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ORIGINAL PAPER

Changes in serum parameters associated with iron metabolism in male rat exposed to lead

Minoo Moshtaghie · Pedram Malekpouri · Mohammad Reza Dinko · Ali Asghar Moshtaghie

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Abstract Due to the severe hazardous influences of lead (Pb^{2+}) on iron-related diseases, the effects of Pb^{2+} on serum parameters associated with iron metabolism have been studied in this project. Male Wistar rats weighing 200–250 g were treated with Pb^{2+} for the short and long period of times. The animals received daily intraperitoneal injection of 100 mg Pb^{2+} kg^{-1} body weight (BW) for 5 days and 4 mgkg^{-1} BW of Pb^{2+} for 30 and 45 days, respectively. The results show that when animals were treated with both low and high concentrations of Pb^{2+}, serum iron concentration decreased markedly, by 23.2, 32.8, and 39.9 %, while the sera TIBC and transferrin concentrations increased significantly ($p<0.05$). Following short- and long-term exposures to Pb^{2+}, the percentage of serum transferrin saturation was also decreased in comparison with the untreated control group ($p<0.05$). Concentrations of serum copper and ceruloplasmin following Pb^{2+} treatments also reduced significantly ($p<0.05$). The percentage of hematocrit and hemoglobin levels was reduced ($p<0.05$) in all Pb^{2+}-treated animals in comparison with the controls. These results suggest that Pb^{2+} changes the serum parameters related to iron metabolism, which may play an important role in producing iron-related diseases.

Keywords Lead · Iron · Copper · Transferrin · Ceruloplasmin

M. Moshtaghie
Department of Environment and Energy,
Science and Research Branch, Islamic Azad University,
Tehran, Iran

P. Malekpouri (✉)
Young Researchers Club, Science and Research Branch,
Islamic Azad University,
Tehran, Iran
e-mail: p.malekpouri@gmail.com

M. R. Dinko
Department of Biochemistry, School of Pharmacy,
Isfahan University of Medical Science,
Isfahan, Iran

A. A. Moshtaghie
Department of Biochemistry, School of Basic Science,
Falavarjan Branch,
Islamic Azad University,
Isfahan, Iran

Introduction

Lead (Pb^{2+}), one of the most highly toxic elements, is used in industrial activities, and it therefore is present considerably in our environment [25]. For many years, the toxicity of Pb^{2+} in humans and animals has been demonstrated frequently [13, 35, 40, 44]. It is now well documented that Pb^{2+} may easily enter the body via several routes and causes many disturbances; amongst them, anemia is one of the most prevalent diseases, which may occur following Pb^{2+} toxicity [15, 37].

Iron is an essential trace element and plays an important role in the synthesis of many heme and non-heme metalloproteins, including hemoglobin,

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myoglobin, transferrin (Trf), ferritin, etc. [41]. Iron enters the blood circulation and binds to apotransferrin [27]. This protein is responsible for transporting iron from the site of absorption to the site of utilization for heme and non-heme protein synthesis [5, 46]. The last step of heme biosynthesis is associated with the activity of the mitochondrial ferrochelatase enzyme, which catalyzes iron movement across the mitochondrial membrane for heme synthesis [7]. This enzyme might be inhibited by some toxic metals [18] and then lead to hypochromic microcytic anemia [42].

Reduction in serum iron concentration might also be engendered by other factors like bacterial growth in infectious diseases [42]. The measurement of serum total iron-binding capacity (TIBC) level, besides serum iron, may assist in investigation of iron status in the serum of those with iron disturbances. Therefore, measurements of serum iron, TIBC, and the percentage of Trf saturation for clinical diagnosis of iron-related disease are required [10, 17]. Ceruloplasmin (Crp) is a ferroxidase containing copper (Cu), which is synthesized by hepatocytes [21]. The major role of Crp in iron metabolism is related to the oxidation of ferrous ion to ferric for binding to Trf and probably to other iron-containing proteins, including ferritin in the cells [16]. On the other hand, Cu deficiency may cause a reduction in Crp concentration and subsequently disturb iron metabolism [2]. In this regard, the relationship between Pb^{2+} and Cu deficiency has not been fully elucidated yet [24].

