Toxicity of zinc oxide nanoparticles on adult male Wistar rats

Roghayeh Abbasalipourkabir, a, *, Hemen Moradi b, Sadegh Zarei c, Soheila Asadi a, Aref Salehzadeh d, Abolfazl Ghaforikhosroshahi e, Motahareh Mortazavi f, Nasrin Ziamajid a

Department of Clinical Biochemistry, School of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran

Department of Clinical Biochemistry, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran

Department of Clinical Biochemistry, School of Medicine, Yazd University of Medical Sciences, Yazd, Iran

Department of Entomology and Toxicology, School of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran

Department of Medicinal Chemistry, School of Pharmacy, Hamadan University of Medical Sciences, Hamadan, Iran

Department of Anatomical Sciences, School of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran

**A R T I C L E   I N F O**

Article history:
Received 12 July 2015
Received in revised form 17 August 2015
Accepted 20 August 2015
Available online 24 August 2015

Keywords:
ZnO nanoparticles
Oxidative stress system
Liver enzyme
Sperm analysis
Histopathology

**A B S T R A C T**

The purpose of this study was to investigate the effects of zinc oxide nanoparticles (nZnO) on adult male Wistar rats. Thirty male Wistar rats divided into five groups of six animals each were used for this study. For ten days, Groups one to four continuously received 50, 100, 150 and 200 mg/kg nZnO, respectively. Group five served as the control group. At the end of the study, the rats were sacrificed and histopathological study of the liver and renal tissue, sperm analysis, serum oxidative stress parameters and some liver enzymes were done.

The results of this study showed that nZnO at concentration more than 50 mg/kg lead to significant changes in liver enzymes, oxidative stress, liver and renal tissue and sperm quality and quantity.

In conclusion, the toxicity of nZnO is more significant when the concentration is increased; however, the use of low doses requires further investigation.

© 2015 Elsevier Ltd. All rights reserved.

1. Introduction

Nowadays, nanotechnology has a vast range of application in diagnosis, drug delivery, food industry, paints, electronics, sports, environmental cleanup, cosmetics, and sunscreens (Al-Suhaibani and El-Morschidi, 2014). Zinc oxide nanoparticles (nZnO) are semiconductor metal oxide nanoparticles that are widely used in biomedical fields as an anticancer drug, a tool for imaging biological systems, and also in cosmetics. Due to its antimicrobial and fungicidal properties, it is used in the food industry and agriculture. Interaction of nanoparticles with biological systems has some unpredictable results, thus understanding their toxicity is essential to prevent their harmful effects on the human body (Sharma et al., 2012; Gerloff et al., 2009; Jin et al., 2009; Rasmussen et al., 2010; John et al., 2010).

Although there are some reports about oxidative stress of nanoparticles, yet it is not clear how oxidative state could make cells more sensitive to cytotoxic nanoparticles. Oxidative stress would be increased in some pathological situations such as inflammation. Hence, it is important to know how oxidative stress could change the sensitivity of cells to cytotoxic nanoparticles (Heng et al., 2010). Some special features such as high surface area, having 1–100 nm in size, and easy penetration into the cells and proteins, sensing, and detection of biological environments, make inorganic nanoparticles as potential candidate for applications in biomedical fields (Wahaba et al., 2009). As a result of the vast usage of nZnO in different areas, investigating its toxicity is critical, especially in bacterial and mammalian cells (Wang et al., 2010).

In vitro and in vivo studies have shown that ZnO nanoparticles have the following toxic effects on the mammalian cell: membrane damage, inflammation, DNA damage, apoptosis and the other effects including the complex cell-cell and cell-matrix interactions and changes in some hormones (Gojova et al., 2007; Jeng and Swanson, 2006; Yang et al., 2009; Osman et al., 2010; Sharma et al., 2011; Fischer and Chan, 2007). ZnO nanoparticles can enter
the body through the gastrointestinal or respiratory system and
reach the blood or organs such as the liver (Hussain et al., 2001;
Ober dorster et al., 2005). In vivo study of ZnO nanoparticles is
necessary to understand their long term effects on biological sys-
tems (Fischer and Chan, 2007).

