pathological changes were also observed in the blood films of direct effect groups of mice in erythrocytes such as crenation, distortion, contraction and irregular pear-shaped RBCs. However, the late effect pathology showed a little improvement for exposed groups for 5 days and nearly complete recovery for exposed groups for 20 days.

KEYWORDS: Magnetic filed, red blood cells, hemoglobin, electrophoresis .

INTRODUCTION

Some studies and researches reported that the increase of the magnetic field level in the environment of man and animals is one of the basic physical factors disturbing the physiological processes in organisms (Ketchen *et al.*,1978).

Blood is one of the first substances ever tested for magnetic field influence. This is due to the major role played by erythrocytes, leukocytes and hemoglobin in the body. Blood proteins being considered a mirror reflecting changes occur within the body. The experimental results on animals proved significant decrease in hemoglobin (Hb) content due to the destructive action of the magnetic field on the membrane of the oldest erythrocytes, and generally erythrocytes lose their sense of existence and their metabolic activity. The direct effect of magnetic fields of relatively high strength led to different Hb conformations accompanied by changes in intermolecular interactions (Chernikov, 1990 and Atef *et al.*,1995). Fiorani *et al.*(1997) studied the effects of 50 Hz MF(2 mT) on rabbit red blood cells. They found increasing in the RBCs damage and arising in the fractionation of Hb, especially methemoglobin production

The work of Mikhail *et al.* (1998) revealed that exposure of animals to EMF has a deleterious effect on the RBCs resulting in their hemolytic tendency. Stashkov and Gorokhov (1999) studied the effect of weak alternating magnetic field of low frequency during many days. The results showed insignificant decrease of number of RBCs in blood, cytopaenia of red blood element of bone marrow, and an increase of

volume of liver and spleen. This leads to reduction of gas transportation function of blood.

Zhernovoi *et al.* (2001) proved that the rate of oxyhemoglobin drop and carbon dioxide release in dogs exposed to MF, enhanced blood oxygen capacity and formation of bioxyhemoglobin molecules. Meanwhile, Chakeres *et al.*, (2003) observed some distortion of the human ECG (electrocardiogram) with increasing magnetic field strength.

The degree of macrophage differentiation in primary cell culture of human was decreased by exposure to (6 mT) MF for 24 or 48 hrs with a consequent fall in cell adhesion and increased polarization of pseudopodia and cytoplasmic protrusions (Dini and Abbro, 2005).

According to the previous introductory results the present study was designed to investigate the effect of exposure of male Swiss albino mice to 50 Hz MF(2 mT) on the red blood cells and hemoglobin proteins electrophoretic pattern.

MATERIALS AND METHODS

Experimental animals:

80 male Swiss albino mice between 6-8 weeks weighting 35-50 g. were used. Mice were divided into nine groups, a control group and eight exposed groups.

In exposed groups whole bodies of mice were exposed to AMF (2 mT, 50 Hz 8 hrs/day), for different time intervals. These groups were divided as follows:

Group A: Control group (40 mice unexposed)

Group B: 5 mice exposed to AMF for 5 days (8 hrs/day).

Group C: 5 mice exposed to AMF for 10 days (8 hrs/day).

Group D: 5 mice exposed to AMF for 15 days (8 hrs/day).

Group E: 5 mice exposed to AMF for 20 days (8 hrs/day).

Group B1: 5 mice were exposed to AMF for 5 days (8 hrs/day) and the duration period was 30 days post exposure to MF.

Group B2: 5 mice were exposed to AMF for 5 days (8 hrs/day) and the duration period was 45 days post exposure to MF.

Group E1: 5 mice were exposed to AMF for 20 days (8 hrs/day) and the duration period was 30 days post exposure to MF.

Group E2: 5 mice were exposed to AMF for 20 days (8 hrs/day) and the duration period was 45 days post exposure to MF.

Groups B, C, D, and E were considered as the direct effect groups while groups B1, B2, E1 and E2 were considered as the late effect groups.

