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Assessment of nickel nano particles induced spleenotoxicity in male sprague dawley rats

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Abstract

Nickel compounds are classified as carcinogens therefore; there is a need to determine the toxic effects of nickel nanoparticles (Ni-NPs) on organisms' health. With enhancement in nanotechnology, Ni-NPs are widely used in various fields of daily life. In the present study, Ni-NPs were used to determine their accumulation and toxic effects on histological profiles of spleen of male Sprague Dawley rats. For this purpose twenty five male Sprague Dawley rats weighing (200-250g) were procured from the animal house of Government College University Faisalabad after approval of the ethical committee on animal experimentation of Government College University Faisalabad. Rats were divided into five groups (n=5) as control (without any treatment), saline (treated with 0.9% sodium chloride for the equivalency of shock) and three nano treated groups (i.e., Ni-NPs @ of either 15 or 30 or 45mg/kg b.wt) with five replicates in each group were used to determine the accumulation and toxic effects of Ni-NPs on histological profile of spleen. At the end of the experiment, histological observations showed abnormalities in spleen structure with the formation of macrophage and increased megakaryocytes number, blood vessel damage and alteration in splenic capsule thickness. Fibrosis and necrosis was also observed. All these histological changes along with bio-distribution of Ni in spleen were dose dependent. Histological alterations and accumulation was more adverse at high dose (45mg/kg b.wt) in comparison with low and medium dose as well as control. Therefore, it is concluded that at higher concentration, exposure to Ni NPs is hazardous and should be handled with care.

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Introduction

Nanoparticles (NPs) production is increasing with the advancement in the field of nanotechnology (Glista-Baker *et al.*, 2014). NPs are well known due to their size (less than 100 nm) that enables them to penetrate through the skin, interact with biological system and interfere with the function ability of the cell (Jiang *et al.*, 2008). NPs with smaller size induce more toxic effects than with larger size particles (De-Haar *et al.*, 2006; Nurkiewicz *et al.*, 2008).

Nanotechnology has great potential to improve medical industry for human health care but use of NPs in medical sciences poses many problems related to human health and also for the environment. NPs along with its toxic properties have potential to be used in various biological applications such as nickel nanoparticles (Ni-NPs) are important for their magnetic property and are used in magnetically guided drug delivery system for therapy of different diseases (Prijic and Sersa, 2011). Such NPs have the ability to be used for chemotherapy for cancer treatment (Guo *et al.*, 2009 a) while anticancer drug named "daunorubicin" accumulates in leukemia cells. There is need to investigate the toxic effects of Ni-NPs used for chemotherapeutic treatment (Zhang *et al.*, 2006; Guo *et al.*, 2009 b). Ni-NPs can easily enter through the skin and can also be inhaled and induce toxicity in spleen and lungs (Jacob *et al.*, 2009; Vemula *et al.*, 2011), while some studies showed that Ni-NPs have carcinogenic properties as well as genotoxic potential (Kasprzak *et al.*, 2003) and respiratory problems in human (Phillips *et al.*, 2010; Ahamed, 2011).

Spleen is important secondary vertebrate organ of immune system; it plays its role in blood filtration and provides protection against any type of infection (Ajdari and Ghahnavieh, 2014). Phagocytes in spleen provide defense and slow down the transmission of pathogen in organ (Owolabi *et al.*, 2014). Exposure to Ni-NPs caused alteration in macrophages number, dilation of sinusoids and red pulp area congestion (Ajdari and Ghahnavieh, 2014). Still there is need to demonstrate the toxic effect of Ni-NPs on spleen as it is involved in filtration of blood and depends on dose and duration of Ni-NPs exposure. Therefore, this study was designed to investigate Ni-NPs induced toxicity at different doses injected intraperitoneally on alternate day for 28 days in male Sprague Dawley rats.

Materials and methods

Preparation of Ni-NPs solution

Ni-NPs (black powder) were purchased from Richest Group Ltd, Shanghai 201202, China with size 50 nm and 99.99% purity. Fine suspension of Ni-NPs was prepared in normal saline (0.9%) and for fine dispersion; it was ultrasonicated for 1 hr after vortex before dosing. Every time fresh dose was prepared under sterile condition.

Animal husbandry

Male Sprague Dawley rats were selected as animal model to assess the Ni-NPs induced toxicity and twenty-five post weaning Male Sprague Dawley rats of 200-250g weight were purchased from animal house of Government College University Faisalabad after approval of the ethical committee on animal experimentation of Government College University Faisalabad.

Animals were kept under favorable laboratory conditions for 1 week for adaptation in stainless steel cages with free access to natural water and commercial animal feed. Bedding of stainless steel cages was changed on daily basis to ensure clean environment. Humidity and temperature was controlled along with lightness/darkness system of 12/12 hrs. Number of rats in each cage was 5 to avoid crowding.

