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Effects of Nano-zinc on Biochemical Parameters in Cadmium-Exposed Rats

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Abstract Cadmium (Cd) is a toxic environmental and occupational pollutant with reported toxic effects on the kidneys, liver, lungs, bones, and the immunity system. Based on its physicochemical similarity to cadmium, zinc (Zn) shows protective effects against cadmium toxicity and cadmium accumulation in the body. Nano-zinc and nano-zinc oxide (ZnO), recently used in foods and pharmaceutical products, can release a great amount of Zn²⁺ in their environment. This research was carried out to investigate the more potent properties of the metal zinc among sub-acute cadmium intoxicated rats. Seventy-five male Wistar rats were caged in 15 groups. Cadmium chloride (CdCl₂) was used in drinking water to induce cadmium toxicity. Different sizes (15, 20, and 30 nm) and doses of nano-zinc particles (3, 10, 100 mg/kg body weight [bw]) were administered solely and simultaneously with CdCl₂ (2–5 mg/kg bw) for 28 days. The experimental animals were decapitated, and the biochemical biomarkers (enzymatic and non-enzymatic) were determined in their serum after oral exposure to nano-zinc and cadmium. Statistical analysis was carried out with a one-way ANOVA and t test. P < 0.05 was considered as statistically significant. The hematocrit (HCT) significantly increased and blood coagulation time significantly reduced in the nano-zinc-treated rats. AST, ALT, triglyceride, total cholesterol, LDL, and free fatty acids increased significantly in the cadmium- and nano-zinc-treated rats compared with the controls. However, albumin, total protein, and HDLc significantly decreased in the cadmium- and nano-zinc-treated rats compared with the controls (P < 0.05). It seems that in the oral administration of nano-zinc, the smaller sizes with low doses and the larger sizes with high doses are more toxic than metallic zinc. In a few cases, an inverse dose-dependent relationship was seen as well. This research showed that in spite of larger sizes of zinc, smaller sizes of nano-zinc particles are not suitable for protection against cadmium intoxication.

Keywords Zinc nano-particle · Cadmium · Biochemical parameters · Rats

Introduction

Cadmium is one of the toxic heavy metals that pose serious health hazards. Cadmium toxicity was diagnosed in many organs, such as the kidneys and liver, and the respiratory, reproductive, brain, and skeletal systems [1, 2]. Cadmium exposure occurs by the ingestion of contaminated food and water, or the inhalation of polluted air [3, 4]. The mechanism of cadmium toxicity consists of binding to sulphur (–SH) groups of proteins, enzymes, and glutathione, inhibiting the function of these biomolecules, generating oxidative stress, decreasing anti-oxidant and thiol group status, binding to Ca²⁺ binding proteins, inhibiting DNA methylation and DNA repair, and damaging cells and carcinogenicity [5–7]. The International Agency of Research on Cancer has classified this metal and its compounds as group 1 human carcinogen [3]. Cadmium and zinc are closely related metals with similar chemical properties. They probably have a common kinetic pathway—i.e. the absorption, distribution, and excretion—in terms of biological effects [8, 9]. In spite of cadmium, zinc is an essential element needed for the activity of many

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enzymes and hormones. Numerous studies have shown that treatment with some doses of zinc supplement in cadmium-intoxicated animals decreases cadmium absorption and accumulation and ameliorate some of its toxic effects [2, 10–16]. Moreover, zinc is a potent inducer of metallothioneins (MTs), a class of low molecular weight (6–7 kDa) and cytosolic cysteine-rich proteins, which are considered to play key roles in cellular defence against cadmium toxicity. Suzuki et al. [17] showed that treating animals with cadmium, followed by zinc infusion, can significantly increase the synthesis of MTs.