Mechanism of exposure:

Mice were housed in 9 plastic cages and fed with a standard diet. The device of exposure consists of coil placed on a wooden rack, which has 320 turns of 8 mm cupper wire wounded around a cupper cylinder of 2 mm thickness, 40 cm in diameter and 40 cm in length. The cylinder wall was earthed to eliminate the effects of electric field. The ends of the coil are connected to variac fed from the mains (220 Vpp and 50 Hz to produce AMF). The magnetic field strength inside magnetic chamber (where the animals were housed) was adjusted by changing the voltage across the coil by the variac. During the exposure, the mice cages were placed in the middle of the coil to get a homogenous MF.

Blood samples were drawn from mice by heart puncture after anaesthesia using heparinized syringes then transferred into heparinized tubes and reserved at 4°c. Hemoglobin (Hb) was extracted from the collected blood according to the method of Trivelli *et al.*(1971).

Blood films were prepared by spreading a drop of blood in a thin layer on microscopic slides and then routinely stained with special mixtures of red (acidic) and blue (basic) dyes, Harris's Hematoxylin and Eosin (Harris and May, 1985).

Hb electrophoretic analysis was made using Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) according to the method of Laemmli (1970). Similarity analysis was conducted using *Statistica* statistical software.

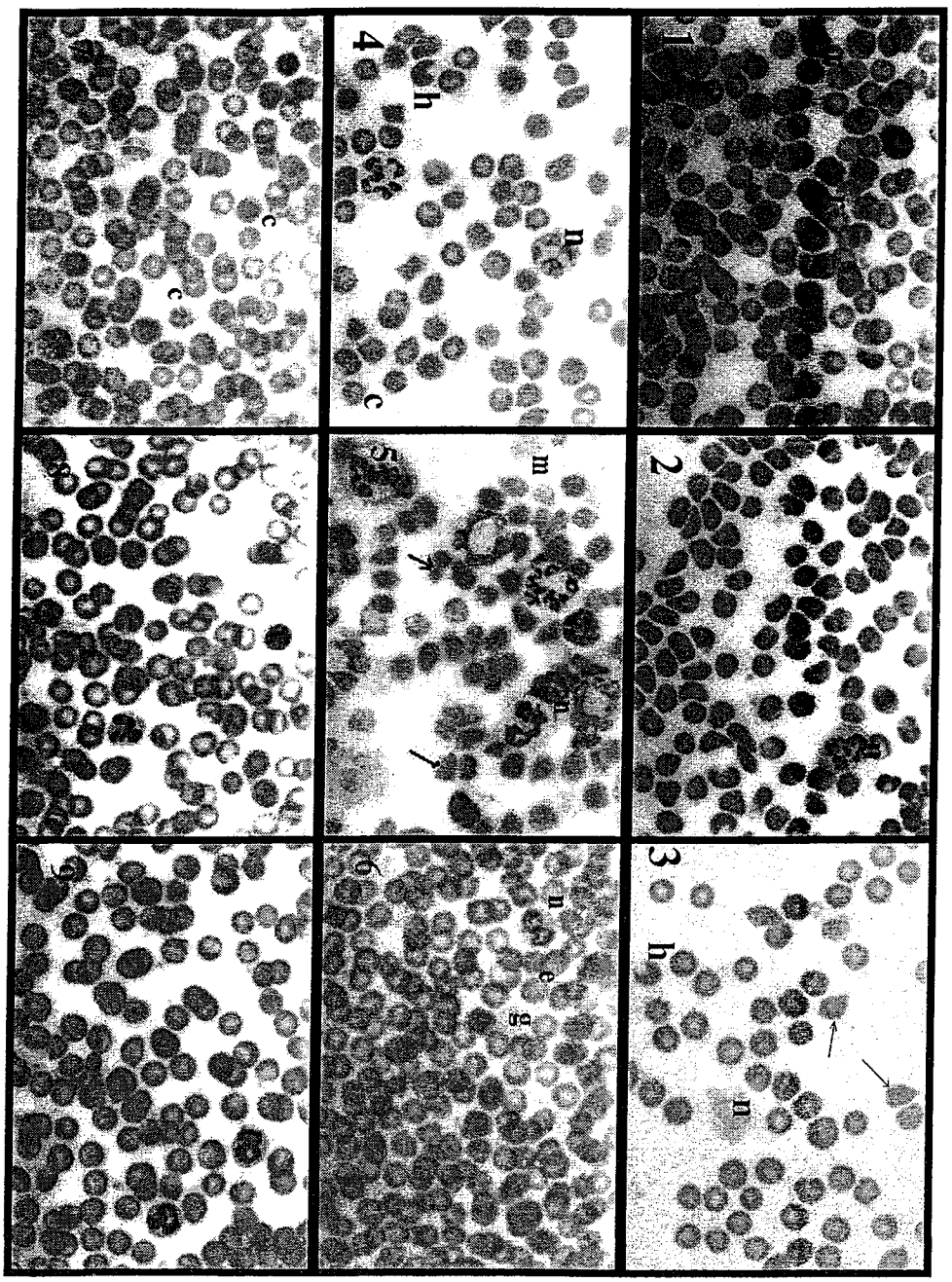
RESULTS

Histopathology of the blood:

Normal control mice blood film, group A showed discoid erythrocytes and normal neutrophils (Fig.1), while histopathological changes in mice blood film, group B were shown in the form of poikilocytes, contracted and slightly distorted erythrocytes (Fig.2). In group C, lesions progressed to show pale stained neutrophils, many of the erythrocytes became pear-shaped and the hemoglobin of some of them appeared accumulated to one side of the cell (Fig.3).

