PROTECTIVE EFFECT OF MELATONIN ON HEMOGLOBIN DAMAGE INDUCED BY GAMMA IRRADIATION

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Abstract. Ionizing radiation causes serious damage in a biological system. Some drugs and antioxidants are used to prevent such damage. In the present study two doses of melatonin (10 mg/kg and 30mg/kg) were selected to be used for such purpose. This study was carried through UV absorption spectrum, ESR spectroscopy, dielectric measurements and relative viscosity. The data obtained indicate that there is some decrease in the absorbance of sort band, and in the absorption ratio A578 / A540. The intensity of ESR signal for hemoglobin extracted from animals exposed to γ-irradiation is greater as compared with normal hemoglobin. An increase in dielectric permittivity (ε'), the dielectric loss (ε'') and the a.c. conductivity (σ_{ac}) while some decrease in the viscosity measurements are noticed after exposing to irradiation. The data obtained from the whole studied parameters after treating animals with melatonin become closer to those for unirradiated samples.

Key words: hemoglobin, γ-irradiation, antioxidant, dielectrics, ESR spectra, UV absorption spectra.

INTRODUCTION

Radiation produces numerous biological perturbations in cells by direct ionization of DNA and other cellular targets such as proteins and lipids and by indirect effect through free radical production. Exposure to ionizing radiation produces oxygen derived free radical (ROS) in the tissue environment. These radicals include hydroxyl radicals (the most damaging radical), superoxide anion and other oxidants such as hydrogen peroxide. The intracellular generation and accumulation of these free radicals causes change in molecular structure of proteins such as hemoglobin. According to this induced radiation effect, numerous antioxidants could be used for biological and medical safety.

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Biological effect of both gamma rays and alpha particles on human erythrocytes to assess radiation induced membrane damage and hemoglobin oxidation and denaturation was studied [11], while the effect short duration exposure to moderate and interest static magnetic field was studied by measuring absorption spectra of hemoglobin molecule and electrical conductivity [3]. Also the effect of ionizing radiation on some bovine hemoglobin characteristics using electron paramagnetic resonance (EPR) spectroscopy and (IR) spectroscopy was studied [8].

Protective effect of ascorbic acid on molecular behavior changes of hemoglobin induced by magnetic field was studied through measuring the relative permittivity, dielectric loss, relaxation time, conductivity, radius and diffusion coefficient of hemoglobin solution [4]. The result indicated that exposure to magnetic field resulted in changes in the molecular behavior of Hb molecule while treatment with ascorbic acid afforded comparatively more significant amelioration in these molecular changes. Protective effect of ascorbic acid against the degradation of protein molecule by scavenging oxygen free radicals that are produced by irradiation is also studied [7].

Protective effect of melatonin on the ionizing radiation induced DNA damage in the rat brain was studied [17]. A significant increase in DNA damage was found in the radiation treated rat brain. Pre-treatment of rats with intraperitoneal dose of 100 mg/kg melatonin provided a significant decrease in the DNA strand breakage and lipid peroxidation. Melatonin was also used in preventing spinal cord damage against radiation toxicity due to its potential of free radical scavenging [1]. The antioxidative properties of melatonin resulting in its prophylactic property against radiation, which induced biochemical and cellular alteration in the cerebellum, were also studied [15]. The findings support the idea that melatonin may be used as an anti irradiation drug.

The aim of this study is to examine radioprotective effects of melatonin against oxidative damage induced by γ-irradiation. This study will be achieved by investigating the change in biophysical properties of hemoglobin from female mice through UV absorption spectrum, ESR spectroscopy, dielectric measurements and relative viscosity.

MATERIALS AND METHODS

ANIMALS

In this study 60 female mice having weight ranged between 20–25g maintained at animal house laboratory, National Research Center under the normal conditions of water and diet supply. The animals were divided into two main groups each group divided into three subgroups:

1– Control group (A):

A1: Normal animals.

A2 and A3 animals treated with 10 mg/kg and 30 mg/kg melatonin.

2– Irradiated group (C):

C1: Animals exposed to γ-irradiation.

C2 and C3 Animals treated with 10 mg/kg and 30 mg/kg melatonin one day before exposing to γ–irradiation.

