SPECTROSCOPIC STUDY OF THE EFFECT OF ALPHA TOCOPHEROL ON ERYTHROCYTES IRRADIATED WITH NEUTRONS

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Abstract. The data presented in this *in vivo* work aimed to evaluate the hazards of exposure to very low doses of fast neutrons using biophysical parameters as well as spectroscopic techniques. This study concerned one of the most important organs, the blood. Furthermore, the study aimed to investigate the effect to IP injection with the natural antioxidant, vitamin E, on the neutron irradiated animals. Vitamin E is known as a scavenger for free radicals. Moreover, we followed the delay effect of neutron irradiation on the structure of hemoglobin and membrane elasticity.

Key words: neutrons, RBCs, FTIR, UV/visible spectroscopy, Hb, membrane elasticity.

INTRODUCTION

Hemoglobin is a 65,500 M.W. metalloprotein and comprises four subunits, each one having one polypeptide chain and one prosthetic group, the heme. The heme group is a protoporphyrin ring at the centre of which there is an iron atom in the ferrous state [2]. Oxyhemoglobin has remarkable oxygen transportation properties where it can change its conformation to accept oxygen. This process can be inhibited by carbon monoxide, which has a 200 times stronger affinity for hemoglobin (carboxyhemoglobin) than oxygen, resulting in severe respiratory problems and death in cases of carbon monoxide poisoning. Hemoglobin possesses an iron atom core in its ferrous (Fe^{2++}) state. If the iron is oxidized to its ferric (Fe^{3++}) state, its oxygen transport capabilities are diminished and the hemoglobin molecule is then called methemoglobin (metHb). The main role of the hemoglobin subunit is to provide a fixed environment for the heme group so that a reversible reaction with oxygen can take place. This role is reflected in the tertiary structure of the subunit [21].

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Several works have been done to study the structure of hemoglobin and its biological functions [27]. Detailed information has been provided about the molecular origin of the heme-heme interaction (the increase in the affinity of the fourth bound oxygen relative to that of the first bound oxygen). Simon and coworkers compared the structures of the high and low oxygen affinity states of hemoglobin. They suggested that in the low oxygen affinity state, the protein pulls the iron away from the heme plane, and opposes the transition to the low spin state which is needed for combination with oxygen [20].

Several works were done to study the effect of radiation on blood and hemoglobin. Szweda-Lewandowska, 1976 [22] studied the effects of gamma radiation on the aqueous hemoglobin solution and demonstrated that radiation injuries involve both the heme prosthetic group and the protein part of the macromolecule. The investigations mainly concerned the processes of iron oxidation and reduction as well as the degradation of the porphyrin ring, which have been studied by examination of the absorption spectra in the visual range. In 2003, Rajinder Pal [13] predicted that the decrease of the particle rigidity reflects a decrease in relative viscosity. O.S. Desouky *et al*., 2009, have studied the effect of gamma radiation on the whole blood; the study proved that the exposure to different doses of radiation increases the blood viscosity and decreases the membrane surface charge density [16]. Again, in 2010, O.S. Desouky *et al.* have used electron microscope and dielectric properties to prove that the gamma irradiation of red blood cells cause the decrease of the conductivity and relative permittivity [17]. Whereas, the investigation of radiation induced Hb structure changes attracted investigators, little has been done to study the effect of neutrons on the blood, however, Grahn has pointed out that the Skylab crews received an average of 75 millirads per day of radiation of mixed qualities [8]. According to Grahn [8], the number of humans exposed to space radiation is expected to increase over the next decade.

This *in vivo* work was tailored to study the effect of very low neutron doses on the electronic and vibrational structure of Hb and the fragility of red blood cell membrane.

MATERIALS AND METHODS

IRRADIATION FACILITY

The neutron source was ^{241}Am-Be source. It contains about 0.2 TBq (5 Ci) ^{241}Am. Also, it has a neutron output of $q = (1.1–1.4)\times10^7$ s^{-1} [15]. This source is made as a compact mixture of americium oxide and beryllium powder. The ^{241}Am-Be spectrum has been reviewed by Weise and Kluge [27], 1982. The neutron spectrum extends over the range from approximately 1 to 10 MeV, with most of the neutrons between about 3 and 6 MeV.