The objective of the current study was to investigate the effect of
nano ZnO on oxidative stress status in adult male Wistar rats by
measuring Malondialdehyde (MDA) as an index of lipid peroxi-
dation, total oxidant status (TOS), total antioxidant capacity (TAC),
superoxide dismutase (SOD), glutathione peroxidase (GPX), liver
enzymes, alanine aminotransferase (ALT) and aspartate amino-
transferase (AST) as a function of the liver, sperm quality and
quantity and histopathological changes in the liver and renal tissue.

2. Materials and methods

2.1. Materials

Zinc oxide nanopowder was purchased from Iranian Nano-
materials Pioneers Company, NANOSANY ( Mashhad, Iran). Its
characteristics are presented in Table 1 and transmission electron
micrograph (TEM) and crystal characteristics of ZnO nanoparticles
are shown in Figs. 1 and 2, respectively.

2.2. Preparation of nanoparticle suspension

A stock suspension was prepared by suspending ZnO nano-
particles in bi-distilled water.

2.3. Experimental design and procedure

Thirty adult male Wistar rats aged 6–8 weeks, weighing
180–200 g were purchased from Pasteur Institute of Iran, IRAN. The
animals were housed two rats per plastic cage and allowed to ac-
climatize under standard conditions (12 h light/dark cycles) for one
week. The rats were given free access to distilled water and
commercialized food throughout the experiment. The animals
were divided into five groups of six animals each. Groups one to
four received a dose of 50, 100, 150 and 200 mg zinc oxide nano-
particles (nZnO)/kg body weight and assigned nZnO50, nZnO100,
nZnO150 and nZnO200 groups, respectively. The rats were injected
intraperitoneally daily for ten days. The control group was injected
with bi-distilled water.

2.4. Measurement of superoxide dismutase activity

Superoxide dismutase (SOD) catalyzes the breakdown of
the superoxide anion into oxygen and hydrogen peroxide. The method
was carried out following the procedure of Marklund and Marklund
(1974). In this method, one unit of SOD activity is defined as the
amount of enzyme required to inhibit the autoxidation of pyro-
gallol by 50%. Autoxidation of pyrogallol was quantified at 420 nm
for 3 min. Therefore, the rate of decreased optical density between
the 1st and 3rd min was expressed as enzyme activity in a UV/Vis
Spectrophotometer (Unico S2100, USA) with recorder.

2.5. Measurement of glutathione peroxidase activity

Glutathione peroxidase (GPX) activity was determined based on
the potential of the enzyme to reduce H2O2. According to the study
of Paglia and Valentine (1967), GPX converts H2O2 to H2O and
catalyzes GSH to GSSG simultaneously. The optical density of the
final mixture was read at 340 nm in UV/Vis Spectrophotometer
(Unico S2100, USA) with recorder. Decrease of OD was expressed as
enzyme activity, U/L.

2.6. Measurement of MDA, TAC and TOS

Total antioxidant capacity (TAC) in serum samples was assessed
using ferric reducing antioxidant power assay (FRAP) (Benzie and
Strain, 1999). Malondialdehyde (MDA) as a lipid peroxidation in-
dex was determined using fluorometric thiobarbituric acid method
(Botsoglou et al., 1994). The oxidation of ferrous ion to ferric ion
accompanied with a number of oxidant species in acidic pH was
used for the measurement of total oxidant status (TOS) in serum.
The ferric ion was determined by xylenol orange (Erel, 2005).

