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The Nickel nanoparticles induced renal pathophysiological and histopathological alterations in male sprague dawley rats

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Abstract

Owing to nickel nanoparticles (Ni NPs) extensive utilizations in various industries, its toxic effects might not be neglected. The recent study considered Ni NPs induced renal physiological and histological alterations in male Sprague Dawley rats. The present study involved 20 rats and randomly divided into four groups (n=5 per group) included control group (C) and treated groups [low dose (N-G1), medium dose (N-G2), high dose (N-G3)]. The rats of treated groups intraperitoneally (i.p) injected with Ni NPs doses either 15mg/kg or 30mg/kg or 45mg/kg for 28 days on alternate day. The rats were sacrificed at 29th day and the samples were collected for subsequent biochemical analyses included hematological analysis, renal function test and histopathology. The results of hematological analysis revealed dose dependent reduction of RBCs and HB. Meanwhile, significant reduction of following RBCs parameters were observed such as MCH, MCV, MCHC, and HTC than control ($P< 0.05$). The results also depicted that the accumulation of Ni NPs caused inflammation and turned on autoimmune system which increased PLTs and WBCs production included granulocyte, monocyte and lymphocyte in treated groups than control ($P<0.05$). The kidney function test of treated rats showed significant production of renal biomarkers (BUN, uric acid and creatinine) in dose dependent manner which induced various histopathologies included necrosis (NIC), cloudy swelling (CS), vascular dilation (VD), and pycnotic nuclei (PN) while the ordinal scoring showed dose dependent severity of histopathologies. So, the study suggested the dose dependent response in renal pathophysiologies and histopathologies due to Ni NPs exposure.

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Introduction

Nanoparticles (NPs) are varying state of aggregation of matter beside NPs other forms such as plasma, liquid, solid, and gas. NPs have various mechanical, physical and chemical characteristics from their bulk state. Owing to NPs different properties, NPs have presented phenomenal mechanical, chemical, electrical, magnetic, and optical characteristics than other bulk forms. If NPs are not appropriately used and reprocess that may cause environmental hazards. A number of engineered NPs are commercially being used in a various products for instance sunscreens, toothpaste, cosmetics, food additives, clothing, tires and numerous building supplies (Buzea *et al.*, 2007).

The utilization of NPs are enormous that initiate the focus on nanotoxicology. The main purpose of nanotoxicology is to create the principles of NPs safe synthesis. Meanwhile, owing to growing of nanobiotechnology, there is also dire need for the cautious evaluation of nanoparticle toxicity. Generally, *in vivo* studies have been used for determination of NPs induced toxicity and following exposure routes were involved such as dermal, oral and pulmonary system (Donaldson *et al.*, 2004; Aillon *et al.*, 2009).

Nickel nanoparticles (Ni NPs) are extensively being used in industries due to their unique properties. Following properties enhance the bioactivities of Ni NPs such as large surface area to volume ratio, low melting point, low burning point and high surface energy (Zhang, 2003; Maynard *et al.*, 2006). Ni NPs have potential to interrupt physiology that may directly disrupt the constitution of organs and tissues of healthy humans and animals. The toxicity relies on various biochemical parameters for instance shape, particle size, surface, composition, charge, and chemistry, as well as subsequent NPs constancy. One recent study noted histoarchitectural alterations in liver of rats at dose dependent manner after Ni NPs intravenous injection and also recoded the biochemical changes in liver biomarkers such as ALP as well as total bilirubin (Magaye *et al.*, 2014). Ni NPs disrupts the membrane by adhering to the negative charges of ions and provoke the destruction of important molecules (Díaz-Visurraga *et al.*, 2011; Kumar *et al.*, 2015) and intravenous administration in mice lead to necrosis of liver, spleen, kidney and lungs of male mice (Ajdari and Ghahnavieh, 2014). Ni NPs trigger ROS mediated toxicity that lead to reduction of GSH level and DNA as well as RNA damage. It also decreased mitochondrial membrane potential (MMP) in dose dependant manner in Ni NPs induced toxicity of HepG2 cells.