EXPERIMENTAL SET-UP

32 male rats were used in this experiment; they were born and housed in NRC's animal house. Rats' ages, at the beginning of the experiment, were between 4–5 months and their weights were 250±30 g. Rats were divided into 2 main groups: group one is control non-irradiated, and group two is irradiated with three different doses of fast neutrons. Each group is subdivided into two categories as follows: control, not injected with vitamin E (alpha tocopherol) and intraperitoneal (IP) injected with vitamin E dissolved in chloroform (alpha tocopherol, Sigma Aldrich) 25 mg/g rat weight, once 12–14 hours prior to exposure [12, 23].

The irradiated group is subdivided into 3 subgroups A, B, and C, which were irradiated with neutrons from the ^{241}Am-Be source with doses 0.1, 2, and 10 mSv respectively. These groups are kept alive for 45 days to study the delay effect of radiation on the bone marrow. The blood of animals was collected at two time points: immediately after irradiation, and after 45 days of irradiation.

PROCEDURES OF HEMOGLOBIN EXTRACTION

Animals were anaesthetized with ether. Blood samples are collected on heparinized tubes using capillary tubes from a vein near the eye. After the collection of blood, it is centrifuged for 20 minutes at 3000 rpm at 4°C.

Hemoglobin was extracted following Trivelli method. RBCs are washed using 5 volumes saline solution (0.9% NaCl) then centrifuged at 3000 rpm for 10 minutes. These washed RBCs were lysed using 2 volumes of distilled water and kept at –20 °C over night. Hemoglobin is extracted and kept frozen for further investigation.

UV/VIS MEASUREMENTS

Model V-570 UV/VIS/NIR Spectrophotometer is used to measure the absorption spectra. The instrument is specified by resolution 0.1 nm and wavelength accuracy ±0.3 nm (at a spectral bandwidth of 0.5 nm in the UV/VIS region), single monochromator, UV/VIS region 1200 lines/mm plane grating, NIR region 300 lines/nm plane grating, Czerny-Turner mount, double beam type. This model measures the absorption spectrum in the wavelength of range 190 to 2500 nm. A deuterium discharge tube (190 to 350 nm) emits light in the ultraviolet region and a tungsten lamp (340–2500 nm) emits in the VIS / NIR region as light source. The light from the light source is converged and enters the monochromator. The grating in the monochromator disperses it and the light passing through the exit slit is monochromatic. This light is split into two light paths by a sector mirror,

one incident on the sample to be measured and the other on the reference sample such as solvent or other. The light that passed through the sample or reference sample is incident on the photomultiplier tube or PbS photoconductive cell. Ultrasonically dispersed solution was used for transmission measurements.

OSMOTIC FRAGILITY TEST

In the osmotic fragility test, whole blood was added to varying concentrations of buffered sodium chloride solution and incubated at room temperature. The amount of hemolysis was then determined by reading the supernatants at wavelength 550 nm on the spectrophotometer (UV/Visible spectrophotometer JASCO V-530, Japan). A normal control blood as well as the irradiated blood were tested at the same time [18].

The present hemolysis was calculated for each supernatant as follows:

$$H\% = \frac{OD \text{ of supernatant}}{OD \text{ of supernatant no.14}} \times 100 \quad (1)$$

where OD = optical density and $H\%$ = percent hemolysis.

The percent hemolysis ($H\%$) was then plotted as a function of the percentage of sodium chloride concentration (NaCl%).

UV-VISIBLE SPECTROSCOPIC STUDIES

The Hb samples were diluted using de-ionized water to a Hb/water ratio of 1/100. Then the spectra of the samples under investigation were recorded using spectrophotometer model JASCOW UV/VIS/ NIR V-570 at National Research Center (NRC). The range of spectrum is between 250 to 650 nm with a reference sample of de-ionized water. The absorbance values were determined using the baseline method. In this method, a vertical line was dropped from the absorption maximum to the arbitrary tangent line drawn between the two wings of the band. The height of this line represents the actual absorbance value of the absorption band.

