EFFECTS OF FAST NEUTRONS AND GAMMA RADIATION ON SOME BIOPHYSICAL PROPERTIES OF RED BLOOD CELLS MEMBRANE OF ALBINO RATS (IN VIVO STUDY)

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The present work aim to study the radiation hazard through measurements of possible changes on some biophysical properties of red blood cell membrane of albino rats in vivo study. The traditional methods for evaluation of the radiation risk for occupational workers includes medical examination in which, blood counts are included. Routinely controlled by personal dosimeters exposures of individuals are not permitted to be higher than the limits recommended by **ICRP 60**.Counting of red blood cells is unsatisfactory to represent the radiation hazards. The biophysical structural functions of blood plays the major role to represent the injury occurred to the system. Sixty male albino rats were equally divided in to three groups namely A, B, C. Animals of group A used as a control group and didn't receive any treatment and housed at normal environmental conditions. Animals of groups B was used for the study of γ-rays effects. The γ-dose rate from the Am^{241} source was 16 μ Sv/h at the irradiation facility. Animals of groups C was used for study of neutrons and gamma effects. The average neutron component of the dose rate was 3 μ Sv/h. Osmofragility of the r.b.cs, blood film were carried for each collected blood samples. The blood viscosity and solubilization of the membrane by non ionic detergent (octylglucoside) were also measured. The results showed decrease in the average osmotic fragility and average membrane solubilization. The effects of radiation on the red blood cell membrane were discussed.

Key words: Red blood cell membrane, radiation, osmotic fragility, blood morphology, blood viscosity, membrane solubilization.

INTRODUCTION

There is an increasing interest about the possible health effects associated with exposure to ionizing radiations. Most human exposures are to γ-radiation and fast neutrons or mixed radiation fields, which are present in both residential and work place environment. It was found that the radiation effects on the red cell membrane from three different but correlated properties: electrical, mechanical and chemical, and to derive useful parameters for the evaluation of radiation effects. AC conductivity of cell suspension was measured in the frequency range 40 kHz to 5 MHz, the osmotic fragility of the membrane and solubilization of the membrane by detergent were also measured. Adult male rats were exposed to 1, 2 .5, 3.5, 5, 7 and 9Gy gamma radiation from Cs^{137} source. The results showed decrease in the AC conductivity, average osmotic fragility and average membrane solubilization [1].

On the other hand, Fadel et al. [2] investigated that radiation exposure can damage living cells, causing death in some of them and modifying others. Potential applications have been reviewed by Green stock et al. [3] to correlate membrane damage to observed doses of radiation Shish kina et al. [4] concluded that exposures to low doses of gamma

and X-rays showed a high sensitivity of characteristics of the lipid metabolism in erythrocyte. Allehyanim et al.[5] studied the effects of γ-rays in the dose rate range 0-5.6Gy on RBCs membrane solubilization, of rats erythrocytes using sodium dodecyl sulfate (SDS). The results showed that shift in the detergent critical concentration of irradiated dose Blood films for in-vivo rats showed irregular shaped red blood cells, while those photographed 10 days post irradiation showed some sort of repair. Red blood cell is not a very radiosensitive cell, thus choosing it is not a reflection of cellular radiation damage *in vivo* Kergounou et al. [6].

Gamma irradiation of red blood cells induces alterations at three different functional units of the membrane: lipid bilayer, protein components and cytoskeleton at the membrane surface [7]. In addition, radiation induces shortening in the lipid fatty acid chains by lipid peroxidation [8]. The production of hydro peroxides and cross-linkages in the membrane lipids can disorder the upper region of the bilayer favoring penetration of water and ending by hemolysis [9]. The osmotic fragility of the membrane can be measured by placing the red blood cells in hypotonic salt solutions, the osmotic pressure exerted by the diffusion of water into the cells, makes them first swell and then hemolyse. The osmotic fragility measures the capacity of the cells to withstand hypotonicity and resist hemolysis, which is determined by their volume to surface area ratio [10]. The solubilization of the membrane by detergent is an induced transformation of the phospholipids bilayer and the proteins into mixed micelles of composed detergent, phospholipids and membrane-bound proteins. This phase transformation depends on the molecular structure of the detergent and the composition of the membranes Lichtenberg et al. [11].