Experimental design and dose selection

Twenty-five healthy rats were randomly divided in to 5 groups (Table 1); 1 control group without treatment, 1a placebo group (saline group) treated with normal saline for the equivalency of shock, and other 3 groups were treated intraperitoneally with either 15 or 30 or 45mg/kg b.wt of Ni NPs on alternate day for 28 days.

Sample collection

At the end of experimental period, after 24 hours from the last dose animals were anesthetized with chloroform inhalation before sacrifice and spleen was collected for further analysis of Ni accumulation and histological observation.

Atomic absorption Spectrophotometry

This technique was used to determine the organ specific accumulation of Ni in target organ (spleen) in Male Sprague Dawley rats after Ni-NPs exposure with different doses of Ni-NPs on alternate day for 28 days. 0.1-0.5g spleen tissue sample from control and treated groups were taken and oven dried by using Micro Accelerated Reaction system (MARS, CEM) for 15 minutes at 180°C.

Then digested the tissues by adding 5ml Nitric Acid for 24 hours and after completion of digestion it was filtered and added distilled water and made filtrate volume upto 15ml. Finally concentration of Ni was determined through atomic absorption spectrophotometer against standards absorption. Results were interpreted by using following formula:

$$\text{Metal concentration } (\mu g/g) = \frac{\textit{Final Volume of Solution} \times \textit{Metal concentration}}{\textit{Sample Weight}}$$

Histological Studies

After collection of sample, spleen was kept in normal saline for the removal of attached adipose tissues. Its small pieces of 2-3 mm were fixed in 10% formalin immediately and then stored for further examination. Tissues were treated under standard histological laboratory tools. Tissues were then dehydrated with different concentrations of alcohols (70-100%) overnight. In paraffin, tissue blocks were made and 3-4 µm tissue was cut by using microtome (SLEE Rotary Microtome CUT5062 by Nikon Instruments Europe) and then stained with hematoxylin and eosin stain with standard staining technique (Hussein, 2015). The slides were studied under light microscope (Nikon E200 POL) and photographed by a digital camera attached with the microscope.

Results

Control group (without treatment) and placebo group (treated with normal saline) showed no significant differences in any assay. Therefore, both of these were considered single control group.

Table 1. Grouping of control and treated groups.

Groups	Treatment
Group 1	Control group (Without treatment)
Group 1 a	Placebo group (intraperitoneally injected with 0.9% normal saline for the equivalency of shock
Group 2	Ni-NPs1: 15mg/kg b.wt of Ni-NPs (intraperitoneally injected)
Group 3	Ni-NPs2: 30mg/kg b.wt of Ni-NPs (intraperitoneally injected)
Group 4	Ni-NPs3: 45mg/kg b.wt of Ni-NPs (intraperitoneally injected)

Accumulation of Nickel in Spleen

Concentration of Ni after exposure to Ni-NPs on alternate day for 28 days with various doses of Ni-NPs (15, 30 and 45 mg/kg bwt) in spleen of male Sprague Dawley rats was examined. There were significant differences in concentration of Ni in spleen in all treated groups. Maximum concentration was observed at high dose 45mg/kg bwt (Table 2 & 3). Table 3 shows the mean concentration of Ni accumulation in control, placebo and different treated groups (Ni-NPs 1, Ni-NPs 2 and Ni-NPs 3) of male Sprague Dawley Rats. Ni accumulation was observed in a dose dependent manner i.e., Ni-NPs3> Ni-NPs2 > Ni-NPs1 (Table 3). Results were significantly different from control and place groups at $P<0.001$ (Table 2).

Histological Studies

Histological sections of spleen from control group showed normal spleen architecture with red and white pulp area, blood vessels, trabeculae and splenic capsule while Male Sprague Dawley rats treated with different doses of Ni-NPs @ either 15 or 30 or 45mg/kg b.wt showed significant differences in

histological structures including increase in number of macrophages (Mp) and megakaryocytes (Mk) with increased blood vessel damage (BVD) and congestion in red pulp area with increased splenic capsule (Sc) thickening. With increasing Ni-NPs dose, severe histological alterations were observed such as necrosis, angiectasis and fibrosis in different treated groups (Fig. 1).

Table 2. Analysis of variance for Ni accumulation in spleen of male Sprague Dawley rats.