Moreover, the cell are arrested at various cell phases either at G1 or S or G2 phases due to apoptosis. The previous study reported that Ni NPs treated HepG2 cells were arrested at G1 phase and upregulated mRNA expression of casepase-3, bax gene and tumor suppressor gene p35 while downregulated the anti-tumor gene expression (Ishikawa *et al.*, 2006; Sharma *et al.*, 2017). Ni NPs found more toxic than bulk form and observed to cause thinning of intestine in Zebra fish at 30, 60 and 100nm (Ispas *et al.*, 2009). Ni NPs promoted the necrosis, edema, lamellar fusion, nuclear degeneration, rupture epithelial in liver, gills and skin of *O. mossambicus* adult fish and reduced the antioxidant enzymes at 10.0mg/L (Jayaseelan *et al.*, 2014). The main source of NPs induced toxicity is oxidative stress which lead to decreasing of GSH level meanwhile increasethe lipid peroxidation and reactive oxygen species (ROS) (Wang *et al.*, 2009; Akhtar *et al.*, 2010). Few studies tried to explore the mechanism of Ni NPs induced toxicity and exact process of Ni NPs induced toxicity yet to be understand, though literature reported that toxicity induces due to onset of pro-inflammatory gene and oxidative stress (Hussain *et al.*, 2005; Xia *et al.*, 2006). However, Ni NPs are significantly more reactive and causative of reactive oxygen species (ROS) as compared to the other nickel compounds (Latvala *et al.*, 2016). Beside that Ni NPs induced the inflammatory response by production of interleukin-4 level and interferon-Ɣ in Wastar rats (Behnammorshedi *et al.*, 2014). One recent study produced Ni NPs induced toxicity in female rats and the biomarkers indicated the mitochondrial swelling, ROS generation, increase malondialdehyde (MDA) and nitric oxide (NO) production. Ni NPs also reported to upregulate

caspase-9, caspase-8, caspase-3 while downregulated BACL-2 protein that brought about apoptosis and oxidative stress (Kong *et al.*, 2016).

Material and methods

Twenty post weaning male Sprague Dawley rats with average weight of 79-81g were purchased from breeding center of University of Agriculture and placed in clean steel cages of Animal house after the approval from the local ethical committee of Government College University Faisalabad. Rats were house at 25±2°C, 60–70% relative humidity and provided the commercially available feed and tap water *ad labium*. Before commencing the trials, rats were acclimatized for about 7 days.

Experimental design

After acclimatization male Sprague Dawley rats of equal weights were divided into five groups (1control, 1 placebo and 3 treated groups) each with five rats and intraperitoneally (i.p) injected with Ni NPs doses either 15mg/kg or 30mg/kg or 45mg/kg. The rats were weighed and sacrificed at 29th day then samples were collected for further analysis which included hematological analysis, biochemical analysis of kidney biomarkers, and histology of kidneys.

Chemicals

Ni NPs of size 50nm with 99% purity were purchased from Richest Group Ltd, Shanghai 201202, China.

Preparation of Ni NPs dose

Ni NPs were added in 0.9% saline solution and ultrasonicated (Universal Ultrasonic cleaner, DSA100-SK-2,8L) for at least 1 hour. Ni NPs were ultrasonicated to evenly distribute Ni Ns in saline solution and vortex before dose.

Sample collection

Blood was collected from Sprague Dawley rats in EDTA. k3 tubes (IMPROVACUTER®) for haematological analysis to evaluate following parameters included haemoglobin (HB), red blood cells (RBCs), mean cell haemoglobin (MCH), mean cell volume (MCV), mean cell haemoglobin concentration (MCHC), haematocrit (HTC), platelets (PLT), and white blood cells (WBCs) using autoanalyser (Mythic™ 22). The blood collected in clot activator tubes (IMPROVACUTER®) for renal function test (RFT) to assess creatinine (CRT), blood urea nitrogen (BUN), and uric acid using autoanalyzer (Chemistry Analyser Techno 786). The targeted organs (kidneys) were preserved in 10% formalin for further histopathological analysis.