1. MATERIALS AND METHODS

1.1. Experimental animals. Sixty male albino rats of average weight 200±250g each and age ranging from 2 to 2.5 months were divided to three equal groups. Rats were kept under standard conditions along the experimental period, 12/12h light-dark regimen. Food and water were supplied daily. Group A was used as control. Group B were exposed to gamma radiation from Am^{241}source for a period 8h/day 7day/week for three weeks, at rate16μSv/h. Group C were exposed to neutrons from Am-Be source at rate 3μSv/h.

1.2. Exposure facility. Neutron source. For the production of neutrons from the Am^{241} source, the source was covered from one face by a beryllium target (Be). As a result of the interaction of the α - particle emitted from the Am^{241} source fast neutrons were produced with average energy 4.5Mev. The reaction rate of the α - particles with Be for the production of fast neutrons is each 10^6 α - particle can interact with Be nuclei to produce 70 neutron [12]. Fig.1. presents sketch diagram for the irradiation facility. The average dose rate of fast neutrons inside the cage was found to be 6μSv/h.

1.3. Blood sample collection method. At the end of exposure periods, each group of animals was anaesthetizes with ether, and then blood samples were collected by heart puncture on heparin containing tubes. Whole blood was divided into two parts; the first part was taken for whole blood viscosity measurements and blood film morphology. Blood film was visualized through the use of image analyzer type Nikon Photo Head V-TP with camera Nikon Ellipse E800 manufactured by Japan, the magnification of the image was chosen to be 1000 times. The second part of blood was centrifuged at 3500 r.p.m for 15 minutes, and the plasma was removed then the thin layer of white blood cells was

separated. The remaining red blood cells were washed by saline solution three times to have clear red blood cells, which was divided into two parts for further experimental (osmo-fragility and solubilization) studies.

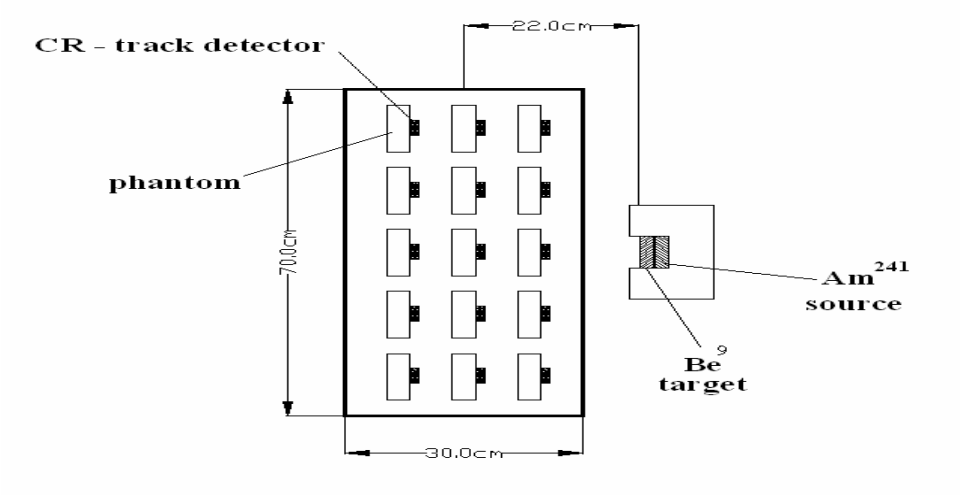


Fig.1. Sketch diagram for the irradiation facility

1.4. Normal red blood cell hemolysis. Normal red blood cells hemolysis was determined by measurement of hemoglobin released from the cells relative to the total cellular hemoglobin content. Ten μ L of whole fresh blood was incubating in 5mL normal saline for 30min.The samples were centrifuged at 3500 rpm for 15 min, and the supernatant was measured spectrophotometrically at 540nm. The percentage of hemolysis was taken against complete blood hemolysis [13].