More aggravated changes were observed in group D, such as crenation, marked irregular contraction of erythrocytes and also contraction of hemoglobin to one part of the cell(Fig.4). Blood film of group E was characterized by an increased mitotic figures of leukocytes, with aging of neutrophils, moderate anisocytosis, macrocytosis, polychromasia, contraction and distortion of erythrocytes (Fig.5).

List of Figures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Groups B1 & B2 showed mitotic neutrophils and some crenated erythrocytes while others were completely empty from Hb (Figs.6&7). Blood films of groups E1 & E2 showed almost complete recovery where, the appearance of erythrocytes and leukocytes was similar to the control blood film (Figs.8&9).

Hemoglobin Electrophoresis:

The results of SDS-PAGE gel scanning for separation of hemoglobin proteins and its corresponding electrophoregrams are shown in table (1) and figs. (10&11).

Scanning of the SDS-PAGE of control male mice Hb proteins (group A) revealed 10 distinct bands (Table 1&Fig.10A). The results exhibited major changes in the protein pattern as shown in table (1) & fig. (10), all over the experimental groups which included changes in the relative mobilities, represented as migration distance (MD) and relative percentages of protein fractions as well as the total number of bands, as a result of disappearance of some original bands and appearance of other new ones.

Hemoglobin protein fractions revealed an increase in the total number of protein fractions, being 13,16,13,16,19,23 and 15 bands for groups B,C,E,B1,B2,E1 and E2, respectively, except group D which had only 9 bands as shown in table (1) & fig. (10). However, the most marked increase in the total number of Hb protein bands was observed in group E1 (23 bands) as a result of the appearance of 20 new bands (Fig.10 E1), while 7 protein bands were lost.

Table (1):

The relative percentages of hemoglobin protein fractions, separated electrophoretically, of control mice group A and mice exposed to 2 mT, 50 Hz (MF) 8 hrs/day for direct and late effect groups (B, C, D, E, B_1B_2,E_1, and E_2).