Histological analysis

Fixation: Liver and kidney tissues were fixed in 10% formalin and composition of 10% formalin was 100% ethanol (Sigma-Aldrich®, CAS No: 64-17-5), 60ml, formaldehyde (Sigma-Aldrich®, CAS No: 50-00-0) 30ml, glacial acetic acid (Sigma-Aldrich®, CAS No: 64-19-7) 10ml then followed by dehydration for1 hour at 20°C from 65% to 100% ethanol then placed samples into cedar wood oil (Sigma-Aldrich®, CAS No:8000-27-9) until became clear and transparent at 20°C.

Embedding: For embedding, dipped tissues twice into Benzol (Sigma-Aldrich®, CAS No: 301836-41-9) for 10 minutes at 20°C. The tissues then embedded three times in paraplast (Sigma-Aldrich®, CAS No:145686-99-3) for 15 hours at 50°C. The tissues then moulted into paraffin wax in plastic caster then allowed to solidify and removed from the caster for sectioning from microtome (SLEE Rotary Microtome CUT5062) and cut the tissue about 3-4µm. The tissue sections were placed on glass slides to deparafinzed the slides by using xylene Rehydration and Haematoxylin-Eosin staining: The tissue section were rehydrated with ethanol from 50% to 100% in ascending manner and stained with haematoxylin-eosin (Sigma-Aldrich® CAS No: 517-28-2).Each slide was washed with 100% ethanol for dehydration then added few drops of Canada Balsam before the mounting of slides and incubated for 12 hours and cover slips were placed on each slide to check under microscope (Meiji Techno. MT4300H).

Statistical analysis

The data was statistically analyzed by using ANOVA in general linear model using software (Minitab 17) and Tukey's test used to compare the means within different groups at the significance level of $P<0.05$.

Results

Hematological analysis

Table 1 presented hematological analysis which revealed significant difference (*P>0.05*) in red blood cells (RBCs), hemoglobin due to Ni NPs exposure for 28 alternate days than control. While mean corpuscular hemoglobin concentration (MCHC), mean cell mean corpuscular hemoglobin (MCH), and mean corpuscular volume (MCV), hematocrit (HTC) were lowered (*P>0.05*) compared to control. The Platelets (PLT) and white blood cells (WBCs) were significantly high (*P>0.05*) in each treated groups in dose dependent manner.

Kidney function test

Table 2 showed the results of kidney function tests that exhibited dose dependent increased of blood urea nitrogen (BUN), creatinine (CRT) and uric acid compared to normal control (*P< 0.05*).

Histopathology of kidneys

Fig. 1 expressed the kidney histopathology of control as well as treated rats. The photomicrograph (A & B; 400X) depicted the typical histoarchitecture array of kidneys in control rats. The micrograph (C & D; 400X) exhibited following histopathologies at low dose (15mg/kg) included pycnotic nuclei (PN), sinusoidal dilation (SD), vascular dilation (VD), necrosis (NIC), and glomerulus sclerosis (GS). The photomicrograph (E & F; 400) observed the medium dose (30mg/kg) response and marked cloudy swelling (CS), glomerulus sclerosis (GS), pycnotic nuclei (PN), vascular dilation (VC) whereas micrograph (G & H; 400X) showed that the high dose (45mg/kg) attributed significant necrosis (NIC), pycnotic nuclei (PN), glomerulus sclerosis (GS), cloudy swelling (CS), and vascular dilation (VD).

Table 1. The blood biochemistry of control (C) and treated groups (N-G1, N-G2, N-G3) treated with either 15mg/kg or 30mg/kg or 45mg/kg.

