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Renal toxicity of nanoparticles of cadmium sulphide in rat

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HIGHLIGHTS

- Bioaccumulation of CdSNPs in kidney is lower than CdS bulk particles. However, metallothionein induction is greater than bulk CdS.
- CdSNPs induce higher LPO than CdS in kidney. Shape of CdSNPs is more important than composition.
- CdSNps cause greater inhibition of SH group than CdS.
- Extensive proximal tubular necrosis and ultrastructural changes in the kidney of CdSNPs treated rats were observed.
- Loss of alkaline phosphatase occurs in cortex of CdSNPs treated rats.

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ABSTRACT

During present investigations, renal toxicity of CdSNPs (cadmium sulphide nanoparticles) (ranged 5 −9 nm) was estimated in rat employing specific parameters. Treatment on each alternate day for 45 days with CdSNPs (10 mg/kg b.w.) yielded significant increase in Cd-MT (cadmium metallothionein), lipid peroxidation and H_2O_2 generation in the kidney of rat in comparison to bulk cadmium. Concentration of creatinine also increased in urine of CdSNPs treated rats. Histopathological observations revealed extensive damage in proximal tubules. Ultrastructural studies showed mitochondrial, nuclear and ER (endoplasmic reticulum) changes. Finally, renal toxicity of CdSNPs was confirmed by loss of alkaline phosphatase from the brush border of proximal convoluted tubules. These observations conclude that CdSNPs manifest greater toxicity in kidney than cadmium sulphide bulk particles. These effects have been attributed to their specific physicochemical properties, greater potential to generate ROS and induction of oxidative stress and impairment of renal structure and function. Present observations suggest that commercial/industrial use of CdSNPs may pose serious renal health problems in man.

1. Introduction

Kidney is a critical organ in cadmium (Cd) poisoning. A number of studies have demonstrated that cadmium induced renal damage is characterized by proximal tubular reabsorption dysfunction (Friberg, 1950; Jarup, 2002). Other manifestations of Cd-induced renal damage include increased urinary excretion of β2-microglobulin and α1-microglobulin, depressed glomerular function (Lauwerys et al., 1974) and metallothionein induction (Nordberg and Nordberg, 1987).Cadmium is poorly excreted and has a long biological half life. Toxicity and carcinogenicity of cadmium salts and their ecological aspects have also been aptly studied (Hiatt and Huff, 1975; IARC, 1993; NTP, 2004). At the cellular level, Cd induces oxidative stress by depleting endogenous antioxidants such as glutathione and is associated with mitochondrial damage, induction of apoptosis and disruption of intracellular calcium signaling. (Rana et al., 1997; Matsuoka and Igisu, 1998; Stohs et al., 2000; Bagchi et al., 2000).

In recent years, cadmium containing nanomaterials have been developed for their application in biology, medicine, engineering and consumer products. (Woodrow, 2010; Esmaeili et al., 2016;

Abbreviations: CdSNPs, cadmium sulphide nanoparticles; CdS, cadmium sulphide; Cd, cadmium; b.w., body weight; CPCSEA, Committee for the purpose of control and supervision of experiments on animals; NPs, nanoparticles; Cd-MT, cadmium metallothionein; μg/g., micrograms per gram; μg MT/ g., microgram metallothionein per gram; Wt%, Weight in percent; RT, room temperature; RH, relative humidity; ROS, reactive oxygen species; H_2O_2, hydrogen peroxide; LPO, lipid peroxidation; GSH, reduced glutathione; TEM, transmission electron microscope; SEM, scanning electron microscope; XRD, X-ray diffraction; JSPDS, Joint Committee on Powder Diffraction Standards; EDAX, Energy Dispersive X- Ray; GL, glomerulus; PT, proximal tubules; EP, epithelium; NEC, necrosis; CP, capsule; VC, vacuole; MT, mitochondria; ER, endoplasmic reticulum; RER, rough endoplasmic reticulum; LY, lysosome; NU, nucleus.

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Morassaei et al., 2016; Shakouri-Arani and Salvati-Niasari, 2014; Emadi et al., 2017; Zinatloo-Ajabshir et al., 2017).The evolution and commercial use of these cadmium containing nanoparticles (NPs) have started posing new challenges before toxicologists. On entering human body, through different routes they may be transported to secondary major targets of interaction. The kidneys are particularly susceptible to them owing not only to its high blood supply but due to its ability to concentrate toxins. A comparative study between the toxicity of NPs with their microsize counterparts confirms higher toxic potential of nanoparticles (NPs) (Donaldson et al., 2002; Jeng and Swanson, 2006; Lewinski et al., 2008). This effect has been attributed to their small size, large surface area and high reactivity. Due to these properties, they are capable of producing higher amount of reactive oxygen species (ROS) (Moller et al., 2010) and severe toxicity.