		Relative fraction percentages								
FRACTION	MD mm	A	B	C	D	E	B_1	B_2	E_1	E_2
1	4.9	---	---	---	---	---	3.9	---	4.8	5.4
2	5.4	---	---	---	3.5	---	---	1.3	---	---
3	5.8	4.7	3.7	---	---	3.4	---	---	---	1.6
4	6.5	---	---	---	---	---	---	2.4	---	2.4
5	7	---	---	2.7	---	---	---	---	2.4	---
6	8.3	---	---	9.1	---	---	---	---	---	---
7	11.9	---	---	---	---	---	---	---	---	2.4
8	25.7	---	---	---	---	---	---	---	5.5	---
9	26.7	---	---	---	---	---	---	6	2.9	---
10	28.1	---	---	---	---	---	---	4.4	---	---
11	31.4	8.8	8	---	---	---	---	---	---	---
12	32.3	---	---	---	---	---	8.7	---	---	---
13	32.9	2.5	---	---	---	11.5	1.1	---	1.4	---
14	34.3	---	1.6	---	---	---	1.5	---	2	---
15	35.7	---	0.9	---	---	---	1.9	---	1.2	6.4
16	36	4.1	---	---	16.7	---	---	---	---	---
17	36.8	---	1.3	---	---	---	0.7	---	---	---
18	37.8	---	---	---	---	---	1.9	2.1	1.3	---
19	38.7	---	---	---	---	---	1.3	1.2	0.8	---
20	39.9	---	---	---	---	---	---	1.7	1.1	0.9
21	40.8	---	---	---	---	---	1.3	---	0.8	---
22	41.4	---	2.3	---	---	---	---	2.9	---	---
23	42.1	---	0.7	---	---	1.6	---	---	---	---
24	42.6	---	---	---	---	1.1	---	---	2.3	---
25	44.1	4.5	---	---	---	---	---	1.7	---	2
26	45.1	---	---	---	---	---	5.8	---	1.3	1
27	46.3	---	---	---	4.8	---	---	---	1.2	---
28	47.9	17.9	9	---	---	---	---	5.3	---	5.7
29	48.5	---	---	19.4	---	6.1	14.2	---	---	---
30	50.1	---	11.7	5.9	17.4	---	---	11.3	8.6	2.8
31	51.4	---	---	3	---	12.9	---	---	9.8	14.9
32	52.4	14.1	9.2	3.9	---	---	7.3	---	---	---
33	53.4	---	---	0.7	9.8	---	---	5	---	---
34	54.2	---	---	1.4	---	---	---	---	---	---
35	54.3	---	---	---	---	---	---	1.9	5.9	6.5
36	55.4	---	---	2.2	---	4.2	---	---	---	---
37	55.7	---	---	---	---	1.9	---	---	1.6	---
38	56.5	---	---	---	6.6	2.2	---	7	---	---
39	57.5	8.7	---	---	---	---	16.1	---	3.4	2
40	58.4	---	17.1	3.9	8.9	8.2	---	9.2	---	---
41	60.4	3	---	1.2	---	9	6.5	---	5.7	---
42	61.7	---	---	6.4	4.5	---	---	1.8	---	---
43	62.1	---	7.3	3.6	27.8	---	---	---	---	---
44	63.3	---	---	2.6	---	---	16.2	7.3	4.5	13.7
45	65.7	31.8	27.1	7.3	--	2.9	11.7	18.4	--	32.1
46	66.1	--	--	--	--	--	--	--	16.7	--
47	66.7	--	---	--	--	35	--	9	--	--
48	67.9	--	--	26.9	--	--	--	--	14.8	--
No. of fractions		10	13	16	9	13	16	19	23	15

Furthermore, the least number of new protein bands (8 bands) and in the same time the least number of lost bands (5 bands) were found in group B (Table 1&Fig.10 B).

Similarity analysis:

Data concerning similarity analysis of Hb protein pattern of male mice between control group and groups exposed to 2 mT, 50 Hz MF (8 hrs/day) direct and late effect groups are presented in table (2). The percentages of similarity in Hb protein pattern between control group and direct effect groups decreased with the increase of time of exposure, where it reached the maximum similarity (57%) in group B. Only 12.4% & 11.8% similarities were recorded in groups C & D while the least similarity was observed in group E (4%) compared to the control group A of Hb protein pattern.

Concerning the changing in duration of late effect groups and its effect on Hb protein pattern, it was found that in the group exposed for 5 days to 2 mT, 50 Hz MF, there was a decrease in the degree of similarity to control group A being 57, 31.8 and 25.4% at 0, 30 and 45 days for groups B, B1 and B2 respectively.

The data in table (2) show an increase in the degree of similarity with control group A in the groups exposed for 20 days, being 4, 7.8 and 43% after 0, 30 and 45 days for groups E,E1 and E2 respectively. Effect of exposure (percentages of dissimilarity with control group A = 100-percentage of similarity with control group A) is presented in table (2).

Table (2) Pecentages of similarity and dissimilarity between Hb protein pattern of control mice group A & mice groups exposed to 2mT, 50Hz MF (8hrs/day),for direct& late effect group (B,C,D,E,B1,B2,E1,& E2).