Nano-zinc is a relatively new product, which is utilized in many commercial products, including cosmetics, personal hygiene products, anti-bacterial agents, paints, textiles, and food packaging. It has also been used as an additive in both human and livestock food while zinc by itself can impress the immunity system. It also has anti-inflammatory properties [18]. Nano-materials are theoretically expected to be more potent than their bulk equivalents because of their greater surface reactivity and the ability to penetrate into cells and organisms [19, 20]. It has been previously shown that some potent effects of nano-zinc particles were related to their pH-triggered release of Zn²⁺ ions in the culture medium or inside cells or in the aquatic mediums [21–25]. Nano-zinc ions induce metallothionein synthesis. Ding et al. [26] revealed that nano-sized ZnO particles are more potent MT inducers than zinc in the liver of Arbor Acres chickens. According to the results of another of our studies, the larger sizes and intensive doses of zinc nano-particles can upregulate MT expression. Renal MTs were induced significantly in response to the simultaneous use of cadmium and different doses of nano-zinc (20 and 30 nm) exposure [27]. The present research assessed the impact of different sizes and doses of nano-zinc particles, used solely and/or in combination with cadmium on some biochemical parameters in male Wistar rats.

Materials and Methods

Characterization of Nano-zinc Particles Three sizes of nano-zinc metal powder were bought from Intelligent Materials Pvt. Ltd., Nanoshel LLC, Wilmington, DE, USA. The mediocre particle sizes of the nano-scale zinc particles were 15 nm (10–15 nm), 20 nm (15–20 nm), and 30 nm (20–30 nm), identified by transmission electron microscopy (TEM; Fig. 1). These three sizes of nano-zinc metal particles possessed more than 99.99% purity.

Preparation of Particle Suspension The particles were administrated in three sizes and three doses (3, 10, and 100 mg/kg bw). Prior to use, the particles were suspended in 1% sodium carboxymethyl cellulose. The particles were dispersed by ultrasonic vibration for 15 min, and glass beads were added to avoid the aggregation of the particles in the suspension [28].

Chemicals All reagents and chemicals used in this experiment were selected from a high analytical grade or were of higher purity.

Animals and Treatment Seventy-five adult (10-week-old) male Wistar rats, weighing 253 ± 25 g, were collected from the Pasteur Institute of Iran. During the experiment, the animals were housed in controlled conventional conditions (temperature 22 ± 2 °C, relative humidity 50–70%, 12-h light-dark cycle). They were given free access to water and a conventional rodent pellet (2390 kcal/kg metabolic energy and 10,320 kcal/kg digestible energy; 19.5% crude protein; 10% crude fibre; 0.69% phosphor; and 0.76% calcium). The local ethics committee approved the design of the experiments and the protocol conformed to the guidelines of the National Institute of Health. After 2 weeks of acclimation, the rats were
randomly divided into 15 experimental groups, each containing five animals (n = 5 each).

**Experiment Design** The control group included the animals that drank the water with no contaminant. Nine groups were exposed to nano-zinc of 15, 20, and 30 nm with doses of 3, 10, and 100 mg/kg bw by daily gavages for 28 days. These animals were additionally exposed to CdCl₂ via their drinking water (2.5–5 mg/kg bw, 1/15 LD₅₀) for 28 consecutive days [29]. One group was exposed to CdCl₂ via drinking water (2.5–5 mg/kg bw), and the other one was exposed to CdCl₂ (2.5–5 mg/kg bw) and bulk zinc chloride (ZnCl₂) (10 mg/kg bw) via drinking water for 28 days. Three groups were treated with nano-zinc 20 nm (3, 10, and 100 mg/kg bw) by daily gavages for 28 days as the control groups of nano-zinc. At the end of the administration period, the rats were anaesthetised by chloroform and decapitated. Blood samples were collected; the serum was separated after centrifugation and preserved at −80 °C until the due date of the tests. All animal procedures used in this study were approved by the University of Tabriz standards for human care and use of laboratory animals, conforming to the Ethical Research Committee of the Ministry of Health and Medical Education of Iran (adopted on 17 April 2006) based on the Helsinki Protocol (Helsinki, Finland, 1975).

**Biochemical Serum Assay** The serum was derived by the centrifugation of the whole blood at 3000 rpm for 15 min. The serum biochemical levels, including blood urea, creatinine (CR), alanine aminotransferase (ALT), aspartate aminotransferase (AST), serum albumin, serum total protein (TP), total cholesterol (TC), triglyceride (TG), LDLc, and HDLc, were assayed by an automatic biochemical analyser (7170A, Hitachi, Tokyo). Zinc and cadmium were measured using graphite furnace atomic absorption spectroscopy.

