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Research Article

Acute Hepatotoxicity of Nano- and Micro-sized Iron Particles in Adult Albino Rats; Histopathological changes

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Abstract

Nanotechnology is the science of today and tomorrow. In the coming future, nanotechnology will be intensively used in an unexpected large scale way. Actually nowadays there are well known applications that start to appear in many life aspects like medicine, energy, sports and much more other things. For such reason, the health and safety aspects of nanoparticles should be strongly studied. As nano iron particles started to play an important role in remediation of water and in medicine so we aimed in this study to clarify its possible hazardous effects on liver. In this study, the acute hepatotoxicity assessment specifically the histopathological changes in livers of adult Wister rats due to oral ingestion of high single dose (2000 mg/kg) of iron particles in both nano and micro sizes. Across this work it was found that significant alterations in histopathological characters occurred more prominently in the nano iron particle treated group.

Keywords: Nanotechnology, Nanoparticles, Nano iron, Micro iron, Hepatotoxicity

1.Introduction

Nanotechnology involves the onset and connivance of details at nanoscale levels to arise goods that have novel properties. Modern, nanomaterials such as nanotubes, nanowires, fullerene derivatives (buckyballs) and quantum dots crack normal huge utilization to create new types of analytical tools for biotechnology and life sciences[1].The manipulation at the nanoscale may open opportunities for favourable uses of many familiar substances. The nanoscale structure of the carbon atoms is the solitary differnce between graphite, and diamonds, or Photostat nanotubes that are capable of conducting electricity. The impulse, solubility, conductivity and magnetic properties of nanoparticles truly be surely make them differ from larger particles of the same chemical composition. For example, in nanoparticle the carbon nanotubes can transmit electricity as well as copper and aluminium explodes [2].

Nonetheless nanomaterials are currently carnal broadly used in the modern technology; there is a serious lack of information concerning the human health and environmental implications of manufactured nanomaterials. The greatest toxicological proceeding is the undeniably wind various of the manufactured nanomaterials are redox active and some particles transport across cell membranes and especially into mitochondria [3]. The interest of zero-valent frightful [Fe0 (ZVI)] for the treatment of toxic chemicals in waters has received wide attention. Zero-valent stubborn is a lion-hearted reducing agent; it is cheap and easy to produce. It has in the presence of been proven physical in reducing chlorinated solvents including chlorinated organic compounds, nitroaromatic compounds, pesticides, nitrate, and metal ions [4].

Mu et al. (2004) studied the reductive degradation of chlorinated organic compounds (COCs) and nitro aromatic compounds (NACs) by synthesized nano-scale ZVI in combination with batch anaerobic treatment systems. They demonstrated that the ZVI could transform the hazardous organic compounds into less harmful or harmless chemicals during in situ treatment of contaminated soils and groundwater. However, they found that compounds with more functional groups were difficult to degrade [5]. The reticulo-endothelial system that located in the liver getting exposed to surrounding nanoparticles coming from the gastrointestinal tract (GIT) to the cardiovascular system. Some Nanoparticles stimulate the macrophages through reactive oxygen species and calcium signaling, to generate some pro inflammatory cytokines such as tumour necrosis factor alpha. The oxidative stress is likely affect both hepatocyte function and bile formation, while pro-inflammatory cytokines are also associated with the pathology of liver disease [6].

2. Materials and Methods

2.1. Nano scale iron particles

Iron nanoparticles solution 5 mg/ml in toluene it was from Sigma-Aldrich Chemical Co. (St. Louis, Missouri, USA). The size of the nano scale iron particle was determined to be 18-22 nm diameters by transmission electron microscopy.

2.2. Micro-scale iron particles

The micro scale iron powder was purchased from Hepure Technologies Inc, Wilmington, DE, USA. The size of the micro scale iron particle was determined to be 70-80 m diameter by transmission electron microscopy.

2.3. Particles suspension

The particles of micro iron were dissolved in Toluene as it was in the powder form, while the nano iron was used as such as it was in suspension form. The element impurities were determined by Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES). The analytical results show that the purity of the two size iron suspensions was more than 99.99 %.

