

Influence of Ultraviolet on Morphology Structure, Blood Structure and Rheological Properties of Blood Serum of Mice

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Abstract. The present study aimed to investigate morphology structure, blood structure and the rheological properties, (viscosity, torque, shear stress and shear rate), of mice blood serum at wide range of shear rates after whole body exposed to different ultraviolet doses. Eighty healthy male mice were used in this study. A significant increase in the mice blood serum viscosity occurs after irradiated by the ultraviolet C radiation (UVC). The relationship between viscosity and shear rate exhibited a non-Newtonian behavior for all mice group. The relationship between shear stress and % torque and shear rate exhibited a linear behavior for the control and UVC irradiated mice group. The mice blood serum showed a non-significant change in the UVC irradiated mice % torque and shear stress with shear rates (40-500 s⁻¹) compared with the control. There was a noticeable change in the general behavior movement with a significant effect in blood mice structure after exposure to UVC radiation.

Keywords: Blood serum, rheological properties, viscosity, torque, shear stress, shear rate, morphology structure, blood structure.

1 Introduction

The reduction of ozone in the stratosphere as a consequence of human activity led to an increase in the level of ultraviolet radiation (UVR) at the ground. UVR is a part of the spectrum of electromagnetic radiation emitted by the sun. It is arbitrarily divided into 3 categories of different wavelength: Ultraviolet A (UVA) 400-320 nm, Ultraviolet B (UVB) 320-290 nm and Ultraviolet C (UVC) 200-290 nm, and it has long been known to cause adverse effects on all organisms [1]. Plenty of evidence has been gathered concerning the harmful effects of exposure of animals even to current levels of UV radiation [2] including a destruction of the immune system [3, 4] and alteration of the hematological and histopathological characteristics of animals. UVA rays cause light brown tan in a short time; the subsequent darkening is due to melanin, which accumulates in the skin. UVB rays cause is delayed but long-term tan mostly resulting in melanin synthesis in the skin. It causes serious sunburn, associated with intensified erythema and edema, ache, and blister formation in less than one day of exposure. UVC rays, which have sterilization and biocidal properties, are especially harmful for the eyes. In general, they cannot reach the earth surface due to absorption in the ozone layer [5, 3, 6]. The role of UVC radiation as a noxious environmental agent has been much less studied despite the fact that it comprises the main component of solar ultraviolet radiation and has greater effects than UVA and UVB radiation. Hematological parameters and blood variables are closely related to the response of the animal to the environment and used as reliable indicators of health status to detect physiological changes following different stress conditions [1]. Though biological effects of UVA and UVB radiations have been studied on several animal species [7-12] but the effects of UVC radiation are poorly studied. Therefore, the current study was conducted to show the biological changes of mice exposed to artificially produced UVC radiation for different periods. The objective of this work was to quantify several rheological parameters for blood serum at a wide range of shear rates, morphology structure and blood construction of mice after exposure to UVC radiation dose.

2 Materials and Method

2.1 Animals

A total of 80, male mice, weighing 20-25 g were used in this study and divided into four main groups: Control group (A): Normal animals, UVC irradiated groups (B and C): B: Animals constantly exposed to UVC-radiation (1hour daily) for one month, C: Animals constantly exposed to UVC radiation (2hour daily) for one month and Recovery group (D): Recovery animals after constantly exposed to UVC radiation (2 hour daily) for one month. These male mice were approved by Institutional Animal Care and Use Committee of our Zoology Department, Faculty of Science, Mansoura University, Egypt.

2.2 Irradiation Facilities

The mice were placed in a special wood box (60x60x60 cm) which has many small holes in the box sides to enable the mice to alive during the experiment of irradiation.

2.3 UV-C Source

Three “Sylvania G15W” ultraviolet lamps in 15 Watt powers for each one and in 45 cm length were placed to the internal top surface of the wood box. These lamps consist of a tubular glass envelope and emit more than 85% of their energy in the UVC with a peak at 253.7 nm for germicidal action. Shape, electrical characteristics and lighting circuits are similar to general fluorescent lamps. The majority of germicidal lamps operate most efficiently in still air at an ambient temperature of 25°. All lamps are ozone free. A protective coating on the inside of the lamp limits the depreciation of the UVC output.

Experiments were performed on mice at the animal house of Zoology Department, Faculty of Science, Mansoura University, Egypt under conventional laboratory conditions. All animals in control and experimental group were housed collectively in polycarbonate cages 30x40x40 cm (W x L x H) and given access to standard laboratory food and water as shown in Figure (1). The dose delivered to the mice was calculated and adjusted at 20 cm from the lamps in the middle of the irradiation shell in order to be sure that all the mice receive a uniform and homogenous field of irradiation. A timer was used to standardize UVC exposure times.

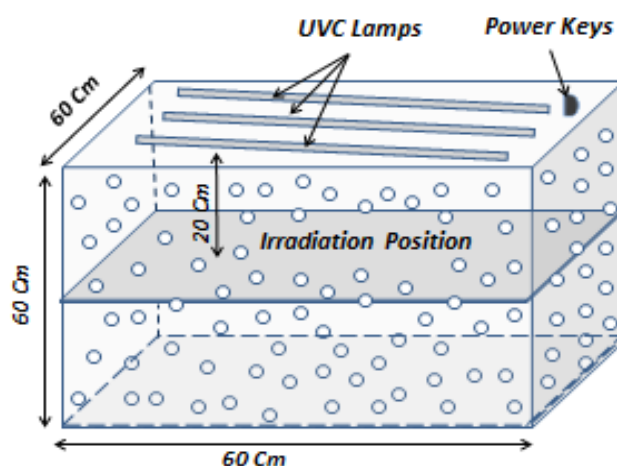


Figure 1. The special wood box

2.4 Blood Samples Collection

Following the end of exposure and post exposure periods, each animal from the groups was slightly anaesthetized with ether. After the mice have been executed, blood samples were collected from a neck vein in heparin containing tubes and used for hematological analysis and osmolality experiments. Other

blood samples were similarly collected in unheparinized plastic tubes and allowed to clot at room temperature then centrifuged at 3000 r.p.m for 15 minutes. Serum samples were separated and stored frozen at -20°C for later biochemical analysis.

2.5 Rheological Parameters Measurements

After irradiating the mice with UVC radiation dose and extracting the serum, several rheological parameters for the blood serum of mice were measured. These parameters, viscosity (cp), % torque, shear stress (dyne/cm^2) and shear rate (s^{-1}), were measured using Brookfield DV-III Ultra Viscometer Programmable rheometer (cone-plate viscometer; Brookfield Engineering Laboratory, Incorporation, Middleboro, USA) supplied with temperature bath controlled by a computer. The rheometer was guaranteed to be accurate within $\pm 1\%$ of the full-scale range of the spindle/speed combination in use reproducibility is within $\pm 0.2\%$. Rheological parameters were measured at temperature of 30.7°C . The temperature inside the sample chamber was carefully monitored using a temperature sensor during the rheological parameters measurement.

A cone and plate sensor having a diameter of 2.4 cm with an angle of 0.8 was used. The rheometer was calibrated using the standard fluids. This viscometer has a viscosity measurement range of 1.5-30,000 mPa.s. The spindle type (SC-40) and its speed combinations will produce results with high accuracy when the applied torque is in the range of 10% to 100% [13].

2.6 Statistical Analysis

Data were analyzed using a commercially available statistics software package (SPSS® for Windows, v. 9.0, Chicago, USA). Results were presented as means \pm SD. Statistical significance was determined at level of $p < 0.05$ using the Student's t-test.

3 Results and Discussion

3.1 Effect of UVC on Body Weight (BW)

The effect of UVC radiation on body weight (BW) of mice is listed in Table (1) and presented in Figure (2). The results show that, BW of mice decreased after exposure to UVC radiation for one hour. Also significant decrease in BW of mice occurs after exposure to UVC radiation for two hours and recovery. That is meant, UVC radiation caused a destruction of the immune system, alteration of the hematological and histopathological characteristics of mice body which appeared in its weight and characteristics.

Figure (3) shows a morphology of control and different irradiated mice groups. Control mice showed normal morphological appearance with a healthy skin and eyes. There was a noticeable change in the general behavior movement of the mice after exposure to UVC radiation in the form of agitation. There are inflammations associated with hair removal and appearance of the wound after exposure to UVC radiation for two hours. Red line has been observed in mice eye.

Table 1. Body weight of mice before and after exposure to UVC radiatio

Group	Group average body weight \pm SD	
	Before exposure	After exposure
Control	22.9 ± 0.1	-----
Group (B)	23.1 ± 0.1	19.3 ± 0.1
Group (C)	23.4 ± 0.1	16.2 ± 0.1
Group (D)	23.5 ± 0.1	17.8 ± 0.1

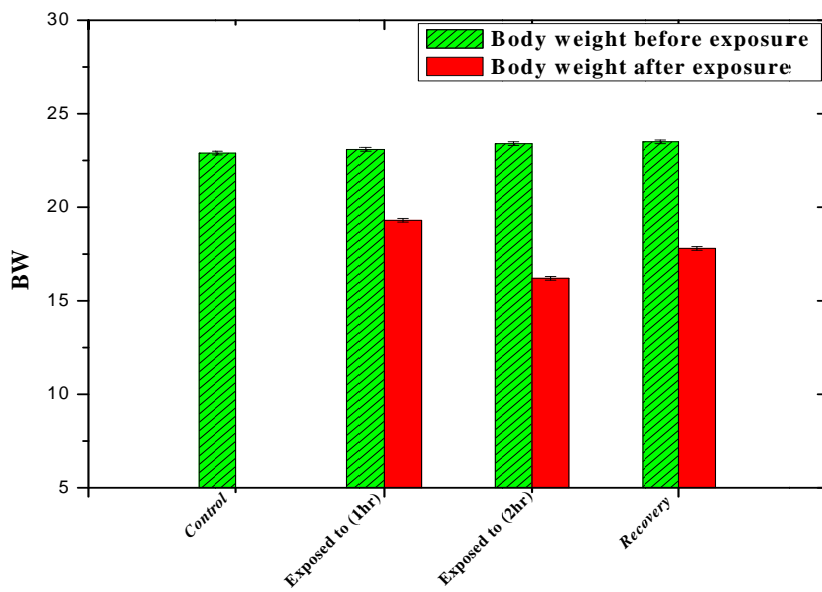


Figure 2. Body weight of mice before and after exposure to UVC radiation



Control



Group (B)



Group (C)



Group (D)

Figure 3. Morphology of control and different irradiated groups

3.2 Effect of UVC Radiation on Blood Structure

From a biological point of view, blood can be considered as a tissue comprising various types of cells (RBCs, WBCs and platelets) and a liquid intercellular material (plasma). There is a significant effect in blood mice structure due to exposure to UVC radiation as presented in Table 2 and Figure 4. Values of RBCs, WBCs and platelets are decreased with increasing time exposure. That means blood components are broken due to exposure to UVC radiation. Also there is a significant effect in hemoglobin molecule structure. The weight of hemoglobin molecule is decreased after exposure to UVC radiation due to damage/breaking as seen in Table 2.

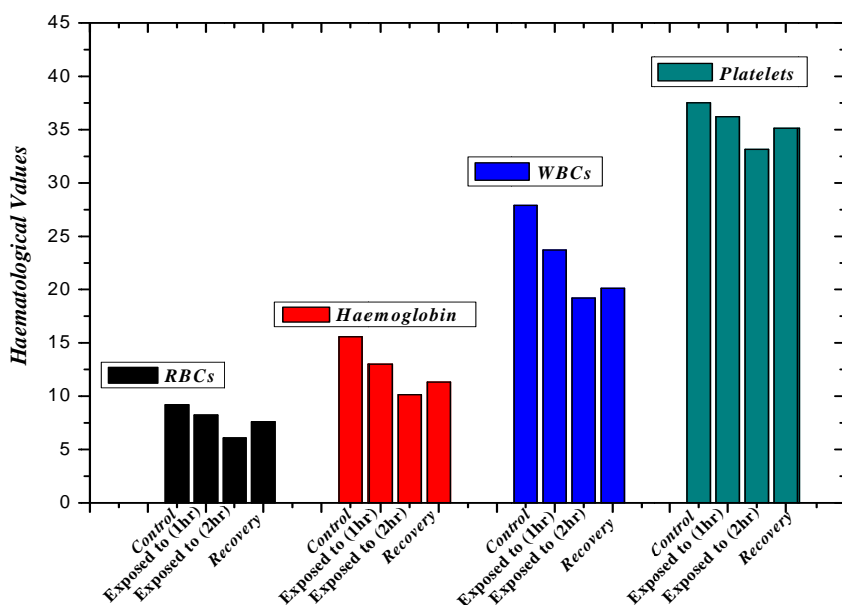


Figure 4. Hematological parameters before and after exposure to UVC radiation

Table 2. RBCs, WBCs, Platelets and Hemoglobin before and after exposure to UVC radiation

Group	RBCs ($10^6/\text{mm}^3$)	Hemoglobin (g/dl)	WBCs ($10^3/\text{mm}^3$)	Platelets ($10^4/\mu\text{L}$)
Control	9.19±1.44	15.57±0.92	27.90±11.87	37.516±2.50
Group (B)	8.24±1.12*	13.02±0.45*	23.7±13.32*	36.217±4.30*
Group (C)	6.10±1.20*	10.12±0.1*	19.2±12.4*	33.151±2.61*
Group (D)	7.60±1.21*	11.31±0.6*	20.1±11.5*	35.113±5.18*

The mice blood serum viscosity at wide range of shear rates (from 40 to 500 s^{-1}) and fixed temperature of 30.7 °C was measured for control, recovery and UVC irradiated mice groups and presented in Figure (5). Figure (5) shows a significant increase in viscosity of the UVC-irradiated mice group compared with the control. The increase in blood serum viscosity might be attributed to changes in non-clotting proteins, glucose, nutrients, electrolytes, hormones, antigens, antibodies and other particles. The precipitation seen in blood serum after treatments with high doses of UVC-irradiation may be partially due to the precipitation of serum proteins [14]. The conformational changes and unfolding of serum proteins following UVC-irradiation were observed as revealed by Protein Fluorometry. In addition, there are changes in the secondary structures of serum proteins [14]. The above changes lead to an increase in the blood serum viscosity.

It is clear from the figure that non-significant change in the recovery group (D) blood serum viscosity compared with the UVC-irradiated mice group (C). Also, the relationship between viscosity and shear rate exhibits a non-Newtonian behavior. The mice blood serum % torque (Figure 6) and shear stress (dyne/cm²) (Figure 7) at wide range of shear rates (from 40 to 500 s^{-1}) and fixed temperature of 30.7°C were measured for control, recovery and UVC-irradiated mice groups. Figures (6 and 7) show a significant change in the UVC-irradiated mice % torque shear stress compared with the control. The relationship between shear stress and % torque and shear rate exhibited a linear behavior for the control and UVC irradiated rats group. Also, it is indicated that non-significant change occurs in the recovery group (D) shear stress and % torque compared with the UVC-irradiated mice group (C).

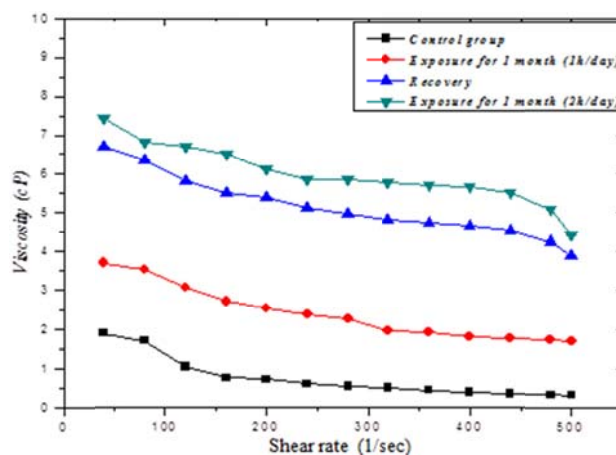


Figure 5. Blood serum viscosity versus shear rate for used mice groups

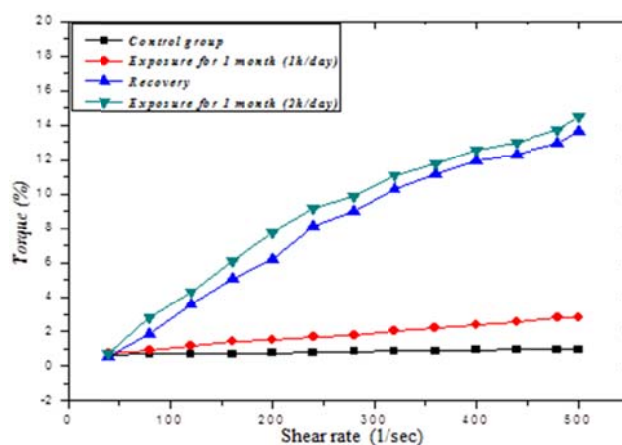


Figure 6. Blood serum torque (%) versus shear rates for used mice groups

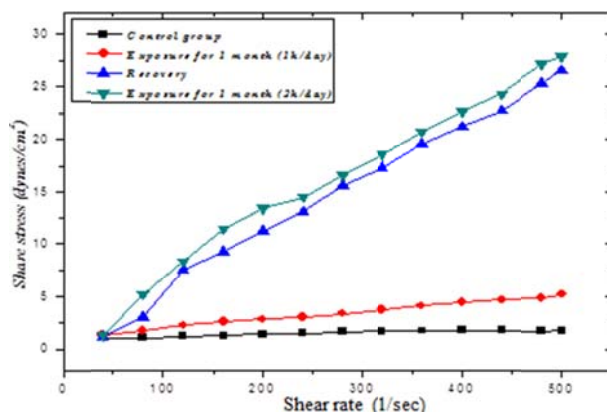


Figure 7. Blood serum shear stress (dyne/cm²) versus shear rates for used mice groups

The decrease in blood viscosity may be attributed to decrease in hematocrit and cytoplasmic hemoglobin concentration of erythrocytes, and to high erythrocyte deformability; while the higher pH of protein has higher serum viscosity. The tissue injuries resulting in serum protein changes can increase its value with high sensitivity [1]. The effect of protein on serum viscosity depends on its molecular weight, pH and structure. The less spheroid shape is the higher molecular weight and aggregating capacity. The higher temperature or pH sensitivity of protein has higher serum viscosity results. Far ultraviolet radiation with a wavelength of 100-1700 Å has enough energy for ionization. This is the type of ultraviolet radiation that is effective on living beings. The longer exposure time of UV radiation shows more harmful effects on living beings. Loss in ozone layer will result with increased UVC exposure that will cause skin cancer, cataract and immune deficiency and impairs blood parameters in the next [1].

4 Conclusions

Biohazard detection technologies use ultraviolet light sources. Couple this with the increasing need for UV illuminators in the medical research and biological fields, and it becomes readily apparent that UV light source design may soon be a part of a lighting engineer's project portfolio. Exposure to ultra-violet radiation causes harmful effects such as a destruction of the immune system and alteration of the hematological and histopathological characteristics. This work gives a brief overview of the main characteristics and health effects of ultraviolet radiation by studying the effect of it.

1. The morphology structure of mice is changed after exposure to UVC radiation and recovery.
2. Blood components of mice are broken due to exposure to UVC radiation with a significant effect in hemoglobin molecule structure.
3. A significant increase in mice viscosity occurs after exposure to UVC radiation. Also, the relationship between viscosity and shear rate exhibits a Non-Newtonian behavior.

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