FT-IR SPECTROSCOPIC STUDIES

The FT-IR spectra were recorded for the lyophilized [using lyophilizer model Snijders single-stage LY5FM-RB (Snijders Scientific b.v., made in Holland)] hemoglobin using KBr pellet technique. In this technique, 2 mg of the hemoglobin samples were roughly mixed to 198 mg of KBr to get 1% sample concentration suitable for obtaining a good spectrum. The mixing takes place for considerable time in an agate mortar with pestle. The mixture was pressed in a special die under

vacuum hydraulic press to form a transparent disc. A pure KBr disc of the same sample weight and thickness was used as reference.

In this work, a JASCO FTIR 420 SPECTROMETER was used for recording the spectra of the samples. The spectrum measures the absorbance of the samples against the wave number 4000–400 cm^{-1}. For quantitative analysis, the precise absorbance values were determined using the baseline method. In this method, a vertical line was dropped from the absorption maximum to the arbitrary tangent line drawn between the two wings of the band. The height of this line represents the actual absorbance value of the absorption band.

RESULTS AND DISCUSSION

UV/VISIBLE SPECTRUM OF NEUTRON IRRADIATED HEMOGLOBIN

The absorption spectroscopy is one of the useful tools that can be used to study the molecular energy level at ground and excited states. Furthermore, it can give some information about molecules conformational changes. The detailed information of the energy band structure of hemoglobin molecule will depend on its conformation when located in the exact environment [19].

Figure 1 shows the UV/visible spectrum of normal hemoglobin. The spectrum shows the presence of six peaks at 225 nm, 270 nm, 350 nm 415, 550 nm and 570 nm. The two peaks at 225 and 270 nm are the characteristic aromatic absorption bands, both bands probably arising from $\Pi \rightarrow \Pi^*$ of aromatics. The absorption band at about 350 nm is due to the absorption by the non-covalent bond between iron and histidine of the protein part. The Soret band which appears at about 415 nm is attributed to $\Pi \rightarrow \Pi^*$ transition of the porphyrin ring. The two bands at about 570 and 550 nm are attributed to the occurrence of the oxy-hemoglobin (HbO_2) [6, 9, 14, 19].

At the first glance the absorption spectrum of hemoglobin molecule, a significant hyperchromicity appears for all the hemoglobin bands for all doses of direct and delay effect of neutron irradiated rats treated and non-treated with vitamin E, Table 1. A primary test of the hemoglobin spatial distribution is to test the position of the porphyrin ring relative to its globulin. This can be done by calculating the ratios of the Soret band (recorded at 414 nm) to the band of aromatic side chain (recorded at 271 nm) and to the Q_o band (recorded at 574 nm) as shown in Table 2. The direct effect of radiation on the A414/A270 ratio of the hemoglobin of non- treated with vitamin E animals, a significant increase appears in the ratio of the Soret band relative to the aromatic band, compared to the control non-treated. Moreover, the value of the ratio for the 0.1 mSv group is significantly higher than both the 2 and 10 mSv groups; while the A414/570 ratio for the same group shows a significant increase with 0.1 and 2 mSv doses and a significant decrease with 10 mSv.

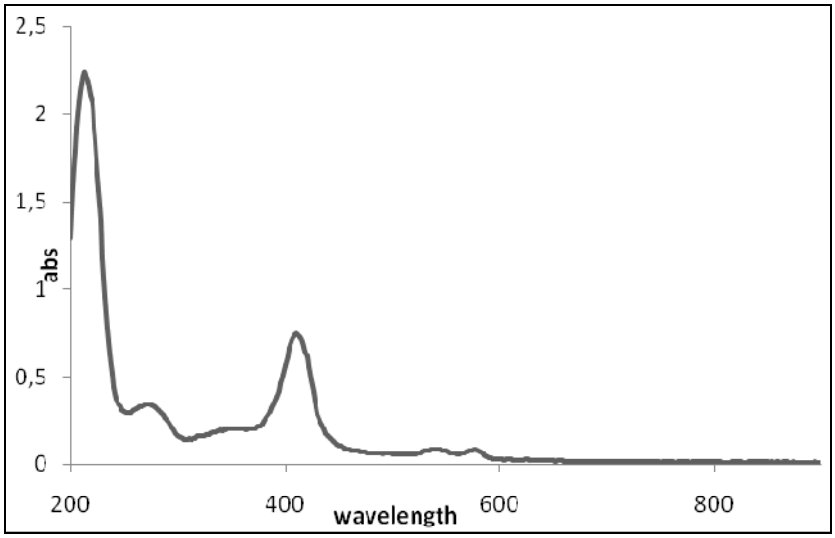

Fig. 1. UV/Visible spectrum of hemoglobin.