Groups of mice	B	C	D	E	B1	B2	E1	E2
Similarity with control group	57	12.4	11.8	4	31.8	25.4	7.8	43
Dissimlarity with control group	43	87.6	88.2	95	68.2	74.6	92.2	55

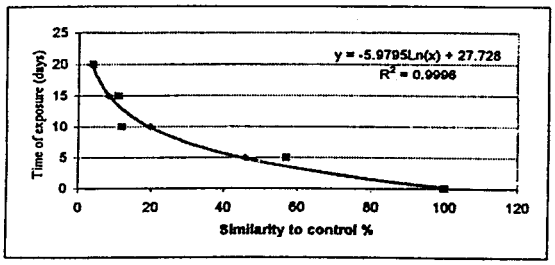

Fig. 12. Graph for the direct effect groups of mice exposed to 2 mT-50 Hz MF (8 hrs/day) and the similarity with control group A.

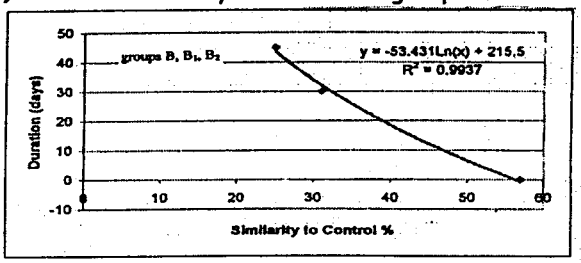

Fig.13. Graph for the duration in late effect groups of mice exposed for 5 days to 2 mT 50 Hz MF (8 hrs/day) and the similarity with control group A.

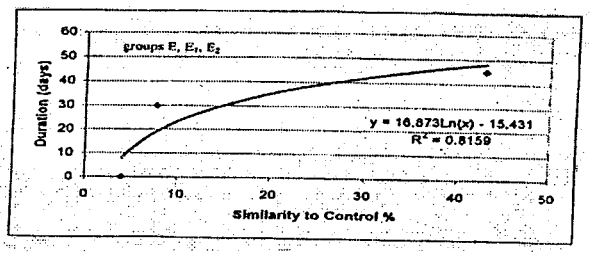

Fig. 14. Graph for the duration in late effect groups of mice exposed for 20 days to 2 Mt 50Hz MF (8hrs/day)& the similarity with control group A

The relationship between similarity and time of exposure:

The relationship between the degree of similarity in Hb protein pattern to the control group A and the time of exposure is shown in fig. (12). The analysis of the data revealed a negative logarithm correlation between the two variables which mean that the increase in exposure time reduces the similarity to control or cause more changes in Hb pattern. Such relation was found to be fulfilled by the following equation:

Time of exposure= -5.98 Ln(similarity to control group A%) + 27.728

with regression coefficient R^2=0.9996.

For studying the effect of the recovery period on the regaining of the Hb original protein pattern the relation between similarity to control pattern and the different treatments of groups B and E were studied.

The data in fig. (13) show also, a negative logarithmic relationship between duration of the late effect of exposure to 2 mT, 50 Hz ELF magnetic field for 5 days and similarity with control group expressed by the following equation:

Duration = -53.431 Ln (similarity to control group A) + 215.2,

with regression coefficient R^2 = 0.9937.

However, in case of exposure for 20 days to 2 mT, 50 Hz ELF magnetic field, the relationship was found to be a positive logarithmic one (Fig.14), where it is expressed by the following equation:

