The Ameliorating Effect of Vitamin C against Cardiopulmonary Toxicity of Zinc Oxide Nanoparticals

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ABSTRACT: It has demonstrated that nanoparticles may cause more inflammation than larger particles of the same materials at a same mass dose. The aim of this study was to investigate the ameliorating effect of vitamin C against cardiopulmonary toxicity of zinc oxide nanoparticles. Animals were divided into 4 groups, group I received vehicle orally for four weeks. Group II received vitamin C orally in a dose of (30 mg/kg) daily orally for four weeks. Group III received zinc oxide nano-particles orally in a dose 1 g/kg body weight/day for two weeks. Group IV received zinc oxide nano-particles orally in a dose 1 g/kg body weight/day for 14 days, co-administered vitamin C (30 mg/kg) daily orally, to be followed by two weeks vitamin C only (30 mg/kg) daily. Cardiac biomarkers troponin-T, lactate dehydrogenase (LDH), creatinine kinase (CK), and aspartate transaminase (AST) were measured. Finally, histopathological studies were performed from hear and lung sections of all rats of all groups. The results showed that administration of repeated doses of ZnO-NPs to rats significantly increased heart function biomarkers. Co-administration of vitamin C significantly reversed these changes to almost normal. Apart from these, histopathological changes also revealed the protective nature of vitamin C against ZnO-NPs induced necrotic damage of the heart and lung tissues.

CONCLUSIONS: Vitamin C proved to be a protective agent against ZnO-NPs heart and lung toxicity.

Keywords: ZnO-NPs, Vitamin C, Heart damages, Heart enzymes, Lung damages

INTRODUCTION

Recent advances in nanotechnology have spurred increases in the use of nanoparticles (NPs), and concerns over the possible detrimental effects with exposure to NPs (1). Zinc oxide nanoparticles (ZnO-NPs) are currently engineered and most widely used NPs. Most applications with ZnO powder exploit the reactivity of the oxide, as a precursor to other zinc compounds (2). The applications in material science utilize the high refractive index, high thermal conductivity, and binding properties of ZnO. Possible exposure to ZnO-NPs could occur in the industrial settings and through everyday consumer products. ZnO-NPs are added into diverse materials and products, such as plastics, ceramics, glass, cement, rubber, lubricants, paints, ointments, adhesives, sealants, pigments, batteries, ferrites, and fire retardants. In addition, ZnO nanomaterials possess ultraviolet (UV) shielding, antibacterial properties, deodorizing effects, and heat and UV light resistance, which could provide many great potentials for a wide range of applications in many fields: cosmetics and sunscreens, food additives, additives in packaging, fungicides in agriculture, and biomedical applications such as anticancer drugs. However, the human risk and toxicity mechanism are not well known.

Since zinc is an essential trace element in the human body and is commonly present in foods or added as a nutritional supplement, ZnO is generally considered to be a material with low toxicity (9). However, ZnO could turn into hazardous material upon inhalation as a gas (for example, metal fume fever), since fumes could be generated from melting and oxidizing at high temperature from zinc or zinc alloys (9). Drinker et al (10) and Balance et al (11) reported that higher concentrations of freshly generated ZnO, as given in previous human inhalation exposure studies, can produce symptomatic, physiologic, and hematologic effects, as well as elevations in certain peripheral blood and bronchoalveolar lavage cytokines. It should be noted that small-sized particles are
more reactive and responsive than bulk-sized particles, because they have a higher proportion of atoms on their surface (12). Also, due to the lower surface energy of ZnO NPs, they can be well dispersed in various solvents as well as in air (13). Therefore, exposures and uptakes of ZnO-NPs could occur through various routes.

There are few number of toxicity reports on ZnO-NPs although their widespread use and potential for use in various applications. Toxicity studies of ZnONP have mainly focused on dermal toxicity due to the inclusion of ZnO-NPs within materials that are directly applied to skin.

The objective of this study was to assess heart and lung cell responses to the manufactured ZnO-NPs to show their potential toxic biological responses and investigate the ameliorating effect of vitamin C.

MATERIALS & METHODS

A-Animals
60 Adult albino rat of either sex weighing 180 ±20 gm will be used throughout the experiments. Animals were kept in raised mesh bottom cages to prevent coprophagy. The animals were maintained in colony cages at 25 ± 2°C, relative humidity 50-55% maintained under 12:12 h light and dark cycle. The animals were fed with standard animal feed and water ad libitum. Animals divide into 4 groups 15 animals each.