The results presented in the table for the delay effect of neutron irradiation on the A414/A270 ratio of the hemoglobin of non-treated with vitamin E animals, shows a significant increase in the 0.1 and 2 mSv doses comparing with the control non irradiated and non treated Hb, whereas, a significant decrease in the value of the 10 mSv irradiated animals appear. In the same while, the A414/570 ratio for the same group shows an increase in the value of the 0.1mSv of irradiated animals, whereas the 2 and 10 mSv show a decrease in their values comparing to the control non treated animals.

Looking at the effect of treating animals with vitamin E. Vitamin E caused a significant decrease in the value of A414/A270 ratio for the 0.1 and 2 mSv doses comparing to the irradiated non-treated and a significant increase for the 10 mSv dose. The same trend appears for both direct and delay effect of radiation. On the other hand, the results of early effect of neutron irradiation show that vitamin E causes the increase in the A414/A270 ratio with all doses. Whereas, the vitamin E effect on the A414/570 ratio shows a decrease comparing to the delay non-treated hemoglobin.

Table 2 also shows that the ratio of the two bands of oxy-hemoglobin Q_o/Q_n is greater than 1 for all doses and for treated and non treated rats.

RBCs MEMBRANE ELASTICITY (OSMOTIC FRAGILITY)

From the UV spectrum, the peak at 550 nm was chosen to indicate the hemolysis of red blood cells in the osmotic fragility test because it is characteristic to the red color of oxy-hemoglobin, as the hemolysis increases the intensity of the

peak increases. In the osmotic fragility test, 50μL of whole blood was added to different concentrations of buffered sodium chloride solution varying from 1% 0.95 of NaCl solution to 0% and then the tubes were allowed to incubate at room temperature. As the pH of NaCl decreases, the RBCs ruptures and the hemoglobin emerges out to the solution, the amount of hemolysis is then determined by reading the supernatants, hemoglobin content in the solution, on a spectrophotometer at wavelength 550 nm. The osmotic fragility study can give an idea about the RBCs membrane permeability to water molecule under the influence of osmotic pressure. Moreover, the sharp dependence of the percentage hemolysis on the extracellular NaCl concentration is a function of the cellular membrane elasticity.

Table 1

The UV/Vis peak intensities for the hemoglobin of control and fast neutron low doses of vitamin E treated and non treated rats

St. analysis	Dose (mSv)	A 270 (cm^{-1})		A 340 (cm^{-1})		A 414 (cm^{-1})		A 540 (cm^{-1})		A 574 (cm^{-1})	
		mean	St	mean	St	mean	St	mean	St	mean	St
Early	0	0.11	±0.03	0.03	±0.01	0.53	±0.2	0.03	±0.01	0.043	±0.02
	0.1	0.123	±0.06	0.07	±0.05	1.04	±0.65	0.06	±0.04	0.080	±0.06
	2	0.113	±0.02	0.053	±0.01	0.85	±0.12	0.05	±0.01	0.07	±0.09
	10	0.13	±0.02	0.07	±0.01	1.05	±0.19	0.06	±0.01	0.09	±0.01
Early + Vit E	0	0.083	±0.03	0.013	±0.01	0.46	±0.24	0.0215	±0.03	0.026	±0.02
	0.1	0.11	±0.04	0.057	±0.02	0.86	±0.36	0.05	±0.02	0.07	±0.03
	2	0.13	±0.04	0.065	±0.03	0.99	±0.43	0.05	±0.03	0.07	±0.03
	10	0.1	±0.04	0.06	±0.02	0.91	±0.37	0.05	±0.02	0.065	±0.04
Delay	0	0.09	±0.05	0.03	±0.02	0.72	±0.45	0.04	±0.02	0.052	±0.04
	0.1	0.115	±0.04	0.056	±0.03	0.86	±0.36	0.043	±0.02	0.06	±0.03
	2	0.14	±0.04	0.05	±0.02	1.02	±0.4	0.054	±0.02	0.07	±0.02
	10	0.17	±0.05	0.07	±0.03	1.09	±0.5	0.069	±0.03	0.1	±0.04
Delay + Vit E	0	0.1	±0.02	0.041	±0.01	0.77	±0.9	0.04	±0.01	0.055	±0.01
	0.1	0.14	±0.04	0.062	±0.02	0.94	±0.31	0.05	±0.02	0.07	±0.03
	2	0.21	±0.05	0.101	±0.03	1.6	±0.3	0.1	±0.02	0.13	±0.03
	10	0.14	±0.06	0.06	±0.03	1	±0.5	0.06	±0.03	0.08	±0.04