MELATONIN TREATMENT

Melatonin (N-acetyl-5-methoxytryptamine), MW 232.3 and M.P.117-120°C from Mallinckrodt Inc., Paris, Kentucky was used. The animals were injected with freshly prepared melatonin dose of 10 mg/kg and 30 mg/kg body weight one day before γ-irradiation. Melatonin was prepared in 0.9% NaCl/ethanol (vol/vol, 20/1) about 0.4 mg of melatonin dissolved in 1 mL of 0.9% NaCl/ethanol. All injections were administered intraperitoneally in a volume of 0.025 mL/g body weight.

IRRADIATION FACILITIES

The animals were placed in a carton box W 20 × L 20 cm and depth of 5 cm many small holes were made in the box sides to enable the animals to be alive during the experiment of irradiation. The dose delivered to the animals were calculated and adjusted in the middle of the box width (2.5 cm from the surface) in order to be sure that all the animals receive a uniform and homogeneous field of irradiation the whole body animals were exposed to 6 Gy γ-irradiation emitted from a linear accelerator cited in the Radiotherapy and Nuclear Medicine Department of the National Cancer Institute, Cairo University. The irradiation machine was supplied from Elekta Company and equipped to 6 MV photon rays production at different field areas at a 100 cm distance perpendicular to the skin. Dose rate was about 400 MV per monitor unit.

HEMOGLOBIN EXTRACTION

The heparinized blood was centrifuged at 3500 r.p.m. for 10 minutes at 4 °C. The packed red blood cells were washed with 5 volumes saline solution at 20 °C. Recentrifuge to separate the washed red blood cells. Steps 2 and 3 were repeated 3 times. The clean red blood cells were lysed with four volumes of deionized water finally; the mixture was centrifuged at 7000 r.p.m. for 40 minutes at 4 °C to separate the hemoglobin.

HEMOGLOBIN SPECTRA

Optical absorption spectra of Hemoglobin diluted with water was done through the use of the Jasco spectrophotometer (UV-vis-type V-NIR 570, Japan) in the range from 250 nm to 700 nm.

ESR SPECTROSCOPY

DMPO (5,5-dimethyl-1-pyrroline N-oxide) from Sigma-Aldrich Chemie-Jmbh, Steinhein, Germany was added to hemoglobin solution. 1 μL of DMPO was added to 0.5 mL of hemoglobin solution in a quartz ESR flat cell and placed into the Tm cavity of a Bruker Electron Paramagnetic Resonance, E-500, Elxsys using super-X-band 9 GHz ESR Spectrometer, Germany. The sample was scanned within 2 min of the addition of DMPO using the following instrument parameters: modulation amplitude 4.0 G; modulation frequency 100 k Hz; microwave power 20 mW ; gain 60; scan time 40 s; time constant 0.02 s.

DIELECTRIC PARAMETERS

Dielectric measurements were done in the frequency range from 100Hz to 1 MHz using computerized RLC HIOKI 3531 Z Hitester (E.E. Corporation, Japan)

The sample cell has two squared platinum black electrodes of area (1 × 1) cm^2 each with an interelectrode distance of 1 cm.

VISCOSITY

The measurements were performed at a certain concentration (3.4×10^{-5} M) and constant temperature (25 °C) with an AVS 350 automatic Ubbelohde-type capillary viscometer from Schott-Geraete, Hofheim, Germany, which allows reproduction of the flow times with an accuracy of 0.03 s. The instrument was also equipped with a model CT 1450 thermostat bath.

RESULTS AND DISCUSSION

ABSORPTION SPECTRA

Absorption spectra for hemoglobin (Hb) extracted from the animals of different subgroups are illustrated graphically in Figures 1 and 2. The obtained bands which characterize hemoglobin are as follows: 578 nm (hem-hem interaction band), 540 nm (Fe–N in porphyrine) nitrogen iron bonds in porphyrine, 414 nm (sort band), 340 nm (globin-hem interaction band) and 275 nm (protein band). These wavelengths are comparable with those found in literature [3, 10, 14].

The average values of peak height, peak position and the width at half maximum (Whmax) of sort band and the absorption ratios of A578 / A540 in the absorption spectra for hemoglobin extracted from the animals of the two groups A and C are calculated and given in Table 1.