Effective tissue distribution after administration of NPs has been shown for lung, vascular system and lymph (Dahan et al., 2000; Akerman et al., 2002). However, studies on the impact of NPs in kidney has attracted little attention yet glomerular structure and tubular cells are very likely to be exposed to NPs. Wang et al. (2008) reported glomerulitis and degeneration of proximal convoluted tubular cells caused by oral administration of titanium dioxide NPs. Although morphological and cytohistochemical effects of cadmium doped silica nanoparticles have recently been studied by Coccini et al. (2013), renal effects of cadmium sulphide nanoparticles (CdSNPs) remain to be studied.

The aim of the present investigations was to determine the nephrotoxicity of CdSNPs in rat employing kidney function tests, study on its bioaccumulation in renal tissue, metallothionein inducing property, effect on lipid peroxidation and oxidative stress. Histopathological, ultrastructural and histochemical studies have also been made to gather support, if any, for above mentioned observations.

2. Materials and methods

2.1. Chemicals and reagents

Cadmium Sulphide nanoparticles were procured from a commercial supplier M/S Nanobeach, Delhi (India) who claimed that their preparation falls under the nanosize category. Cadmium sulphide was supplied by Hi-Media (Mumbai). Thiobarbituric acid, 1, 1, 3 tetramethoxy propane, bovine serum albumin, 5′, 5' dithiobis-2-nitrobenzoic acid, osmium tetraoxide, and glutraldehyde were purchased from sigma chemical company (U.S.A). Other chemicals/reagents were procured from Hi-media (Mumbai).

2.2. Characterization of nanoparticles

The size and shape of CdSNPs were confirmed using transmission electron microscope at Sophisticated Analytical Instrument Center of Punjab University Chandigarh. Scanning electron microscopy observations and Energy Dispersive X- Ray analysis (EDAX) were made at Department of Physics, Ch. Charan Singh University, Meerut. Size distribution, zeta potential and XRD analysis of CdSNPs were done at Indian Institute of technology, Roorkee. Mean crystallite size of the nanoparticles was calculated following Scherrer's formula $D = K\lambda/(\beta \cos \theta)$.

Where D = crystallite size, K= Shape factor (0.9), λ (X-ray wavelength) = 1.54 Å or (1.54×10^{-10} nm), β (full width half maxima) = 0.864, θ (angle of diffraction) = 12.97 (2 θ = 25.95).

2.3. Animals and treatments

Due permission from Institutional Ethical Committee was sought before making these investigations, CPCSEA guidelines for laboratory animal care and maintenance were followed.

Male Wistar rats (150 g ± 30 g) were procured from animal facility of Jamia Hamdard University, Delhi. They were housed individually in polypropylene cages under standard laboratory conditions (RT 25 °C ± 5 °C and RH 50% ± 10% and 12 h dark/light cycle). Each rat was allowed free access to food pellets (Golden Feeds, Delhi) and tap water.

Rats were divided into three groups each containing five rats. Rats of group A were administered a predetermined sublethal dose of CdSNPs (10 mg/kg b.w.) mixed in saline through gavage on each alternate day for 45 days. Similarly rats of group B were treated with predetermined sublethal dose of CdS microparticles (10 mg/kg b.w.) mixed in saline on each alternate day for 45 days.

Whereas rats of group C were administered saline only through gavage on each alternate day for 45 days to serve as control. No mortality occurred during these investigations. Record of their body weight gain or loss was maintained.

2.4. Renal function tests

2.4.1. Creatinine

On completion of treatments, urine samples were collected from each rat through metabolic cages. The specific gravity was determined using urinometer (Atago, Japan). Creatinine was estimated applying alkaline picrate method of Toro and Ackerman (1975), using a commercial kit supplied by excel diagnostics Pvt. Ltd. Hyderabad, (India).

2.4.2. Uric acid

After scheduled treatments rats were sacrificed by light ether anesthesia. Blood samples from each rat were collected directly through cardiac puncture. Serum was separated by centrifugation and used for the estimation of uric acid following the method of Henry et al. (1957) using a commercial kit supplied by excel diagnostics Pvt. Ltd, (India).

2.5. Cadmium concentration in kidney

Small pieces (1 g) of kidney from each rat were collected immediately after sacrifice and digested in 10 ml of concentrated nitric acid at 80 °C for 16 h. After digestion samples were diluted with double distilled water to 100 ml. 2 ml aliquot was subjected to cadmium analysis through atomic absorption spectrophotometer (Electronic Corporation of India, Hyderabad). Hollow cathode lamp for cadmium was used and absorption was recorded at 228.8 nm. Metallic cadmium (Himedia, India) was used as the standard.