From the data of the osmotic fragility concerning the animals exposed to low doses of fast neutrons for direct effects, and for late effects, it is possible to calculate $W_{H,\,max}$. The $W_{H,\,max}$ parameter represents the differences in concentration of %NaCl at the half maximum of the differential main peak plot for the hemolysis curve. Therefore, $W_{H,\,max}$ is the average rigidity maximum value of the cellular

membrane. The increase of $W_{H,\ max}$ indicates the increase of the average rigidity range of the cellular membrane. It is well known that blood is considered as a non-homogenous fluid of at least two phases when flowing through blood capillaries. When blood flows in capillary vessels of diameter smaller than or equal to that of the RBCs, it has to be squeezed, deformed, and moves in a single file. The amount of squeezing of the RBCs will depend mainly on their membrane elasticity. Therefore, decrease in the membrane elasticity will lead to the increase of the blood capillary resistance to the flow of red blood cells [16, 18].

The calculated values from the experimental osmotic fragility results indicate a significant increase of $W_{H,\ max}$ with dose comparing to the non irradiated control (Table 3), furthermore, the value of $W_{H,\ max}$ of the 2 mSv dose is higher than that of 0.1 mSv and of 10 mSv dose for non treated animals comparing with the control non treated animals. Although the delay values of $W_{H,\ max}$ significantly decrease comparing to the early effect of radiation data, we can notice that the value corresponding to 0.1 mSv is slightly higher than that of 2 and 10 mSv doses, whereas, the delay treated and non treated animals show a significant decrease of $W_{H,\ max}$ comparing to control and early irradiated animals.

Table 2

The ratio of Soret band to the aromatic side chains (A270) and to the α (A570) and the ratio of the two peaks of oxy-hemoglobin Qo and Qn

	Dose (mSv)	A414/270	St	A414/570	St	A570/540	St
Early	0	4.3	±0.8	12.37	±0.7	1.48	±0.10
	0.1	8.2	±0.65	12.5	±2.3	1.26	±0.2
	2	7.6	±0.73	12.59	±0.45	1.33	±0.08
	10	7.7	±0.45	12.06	±0.6	1.34	±0.04
Early +Vit E	0	4.7	±1.1	16.1	±1.66	1.23	±0.14
	0.1	7.42	±0.5	12.85	±1.81	1.36	±0.13
	2	7.23	±1.3	13.22	±0.84	1.36	±0.12
	10	8.5	±0.9	15.33	±3.481	1.24	±0.20
Delay	0	7.27	±1.0	14.88	±2.1	1.21	±0.21
	0.1	7.45	±1.04	14.98	±2.1	1.36	±0.13
	2	7.7	1.15	13.66	±0.12	1.21	±0.12
	10	6.2	±1.00	11.58	±0.62	1.36	±0.08
Delay +Vit E	0	7.82	±0.1	14.15	±0.7	1.28	±0.06
	0.1	6.65	±0.6	14.59	±3.21	1.29	±0.17
	2	7.5	±0.4	12.55	±0.54	1.33	±0.03
	10	6.8	±0.75	12.41	±0.83	1.4	±0.012

Table 3

Width at half maximum ($W_{h, max}$) for osmotic fragility test. The t-test was performed using origin 6 program, the significant level was chosen to be 0.05 and the test type is paired