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Groups	4	0.005012	0.001253	4510.29	0.001
Error	20	0.000006	0.000000		
Total	24	0.005017			

Discussion

In current study level of Ni accumulation was increased with increasing dose concentration in spleen of male Sprague Dawley rats. The results of current study are in good agreement with the findings of a previous study in which dose dependent exposure of Ni chloride was given with concentrations of 300 and 1200 ppm for 90 days in rats. Ni level was enhanced in kidney, lungs and serum while its accumulation was low in liver, spleen and brain (Cempel and Janicka, 2002). Ni accumulation was 2.8 to 3.7 times more in 1200 ppm treated group than 300 ppm treated group. Nickel is considered to be human carcinogen (Zhao *et al.,* 2009). Nickel accumulation through inhalation is mostly occur in cerebral cortex and in whole brain (He *et al.,* 2013), while intraperitoneal administration of Ni causes low accumulation in brain (Gao *et al.,* 2015). When rats treated with 2.5mg/kg b.wt NiO NPs intraperitoneally, it caused low neurotoxicity because they detected NiO NPs in spleen and liver not in brain (Minigalieva *et al.,* 2015).

Table 3. Mean concentration (±SD) of Ni in spleen of control and treated groups of male Sprague Dawley Rats.

Sr. No	Group	Mean Concentration (µg/g)
1	Control	0.102 ± 0.012^{d}
2	Placebo	0.102 ± 0.012^{d}
3	Ni-NPs 1	0.104 ± 0.013^{c}
4	Ni-NPs 2	0.113 ± 0.014^{b}
5	Ni-NPs 3	0.139 ± 0.016^{a}

Current study is an agreement with the previous study in which oral exposure of NiO-NPs in rats with different doses (125, 250 and 500mg/kg b.wt) caused biodistribution of nickel in spleen. They also investigated that with increasing dose concentration and time, uptake level of nickel in spleen was also enhanced such as its accumulation was more in 500mg/kg b.wt dose than 250 and 125mg/kg b.wt of NiO-NPs (Dumala *et al.,* 2017) which is in good agreement with the current study.

Our histological results in current study are in harmony with previous studies and explained dose dependent (little to severe) abnormalities in spleen when treated with 15, 30 and 45mg/kg b.wt of Ni-NPs because spleen is secondary organ in the body and play important role in filtration of blood along with removal of useless materials and provide immunity by red and white pulp area of spleen, respectively (Dantey and Cooper, 2016). Control group showed normal histological structure of spleen with white and red pulp area and splenic capsule (fig 1 A-D).

Current study is in accordance with the earlier study on Ni-NPs induced toxicity at different doses 1 or 10 or 20mg/kg b.wt which showed remarkable changes at dose 10 and 20mg/kg b.wt in splenic red pulp area than at 1mg/kg b.wt. Splenic changes were due to Ni-NPs exposure that caused inflammation (extramedullary hematopoiesis) in spleen with

increasing dose of NPs (Magaye *et al.,* 2014; Ajdari and Ghahnavieh, 2014). Investigation of toxic effect of nickel nanoparticles on lungs, liver and spleen in male mice showed some significant results such as increased macrophages and intense congestion in red pulp area of spleen due to 75 ppm concentration exposure of Ni-NPs for 7 days. Same changes were seen in current study, number of macrophages and congestion in red pulp area was significantly increased with increasing Ni- NPs dose.

