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The influence of zinc on the blood serum of cadmium-treated rats through the rheological properties

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Abstract. The blood rheological properties serve as an important indicator for the early detection of many diseases. This study aimed to investigate the influence of zinc (Zn) on blood serum of cadmium (Cd) intoxication-treated male rats through the rheological properties. The rheological parameters were measured in serum of control, Cd, and Cd+Zn groups at wide range of shear rates (225–1875 s^{-1}). The rat blood serum showed a non-significant change in cadmium-treated rats' %torque and shear stress at the lower shear rates (200–600 s^{-1}) while a significant increase was observed at the higher shear rates (650–1875 s^{-1}) compared with the control. The rat blood serum viscosity increased significantly in the Cd-treated group at each shear rate compared with the control. The viscosity and shear rate exhibited a non-Newtonian behavior for all groups. The increase in blood serum viscosity in Cd-treated male rats might be attributed to destruction or changes in the non-clotting proteins, and other blood serum components. In Cd+Zn-treated rats, the rat blood serum viscosity values returned nearer to the control values at each shear rate. Our results confirmed that Zn displaced Cd or compete with the binding sites for Cd uptake.

Key words: Rheological properties — Cadmium — Zinc — Viscosity — Rats

Introduction

The cadmium (Cd) toxicity has a scientific focus during the last century. The Cd is widely used as an anticorrosive agent, as a color pigment, and a neutron absorber in nuclear power plants (Jarup 2003). The main sources of daily exposure to Cd are food, drinking water and inhalation of smoke from tobacco products. It accumulates in the living organism causing severe DNA damage, renal and hepatic dysfunction, anemia, etc. (Gluhcheva et al. 2011). The blood serum hyper-viscosity may cause neurologic or ocular disorders which results in reduced blood flow or capillary perfusion and increased organ congestion. The hyper-viscosity values are produced by the deformability of red blood cells (RBCs). The deformability of RBCs is an intrinsic cellular property, it is determined by the geometric and material properties of the cell membrane, and it is an important determinant for the blood viscosity values (Chien 1987; Baskurt et al. 2003). The hyper-viscosity values are more common, and clinically important, thus the serum and plasma viscosity values are more frequently measured (Bekelman et al. 2006). It is well known that the presence of Cd in the organism decreases the level of iron (Fe) in the blood, kidney and liver (Jurczuk et al. 2004). Since the catalase contains Fe in its active form, the decreased activity of the enzyme in the blood of the rats exposed to Cd might be a result of Fe deficiency (Messaoudia et al. 2010).

The zinc (Zn) can interact with the radical of several compounds (Bertini et al. 1994). It functions as a complex antioxidant, inhibited the oxidative stress induced by the Cd (Li et al. 2000), alloxan (Moustafa 2004), malathione (Brocardo et al. 2005), and chromium (Rudolf et al. 2005). Moreover, the Zn deficiency results in an increase in the oxidative damage in the cells (Yousef et al. 2002) as well as the alterations in components of the antioxidant defense system (Virgili et al. 1999).

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The Zn is essential element in almost all biological systems and well-established antioxidant (Klotz et al. 2003). It is well documented also that Zn can prevent or decrease the harmful effects induced by Cd in the different tissues. It has shown that the Zn supply in conditions of exposure to Cd can protect against Cd-induced oxidative stress (Chowdhury et al. 1987; Jacquillet et al. 2006; Jemai et al. 2007; Brzoska et al. 2008). The Zn has a direct antioxidant activity through scavenging of free radicals or indirect effect by interfering with Cd transport into the cell rather than its activity to stimulate metallothionein synthesis (Sato and Bermner 1993). The literature data are insufficient in regards to the influence of Zn on the blood serum of the Cd-treated rats with thorough investigation of the rheological properties. Thus, the aim of this study was to investigate the changes in blood serum rheological properties firstly in the Cd-treated and secondly in the Cd+Zn-treated rats.

Materials and Methods

Animals

Thirty six male Wister rats were used in the present study. The age of rats was five months and their weights ranged from 180 to 220 g. The animals were maintained under standard laboratory condition (12 h light, temperature 23 ± 1°C). They fed dry ration *ad libitum*. All experiments were conducted in accordance with the guidelines approved by King Saud University Local Animal Care and Use Committee.