2.4. Preparation of particle suspension for injection

The administrated particles were suspended in Toluene dispersed by ultrasonic vibration for 15 min. The concentration of Fe in the suspension was 50 mg/mL. In order to avoid the aggregation of the particles, a few glass beads were added in the suspension and then stirred on vortex agitator before every use.

2.5.Animals

In the study, 90 adult albino rats about 90–120 gm weight were supplied by the Experimental Animal Center, Cairo University. The animals were housed in standard polypropylene cages with a stainless steel top grill and maintained on commercial pellet diet, given deionized water and libitum and kept in cages in a 20±2 □C, 50–70% relative humidity room with a 12-h light/dark cycle. The study design was approved with approval from the local ethics committee.

After acclimatizing, the rats were randomly divided into 4 groups:

a) Group 1 (-ve control group) consists of 15 rats given 1% sodium carboxy methyl cellulose solution.

b) Group 2 (+ve control group) consists of 15 rats given toluene.

c) Group 3 (Nano group) consists of 30 rats given nano scale iron suspension

d) Group 4 (Micro group) consists of 30 rats micro scale iron suspension in toluene at a dose of 2000 mg/kg.

The dose used in the study was 2000 mg/kg for both mico and nano groups, this dose was conducted following Organization for Economic Cooperation and Development guidelines 420 (OECD, 2001) [7].

All the animals were kept fasting over night before giving them the iron. The micro group rats was orally administered single dose of micro scale iron suspension in toluene at a dose of 2000 mg/kg, and the nano group was given single dose of nano scale iron suspension in toluene at a dose of 2000 mg/kg. The negative control group given 1% sodium carboxy methyl cellulose solution, and the positive control group was given Toluene in same volume given to the nano and micro groups. The rats then get observed daily for total 7 days. The body weight of animals was measured before the study, and their behavior was carefully recorded daily during the course of the experiment. At the end of the study (after 1 week), the animals get weighted again then sacrificed. The livers of all animals in each group get collected and were kept in 10% formalin for histopathological examination. Fresh portions of the lateral lobes of the liver from each rat were cut rapidly, fixed in neutral buffered formalin (10%), then dehydrated, with grades of ethanol (70, 80, 90, 95 and 100%). Dehydration was then followed by clearing the samples in 2 changes of xylene. Samples were then impregnated with 2 changes of molten paraffin wax, then embedded and blocked out. Paraffin sections (4-5 μm) were stained with hematoxylin and eosin and get examine under light microscope with high power ($\times$40). Stained sections of control and treated rats were examined for alterations in the architecture, portal triads, hepatocytes, sinusoids and for the presence of degeneration, necrosis, and portal fibrosis.

3.Results

3.1. Gross pathological changes in rats' livers

The mean weight of the livers in all four groups was normal in all of them. The livers show normal coloration in all animals in group 1 and group 2 (control groups), while the color of the liver was obviously darker in the group 3 and group 4 (nano group and micro group). The liver shows normal sharp borders in all animals in group 1 and group 2 (control groups), while the liver borders were rounded in the group 3 and group 4 (nano group and micro group) (Fig. 1a-b).

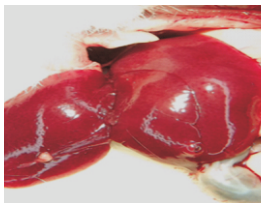

Fig. 1a: Gross appearance of liver in control groups, showing normal color, size and sharp borders

Fig. 1b: Gross appearance of liver in iron treated groups, showing dark color due to congestion with rounded borders

3.2. Histopathology changes in rats' liver in all groups

3.2.1. Hepatic lobules

The hepatic cords were normally arranged in group 1 and group 2 (control groups) while they show complete disarrangement in group 3 and group 4 (nano and micro groups) (Plate 1).