Drugs and Chemicals
Zinc oxide nano-particles
Vitamin C
Fine chemicals
All chemicals will be obtained from Sigma Chemical Co

Experimental Design
Animal Grouping
The study animals are divided into 4 groups (15) rats each
Group I (normal control) will receive vehicle orally for four weeks
Group II will receive vitamin C orally in a dose of (30 mg/kg) daily orally for four weeks.
Group III will receive zinc oxide nano-particles orally in a dose 1 g/kg body weight/day for two weeks. Sharma et al. (14)
Group IV will receive zinc oxide nano-particles orally in a dose 1 g/kg body weight/day for 14 days, co-administered vitamin C (30 mg/kg) daily, to be followed by two weeks vitamin C only (30 mg/kg) daily.

METHODS

Rats of all groups will be subjected to
Blood samples will be obtained from the retro-orbital vein plexus under light ether anesthesia. Serum was separated from the blood for the estimation of cardiac biomarkers troponin-T, lactate dehydrogenase (LDH), creatinine kinase (CK), and aspartate transaminase (AST) ) according to manufacture procedures

Histopathological studies by light microscope
After the end of the tested period, rats were scarified; lungs and hearts were excised and fixed in 10% formalin saline. After fixation tissues were embedded in paraffin-wax, and thick sections were cut into thin sections and stained with hematoxylin and eosin. These slides were then observed under light microscope for histopathological changes.

Statistical analysis
Values are expressed as mean ± SD and analyzed using Graph Pad prism version 5.1 using ANOVA followed by Tukey's multiple comparison test. P < 0.05 was considered significant.

RESULTS

There was no significant difference in the level of troponin-T, LDH, CK, and CKMB between control (G1) and Vitamin C (G2) alone treated rats. Rats that were treated with ZnO-NPS (G3) significantly (P < 0.0001) increased the level of troponin-T, LDH, CK, and AST when compared with control (G1). Rats that were treated by
ZnO-NPs with vitamin C (G4) ($P < 0.0001$) significantly decreased the level of troponin-T, LDH, CK, and AST when compared with ZnO (G3) treated rats (Table 1)

<table>
<thead>
<tr>
<th>Group</th>
<th>LDH IU/L</th>
<th>CK IU/L</th>
<th>AST IU/L</th>
<th>Troponin-T µg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1: control</td>
<td>108.2 ± 6.482</td>
<td>113.3 ± 6.766</td>
<td>122.5 ± 2.9</td>
<td>0.49 ± 0.21</td>
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<tr>
<td>G2: Vitamin C (30mg/kg)</td>
<td>118.0 ± 2.208</td>
<td>127.3 ± 1.7</td>
<td>139.0 ± 0.89</td>
<td>0.58 ± 0.34</td>
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<tr>
<td>G3: ZnO (1g/kg)</td>
<td>299.0 ± 19.66</td>
<td>248.3 ± 4.2</td>
<td>346.0 ± 17.8</td>
<td>1.6 ± 0.63 &lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>G4: ZnO + Vit C</td>
<td>106.5 ± 5.696</td>
<td>134.7 ± 1.88</td>
<td>120.0 ± 16.9</td>
<td>0.80 ± 0.14 &lt;sup&gt;b&lt;/sup&gt;</td>
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All values expressed as mean ± SD. <sup>a</sup>$P < 0.0001$ versus G1. <sup>b</sup>$P < 0.0001$ versus G3.

Histopathology

Light microscopical section of heart and lung tissues of saline treated rat (G1) and vitamin C treated rat (G2) show no histopathological alteration and the normal histological structure of the myocardial bundles was recorded in (Fig. 1). In lung tissues there was no histopathological alteration and the normal histological structure of the bronchiol, blood vessels and surrounding air alveoli were recorded in (Fig. 2).

Compared with the control group, the heart of the group treated with zinc oxide showed focal inflammatory cells infiltration in the degenerated dispersed myocardium (Fig. 3), associated with congestion in the myocardial blood vessels (Fig. 4). While in the lung of the same group peribronchiolar and perivascular inflammatory cells aggregation as well as infiltration were detected surrounding the bronchiols and dilated blood vessels (Fig. 5 & 6). Rats treated with vitamin C + ZnO-NPs show well-preserved myocardium and lung tissues when compared to ZnO-NPs (G3) treated group.
Figure 2. control rat lung tissues (group 1) normal histological structure of the bronchiol (b), blood vessels (v) and surrounding air alveoli (a).

