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Original Research

Manganese-induced hematological alteration in Wistar rats

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ABSTRACT

Aim: Manganese (Mn)is an essential trace metal that acts as cofactor in many cellular enzymes. The present study was designed to evaluate toxic effects of manganese chloride ($MnCl_2.4H_2O$) on marker hematological parameters in rats after subchronic exposure and after 60 days of treatment withdrawal. **Materials and Methods:** Adult male Wistar rats were randomly divided into five groups. In Group I, the rats were treated with vehicle (0.5 mL distilled water) and served as control. The rats in Group II, III, and IV were exposed to $MnCl_2$ (50, 100, and 150 mg/kg b.wt./day, p.o. respectively)for 120 days. Half of the rats of Group IV were followed by 60 days post-exposure recovery period and served as Group V. **Results:** The results of the study showed significant dose-dependent decrease in red blood cell (RBC) count, hemoglobin (Hb), hematocrit (Hct) value, platelet count, and significant increase in white blood cell count after $MnCl_2$ exposure. Whereas no significant changes were observed in mean corpuscular volume, mean corpuscular hemoglobin (MCH) and MCH concentration (MCHC) after treatment. Scanning electron microscopic study of blood showed a dose-dependent increase in abnormal-shaped RBCs in $MnCl_2$ treated rats. Most of the effects in these parameters were recovered after 60 days of treatment withdrawal. **Conclusion:** The results of the study reveals that $MnCl_2$ exposure resulted in hematological toxicity in rats and most of the changes in these parameters recovered when Mn exposure was ceased.

KEY WORDS: Hematological parameters, manganese, rat

INTRODUCTION

Manganese (Mn)is one of the most abundant transition metals and is widely distributed in soil, air, water, and food [1,2]. It is an essential element for humans in small quantities, playing important roles for normal mammalian physiology [3]. Several enzyme systems interact with or depend on Mn^{2+} for their optimal catalytic or regulatory function such as arginase, superoxide dismutase (SOD), pyruvate carboxylase, alkaline phosphatase, and glutamine synthetase [4]. Mn is also necessary for normal growth and development of bone and cartilage [5]. It has been reported that a deficiency in intake of Mn can impair normal human growth and functioning and cause birth defects [6]. It was suggested that Mn^{2+} has dual behavior depends on the quantity of administration since low doses have an antioxidant effect while high doses cause oxidative injury [7,8]. Heavy industrial use of Mn and Mn-containing compounds in the production of paint pigments, pesticides, dry cell batteries, glass and ceramics, gas additives (methylcyclopentadienyl manganese tricarbonyl)as well as mining of Mn ores and welding of mild steel may expose human population to an excessive amount of this element [9,10]. Excessive exposure of Mn^{+2} produces several toxic effects on various organs and body systems [1,11,12].

Blood parameters are generally considered physiological indicators of the whole body functioning and therefore are important in diagnosing the structural and functional status of the humans exposed to toxicants [13]. A number of hematological indices such as hematocrit (Hct), hemoglobin (Hb), red blood cells (RBC), and so on, are used to access the functional status of the oxygen-carrying capacity of the blood stream and have been used as an indicator of metal pollution [14]. As earlier known, Mn accumulates in bone, liver, and kidney tissues [15,16], therefore, it may alter the normal process of hematopoiesis. In addition, Mn^{2+} also induces disruption of the homeostasis of Fe, Ca and trace minerals in the body [17,18]. There are several reports that clearly indicate that Mn alters iron homeostasis through competition with iron for binding protein and subsequent transport system [19,20]. Under conditions of either overload due to high exposure or disturbed homeostasis Mn can disturb the cellular response to DNA strand break [21]. In the past decade, various independent studies have been conducted on Mn, which have reported toxicity of Mn, but the majority of them have focused on 7724], hepatic toxicity [25,26]and developmental toxicity [27,28]. However, adverse effects of Mn on hematological parameters have been rarely studied. Thus, there is a need for better understanding of the toxic

effects of Mn on hematological parameters in the body. The present investigation was undertaken to assess the effects of Mn exposure on hematological parameters in Wistar rat.