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**Fig. 3** Blood creatinine in controls, cadmium (Cd), cadmium and zinc (Cd + Zn), nano-Zn 20 nm (3 mg/kg) (Zn20–3), nano-Zn 20 nm (10 mg/kg) (Zn20–10), and nano-Zn 20 nm (100 mg/kg) (Zn20–100)-treated rats. Significant compared to controls (α).

**Fig. 4** Blood creatinine in controls, cadmium (Cd), cadmium + nano-Zn 15 nm (3 mg/kg) (Cd + Zn15–3), cadmium + nano-Zn 15 nm (10 mg/kg) (Cd + Zn15–10), cadmium + nano-Zn 20 nm (3 mg/kg) (Cd + Zn20–3), cadmium + nano-Zn 20 nm (10 mg/kg) (Cd + Zn20–10), cadmium + nano-Zn 30 nm (10 mg/kg) (Cd + Zn30–10), and cadmium + nano-Zn 30 nm (100 mg/kg) (Cd + Zn30–100). Significant compared to controls (α).

**Fig. 5** Serum albumin in controls, cadmium (Cd), cadmium and zinc (Cd + Zn), nano-Zn 20 nm (3 mg/kg) (Zn20–3), nano-Zn 20 nm (10 mg/kg) (Zn20–10), and nano-Zn 20 nm (100 mg/kg) (Zn20–100)-treated rats. Significant compared to controls (α).

**Fig. 6** Serum albumin in controls, cadmium (Cd), cadmium + nano-Zn 15 nm (3 mg/kg) (Cd + Zn15–3), cadmium + nano-Zn 15 nm (10 mg/kg) (Cd + Zn15–10), cadmium + nano-Zn 15 nm (100 mg/kg) (Cd + Zn15–100), cadmium + nano-Zn 20 nm (3 mg/kg) (Cd + Zn20–3), cadmium + nano-Zn 20 nm (10 mg/kg) (Cd + Zn20–10), cadmium + nano-Zn 20 nm (100 mg/kg) (Cd + Zn20–100), cadmium + nano-Zn 30 nm (3 mg/kg) (Cd + Zn30–3), cadmium + nano-Zn 30 nm (10 mg/kg) (Cd + Zn30–10), and cadmium + nano-Zn 30 nm (100 mg/kg) (Cd + Zn30–100). Significant compared to controls (α).
Statistical Analysis  For statistical analysis, the experimental values were compared with their corresponding control ones. Statistical analysis was done with a one-way analysis of variance (ANOVA) using SPSS software (version 11.0) and t-test. The significant difference was considered to be $P < 0.05$.

Results  

In this study, HCT was significantly increased and blood coagulation time was significantly reduced in all rats treated with nano-zinc. As Fig. 2 shows, cadmium significantly increased blood urea compared with the controls ($P < 0.001$), whereas the simultaneous administration of ZnCl$_2$ and cadmium decreased blood urea ($P = 0.019$) compared with the cadmium-treated groups. Nano-zinc (20 nm) at the doses of 3, 10, and 100 mg/kg bw significantly increased blood urea ($P < 0.05$). Cadmium also significantly increased serum creatinine ($P = 0.024$). Although ZnCl$_2$ decreased the creatinine levels, the reduction was not statistically significant. The co-administration of cadmium and nano-zinc (15, 20 nm) dose-dependently increased the serum creatinine compared with the controls ($P < 0.05$) (Figs. 3 and 4).

The serum albumin decreased in both cadmium-treated groups and nano-zinc-treated groups significantly ($P < 0.05$) (Figs. 5 and 6). The cadmium-treated rats showed lower total protein levels compared with other treated rats and the controls, probably related to cadmium-induced liver injury and/or glomerulonephritis ($P < 0.05$) (Fig. 7). Cadmium also
significantly elevated serum triglyceride compared with the controls ($P < 0.05$) (Figs. 8 and 9).

The total cholesterol significantly increased in the groups treated with nano-zinc 20 nm (3, 10 mg/kg) compared with the controls ($P < 0.05$). However, in a higher dose (100 mg/kg), total cholesterol decreased compared with the nano-zinc 20 nm (3 mg/kg)-treated group ($P < 0.05$) (Fig. 10). As Fig. 11 shows, in simultaneous cadmium- and nano-zinc (20 nm)-treated groups, the total cholesterol increased dose-dependently. HDL cholesterol changes were not significant in the treated groups (Figs. 12 and 13). Figures 14, 15, 16, and 17 show the serum LDL cholesterol and serum-free fatty acid levels in the experimental and control groups. The serum cadmium level in all groups was less than 5 ppm. Figure 18 shows the serum zinc content in all treated groups.