It is now well evidenced that due to the chemical similarities between iron and some other trace elements such as chromium, cadmium, indium, and aluminum, they enter blood circulation and bind to serum Trf and interfere with the biochemical pathways of iron [1, 3, 31, 33, 34]. On the other hand, the inhibitory effects of Pb^{2+} on other molecules have been already reported [35, 47]. For example, first key enzyme for the heme synthesis, aminolevulinate synthase, which is able to catalyze the conversion of succinyl-CoA and glycine to aminolevulinic acid [11], might be inhibited by some toxic metals, including Pb^{2+}, Cd, etc. [27]. With regard to the above explanations, we encouraged to carry out the present project to clarify the possible involvement of Pb^{2+} on iron metabolism parameters, including serum iron, TIBC, Trf, Crp, Cu, hemoglobin (Hb), hematocrit (Hct), and percentage of Trf saturation. Rat was used as an experimental animal model in this project.

Materials and methods

Animal treatments

All chemicals used in this study were reagent grade and obtained from Sigma Chemical Company (Germany). Eight-month-old male Wistar rats weighing 200–250 g were used to carry out this project. All animals were fed with standard food and water ad libitum. Animal's room, which is used for maintaining all experimental rats throughout the study, encompassed 20±3 °C in temperature, 40–60 % in relative humidity, and had no changes in normal light–dark cycles as well. Various concentrations of Pb^{2+} as Pb $(CH_3COO)_2{\cdot}3H_2O$ were prepared in normal saline (NaCl 9 gL^{-1}). Controls were treated with only the same volume of normal saline (0.3 ml) as used for experimental animals.

To achieve this project, the animals were divided into two separated groups entitled short-term and long-term exposures of Pb^{2+}. For each group, a specific control group which was untreated with Pb^{2+} was selected. For the investigation of Pb^{2+} exposure on iron metabolism in short term, animals were injected intraperitoneally with 100 mg Pb^{2+} kg^{-1} body weight (BW) daily for five continuous days (lower than LD50 for intraperitoneal injection in rat, which is 150 $mgkg^{-1}$ [26]). In long-term study, the animals were treated by daily intraperitoneal injection of 4 mg Pb^{2+} kg^{-1} BW for 30 days and also 45 days. All control groups received only the above-mentioned normal saline.

Sample preparation and serum biochemical determination

At the end of experimental times, all rats were killed by decapitation and blood samples were collected carefully in acid pre-washed tube and then centrifuged at 2,000 rpm for 5 min to separate blood cells from serum. The residual blood was collected separately and prepared for Hb and Hct measurements. Hb was measured by the method reported by Fairbanks [10] and Hct was determined by using total blood and microhematocrit set (Adams Autocrit Centrifuge model). Serum iron and TIBC were measured by using phenanthroline as the chromogen [49]. The amount of Cu in serum was determined by atomic absorption (Perkin Elmer). Crp concentration in the serum was estimated at 525 nm by using

paraphenylendiamine dihydrochloride as a substrate [20]. The percentage of Trf saturation calculated by the ratio of serum iron to TIBC as indicated in following equation [48]:

$$\frac{Serum\ Fe \times 100}{TIBC} = Trf\ saturation\%.$$

Serum Trf was also calculated using the equation below [48]:

$$Serum\ Trf = 0.7 \times TIBC.$$

Statistical analysis

The *independent samples t test* was used for comparing each treatment's mean value with their specified control using SPSS software (version 18). Level of significance was also established less than 0.05 ($p<0.05$) in all cases.

