Effect of ionizing and non-ionizing radiation fields on the structural properties of rats RBCs for the evaluation of the radiation hazards (in vivo study)

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Abstract

The aim of the present study is to evaluate the radiation hazards from exposures to 50Hz-0.05 mT magnetic field, fast neutrons within the permissible dose limit and mixed radiation fields. One hundred albino rats were used in this work. The rats of similar age and weights (~150 g ±10%), were divided into 4 equal groups namely A, B, C and D. Group A was used as control and was not exposed to any type of radiation, Group B animals were exposed to 50 Hz, 0.05 mT magnetic field for different periods up to 4 weeks. Group C was exposed to fast neutrons from ^{252}Cf source and received a total dose of 1.6 mSv distributed over a period of 4 weeks at a rate of 5 days/week, 8 h/day, 10 µSv/h. Group D was exposed to a mixed field of 50 Hz, 0.05 mT magnetic field together with fast neutrons from ^{252}Cf at the same dose rate as group C. At the end of the exposure period the animals were sacrificed, and blood was collected from each animal for further investigations.

The mechanical properties of the RBCs membrane were measured using osmo-fragility experiments, solubilization by nonionic detergent, and histological examination. The results indicated changes in all measured parameters after irradiation and exposures to single and mixed radiation fields. It was concluded from the results that on the calculation of the radiation dose it is recommended to differentiate between doses received from radioactive isotopes and from nuclear generating facility taking in consideration the higher biological effect of mixed radiation field.

Keywords: **radiation dose, ionizing and non-ionizing radiation fields, radiation injury.**

1 Introduction

On one hand there is long scientific history on the biological effects of ionizing radiation and the safe limits of exposures [1]. On the other hand, great attention has been focused on non–ionizing radiation which can induce a number of changes in biological systems of different living species.

The fast-growing uses of nuclear accelerators in medicine, manufacture and research in addition to the use of nuclear reactors for generating electric power, all increased the health risk associated with mixed exposures to ionizing and non-ionizing radiation.

Contrary to high frequency and high energy ionizing electromagnetic radiation, the harmful features of extremely low frequency magnetic fields ELF-MFs have not been proven unambiguously although biological effects were demonstrated in many studies of different sort [2]. Extremely low-frequency magnetic fields (ELF-MFs) are produced wherever electricity is generated, transmitted, or distributed, such as power lines, cables, subways, and electrical appliances. ELF-MFs include alternating current (AC) fields and other forms of non-ionizing radiation with frequencies ranging from 1 to 300 Hz. The major frequencies of ELF-MFs are 50/60 Hz, and humans in industrialized nations are continuously exposed to a few milli-teslas (mT) of ELF-MFs at these frequencies everyday [3, 4].

As a consequence of such effects the international Commission on Non–ionizing Radiation Protection (ICNIRP) published guidelines (14) for the permissible safe limits of the public and occupational exposures. Based on the ICNIRP guidelines in the spring 2004, the European parliament and council adopted a directive 2004/40/EC on minimum health and safety requirements regarding the exposure of workers to electromagnetic fields (13). So far, there is no legislation covering occupational exposure to ELF-EMF (2). In the USA 5 years program of the National Institute of Environmental Health Science was designed to conduct mostly laboratory research to fill in data gaps by replicating isolated studies that had raised concern and to examine the effects of chronic, near lifelong exposures of rats and mice to multiple levels of electromagnetic field [5]. These studies have provided risk assessment of electromagnetic fields [6,7].

Since 1979 there has been concern that these fields may be associated with cancer. Several epidemiological studies have investigated the biological effect of MFs with a frequency of 50/60 Hz [8, 9]. In particular, increased risk of brain tumors, leukemia, and breast cancer has been reported to be associated with exposure to electromagnetic fields from overhead power lines and various consumer devices [10, 11]. In fact, some studies have demonstrated that MFs from power lines pose a potential carcinogenic risk to humans. Recent researches indicated that exposures to 50 or 60 Hz magnetic fields are possibly carcinogenic for childhood, the risk factor of childhood leukemia is about 1.69 at 50 Hz magnetic fields as low as 0.3 mG [12].

Some reports demonstrated that exposure to 50-Hz MF (1mT) for 1–24h induces DNA strand breaks and chromosomal damage [15, 16]. Other investigations showed that repetitive exposure to MF with extremely low frequency can induce DNA double-strand breaks and apoptosis[17]. Moreover, exposures to ELF-EMF may alter the transcription and translation of genes [18] leading to generation of free radicals that affect both cell proliferation rate and enzymes activities [1].

Other studies support a possible link between exposure to ELF-EMFs and modification of the cellular redox state, which could, in turn, increase the level of intracellular Ca2+and thus modulate the metabolic activity of C2C12 cells [19].