Duration =16.873 Ln (similarity to control group A) -15.431,

with regression coefficient R^2 = 0.8159

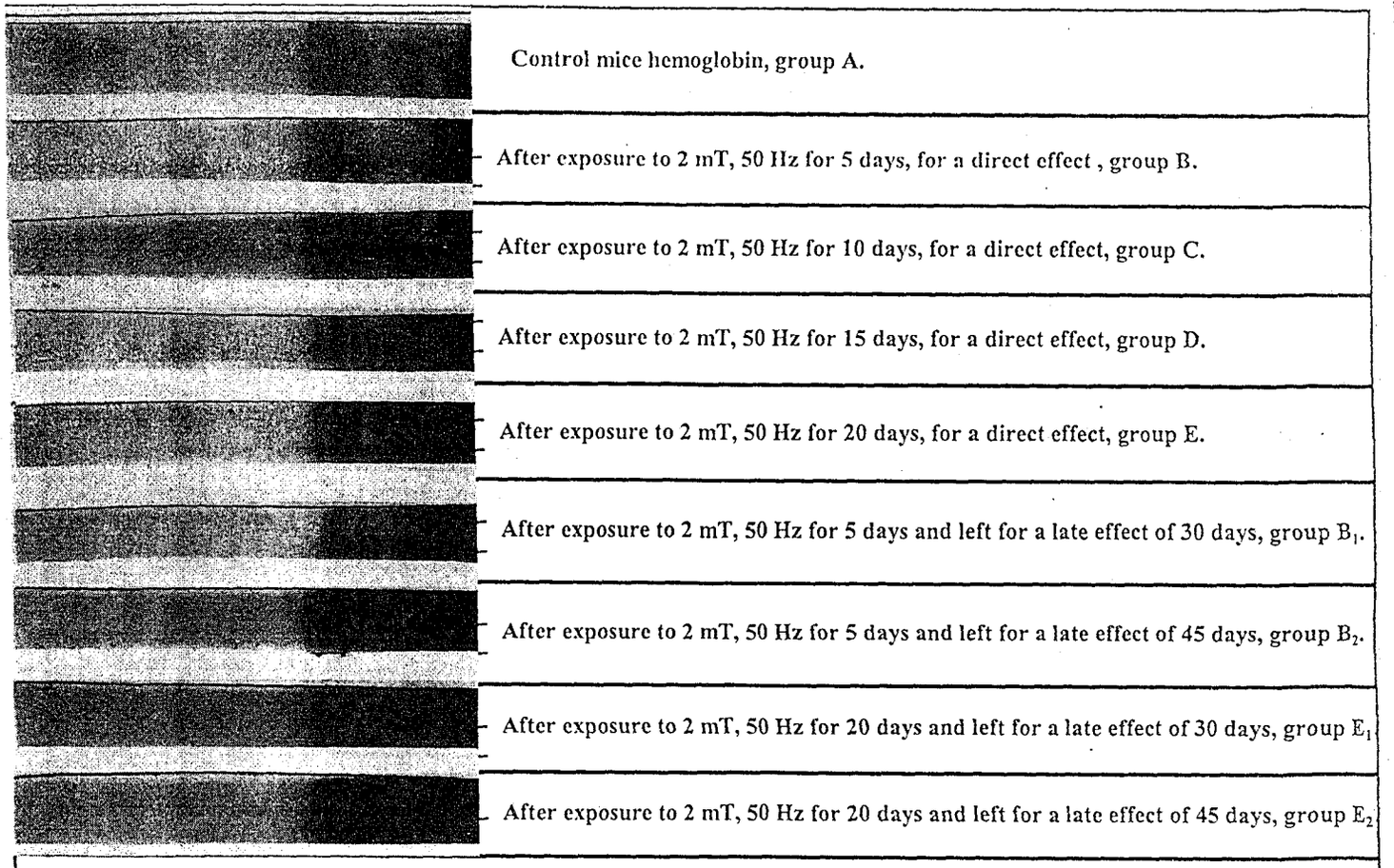

Fig. 11. SDS-PAG gel of hemoglobin of control mice and after exposure to 2 mT, 50 Hz MF (8 hrs /day) for different time intervals.

