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Rheological properties of blood serum of rats after irradiation with gamma radiation dose in vitro

Rheological properties of blood of rats after irradiation with gamma radiation

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Abstract* – The blood serum rheological properties can open the door to many investigators to find the 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 75Gy gamma radiation dose in vitro. Thirty healthy male Wistar-Kyoto rats weighing 200 gm were used in this study. Animals were randomly divided into gamma-irradiated rat group (irradiated with 75Gy) and control rats group. The irradiation process was carried out using Co 60 source with 0.883 cG/sec dose rate. The rheological parameters measured were viscosity (cp), %torque, shear stress (dyne/cm2) and shear rate (s-1). The rat blood serum viscosity increased significantly in the gamma-irradiated rats group corresponding to each shear rate compared with the control. The relationship between viscosity and shear rate exhibited a Non-Newtonian behavior for the control and gamma-irradiated rats group. The relationship between shear stress and % torque and shear rate exhibited a linear behavior for the control and gamma-irradiated rats group. The rat blood serum showed a non-significant change in the gamma-irradiated rats' % torque and shear stress at the lower shear rates (200-1200s-1) while a non-significant increase was observed at the higher shear rates (1400-1875s-1) 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 blood serum components, which might interfere with the antioxidant defense mechanism, leading to reactive oxygen species (ROS) generation. *(Abstract)

Keywords— **Rheological properties; gamma radiation doses; blood serum; rats**

I. *Introduction*

The blood serum is fluid exposed to different shear rates during its lifetime. Rheology deals with the flow and deformation behavior of materials being solids or fluids, including liquids and gases (1,2). The deformation can be defined as the relative displacement of material points within the body. Fluids continuously deformed because of the application of applied forces (3).

In studying the degree of deformation (or flow) of a material, the force applied per unit area must be considered (3). This deforming force, is termed stress, it may have several components including: 1) shear stress, the force per unit area acting parallel to the surface; 2) normal stress, the force per unit area acting perpendicular to the surface. The latter defined as pressure in a fluid. The degree of deformation is termed strain, which also has various components associated with the different stress components (3). The shear stress results in shear strain which often termed shear rate in which the layers of material move parallel to each other in a progressive manner (4).

Serum and plasma used frequently while performing blood tests and disease diagnostic purposes. 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 the fibrinogens removed. Serum includes all proteins not used in blood clotting (coagulation) and all 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 often checked as well during a lipid profile.

The blood viscosity increased and the membrane surface charge density decreased after exposure to different doses of radiation (5). Again, Selim et al. (2010) have used the electron microscope and dielectric properties to prove that the gamma irradiation of red blood cells cause the decrease of the conductivity and relative permittivity (6).

Adult male rats were exposed to different gamma radiation doses (1, 2.5, 3.5, 5, 7 and 9 Gy) from Cs137 source. The conductivity of cell suspension in the frequency range 40 kHz to 5 MHz, the osmotic fragility of the membrane and solubilisation of the membrane by detergent were measured. The results showed decrease in the conductivity, average osmotic fragility and average membrane solubilisation (5).

The rheological properties of blood serum after irradiating with gamma radiation dose have still to be fully understood. The rheological properties of blood can be used as an indication of several diagnostics 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 (4).

The objective of this study was to quantify several rheological parameters for blood serum of rats after exposure to gamma radiation dose at a wide range of shear rates.

MATERIALS AND METHODS

Animals:

Thirty healthy male Wistar-Kyoto rats weighing 200 gm were used in this study. Animals were randomly divided into 2 groups; gamma-irradiated rat group: this group irradiated with 75 Gy (n = 15) and control rats group (n =15). The rats were maintained on standard laboratory rodent diet pellets, housed in humidity and temperature-controlled ventilated cages for a period of 24 h day/night cycle.

Gamma irradiation and blood serum:

The irradiation process carried out at Research Center, King Saud University using Co 60 source with 0.883 cG/sec dose rate at the beginning of the experiment. The gamma-irradiated rat group exposed to 75Gy dose. The rats dissected 24 hours after the exposure. The rats anesthetized by inhalation of 5% isoflurane until muscular tonus relaxed. Blood sample of nearly 2 ml collected into gel tubes. The blood samples withdrawn from the left ventricle of the heart using needles. For isolation of serum, a blood sample is allowed to clot and then the coagulated blood was centrifuged (Eppendorf Centrifugation; 5804 R with 6 x 85 ml Rot, Germany). In order to separate the serum, the whole blood centrifuged at 3000rpm for 10min. All experiments 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 75Gy gamma radiation dose and extracting the serum, several rheological parameters for the blood serum of rats measured. The rheological parameters were viscosity (cp), % torque, shear stress (dyne/cm^2) and shear rate (s^{-1}). These rheological parameters measured using Brookfield LVDV-III Programmable rheometer (cone-plate viscometer; Brookfield Engineering Laboratory, Incorporation, Middleboro, USA) supplied with temperature bath controlled by a computer (7). The rheometer was guaranteed to be accurate within ± 1% of the full-scale range of the spindle/speed combination in use reproducibility is within ± 0.2%.