Epidemiological studies suggested an association between chronic ELF-MFs exposure and physiological disturbance, [20] showed that exposure to ELF-EMF may slightly induce alterations in some hematological parameters of rats. Meanwhile [21] reported that animals exposed to MFs of 3 mT suffered from an increase in RBCs osmotic fragility and a decrease in

its membrane elasticity, in addition to partial change in Hb molecular structure without change in electrophoretic band of Hb. Also, there was an increase in ALT and AST levels in plasma indicating some damage in liver cell membrane. Moreover, an elevated level of MDA and catalase activity is considered as evidence for the increase in free radicals level. Whole body exposures of rats to 50 Hz, 0.2 mT magnetic fields for a period of one month caused a decrease in erythrocyte's membrane elasticity and permeability and induced changes in the molecular structure of hemoglobin (Hb). Besides, heart injuries were observed [22].

Similar effects have been observed on membrane solubilization of erythrocytes for rats irradiated with ^{252}Cf fast neutron with fluences of 106, 107 and 5×107 n/cm2. The study showed a decreasing trend of erythrocytes osmotic fragility with increasing radiation fluence [24]. Structural changes were also observed in the membrane lipid bilayer macromolecules exposed to fast neutrons. The data represented the damaging effects of fast neutrons for both hydrophobic and hydrophilic regions of lipid bilayer [23].

One may conclude from the reported data that exposures to either neutrons or non-ionizing electromagnetic fields can alter the physical properties of the erythrocyte membrane.

The permissible doses recommended by the ICRP depend on the exposures to ionizing radiation only. The question now does these recommended safe limits of exposures can be valid for mixed radiations of non-ionizing and ionizing fields?

Therefore, the aim of the present work is to study the effects of whole-body exposure of rats to 50 Hz – 0.5mG magnetic field, fast neutrons within the permissible dose limits and mixed effects of both magnetic field and fast neutrons on the erythrocytes biophysical characteristics as a step forward to evaluate the risk associating exposures to mixed radiation fields.

2 Materials and methods

2.1 Animals

One hundred adult male Albino rats of weights (~150 g ±10%), 7-8 weeks old, were obtained from the animal house of National Center of Researches in Egypt. They were maintained for one week in the laboratory for adaptation. Rats (10 rats per cage) were housed in cages with free access to drinking water and standard chow diet. 4)' All animals' procedures and care were performed using guidelines for the Care and Use of Laboratory Animals, (1996) and approved by the animal Ethics Committee at Cairo University.

The rats were divided into four equal groups namely A, B, C and D. Animals of group A were used as control (sham) group. Group B was whole body exposed to 50 Hz, 0.05 mT magnetic field for different periods up to 4 weeks, 5 days/week, 8 hours/day. Group C was whole body exposed to fast neutrons from 252Cf source to receive a maximum dose of 1.6 mSv distributed over a period of 4 weeks,10 µSv/h, 8 hours/day, 5 days/week. Animals of group D were exposed to fast neutrons from 252CF source and a magnetic field with the same exposure conditions as for groups B and C at the same time, i.e. two exposure fields. Animals of group A were kept (as sham group) inside a similar solenoid without any electric or magnetic field for periods similar to other groups.

The animals were kept in special cages that permit normal ventilation and daylight. Animals from all groups were kept under similar environmental conditions of temperature, illumination, acoustic noise, and ventilation, and received the same diet during the course of the experiment.

Food and water were kept in special open containers fixed on the walls inside the cages. The cages with the animals from all groups were fixed on supports inside the irradiation system. Cleaning and changing water and food was done for all animals twice daily. The irradiation system was switched off during cleaning the cage. During the experimental period, all animal groups were maintained in clean firsthand cages under standard condition in a separate laboratory which belongs to animal care unit. At the end of the exposure period the animals were sacrificed, and blood samples were collected from an eye vein in heparinized tubes then used for osmolarity and solubilization experiments.

2.2 Experimental setup

The animals were housed freely in a plastic cage at the center of a solenoid carrying current of 50 Hz from the main supply using a variac. The solenoid consisted of four coils connected in parallel of 320 turns from electrically insulated 0.8 mm copper wire. It was wounded around a copper cylinder of 2 mm thick, 45 cm diameter and 60 cm length. The cylinder wall is earthed to eliminate the electric field component inside the solenoid. The magnetic field at the animal exposure area was measured by a gauss tesla meter model 4048 with probe T-4048 (USA), manufactured by Bell Technologies Inc. (USA).252Cf point source of 50 µ gm (original activity 27 m Ci), purchased from Amersham radiochemical center (U.K), was used. The yield of the source in the date of the experiment is 5.4x104 n/s. The source was put in a special designed drawer that was fixed on the inner top wall at the center of the solenoid chamber to allow average homogeneous field to neutrons during magnetic field exposure, for group D. The average neutron dose rate at the animals was 10µSv/h as measured by a neutron monitor model NM2, manufactured by Nuclear Interprises, England from National Institute of Standards (NIS) of Egypt. The irradiation facilities of the animals were done in the Biophysics Department, Faculty of science, Cairo University.