Results

In the first series of experiments, the short- and long-term effects of Pb^{2+} on serum iron concentrations were investigated. Data presented in Fig. 1 show that daily Pb^{2+} administration (100 mg Pb^{2+} kg^{-1} BW for 5 days) to rat has reduced serum iron level by 23.2 % when compared to control. Significant reductions in iron levels by 32.8 and 39.9 % were seen when rats were treated with 4 mg Pb^{2+} kg^{-1} BW for 30 and 45 days, respectively (Fig. 1). Significant ($p<0.05$) increases in the serum TIBC by 26.7, 45.8, and 55.4 % in comparison with control were found when animals treated with all Pb^{2+} doses, whereas there was an obvious decrease ($p<0.05$) in the percentage of Trf saturation in animals treated with same doses of Pb^{2+} (Figs. 2 and 3). The amount of serum Trf has been calculated as described in "Materials and methods." It can be seen from Fig. 4 that short-term Pb^{2+} administration (100 mgkg^{-1} BW) raised Trf concentrations by 26.7 % and long-term (30 and 45 days) administration of 4 mg intraperitoneal Pb^{2+} raised serum Trf by 45.8 and 55.4 % compared to the control, respectively. The percentage of Trf saturation in Pb^{2+} treated animals and in control was also investigated following daily (5 days) interpretational administration of Pb^{2+} (100 mgkg^{-1} BW). It was found that Trf saturation

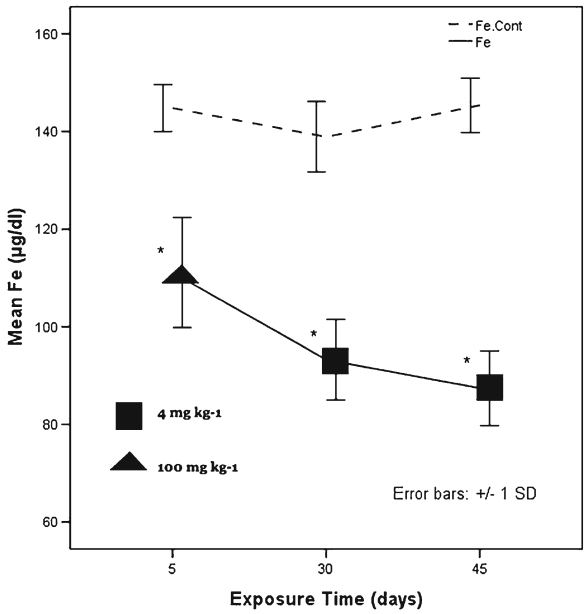

Fig. 1 The effect of Pb^{2+} on serum iron concentration in rat. Doses of Pb^{2+} were injected daily for 5, 30, and 45 continuous days to rats. Animals were killed by decapitation at indicated times. Sera were prepared and iron concentrations were determined as mentioned in "Material and methods." Each point is the mean of five observations. *Asterisks* indicate significant differences ($p<0.05$) between treatments and their specified controls

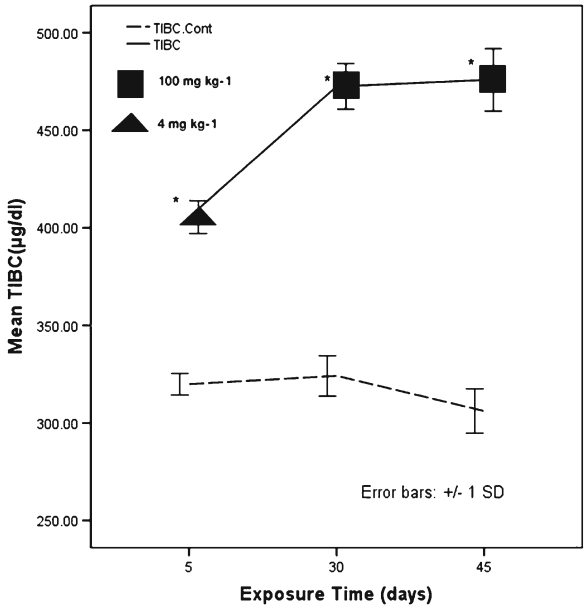

Fig. 2 The effect of Pb^{2+} on serum TIBC during short and long periods. Rats were injected daily with doses of Pb^{2+} as mentioned in the legend of Fig 1. Sera were obtained and TIBC level was measured. Values represent mean of five random samples± SD. Significances are expressed by *asterisk* at $p<0.05$

