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Hepatotoxicity of nickel nanoparticles in rats

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ABSTRACT

Nanoparticles (NPs) are increasingly used nowadays for nanomedicine purposes, although they have been found to induce harmful effects on human health and living species in the environment. The current study investigated the impact of different doses of nickel nanoparticles (NiNPs) on the rats liver. Intraperitoneal (i.p.) administration of (0, 5, 20, 100mg/kg/BW/day) NiNPs for four weeks showed dose dependent elevation of malondialdehyde (MDA, lipid peroxidation marker), liver function enzymes (ALT, AST and ALP) and bilirubin. While, superoxide dismutase (SOD), reduced glutathione (GSH), glutathione peroxidase (GPX) and catalase (CAT) recorded significant reduction in their activity. NiNPs were found to cause several histopathological changes and ultrastructural alterations in liver such as appearance of inflammatory infiltrated leucocytes, sinusoid dilatation, fatty changes and degeneration of hepatocytes. The present findings suggest that administration of NiNPs may induce dose dependent hepatotoxicity.

Key words: Hepatotoxicity, Nickel nanoparticles, Rats.

INTRODUCTION

Nanoparticles (NPs) are materials or structures with a dimension at 1–100 nm range (Nakamura and Watano 2018). Metal NPs are widely used in industry such as chemical catalysts, ceramic capacitors, sensors, hydrogen storage, conductive pains and biological nanomedical applications due to their physicochemical characteristics (Mo *et al*., 2019).

The nickel nanoparticles (NiNPs) have specific characteristics, such as low melting point and high magnetism, reactivity and surface area (Zhang *et al.*, 2003). The adverse effects of NiNPs to human health have been paid attention by many researchers (Zhao *et al*., 2009). NiNPs were found to induce neurotoxicity, hepatotoxicity, nephrotoxicity and reproductive toxicity (Patlolla *et al*., 2019; Abudayyak *et al*., 2017).

The main mechanism for the cell damage caused by NiNPs is through inducing oxidative stress (Patlolla *et al*.,2019; Ahmad *et al*., 2015). Specifically, NiNPs were found to induce DNA damage (Abudayyak *et al*., 2017) inflammation, cell degeneration (Razavipour *et al*., 2015),cell cycle arrest (Ahmad *et al*., 2015) and cytogenetic alterations(Saquib *et al*., 2017). The present investigation aimed to focus on the hepatotoxicity of NiNPs in rats using different doses.

MATERIALS AND METHODS

NiNPs: The powdered 20nm nickel nanoparticles (NiNPs) procured from Sigma-Aldrich Co.USA were used.

Three different doses of NiNPs suspensions were prepared (5, 20, 100mg/kg body weight). The NiNPs suspensions were ultra-sonicated for 3 h in deionized water, using biologic ultrasonic homogenizer (Model 150VT, BioLogics, Inc., USA), to disperse the NiNPs in a homogenous stable. The NiNPs were vibrated for 2 min, immediately prior to administration in animals.

Experimental animals: Thirty two male Wistar rats, weighing 200-220g, kept on commercial pellet feed and water *ad libitum* were used. The rats were maintained at 21ºC±2ºC.

The rats were randomly divided into 4 groups. The 1st group represents the control and they were administrated intraperitoneally (i.p.) with normal saline. The groups 2, 3 and 4 (treatment groups) were injected with ultrasonicated NiNPs suspension at 5, 20 and 100 mg/kg body weight respectively. The injections were given once daily for five days a week and it continued for four weeks. All rats were dissected, then liver and blood samples were obtained for histological, biochemical and ultrastructural investigation. The study had approval from Animal Care Ethical Committee protocols in Salahaddin University-College of Science.

Biochemical assays: Liver tissues were washed in ice-cold normal saline solution, and homogenization was achieved in 20mM phosphate buffer (pH=7.4) using a glass hand homogenizer. The homogenates were centrifuged at 4000 g at 4ºC for 10min. The supernatants were collected and stored at -80ºC until assayed. The level of liver homogenate MDA was determined by thiobarbituric acid (TBA) method using manual commercial reagents kits (Alnahdi *et al*., 2018).

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The liver function measures including ALP, ALT, AST and bilirubin were estimated in blood serum using kits from BIOLABO SA, Maizy, France. Similarly, SOD, GSH, GPX and CAT, kits were estimated using specialized kits and the manufacturers protocols were followed.

Histopathology and electron microscopy analysis: Liver pieces were fixed in 10% buffered formaldehyde followed by dehydration, clearing and embedding in paraffin. Hematoxylin and eosin were used for routine histology (Tamizhazhagan and Pugazhendy 2017).

For electron microscopic studies, plastic blocks were prepared using liver samples (≤1mm^3) fixed in glutaraldehyde (2.5%) in 0.1M cacodylate buffer followed by 1% osmium tetroxide, dehydrated, cleared and finally embedded in araldite mixture. The ultrathin sections were stained with uranyl acetate and lead citrate and viewed by JEOL JEM 1400 transmission electron microscope.