2.6. Estimation of metallothionein

Metallothionein concentration in kidney was determined through cadmium saturation method of Onosaka and Cherian (1982). Briefly, kidney samples were perfused with saline, homogenized in 1.15% potassium chloride and centrifuged at 9000 × g. The supernatant was mixed with tris hydrochloride buffer and freshly prepared hemoglobin. Cd-MT (Sigma) was used as the standard. Finally the supernatant was analyzed for Cd through atomic absorption spectrophotometry as described elsewhere (Rana and Kumar, 2000). Validation of these results was also made through ELISA technique as described by Saito et al. (1999).

2.7. Lipid peroxidation

LPO in the kidney samples was determined following the method of Jordan and Schenkman (1982). The formation of

thiobarbituric acid (Sigma) reactive substances was recorded at 532 nm using a spectrophotometer (Systronic, India). 1-1-3 - tetra methoxypropane (Sigma) was used as the standard. Proteins were analyzed following the method of Lowry et al. (1951). Bovine serum albumin (Sigma) was used as the standard.

2.8. Hydrogen peroxide

The basic level of H_2O_2 in kidney homogenates (50% w/v prepared in 0.25 M sucrose) was estimated by ferrithiocynate method as described by Thurman et al. (1972). Presence of H_2O_2 was measured at 480 nm using spectrophotometer (Systronics, India).

2.9. Sulfydryl contents

GSH was measured as acid soluble sulfydryl contents using 5′-5′ dithiobis-2-nitrobenzoic acid (DTNB) as described by Ellman (1959). Absorbance was recorded at 412 nm using a spectrophotometer (Systronics, India).

2.10. Histopathology

Small pieces of kidney were fixed in 10% neutral formalin. After dehydration the samples were embedded in paraffin wax. Six micron thick sections thus prepared and stained with hematoxylin and eosin were examined under a research microscope (Nikon).

2.11. TEM observations

Small sections (1–2 mm^2) of kidney were immersed in 2.5% glutraldehyde, postfixed in 1.0% osmium tetraoxide, dehydrated through a graded series of ethanol and embedded in embedding medium. After several changes in propylene oxide, ultrathin sections stained with uranyl acetate and lead nitrate were observed under a transmission electron microscope (Tecnai, G20-FEI) at sophisticated analytical instrumentation facility, All India Institute of Medical Sciences, New Delhi.

2.12. Histochemical observations on alkaline phosphatase

Small pieces of kidney were fixed overnight at 4 °C in absolute acetone, dehydrated and embedded in paraffin wax. Six micron thick sections prepared from these samples were used for histochemical localization of alkaline phosphatase following calcium cobalt method of Gomori as described by Pearse (1968).

2.13. Statistical analyses

Students't-test was applied to make multiple comparisons amongst different groups. Differences between groups with p values < 0.05 were considered significant.

3. Results

3.1. Characterization of CdSNPs

The physical properties of CdS were determined through a battery of standard methods. These observations show that average size of these particles ranged from 5 to 9 nm. (Fig. 1A). The SEM image indicates the formation of nanoclusters (Fig. 1B). The XRD pattern of CdSNPs showed a hexagonal structure on comparison with the standard data (JSPDS: 00-006-0314) (Fig. 1C). XRD pattern shows that the change in the Full Width Half Maxima (FWHM) and shifting of the 2θ° value. Using the values of FWHM, the crystallite size was calculated applying the Scherrer's formula. It indicated that CdSNPs mean crystallite size was 9 nm. Fig. 2 shows the elemental composition of the sample analyzed by EDAX. EDAX shows peaks of cadmium and sulfide 72.86 wt% and 27.14 wt% proves CdS is free from any impurities. EDAX results also determined the extent of cadmium and sulfide in CdSNPs separately. Zeta potential of CdSNPs was found (− 15.7) mV (Fig. 3A). Intensity-weighed particle sized distribution of CdSNPs is shown in Fig. 3B. SEM, TEM and XRD observations thus confirmed the nanosize of the particles used in this study.

3.2. Biological observations, accumulation of NPs and metallothionein induction

Biological observations showed that treatment of rats with CdS nanoparticles for 45 days expressed no significant effect on their body weight while those treated with cadmium sulphide showed a decrease in body weight of rats (Fig. 4). Results on kidney/body weight relationship showed an increase in cadmium treated rats whereas those treated with CdSNPs exhibited a decline in renosomatic index (Table 1). Cadmium is toxic to several soft tissues but it possesses a strong affinity with renal tissue, therefore, its bioaccumulation in kidney was also determined. It was found that renal concentration of bulk CdS was higher than CdSNPs after 45 days of respective treatments (Table 1). Metallothionein inducing potential of CdSNPs in renal tissue was also determined. Cadmium -metallothionein induction was found to be higher in the kidney of CdSNPs treated rats in comparison to those treated with bulk particles of CdS (Table 1).