<table>
<thead>
<tr>
<th>Certificate of analysis</th>
<th>Content ZnO%</th>
<th>Cu ppm</th>
<th>Mn ppm</th>
<th>Cd ppm</th>
<th>Pb ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Particles size nm</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10–30</td>
<td>≥99%</td>
<td>≤3 ppm</td>
<td>≤5 ppm</td>
<td>≤9 ppm</td>
<td>≤9 ppm</td>
</tr>
<tr>
<td>Specific surface area m2/g</td>
<td></td>
<td>Color</td>
<td>Crystal phase</td>
<td>Crystal Morphology</td>
<td>True density g/cm³</td>
</tr>
<tr>
<td>20–60</td>
<td></td>
<td>Milky white</td>
<td>Single</td>
<td>Nearly spherical</td>
<td>5.606</td>
</tr>
</tbody>
</table>

Table 1
Characteristics of zinc oxide nanoparticles.
groups were immediately fixed in 10% formalin overnight, cut into 5 μm sections, placed on slides and stained with Hematoxylin-Eosin (H&E). The tissue sections were viewed under a light microscope (Nikon Y-S100, German).

2.10. Statistical analysis

The data were expressed as mean ± standard deviation. For statistical analysis, the experimental values were compared to their corresponding control. One-way analysis of variance (ANOVA) in SPSS software (Version 16.0) was used to illustrate the significant difference between the experimental and control groups. The significant difference was considered to be \( p < 0.05 \) or less.

3. Results

3.1. Effect of increasing concentration of zinc oxide nanoparticles on oxidant and antioxidant parameters

In this study, blood samples were obtained from the rats at day 10 upon treatment and were used to determine biochemical parameters. The effect of zinc oxide nanoparticles on the oxidant and antioxidant parameters are shown in Table 2. As shown in the table, although there was no significant increase in SOD and GPX activity in all experimental groups compared to the control and between them, an increment was observed in the SOD and GPX activity, when the concentration was increased.

Based on the data in Table 2, treatment with nZnO150 and nZnO200 showed a significant increased peroxidation index of lipids, MDA in comparison with the control and other nZnO groups. Although, nZnO100 increased the MDA level significantly compared to the control group, treatment with nZnO150 and nZnO200 led to a significant increase in MDA compared to the control and nZnO50 groups. Treatment with nZnO50 indicated negligible increase in comparison with the control group.

Total oxidant status (TOS) results are shown in Table 2. While, group nZnO200 showed a significant increase in TOS when compared only to the control group, there were no significant changes in TOS levels. However, treatment with nZnO, lead to increased total oxidant status by increasing the concentration. According to the obtained data presented in Table 2, total antioxidant capacity (TAC) decreased insignificantly in all groups when the concentration of zinc oxide nanoparticles was increased. However, group nZnO200 showed a significant reduction in total antioxidant capacity compared to the control group.

3.2. Effect of increasing concentration of zinc oxide nanoparticles on two liver enzymes

The effects of the concentration of a variety of zinc oxide nanoparticles on two liver enzymes are presented in Table 3. As shown, although treatment with ZnO nanoparticles increased ALT...
Kupffer cells, congestion, in animals exposed to zinc oxide nanoparticles showed increased in Figs. 3 and 4, respectively. According to Fig. 3 the liver tissue of

3.4. Effect of zinc oxide nanoparticles on the histopathology of liver morphology.

as shown in Fig. 4, there were pathological changes including the proliferation of glomerular cells, inflammation of interstitial tissue and congestion of glomerulus in kidney of all groups treated with zinc oxide nanoparticles at concentrations above 50 mg/kg body weight.

4. Discussion

The histopathological features of the liver and kidney are shown in Figs. 3 and 4, respectively. According to Fig. 3 the liver tissue of animals exposed to zinc oxide nanoparticles showed increased Kupffer cells, congestion, inflammation in the liver parenchymal, ballooning, port inflammation and chromatin condensation as a result of apoptosis. Also as shown in Fig. 4, there were pathological changes including the proliferation of glomerular cells, inflammation of interstitial tissue and congestion of glomerulus in kidney of all groups treated with zinc oxide nanoparticles at concentrations above 50 mg/kg body weight.