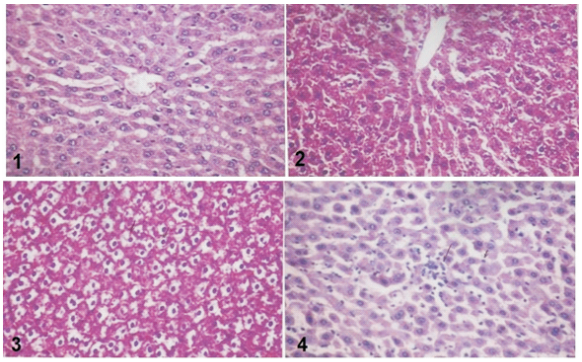

Plate 1. Histological structure difference in the 4 study groups regarding arrangement of hepatic cords (H&E) $\times$40. Key: (1) Liver tissue of –ve control group showing normal histological structure of hepatic lobules; (2) Liver tissue of +ve control group showing normal histological structure of hepatic lobules; (3) Liver tissue of nano iron treated group showing disarrangment of hepatic lobules; (4) Liver tissue of micro iron treated group showing disarrangment of hepatic lobules.

New vascularization appeared in almost all animals in group 3 and in about 50% of group 4. Also some necrotic areas appeared in almost all animals in group 3 and in about 80% of group 4 (Plate 2). Inflammatory cell infiltration appeared in almost all animals in group 3 and in only 40% of group 4 (Plate 2).

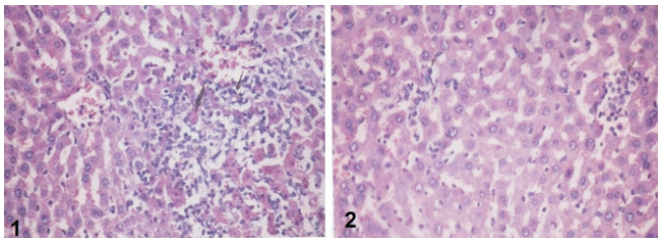

Plate 2. Focal necrotic areas affecting hepatic lobules in iron treated groups (H&E) $\times$40. Key: (1) Liver of nano iron treated group showing multiple focal areas of hepatic necrosis associated with inflammatory cells infiltration; (2) Liver of micro iron treated group showing focal areas of hepatic necrosis with apoptosis associated with inflammatory cells infiltration

The hepatic lobules affection was nil in both group 1 and group 2 (control groups), while sever affection was noticed in group 3 and group 4 (nano and micro groups) (Fig. 2).

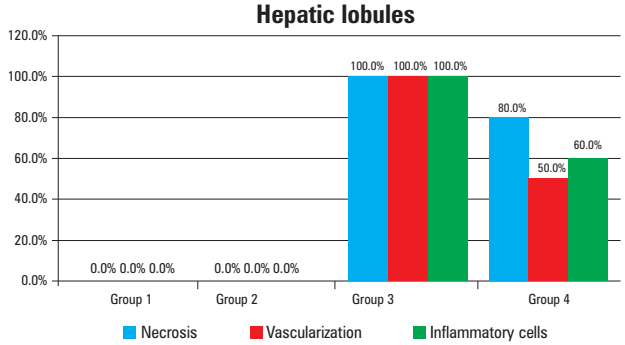

Fig. 2: Comparison between all the study groups regarding hepatic lobules affection

3.2.2. Hepatocyte apoptosis

Both control groups showed normal hepatocyte in almost all animals with no detected apopotosis. In group 3 about 23.3% of animals showed some different degrees of cellular apoptosis, while in the group 4 about 13.3% of animals showed some different degrees of cellular apoptosis (Plate 3).

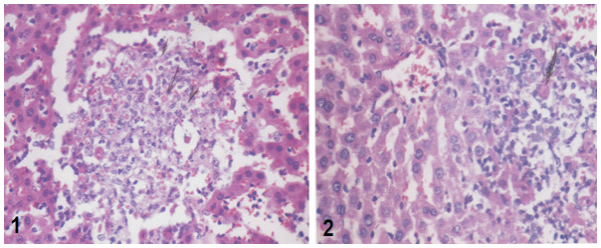

Plate 3. Hepatocyte apoptosis in nano and micro iron treated groups (H&E) ×40. Key: (1) Liver of nano iron treated group showing sever liver cell apoptosis; (2) Liver of micro iron treated group showing milder liver cell apoptosis.

Comparing group 3 and group 4 findings showed no significant difference (p value = 0.5). Comparing the control groups and the group 3, it was found that there no significant difference, and comparing the control groups and group 4, showed no significant difference (Fig. 3).