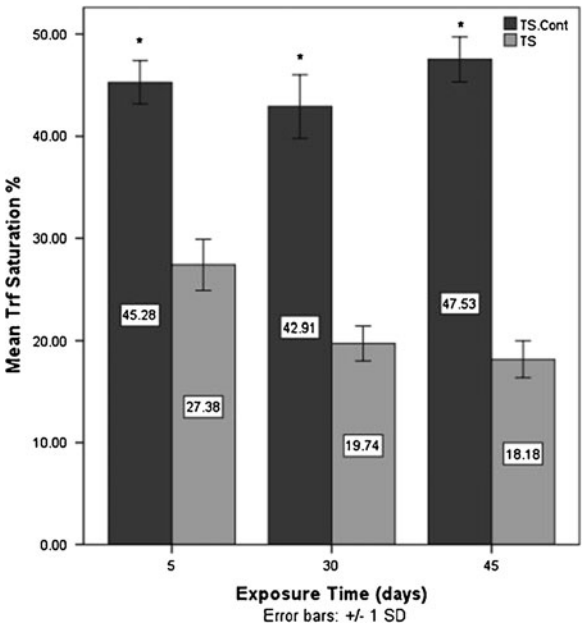

Fig. 3 The percentage of Trf saturation following Pb^{2+} exposures. Intraperitoneal injections of rats have been done daily for 5 days of exposure with Pb^{2+} (100 mg kg^{-1}) and long term (4 mg kg^{-1}) for 30 and 45 continuous days. Mean and SD (N=5) are shown for each individual time. *Asterisks* indicate significant differences (p<0.05) between treatments and their specified controls

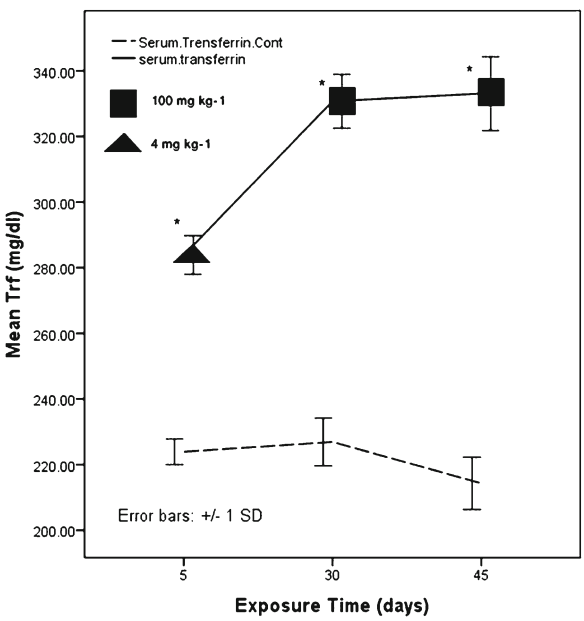

Fig. 4 Sera Trf concentrations after Pb^{2+} exposures. Thirty animals were treated with intraperitoneal injection for short and long periods of time. Values are the averages of five individual measurements (±SD) and compared to control treatment (p<0.05)

was reduced by 39.5 % in comparison with control treatment. The percentages of Trf saturation in controls were 2.2-fold and 2.6-fold higher than their considered treatments after 30 and 45 days, respectively.

Since Crp acts as ferroxidase activity in iron metabolism, the concentrations of Cu and Crp in the sera of both Pb^{2+}-treated and untreated controls were determined next and compared with each other. As indicated in Fig. 5, treatment with Pb^{2+} significantly (p<0.05) lessened Crp in comparison with control in all treated groups. Decreases in serum concentration of Crp following 5, 30, and 45 day of exposures were approximately 26.5, 29.4, and 29.7 % compared to control, respectively (Fig. 5). Levels of Cu in both exposed and unexposed rats were also determined. A reduction in serum Cu by 28.2 % was seen when animals were treated with 100 mg Pb^{2+} kg^{-1} BW for 5 days. Moreover, significant reductions (p<0.05) were also observed when they were treated with the low dose of Pb^{2+} for 30 and 45 days. Cu contents in sera of control were approximately 2.4- and 4.04-fold higher than in rats that had been exposed to 4 mg kg^{-1} BW for 30 and 45 days, respectively (Fig. 6).