Chemicals

The Cd as cadmium chloride ($CdCl_2$) and Zn as zinc chloride ($ZnCl_2$) were purchased from Merk (Dormstadt, Germany). The chemicals used were of highest purity.

Experimental design

The animals were treated four times weekly for eight weeks. The rats were randomly divided into three groups of twelve animals for each group. Group 1 served as control, it received the equivalent volume of saline, Group 2 was injected subcutaneously with 2.2 mg Cd/kg body weight (b.w.) in 0.1 ml saline, and Group 3 injected subcutaneously with 2.2 mg Zn/kg b.w., 1 h prior to Cd injection (it is reported dose, see Bashandy et al. 2006). The subcutaneous administration of Cd causes a rapid effect. When the Cd is administered in food or drinking, the effect of Cd will appear from 3–6 months after the administration. Cd is listed as a carcinogen, and has also been identified as a toxic air contaminant. While when Cd is administered intraperitoneally, the rats will die spontaneously.

Collection of blood and preparation of serum

Twelve blood samples from each group were collected after 8 weeks and the blood serum was separated and kept at –38°C due to our permissible equipment in the laboratory. The blood samples were left to clot. The blood sample was centrifuged at 3000 rpm for 10 minutes, and then the serum was separated from the blood by a needle. Finally, the rheological properties of the blood serum were determined.

Experimental set up and rheological parameters measurement

The rheological parameters, such as viscosity ($N{\cdot}s/m^2$), %torque, shear stress (N/m^2) and shear rate (s^{-1}) in the blood serum of different groups were measured at temperature of 37°C using Brookfield LVDV-III Programmable rheometer (cone-plate viscometer; Brookfield Engineering Laboratory, Incorporation, Middleboro, USA) supplied with temperature bath and controlled by a computer.

After the administration of rats groups with Cd, and Cd+Zn, respectively, extracting 0.5 ml from the blood serum, which was poured in the sample chamber of the rheometer. The spindle was immersed and rotated in this 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 was measured by the deflection of the calibrated spring (Abdelhalim 2011, 2012; Abdelhalim and Mady 2012).

Statistical analysis

The results of this study were expressed as mean ± Standard Error (SE). The significance of difference between the control and other Cd-treated groups and Cd+Zn-treated groups was performed using the one-way analysis of variance (ANOVA) for the repeated measurements, and with the significance assessed at 5% confidence level.

Results

In this study, we used wide range of shear rates from 225 to 1875 s^{-1}, which related to the calibration of the Brookfield LVDV-III Programmable rheometer, in which the %torque was confined within the range from %0 to %100. Thus, the upper shear rate value was around 1875 s^{-1} at the maximum %torque (%100); while the lower shear rate value was around 225 s^{-1} at the lowest %torque (%0) value.

This study aimed to evaluate the changes in blood serum rheological properties firstly in the Cd-treated rats and secondly in the Cd+Zn-treated rats. The blood serum is the liquid fraction of whole blood that is collected after the blood is allowed to clot.

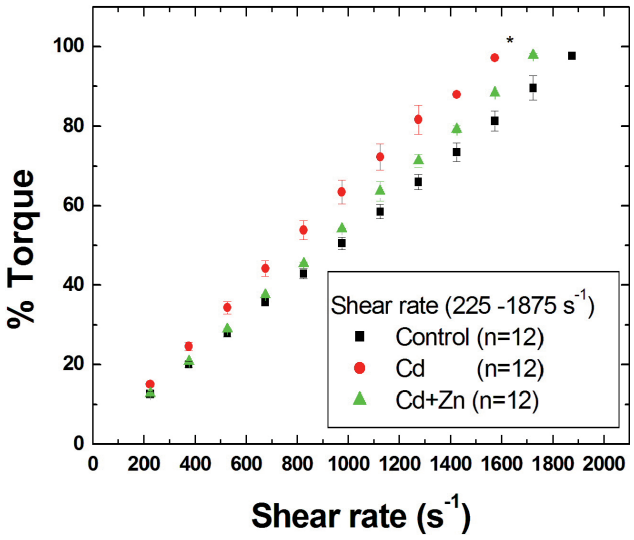

Figure 1. The %torque at wide range of shear rates for Control, Cd and Cd+Zn rats´ blood serum groups (* $p < 0.05$).