Group	HB (g/dl)	RBCS X (10^{12}/L)	WBCS 10^3/µL	HTC (%)	MCV (pg)	MCH (pg)	MCHC (%)	PLT X (10^9/L)	MON (%)	LYM (%)	GRA (%)
C	16.04^{A}± 0.11	8.16^{A}± 0.12	16.04^{D} ±0.18	46.78^{A}± 0.72	56.9^{A}± 0.2	18.08^{A}± 0.08	38.18^{A}± 0.59	926.8^{D} ±2.77	8.04^{D}± 0.06	77.79^{D}± 0.39	4.93^{D}± 0.08
N-G1	14.55^{B}± 0.23	7.00^{B}± 0.63	18.53^{C}± 0.38	43.1^{B}± 0.15	51.18^{B}± 0.97	17.04^{AB}± 0.97	36.44^{B}± 0.33	985.6^{C}± 2.96	9.87^{C}± 0.03	82.82^{C}± 1.21	6.38^{C}± 0.21
N-G2	13.39^{C}± 0.28	6.64^{B}± 0.48	19.36^{B}± 0.032	41.49^{C}± 0.21	49.60^{BC}± 1.07	16.91^{B}± 0.32	36.39^{B}± 0.32	1047.51^{B}± 1.60	10.73^{B}± 0.04	85.31^{B}± 0.34	7.19^{B}± 0.04
N-G3	12.68^{D}± 0.39	5.06^{C}± 0.03	20.06^{A}± 0.12	40.84^{C}± 0.04	48.43^{C}± 1.22	16.542^{B}± 0.74	34.38^{C}± 0.29	1100^{A}± 1.58	11.22^{A}± 0.07	84.53^{A}± 0.35	7.79^{A}± 0.05

Values are represented as mean± standard deviation, letters represents present significantly different (P < 0.05)

RBCS (Red blood cells), HB (Hemoglobin), hematocrit (HTC), MCH (Mean Corpuscular Hemoglobin), MCHC (Mean Corpuscular Hemoglobin Concentration), PLT (Platelets), WBCs (White blood cells), , LMY (Lymphocytes)MON (Monocytes), GRA.

Table 2. The kidney profile of control (C) and treated groups (N-G1, N-G2, N-G3) treated with either 15mg/kg or 30mg/kg or 45mg/kg.

GROUPS	BUN (IU/L)	CRT (g/dl)	Uric Acid (g/dl)
C	13.88^{D}±0.49	0.37^{D}±0.003	1.87^{D}±0.003
N-G1	17.79^{C}±0.25	0.46^{C}±0.003	2.67^{C}±0.017
N-G2	19.44^{B}±0.51	0.52^{B}±0.004	3.57^{B}±0.013
N-G3	22.73^{A}±0.31	0.58^{A}±0.003	4.17^{A}±0.041

Values are represented as mean± standard deviation, letters present significant difference from the control (*P < 0.05*)

BUN =Blood urea nitrogen, CRT =Creatinine

Table 3. Scoring of kidney histomicrographs to evaluate histopathologies in control (C) and treated groups (N-G1, N-G2, N-G3).

GROUPS	% SD	%PN	%NIC	%VD	%CS
C	0	0	0	0	0
N-G1	40	20	20	20	0
N-G2	0	40	40	40	40
N-G3	0	60	80	60	60

Sinusoidal dilations (SD), Pycnotic nuclei (PN), Necrosis (NIC), vascular dilation (VD), Cloudy swelling (CS).

Table 4. Ordinal scoring of kidney histographs to evaluate histopathologies in control and treated groups (N-G1, N-G2, N- G3).

GROUPS	SD	PN	NIC	VD	CS
C	0	0	0	0	0
N-G1	2	1	1	1	0
N-G2	0	2	2	2	2
N-G3	0	3	4	3	3

Sinusoidal dilations (SD), Pycnotic nuclei (PN), Necrosis (NIC), vascular dilation (VD), Cloudy swelling (CS).