Rheological parameters measured at temperature of 37ºC. 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 used. The rheometer was calibrated using the standard fluids. This viscometer has a viscosity measurement range of 1.5-30,000mPas. 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.

After irradiating the rats with 75Gy gamma radiation dose and extracting the serum, 0.5 ml of the blood serum poured in the sample chamber of the rheometer. The spindle immersed and rotated in these blood serum samples in the speed range from 20 to 180 RPM in steps of 20 minutes. The viscous drag of the blood serum samples against the spindle measured by the deflection of the calibrated spring (7).

Statistical analysis:

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

RESULTS AND DISCUSSION

Rheological parameters measurement:

The rat blood serum viscosity at wide range of shear rates (from 225 to 1875s^{-1}) and fixed temperature of 37°C were measured for control and gamma-irradiated rats group (**Fig. 1**). **Fig. 1** shows a significant increase in viscosity of the gamma-irradiated rats group compared with the control. This study suggests that 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 gamma irradiation (50kGy) may be partially due to the precipitation of serum proteins (8). The conformational changes and unfolding of serum proteins following gamma-irradiation were observed as revealed by Protein Fluorometry. In addition, there are changes in the secondary structures of serum proteins (8). The above changes lead to an increase in the blood serum viscosity.

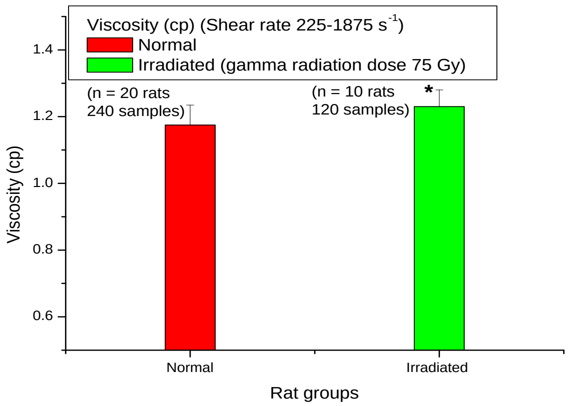

Figure 1: The rat blood serum viscosity for control and gamma-irradiated rats group (*means that the means are significantly different (p<0.05))

Zakrzewski (1973) has showed that gamma radiation induce cross-linkage in the serum albumin (9). Martel et al. (2010) have showed that gamma irradiation (5–50kGy) produces extensive dose-dependent serum protein breakdown as demonstrated by the UV and Visible light spectrophotometry, fluorometry, Fourier-transformed infrared spectroscopy, and gel electrophoresis (8). The gamma rays are consisting of electromagnetic radiation of high-energy values (i.e. above 100keV), and are able to break the covalent bonds in any macromolecule present in the biological fluid (10,11). While most biological molecules can be directly damaged by the gamma rays, this damage is usually attributed to the free radicals hydroxyl (*OH) and superoxide (O_2^-) produced during the hydrolysis of water. These highly reactive free radicals are not only breaking the covalent bonds, but they are also altering the functional groups of biological molecules by mediating oxidation, peroxidation, de-amination, and decarboxylation reactions, among others (10-12). In turn, the ionization and alteration produced by gamma radiation can lead to the degradation of biological molecules or can induce their denaturation, aggregation, or precipitation. In this respect, it should be noted that the reactive oxygen species (ROS) produced by gamma irradiation are known to target proteins (13) which are present in relatively high amounts in the blood serum.

The rat blood serum viscosity (cp) at wide range of shear rates (from 225 to 1875$s^{-1)}$ and fixed temperature of 37°C were measured for control and gamma-irradiated rats groups (**Fig. 2). Fig. 2** shows a significant increase in viscosity of the gamma-irradiated rats (75Gy gamma radiation dose) corresponding to each shear rate compared with the control. **Fig. 2** also indicates that the relationship between viscosity and shear rate exhibits a Non-Newtonian behavior.