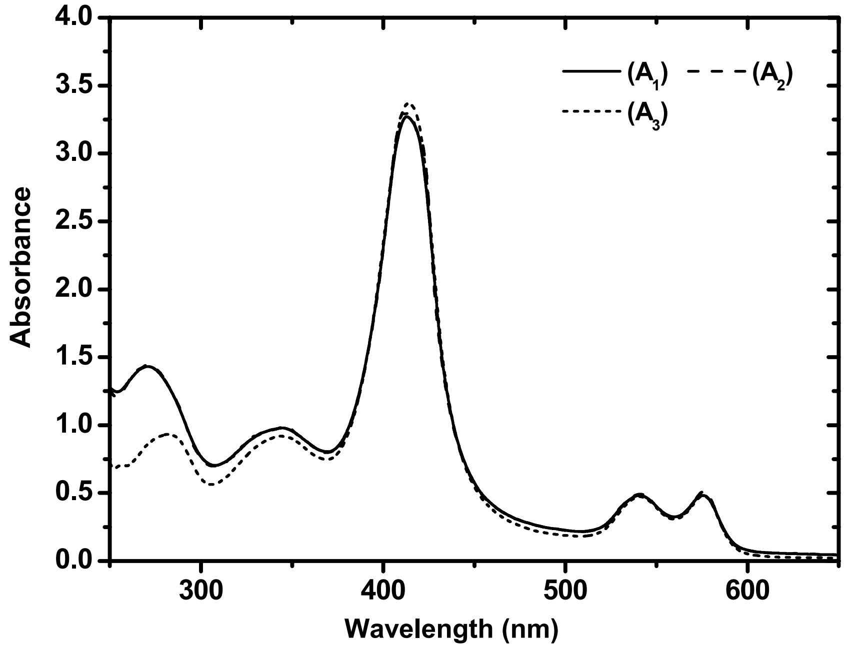

Fig.1. Absorption spectra of hemoglobin extracted from animals of control group A:
(A1) normal animals. (A2) and (A3) animals treated with 10 mg/kg and 30 mg/kg melatonin.

No detectable change is observed in absorption spectra for hemoglobin extracted from the animals of control group A either before or after treating with melatonin. Two doses of melatonin were selected (10 and 30 mg/kg) which are considered to be in the non-toxic range [13].

Great differences are detected in heme parts at visible wavelength for hemoglobin extracted from the animals exposed to γ-irradiation for the subgroups C1, such as relative disappearance of globin band at 280 nm. Moreover, there is an increase in the width at half maximum of the sort band beside decrease of the absorbance at sort band, decrease in heme – heme interaction band and decrease in A578/ A540. These results indicate a partial loss of Hb molecule stability [14].

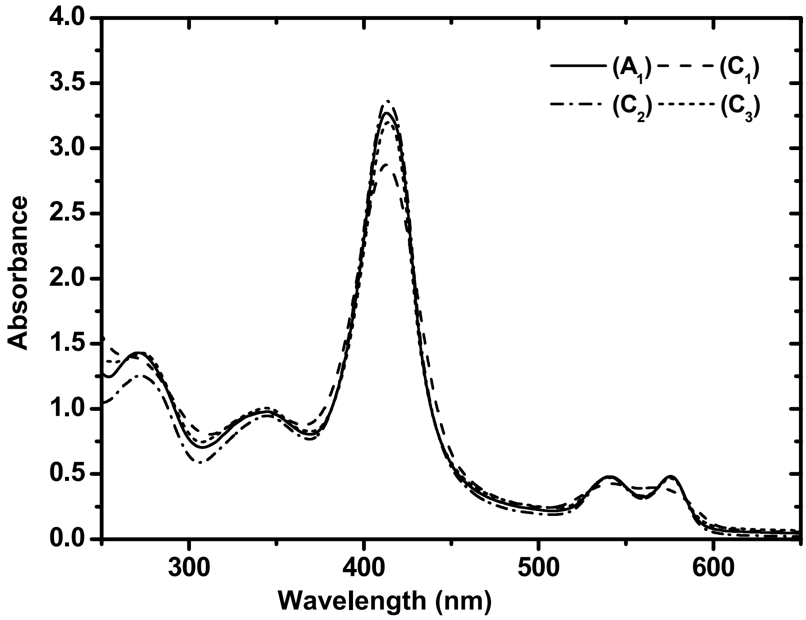

Fig. 2. Absorption spectra for hemoglobin extracted from animals of whole body irradiated group C: (C1) animals with whole body exposed to γ-irradiation, (C2) and (C3) animals treated with 10 mg/kg and 30 mg/kg melatonin.