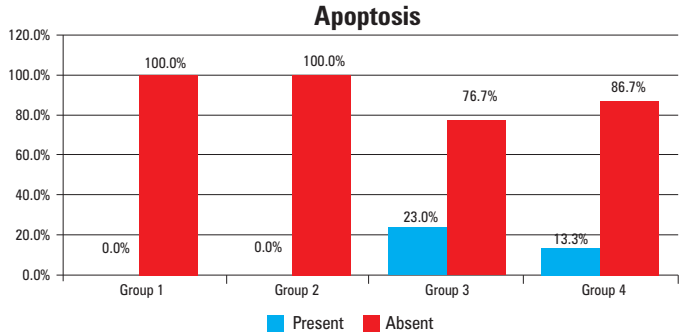

Fig. 3: Comparison between the four study groups regarding the hepatocyte apoptosis

3.2.3. Central vein

Both control groups showed normal central vein in almost all animals with no significant difference (p value = 0.5). In group 3 almost all animals showed both dilatation and congestion in their central veins, while in group 4 about 86.7% of animals showed dilated central veins and about 73.3% showed both dilatation and congestion in their central veins (Plate 4). Comparing the control groups and group 3 regarding the center vein affection, it was found that there high significant difference (p value <0.001), and comparing the control groups and group 4, showed high significant difference (p value <0.001). Comparing group 3 and group 4, showed no significant difference (p value = 0.1).

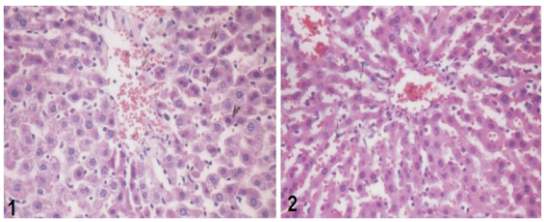

Plate 4. Central vein affection in nano and micro iron treated groups (H&E) ×40. Key: (1) Liver of nano iron treated group showing marked dilatation and congestion of central vein; (2) Liver of micro iron group showing milder degree dilatation and congestion of central vein

3.2.4. Hepatic sinusoids

Both control groups showed normal hepatic sinusoids in almost all animals with no significant difference (p value = 1). In group 3 almost all animals showed both dilatation and congestion in their hepatic sinusoids and about 96.7% showed Kuppffer cell activation, while in group 4 about 76.7% of animals showed both dilatation and congestion with Kuppffer cell activation. Comparing the control groups and group 3 regarding the hepatic sinuosoids affection, we found that there was high significant difference (p value < 0.001), and Comparing the control groups and group 4, showed that there was high significant difference (p value < 0.001). Comparing the group 3 and group 4 there was a significant difference (p value = 0.01) regarding the hepatic sinusoids affection, except regarding Kuppffer cells activation where there was no significant difference (p value= 0.052) (Fig. 4a-b).

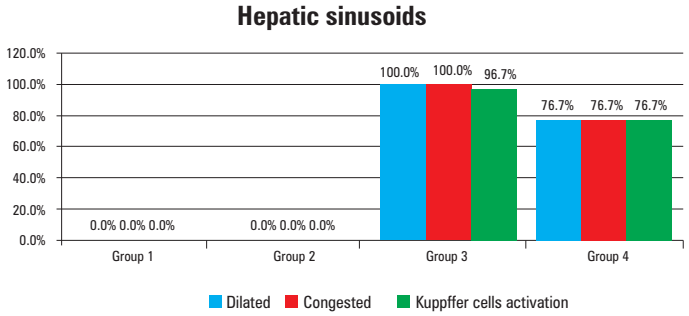

Fig. 4a: Comparison between hepatic sinusoids abnormality changes regarding the dilatation, congestion, and activation of Kuppffer cells in all study groups

