Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
DOI: 10.29261/pakvetj/2019.106

RESEARCH ARTICLE

Nickel Nanoparticles Induced Nephrotoxicity in Rats: Influence of Particle Size

Shang Ziyad Abdulqadir[1*] and Falah Mohammad Aziz[2]

Department of Biology, College of Science, Salahaddin University-Erbil, Kurdistan Region, Iraq
*Corresponding author: shang.abdulqadir@su.edu.krd

ARTICLE HISTORY (18-400)

Received: October 21, 2018
Revised: May 28, 2019
Accepted: August 21, 2019
Published online: October 15, 2019

Key words:
Histopathology
Nephrotoxicity
Nickel nanoparticles
Oxidative stress
Rats

A B S T R A C T

Since nickel compounds are carcinogenic and strong toxicants to the various organs, it is necessary to carry out extra *in vivo* trials on Nickel nanoparticles (NiNPs) to demonstrate their influences on health pragmatically. The current study was executed to scrutinize the expected undesired impacts of various sizes of NiNPs on renal nephrons of rats. To meet the trial requirements, a total of 24 male Wistar rats, each 12 weeks old were allocated randomly into four groups (n=6). Group 1 was designed as the control (only given sodium chloride 0.9%) while the other three groups (2, 3 and 4) of the experimental animals were exposed to intraperitonial NiNPs (20mg/kg B.W) daily at three sizes (20 nm, 40 nm and 70 nm) for 28 days. Inflammatory cells aggregations of infiltrated leucocytes and degeneration of the proximal tubular cells were the most frequent histopathological features in the NiNPs treated groups which indicates a NiNPs-induced nephrotoxicity. Biochemical analysis of tissue malondialdehyde (MDA), superoxide dismutase (SOD) and serum creatinine were performed. MDA level was significantly elevated ($P \leq 0.05$) in all NiNPs treated groups as compared to the control group. All the three NiNPs groups revealed a significant tissue SOD and serum creatinine elevation as compared to the control group ($P \leq 0.05$). Furthermore, a significant increase in the p53 positive kidney tubular cells was detected in NiNPs treated groups as compared to control group ($P \leq 0.05$). All alterations above in the treated rats were size dependent; the smallest NiNPs being more toxic than the largest ones.

To Cite This Article: Abdulqadir SZ and Aziz FM, 2019. Nickel nanoparticles induced nephrotoxicity in rats: influence of particle size. Pak Vet J, 39(4): 548-552. http://dx.doi.org/10.29261/pakvetj/2019.106

INTRODUCTION

Nephrotoxicity is a major risk in pre-clinical toxicological studies due to direct cells and tissues injury, obstruction of renal excretion, hemodynamic changes and inflammation. The kidney plays a major role in infiltrating plasma and maintaining metabolic homeostasis. Nephrotoxicity in response to pollutants can impede the renal excretory activities and cause alteration in kidney physiology and structure (Zhang and Sun, 2015).

Nanoparticles (NPs) are defined as any particles with dimensions at 1–100 nm range (Khan *et al*., 2017). With the emergence of nanotechnology as a modern branch of science, NPs-induced toxicity studies have been increased. Nickel nanoparticles (NiNPs) have many applications in the industrial field. Over the past ten years, these NPs were extensively utilized in ceramic capacitors, hydrogen storages, paints, and chemical catalysts (Ding *et al*., 2018). On the other hand, a large number of recent studies have focused on the NPs-induced cytotoxicity and genotoxicity in vitro and seldom employing experimental animals. Undoubtedly, *in vitro* assays have some benefits, particularly the primary toxicity mechanism analysis. Nevertheless, any conclusion or prediction of obtained results to the whole organism may be built on some hypotheses and doubts. Also, certain significant characteristics of toxicology, mainly dose effect and toxicokinetics, can be gained merely using *in vitro* trials (Minigalieva *et al*., 2015).