MATERIALS AND METHODS

Experimental Animals

Colony-bred adult male Wistar rats weighing 160-180 g were used in the present study. The animals were housed in polypropylene cages under well-regulated light/dark (12 h:12 h) cycle at standard temperature (22±1°C). Animals were provided rat chow (Aashirwad Food Industries, Chandigarh) and tap water *ad-libitum*. The animals were maintained as per guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals regulations. The study was approved by Institutional Ethical Committee, Department of Zoology, University of Rajasthan, Jaipur, India.

Chemicals

Manganese chloride ($MnCl_2.4H_2O$) was purchased from Ranbaxy Fine Chemicals Limited, New Delhi, India. All other chemicals used in the study were of analytical grade. $MnCl_2$ solution was prepared by dissolving it in sterile saline.

Experimental Design

Forty male Wistar rats were involved in this study. Rats were randomly divided into four groups of eight rats in I, II, and III groups and 16 rats in Group IV. Group I served as control and received only vehicle (0.05 mL/rat). Groups II, III, and IV were treated orally with $MnCl_2$ (50, 100, 150 mg/kg b.wt./day, respectively) for 120 days. At the end of the experiment (120 days), all the animals of I, II, and III Group and half of the animals of Group IV were sacrificed. Remaining half animals (Group V) were sacrificed after 60 days of drug withdrawal.

Sample Collection and Parameters Measured

One day after the completion of treatment, overnight fasted rats were anesthetized and blood was withdrawn from the heart chamber using an injector. The blood samples were collected in test tubes contained ethylene diamine tetra acetic acid anticoagulant and used for determination of RBC, white blood cell (WBC), platelets counts, Hb, Hct, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and MCH concentration (MCHC) values by automatic hematology analyzer (AccurexCBC-360 plus).

Scanning Electron Microscopic (SEM) Study

For morphological study of erythrocytes, a drop of fresh blood was fixed in 2% glutaraldehyde (in 0.1 M phosphate buffer) for 30 minutes. It was then centrifuged, washed in 0.1 M phosphate buffer (pH 7.4), washed and centrifuged in double distilled water 5 times and resuspended in double-distilled water. A thin film was then applied onto cut glass slide pieces (1 cm × 1 cm), dried, sputter coated with gold and finally observed under SEM (30 kV, EVO-18 Carl Zeiss).

Statistical Analysis

Data were analyzed by means of one-way ANOVA using the SPSS software statistical program (SPSS for Windows, Version 20.00, USA) followed by Tuckey's multiple comparison procedure as a post hoc test. Data are expressed as the mean ± standard error, and $P < 0.05$ was considered statistically significant.

RESULTS

Hematological Parameters

The results of present study indicate that the RBC count, Hb, Hct, and PLT counts were decreased significantly in medium (100 mg/kg) and high (150 mg/kg) dose $MnCl_2$ exposed rats when compared with control rats. Furthermore, when the values of high-dose group were compared with low-dose (50 mg/kg) group, a significant reduction was observed while on comparison with medium-dose group no significant changes were observed. WBC counts were significantly increased only in high-dose group as compared to control group while no significant change was observed when compared to low- and medium-dose group. The MCV, MCH, and MCHC values

Table 1: Hematological parameters of experimental groups

Parameters	Control Group I	Treatment			
		Group II (50 mg $MnCl_2$)	Group III (100 mg $MnCl_2$)	Group IV (150 mg $MnCl_2$)	Group V (recovery)
RBC ($\times 10^6$/mm^3)	9.60±0.34^a	8.12±0.43^{ab}	7.17±0.60^{bc}	5.67±0.62^c	8.03±0.42^{ab}
Hb (%)	13.04±0.52^a	12.38±0.62^{ab}	10.22±0.67^{bc}	8.99±0.70^c	12.50±0.61^{ab}
Hct (%)	46.63±1.46^a	42.36±1.46^{ab}	38.02±1.40^{bc}	33.68±1.48^c	43.95±1.35^a
MCH (pg)	13.16±0.39^a	15.47±1.17^a	14.10±1.90^a	16.28±2.49^a	15.83±1.28^a
MCHC (g/dL)	27.34±1.71^a	27.20±1.86^a	27.50±2.24^a	26.30±2.24^a	27.83±1.56^a
MCV (fL)	50.59±1.66^a	53.01±2.81^a	55.28±5.23^a	63.57±6.21^a	55.59±4.08^a
WBC ($\times 10^3$/mm^3)	11.79±0.66^a	12.08±0.53^a	13.60±0.73^{ab}	15.19±0.79^b	11.78±0.66^a
Platelets ($\times 10^3$/mm^3)	641.09±23.47^a	610.64±25.67^{ab}	511.69±23.16^{bc}	443.46±24.29^c	582.49±16.15^{ab}