Dose (mSv)	Dose (mSv)	mean	St	*p*-value	t-test
Early non-treated	cont	35	±0		
	0.1	37.5	±3.53	0.29	Significant
	2	42.5	±3.5	0.228	Significant
	10	40	±0	0.70	Significant
Early treated with vitamin E	cont	20	±0		
	0.1	34	±0		
	2	26	±8.5	0.205	Significant
	10	41	±1.4	0.5	Significant
Delayed non-treated	0.1	28	0.54	0.54	Nonsignificant
	2	23	0.155	0.156	Significant
	10	26	±2.82	0.14	Significant
Delayed treated with vitamin E	0.1	34	±0		
	2	27	±9.9	0.42	Significant
	10	24	±5.65	0.20	Significant

VIBRATIONAL SPECTRUM OF NEUTRON IRRADIATED HEMOGLOBIN

Infrared spectroscopy is one of the classical methods for structure determination of small molecules. This standing is due to its sensitivity to the chemical composition and architecture of molecules. The high information content in an infrared spectrum carries over also to biological systems. This makes infrared spectroscopy a valuable tool for the investigation of protein structure [3].

The amide bands widely used in studies of protein secondary structure, which was used extensively to quantify α-helices, β-sheets, turns, and non-ordered structures in proteins [11]. There are two prominent absorption bands, one at 1655 cm^{-1} due to the amide C=O stretching band (the secondary amide I) and another at 1542 cm^{-1} originating from the N–H stretching bands (amide II). The bands at 1452 and 1387 cm^{-1} originate from the bending vibrations of $–CH_2$ and $–CH_3$ groups of amino acids in the protein side chains. The N–H out of plane deformation of secondary amides (amide V) is seen around 700 cm^{-1} [10].

The characteristic band of secondary amide around 3100 cm^{-1} was explained as due to the Fermi resonance of the N–H stretching with the overtone of the amide II band in the trans–amides and due to the Fermi resonance of the N–H stretching with the combination band of C=O stretching and N–H in plane bending in Cis-amides [5, 14]. Figure 2 shows the peak position of FTIR spectrum of normal hemoglobin.

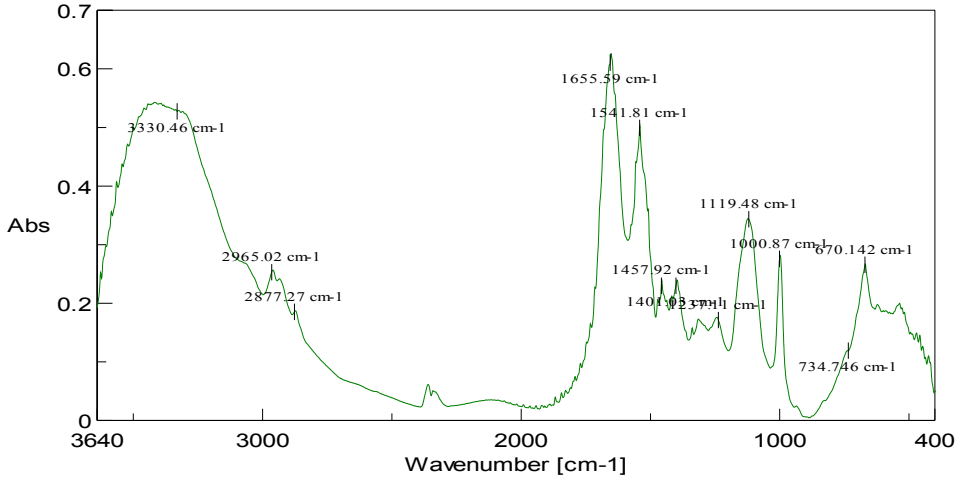

Fig. 2. IR spectrum of control rats.

Figure 3 draws the dose response of the ratios A1650/A1450 and A1540/A1450 to study the change in hemoglobin structure and A1240/A1450 to study the hemoglobin chain. Whereas, Figure 4 illustrates the chosen ratios for the haemoglobin of untreated rats immediately after irradiation as well as the delay effect after 45 days of irradiation of vitamin E treated animals respectively. Data represented reveals that there is a decrease in peak height ratios A1650/A1450 and A1540/A1450 with increasing dose, while A1240/A1450 shows no measurable decrease with dose. Whereas, we can notice that the decrease in ratio with dose is slower in the presence of vitamin E, we can also notice that after 45 days of irradiation the ratio is higher for each individual dose except for 0.1 mSv [5].