Table 5. Ordinal scoring criteria.

Scoring	%	Significance
0	0	no change
1	20	Low
2	40	Mild
3	60	Moderate
4	80	High

Discussion

Nanoparticles (NPs) have widespread applications due to their unique characteristics as compared to the bulk form and many studies involved the NPs induced toxicity. The recent study focused on Ni NPs induced nephrotoxicity by assessment of blood biochemistry, kidney biomarkers, and histopathology. NPs have toxic effects when interact with physiological system due to their large surface area to volume ratio which make NPs permeable to cell membrane and interrupt numerous physiological phenomenon (Oberdorster *et al.*, 2005; Nel *et al.*, 2006; Shang *et al.*, 2014). The reported studies of Ni NPs exposure on Sprague Dawley rats are very few because of that the present study has cited other nanoparticle induced toxicty for comparison.

After exposure of Ni NPs for 28 alternate to male Sprague Dawley rats, the samples were went through various biochemical assays for evaluation of selected parameters such as blood biochemistry revealed reduction of RBCs and HB in dose dependent manner while significant difference ($P<0.05$) was observed in various RBCs parameters included MCH, MCV, HTC, and MCHC in treated groups (N-G1, N-G2, N-G3) compared to control group (C) which illustrated presence of anemia due to RBCs reduction that brought about increased corpuscular volume. These findings are corresponding to the study related to zinc oxide nanoparticles induced toxicity in rats and exposed at doses either 500 or 1,000, or 2,000mg/kg for 14 days which lead to reduction of HB, RBCs and their parameters (MCH, MCV, HTC, and MCHC) on dose dependent manner. The present also corresponds to the study depicted the nickel oxide nanoparticle induced toxicity in Wistar albino rats at various doses that lead to decrease in RBCs as well as HB and these physiological alteration related to disturbance in the process of erythropoiesis (Ko *et al.*, 2015; Dumala *et al.*, 2018). Platelets (PLTs) and white blood cells (WBCs) are also highly significant ($P<0.05$) in all the treated groups as compared to the control. In the WBCs, the percentage of monocytes (MON), lymphocytes (LYM) and granulocytes (GRA) was significant ($P>0.05$) and observed dose dependent relation. These physiological alterations indicated the activation of autoimmune system and inflammation of kidney. These results consistent to one recent study that reported increased production of WBCs and PLTs on intravenous injection of Ni NPs once on day 1st then at day 14th later on biochemical analysis reveal increased production of WBCs and PLTs than control (Magaye *et al.*, 2014).

The kidney biomarkers (BUN, CRT, Uric acid) act as significant diagnostics for determination of pathophysiological alterations due to deleterious effects on exposure of toxic elements. In the present study, the kidney function test revealed significant ($P<0.05$) increase of biomarkers (uric acid, BUN and CRT) as compared to control and exhibited dose dependent relationship. These changes in kidney biomarkers lead to various histopathological alterations. These findings are consistent to previous study of titanium nanoparticle induced hepatic and kidney toxicities in rats which marked considerable changes in renal biomarker at high doses (Valentini *et al.*, 2019).

The photomicrograph (Fig 1, A & B; 400X) exhibited typical histoarchitecture of cortical as well as medulla in control rats. In treated groups, the ordinal grading marked dose dependent histopathological effects as the photomicrograph (Fig. 1, C & D; 400X) on exposure of low dose (15mg/kg) exhibited mild sinusoidal dilation (SD), less significant pycnotic nuclei (PN), vascular dilation (VD) and necrosis (NIC), the micrograph (Fig 1, E&F; 400X) at medium dose (30mg/kg) expressed