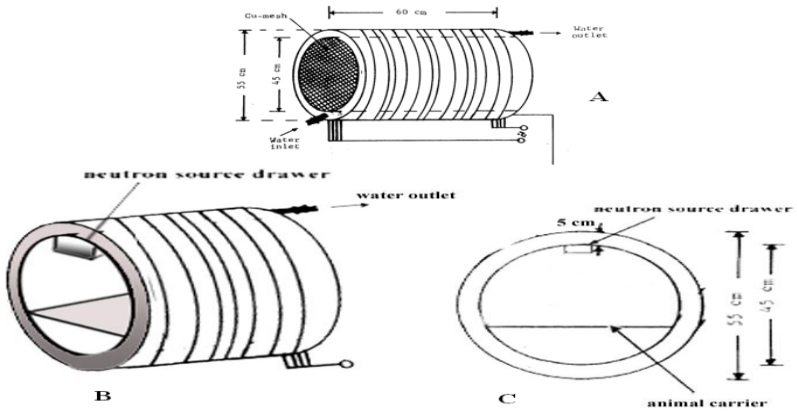

Figure (1): Irradiation facility for mixed neutron and magnetic fields (A) show the solenoid, (B and C) show cross section at the mid of the solenoid showing the position of 252Cf source.

2.3 Erythrocytes osmofragility

For the determination of osmotic fragility of erythrocytes, 5 blood samples were collected from 5 rats of each group. Blood was collected in heparinized tubes and the test was carried out within 2h of collection at room temperature according to the method described in Sir John and Lewis 1991. Fourteen test tubes each containing 5ml saline solution with a concentration range of 0.0-9.0 gm Nacl/L were prepared. Twenty-five µL of well mixed blood were added to

each tube and they were incubated for 30 min at room temperature. After incubation time, the suspensions were centrifuged for 5 min at at 3000 rpm for 10 min at room temperature (25±10C). Then, the absorbance of supernatant of each tube was measured at wavelength 550 nm using a spectrophotometer (6405UV/Vis (ultra-violet/visible) spectrophotometer JENWAY England). The lysis percentage was calculated by the relation

$$\% \text{ Hemolysis} = \frac{A_{sample}}{A_{100\%lysis}} \times 100. \quad (1)$$

Where Asample and A100%lysis are the absorbances of the hemoglobin released from erythrocytes incubated with different concentration of saline and that incubated with distilled water (100% lysis) respectively.

2.4 Erythrocytes solubilization

Whole blood was drawn on heparin anticoagulant tubes then 0.05 ml of the blood was transferred to a cuvette. The blood in the cuvette was then diluted by adding 1 ml of 0.9 % Nacl isotonic solution. The detergent solution was introduced to the cuvette through a micropipette, then stirring gently.

Solubilization of RBCs membrane was done using the nonionic detergent octylglucoside (OG) (SIGMA, U.S.A). The absorbance at 620 nm of the RBCs membrane as a function of detergent concentration was measured using the spectrophotometer (type 6405 UV/Vis, JENWAY, U.K). The absorbent wavelength was chosen since there is no absorption band for proteins, lipids or detergent at this wavelength and the change in the optical density of the sample is mainly due to light scattering at the surface of RBCs membrane which is a function of their size. The readings were taken after 15 seconds at each concentration.

2.5 Blood film

Blood films were prepared according to Brown [25]. The blood film was photographed by using an Automatic Image Contour Analysis system (SAMICA) (ELBEK GmbH, Zeiss Germany). The SAMICA system is provided with an electronic camera connected to a computer through an interface built in card and the image can be magnified up to 1200 times and displayed by the computer. The examination was done in National Institute of Standards (NIS) of Egypt.

2.6 Statistical treatments

The statistical analyses of the data were used according to Harnet [26]by calculating arithmetic means and standard deviations for RBC's osmotic fragility and solubilization data. All these measurements were done for the animals from all groups and the average reading of 5 runs were used to calculate the mean and standard deviations for each group. The differences between control and exposed samples were determined using ANOVA test.