Figure 3. Myocardium of ZnO-NPs treated rat (group 3) depicting focal inflammatory cells infiltration (arrow) in the degenerated dispersed myocardium.
Figure 4. Myocardium of ZnO-NPs treated rat (group 3) showing congestion in the myocardial blood vessels (v).
Figure 5. and 6 lung tissues of ZnO-NPs treated rat (group 3) showing peribronchiolar and perivascular inflammatory cells (m) aggregation as well as infiltration were detected surrounding the bronchioles (b).

Figure 7. Lung tissues of ZnO-NPs treated rat (group 3) showing perivascular inflammatory cells (m) and dilated blood vessels (v).
DISCUSSION

In the present study, ZnO-NPs-treated rats showed significant elevation in the levels of these diagnostic cardiac marker enzymes (LDH, CK and AST). Moreover, elevated levels of these enzymes are an indicator of the severity of ZnO-NPs-induced myocardial damage.

ZnO-NPs destructed myocardial cells. As a result of this, lactate dehydrogenase (LDH), transaminase (AST), and creatine kinase (CK) were released into blood stream and served as the diagnostic markers of myocardial tissue damage. The amount of these cellular enzymes present in the blood reflects the alteration in plasma membrane integrity and/or permeability. These results were consistent with the previous studies reported by other investigators (15). Oxidative stress may play a major role in ZnO-NPs induced cardiopulmonary toxicity by generation of lipid peroxidation. Myocardial and lung tissues are susceptible to free radical damage due to fewer amounts of antioxidants like SOD and catalase present in the heart and lung (16). So administration of ZnO-NPs increases the lipid peroxidation and depleted the endogenous antioxidants in the myocardium and lung tissues.

The administration of vitamin C showed significant reduction in ZnO-NPs induced elevated serum marker enzymes. This reduction in the enzyme level confirms that vitamin C is responsible for maintenance of normal structural and architectural integrity of cardiac myocytes, thereby restricting the leakage of these enzymes, which can be accounted for membrane-stabilizing property of vitamin C. Cardiac markers level decreased by vitamin C indicated the protective role of vitamin C via reduction of oxidative stress. Previous studies have demonstrated that vitamin C exhibits antioxidant property in various oxidative conditions that cause tissue injury (17).

Another important findings observed in the present study are that vitamin C significantly decreases the elevated level of serum troponin-T. Troponins are myocardial regulatory proteins, which regulate the calcium mediated actin and myosin interaction. Troponin-T is widely used as specific marker to diagnose myocardial infarction. Vitamin C treatment significantly decreased the elevated level of serum troponin-T near to normal level which conformed the membrane stabilizing effect of vitamin C against ZnO-NPs-induced myocardial damage.

The biochemical data was supported by histopathological report, which showed severe myocardial necrosis with subendocardial loss of muscles in ZnO-NPs administered rat. While co-administration of vitamin C with ZnO-NPs attenuates the cardiotoxic effects induced by ZnO-NPs.

Histopathological examination of the lung of ZnO-NPs group showed peribronchiolar and perivascular inflammatory cells aggregation as well as infiltration were detected surrounding the bronchioli and dilated blood vessels. This finding is accordance with Cho et al. (18) who reported ZnO-NPs-induced eosinophilia, fibrosis, and goblet cell hyperplasia mediated by the soluble Zn2+. Therefore, ZnO-NPs pose a unique and substantial hazard to the lungs and hygiene precautions and control of airborne exposure should be instituted in any situation with the potential for exposure in order to reduce the risks of the kinds of lung pathology described here. Another research suggested that oxidative stress might be important for inducing the respiratory immunotoxicity of nanoparticles and ZnO nanoparticles induced greater cytotoxicity than the silver and titanium-dioxide nanoparticles (19).

Rats treated with vitamin C+ZnO show well-preserved lung tissues when compared to ZnO (G3) treated group.

The results demonstrate that ZnO-NPs cause cardiopulmonary impairments. These findings highlight the occupational health effects for ZnO-NPs-exposed workers.

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