3.3. LPO, H_2O_2 and sulfydryl content

LPO is an important mechanism through which cadmium is known to manifest its toxicity. The objective of present study was to know whether LPO plays any role in CdSNPs toxicity. Its measurement in terms of malondialdehyde showed higher values in the kidney of CdSNPs treated rats in comparison to those treated with CdS (Table 1). Thus it was confirmed that LPO plays a pivotal role in renal toxicity of CdSNPs. Subsequent observations on the concentration of hydrogen peroxide (H_2O_2) also showed that it was significantly higher in kidney of CdSNPs treated rats than the CdS treated rats (Table 1). These observations supported the results on malondialdehyde. Results on sulfydryl content supported the observations on LPO. Treatments with CdS and CdSNPs inhibited thiols in the kidney. However, diminution was higher in CdS treated rats than CdSNPs treated rats. Nonetheless, these results confirm that oxidative stress and related mechanisms play a significant role in renal toxicity of CdSNPs.

3.4. Bioindicators of kidney function

Kidney function was also affected by CdSNPs. Results on two parameters viz. creatinine and uric acid showed interesting results. Whereas, creatinine values were higher in CdSNPs treated rats, uric acid was found to be higher in serum of CdS treated rats (Table 2).

3.5. Histopathological observations

These observations comprised changes caused by Cd particles in three basic components of the kidney i.e. glomerulus, tubules and medulla. Pathological changes in glomerulus caused by bulk CdS included glomerulonephritis, glomerular necrosis, shrinkage and degeneration (Fig. 5A). Blurred tubular epithelium, atrophic brush border and partial fragmentation of cells were also observed (Fig. 5B). CdSNPs treatments also caused glomerulonephritis exhibited by degenerative changes, enlargement of glomeruli and

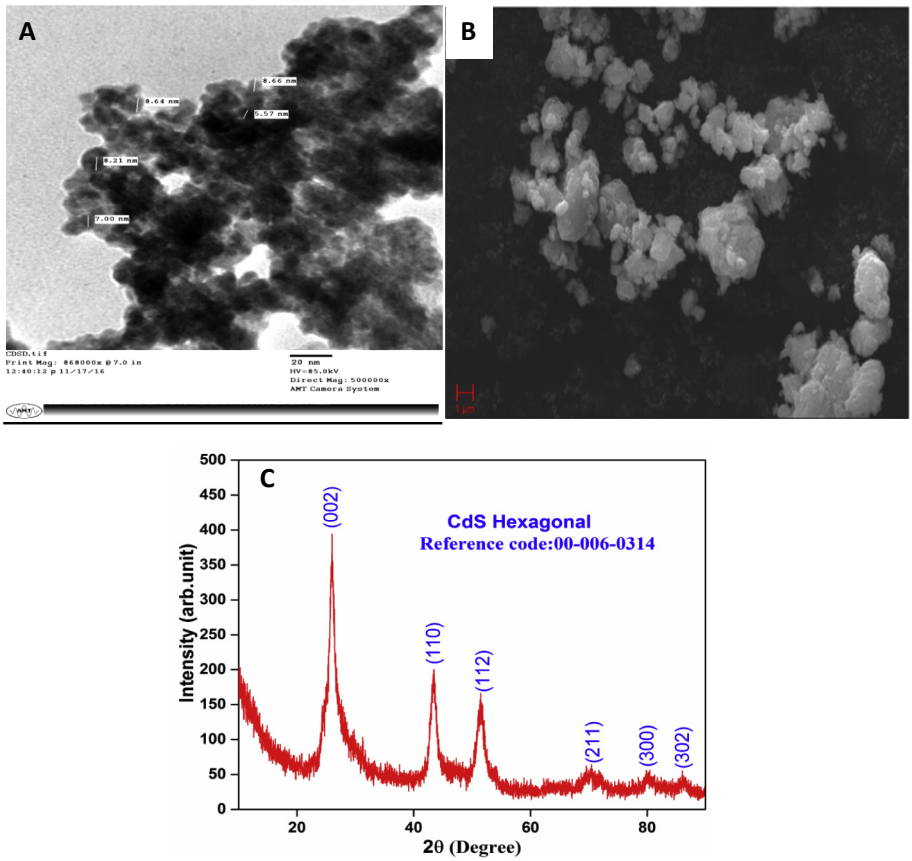


Fig. 1. Characterization of CdS nanoparticles (A) Transmission electron microscopic observations exhibit the size of CdSNPs. (B) Scanning electron microscopy observations of CdS NPs. (C) X- ray powder diffraction (XRD) analysis of CdS NPs.

presence of particle inclusions (Fig. 5C). Proximal tubular necrosis and epithelial degeneration were recorded in both CdS and CdSNPs treated rats (Fig. 5D and E). Medullary changes were mainly in the form of necrosis. While CdS treated rats showed medullary pycnosis, necrosis, apoptosis and sclerosis (Fig. 5F). Only necrosis and apoptosis were observed in the renal medulla of CdSNPs treated rats (Fig. 5F). A comparison of these observations was made with those of a control rat as shown in Fig. 5G, H, I.