Following 5 days of Pb^{2+} administrations, Pb^{2+} reduced the Hb levels by 34.1 % when compared to control. Higher concentrations of Hb, about 2.7- and

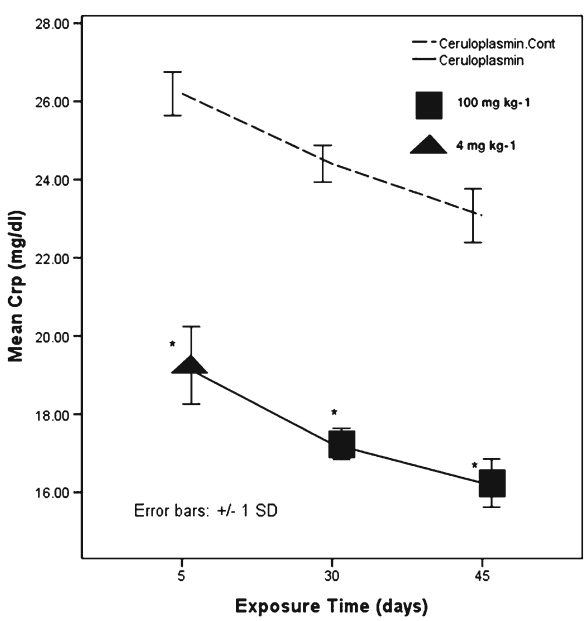

Fig. 5 Effect of daily injection of Pb^{2+} on Crp levels. Rats were injected daily with doses of Pb^{2+} as mentioned in the legend of Fig. 1. Data are expressed as mean±SDs. *Asterisks* indicate significant differences (p<0.05) between treatments and their specified controls

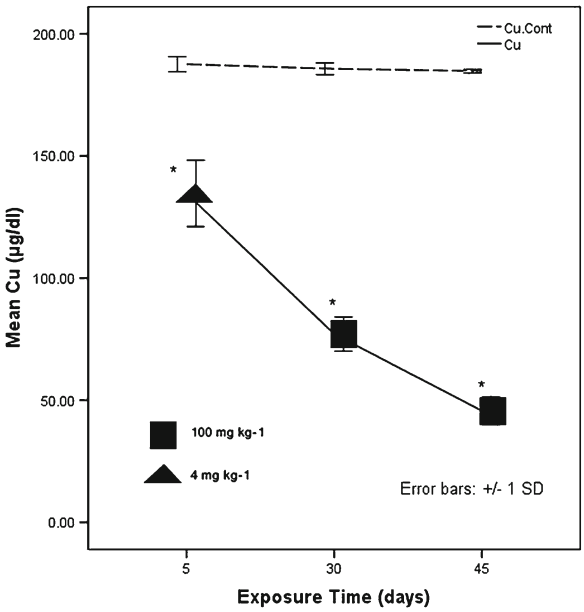

Fig. 6 Changes in serum Cu following short- and long-term Pb^{2+} exposures. Rats were injected daily with doses of Pb^{2+} as mentioned in the legend of Fig. 1. Mean±SDs are represented for each treatment. *Asterisks* indicate significant differences ($p<0.05$) between treatments and their specified controls