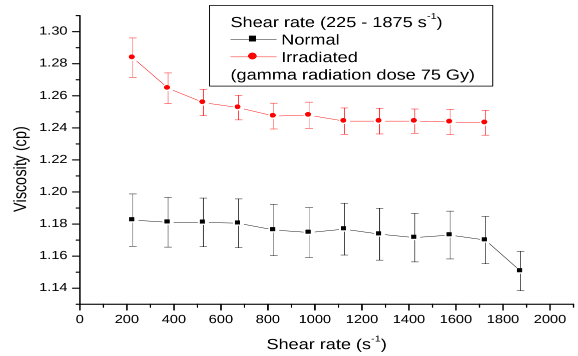

Figure 2: The rat blood serum viscosity at wide range of shear rates for control and gamma-irradiated rats group (*means that the means are significantly different (p<0.05))

The rat blood serum %torque **(Fig. 3)** and shear stress (dyne/cm^2) **(Fig. 4)** at wide range of shear rates (from 225 to 1875$s^{-1)}$ and fixed temperature of 37°C were measured for control gamma-irradiated rats groups**.** Figs. 3 and 4 show non-significant changes in the gamma-irradiated rats' % torque shear stress at the lower shear rates (200-1200s^{-1}) while a non-significant increase observed at the higher shear rates (1400-1875s^{-1}) compared with the control. The relationship between shear stress and % torque and shear rate exhibited a linear behavior for the control and gamma-irradiated rats group.

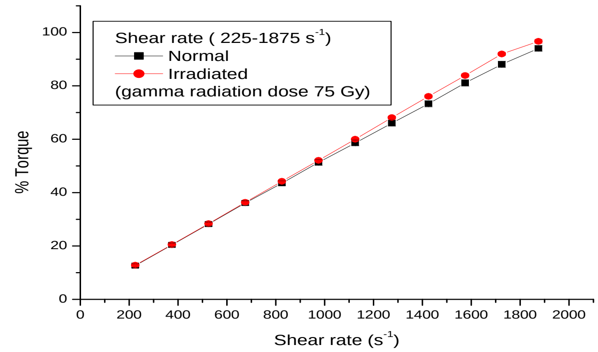

Figure 3: The rat blood serum %torque at wide range of shear rates for control and gamma-irradiated rats group

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 results. The tissue injuries resulting in serum protein changes can increase its value with high sensitivity (14-17).

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 (18,19).

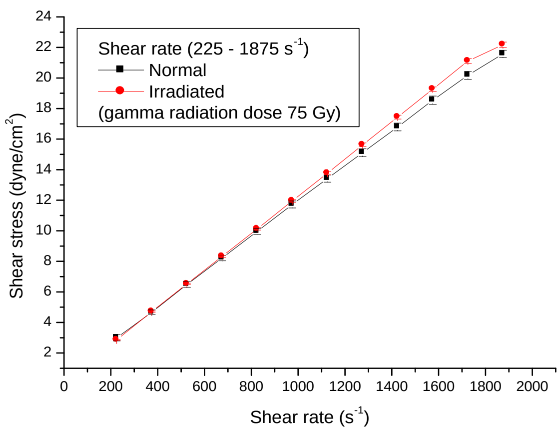

Figure 4: The rat blood serum shear stress (dyne/cm^2) at wide range of shear rates for control and gamma-irradiated rats group

This study suggests that further experiments should be taken into consideration the role of blood serum non-clotting proteins, glucose, nutrients, electrolytes, hormones, antigens, antibodies, other particles and pH of proteins on the rheological properties**.**

Conclusions

The present study aimed to investigate the rheological properties of gamma-irradiated rat blood serum 5Gy gamma radiation dose) at wide range of shear rates (225 to 1875s-1) and fixed temperature of 37°C compared with the control.

Thirty healthy male Wistar-Kyoto rats were used in this study. The animals were randomly divided into 2 groups, control and gamma-irradiated rat groups. The irradiation process was carried using Co 60 source with 0.883 cG/sec dose rate at the beginning of the experiment.

The rat blood serum viscosity increased significantly in the gamma-irradiated rats group (75Gy gamma radiation dose) corresponding to each shear rate compared with the control. The relationship between viscosity and shear rate exhibits a Non-Newtonian behavior for the control and gamma-irradiated rats group.

The rat blood serum showed a non-significant change in the gamma-irradiated rats' % torque and shear stress at the lower shear rates (200-1200s-1) while a non-significant increase observed at the higher shear rates (1400-1875s-1) compared with the control. The relationship between shear stress and % torque and shear rate exhibited a linear behavior for the control and gamma-irradiated rats group.

This study demonstrates that the exposure to a 75Gy gamma radiation dose may induce destruction in the non-clotting 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.

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Authors' contributions

Authors have contributed equally to the manuscript.

Conflict of interest

Authors declare no conflict of interest.

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