3.6. Histochemical observations

Damage to renal components, specially the proximal renal cortex induced by bulk and nanoparticles of Cd were studied by localizing an enzyme biomarker i.e.alkaline phosphatase. In healthy rats, brush border of proximal convoluted tubules exhibited a strong activity for alkaline phosphatase (Fig. 6A). In CdS treated rats a weak enzyme activity was observed in a few proximal tubules (Fig. 6B). Whereas, in CdSNPs treated rats, significant loss of enzyme activity was observed. Tubules below the capsule only showed the presence of alkaline phosphatase activity (Fig. 6C) indicating significant damage to proximal tubules.

3.7. Ultrastructural studies

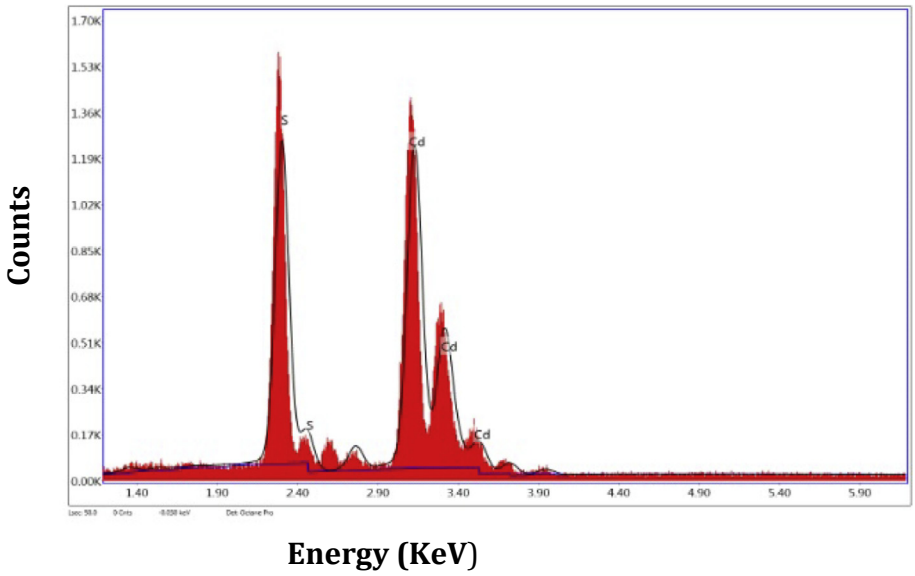
These observations showed the presence of NPs in vesicles. CdSNPs caused changes in nuclear shape and size and mitochondrial shape and size. Fragmentation of chromatin and proliferation of ER were also recorded (Fig. 7A). In the renal cells of CdS treated rats also, these changes were observed but there were no particle inclusions (Fig. 7B). Fig. 7C shows the normal ultrastructure of renal tubule of a control rat.

4. Discussion

Recent upsurge in the usage of nanomaterials/nanoparticles in science, engineering, medicine and consumer products has raised serious human health concerns. The biomedical applications of inorganic nanoparticles make it necessary to assess their toxic potential employing suitable parameters. Common mechanisms of NPs cytotoxicity include their uptake, distribution, bioconcentration, disruption of cellular/ organ function, generation of ROS, oxidative stress and consequent morphological changes in the target organ. During present investigations, renal toxicity of CdSNPs was studied using this general scheme of NPs toxicity.

Since biocompatibility of NPs depends upon their physicochemical properties i.e. diameter, surface charge, surface topograph, surface area and solubility, it was ascertained through TEM, SEM, XRD observations that CdSNPs measure in nano range. The NPs used in present investigation were crystalline and formed agglomerates. Structural characterization confirmed their nano size ranged from 5 to 9 nm. In general, NPs below 5 nm diameter are considered most hazardous due to their strong nuclear penetration capacity. Whereas, NPs bigger than 40 nm in diameter are known to be less toxic (Soenen and De Cuyper, 2009).

Biological observations showed no adverse effects of CdSNPs on the growth of rats whereas, CdS treated rats showed decline in their body weight. Contrarily, reno-somatic index declined in CdSNPs treated rats and increased in CdS treated rats. Similar observations



Element	Wt %	Atomic %	Error %	Net Int.	Net Error %	K Ratio	Z	R	A	F
S K	27.14	56.63	6.41	498.41	0.94	0.1907	1.2202	0.8761	0.5614	1.0258
Cd L	72.86	43.37	4.07	563.04	1.50	0.5772	0.9186	1.0670	0.8508	1.0137

Fig. 2. Energy dispersive X-ray analysis (EDAX) shows the quantification of elemental composition of CdSNPs.