Statistical analysis: All the data were expressed by means±standard error (M±SE) and the statistical analysis was achieved by SPSS version 22. One-way analysis (ANOVA) was performed for testing the significant of the treatment followed by Duncan's multiple range comparison between the groups. P values ≤ 0.05 were considered to be significant.

RESULTS AND DISCUSSION

NiNPs-induced hepatotoxicity was reflected by the elevated serum bilirubin, ALT, AST and ALP levels ($P \leq 0.05$), (Table 1). Increase in bilirubin level and liver function enzymes activity is known to accompany the hepatobiliary damage and leakage of these liver enzymes from hepatocytes (Yaqub *et al*., 2018; El Shahat *et al*., 2017). NiNPs accumulation due to increased doses may change the phosphate and ATP metabolism. This change leads to cellular energy depletion and disturbance in the potential of membrane causing hepatocytes necrosis and this consequently may lead to transaminases leakage into the bloodstream (Morsy and Elkon 2014).

The NiNPs treatment caused a significant increase ($P \leq 0.05$) of MDA in the liver homogenates of the exposed rats in a dose dependent manner (Fig 1). Probably, this reflected a nanoparticles induced oxidative stress since MDA was considered a marker for lipid peroxidation or oxidative stress and tissue damage (Aitken and Roman 2008). A generation of free radicals and inducing oxidative stress by NiNPs have been recorded previously (Dumala *et al*., 2018).

On the other hand, a significant decrease ($P \leq 0.05$) in SOD, GPX, GSH and CAT level was recorded in NiNPs treated rat liver homogenate compared to the control (Table 2). Several previous studies have shown that the accumulation of NiNPs in tissues may cause significant dose dependent cellular and biochemical changes such as enhancing excessive release of free reactive radicals and altering the endogenous antioxidants, leading to induction of lipid peroxidation (Dumala *et al*., 2018; Morsy and Elkon 2014).

The most important histopathological change in NiNPs treated rat liver was the dose dependent increase of the infiltrated inflammatory leucocytes cells in the regions adjacent to the blood vessels especially the portal area (Fig 2). Other histological changes included congestion of blood vessels, fibrosis around the blood vessels, degenerated hepatocytes (with dark condensed nuclei, shrunken cells and hypereosinophilic cytoplasm) and accumulation of lipid droplets in the cytoplasm of some hepatocytes (Fig 2). The normal cellular structure of hepatocyte in control group with normal mitochondrial structure and density is shown in Fig (3A). The histological changes following NiNPs treatment were confirmed by electron microscopy in which lipid accumulated hepatocytes (Fig 3B), apoptotic cells with fragmented nuclei (Fig 3C) and bundles of collagen fibers (Fig 3D) were clearly detected especially in the higher NiNPs doses treated rats.

Table 1: Effect of different doses of NiNPs on liver function markers.

NiNPs(nm)	Bilirubin(mg/dL)	ALP(U/L)	ALT(U/L)	AST(U/L)
0(Control) (G1)	63.23±4.2	7.56±0.3	293.2±7.2	0.32±0.04
5 (G2)	80.21±8.2*^{a}	9.23±0.9^a	303.3±7.6*^{a}	0.51±0.03*^{a}
20 (G3)	112.31±3.22^b	13.33±1.7^b	353.2±9.2^b	0.74±0.08^b
100(G4)	139.47±11.3^c	19.25±2.6^{ab}	404.3±9.4*^{c}	0.92±0.02*^{c}

Different letters indicate statistically significant differences (P values ≤ 0.05), (M±SE).
*indicates significant different compared to control at ($P \leq 0.05$).

Table 2: Effect of different doses of NiNPs on antioxidant status.

NiNPs(nm)	SOD(U/mg Protein)	GSH(nmol/mg Protein)	GPX(U/mg Protein)	CAT(U/mg Protein)
0(Control) (G1)	41.43±4.12	4.54±0.51	47.41±1.08	43.78±4.45
5 (G2)	36.15±3.84*^{a}	3.16±0.33^a	40.62±3.68*^{a}	19.98±2.32*^{a}
20 (G3)	32.62±3.22^b	3.56±0.53^b	35.88±4.89^b	14.45±5.45^b
100(G4)	27.20±2.88^c	2.54±0.43^{ab}	28.9±2.28*^{c}	11.37±6.54*^{c}

Different letters indicate statistically significant differences (P values ≤ 0.05), (M±SE).
*indicates significant different compared to control at ($P \leq 0.05$).