In general the results of the UV spectrum of hemoglobin absorbance spectrum show a significant increase in the non-covalent bond of iron and the histidine at 340 nm, the Soret band at 414 nm and the α band at 570 with dose, moreover, a significant increase in the ratio A414/A570 with doses was observed. The former result is not in agreement with the result published by Nabila S. Selim *et al.* [17], 2010, and H.A. Ashry *et al*. [1], 1994, but the latter result is consistent with their results.

The heme part of the hemoglobin molecules has three bands, a strong band at 414 nm and two bands at 570 and 540 nm (α and β) in the visible region; these bands are attributed to the Π → Π* transition of the porphyrin ring, a transformation in the iron state disturbing the three bands. Free radicals that result from the interaction of radiation with the water molecules may affect the hemoglobin molecule [1]. The disturbance in the globin part was known to greatly affect the electron cloud of the porphyrin ring. The increase in the A414/A270 ratio and the increase in its total absorption cross-section reveal slight displacement of the porphyrin ring out of its globin pocket. The ring out of its pocket may result in

increase in the length of this bond and its weakness, which affect the iron atom coordination inside the porphyrin ring. This rearrangement enhances the increase in the charge transfer between the porphyrin ring (ligand) and the iron atom (metal) [17, 25].

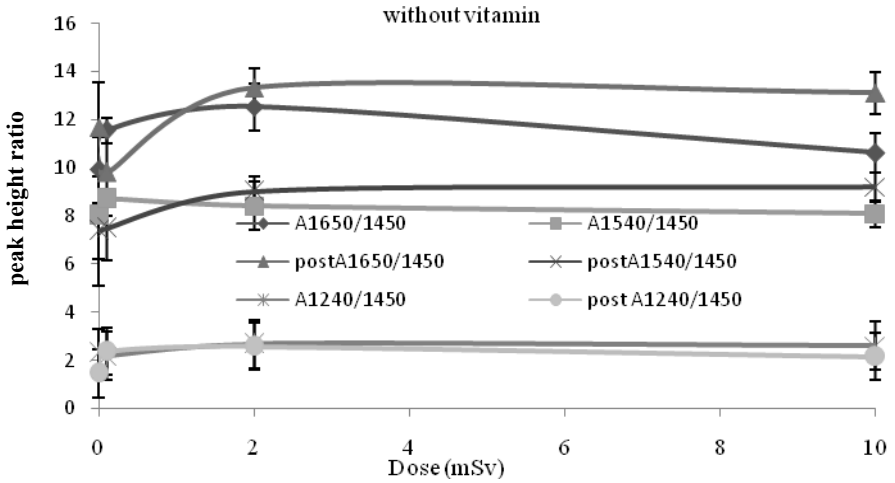

Fig. 3. Radiation-dose response of A1650/A1450, A1540/A1450 and A1240/1450 ratios of vitamin E-untreated rats.

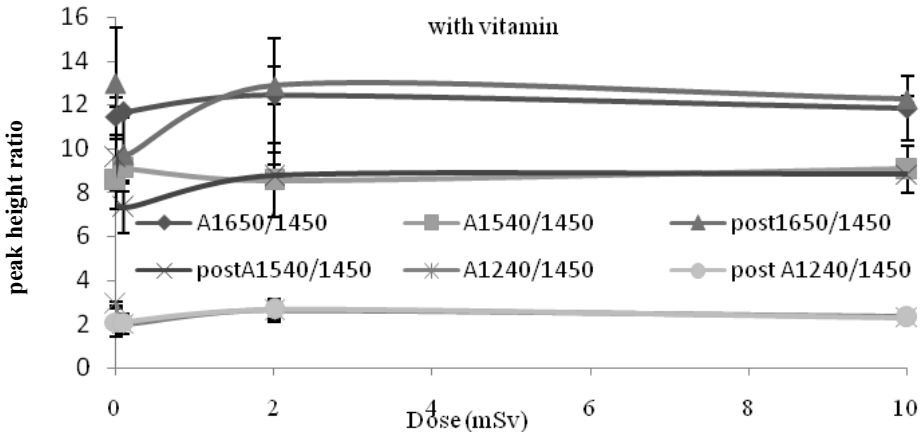

Fig. 4. Dose responses of A1650/A1450, A1540/A1450 and A1240/1450 ratios of vitamin E-treated rats.