3 Results and discussion

Figure (2) a, b, c shows plots for the osmotic fragility curves for one animal from each group A, B, C and D where the percentage hemolysis is plotted as a function of the percentage of sodium chloride concentration (NaCl%). For analysis of these results, the curves were

differentiated (%Hemolysis) and plotted as a function of NaCl concentration percentage as shown at the top of the right corner of each figure. The osmofragility differential curves for erythrocytes collected from animals of groups A, B, C and D appeared in main peaks whose half maximum width (Wh,max) represent the elastic range of the rbc's cellular membrane (22). The position of this peak (Cm) showed shifts for radiation exposed animals towards lower NaCl concentrations as compared with control A.

The average values with the statistical evaluation for Wh,max, Cs and Cm for the blood samples from the animals of groups A, B, C and D are given in Table (1-1,1-2,1-3) where Cs is the starting of the osmofragility. It is clear from the data that exposure to the magnetic, neutrons or mixed fields caused highly significant changes in the average values of Wh, max, Cs and Cm. The profile of the osmofragility curves for samples from group B, C and D differ from control.

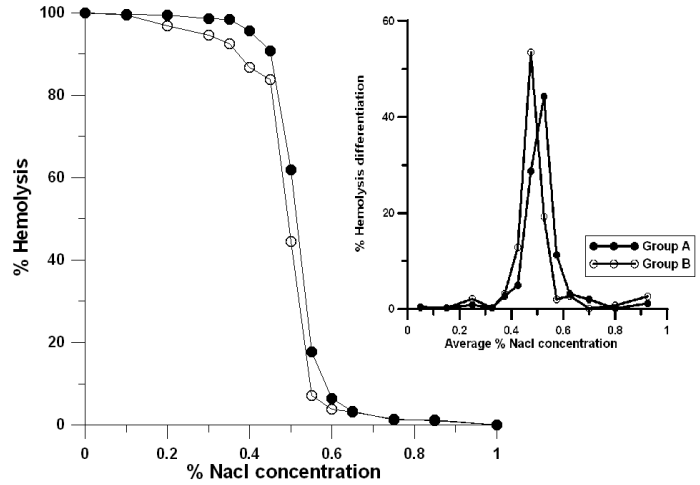

Figure (2-a): The variation of the percentage hemolysis of erythrocytes membrane as a function of the percentage Nacl concentration of one animal for group B vs. group A.

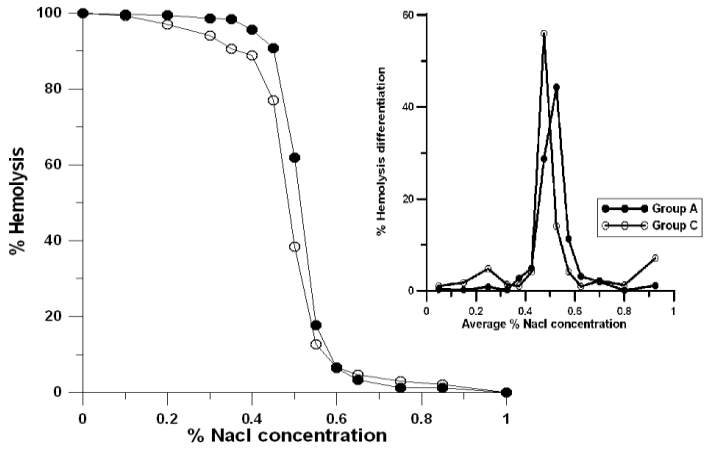

Figure (2-b): The variation of the percentage hemolysis of erythrocytes membrane as a function of the percentage Nacl concentration of one animal for group C vs. group A.

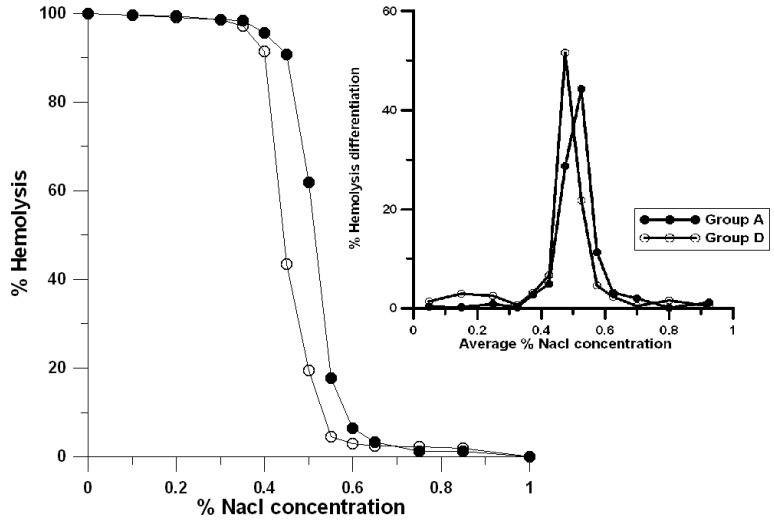

Figure (2-c): The variation of the percentage hemolysis of erythrocytes membrane as a function of the percentage Nacl concentration of one animal for group D vs. group A.