mild pynotic nuclei (PN), necrosis (NIC), vascular dilation (VD), cloudy swelling (CS) while the high dose (45mg/kg) illustratedsignificantly high tubular necrosis (NIC), vascular dilation (VD) and cloudy swelling (CS). The Ni NPs brought about ROS generation which caused lysis of cell membrane and lead to kidney malfunctioning. In present study, the glomerular sclerosis and tubular necrosis of kidney were observed which indicated immunological alteration and infiltration of inflammatory cells (Sardari *et al.*, 2005; Lam *et al.*, 2006; Chen and Schluesener, 2008). The neutrophils and monocytes also provoked inflammatory cytokines which promoted the cloudy tubular swelling. Moreover, kidneys are main organ for metal excretion and Ni NPs accumulation also brought about destruction of podocytes and glomerular sclerosis (Abdelhalim and Jarrar, 2011; Iavicoli *et al.*, 2016).

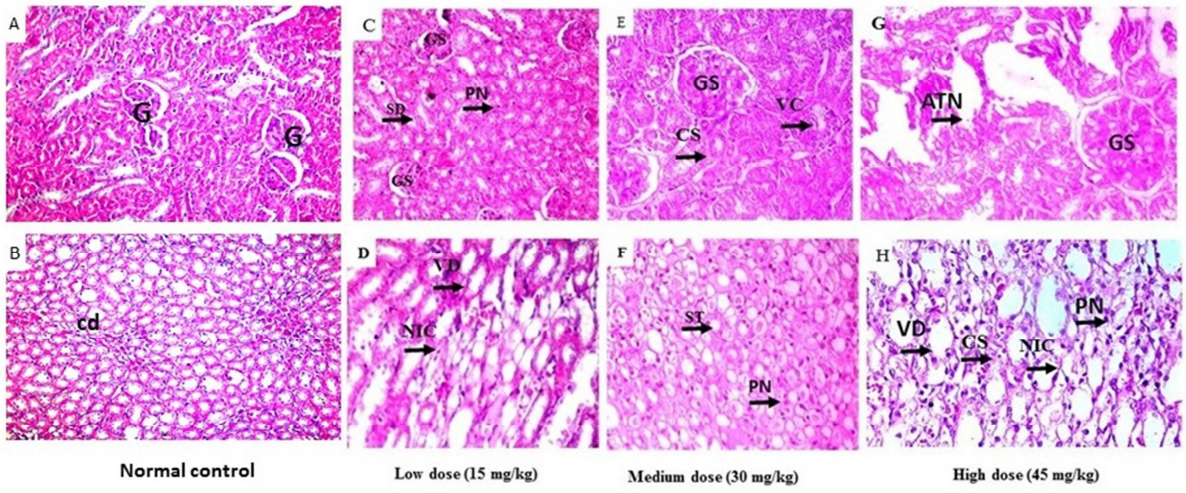

Fig. 1. The photomicrograph (A &B; 400X) showed normal histoarchitecture in cortex and medulla regions of kidney. Micrograph (C & D; 400X) marked glomerulus sclerosis (GS), pycnotic nuclei (PN), sinusoidal dilation (SD), vascular dilation (VD) and necrosis at low dose. The photomicrograph (E & F; 400) revealed that medium dose attributed glomerulus sclerosis (GS), cloudy swelling (CS), vascular dilation (VC), and pycnotic nuclei (PN). Photomicrograph (G & H; 400X) showed that high dose induced necrosis (NIC), glomerulus sclerosis (GS), cloudy swelling (CS), vascular dilation (VD), and pycnotic nuclei (PN).

Conclusion

The data depicted dose dependent reduction of RBCs and HB on Ni NPs exposure as well as significant alteration in RBCs parameters (HTC, MCH, MCV, and MCHC) which indicated anemia. The WBCs and PLTs were also increased due to activation of autoimmune system and inflammation to combat infection. The kidney biomarkers (Creatinine, BUN, and uric acid) were also increased in dose dependent relation that led to occurrence of various histopathologies. The study concluded that Ni NPs induced renal associated pathophysiologies and histopathologies in dose dependent manner. Moreover, the present study would pave new avenues for considering Ni NPs related toxicities at long term exposure.

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