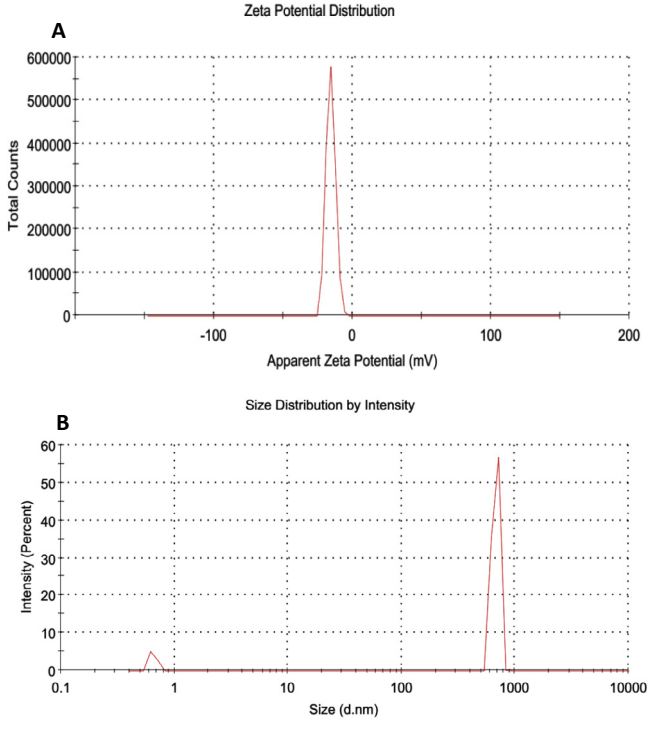


Fig. 3. (A) Zeta potential of CdS NPs (B) Size distribution (by intensity) of CdS NPs.

were made by Papp and Sarkozi (2008) in rats after intratracheal exposure of CdS nanoparticles. The selected dose and duration of exposure/treatment determines the trend in body weight gain and loss. Nonetheless, accumulation of bulk cadmium in kidney was higher than its nanoparticles. Since NPs possess distinct properties than the bulk form of the same material, their bioaccumulation will depend upon their interaction with proteins. Proteins interacting with NPs undergo conformational changes. The immune system may not recognize these proteins as naive but as foreign object and may try to eliminate them as also suggested by Lacerda et al. (2010) and Mahmoudi et al. (2013). Second hypothesis suggests the endocytosis of NPs after binding with serum proteins (Lynch and Dawson, 2008). These differences in uptake and transport mechanisms of NPs and bulk particles determine their bioaccumulation and subsequent cytotoxicity (Lin et al., 2009). Cytotoxicity also depends upon intracellular degradability (Soenen et al., 2010; Lehmann et al., 2010).

Metallothionein induction also becomes important specially when CdSNPs toxicity are under investigation. Cadmium compounds are poorly excreted and have long biological half life. Accumulating mainly in the liver and kidney, cadmium is bound to metellothionein which protects from its toxicity (Waalkes, 2000). Several other reviews on Cd induced metallothionein, their biochemistry and pharmacology are available in literature (Kagi and Schäffer, 1988; Brady, 1991; Kuwahara et al., 2002; Sato and Kondoh, 2002). However, metallothionein induction by NPs is an emerging area of research (Sharma et al., 2013). Our observations show that, bioaccumulation of CdSNPs may be lower than CdS bulk particles but they can induce higher amount of metallothionein in renal tissue. L'Azou et al. (2014) suggested that CdSNPs might induce two metallothionein (MT1) isoforms in renal cells. These metallothioneins offer protection to target molecules against Cd ions. Gene expression studies to be made in future may perhaps reveal dissimilarity of genes, if any, in these metallothioneins

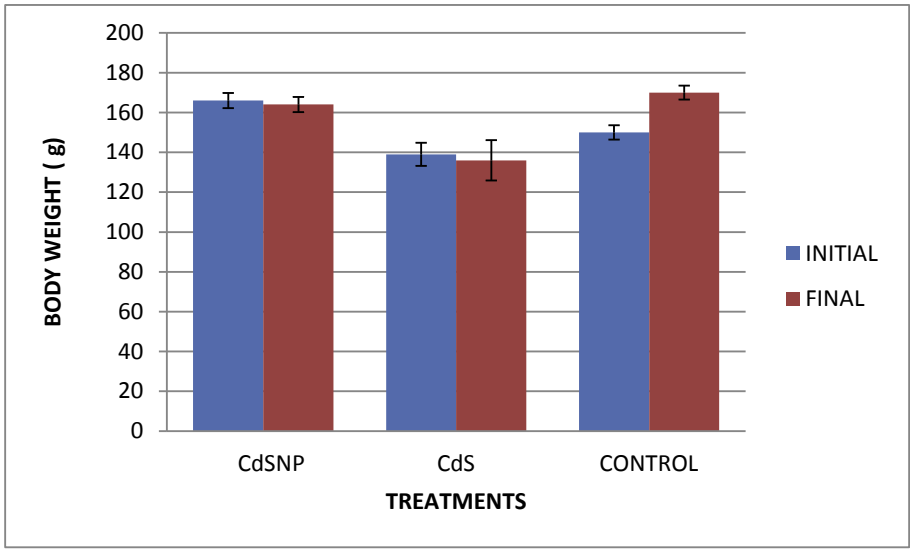


Fig. 4. Body weight of rats treated with CdS and CdSNPs.