$$\%H=\frac{A_{sample}}{A_{100\%lysis}}\times 100,$$

where A_{sample} and $A_{100\%\text{lysis}}$ are the absorbances of the hemoglobin released from red blood cells (RBCs) in normal saline and after complete hemolysis in distilled water respectively

1.5. Osmotic fragility measurements. The degree of hemolysis can be quantitatively evaluated from the osmotic fragility test [14]. Whole blood was added to varying concentrations of buffered sodium chloride and allowed to incubate at room temperature for 30 min and centrifuged at 3500rpm for 15min, spectroscopically using a spectrophotometer (model 6400, Jenway, England) to precipitate the nonhemolyzed red blood cells. The osmotic lysis of red blood cells is detected by the release of hemoglobin into the extracellular fluid. The amount of hemoglobin appearing in media was determined colorimetrically according to the method reported by Dacie and Lewis [15].

1.6. Membrane solubilization test. Solubilization of RBCs membrane was done using the nonionic detergent ctylglucoside (OG from SIGMA) . Its high solubilizing capacity is related to its ability to form mixed micelles with membrane lipid and proteins. The absorption of the RBCs membrane as a function of detergent concentration was measured using an UV/visible spectrophotometer LKB at 620 nm. The detergent solution is introduced to the cuvettes (containing 1ml RBCs. at the desired concentration) through a

micropipette, then stirring gently. The reading was taken after 30 seconds to permit the stability of the absorbance) [16].

1.7. Blood viscosity. A viscometer type Wells-Brookfield Cone/Plate DV-II manufactured by Brookfield Laboratories, Stoughton,MA). The temperature of the sample controlled by a refrigerated circulating fluid bath during measurement of the viscosity over a range of different temperatures [17].

1.8. Preparation and staining of blood film. The films were stained with Leishman stain for 5 min. The slides were then rinsed with phosphate buffer solution until they were acquired pinkish color. Blood film was then examined with the microscope at a magnification x 1000 (using Olympus Binuclear Microscope provided with digital camera type Ms.t 11-4d made in Japan with interface to be viewed by computer).

1.9. Statistical evaluation. Statistical analysis for evaluation of the results was done by calculating arithmetic mean and standard deviation for red blood cells and hemoglobin measurements. All these measurements had been done for all groups. Results were expressed as mean ± standard deviation for each group. The results were evaluated by Student's unpaired t-tests.

2. RESULTS AND DISCUSSION

There is a growing concern about the hazards associating exposures of radiation workers to γ-rays, fast neutrons, or mixed radiation fields of them. This is due to the pronounces increase of uses of radioisotopes and nuclear generators in medicine and manufacture. Received doses of radiation workers are normally controlled by personal and area monitoring system as the allowable maximum annual and quarter dose limits are controlled according to the ICRP-60. Periodical medical examination is one of the requirements after the safety of the radiation workers which included counts of blood. The quiry is that, do counting of blood gives a satisfactory evaluation for the radiation hazard? The procedure followed in this work was all focused on red blood cell functions and morphology. The used radiation doses were all in the allowable limits recommended by the ICRP. No change in blood counts for animals received 3.56mSv as compared with control group. However, the data concerning ,osmofragility studies ,solubilization of RBCs with nonionic detergent, viscosity and RBCs morphology showed dramatic changes in all the measured properties. The interaction of γ radiation with a live biological tissue results in the formation of chemically energetic ions and molecules which may recombine again at random causing new molecular forms may cause changes in the structure of the cellular membrane and interconstituents of the cells and hence their physiological properties .The interaction of fast neutrons with biological material is the formation of nuclear recoils, protons (from hydrogen atoms), carbon, nitrogen and oxygen nuclei. Since the neutron scattering cross-section with hydrogen is relatively high, most of the recoiled nuclei will be the protons. Such recoiled protons with receive high energies from the recoiled fast neutrons and they will migrate in the hydrocarbon net work causing deficient regions. Since the logarithmic energy decrement per collision for hydrogen is equal to unity there is a probability for the recoiled proton to receive the whole neutron energy during a head on collision process. Since hydrogen form the main binding system for the structure of all cellular contents (proteins and phospholipids), the recoil of hydrogen nuclei will cause