According to Katsnelson *et al*. (2015), NiNPs could induce higher toxicity in comparison to the larger particles. The most exposed organs to Nickel (Ni) are the liver, kidneys, brain, lungs, and testis (Capasso *et al*., 2014; Razavipour *et al*., 2015; Dumala *et al*., 2017; Yu *et al*., 2018). The most critical mechanism proposed for NiNPs-induced cytotoxicity is the increase in reactive oxygen species (ROS) level (Yu *et al.*, 2018). NiNPs were also found to stimulate cell cycle arrest (Ma *et al*., 2014), cell degeneration and inflammation (Razavipour *et al*., 2015). Furthermore, these nanoparticles were found to

induce cytogenetic alterations, oxidative stress and apoptosis (Abudayyak *et al*., 2017). Having said that, the increasing commercialization of Ni-containing nano-particles has emboldened researchers to assess their toxicological properties using advanced techniques (Latvala *et al*., 2016). Hence, the current investigation was aimed at studying the nephrotoxic impact of different sizes of NiNPs in rats and their relation to the oxidative state utilizing basic diagnostic tools such as biochemical assays, histopathological and immunohistochemical techniques.

MATERIALS AND METHODS

Experimental design: Adult, 12-week-old male Wistar albino rats (n=24) weighing 200-220g were obtained from the animal house of the Science College of Salahaddin University, Iraq. The animals were housed in stainless steel cages at a mean temperature of 21±2ºC and a mean humidity 35% with free access to standard laboratory animal diets and tap water. The rats were randomly divided into four groups (n=6 for each group). Rats in group 1, the control group, were intraperitoneally injected (i.p.) with 0.5 mLsaline (sodium chloride 0.9%) daily, while the groups 2, 3 and 4 received 20nm, 40nm and 70nm intraperitonial ultrasonicated NiNPs, respectively. Injection of all groups continued for 28 days. Twenty hours after the last injection, the rats were euthanized and dissected. Blood samples were collected and the kidneys were kept for histopathological, biochemical and immunohistochemical analysis.

NiNPs preparation: NiNPs powders (20, 40 and 70nm in diameters) were purchased from Sigma-Aldrich Company, (St Louis, MO, USA). All essential reagents and other chemicals used in this process were of laboratory grades and obtained from the company mentioned above. The three separate NiNPs stock solutions of the aforementioned sizes were sonicated (using ultrasound sonicator Mod.150VT) in saline solution (10mg/mL) for 30 seconds. Then they were left in ice for 15 sec., and again sonicated for 3min at 400W in ice. The suspensions need to be vibrated for two minutes prior to injection. A dose of (20mg/kg B.W.) for each size of NiNPs were calculated.

Biochemical assays: After washing in ice-cold saline, the samples of a kidney from each rat were homogenized in 20mM phosphate buffer saline (pH=7.4) followed by centrifugating the homogenates at 3000 rpm at 4ºC for 10min, the supernatants were stored at -80ºC. Malondialdehyde (MDA) level in the homogenates was spectrophotometrically estimated following the thiobarbituric acid method. In short: 150 µl of supernatant was added to the followings: 1 ml trichloroacetic acid (17.5%) and 1ml thiobarbituric acid (0.66%). After mixing well by vortex, the solution was left in a boiling water bath for 15min. Once it cooled, 1ml of trichloroacetic acid (70%) was added and allowed to cool for 20 minutes. This was followed by centrifugation for 15minutes at 2000 rpm to obtain the supernatant which was used for the estimation of MDA.

To determine the superoxide dismutase (SOD) (Cat. NoE-BC020) activity, the kits purchased from Elabscience Biotechnology (Bethesda, MD, USA) were used in accordance with the manufacturer protocols.

Estimation of serum creatinine: The sera were obtained by centrifugation the collected rat blood samples at 3000 rpm for 15 minutes then stored at -80ºC for the estimation of creatinine. Serum creatinine was spectrophoto-metrically estimated (at 500nm) using BIOLABO kits (France).

