Toxipathologicalexaminationsofterpeatedintraperitonealadministrationofsilvernanoparticlesinhweistrainmice

Ehsan Zayenazardeh1 M e im Shabaninan Mohammado Kaarm Kooch2
1Deparmin the Food Industry and Agriulture Research Institute, Iran.
2Deparmin the Livestock and Petrochemical Industries Research Institute, "Iran.

ARTICLE

ABSTRACT

Objective: The objective of this study was to investigate the cytotoxicity and genotoxicity of silver nanoparticles on the liver of mice.

Methods: The study was carried out in the period from February to June 2019. A total of 40 Wistar rats were divided into five groups of 8 rats each. The rats were injected with saline solution, 5, 10, 20, or 40 mg/kg of silver nanoparticles for 4 weeks. The liver was examined histologically after necropsy.

Results: The results showed that the liver weight of the rats was significantly increased in the group injected with 40 mg/kg of silver nanoparticles. The histological examination of the liver revealed congestion of the hepatic sinusoids, vacuolization of the liver cells, and necrosis of the liver cells.

Conclusions: The results of this study indicate that silver nanoparticles can cause liver damage at high doses and are toxic to the liver. Further studies are needed to investigate the mechanism of action of silver nanoparticles on the liver.
for supporting NSPs safety. However, despite such widespread use of these products, potential toxicity of NSPs still remains controversial. Most of toxicity investigations on NSPs have been done on bacteria, cell lines and non mammalian animal species. These in vivo investigations disclosed clear mechanisms of NSPs toxicity, such as ROS production, with subsequent oxidative stress; interaction with cellular proteins and enzymes inducing some complications. These mechanisms finally lead to cytokine generation, cellular damage, and finally apoptosis or necrosis. However, the in vivo toxicity of NSPs has been analyzed in a wide range of investigations. Previous studies demonstrated that NSPs may have adverse effects on the lungs, liver, intestine and immune system, following single or repeated administration with various routes of exposure. In contrast, some other studies indicated no relevant toxic effects in these organs. These contradictory findings may depend on the high variability of the tested NSPs properties such as source, size, dispersion state and concentration. In addition, the animal species, sex, age, and different experimental designs may have affected on results of the investigation. However, there is still a lack of consistent and reliable data about pathological complications that may be induced by different sizes of NSPs in laboratory animals. Up to our knowledge, there is no study about toxicopathological effects of this size 20 nm of NSPs that were produced in Iran in the Wistar rat model with intraperitoneal injection route. Hence, in the present study here, we characterized NSPs before use and evaluated clinical observations, mortality and pathotoxicological complications of NSPs following intraperitoneal injection at different doses in experimentally rats.

Materials and Methods

Nanoparticles

Nanosilver particles produced by the chemical vapor deposition (CVD) method, with an average diameter 20 nm purity = 99.99% were purchased from Iranian Nanomaterials Pishgam an Company and used in the present study without further purification or sieving (Fig. 1).

Annals of the Biosciences

Twenty-four male Wistar rats were used in this study. They were obtained from Razavi Vaccine and Serum Research Institute and allowed to acclimate for one week before treatment. Animals were maintained in a controlled environment with a 12-hour light/dark cycle, a temperature of 22±3°C and 60±10% relative humidity. Sterile standard pellets diet for the rats and fresh tap water were available ad libitum. All animals were taken care in accordance to the advice of the animal care committee of the Tehran University based on the Guide for Care and Use of Laboratory Animals' National Research publication 86-23, revised 1985.

Experimental design

Nanosilver particles were suspended in a sterile saline solution containing 1% Tween-80 and were dispersed about 30 m in an ultrasonic liquid processor at 4°C and 30% amplitude to read pulses. Twenty-four rats were randomly divided into four groups, six for each group. One group was selected

Fig. 1 Scanning electron microscope (SEM) image of NSPs.
as the control group, and the rest groups were used as experimental groups. Rats were injected intraperitoneally over a period of 5 days with repeated doses at different dose levels 20, 80, 320 m g/kg of NPs.