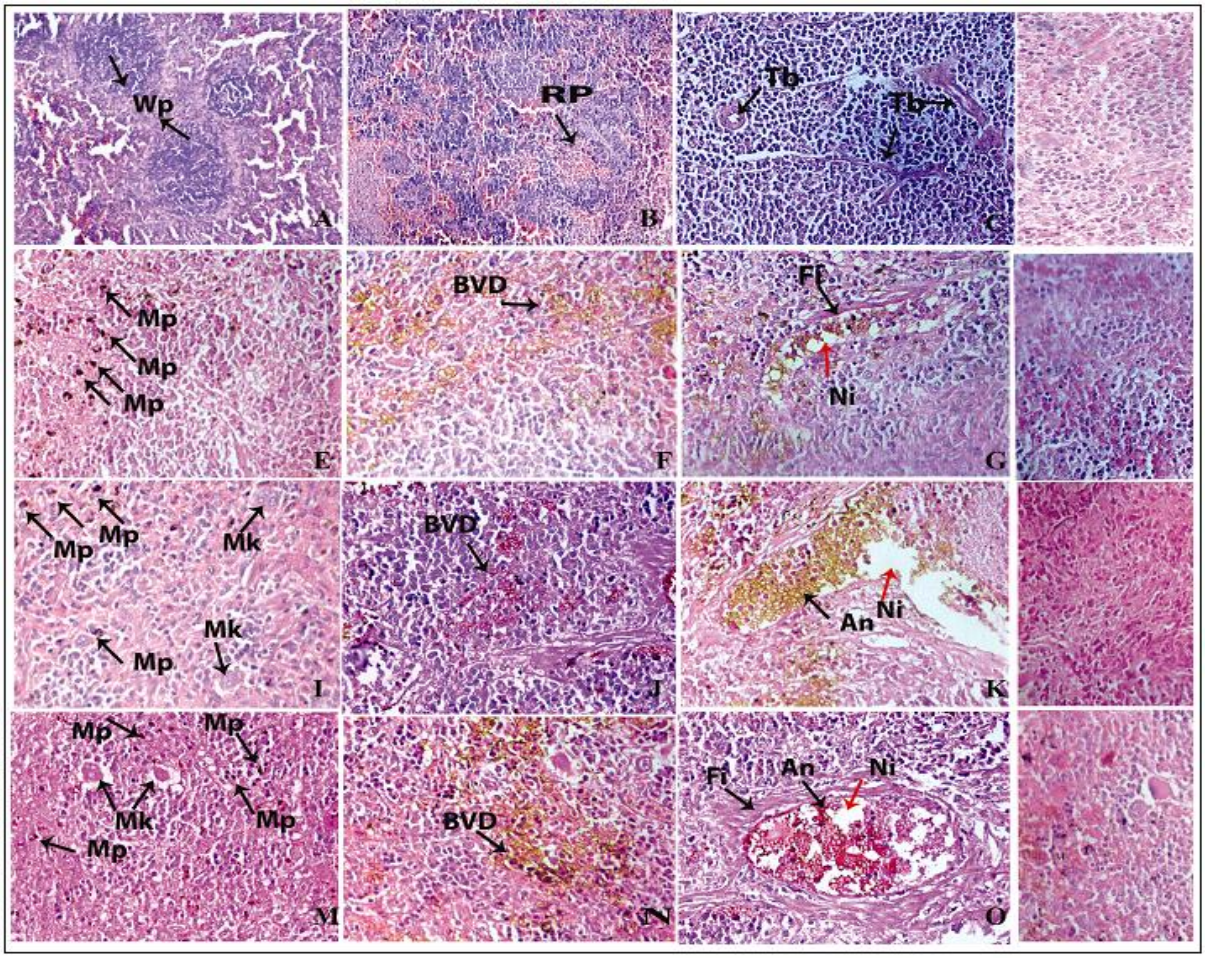

Fig. 1. Photomicrograph (400X H & E stain) showing normal architecture of splenic tissue in control group of male Sprague Dawley rats with white pulp area, red pulp area, cords and sinuses, trabeculae and normal splenic capsule (A-D). Sprague Dawley rats treated with Ni NPs @ of 15mg/kg b.wt (Group 2) showed mild histological alterations: macrophages (Mp), damage in blood vessels (BVD), necrosis (Ni), fibrosis (Fi) and thickness of splenic capsule (Sc) (E-H). Group 3 (treated @ 30mg/kg b.wt) showed moderate histological abnormalities: macrophages (Mp), presence of megakaryocytes (Mk), blood vessel damage (BVD), necrosis (Ni), angiectasis (blood filled spaces lined by endothelium in splenic parenchyma) (An) and thickness of splenic capsule (Sc) (I-L). Group 4 (treated @ 45mg/kg b.wt) caused severe histological changes: macrophages (Mp), megakaryocytes (Mk), increased blood vessel damage (BVD), severe necrosis (Ni), angiectasis (An) and intense fibrosis (Fi) and thickness of splenic capsule (Sc) (M-P).

Current findings are in agreement with ZnO-NPs exposure for 4 weeks induced toxic effects on spleen of albino rat showed markable abnormalities included white pulp area reduced while congestion in red pulp area. Significant number of megakaryocytes was detected in splenic red pulp area and thickening of splenic capsule due to ZnO-NPs exposure (Abass *et al.,* 2017). Thickening of capsule was also observed when Tilapia was exposed to 100mg/kg b.wt of Ag-NPs (Thummabancha *et al.,* 2016). Splenic

abnormalities were seen when exposed to different concentration of Bisphenol A (BPA), 50, 100 and 200mg/kg b.wt it showed atrophy in white pulp area while presence of macrophages in red pulp area along with congestion in sinusoids with increasing dose exposure (Hussein, 2015). Increase of macrophages was also observed in spleen when mice was exposed to 20mg/kg b.wt of lead acetate. It caused distortion in spleen architecture, lymphoid necrosis, diffusion of splenic white pulp area into red pulp and fibrosis (Aldahmash and El-Nagar, 2016).

Conclusion

From the current study, it is concluded that administration of Ni-NPs caused toxicity in spleen and also Ni accumulation in a dose dependent manner because of their severe toxicological effects. Toxicity of Ni-NPs caused distortion of normal architecture of spleen and also resulted in their tissue accumulation. Moreover, there is need for more research to elucidate the toxic and safe doses for Ni-NPs application in various fields including nanomedicine.

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