Table 3

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
<th>Control</th>
<th>nZnO50</th>
<th>nZnO100</th>
<th>nZnO150</th>
<th>nZnO200</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AS (U/L)</td>
<td>64.25 ± 2.21</td>
<td>96.00 ± 3.65&lt;sup&gt;a&lt;/sup&gt;</td>
<td>104.00 ± 3.36&lt;sup&gt;a&lt;/sup&gt;</td>
<td>109.25 ± 2.21&lt;sup&gt;a&lt;/sup&gt;</td>
<td>117.50 ± 3.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>77.00 ± 2.27</td>
<td>104.50 ± 12.60</td>
<td>104.50 ± 5.25</td>
<td>111.00 ± 4.96&lt;sup&gt;a&lt;/sup&gt;</td>
<td>117.00 ± 5.71&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&lt;0.05</td>
<td></td>
</tr>
</tbody>
</table>

All values are expressed as mean ± standard deviation. *p < 0.05; Δp < 0.01; #p < 0.001. AST – Aspartate aminotransferase; ALT – Alanine aminotransferase.

<sup>a</sup> Comparing with Control.
<sup>b</sup> Comparing with nZnO50.
<sup>c</sup> Comparing with nZnO100.

Effect of zinc oxide nanoparticles concentration on sperm quality and quantity

The effect of zinc oxide nanoparticles on sperm quality are presented in Table 4. According to the obtained results the sperm count and vitality of all groups decreased significantly compared to the control group. Furthermore, while sperm motility decreased by increasing the concentration of zinc oxide nanoparticles, the reduction was not significant at the 50 mg concentration. All levels of zinc oxide nanoparticles had a significant impact on sperm morphology.

Table 4

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
<th>Control</th>
<th>nZnO50</th>
<th>nZnO100</th>
<th>nZnO150</th>
<th>nZnO200</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Count (10&lt;sup&gt;6&lt;/sup&gt;/ml)</td>
<td>201.6 ± 13.14</td>
<td>136 ± 24.56&lt;sup&gt;a&lt;/sup&gt;</td>
<td>142.4 ± 13.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>143.2 ± 23.73&lt;sup&gt;a&lt;/sup&gt;</td>
<td>134 ± 8.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Motility (%)</td>
<td>70.8 ± 6.7</td>
<td>51.8 ± 5</td>
<td>35.6 ± 14.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>30.6 ± 5.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>32.25 ± 11.34&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Normal Morphology (%)</td>
<td>98 ± 1.4</td>
<td>84.8 ± 6.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>82.4 ± 1.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>81.2 ± 5.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>74.75 ± 6.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Viability (%)</td>
<td>95 ± 2.7</td>
<td>71.6 ± 6.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>74.8 ± 8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>68.8 ± 2.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>71.25 ± 6.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&lt;0.001</td>
<td></td>
</tr>
</tbody>
</table>

All values are expressed as mean ± standard deviation. *p < 0.05; Δp < 0.01; #p < 0.001.

<sup>a</sup> Comparing with Control.
<sup>b</sup> Comparing with nZnO50.
abnormal increase of serum ALT concentrations, may suggest liver hepatotoxicity (Lynch et al., 2005). The results of this study showed that apart from the nZnO50, and nZnO100 groups, the activity of ALT increased significantly in all groups compared to the control and nZnO50 groups. Although treatment with ZnO nanoparticles at 50 mg/kg body weight had no toxic effect on the liver, its concentration at 100 mg/kg body weight and above produced a dose-dependent toxicity in the liver. Therefore, a high dose of ZnO nanoparticles might decrease its efficiency by increasing its toxicity. Sharma et al. (2011) reported significantly (p < 0.05) higher levels of ALT compared to the control mice followed by treatment with 300 mg/kg Zinc oxide nanoparticles. It was suggested that to find liver damage, ALT activity is usually determined with AST (Wang et al., 2006). The current study also reported the impact of ZnO nanoparticles on AST activity in the treatment groups. The statistically significant increased activity of AST in all groups in comparison with the control group may be pointed to the liver damage. Similar results were reported by Wang et al. (2008) that there were insignificant differences in plasma biochemical parameters between the group that was treated with micro-scale zinc powder and the group treated with nano-scale zinc powder. However, this result may be due to the high doses of zinc powder that cause toxic effects in organs such as liver.