Values are mean±SE of eight rats, values in a row bearing different superscripts are significantly different from each other ($P<0.05$). RBC: Red blood cell, Hb: Hemoglobin, Hct: Hematocrit, MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration, MCV: Mean corpuscular volume, WBC: White blood cell

were non-significantly changed in comparison with control and $MnCl_2$ exposed groups. In recovery group, there was a significant increase in RBCs, Hb, Hct, PLTs values and a reduction in WBCs count when compared with all three $MnCl_2$ treated groups. The values of these parameters in recovery group were almost comparable to that of the values of these parameters in control group [Table 1].

SEM Results

SEM micrographs of blood sample of control rat showed normal morphology of erythrocytes having smooth surface with concavity and rounded shape. On the other hand, $MnCl_2$ administered rats showed deformed erythrocytes with wavy appearance and spicule-like projections on cell surface. Visual observation of SEM picture of blood showed an increase in abnormal RBC in dose-dependent manner. Photomicrograph of blood sample of recovery group showed significant improvement in erythrocyte morphology as compared to Mn-treated rats. [Figures 1-5].

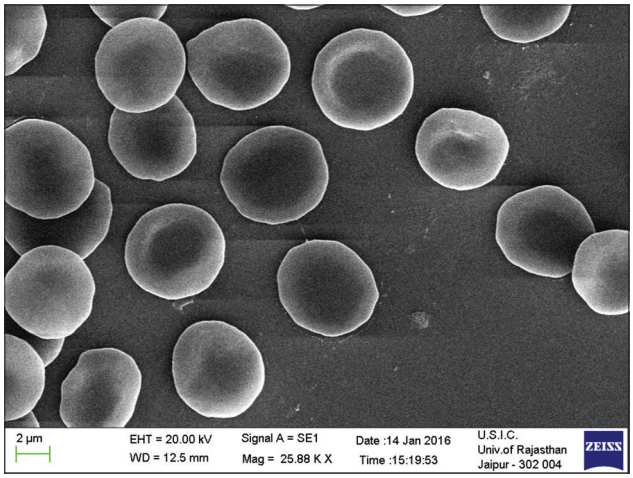

Figure 1: Scanning electron photomicrograph of blood sample of control rat showing normal red blood cells with rounded shape and concavity

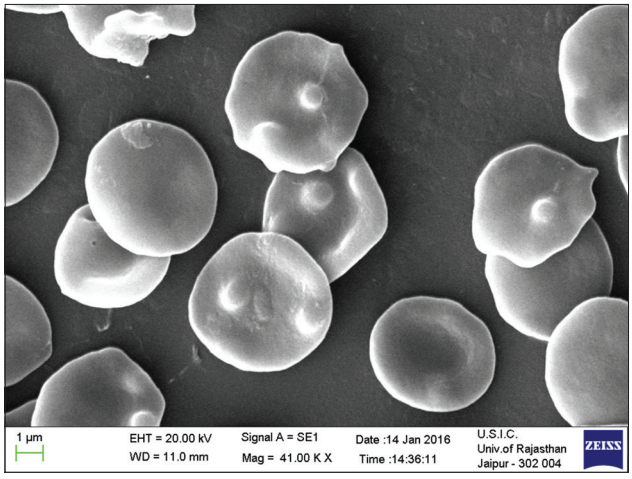

Figure 2: Scanning electron photomicrograph of blood sample of $MnCl_2$ (50 mg/kg b.wt.)treated rat showing altered shape of erythrocyte