The ratio A_{578}/A_{540} is >1 for all doses, and there is no appearance of met-hemoglobin peak at 630 nm, which means that there is no conversion of oxy-hemoglobin into met-hemoglobin under the irradiation by those doses which are consistent with the results of Ashry *et al.* [1].

The calculated values from the experimental osmotic fragility results indicated a significant increase of $W_{H,\,max}$ with dose comparing to the non irradiated control, furthermore the value of $W_{H,\,max}$ of the 2 mSv dose is higher than that of 0.1 mSv and of 10 mSv doses for non treated animals comparing with the control non treated animals. Although, the delay values of $W_{H,\,max}$ significantly decrease comparing to the early data, we can notice that the value corresponding to 0.1 mSv is slightly higher than that of 2 and 10 mSv, whereas, the delay treated and non treated animals show a significant decrease of $W_{H,\,max}$ comparing to control and early irradiated animals.

Neutron irradiation affects membrane in many ways; the hemoglobin release from incubated cells in saline gives rough indication about membrane damage. Free radicals formed during irradiation can cause several membrane changes such as lipid peroxidation, hydrolysis of phospholipids head group, lipid-lipid cross linking, disulfide bridge formation and amino acid residue damage in membrane protein and lipid protein cross-links. Changes in membrane structure can also affect the cytoskeleton. The combined effect of free radicals on the membrane structure and the cytoskeleton can cause hemoglobin leakage from the red blood cells. The hemolysis of red blood cells can reflect the loss of integrity of the cell which can lead to the liberation of hemoglobin [16, 18]. Our results reveal that the low doses of neutron increase the rigidity of red blood cells which is in agreement with results of Selim *et al.* [16, 18] that showed an increase in membrane fragility with increasing dose.

FTIR spectrum of hemoglobin of irradiated rats showed no peak positions shift compared to the control spectrum of hemoglobin. Whereas, by calculating the early effect of radiation on A1650/A1450 and A154/A1450, we have observed a decrease of both ratios with doses for hemoglobin of untreated rats. But, the dose response of hemoglobin of rats after 45 days of irradiation showed that the ratio decreases at dose 0.1mSv, then an increase at 2mSv and 10 mSv occurs. The decrease in the amides peak height ratios could be dedicated to a partial breakage in the C=O bond, the N–H bond leading to a change in the secondary structure of the hemoglobin.

The presence of vitamin E seems to play a role in the protection of bond breakage; moreover, results of delay effect show that a repair in amide I and amide II has occurred. Results of the ratio A1240/A1450 showed that there was no breakage in PO_2 bond [11].

Previous studies proved that vitamin E protects against reactive oxygen metabolites (ROM)-mediated cellular damage through its free radical scavenging properties, thus preventing the oxidation of low density lipoprotein and inhibits the propagation of lipid peroxidation [4, 7, 24]. Results of the vitamin E IP injected animals showed that the red blood cells are more resistant to the change induced by the neutron irradiation in non injected animals, revealing that vitamin E mediates a protective effect against the formation of free radicals induced damage.

CONCLUSION

To sum up, the *in vivo* studies of hemoglobin spectrum revealed a hyperchromicity in the 340, 414, and 570 nm bands and an increase in the ratio A414/A270 with all used doses. Also, the study revealed no transformation change of oxy-hemoglobin to met-hemoglobin in response to fast neutron low doses. The RBCs rigidity of the studied untreated rats increases with increasing radiation; while the IP injection of vitamin E proved to improve the elasticity of rats RBCs, and protect the hemoglobin molecule structure exposed to very low doses of fast neutrons. Whereas, the elasticity of the irradiated cells improved after 45 days of irradiation. Finally, the study showed that the amide I, amide II of hemoglobin intensities decreases with dose, and the vitamin E protects hemoglobin amides I and II from fast neutron induced breakage.

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