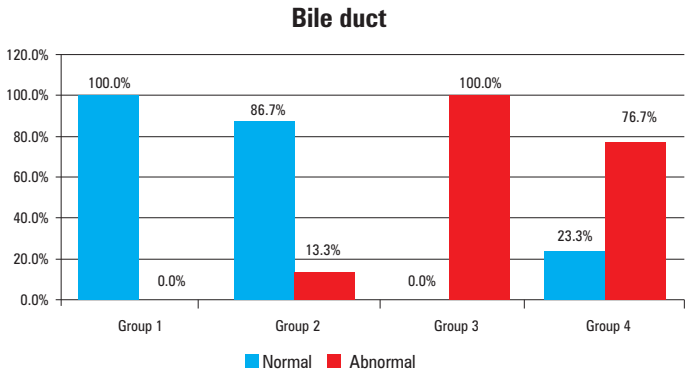

Fig. 4b: Comparison between the study groups regarding the bile duct affection

3.2.5. Portal area

Both control groups showed normal portal area in almost all animals with no significant difference between both groups (p value = 0.5). In group 3 almost all animals showed inflammatory cells infiltration in portal area and about 93.3% showed congested portal area with some degree of fibroplasias, while in group 4 about 80% animals showed inflammatory cells infiltration in portal area, about 70% showed congested portal area and about 63.3% showed some degree of fibroplasias (Plate 5).

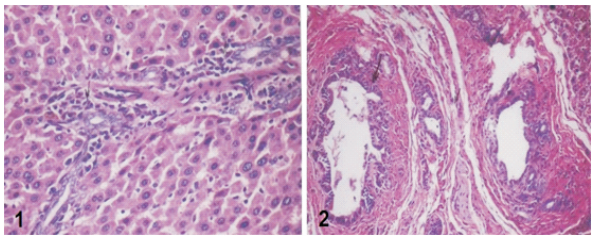

Plate 5. Pathological changes in portal area of nano and micro iron treated groups (H&E) ×40. Key: (1) Liver of nano iron treated group showing portal area sever infiltration with marked leucocytosis; (2) Liver of micro iron group showing fibrous connective tissue proliferation in the portal area associated with hyperplasia of epithelial lining of the bile duct bile duct

Comparing the control groups and group 3 regarding the portal area affection, it was found that there was a high significant difference (p value < 0.001), and comparing the control groups and group 4, showed high significant difference (p value < 0.001). Comparing group 3 and group 4, there was a significant difference regarding inflammatory cell, congestion and fibroplasias of the portal area (Fig. 5).

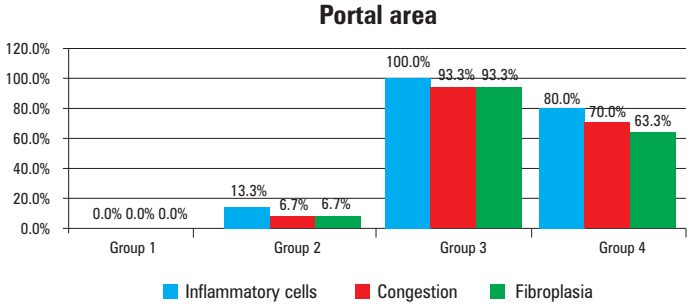

Fig. 5: Comparison between the four study groups affection of portal area regarding inflammatory cell, congestion and fibroplasias of the portal area

4. Discussion

In the present study, the concern of this study was whether the smaller nano-scale sized iron NPs were capable of producing more prominent histopathological alterations than the micro-sized iron or not?. The present study showed that the nano sized treated group livers shows a clear gross pathological picture of hepatic congestion with many areas of hemorrhagic areas and with obvious rounded borders, the picture simulate this in the micro treated group but in milder way. In consistent to this study results, the study conducted by Chen et al. (2006) on the acute toxicological effects of copper nanoparticles in vivo, the liver of the studied animals showed gross pathological affection in the form of congestion, dark coloration and rounded edges [8].

The present study showed that the histopathological picture of the liver of the nano sized treated groups was showing severe affection regarding; the hepatic lobules arrangement and infiltrations with inflammatory cells, the hepatocyte cytoplasm and nucleus, the central vein, the hepatic sinusoids, the portal area and the bile duct, while the micro sized particles treated group showed the same affection but in milder form.

In consistent to the present study results, study done by Wang *et al*. (2006) on the acute toxicity of nano- and micro-scale zinc powder in healthy adult mice showed pathological lesions in the liver more in nano group than micro group [9]. Similar results was shown by Wang et al. (2007) on study the acute toxicity and biodistribution of different sized titanium dioxide particles in mice after oral administration, they noticed that liver tissue, the hydropic degeneration around the central vein was prominent and the spotty necrosis of hepatocyte was also found in the female mice post-exposure 2 weeks to the 80 nm and fine TiO2 particles and it was obvious that the more smaller the particles the more the cellular hepatic damage [10].