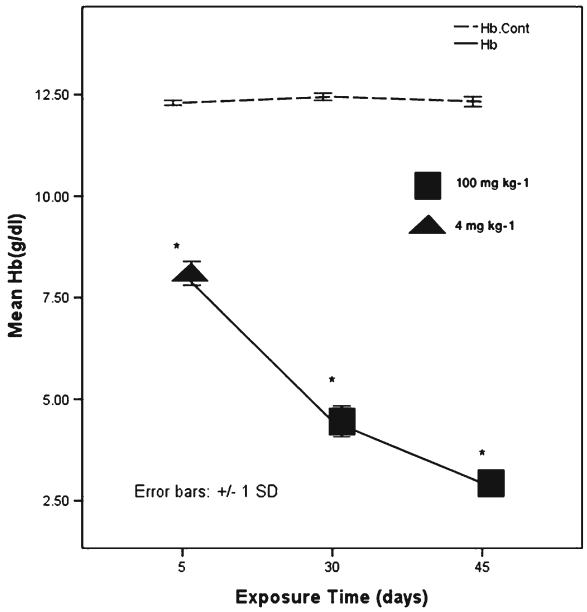

Fig. 7 Effect of daily injection of Pb^{2+} on Hb levels. Rats were injected daily with doses of Pb^{2+} as mentioned in the legend of Fig. 1. Data are expressed as mean±SD. *Asterisks* indicate significant differences ($p<0.05$) between treatments and their specified controls

4.2-fold in control, have been seen following 30 and 45 days of Pb^{2+} exposure, respectively (Fig. 7). Treatment of animals with Pb^{2+} resulted in significant ($p<0.05$) diminishing of Hct percentage. Following 5 days of exposure, Hct was reduced by 36.3 % in comparison with control. Rats, which were injected daily for 30 days with Pb^{2+} (4 $mg kg^{-1}$ BW), showed a marked reduction in the percentage of Hct. In other word, the percentage of Hct in control was 2.7-fold higher than Pb^{2+} treated. In the end, the percentage of Hct in the control group was 3.98-fold higher than 45 days of Pb^{2+} treatment (Fig. 8).

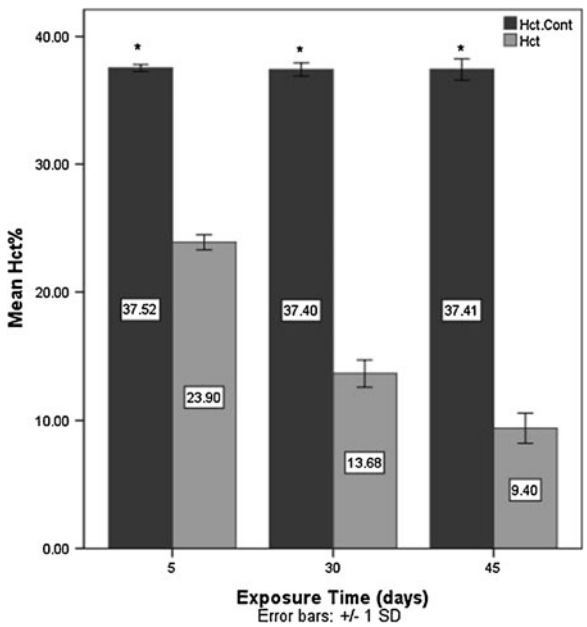

Fig. 8 Effect of different doses of Pb^{2+} on Hct percentage. Intraperitoneal injections of rats with Pb^{2+} have been done daily for 5 days of exposure (100 $mg kg^{-1}$) and long term (4 $mg kg^{-1}$) for 30 and 45 continuous days. All data appear as mean and SD ($N=5$). Significances are expressed by *asterisk* at $p<0.05$ when compared with control treatment

Discussion

The data presented in this study indicated a significant reduction in the concentration of serum iron and an elevation of serum TIBC following short- and long-term Pb^{2+} administrations to rats (Figs. 1 and 2). Since iron in the plasma binds to transferrin molecules, this binding activity might be disturbed by Pb^{2+}, and, therefore, unbound iron might be excreted through the kidneys of animals [38]. The elevation of serum TIBC might confirm this hypothesis. In agreement

with our hypothesis, Hegazy et al. [15] and Jain et al. [19] demonstrated that there was a direct relationship between a high serum level of Pb^{2+} and low serum iron concentrations in children who suffer from anemia.