Table 1

The average values of peak height, peak position and the width at half maximum (Whmax) of sort band and the absorption ratios of A578 / A540 in the absorption spectra of hemoglobin

Group	Peak height	Peak position	Whmax	A578/A540
Group A				
A1	3.3	414	40	1.007
A2	3.33	414	40	1.010
A3	3.36	414	40	1.020
Group C				
C1	2.85	413	52	0.840
C2	3.20	414	41	1.000

Irradiation disrupted the heme groups, resulting in decrease of the absorbance at sort band. It causes a slight breakdown of the polypeptide chain break covalent bonds and disrupts the ordered structure of proteins [7, 13] as a result of the increase in the free radical production [2, 8, 15]. These free radicals contribute to hemoglobin denaturation and precipitation, leading to anemia [6, 14]. Also these free radicals (reactive oxygen species) induce damage effect on antioxidant defensive system, decreases antioxidant capacity of the organism and depletes levels of known antioxidant [2]. This promoted oxyhemoglobin to meet hemoglobin [13]. In the presence of melatonin a protective effect against γ-irradiation damage is observed. This finding is achieved by the studied parameters given in the table for the investigated animals treated with both doses of melatonin 10 mg/kg and 30 mg/kg before exposing to γ-irradiation (C2, C3). These parameters are found to become closer to those given of the control group A. Melatonin acts as direct free radical scavenger and detoxifies the highly cytotoxic OH• and other radicals produced by ionizing radiation [8, 13, 14]. Because of its rather small size and high lipophilicity, melatonin crosses biological membranes easily, thus reaching all compartments in the cell [16].

ESR SPECTRA OF DMPO SPIN ADDUCTS

The free radicals which are expected to be formed by exposing to γ-irradiation are studied through the electron spin resonance ESR spectroscopy. It can detect spin adducts of spin-trapping agent 5,5-dimethyl 1-pyrroline n-oxide (DMPO) with reactive oxygen radicals which are expected to be generated in hemoglobin extracted from the animals of two groups A and C, as shown graphically in Figures 3 to 5.

As shown in Figure 3, no signal is detected for hemoglobin of control group except after the addition of DMPO to hemoglobin as shown in Figure 3c as compared with Figures 3a and 3b.

When the spin-trapping agent (DMPO) is added to hemoglobin in the presence of hydrogen peroxide radical (H2O2), DMPO radical adduct has been detected which is assigned to peroxyl radical at tyrosin-103. This provides an evidence for unpaired electron density [9].

As shown in the same figure, ESR spectra consist of the three sharp, differentially broadened lines, which is characteristic of nitro oxide spin labels [5].

DMPO spin adducts signal intensity of oxygen radical in hemoglobin extracted from animals of control subgroups (A1, A2 and A3) are not changed by treating with melatonin doses 10 mg/kg and 30 mg/kg as shown in Figure 4.

Figure 5 illustrates that the DMPO spin adducts signal intensity of oxygen radicals in hemoglobin extracted from animals of irradiated subgroups (C1) is much greater than the control subgroup (A1) and those for the subgroups (C2 and C3) which are treated with melatonin doses before exposing to γ-irradiation. This could be attributed to the increase in the free radical which is expected to be formed as a result of irradiation.

From this figure it is also seen that treatment with melatonin decreases the signal intensity. So it could be concluded that melatonin is a powerful scavenger for O_2 and H_2O_2 [2, 12, 15].