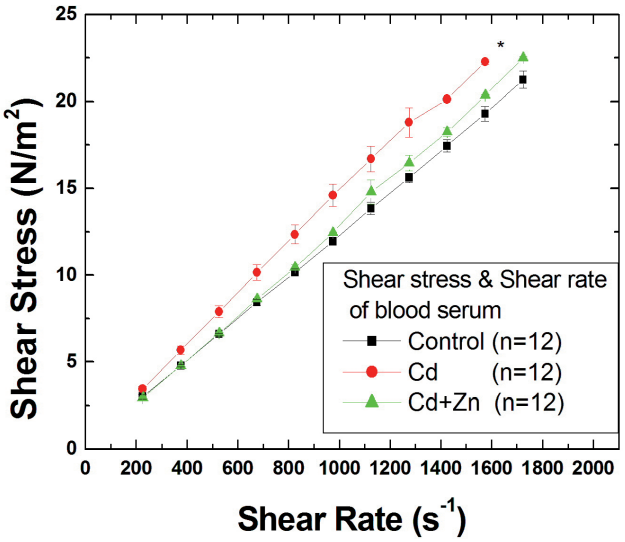

Figure 2. The shear stress (N/m^2) at wide range of shear rates for Control, Cd and Cd+Zn rats´ blood serum groups (* $p < 0.05$).

The rat blood serum %torque (Fig. 1) and shear stress (N/m^2) (Fig. 2) at wide range of shear rates (225–1875 s^{-1}) and fixed temperature of 37°C were measured for control, Cd, and Cd+Zn groups. The %torque (Fig. 1) and shear stress (N/m^2) (Fig. 2) exhibited a linear behavior with the shear rate values (225–1875 s^{-1}) in all three groups. The rat blood serum showed a non-significant change in the cadmium-treated rats' %torque and shear stress at the lower shear rates (200–600 s^{-1}); while a significant increase was observed at the higher shear rates values (650–1875 s^{-1}) compared with the control. However, a non-significant change in the %torque and shear stress values was observed in the Cd+Zn-treated group compared with the control group.

Fig. 3 shows the rat blood serum viscosity values at wide range of shear rates (225–1875 s^{-1}) and fixed temperature of 37°C for control, Cd, and Cd+Zn groups. Fig. 3 shows a significant increase in the viscosity values of the Cd-treated group at each shear rate value compared with the control and the Cd+Zn group.

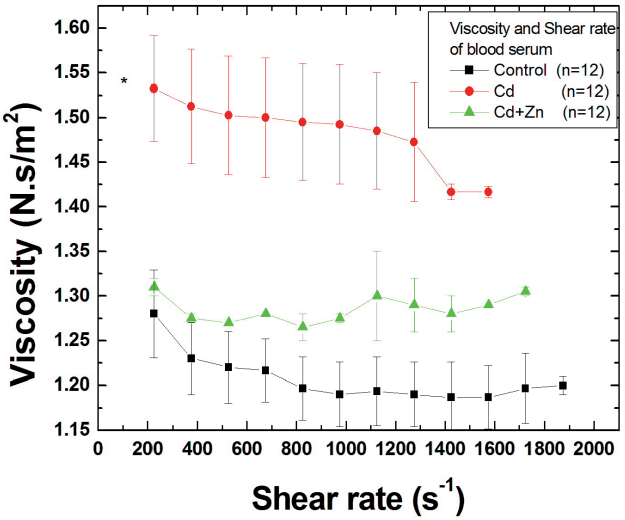

Figure 3. The viscosity at wide range of shear rates for Control, Cd and Cd+Zn rats´ blood serum groups (* $p < 0.05$).