Table (1-1): *The average values of Wh, max, Cs, and Cm for blood samples from all animals of group B as compared with control.*

Exposure time (weeks)	Wh, max	Cs	Cm
1	0.065±0.005***a	0.600±0.039ab	0.525±0.018d
2	0.120±0.012d	0.600±0.066ab	0.485±0.042abc
3	0.105±0.013bcd	0.585±0.022ab	0.475±0.001*abc
4	0.085±0.001***a	0.585±0.022ab	0.475±0.001*abc
Time effect (ANOVA)	<0.0001	>0.05	<0.001
Group A	0.103±0.011	0.656±0.085	0.513±0.022

Table (1-2): *The average values of Wh, max, Cs, and Cm for all blood samples from all animals of group C.*

Exposure time (weeks)	Wh, max	Cs	Cm
1	0.07±0.0007***a	0.565±0.042a	0.445±0.027*a
2	0.119±0.021cd	0.575±0.035ab	0.465±0.022*ab
3	0.106±0.026bcd	0.615±0.022ab	0.465±0.022*ab
4	0.122±0.007***d	0.625±0.001ab	0.495±0.027bcd
Time effect (ANOVA)	>0.05	<0.05	<0.05
Group A	0.103±0.011	0.388±0.022	0.513±0.022

Table (1-3): *The average values of Wh, max, Cs, and Cm for all blood samples from all animals of group D.*

Exposure time (weeks)	Wh, max	Cs	Cm
1	0.123±0.010*d	0.6±0.056ab	0.455±0.027*ab
2	0.096±0.018*bc	0.595±0.027ab	0.485±0.042abcd
3	0.108±0.023bc	0.565±0.042a	0.475±0.035abc
4	0.085±0.008**a	0.585±0.042ab	0.455±0.045*ab
Time effect (ANOVA)	>0.05	>0.05	>0.05
Group A	0.103±0.011	0.388±0.022	0.513±0.022

The variation of the turbidity of erythrocytes membrane as a function of the OG in mMol of one animal from groups B, C and D as compared with control (group A) are shown in figures 3 a, b, c and d respectively. The solubilization curve passes through different stages which can be represented by the differential curves as being plotted at the top right corner of the curves. The solubilization results indicate the modes of interaction of the detergent at its different concentrations with the molecules forming the erythrocytes membranes. At low concentrations (region I) of the detergent, the extrinsic proteins are dissolved which causes the decrease in the intensity of the scattered light. At higher concentrations (region II) the detergent can dissolve the intrinsic proteins and then at region III it can co-corporate in between the phospholipid macromolecules forming the bilayer membrane. At this stage complete mecilization occurs to the membrane. These stages can be represented by the peaks in the differential curves in each plot. As can be noticed from the data, there are changes in the peak position for all irradiated groups. These changes reflect the structural changes in the packing properties of the molecules forming the membranes bilayer.

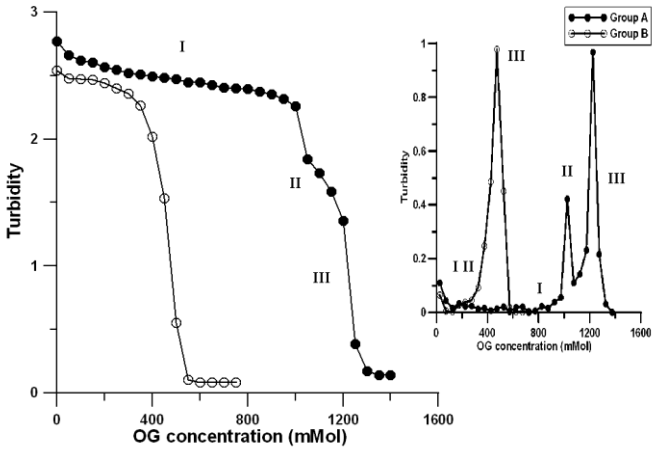

Figure (3-a): *The variation of the turbidity of erythrocytes membrane as a function of the OG in mMol of one animal from group B and group A.*