pronounced damage to the structure of the macromolecules forming the biological cells, especially the cell membrane. Therefore, one may expect as a result of neutron interactions with biological system is the formation of free high active species which may recombine with each other or with other's macromolecules forming new structural sites. One may not neglect the formation of deficient regions, resulting from the lose of hydrogen protons. All these effects may give a clear idea about the changes in the structural properties of RBCs following the exposure to ionizing radiation. The formation of the highly active species in the hydrocarbon chains in the bilayer membrane of the RBCs will disturb the packing properties of the phospholipid bilayer macromolecules forming the cell membrane. Changes in the packing properties of these molecules will result in changes of the intermolecular Vander Val adhering forces forming the cellular membrane and hence the membrane permeability and morphology. There changes in the intermolecular forces will form something tibe separated islands of forces and arrangements throughout the cell membrane and hence changes in the membrane permeability which can be noticed from the Fig.2 which shows the results of osmotic fragility measurements for the RBCs collected from one animal randomly chosen from each group for analysis of these results ,the curves were differentiated and plotted as a function of NaCl concentration percentage as shown in Fig.3.

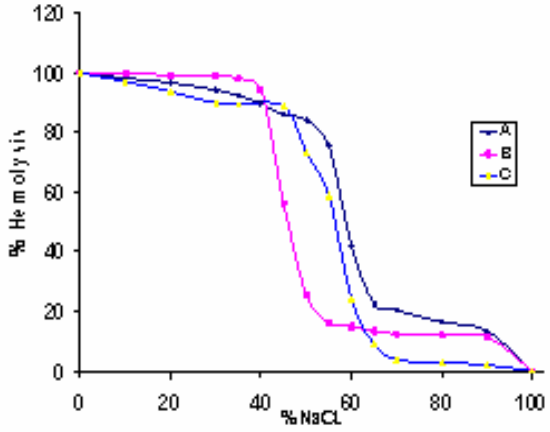


Fig.2. Variation of the hemolysis percentage of the RBCs from group A, B and C as a function of NaCL Concentration percentage