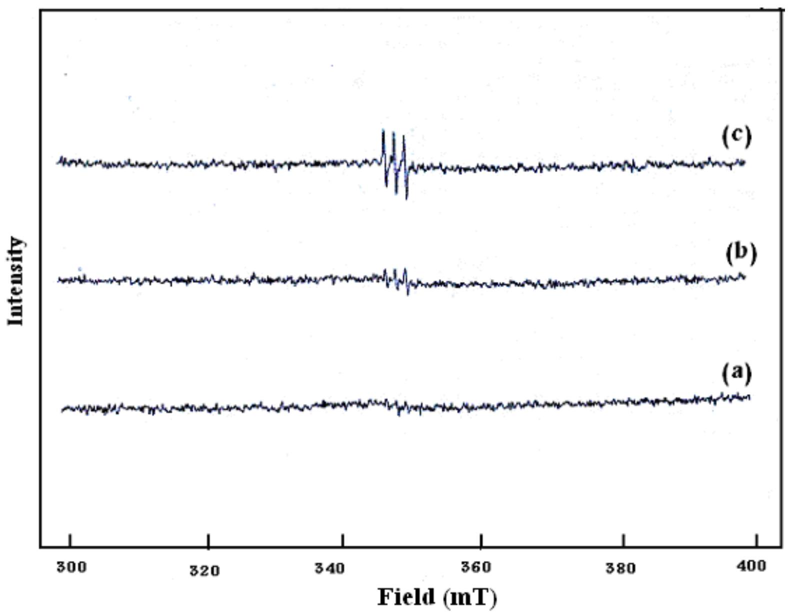

Fig. 3. ESR spectra of hemoglobin extracted from animals of control group (a), DMPO (b) and DMPO-hemoglobin mixture (c).

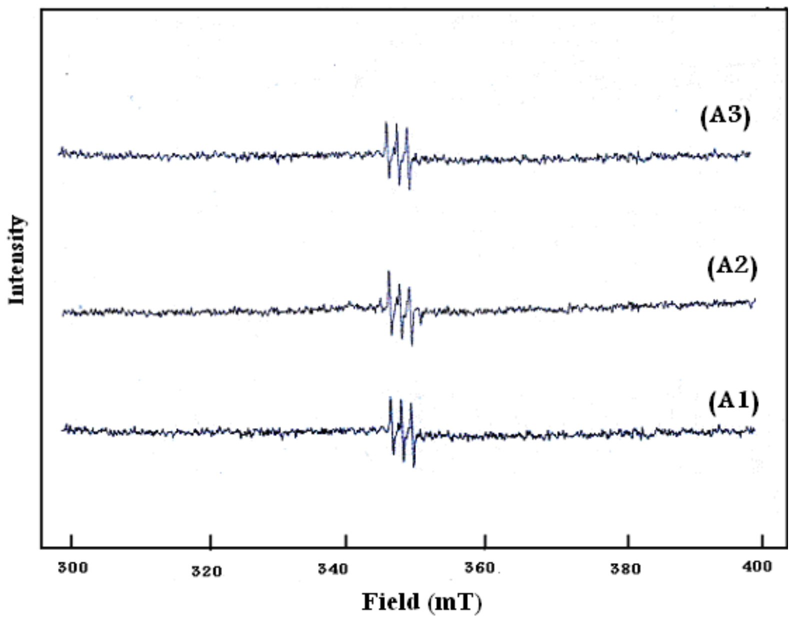

Fig. 4. ESR spectra of hemoglobin extracted from animals of control group (A): (A1) normal animals, (A2) and (A3) animals treated with 10 mg/kg and 30 mg/kg melatonin.