*H. isthmus*

Organs including heart, lungs, liver and kidneys extracted from animals were immersed in 10% buffered formalin for 2 weeks. Then, they were transversely sectioned in 3-4 mm slices. Sections were dehydrated in a graded series of alcohol and xylenes. After that, they were embedded in paraflin and multiple slices were produced and stained by hematoxylin and eosin stains. Sections were observed and photographed by a light microscope. Nikon E 200 Japan.

*St. tata* in *ana* *ghas*

All data are expressed as mean ± SD. The mean of all parameters between groups was compared using the Student's t-test. Data were analyzed using the SPSS software version 19, and a p<0.05 is considered statistically significant.

**R E S U L T A N D D I S C U S S I O N**

Silver nanoparticles have various applications in the field of biotechnology and in several nanoparticles have extensive antibacterial effects on a range of bacteria and antibiotic-resistant bacteria strains [20]. Antibacterial efficacy of NPs depends on their size and concentration. Nanosilver is also a potent antifungal agent against an extensive spectrum of fungi [21]. NPs are also an antiviral agent [22, 23]. NPs also display anti-inflammatory properties in both animal models and in the clinic [24]. In contrast with NPs' benefits, they may have potential toxicities at same concentrations and can induce different health problems if used incorrectly. The toxicological database and the potential for probable adverse effects in human and the environment have not yet been established for NPs. Therefore, it is important to address the biosafety of NPs in human and animal health. In our study, some indexes including eating, drinking and physical activity were decreased in the 320 mg/kg dose group. However, mortality was not observed in any groups. The behavior of the animals in the other groups was normal throughout the investigation. Body weight of the fourth group of experimental animals with NPs was significantly decreased in comparison with control group. On the other hand, body weight of the second and third groups of experimental rats with NPs did not show any significant change in comparison with control group. Table 1. (The histological examination of the heart, lungs, liver and kidneys in the control group following treatment revealed no observable changes. The fourth group of animals, intraperitoneal administering NPs induced multi-organ histopathological lesions such as severe alveolar edema, hemorrhage and inflammation at lungs, miosis, congestion and edema in heart, congestion and inflammation in kidneys and liver. However, there were not observed any significant histopathological abnormalities in the second and third groups.)

Two factors can induce pulmonary edema, including a cardiogenic factor due to dysfunction of the left ventricle and a non-cardiogenic factor related to inflammation response. In agreement with our results, Santhan and Husseim reported intraperitoneal injection of silver nanoparticles 2000 mg/kg (in albino rats) induced pathological complications such as renal tubules vacuolization and mitochondrial destruction in kidneys and swollen hepatocytes and accumulation of fat globules in the nucleus and cytoplasm in liver [25]. Previous epidemiological and nanotoxicological studies demonstrated that NPs can transcytose epithelial/endothelial cells into the systemic circulation to reach target tissues such as liver and kidney. Findings of these investigations also demonstrated that NPs are capable of generating

<table>
<thead>
<tr>
<th>Study Groups</th>
<th>Heart body weight (g)</th>
<th>Fimal body weight (g)</th>
<th>Body weight difference (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>First</td>
<td>201 ± 22</td>
<td>248 ± 23</td>
<td>47</td>
</tr>
<tr>
<td>Second</td>
<td>198 ± 21</td>
<td>247 ± 22</td>
<td>49</td>
</tr>
<tr>
<td>Third</td>
<td>203 ± 20</td>
<td>251 ± 24</td>
<td>48</td>
</tr>
<tr>
<td>Fourth</td>
<td>199 ± 19</td>
<td>223 ± 21*</td>
<td>24</td>
</tr>
</tbody>
</table>