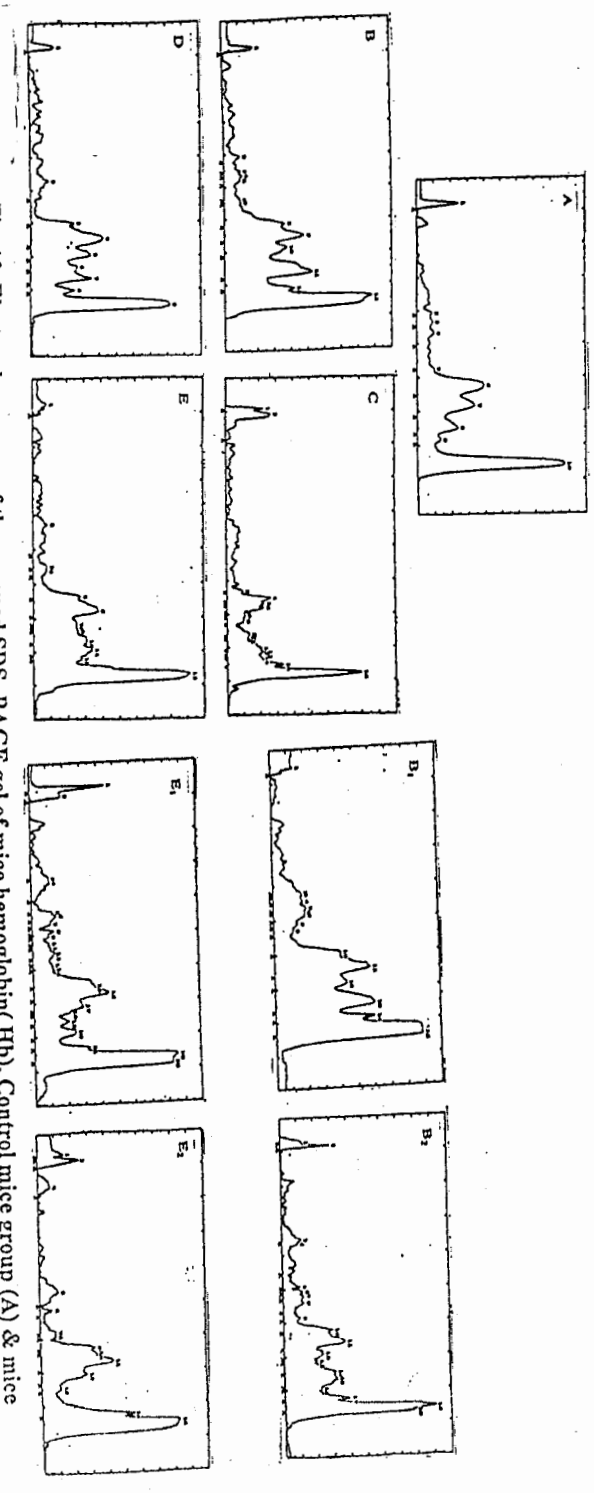

Fig. 10. Electrophoregram of the scanned SDS- PAGE gel of mice hemoglobin(Hb), Control mice group (A) & mice exposed to 2 mT, 50 Hz MF (8hrs/day) for direct and late effect groups (B,C,D,Eand B1,B2,E1,&E2).

DISCUSSION

The experimental results of the present study on the blood cells detected progressive pathological changes in the direct effect groups exposed to 2 mT, 50 Hz ELF magnetic field, (8 hrs/day). The pathology of late effect groups B1 & B2 showed a little improvement for the general blood pictures and no repair was detected in erythrocytes, while groups E1 & E2 revealed nearly complete recovery.

However, these pathological changes of blood films of all exposed groups especially those with the direct effect were similar to blood films of a patient who developed hemolytic anemia induced by different drugs (Dacie, 1967). This conclusion is similar also to Mikhail *et al.*(1998) who found that the exposure of albino rats to electromagnetic field had a deleterious effect on the red blood cells resulting in their hemolytic tendency.

Ichioka *et al.*(1998) found that static magnetic fields induced hyperaemia in rat following reduced blood flow during exposure. In this regard, Stashkov *et al.*(1999) assumed that weak alternating magnetic field of low frequency caused a decrease in number of erythrocytes in blood, cytopaenia of red blood elements of bone marrow and increased volume of liver and spleen which lead to reduction of gas transportation function of blood.

The present study also support the observation made by Lisi *et al.*(2000) who found several modifications in plasma membrane morphology of the lymphoid cells exposed to 50 Hz sinusoidal magnetic field.

Hemoglobin electrophoresis is performed when a disorder with abnormal hemoglobin is suspected (Wintrobe, 1967 and Smith, 2005). Each of the major hemoglobin types has an electric charge of a different degree, so the most useful method for separating and measuring normal and abnormal hemoglobin is electrophoresis. This process involves subjecting hemoglobin components to an electric field. The components then move away from each other at different rates (according to their molecular weights), and when separated they form a series of bands. The bands of exposed groups were then compared with those of control group. Each band can be further assessed as a percentage of the total hemoglobin, thus indicating the severity of any abnormality (Pagama, 1998).

The present study showed that the use of electrophoresis for Hb protein separation has been a good tool in the diagnosis of protein changes induced by exposure to 2 mT, 50 Hz MF (8 hrs/day). The electrophoretic protein profiles revealed alteration in Hb protein patterns. These alteration included changes in the relative percentages of protein fractions as well as the total number of protein bands (due to the loss of some bands and the appearance of other new ones). It was noticed that the low molecular weight protein bands were more in number in most of the exposed groups direct and late effects, compared to control.

The most of the lost protein fractions of almost all exposed groups, direct and late effects, were in high molecular weight regions. These changes in the low and high molecular weight areas could be attributed to the changes in the mobility of Hb protein and its ability to

bind with oxygen or carbon dioxide, to transfer them to or from circulation.

This may indicate some abnormalities in hemoglobin structure and appearance of abnormal types of hemoglobin which may lead to functional disorders concerning mainly the Hb principle function as oxygen carrier. This result agreed with Szweda, *et al.* (1976) & Szweda and Leyko (1987) who mentioned that the radiation modified Hb molecules, exhibited a decrease of co-operative binding of oxygen.

The present observations about the abnormalities of Hb and RBCs of direct and late effect exposed groups were similar to Wintrobe (1967) and Smith (2005) who referred to the abnormalities of Hb synthesis as being a result of production of abnormal protein molecules (e.g in sickle cell anemia with red blood cells abnormalities).

In conclusion, the present observations agreed with the previous investigators who suggested that following exposure to extremely low frequency magnetic field have shown major pathological changes concerning red blood cells and Hb. Further experimental researches are necessary to elucidate the underlying biophysical principles and evaluate appropriate safety factors.

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تقييم التأثير البيولوجى للمجال المغناطيسي المترددعلى خلايا الدم الحمراء وشكل الفصل الكهربي لهيموجلوبين الفئران السويسرية البيضاء.

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*- قسم علم حيوان **-قسم الفيزياء (شعبة الفيزياء الحيوي

مع ازدياد تقدم التقنيات الحديثة في المجتمعات الصناعية و أيضا تقدم الوسائل الحديثة في تشخيص الأمراض ازداد تعرض الإنسان لمستويات مختلفة من المجالات المغناطيسية. و قد توصلت هذه الدراسة إلي توضيح بعض التأثيرات للمجال المغناطيسي المنخفض التردد على هيموجلوبين الدم و على خلايا الدم الحمراء خلال فترات زمنية معينة على ذكور الفئران السويسرية البيضاء.

اشتملت هذه الدراسة على عدد ثمانين فأرا قسموا إلى مجموعة ضابطة و ثماني مجموعات معرضة،حيث تم تعريض كل أجسام الفئران للمجال المغناطيسي منخفض التردد (٥٠هرتز- ٢ملي تسلا) لمدة ثماني ساعات يوميا، ثم قسمت بعد ذلك مجموعات الفئران المعرضة إلي مجموعات تأثير مباشر و أخرى تأثير متأخر.

مجموعات التأثير المباشر ذبحت بعد نهاية فترة التعرض مباشرة بينما مجموعات التأثير المتأخر ذبحت بعد فترة زمنية معينة من نهاية التعرض للمجال.

دلت صورة الدم لمجموعات التأثير المباشر على تغيرات معظمها في كرات الدم الحمراء مثل تسنن و ثني و انقباض الغشاء و تباين في الحجم و درجة اللون و أيضا وجود بعض الخلايا الحمراء الكمثرية الشكل. كما حدث تحسن بسيط في شكل هذه الخلايا في مجموعات التأثير المتأخر و التي تعرضت خمسة أيام بينما حدث تقريبا شفاء تام في مجموعات التأثير المتأخر التي تعرضت عشرون يوما و مكثت بعيدا عن المجال خمسا و أربعين يوما.

و قد أظهر الفصل الكهربي لهيموجلوبين الفئران المعرضة للمجال المغناطيسي تغيرات كبيرة في النمط البروتيني اشتملت على تغيرات في الحركة النسبية و العدد الكلي لشرائط البروتينات المفصولة نتيجة لظهور شرائط جديدة من البروتينات و اختفاء أخرى مقارنة بالمجموعة الضابطة و المجموعات المعرضة للمجال و ذلك مع زيادة زمن التعرض للمجال و ارتفعت نسبة التشابه بين المجموعة الضابطة و مجموعات التأثير المتأخر مع زيادة فترة التأثير المتأخر.