DISCUSSION

In recent years, hematological variables have been used to determine the sublethal concentration or toxicity of environmental pollutants and drugs in humans and animals [29]. Results of the present investigation showed that the $MnCl_2$ treatment inflicted a drastic dose-dependent reduction in the total RBC count, Hb, and Hct values. The decrease in hematological parameters (RBC, Hb and Hct)observed in the present study is in agreement with some earlier studies who also showed a decrease in blood indices in Mn-exposed animals [20-32]. A similar decline of these blood parameters has also been reported in animals exposed with other metals such as Cd, Hg [13,33]. The decline of blood parameters value might be the result of disturbed activity of hematopoietic system as hematopoietic system, is one of the most sensitive systems to assess the toxicity of environmental toxins in animals [29]. Due to metal toxicity, hematopoietic organs may get affected and became unable to release normal RBCs in general

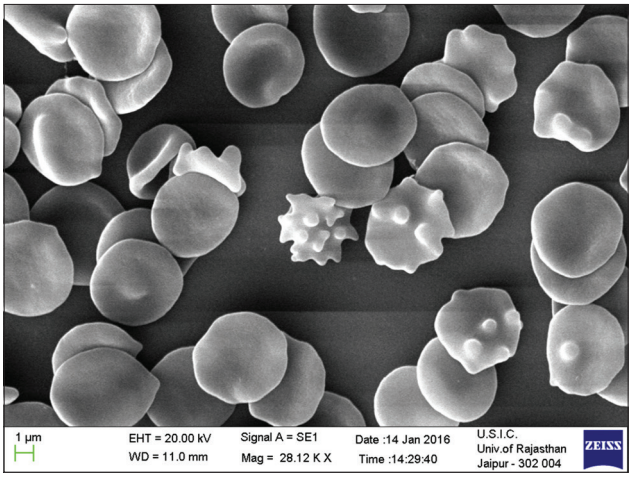

Figure 3: Scanning electron photomicrograph of blood sample of $MnCl_2$ (100 mg/kg b.wt.)treated rat showing increased alteration in shape of erythrocytes

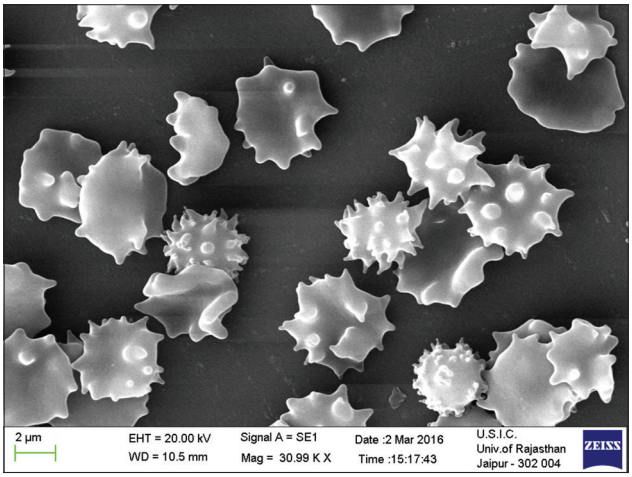

Figure 4: Scanning electron photomicrograph of blood sample of $MnCl_2$ (150 mg/kg b.wt.)treated rat showing highly deformed and shrinked erythrocytes with increased number of leucocytes

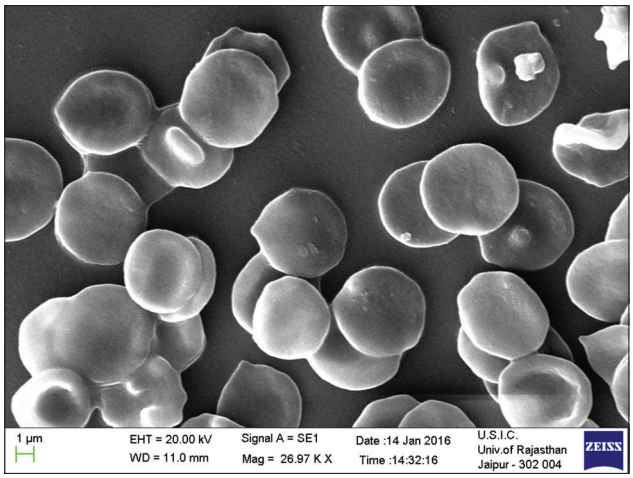

Figure 5: Scanning electron photomicrograph of blood sample of recovery rat (60 days after treatment withdrawal)showing increased number of normal erythrocytes