Immunohistochemical analysis: Immunohistochemical kits for detection of p53, manufactured by Leica Biosystems Newcastle Ltd, were performed on an automated immunostainer (AutostainerLink48 DAKO). Two slides were randomly taken to count the p53 positive and negative hepatocytes, and the result was expressed as the percentage of p53 positive cells [calculated by dividing the number of the apoptotic cells by the total number of renal tubular epithelial cells (including the positive plus the negative p53 cells) and multiply by 100]. Positive p53 cells were determined by brown colour, while the negative p53 cells showed only the counterstain, indicated by the color blue (Milićević *et al*., 2014). The cells were counted at 400x magnification (Olympus BX 43, Olympus, Japan).

Histological preparation: Pieces of kidney were fixed in a buffered formaldehyde solution (10%) for 24 hr, dehydrated in ethanol, cleared in xylol, infiltrated with paraffin wax and finally embedded in paraffin wax. The paraffin sections (5µm thick) were stained with hematoxylin and eosin (H&E).

For the preparation of plastic sections, kidney pieces ($\leq$1mm^3) were primarily fixed in a 2.5% glutaraldehyde in 0.1M cacodylate buffer, post fixed in 1% osmium tetroxide, dehydrated in ethanol, cleared in propylene oxide, infiltrated with epoxy resin mixture plus propylene oxide then finally embedded in pure epoxy resin. The semithin sections were stained with 1% toluidine blue in 1% borax (Suvarna *et al*., 2018).

Statistical analysis: Statistical analysis of the obtained data (expressed as mean±SE) was conducted using available software (SPSS version 22). To find the significance of treatments, one way variance analysis (ANOVA) was followed. For comparison between groups, the Duncan's test was applied. $P \leq 0.05$ was considered statistically significant.

RESULTS

The shape and size of the three used sizes of NiNPs are shown in Fig. 1. The sizes of 20, 40, and 70nm were very close to that indicated by the manufacturer. In Fig. 2, MDA level in the NiNPs treated groups was significantly increased ($P \leq 0.05$) in comparison with the control. However, activity of tissue SOD in kidney homogenate of NiNPs administrated rats was significantly ($P \leq 0.05$) decreased in comparison to the rats in the control group (Fig. 3). Serum creatinine level was recorded a significant elevation in the NiNPs groups in comparison to control (Fig. 4). Concerning the stated biochemical parameters, the smallest NiNPs size showed higher effects compared to the larger sizes.

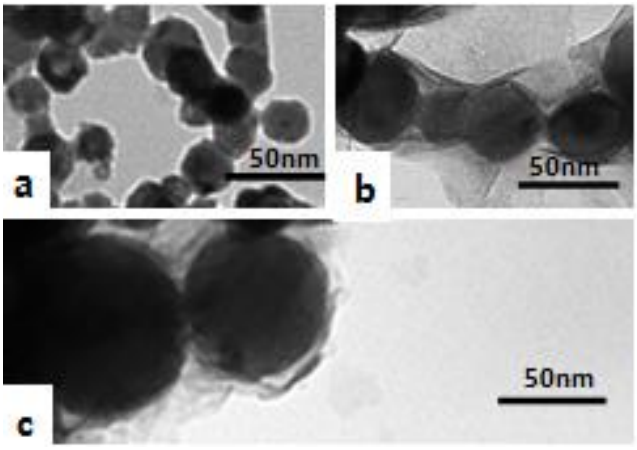

Fig. 1: Transmission electron micrographs of NiNPs different sizes used in the experiment, (a) 20nm, (b) 40nm, (c) 70nm.