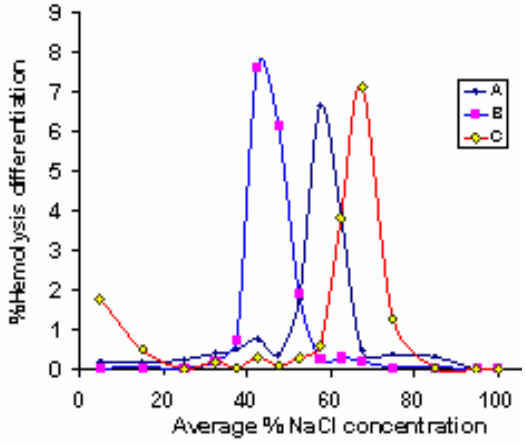


Fig.3. Osmofragility differential curves for samples from group A, B and C

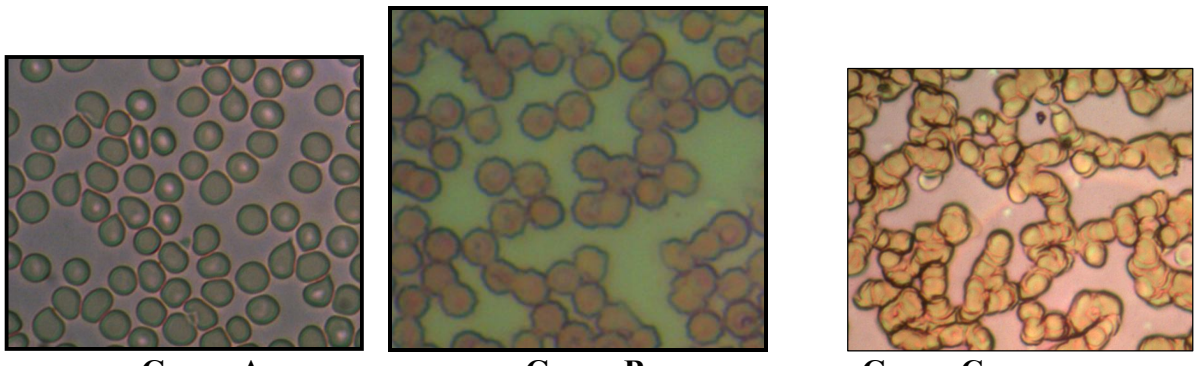
As a result of this treatment each characteristic plot in Fig.2. was represented by a peak in figure.3. Whose width indicates the elastic range of the RBCs cellular membrane. The percentage of the NaCL(C_S) at which hemolysis starts to occur characterizes the transport of water molecules through the RBCs membrane and hence its permeability. The decrease of the value of C_S indicates the decrease in the membrane permeability. The values of widths at half maximum W_{hmax} of the osmofragility from groups B and C as given in table(1). Can be indicated a decrease in the values of W_{hmax} which indicates a decrease of the cellular membrane elasticity which resulted from the lose of the intermolecular adhering forces in the cellular membrane [18]. Therefore decrease of the RBCs membrane elasticity will lead to the increase of the of the blood capillary resistance to passage of the RBCs to the cell of the body for caring normal metabolism. The remarkable decrease in the

elasticity of the RBCs membrane may lead to a growing resistance of the blood capillaries to the passage of the RBCs and hence toxicity in some organ could occur. In **Table 1** the average value of C_s and W_{hmax} for RBCs for each group is given. The result indicates that both RBCs membrane elasticity and permeability had been decreased due to exposure of the animals to ionizing radiation.As it is will known, RBCs cellular membrane carry a positive electric charge upon their external surface for their normal metabolic activity. These positive charges form electric coulomb repulsive forces between adjacent cells from being stucked together as shown for alls from control group. The formation of these positive charges on the surfaces of the RBCs are a result of the natural ionic pumping of the positive potassium ions from inside of the cell to the surface leaving the inner surface with an electrically negative Cl^- charge. This is the normal resting potential of the cell with normal cellular membrane.` Changes in the cellular membrane permeability as a result of the changes in the packing properties of the macromolecules forming the cell membrane will result in the increasing possibility of the passage of the whole KCl molecules from inside the cell (high concentration) to the surface (lower concentration), which will cause the decrease of the resting potential and even its disappearance. All these will cause the disappearance of the surface coulomb repulsive forces between the cell membrane, and permits the stick of adjacent cells together to form group of sticked cells with common membrane. These results are supported by the blood film image in Fig.4.

Table 1. **The average value of the Cs, C_{max}, $W_{h\,max}$, $H_{50\%}$ were calculated for all animals from each group**

Parameters		**Control group (A)**	**Gamma exposed group (B)**	**Gamma-neutron exposed group (C)**
C_s	Mean	72.1978 ± 4.9812	64.045 ± 4.9619	59.3243^b ± 3.2173
	Max	76.50	74.56	66.26
	Min	62.39	59.26	55.56
C_{max}	Mean	51.7560 ± 1.6964	58.3160^a ± 4.8667	44.4220^b ± 2.5292
	Max	54.02	62.29	41.92
	Min	50.15	52.57	47.43
$H_{50\%}$	Mean	57.5967 ± 0.9537	$44..0729^a$ ± 3.4324	35.2540^b ± 5.7990
	Max	75.28	54.12	35.09
	Min	49.31	31.73	19.92
W_{hmax}	Mean	10.6300 ± 1.2810	7.5829^b ± 1.9157	6.3743^b ± 1.3625
	Max	12.27	10.86	8.92

a Statistical significance is considered to be statically significant.
b Statistical significance is considered to be very statically significant.