circulation and thus can be held responsible for drastic decline in RBC's count [33,34]. There are several studies that indicate accumulation of Mn in bones [15], liver and kidneys [16]where it might suppress the activity of these important hematopoietic tissues. It has been reported that Mn can induce hepatic and renal damage which may lead to the defect of secretion of the erythropoietin and consequently the formation of RBCs in the bone marrow [35]. Mn is generally believed to exert cellular toxicity through a number of mechanisms, including the induction of free radical production, direct or indirect formation of reactive oxygen species (ROS), enhancing the rate of lipid peroxidation [23]. Fernsebner *et al*., [36]reported that overexpose of Mn might induce the shift of the Fe (II)/(III) ratio showing an increased level of Fe (II)in the rat brain which could then participate in different oxidative reactions such as the Fenton reaction or lipid peroxidation. Excessive generation of ROS can lead to anemia as a result of accelerated erythrocyte destruction due to altered erythrocyte membrane permeability and increased mechanical fragility in RBCs [33].

Mn-induced toxic effects on RBCs were also confirmed by morphological studies of blood cells. SEM study of the blood of Mn-treated rats clearly revealed dose-dependent increase in number of distorted RBCs suggesting that Mn exposure lead decline in both quantity and quality of RBCs. Distorted shape of RBCs can lead to tissue hypoxia by reducing the oxygen carrying capacity of RBCs [32].

Hb is the iron-containing oxygen-transport metalloprotein in the RBCs, and the Hct is the volume percentage (%)of RBCs in blood which is major determinant of viscosity [37]. The decreases in Hb concentration in Mn-exposed rats in the present study represent impaired supply of adequate oxygen to the tissues consequently resulting in decline of physical stir. It has been reported that Mn interferes with absorption of the dietary iron sharing the similar metabolic pathway as that of iron [20]. Thus, long-term exposure to excess levels of Mn may result in iron deficient anemia and decline in production of Hb [38]. Smith *et al*., [39]suggested that after absorption Mn enters the circulation and binds to transferrin. This conjugate, later on, binds to transferrin receptors on erythroid cells. These erythroid cells potentially may incorporate Mn into the protoporphyrin ring in place of iron. The formation of hybrid Hb may result in an altered oxygen caring capacity of Hb [40]. Moreover, the significant decline in Hb and Hct values in Mn-treated group might be attributed to the formation of methemoglobin, a form of Hb, which has a decreased ability to bind oxygen [30]. In the present study, no significant changes were observed in MCH, MCHC, and MCV after Mn administration.

WBCs play a major role in the defense mechanism of the animal. In the present investigation, WBC count showed a significant increase in Mn-exposed rats when compared with that of the control group. These results are in accordance with earlier workers [31,32,41]who also reported an increase in WBC count after exposure with Mn compounds. In contrast to our findings, some workers have reported decrease in WBC count after Mn exposure [30,42]. This discrepancy in the results might be due to different route of administration and short duration. The increase in WBC count observed in Mn-treated groups in the present study may indicate an activation of the animal's immune system in response to tissue damage caused by any toxicant [43].

Blood platelets play an important role in blood clotting and prevent blood loss from hemorrhaging. Our results showed decrease in platelet number after exposure with $MnCl_2$. This might to be due to disturbance in hematopoiesis process. A similar decline in platelets count has been reported in fishes after $MnSO_4$ administration [32]. In contrast to this, Khan *et al*. [42]reported an increase in platelet number in $MnCl_2$ exposed dogs. This might be due to short duration and intravenous route of administration. In the present study, after 60 days of cessation of Mn exposure, all these altered hematological parameters showed recovery indicating resumption of normal functioning of hematopoietic tissues. This is likely due to redistribution and excretion of Mn from the body as Mn has half-life of 10-42 days [9].

On the basis of the present study, it can be concluded that $MnCl_2$ exposure showed an adverse impact on hematological parameters in rats. Most of the changes in these parameters returned towards normal side after 60 days of Mn exposure withdrawal. Therefore, further studies are suggested to better understand the mechanism(s)of Mn-induced hematological toxicity and possible mechanism behind the recovery.

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