*Significant difference p<0.05 (mean comparison with control group).
Fig. 2. Photomicrographs of heart, lung, liver and kidney sections obtained from mice exposed to different concentrations of NSPs. A1 B1 C1 D1 (control), A2 B2 C2 D2 (mice received 20 mg/kg of NSPs), A3 B3 C3 D3 (mice received 80 mg/kg of NSPs) and A4 B4 C4 D4 (mice received 320 mg/kg of NSPs). Panels A1 A2 A3 (mice: heart): A4 (mice: kidney): congestion, myocyteysis and edema in heart. Panels B1 B2 B3 (mice: lung): B4 (mice: liver): hemorrhage and inflammation in lungs. Panels C1 C2 C3 (mice: liver): C4 (mice: kidney): congestion and inflammation in liver. Panels D1 D2 D3 (mice: kidney): D4 (mice: kidney): congestion and inflammation in kidney.
Fig. 2. Photomicrographs of heart, lung, liver, and kidney sections obtained from mice exposed to different concentrations of NSPs. A1 B1 C1 D1 (controls), A2 B2 C2 D2 (mice received 20mg/kg of NSPs), A3 B3 C3 D3 (mice received 80mg/kg of NSPs) and A4 B4 C4 D4 (mice received 320mg/kg of NSPs). Panels A1 A2 A3 (normal heart): A4 (congestion and edema in heart). Panels B1 B2 B3 (normal lung): B4 (severe alveolar edema, hemorrhage, and inflammation in lungs). Panels C1 C2 C3 (normal liver): C4 (congestion and inflammation in liver). Panels D1 D2 D3 (normal kidney): D4 (congestion and inflammation in kidney). Staining with hematoxylin and eosin (magnification: 100x for panels).
reactive oxygen species, which have been related to inflammatory lung diseases and cardiac complications [26]. Sung et al also demonstrated that inhaled silver nanoparticles induced lung inflammation. They also reported that the target organs for silver nanoparticles were the liver and lungs in a subchronic inhalation investigation [27]. These results are consistent with our results. Inhalation and instillation experiments in rats demonstrated that low concentration of nanosilver appeared in the lung and was finally entered to the blood and other organs including heart, liver and kidney [28]. Myocytolysis is a specific pathologic marker of congestive heart failure without relation to coronary blood flow, myocardial hypoxia and myocardial fibrosis [29]. Previous studies have shown that carbon nanotubes can also induce histopathological changes in heart and lungs following oral administration in rats [30]. Findings of our investigation have shown no NPs accumulation in heart, lungs and kidneys of experiment animals. In a recent oral toxicity study of rats, in contrast with our results, Kim et al discovered that silver nanoparticles appeared in blood, liver, lungs, kidneys, stomach, testes, and brain at different doses exposure after 28 days [14]. Inflammation and congestion, in the kidneys and livers of animal without a group of our study were observed. In consistent with our findings, Spedding et al reported that oral administration of NPs to mice did not induce severe hepatocellular necrosis and hemorrhage, multifocal peribiliary micro hemorrhages, occasional portal vein endothelial damage. In this study, periportal coagulative necrosis, scattered hepatic single cell necrosis, and gall bladder severe mural and intraluminal gall bladder hemorrhage were observed following 40 mg NPs exposure only in one out of six treated mice. No relevant pathological complications were seen in examined organs of NPs-treated mice, and control mice [31]. There is a hypothesis that cardiac in-pacts are an outcome of pulmonary inflammation, which interferes with coagulation and stability of atherosomatic plaques. In vivo, NPs may also have impacts on cardiovascular physiology if they enter to the bloodstream. The possibility of transferring of particles from blood to the heart can induce cardiac direct impacts [32-34]. Combining with our findings; it can be concluded NPs can induce heart and lung injury via directly or indirectly oxidative stress (chronic inflammation) [35].

CONCLUSIONS
The toxicity of NPs is a controversial issue in the nanotoxicology science. In summary, two weeks after five days intraperitoneal injection of different doses of NPs, clinical observations, animal mortality and histopathology were evaluated. Our study demonstrated that five days intraperitoneal injection of 320 mg/kg NPs induced clinical abnormalities including decreased body weight, decreased food and water intake and decreased physical activity but no mortality in the Wistar rats. NPs injection also induced histopathological complications such as hemorrhage and inflammation in vital organs. However, NPs high dose may be toxic for human and animal. However, more nanotoxicological investigations need to clear essential elements of pathological changes following exposure with NPs.

ACKNOWLEDGMENT
The work was financed by the National Research Institute.

CONFLICT OF INTEREST
The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

REFERENCES


16. Stebounova IV, Adm cakova D od A M, Kim J, Park H, O'Shaughnessy PT, G rassian VH, et al. In mice, induces m in the lung toxicity or inflammation in a subacute m urine inhaled m od. *Particle and Fibre Toxicology*. 2011;8(5).