Cadmium significantly elevated the serum ALT compared with the controls ($P = 0.009$) and ZnCl$_2$ significantly decreased serum ALT in the cadmium-treated rats ($P = 0.034$). At the dose of 100 mg/kg bw of nano-zinc (20 nm), the serum ALT showed significantly increased levels compared with the controls ($P < 0.05$) (Figs. 19 and 20). The serum AST level increased in the cadmium-treated group ($P > 0.004$) whereas ZnCl$_2$ reduced the serum AST levels compared with the controls ($P = 0.007$) (Fig. 21).

Discussion

Exposure to cadmium is known to induce a variety of toxicity symptoms in the exposed population and experimental
studies. The protective effect of zinc against the acute and chronic toxicity of cadmium, as well as its enhancing metallothioneins (MT) synthesis induction, has been well documented [30–37].

This study planned to investigate the strong and facilitated protective effects of nano-zinc particles against sub-acute cadmium toxicities. It proved that nano-zinc expressed not only a protective effect on cadmium toxicity but also toxic effects on the exposed rats. HCT and blood viscosity were significantly increased and blood coagulation time was significantly reduced in nano-zinc-treated groups. Partial haemolysis was significant in the blood samples. Wang et al. [28] reported acute haematotoxicity symptoms and severe anaemia in the mice that received 5 mg/kg nano-ZnO orally for 2 weeks. In their study, Plateletes (PLT) and RDW-CV significantly increased, and haemoglobin and HCT significantly decreased compared with the controls. However, another study reported an increase in blood viscosity and PLT, decrease in MCH and MCHC, and anaemia by low and median doses of 20 nm ZnO and high doses of 120 nm ZnO [38]. As Wang et al. [32] reported, an elevated RBC and HCT count may occur due to dehydration, hypoxia, inappetence, diarrhoea, and dehydration or subcutaneous oedema. The previous study has reported significantly shortened PT and increased blood viscosity in rabbits treated with zinc gluconate (150.0 mmol/L) [39]. The significantly increased blood viscosity and decreased blood coagulation time are associated with an increased risk of haemostasis and cardiovascular diseases [40]. Wang et al. [32] showed elevated levels of heart lesion biomarkers, CPK, LDH, and AST, which were in accordance with the fatty degeneration in the cardiovascular cells of the mice exposed to ZnO.
Nephrotoxicity biomarkers, serum urea, creatinine, and albumin analysis showed protective effects of ZnCl₂ against cadmium nephrotoxicity as previously shown [41–43]. However, nano-zinc solely increased nephrotoxicity biomarkers dose-dependently and in combination with cadmium in spite of the significant upregulation of metallothionein synthesis. Rasheed et al. [44] showed a serious increase in urea and creatinine levels in the serum of intoxicated rats acutely treated with 50 nm ZnO nano-particles compared with the controls. They also reported histopathological changes, including massive atrophy and fragmentation of numerous glomeruli, renal casts, renal tubules epithelial exfoliation, degeneration, and necrosis, as well as severe renal interstitium congestion. Proteinaceous casts in the renal tubules and renal tubular dilatation in the mice exposed to nano-zinc at an acute toxic dose of 5 g/kg bw were previously reported [28, 32]. Albumin is the most abundant serum protein synthesized in the liver and is a biomarker for assessing liver and kidney activity. Increased urinary excretion of albumin and suppressed albumin synthesis in liver was previously described in cadmium-induced toxicity [45].

In vitro administration of coated and uncoated ZnO using a hepatocyte cell line significantly decreased albumin production [45]. As shown in this study, Pasupuleti et al. [46] reported reduced serum albumin levels in the acute oral...
administration of nano-ZnO in rats at the doses 1 and 2 g/kg body. However, Wang et al. [28, 32] did not report a decrease in serum albumin levels at the dose of 5 g/kg. Serum albumin levels decreased in glomerulonephritis or liver injury. Cadmium and nano-zinc may induce renal and liver toxicity and, subsequently, lower levels of serum albumin. In our study, serum total protein decreased in cadmium-treated rats compared with the controls. Roels et al. [47] found a statistically significant correlation between the urinary excretion rate of cadmium and the urinary excretion rates of total protein, amino acids, β2-microglobulin, and albumin. In our study, ZnCl2 ameliorated the urinary excretion of total protein.