Table 1
Bioaccumulation, metallothionein induction, lipid peroxidation and oxidative stress in the kidney of CdSNPs and CdS treated rats.

Treatments	Renosomatic Index	Cadmium Concentration (μg/ g kidney)	MT Concentration (μg MT/ g kidney)	LPO (Malondialdehyde) (n moles MDA/mg protein)	H_2O_2 (ml/ 100 ml)	Sulfydryl content (μg/g wet kidney)
Control	1.3 ± 0.070	0.14 ± 0.014	52.01 ± 1.75	0.050 ± 0.0020	0.05 ± 0.01	0.155 ± 0.01
CdS	1.22 ± 0.140[a]	0.370 ± 0.02[a]	310.56 ± 4.78[a]	0.367 ± 0.037[a]	0.08 ± 0.01[a]	0.09 ± 0.003[NS]
CdSNPs	0.959 ± 0.050[NS]	0.267 ± 0.045[a]	336.241 ± 3.91[a]	0.547 ± 0.077[a]	0.592 ± 0.298[a]	0.104 ± 0.132[a]

Results are expressed as Mean ± S.E (n = 5).
NS– non significant.
[a] Denotes values significantly different from control ($p < 0.05$).

Table 2
Renal function of rats treated with CdSNPs and CdS.

Treatments	Creatinine(g/l)	Uric acid (mg%)
Control	0.526 ± 0.056	0.33 ± 0.080
CdS	0.684 ± 0.1022[a]	2.2 ± 0.230[a]
CdSNPs	0.895 ± 1.055[a]	0.643 ± 0.177[a]

Results are expressed as Mean ± S.E (n = 5).
[a] Denotes values significantly different from control ($p < 0.05$).

induced by CdS and CdSNPs.

Cytotoxicity caused by CdSNPs in kidney was assessed by measuring the generation of radical as well as non radical/ species. CdSNPs were found to be potent inducers of LPO. Infact, generation of ROS was comparatively higher in the kidney of CdSNPs treated rats than CdS treated rats. A series of reports including those from our own laboratory have confirmed that Cd toxicity is manifested through ROS (Gabor et al., 1978; Waalkes et al., 1985; Sunderman, 1986). A number of workers attribute NPs toxicity to ROS (Nel et al., 2006; Xia et al., 2008). The large surface area and reactive nature of molecules provide them massive oxidizing capabilities. Pisanic et al. (2007) postulated that NPs can generate ROS by different mechanisms. (1) ROS are generated directly due to an exposure to an acidic environment (lysosomes), either from the surface of the NPs or from leached ions (Stroh et al., 2004; Jain et al., 2008). (2) NPs can interact with organelles such as mitochondria and generate ROS (Soto et al., 2005). (3) NPs can interact with redox active proteins such as NADPH oxidase. (4) They can interact with cell surface receptors and activate intracellular signaling pathways. As the kinetics of ROS induction might largely differ amongst different NPs, it was important to gather support from observations on cytoplasmic redox status i.e. glutathione levels.

Cd is known to manifest its toxicity due to its strong affinity with SH groups (Stohs et al., 2000; Sharma et al., 2014).We recorded a significant decline in GSH in the kidney of Cd treated rats. Similar observations have been made earlier also in our laboratory (Rana and Singh, 1996). In the kidney of CdSNPs treated rat also, GSH values declined indicating that oxidative stress may be one amongst other mechanisms of its toxicity. In general, NPs are potent inducers of oxidative stress (Donaldson et al., 2002). This hypothesis along with the observations from other workers (Sharma et al., 2014; Akhtar et al., 2012) support present results on GSH.

Another effort was made to determine differences in the patterns of localization of alkaline phosphatase, if, any. In the kidney, brush border of the proximal tubules harbours the enzyme. Exposure to Cd^{2+} injures the brush border resulting into loss of alkaline phosphatase (Rana et al., 1981). This effect has been attributed to its direct effects as enzyme poison and ROS production (Rana and Rastogi, 2000). However, very poor activity of this enzyme and that too in the tubules adjacent to capsule only, confirmed that CdSNPs directly inhibit enzyme activity and induce structural and functional changes in the kidney. Histopathological observations demonstrated differences in the morphology of nephron viz. glomerulus renal cortex as well as medulla of CdS and CdSNPs treated rats. After 45 day of exposure to CdSNPs, glomeruli showed oedematous and hemorrhagic conditions with particle inclusions. Tubular epithelium was fragmented. Medulla exhibited necrotic conditions. However, CdSNPs failed to induce renal tumors in rat.

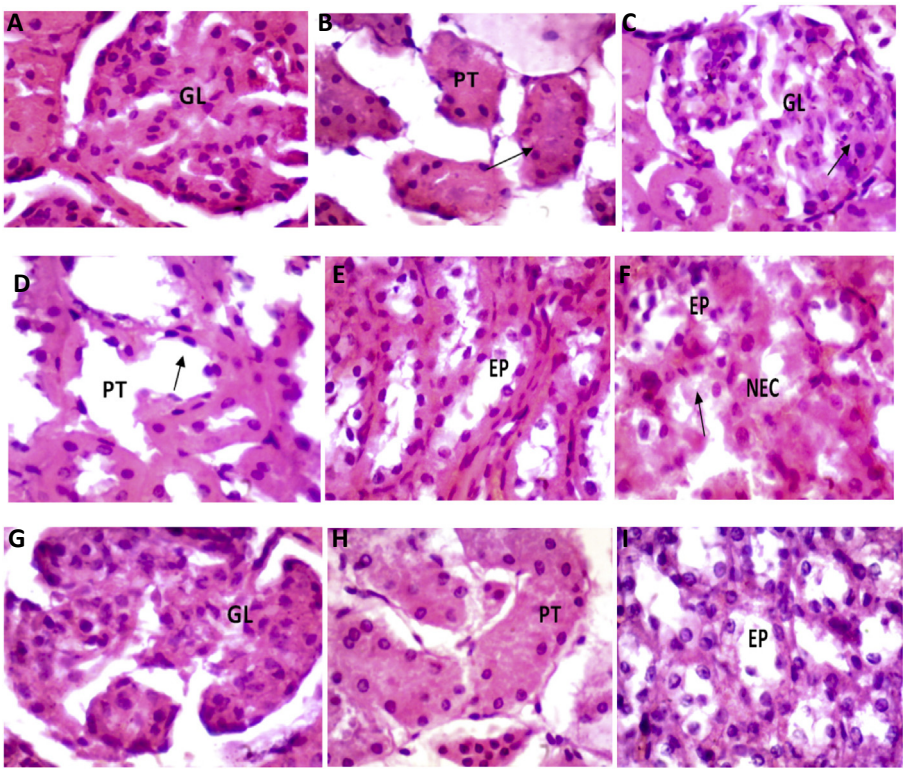


Fig. 5. Histopathological observations. (A) Glomerulus in the kidney of a CdS treated rat shows vacuolar degeneration and nuclear aggregates. ×400. (B) Tubular segregation, blurred epithelium, nuclear disintegration in the cortex of CdS treated rats. ×400. (C) Glomerulus of the kidney of a CdSNPs treated rat shows glomerulonephritis accompanied with particle inclusion. Nuclear changes are also seen. ×400. (D) Fragmented tubular epithelium, loss of brush border and necrotic changes observed in the cortex of CdSNPs treated rat. ×400. (E) Massive tubular damage in the medulla caused by CdS in the kidney of rat. Loss of epithelium and pycnotic nuclei were observed. ×400. (F) Severe injury was caused by CdSNPs in the renal medulla. Loss of tubular integrity and irregular dispersion of nuclei was also seen. ×400. (G) Well organised glomerulus observed in the kidney of control rat.×400. (H) Intact tubular epithelium, well placed nuclei observed in the cortex of the kidney of control rat. ×400.(I) Intact medullary tubules, well placed regular shaped nuclei observed in the medulla of control rat. ×400.

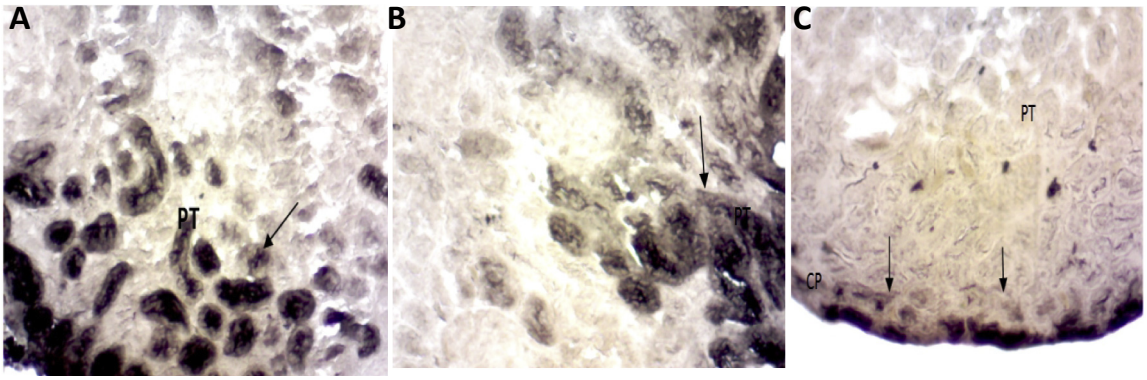


Fig. 6. