

SHORT COMMUNICATION

Rheological properties of blood serum of rats after irradiation with different gamma radiation doses *in vivo*

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Abstract: The blood serum rheological properties open the door to find suitable radio-protectors and convenient therapy for many cases of radiation exposure. The present study aimed to investigate the rheological properties of rat blood serum at wide range of shear rates after whole body irradiation with different gamma radiation doses *in vivo*. Healthy male rats were divided into five groups; one control group and 4 irradiated groups. The irradiation process was carried out using Co60 source with dose rate of 0.883cG/sec. Several rheological parameters were measured using Brookfield LVDV-III Programmable rheometer. A significant increase in viscosity and shear stress was observed with 25 and 50Gy corresponding to each shear rate compared with the control; while a significant decrease observed with 75 and 100Gy. The viscosity exhibited a Non-Newtonian behaviour with the shear rate while shear stress values were linearly related with shear rate. The decrease in blood viscosity might be attributed to changes in molecular weight, pH sensitivity and protein structure. The changes in rheological properties of irradiated rats' blood serum might be attributed to destruction changes in the haematological and dimensional properties of rats' blood products.

Keywords: Rheological properties; gamma radiation doses; blood serum; rats

INTRODUCTION

Serum and plasma are both components of the blood. Thus, it can be used frequently while performing blood tests and disease diagnostic purposes. The branch of medical science that deals with blood serum is called serology. The serum is the component that is neither a blood cell (serum does not contain white or red blood cells) nor a clotting factor; it is the blood plasma with no fibrinogens. The serum includes all proteins that are not used in blood clotting (coagulation), and the electrolytes, antibodies, antigens, hormones, and any exogenous substances (e.g., drugs and microorganisms). Another of the serum components is a group of lipids. Lipids include such things as fats, oils, and triglycerides. Cholesterol is often checked as well during a lipid profile.

The blood viscosity increased and the membrane surface charge density decreased after exposure to different radiation doses (Selim *et al.*, 2009). Again, Selim *et al.* (2010) have used the electron microscope and dielectric properties to prove that the gamma irradiation of red blood cells cause decreases in the conductivity and the relative permittivity.

Adult male rats were exposed to different gamma radiation doses 1, 2.5, 3.5, 5, 7 and 9Gy from Cs¹³⁷ source. The conductivity of cell suspension in the

frequency range 40 kHz to 5 MHz, the osmotic fragility of the membrane and solubilization of the membrane by detergent were measured. The results showed decrease in the conductivity, average osmotic fragility and average membrane solubilisation (Selim *et al.*, 2009).

Many fundamental issues concerning blood serum after irradiating with different gamma radiation doses have still to be fully understood, for instance the blood serum rheology. The rheological properties of blood can be used in diagnostics of several clinical disorders. The viscosity changes up to many pathologic conditions, but its importance has not been fully investigated because the current methods of measurement are poorly suited for clinical applications. Therefore, the rheological properties of blood can be helpful in detecting human diseases as well as designing suitable treatments. Obtained results in these fields can be helpful in our better understanding of diseases and in medical diagnosis and therapy (Annak, 2012). The objective of this study was to quantify array of rheological parameters for blood serum of rats after the exposure to different gamma radiation doses and over a wide range of shear rates.

MATERIALS AND METHODS

Animals

A total of 60 healthy male Wistar-Kyoto rats weighing 200gm were used in this study. Animals were randomly divided into five groups, 4 gamma-irradiated rat groups

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[(The 1st group irradiated with 25Gy gamma radiation (n=10); the 2nd group irradiated with 50Gy (n=10); the 3rd group irradiated with 75Gy (n=10); the 4th group irradiated with 100Gy (n=10)] and one control group (NG: n=20). The 4 gamma-irradiated rat groups were maintained on standard laboratory rodent diet pellets and housed in humidity and temperature-controlled ventilated cages for a period of 24h day/night cycle.

Gamma irradiation and serum biochemical analysis

The irradiation process was carried out at Research Center, King Saud University using Cs-137 source. The dose rate was (0.883cG/sec) at the beginning of the experiment. The animals were exposed to 25, 50, 75 and 100Gy single doses. They were dissected 24 hours after exposure. The rats were anesthetized by inhalation of 5% isoflurane until muscular tonus relaxed. Blood sample of nearly 2ml was collected into gel tubes. The blood samples were withdrawn from the left ventricle of the heart using needles. For isolation of serum, first a blood sample is allowed to clot, after which the coagulated blood is centrifuged. The whole blood was centrifuged twice at 3000rpm for 10min in order to separate the serum using a biochemical Auto analyzer (Type 7170, Hitachi). All experiments were conducted in accordance with the guidelines approved by King Saud University Local Animal Care and Use Committee.

Experimental set up and rheological parameters measurement

After irradiating the rats with 25, 50, 75 and 100Gy gamma radiation doses, several rheological parameters for the blood serum of rats were measured. The rheological parameters were viscosity (cp), torque%, shear stress (dyne/cm²) and shear rate (s⁻¹). These rheological parameters were measured using Brookfield LVDV-III 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 $\pm 2\%$ (Abdelhalim, 2011; Abdelhalim *et al.*, 2011; Abdelhalim, 2012; Abdelhalim and Mady, 2012).

Rheological parameters were measured at temperature of 37°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 2.4cm 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 mPas.

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% and accordingly the spindle is chosen.

0.5 ml of each irradiated blood serum was poured in the sample chamber of the rheometer. The spindle was immersed and rotated in these irradiated blood serum samples in the speed range from 20 to 180 RPM in steps of 20 minutes. The viscous drag of the irradiated blood serum samples against the spindle was measured by the deflection of the calibrated spring.

STATISTICAL ANALYSIS

The results of this study were expressed as Mean \pm Standard Error (Mean \pm SE). To assess the significance of the differences between the control group and the four gamma-irradiated rat groups (25, 50, 75 and 100Gy), a statistical analysis was performed using one-way analysis of variance (ANOVA) for repeated measurements, with significance assessed at the 5% confidence level.

RESULTS

Rheological parameters measurement

The relationship between viscosity and different gamma radiation doses for rat blood serum at wide range of shear rates from 225 to 1875s⁻¹ and fixed temperature of 37°C were measured as shown in fig. 1. It shows a significant increase in viscosity with 25 and 50Gy gamma radiation doses compared with the control while a significant decrease was observed with 75 and 100Gy.

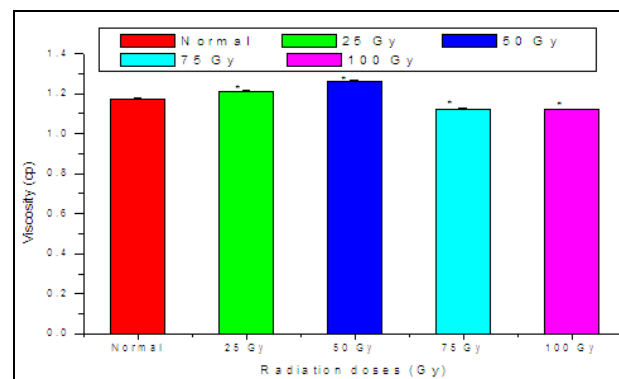


Fig. 1: The relationship between viscosity and the different gamma radiation doses for rat blood serum (* means that the means are significantly different ($p < 0.05$))

The relationship between shear stress and the different gamma radiation doses for rat blood serum at wide range of shear rates (from 225 to 1875s⁻¹) and fixed temperature of 37°C were measured as shown in fig. 2. It shows a significant decrease in shear stress with all gamma radiation doses compared with the control.

The relationship between viscosity (cp) and shear rate (s⁻¹) at different gamma radiation doses for rat blood serum at wide range of shear rates from 225 to 1875s⁻¹ and fixed

temperature of 37°C were measured as shown in fig. 3. It shows a significant increase in viscosity with 25 and 50 Gy gamma radiation doses corresponding to each shear rate compared with the control while a significant decrease was observed with 75 and 100Gy. Moreover, the viscosity exhibits a Non-Newtonian behaviour with the shear rate.

The relationship between shear stress and shear rate at different gamma radiation doses for rat blood serum, wide range of shear rates (from 225 to 1875s⁻¹) and fixed temperature of 37°C were measured as shown in fig. 4. Fig. 4 shows a significant increase in shear stress with 25 and 50Gy gamma radiation doses corresponding to each shear rate compared with the control while a significant decrease was observed with 75 and 100Gy. The relationship between the shear stress and shear rate at the different gamma radiation doses for rat blood serum exhibits a linear behaviour (fig. 4).

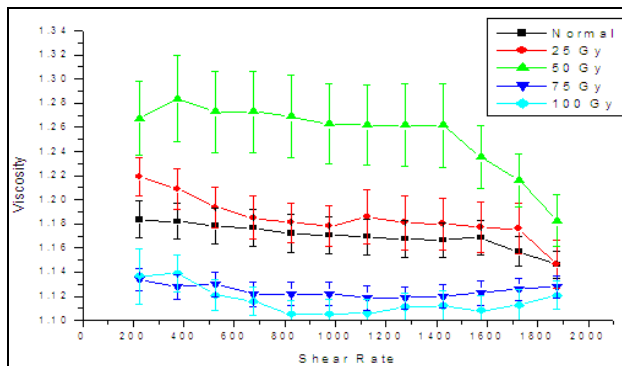


Fig. 3: The relationship between viscosity and shear rate at different gamma radiation doses for rat blood serum (* means that the means are significantly different ($p < 0.05$))