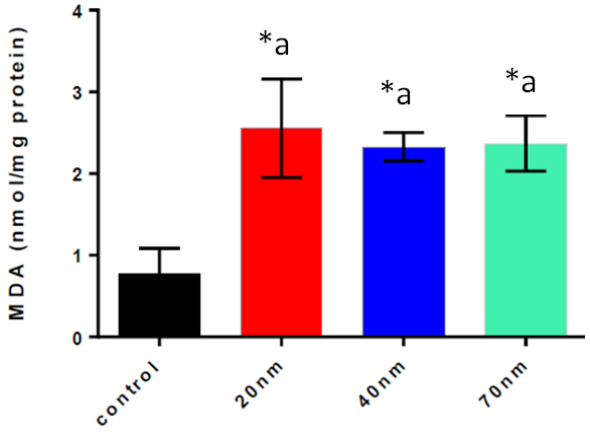

Fig. 2: Each value denotes a mean±SD of MDA level in control and treated rats (n=6 per group), similar letter indicates a statistically non-significant difference among the three NiNPs treated groups (P≤0.05). *indicates a statistically significant difference compared to the control. MDA=Malondialdehyde.

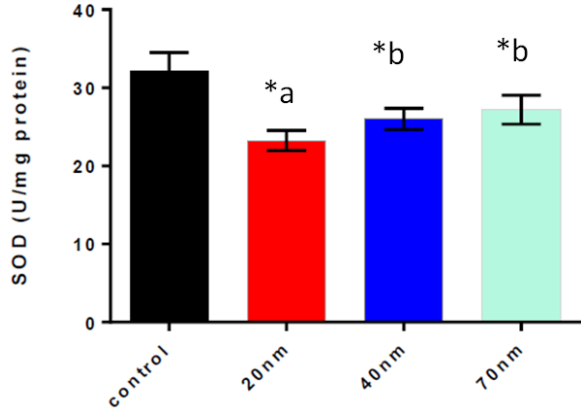

Fig. 3: Each value denotes a mean ±SD of SOD in control and treated rats (n=6 per group), different letters indicate that the values are statistically significant among NiNPs treated groups (P≤0.05). *indicates a statistically significant difference as compared to the control (P≤0.05). SOD= Superoxide dismutase.

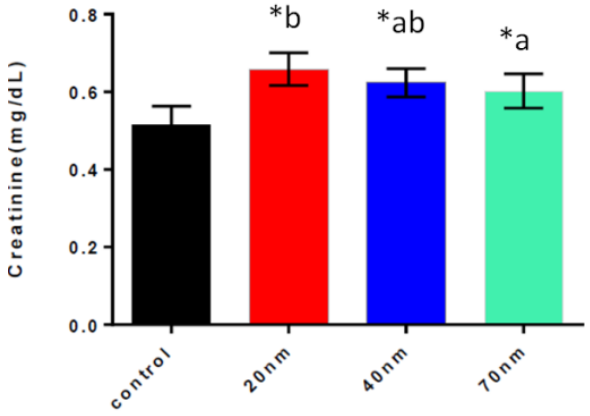

Fig. 4: Each value denotes a mean±SD of serum creatinine in control and treated rats (n=6 per group), different letters indicate a statistically significant difference among NiNPs treated groups (P≤0.05). *indicates a statistically significant difference as compared to the control (P≤0.05).

Histologically, the current study illustrated various alteration in kidney structure, the obvious feature being the infiltrated inflammatory leucocytes (Fig. 5). The main infiltrated inflammatory cells were the lymphocytes, which showed different locations in the interstitial region and around glomeruli.

The plastic sections in Fig. 6 showed the morphology of the healthy and degenerated renal tubules lining cells in control and following NiNPs treatment. The degenerated cells have shrunken irregular shape, dark stained cytoplasmic and condensed nuclei in comparison to the healthy cells. The immunohistochemical demonstration of p53 positive cells showed a significant size-dependent increase in the number of these cells compared with control (Fig. 7). However, the smaller NiNPs size showed higher p53 positive cells number than the other two larger sizes (Fig. 8).