The results of the current study showed the impact of zinc oxide nanoparticles on sperm quality in a dose-dependent manner in rats. This observation is similar to that of Talebi et al. (2013) which showed that dose of 50 and 300 mg/kg zinc oxide nanoparticles significantly reduced sperm count, motility and increased abnormality in mice. It was suggested that the toxicity of ZnO particles may increase from Zn ions, diffusion of particles into and their contact with organs (Manzo et al., 2013). Therefore, the risk of gonadotoxicity of zinc oxide nanoparticles should also be considered.

In this study, the histopathological findings confirmed the results obtained for the effect of zinc oxide nanoparticles on serum oxidant and antioxidant status in the rats. Similar result was also reported in the study of Saman et al. (2013) that the administration of different concentrations of ZnO nanoparticles (100, 200 and 300 mg/kg) to rats for 10 days, caused significant changes in the liver.
400 mg/kg body weight) resulted in apoptosis and congested blood vessel in liver tissue of male adult Wistar rats. It was suggested that the toxic effect of ZnO nanoparticles maybe through their accumulation in the liver and induction of intracellular reactive oxygen species (ROS) production. However, increased levels of ROS results in reduced mitochondrial membrane potential (MMP) as well as increased apoptotic protein, Bax (Esmaeillou et al., 2013). In another study by AL-Taee and Hamdani (2013), it was observed that the pathological damage in the liver may be due to increased cellular oxidative stress including disturbed superoxide dismutase (SOD), glutathione peroxidase activities and increased peroxidation of lipids. These results are concurrent with a study that reported liver damage caused by high-dose of zinc salt such as zinc acetate intraperitoneal administration (Wang et al., 2010). In a previous study it has been reported that the mechanism of dose- and time-dependent effect of ZnO nanoparticles in the liver may be raised from oxidative stress generation, lipid peroxidation, and cell membrane and DNA damage (Najafzadeh et al., 2013).

The histopathology results of the present study also revealed toxicological impact through the kidney. With regard to the excretion of xenobiotic products through the kidney, pathological renal damage is expected (Esmaeillou et al., 2013). However, some biochemical parameters such as BUN and creatinine can be determined to understand the ZnO nanoparticles-induced nephrotoxicity. As a result, it seems the toxicity of zinc oxide nanoparticles is more significant when the concentration is increased. However, the use of ZnO nanoparticles even at low doses requires further investigation.

5. Conclusion

Although, the use of zinc oxide in the form of nanoparticles is very common, but data related to harmful effect in human is not sufficient and requires more researches. Some reports on its toxicological properties have encouraged us to investigate the toxicity of Zinc oxide nanoparticles, as well as the enhanced liver enzymes and oxidative stress index in rat as an animal model. The toxicity of zinc oxide nano particles on adult male Wistar rats treated by peritoneal injection of various concentrations of ZnO nanoparticles was assessed by measuring some liver enzymes, oxidative stress factors, histopathological examination of liver and kidney and sperm analysis. The results showed significant increase in liver enzymes starting from ZnO nanoparticles concentration of 100 mg/kg animal body weight. Also, it was found that ZnO nanoparticles at concentrations above 50 mg/kg resulted in significantly enhanced SOD and non-significant decreased GPX, suggesting oxidative stress in treatment groups compared to the control group. Although the current findings indicate that the significant toxicity effects of ZnO nanoparticles appear at concentrations above 50 mg/kg body weight of animals, it is not yet clear whether the use of a low dose of ZnO nanoparticles (50 mg/kg and below) in industrial materials such as cosmetic products and sunscreen is justified. However, further research is needed to study the impact of ZnO nanoparticles on human health and safety.

Acknowledgements

We would like to thank Hamadan University of Medical Sciences for financial support (grant number; 9206261845) and facilities.

Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.fct.2015.08.019.