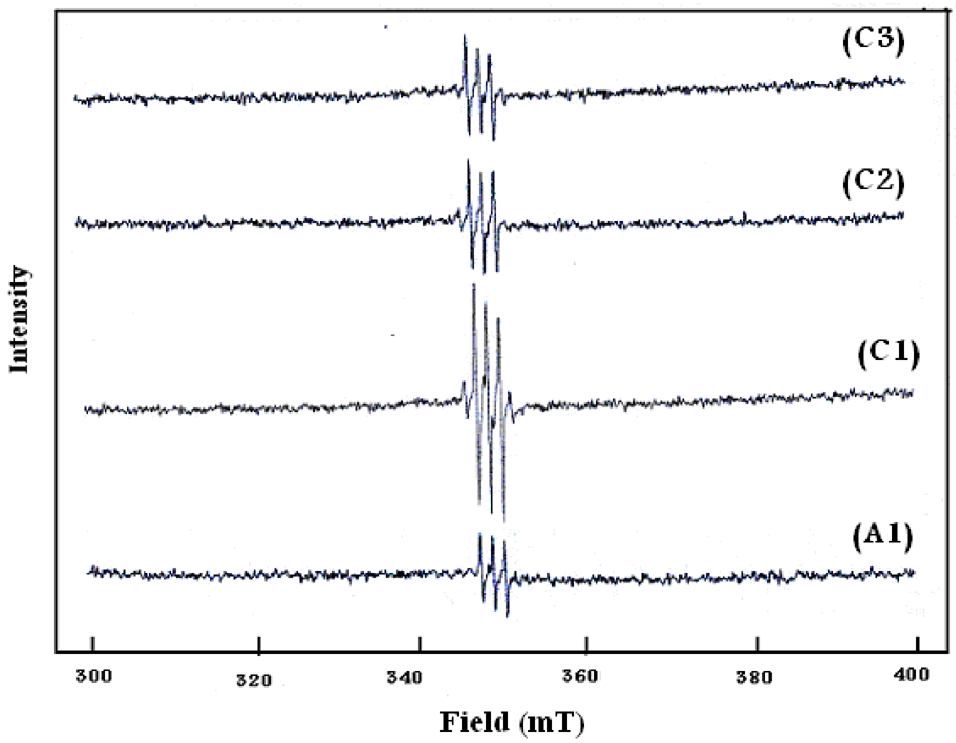

Fig. 5. ESR spectra of hemoglobin extracted from animals of irradiated group (C). (C1) irradiated animals, (C2) and (C3) animals treated with 10 mg/kg and 30 mg/kg melatonin.

Finally, ESR is considered to be a sensitive tool to support the other investigated tools to detect the free radicals.

DIELECTRIC MEASUREMENTS

The dielectric permittivity ε', the dielectric loss ε'' as well as the electrical conductivity σ_{ac} for hemoglobin extracted from the animals of control group (A) and γ-irradiated group were measured in the frequency range 10^2–10^6 Hz and illustrated graphically in Figures 6 and 7. No detectable change is noticed when the animals from the control group (A) are treated with both doses of melatonin. On the other hand, it is noticed that there is some increase in ε', ε'' and σ_{ac} of sub group C1 as compared with those of control sub group A1. This could be a good evidence for the increase of free radicals which are expected to be formed by exposing to γ-irradiation. By treating with melatonin all these values become closer to those of control subgroup A1.

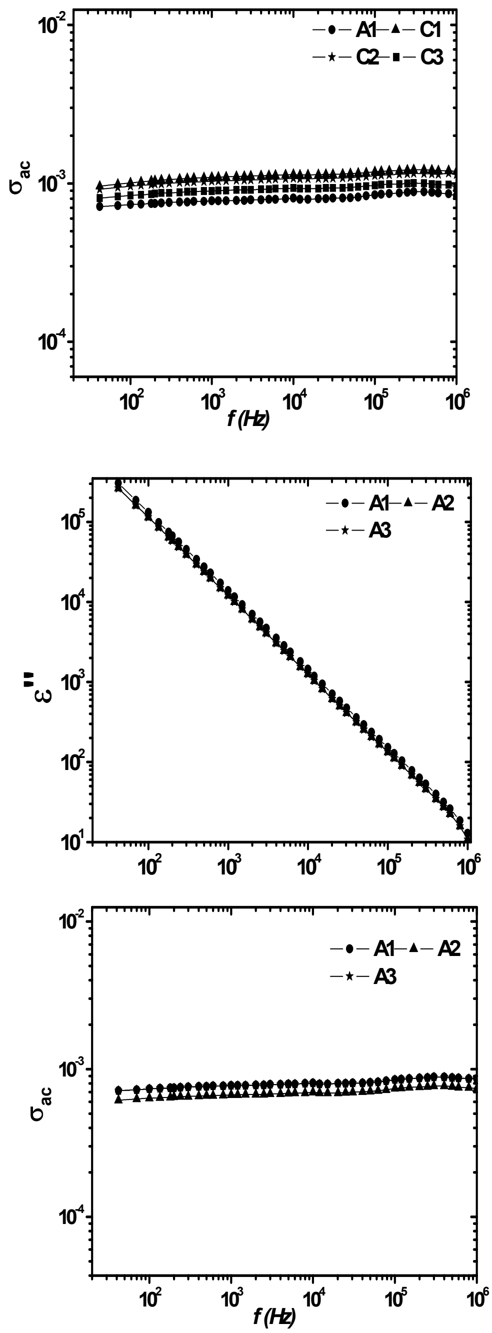

Fig. 6. The variation of dielectric permittivity ε', dielectric loss ε'' and electrical conductivity σ_{ac} as a function of the frequency for hemoglobin extracted from animals of control group A.

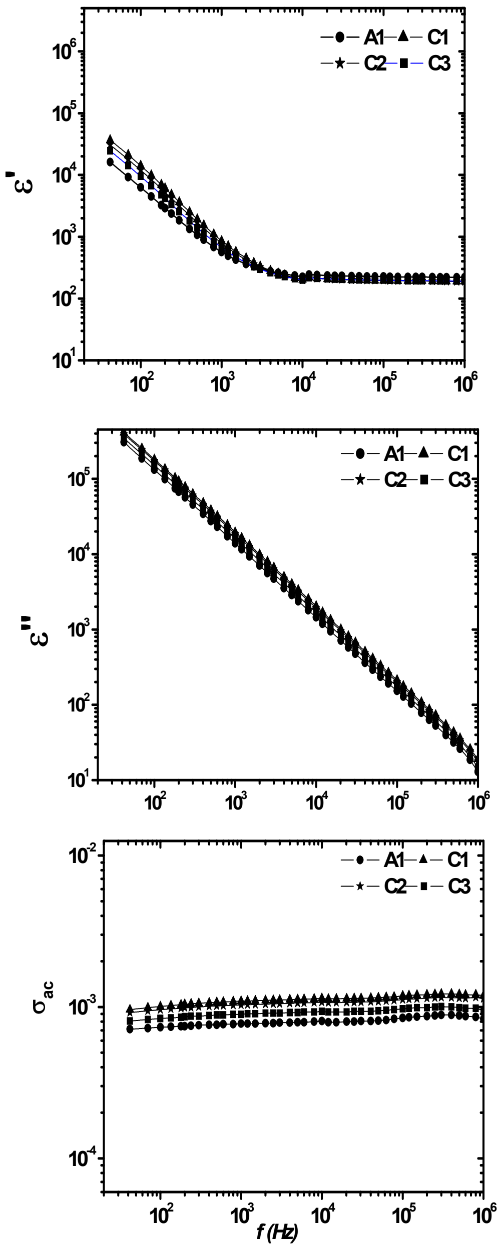

Fig. 7. The variation of dielectric permittivity ε', dielectric loss ε'' and electrical conductivity σ_{ac} as a function of the frequency for hemoglobin extracted from animals of whole body irradiated group C.

So, it could be concluded that the dielectric spectroscopy is considered to be a good tool to support the trend given by UV spectra and confirmed by ESR spectroscopy.

VISCOSITY MEASUREMENTS

The dynamic viscosity (η) of hemoglobin extracted from animals of different subgroups is measured at certain concentration (3.4×10^{-5} M) and temperature (30 °C). The data obtained are given in Table 2.

Table 2

Values of hemoglobin dynamic viscosity (η)

Group	A1	C1	C2	C3
η(CP)	0.77	0.756	0.759	0.764

These values indicate that there is a slight decrease in the dynamic viscosity for hemoglobin of group exposed to γ–irradiation (C1) as it compared with control group (A1). By treating with melatonin (C2 and C3) subgroups, the viscosity values become closer to those of control group (A1). The decrease in viscosity detected by exposing to γ-irradiation (even if it is very small) indicates some changes in dimension and shape of hemoglobin molecule [13].

CONCLUSION

Exposing hemoglobin to gamma irradiation causes disruption in the heme groups, slight breakdown of the polypeptide chain and break covalent bond. The study was carried out on female mice depending on the change in the biophysical and biochemical properties which are expected to happen under the effect of the ionizing radiation. These changes were studied through UV absorption spectra, ESR spectroscopy, dielectric and viscosity measurements. A pronounced increase in the average value of peak position and width at half maximum Whmax followed absorption ratio A578 / A540 was noticed. An increase in the intensity of ESR signal was noticed which is considered to be an evidence for the formation of free radicals. These changes were also studied through the increase in the dielectric parameters and decrease in the viscosity measurements after exposing to irradiation. The studied changes which are obtained as a result of exposing to irradiation are found to be eliminated by treating with 10 mg/kg and 30 mg/kg melatonin.

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