Comparing the effect of Micro/Nano Silica particle feeding for mice was studied by Jeong *et al.* (2008) showed that nano sized particle dieted group indicated some fatty liver pattern unlike the micro silica group [11]. From the results, it was suggested that the nano sized silica particle had a more toxic effect on the liver than the micro silica. Similarly, Najafzadeh *et al.* (2013) in study about Serum biochemical and histopathological changes in liver and kidney in lambs after zinc oxide nanoparticles administration showed that zinc oxide nano-particles caused cell swelling, eosinophilic necrosis of hepatocytes in the histological examination [12].

In consistent to the present study, Karnakar Reddy *et al.* (2014) studied the possibility of nariginin to prevents the zinc oxide nanoparticles induced toxicity in Swiss albino mice, it was shown that n toxic groups, hepatic parenchyma were observed with foamy degeneration of hepatocytes and hepatic cell necrosis [13].

5. Conclusion

Based on the results of histopathological findings in this study, a preliminary conclusion could be drawn that the high dose nano iron particles oral exposure could induce more severe liver damage than micro iron particles with the same dose that means that the nano iron particles ingestion is more hepatotoxic than micro iron particles ingestion with the same dose.

Acknowledgment

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Conflicts of interest

The authors' declare that there is no of conflicts of interest.

References

[1].Cui HF, Vashist SK, Al-Rubeaan K et al. Interfacing carbon nanotubes with living mammalian cells and cytotoxicity issues. Chem Res Toxicol 2010; 19: 1131-47.

[2]. Roco MC. From vision to the implementation of the U.S. National Nanotechnology Initiative. Journal of Nanoparticle Research 2001; 3:5–11.

[3].Foley S., Crowley C., Smaihi M. *et al.* Cellular localisation of a water-soluble fullerene derivative.Biochemical and Biophysical Research Communications 2002; 294: 116–119.

[4]. Matlochová A, Plachá D, Rapantová N. The Application of Nanoscale Materials in Groundwater Remediation. Pol J Environ Stud 2013; 22: 1401-1410

[5]. Mu Y, Yu HQ, Zheng JC et al. Reductive degradation of nitrobenzene in aqueous solution by zero-valent iron. Chemosphere J 2004; 54:789-94.

[6]. Buzea C, Blandino I I, Robbie K (2007): Nanomaterials and nanoparticles: Sources and toxicity. Biointerphases J; 2: MR17 - Mr172.

[7]. Wang TC, et al. Acute toxicity of nano- and micro-scale zinc powder in healthy adult mice. Toxicol Lett 2006; 161:115-23.

[8]. Wang J, Zhou G, Chen C et al. Acute toxicity and biodistribution of different sized titanium dioxide OECD (Organization for Economic Cooperation and Development guidelines 420) (2001): OECD GUIDELINE FOR TESTING OF CHEMICALS, Acute Oral Toxicity – Acute Toxic Class Method. Available on:http://ntp.niehs.nih.gov/iccvam/suppdocs/feddocs/oecd/oecd_gl423.pdf (accessed in Jun 2013)

[9].Chen Z, Meng H, Xing G et al. Acute toxicological effects of copper nanoparticles in vivo. Toxicol Lett 2006; 163:109–20.

[10].Wang B, Feng WYparticles in mice after oral administration. Toxicology Letters 2007; 168:176–185.

[11]. Jeong S, Soon J, Chong H. Effect of Micro/Nano Silica Particle Feeding for Mice. Journal of Nanoscience and Nanotechnology 2008; 8: 5367-5371.

[12].Najafzadeh H, Ghoreishi S, Mohammadian B et al. Serum biochemicaland histopathological changes in liver and kidney in lambs after zinc oxide nanoparticles administration. Vet World J 2013; 6(8): 534-537.

[13]. Karnakar Reddy Y, Saritha Ch, Sridhar Y et al. Nariginin Prevents the Zinc Oxide Nanoparticles Induced Toxicity in Swiss Albino Mice. Central. J Pharmacol Clin Toxicol 2014; 2: 1021.