Group A **Group B** **Group C**

Fig.4. Blood films for control group A and exposed group B and C. The irregularity and morphological changes in the membrane for the exposed groups B & C are clear

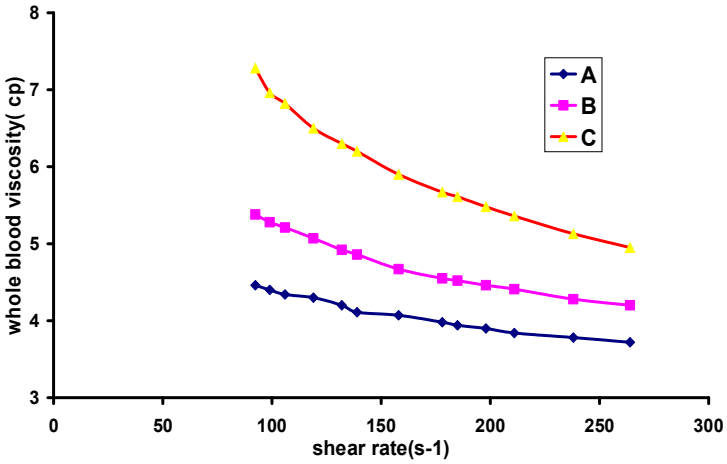


Fig .5. Shows the variation of blood viscosity (cp) as a function of shear rate(s^{-1}) for group A,B and C

Fig.5 illustrates the variation of viscosity for the whole blood collected from one animal from each group A ,B and C as a function of the shear rate(s^{-1}). All these will result in the formation of clots,Moreover, the sticking of adjacent cells together will cause the increase of the blood viscosity, a phenomena,which was markedly noticed in Table 2.

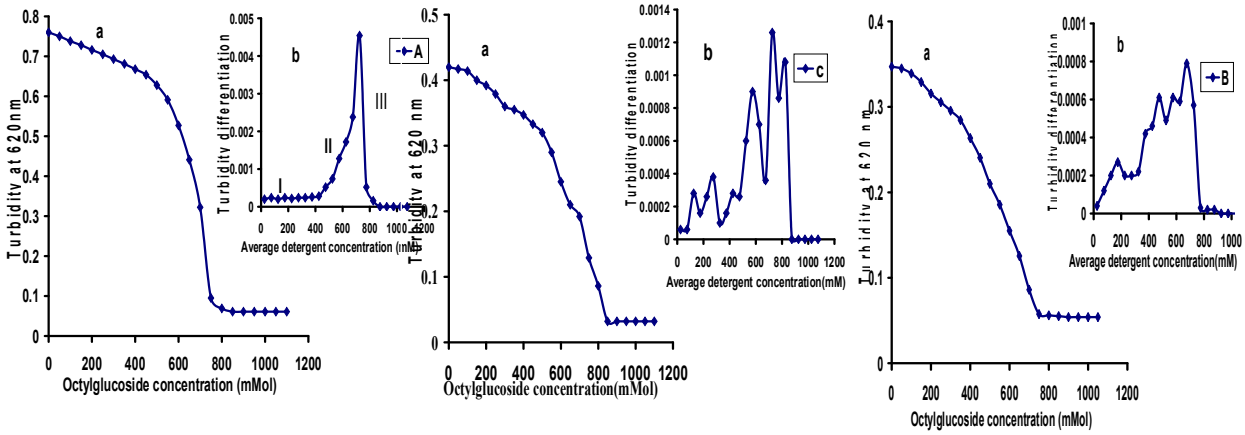


Fig 6.The Turbidity at 620nm as a function of detergent concentration mM (a) and its differential curve (b) for groups A,B, and C respectively

CONCLUSION

Counting of blood in the medical examination for occupational workers is inadequate to evaluate the radiation hazards 2- It is necessary to measure the blood viscosity for radiation workers. Blood clot formation is not only indicated by measurements of the Thrombin level in blood, which is the running technique, but also by the loss of surface electric charge of the cells. 3- Moreover it is necessary to investigate the blood film, RBCs osmofragility and solubilization and use a stander chart for these investigations (e.g. blood samples from healthy control group) as a reference for any changes. 4- Exposures to ionizing radiation in the permissible range(ICRP-60) still have hazardous effects on RBCs cellular membranes biophysical properties and functions also cause blood anemic disease. It is necessary to review the dose limits recommended by the ICRP-60 for radiation workers based on the present findings.

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