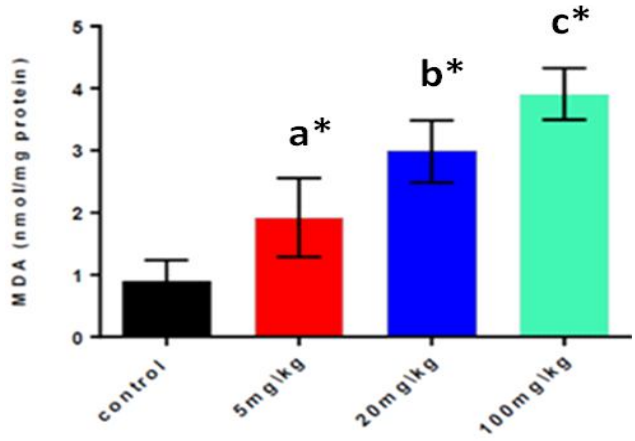

Fig 1: MDA level in the liver of NiNPs treated rats. Different letters indicate statistically significant differences at ($P \leq 0.05$) *indicates statistically significant difference compared to control at ($P \leq 0.05$).

NiNPs induced hepatotoxicity may also be the reason for the observed inflammation which may be caused by Kupffer cells activation (Berrahal *et al*., 2011). Such activation of Kupffer activation may serve as indirect NPs hepatotoxic effect which is associated with apoptosis (Manke *et al*., 2013). Furthermore, NPs are taken up by Kupffer cells in the liver and then by macrophage in other places (Sadauskas *et al*., 2007). In the present work, fibrosis was detected near sinusoid lining after injecting the rats with NiNPs (Fig 3D). Kupffer cells are the main sources of TGâ1 production, which caused the stellate cells transformation into myofibroblasts (Kolios *et al*., 2006).

Thus NiNPs may be considered as a dose dependent inducer of oxidative stress, inflammation and, fatty changes in liver of rats.

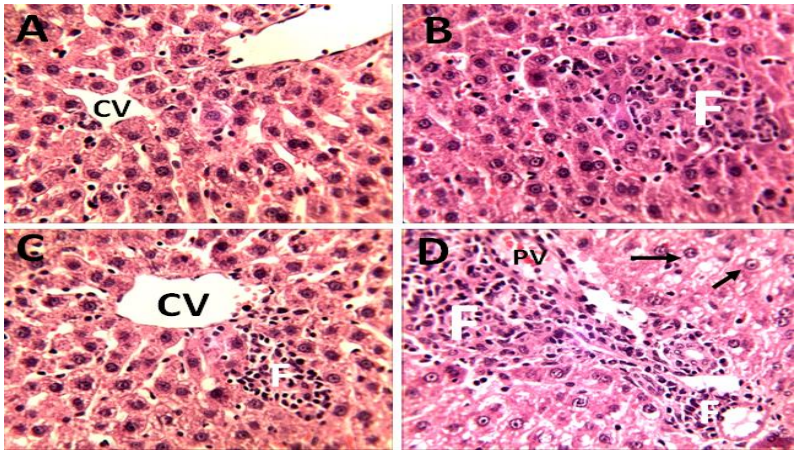

Fig 2: Dose dependent hepatotoxic effect of NiNPs; Appearance of inflammatory infiltrated leucocytes foci (F) near the blood vessels: (A) control group, (B) 5mg/kg, (C) 20mg/kg, (D) 100mg/kg. CV: central vein, PV: portal vein, arrows: fatty changes. H&E, magnification 400X.

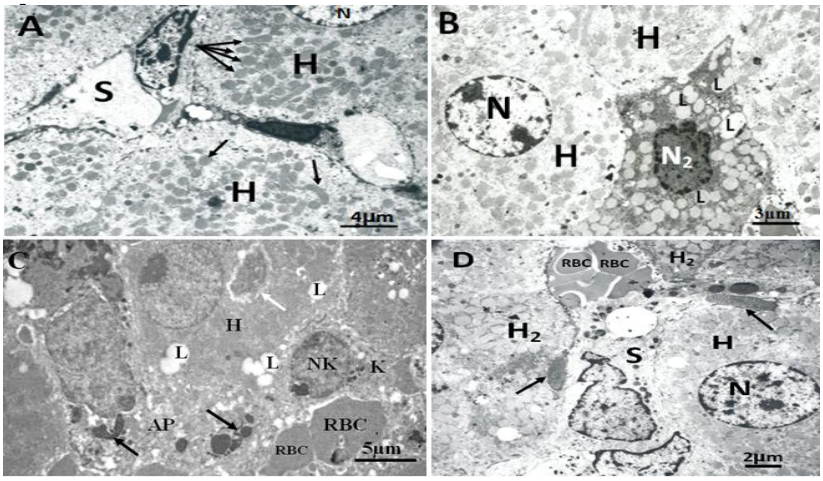

Fig 3: Electron micrographs of the liver of NiNPs treated rats. (A) Hepatocytes in the control group showing a lot of mitochondria (arrows). (B) Healthy hepatocyte (H) and adjacent lipid accumulated hepatocyte. N: Nucleus of hepatocyte. N_2: Nucleus of the lipid accumulated hepatocyte, L: Lipid droplets, S: Sinusoid. (C) Apoptotic cells (AP), Degenerated nucleus (white arrow) and fragmented nuclei (black arrows), K: Kupffer cell, NK: nucleus of Kupffer cell. (D) Bundles of collagen fibers (F) (arrows). H_2: Lipid accumulated hepatocytes, RBC: Red blood cells.

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