Cadmium increased liver injury biomarkers, serum ALT, and AST significantly compared with the controls. The co-administration of ZnCl2 with cadmium notably decreased serum ALT and AST. It can be concluded that zinc might have protective effects on cadmium hepatotoxicity. Nano-zinc increased ALT and AST, as Wang et al. [28, 32] reported oedema and degeneration in hepatocytes and serum increase in ALT, AST, ALP, and LDH in the nano-zinc-treated mice. Esmaeillou et al. [48] reported significant increases in ALT and AST activity and hepatocyte necrosis in all mice exposed to ZnO nano-particles. Pasupuleti et al. [49] reported an inverse dose-dependent increase in the AST and ALT serum levels of nano-size ZnO groups compared with micro-sized ZnO. They reported that histopathological lesions in liver, pancreas, heart, and stomach were higher in the lower doses of nano-sized ZnO compared with the higher doses. They proposed further dose-metric studies to elucidate the effect of size and dose on nano-zinc traits. It seems that the trait of nano-zinc particles in smaller sizes depends on their ionization rate and, in the bigger sizes, on their greater doses.

Different epidemiological and experimental studies have shown that cadmium exposure directly or indirectly alters the lipid metabolism and the serum lipid content of free fatty acids, triglycerides, total cholesterol, HDL, and LDL. Cadmium-induced dyslipidemia increases the risk of cardiovascular diseases [10, 50]. This study showed a significant increase in serum triglyceride content in cadmium-treated rats compared with the controls. It seems that the lipid metabolism disturbance, expressed by cadmium intoxication, was the most significant reaction in the chronic cadmium exposure. The protective effect of zinc on cadmium-induced dyslipidemia was previously shown by Rogalska et al. [10]. However, nano-zinc not only decreased the serum content of serum lipids but also increased the serum content of the serum triglyceride, cholesterol, LDL, and free fatty acids. LDL mostly increased in smaller sizes compared with the bigger ones. Wang et al. [28] reported a significant cholesterol increase in micro-zinc-treated mice. Jenkins and Kramer [21] showed that excess dietary zinc ingestion in calf increased cholesterol esters and cardiolipin. An increased cholesterol esters level, to be expressed by the micro-zinc administration, was caused by a liver esterase inhibition, which the Wang et al. [32] study reported earlier. Blood zinc content analysis in this experiment revealed a slight elevation of the nano-zinc level in the nano-zinc-treated groups. Other studies revealed a significant increase in the nano-zinc levels in the kidneys, liver, spleen, lungs, and heart compared with the micro-zinc-treated groups [32, 51]. Our study and others proposed a great volume of distribution in different organs, even in the oral routes of administration.

Our findings about the nano-sized zinc particles did not show any dose- or size-dependent response in the different analysed parameters in rats. Compared with Wang et al.’s [28, 32] studies, our findings led us to conclude that smaller sizes with low doses, and larger sizes with high doses of nano-zinc particles have more toxic effects on the experimental models. In some cases, inverse dose-dependency was seen. It can suggest that at lower sizes and doses of nano-zinc, the triggered release of Zn2+ ions leads to toxic effects. At higher sizes, nano-particles’ aggregation may lead to a decrease in the oral absorption of nano-zinc. Further doses of larger sizes lead to further toxic responses.

Our findings showed the protective effects of metal zinc against cadmium toxicity. The study revealed that nano-zinc particles do not have any protective effects against cadmium toxicity. Moreover, it showed the toxic effects of the oral administration of nano-zinc particles.

Compliance with Ethical Standards The local ethics committee approved the design of the experiments and the protocol conformed to the guidelines of the National Institute of Health. All animal procedures used in this study were approved by the University of Tabriz standards for human care and use of laboratory animals, conforming to the Ethical Research Committee of the Ministry of Health and Medical Education of Iran (adopted on 17 April 2006) based on the Helsinki Protocol (Helsinki, Finland, 1975).

Conflict of Interest The authors declare that they have no conflict of interest.
References