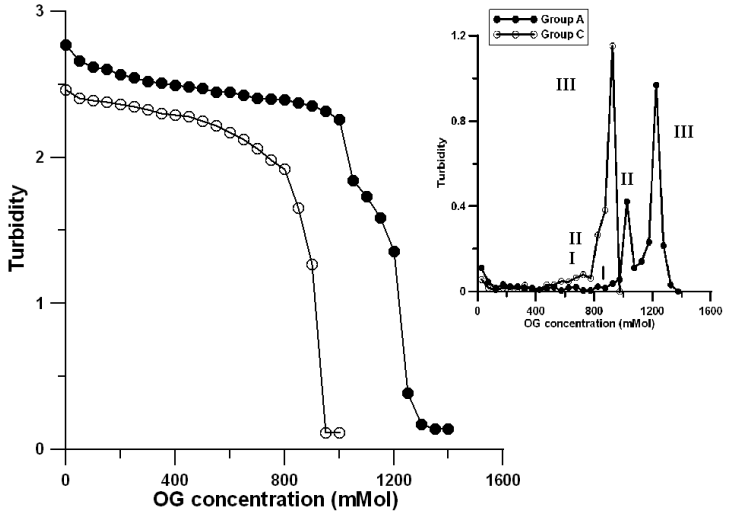

Figure (3-b): *The variation of the turbidity of erythrocytes membrane as a function of the OG in mMol of one animal from group C and group A.*

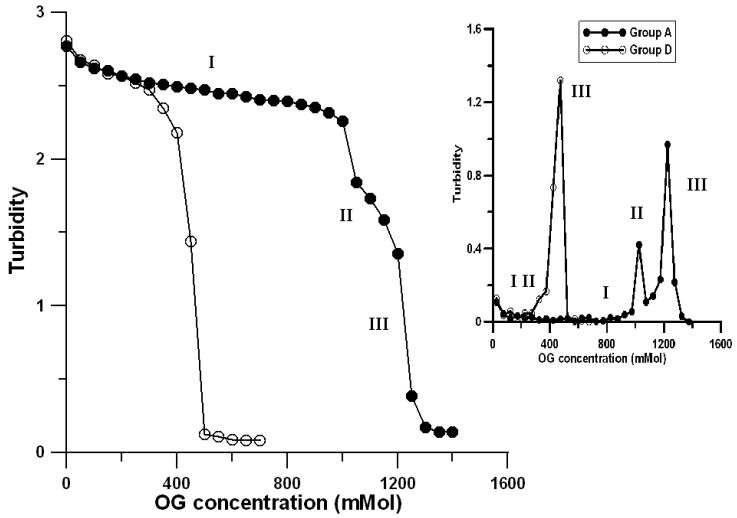

Figure (3-c): *The variation of the turbidity of erythrocytes membrane as a function of the OG in mMol of one animal from group D and group A.*

Table (2-1): *The values of the detergent concentration (mMol) for stages I, II and III for samples from exposed animals to the magnetic field collected at the end of each week.*

Exposure time (weeks)	Stage I (mMol)	Stage II (mMol)	Stage III (mMol)
1	275±10***b	375±10***b	475±25***b
2	225±25***a	330±20***a	475±25*** b
3	275±10***b	375±10***b	475±25*** b
4	225±25***a	325±25***a	475±25*** b
Time effect (ANOVA)	<0.05	<0.05	>0.05
Group A	830±10	1040±20	1225±25

Table (2-2): *The values of the detergent concentration (mMol) for stages I, II and III for samples from exposed animals to the fast neutrons collected at the end of each week.*

Exposure time (weeks)	Stage I mMol	Stage II mMol	Stage III mMol
1	520±10***d	627±2***d	475±25***b
2	527±2***d	627±2***d	475±25*** b
3	427±2***c	527±2***c	475±25*** b
4	727±2***e	827±2***e	475±25*** b
Time effect (ANOVA)	<0.0001	<0.0001	<0.0001
Group A	830±10	1040±20	1225±25

Table (2-3): *The values of the detergent concentration (mMol) for stages I, II and III for samples from exposed animals to mixed radiation field collected at the end of each week.*

Exposure time (weeks)	Stage I	Stage II	Stage III
1	225±5***a	330±20*** a	425±25***a
2	225±5***a	330±20*** a	475±25***b
3	270±20***b	370±20*** b	475±25*** b
4	220±20***a	330±30*** a	475±25*** b
Time effect (ANOVA)	<0.001	>0.05	>0.05
Group A	830±10	1040±20	1225±25

Figures (5) a, b, c and d show the blood film images as recorded by the image analyzer (SAMICA) for the erythrocytes collected from animals of the studied groups. It can be noticed from the blood films that the presence of irregularity in the erythrocytes membranes in addition to an observed sticking of several cells together forming one body in a single common membrane for all irradiated groups. The sticking of adjacent cells is marked by arrows.