It is quite clear that, following the reduction of serum iron and elevation of TIBC, the Trf concentration should be elevated in the same manner, which confirms data presented in Fig. 4. Low levels of serum iron also led to the reduction in the percentage of Trf saturation (Fig. 3). These data are consistent with previous work, which had been performed by Becking et al. [4]. Data presented in Fig. 5 show that Pb^{2+} treatment led to a reduction in the percentage of Trf saturation.

Since iron in the plasma will be oxidized first by the ferroxidase activity of Crp and then bound to the Trf, it seems that the reduction in Crp level following Pb^{2+} treatment reduces the oxidation of Fe(II) to Fe(III) [16] with a subsequent reduction in the percentage of Trf saturation. Consistent with our findings, previous studies have shown that Pb^{2+} has a preventive effect on the liver function, which is responsible for the production of Crp [9, 37] and causes a Crp reduction in serum [12]. The inhibition of Crp following Pb^{2+} has been addressed as a biomarker for Pb^{2+} intoxications [23]. Our findings also showed that short- and long-term administration to rats with Pb^{2+} lead to a significant reduction in serum concentration of Cu. Previous observations have also suggested that Pb^{2+} could alter the levels of some elements especially Cu [45]. Decrease in serum Cu leads to a simultaneous reduction in plasma Crp [39]. The reduction in Crp could be due to the inhibition of Cu binding to apo-Crp in the presence of Pb^{2+}, and thereby, the metabolism of iron will be impaired sequentially.

We have found a significant reduction in Hct% and Hb in all Pb^{2+} treatments. This might be due to the interference effect of Pb^{2+} on some steps of heme synthesis pathways [8, 14]. Pb^{2+} may inhibit any key enzyme in the biosynthesis of hemoglobin, or, alternatively, Pb^{2+} may reduce the production of globin by liver, which is necessary for the production of hemoglobin. Toxic elements may reduce the production of protein by the liver as well. In agreement with our findings, Jain et al. [19] and Hegazy et al. [15] reported reductions in Hct% and Hb in children who were exposed to Pb^{2+}. Kohno et al. [22] have shown that, when erythroleukemia (K562) cells were treated with 0.1 mmol/L Pb^{2+} for 48 h, the rate of cellular iron uptake from transferrin was reduced by 48 %, confirming the reduction of heme synthesis in the presence of Pb^{2+}. In contrary to the above observation, Qian et al. [43] have shown that the inhibitory effect of Pb^{2+} on iron uptake may occur intracellularly, rather than in membrane-binding step, probably inhibitory translocation of iron across the endosomal membrane, which lead to the reduction in Hb synthesis.

The influences of toxic elements on iron metabolism have been reported during last decades. Moshtaghie et al. [33] showed the interaction of cadmium with iron metabolism using in vivo and in vitro studies. They also suggested that the inhibition effect of cadmium on iron binding to Trf may disturb heme synthesis. Cadmium also induced iron deficiency in rats [6]. Changes in iron-related parameters in rats, which were exposed to chromium and/or aluminum, have been also reported [1, 30, 31]. Moreover, other elements such as gallium, nickel, indium, and cobalt may have significant effects on iron metabolism [28, 29, 32–34, 36].

Overall, due to high toxicity of Pb^{2+} in one hand and its abundance in our living environment on the other hand, this nonessential toxic element may enter iron biochemical pathways particularly heme synthesis and disturbs production of hemoglobin. Reduction in Hb, Hct, and iron levels may suggest that this toxic element would be probably able to interfere with any stage of iron metabolism, which included absorption, blood circulation, and/or intracellular movement. This interference may also affect liver function, which is responsible for Trf and Crp synthesis or alternatively affects kidney function by decreasing in erythropoietin synthesis, which is necessary for erythropoietic processes. Therefore, appearance of anemia as a devastating prevalence disease especially due to environmental pollution with Pb^{2+} can occur. We have found that the effect of Pb^{2+} on these parameters is a dose-dependent process as well. We believe that more deep investigation should be done at the cellular and molecular levels to elucidate the exact mechanism of Pb^{2+} toxicity on iron metabolism. By using rat everted gut sac technique in the future, we will be able to study the interaction of Pb^{2+} with iron absorption in intestinal mucosal cells.

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