Discussion

The blood serum includes all the proteins that are not used in the blood clotting (coagulation) and all the electrolytes, antibodies, antigens, hormones, and any exogenous substances. This study suggests that the increase in the blood serum viscosity of Cd-treated group compared with the control and the Cd+Zn group might be attributed to changes in the non-clotting proteins, glucose, nutrients, electrolytes, hormones, antigens, antibodies and other particles. Moreover, the increase in blood serum viscosity values might be attributed to the Cd-protein interaction in the blood serum. It has been reported that the whole blood viscosity significantly elevated in the Cd-treated mice (Gluhcheva et al. 2011). Also, the Cd toxicity may lead to an increase in the blood pressure through increasing the plasma viscosity (Kacar Kocak et al. 2009). Ivanova et al. (2012) have proved that exposure of the rats to the Cd induced hematological alterations and leads to elevation in the whole blood viscosity, and reduced the hemoglobin (Gluhcheva et al. 2011). Koçak

and Akçil (2006) have reported that the chronic Cd-toxicity may produce hypercoagulation and hence increases the risk of thrombosis.

In this study, the Zn was administered prior to the Cd, but we have not studied the possibility of Zn administration to the rats after their exposure to the Cd, because it became evident from the results of this study that Zn might replace Cd, which might decrease the toxicity induced by Cd, through reducing the production of free radicals. Moreover, the Zn may act as an endogenous protective factor perhaps through reducing the production of oxidative stress parameters and increasing the antioxidant enzymes (Abdelhalim et al. 2010).

The treatment of Cd-exposed animals with Zn partially reversed Cd-induced increase in the blood serum viscosity. The available data indicate that the Zn is one of the most important nutritional factors influencing the metabolism and toxicity induced by Cd (Brzoska et al. 2008).

The interactions between Cd and Zn may occur at various stages of both metals' metabolism, i.e. absorption, distribution in the organism and excretion, as well as at the stage of Zn biological functions (Brzoska et al. 2008). The enhanced consumption of Zn may decrease Cd absorption from the digestive tract and its accumulation in the organism, and as a result it may ameliorate the toxic effects of Cd, including oxidative damage in erythrocytes (Jemai et al. 2007; Brzoska et al. 2008).

The role of Zn in protecting the biological structures from the free-radical damage may be due to several factors: firstly, by maintaining an adequate level of metallothionein, which are also free-radical scavengers, then, as an essential structural component of Cu/Zn SOD, and finally, as a protective agent for thiols and other chemical groups (Messaoudia et al. 2010).

The Zn can also act by displacing redox-active metals (Fe, Cu) from membrane-binding sites, and it has been shown to reduce Fe^{2+}-initiated lipid oxidation (Girotti et al. 1985). Also, Zn supplementation could compete with binding sites for Cd uptake.

The literature data are insufficient in regards to the influence of Zn on the blood serum Cd- treated rats through investigation of the rheological properties. In the literature review, we have not found published papers in regards to the rheological properties changes in the presence of other toxic metals (e.g. As, Pb, Hg) and treated by the other biogenic metal (e.g. Mg).

Our results confirmed the antioxidative activity of Zn, which is connected with the enhancement of protective mechanisms against the toxic effects of Cd. This study suggests that further studies are required to be done using Zn and selenium to completely avoid the toxicity effects induced by Cd. Moreover, further experiments regarding to the changes in body weight of the control and treated rats will be performed. Moreover, the rheological properties changes in the presence of other toxic metals (e.g. As, Pb, Hg) and treated by the other biogenic metal (e.g. Mg) will be also performed.

Conclusions

Our results indicate the alterations in the rats' blood serum rheological parameters at wide range of shear rates (225–1875 s^{-1}) in the Cd-treated rats. The %torque and shear stress exhibited a linear behavior with the shear rate values (225–1875 s^{-1}), and it significantly increased in Cd-treated rats group compared with the control and the Cd+Zn-treated rats at all the shear rate values. However, non-significant changes in the %torque and shear stress values was observed in the Cd+Zn-treated group compared with the control group. The viscosity values significantly increased in the Cd-treated group compared with the control and the Cd+Zn group and exhibited a non-Newtonian behavior with the shear rate. The chronic exposure to Cd might induce changes in the blood components and elevation in the blood serum viscosity, and the Zn supply prior to Cd returned the rheological parameters of rats' blood serum to nearly their control values at the all different shear rates.

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