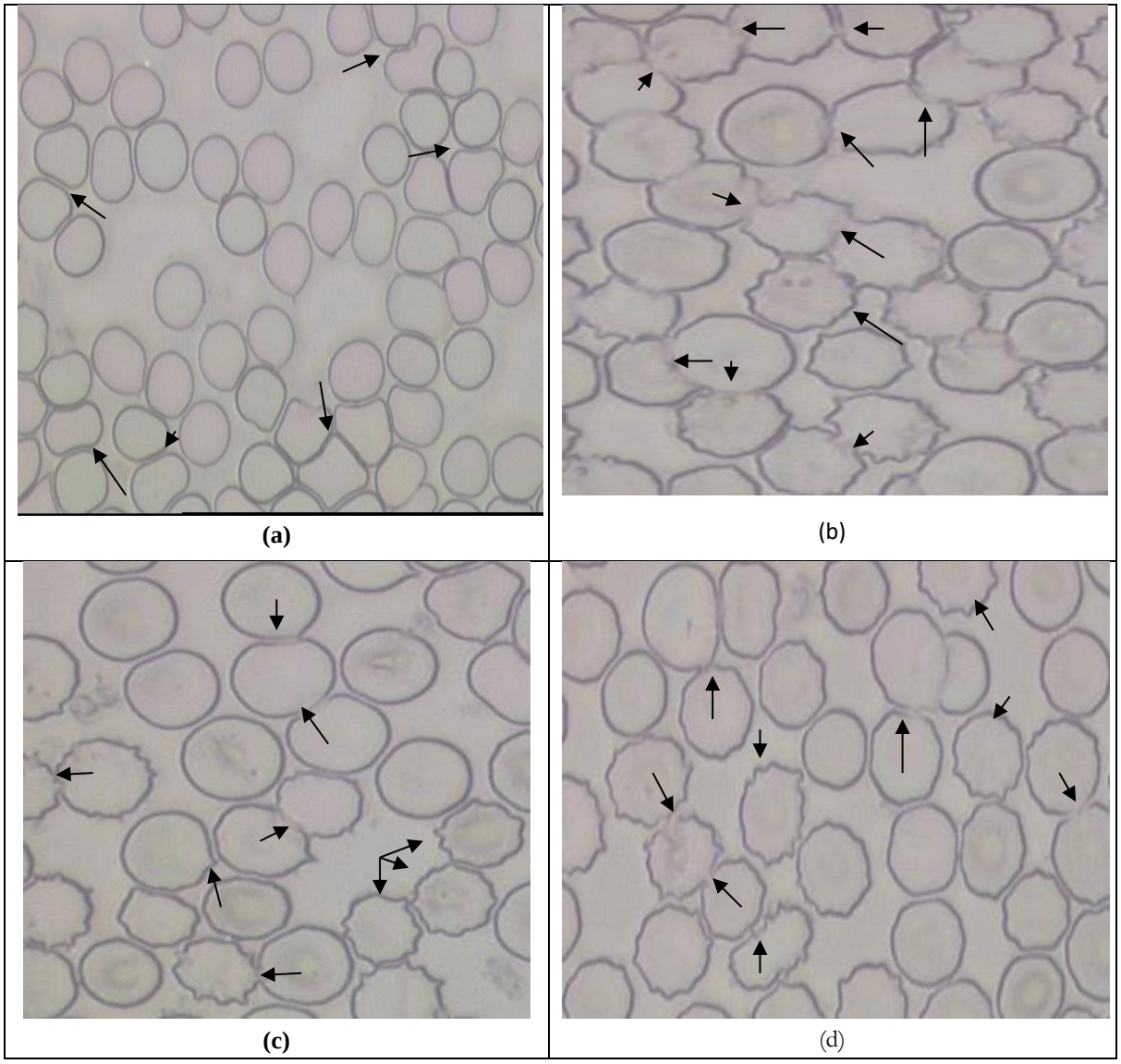

Figure (5): *The blood film studying the effect on the erythrocytes for (a) group A, (b) group B (c) group C and (d) group D.*

The results presented in this work indicated changes in the mechanical and morphological properties of the RBCs membrane of the rat's blood after whole body exposure of the animals to 50 Hz, 0.05 mT magnetic field, 1.6 mSv fast neutrons and combined magnetic and neutron fields as compared with control healthy group. The schedule of animal exposures was deigned to be similar to the working hours of the radiation workers which is 8 h/day and 5 day/week. The dose rate of fast neutrons received by the animals was 10μSv/h which is within the safe limits recommended by the ICRP-60.

The present data can be analyzed based on the basic interactions of radiation with biological systems. It may be presumed that the interaction of fast neutrons with biological systems is the formation of highly energetic nuclear recoils which are mainly the nuclei of hydrogen (protons), carbon, nitrogen and oxygen which are the nuclei of the atoms forming the biological material. One may not neglect the neutron capture reactions especially with chlorine and nuclei of the electrolytes. Since hydrogen atoms play the major role in the structure of the phospholipids macromolecules forming the cellular membrane bilayer and the neutron

scattering cross-section for hydrogen is relatively high (20.4 barns), the most neutron recoiled nuclei are protons. Since the logarithmic energy decrement per collision for hydrogen has the maximum value, which is unity, a neutron can transfer all its full kinetic energy to the recoiled proton in the case of head on collision. These highly energetic nuclear recoils will migrate in the hydrocarbon network forming further damages and highly active species. These chemically active sites will carry random recombination between adjacent molecules leading to the disturbance of the intermolecular forces in the cellular membrane. All these possible changes in the cellular membrane structure will result in changes in the packing properties of the phospholipids bilayer macromolecule and hence the membrane permeability and morphology.

Exposure to 50 Hz, 0.05 mT magnetic field, that accompanied electric field, can result in the formation of induced electric dipoles and the disturbance of the regular free motions of the head group in the phospholipids bilayer macromolecules [27, 14]. Moreover, the recombination reactions of the highly energetic active species which resulted from the neutron interactions can be changed under the influence of the magnetic field.

The remarkable change of the average values of Wh,max given in Table (1) for radiation exposed groups compared with control are considered as indicators for the loss of the intermolecular forces between the membrane phospholipids of macromolecules which caused changes in their packing properties. Such Changes in the cellular membrane permeability will affect the ionic pumps across the cellular membrane and hence disturb the normal electrostatic potentials and charges upon the surface of the cells.

In the normal cell the surface electrostatic charges form coulomb repulsive forces between adjacent RBCs and prevent their sticking. The loss of these charges was the main reason of the sticking of the RBCs and their fusion which was noticed in blood films for the irradiated groups. Since the diameter of the erythrocytes of rats is in the range of 12–16 μm and blood capillaries are in the range of 6–8 μm, the erythrocytes have to be folded to pass through blood capillaries in order to carry metabolic processes (28,29). The sticking of adjacent cells together in a way to have a common membrane and/or the loss of the cellular membrane elasticity that deteriorates the folding mechanisms, all, will not permit erythrocytes to pass through blood capillaries and hence fail to carry metabolic processes. Measurement of the electrophoretic mobility of erythrocytes is now the most convenient method for estimating these charges both in experimental studies and in clinical practice (30).

The changes in RBCs cell membrane were tested chemically by their interaction with detergent. The solubilization process induced the transformation of RBCs membrane into nearly flat phospholipids bilayer containing embedded proteins in the form of mixed micelles composed of detergent, phospholipids and membrane-bound proteins. For the phase transformation (micellization) to occur, the added detergent distributes between the membrane bilayer and the aqueous medium (31). The nonionic detergent at low concentrations will dissolve the extrinsic proteins of the erythrocytes membrane which partially work as signal receptors of the cell. At higher concentration the detergent begins to incorporate within the bilayer macromolecules forming the RBCs membranes and then dissolute their intrinsic proteins. When the incorporation of the detergent molecules is completed, (at relatively high concentrations) solubilization of the macromolecules takes place and followed by membranes rupture that revealed by a pronounced decrease of the scattered light and formation of the main peak (differential curves). As can be noticed from Fig. 5, the profile of the solubilization curves is

changed; these changes indicate the disturbance in the phospholipids packing properties of the cell membranes molecules. The detergent concentration at which the characteristic dissolution peaks occur were calculated for each animal from the studied groups then the average was illustrated (Table 2). As noticed from Table (2) a marked changes of the values of Wh,max indicate the increase of the elasticity range of the cell membranes. The significant changes in the profile of the solubilization curves for erythrocytes collected from all the exposed groups compared to the control showed that receptors on the surface of cells suffered structural changes upon irradiation which led to loss in their functions. Such functional failures of the receptor may affect cell to cell communication and consequently the animal's metabolic processes.

All the fore-mentioned interacting parameters will affect the metabolic functions of erythrocytes which may lead to anemic diseases.

5 Conclusion

It may be concluded from the present findings the following:

1. Exposure to 50 Hz, 0.5 G magnetic field for occupational workers is risky and deteriorates the physiological functions of the red blood cells.
2. On the calculation of the radiation dose, it is recommended to differentiate between doses received from radioactive isotopes and from nuclear generating facility.
3. Periodic medical examination of the radiation occupational workers should include the test of red blood cells morphology and functions. Counting of blood is unsatisfactory to inform about the radiation injury.

6 Declarations

6.1 Acknowledgements

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6.2 Competing Interests

The authors declare that there is no conflict of interest.

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