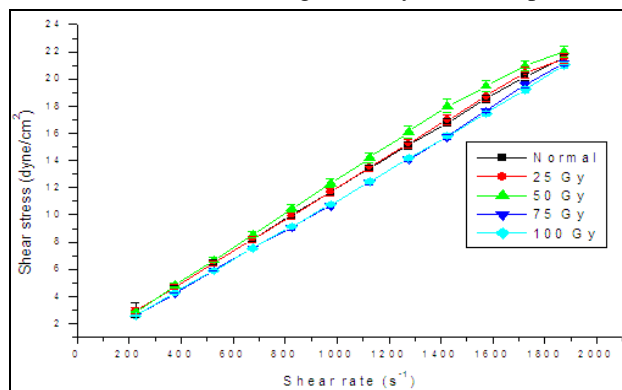


Fig. 4: The relationship between shear stress and shear rate at different gamma radiation doses for rat blood serum (* means that the means are significantly different ($p < 0.05$))

DISCUSSION

The viscosity values exhibited a Non-Newtonian behaviour with the shear rates. Our results are in agreement with those reported by Selim *et al.* (2009). The

decrease in blood viscosity may be attributed to decrease in hematocrit, hemoglobin concentration, count of erythrocytes, and to high erythrocyte deformability (Abdelhalim *et al.*, 2015; Abdelhalim and Sabra, 2015) the higher pH of protein has higher serum viscosity results. Inflammations and tissue injuries results in serum protein changes, increases its value with high sensitivity (Késmárky, 2005; Baskurt, 2009; Steffe, 1996). This study suggests that the changes in blood viscosity values might be attributed to changes in the haematological and dimensional properties of rats' blood products.

The effect of protein on plasma viscosity depends on its molecular weight and structure. A protein which has the less spheroid shape, might has the higher molecular weight, the higher aggregating capacity, and the higher temperature or pH sensitivity, the higher plasma viscosity results (Késmárky *et al.*, 2005; Mohandas, 1980).

Because blood is a non-Newtonian suspension, its fluidity cannot be described by a single value of viscosity. The low shear viscosity depends mainly on the erythrocytes aggregation while the high shear viscosity depends on the erythrocytes deformability. This deformability is responsible for the low viscosity at higher shear rates (Baskurt and Meiselman, 2003; Selim *et al.*, 2009).

Abdelhalim and Sabra, 2015 have investigated the effects of different gamma radiation doses on the blood products of rats' *in vivo*. Different hematological parameters were measured for complete blood count (CBC). Abdelhalim and Sabra, 2015 have investigated the effects of different gamma radiation doses on the blood products of rats' *in vivo*. Different hematological parameters were measured for complete blood count (CBC). This study showed decrease in WBCs count, which might indicate a disturbance in the immune system function; therefore it becomes susceptible to any microbial infection. It has been reported that during exposure to the ionizing radiation, several alterations in the hemorheological properties may take place, such as the generation of free radicals and lipid per oxidation. The lipid per oxidation may decrease RBCs deformability and increase aggregation (Jian *et al.*, 1993). The direct radiation attack cell membrane may induce membrane abnormalities, such as loss of lipid, increase in lipid bilayer rigidity and membrane proteins aggregation (Mohandas *et al.*, 1980; Selim *et al.*, 2009).

Inflammations and tissue injuries leads to changes in plasma protein, which increases its value with high sensitivity. Plasma fibrinogen concentration and plasma viscosity are elevated in unstable angina pectoris and stroke, and their higher values are associated with higher rate of major adverse clinical events. Elevation of plasma viscosity correlates to the progression of coronary and peripheral artery diseases (Késmárky *et al.*, 2005).

A significant decrease in red blood cells (RBCs) count, haemoglobin (HGB), and hematocrit (HCT) after irradiation by gamma has been reported by Abdelhalim *et al.*, 2015. Our results were in agreement with Stohlman *et al.*, 1957, which reported that the decrease in RBCs count may be due to defective hemopoiesis as well as intravascular RBCs damage. According to the kinetics of the RBCs, the fall in RBCs count after irradiation might be due to changes in plasma volume, leakage of cells through capillaries secondary to thrombopenia (Bond *et al.*, 1965).

A significant decrease in white blood cells (WBCs) count and lymphocytes (LYM) was observed when exposed to gamma radiation. While a significant increase in monocytes (MON), neutrophils (NEU), basophils (BAS), and eosinophils (EOS) were observed (Abdelhalim *et al.*, 2015). Our results are coincided with the results of Robert *et al.*, 2001.

This study suggests that the exposure to gamma radiation may induce destruction to proteins, glucose, nutrients, electrolytes, hormones, antigens, antibodies, and interfering with the antioxidant defense mechanism and leading to reactive oxygen species (ROS) generation in the blood serum.

CONCLUSION

A significant increase in viscosity and shear stress was observed with 25 and 50Gy gamma radiation doses corresponding to each shear rate compared with the control while a significant decrease was observed with 75 and 100Gy. The relationship between the viscosity and shear rate exhibited a Non-Newtonian behaviour. The relationship between the shear stress and shear rate at the different gamma radiation doses exhibited a linear behaviour. The changes in blood viscosity values might be attributed to changes in the haematological and dimensional properties of rats' blood products.

ACKNOWLEDGEMENTS

The authors would like to extend their sincere appreciation to the Deanship of Scientific Research at King Saud University for its funding of this research through the research Group Project No. RGP-VPP-285

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