DISCUSSION

The present study highlighted the influence of intraperitonial injection of 20, 40 and 70nm NiNPs on oxidative stress and lipid peroxidation through the estimation of tissue MDA and SOD quantities in rat kidneys. MDA-induced elevation was observed in the groups treated with NiNPs as compared to the control group. MDA, which is the final product of lipid peroxidation process (Abdelhalim *et al*., 2018) continues to be regularly utilized as a reliable indicator for the evaluation of oxidative stress induced by assorted toxicants. In this regard, many studies have scrutinized the approach that enables NiNPs to induce the toxicity *in vitro* (Abdel-Wahhab *et al*., 2017) and *in vivo* (Alidadi *et al*., 2018), which includes the induction of oxidative stress via increasing the levels of ROS. On the other hand, the considerable decline of SOD in the groups that received NiNPs indicates the reduced antioxidant capacity. The study of Yu *et al*. (2018) has revealed that enhanced lipid peroxidation and diminished antioxidant capacity are consider as two main mechanisms of the toxicity in organs treated with different toxicants.

Results from the biochemical analysis demonstrated that the smallest NiNPs (20nm) have higher renal nephrotoxic effects than the other two larger sizes (40 and 70nm). Such size impact was also observed by other investigators in the field of nanonephrotoxicity regarding creatinine level (Dumala *et al*., 2017), non enzymatic and enzymatic antioxidants (Yu *et al*., 2018), ROS induction

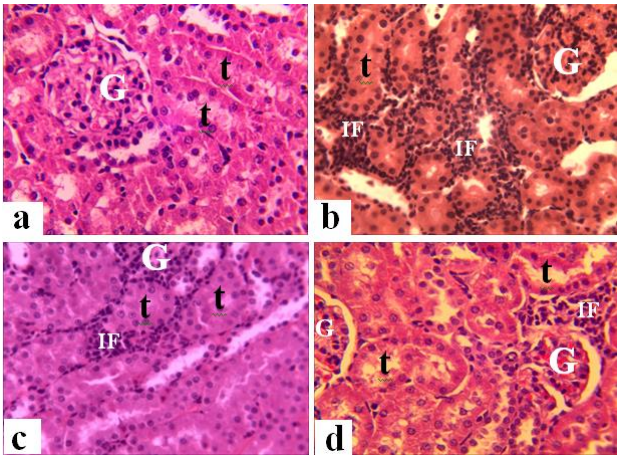

Fig 5: Photomicrographs of kidney of different NiNPs sizes treated rats: a) Control group; b) 20nm NiNPs treated group showing interstitial inflammatory infiltrated leucocytes(IF); c) and d) 40nm NiNPs and 70nm treated groups respectively with lower density of inflammatory leucocytes (IF), all magnifications are 400X, H&E. G=glomerulus; t=renal tubules.

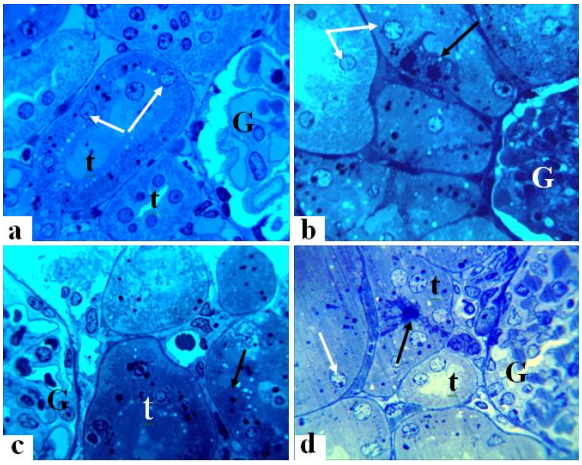

Fig. 6: Plastic sections of the kidney of NiNPs treated rats showing the effect of different sizes: a) Control group, showing normal histological architecture of glomerulus (G), kidney tubues (t) lined by normal epithelial cells (white arrows); b) 20nm group shows degenerating kidney tubule cells with condensed nucleus and irregular plasma membrane(black arrows); c) 40nm group showing degenerating cells in the renal tubules(t) (black arrows); d) 70nm group showing a degenerating cell in the lumen of renal tubules(black arrows). All magnifications are 400X, Toluidine blue.

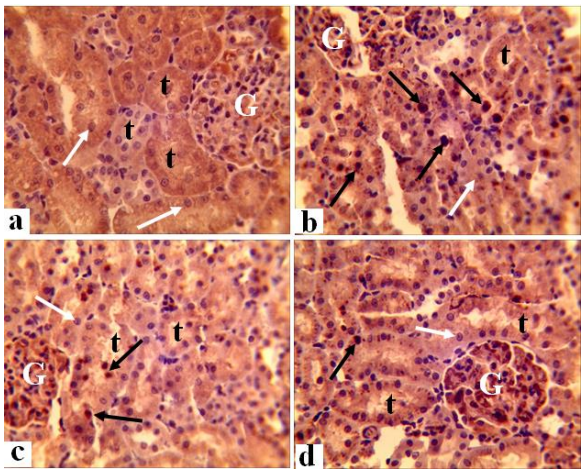

Fig. 7: Immunohistochemical images of p53 positive kidney tubular cells in NiNPs treated rat kidney, a) Control group showing healthy epithelial cells lining the kidney tubules (white arrow), no positive 53 cells are seen; b) 20nm NiNPs treated group showing high p53 positive cells(black arrows). c) 40nm NiNPs treated group showing lower p53 positive cells; d) 70nm NiNPs treated group showing few p53 positive cells. All magnifications are 400X.

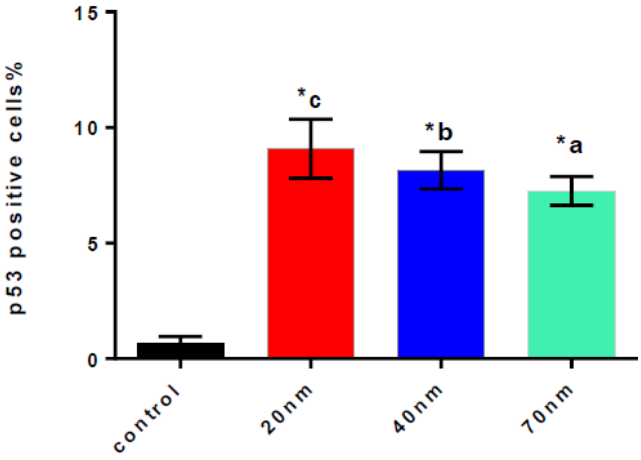

Fig. 8: Percentage of p53 positive cells in different NiNPs treated groups. Each value is a mean of p53 positive cells in rats (n=6 per group) ±SD; different letters indicate a statistically significant difference among NiNPs treated groups. *indicates a statistically significant difference in NiNPs groups in comparison with control (P≤0.05).

and lipid peroxidation production expressed by the elevation of MDA (Razavipour *et al*., 2015; Yu *et al*., 2018). The over production of ROS can have potentially damaging biological responses resulting in oxidative stress. The main mechanism found responsible for the toxicity of nanoparticles is the ROS production that causes cell failing to maintain the normal physiological redox-regulated functions. The oxidative stress, due to ROS production, induces cellular alteration such as DNA damage, unregulated cell signaling, cell motility change, apoptosis and cancer initiation (Kusaczuk *et al*., 2018).

The increased level of creatinine in the serum of the experimental animals treated with NiNPs may refer to the affected kidney functions, such as the glomerular filtration rate, which may be due to the accumulation of NiNPs in the kidney. Such increase in serum creatinine level was observed after exposure to other nanoparticles such as sliver NPs (Tiwari *et al*., 2017).

The size-dependent histopathological changes were observed in the NiNPs treated groups. The most apparent change was the appearance of inflammatory infiltrated leucocytes. NPs used to activate the signaling of the nuclear factor-kappa B(NF-kB), which found to be involved in various cellular processes such as immune responses, inflammation, cell growth and survival (Zhang and Sun, 2015). Nickel oxide nanoparticles were found to induce inflammation in epithelial cells in other organs other than the kidney (Capasso *et al*., 2014).

The degenerated kidney tubular cells which appeared in the NiNPs treated groups have the features of apoptotic cells with shrunken irregular shape; darkly stained cytoplasm and condensed nuclei in comparison to healthy

cells (Xu *et al*., 2019). The Immunohistochemical demonstration of p53 positive cells has confirmed that the main mode of NiNPs-cell death was the programmed cell death, particularly apoptosis. When the damage in the gene is too sever and couldn't be repaired by it's own cell, the induction of apoptosis by p53 is processed (Wawryk-Gawda *et al*., 2014).The significant increase in the number of p53 positive kidney tubular cells in the NiNPs treated rats indicates the increased apoptosis mode of cell death, while the size-dependent difference may be related to the induced oxidative stress since oxidative stress is one of the important factors that are known to induce apoptosis (Sun *et al*., 2018). However, the smallest NiNPs size showed higher p53 positive cells than the other two larger sizes.

The oxidative stress, which has been detected in the present work as a consequence of MDA elevation and decreased antioxidant enzymes in response to toxic NiNPs, is deemed among the main causes of cell death, apoptosis or necrosis (Abudayyak *et al*., 2017). Perhaps, other nanoparticles can also induce apoptosis, an approach being utilized recently by investigators to fight cancerous cells (Jiang *et al*., 2018). In the current study, the smallest sizes of NiNPs showed more toxicity to nephrons than the larger particles. In this regard, Ahmadzadeh *et al*. (2017) have justified this phenomenon as follows; with the decrease of the nanoparticle sizes and increase in the ratio of their surface area to volume, their chemical and biological reactivity rises. This conduces to increased production of ROS beyond the tolerance of biological systems to eliminate or reduce their harmful effects (Thai *et al*., 2019).

Conclusions: In the light of the current *in vivo* study, it was concluded that injected rats with NiNPs intraperitoneally had shown induction of oxidative stress, lipid peroxidation, inflammation, renal tubular cell degeneration. These nephrotoxic effects were size-dependent; the smallest size had exhibited more nephrotoxic effects as compared to the larger sizes.

Authors contribution: SZA has performed the experiments and laboratory analysis. FMA has designed the research protocol, supervised the research procedure and participated in manuscript preparation.

REFERENCES

Abdelhalim MA, Moussa SA and Qaid HA, 2018. The protective role of quercetin and arginine on gold nanoparticles induced hepatotoxicity in rats. Int J Nano 13:2821-5.

Abdel-Wahhab M, Aljawish A, El-Nekeety A, *et al*., 2017. Chitosan nanoparticles plus quercetin suppress the oxidative stress, modulate DNA fragmentation and gene expression in the kidney of rats fed ochratoxin A-contaminated diet Food. Chem Toxicol 99:209-21.

Abudayyak M, Guzel E and Özhan G, 2017. Nickel oxide nanoparticles induce oxidative DNA damage and apoptosis in kidney cell line (NRK-52E). Biol Trace Elem Res 178:98-104.

Ahmadzadeh A, Aghababaei M, Allameh Z, *et al*., 2017. The impact of silymarin extract on oxidative stress induced by gold nanoparticles. Biomed Res 28:6144-50.

Alidadi H, Khorsandi L and Shirani M, 2018. Effects of quercetin on tubular cell apoptosis and kidney damage in rats induced by titanium dioxide nanoparticles. Malays J Med Sci 25:72-81.

Capasso L, Camatini M and Gualtieri M, 2014. Nickel oxide nanoparticles induce inflammation and genotoxic effect in lung epithelial cells. Toxicol Lett 226:28-34.

Ding D, Liu K, Fan Q, *et al*., 2018. Nickel nanoparticles individually encapsulated in densified ceramic shells for thermally stable solar energy absorption. J Mater Chem A 7:3039-45.

Dumala N, Mangalampalli B, Kamal SK, *et al*., 2017. Biochemical alterations induced by nickel oxide nanoparticles in female Wistar albino rats after acute oral exposure. Biomark 22:1-11.

Jiang J, Pi J and Cai J, 2018. The advancing of zinc oxide nanoparticles for biomedical applications. Bioinorg Chem Appl 5:1062562.

Katsnelson BA, Privalova L, Sutunkova MP, *et al*., 2015. Some inferences from in vivo experiments with metal and metal oxide nanoparticles: the pulmonary phagocytosis response, subchronic systemic toxicity and genotoxicity, regulatory proposals, searching for bioprotectors (a self-overview). Int J Nanomed 10:3013-29.

Khan I, Saeed K and Khan I, 2017. Nanoparticles: properties, applications and toxicities. Arab J Chem 12:908-31.

Kusaczuk M, Krętowski R, Naumowicz M, *et al*., 2018. Silica nanoparticle-induced oxidative stress and mitochondrial damage is followed by activation of intrinsic apoptosis pathway in glioblastoma cells. Int J Nanomed 12:2279-94.

Latvala S, Hedberg J, Di Bucchianico S, *et al*., 2016. Nickel release, ROS generation and toxicity of Ni and NiO micro- and nanoparticles. Plos One 11:1-20.

Ma C, Songb M, Zhang Y, *et al*., 2014. Nickel nanowires induce cell cycle arrest and apoptosis by generation of reactive oxygen species in HeLa cells. Toxicol Rep 1:114-21.

Milićević Z, Bajić V, Živković L, *et al*., 2014. Identification of p53 and its isoforms in human breast carcinoma cells. Sci World J 10 pages.

Minigalieva IA, Katsnelson BA, Privalova LI, *et al*., 2015. Attenuation of combined nickel (ii) oxide and manganese (ii, iii) oxide nanoparticles, adverse effects with a complex of bioprotectors. Int J Mol Sci 16:22555-83.

Razavipour ST, Behnammorshedi M, Razavipour R, *et al*., 2015. The toxic effect of nickel nanoparticles on oxidative stress and inflammatory markers. Biomed Res 26:370-4.

Sun YY, Wang C, Wang LG, *et al*., 2018. Arsenic trioxide induces apoptosis and the formation of reactive oxygen species in rat glioma cells. Cell Mol Biol Lett 23:13.

Suvarna SK, Layton C, Bancroft JD, 2018. Bancroft's theory and practice of histological techniques. 8th ed. Elsevier, UK.

Thai S, Jones C, Nelson G, *et al*., 2019. Differential effects of nano TiO2 and CeO2 on normal human lung epithelial cells in vitro. J Nanosci Nanotech 19:6907-23.

Tiwari R, Singh RD, Khan H, *et al*., 2017. Oral subchronic exposure to silver nanoparticles causes renal damage through apoptotic impairment and necrotic cell death. Nanotoxicol 11:671-86.

Wawryk-Gawda E, Chylińska-Wrzos P, Lis-Sochocka M, *et al*., 2014. P53 protein in proliferation, repair and apoptosis of cells. Protop 251:525-33.

Xu X, Lai Y and Hua Z, 2019. Apoptosis and apoptotic body: disease message and therapeutic target potentials. Biosci Rep 39:BSR20180992.

Yu S, Liu F, Wang C, *et al*., 2018. Role of oxidative stress in liver toxicity induced by nickel nanoparticles in rats. Mol Med Rep 17:3133-9.

Zhang H and Sun S, 2015. NF-kB in inflammation and renal diseases. Cell Biosci 5:63-72.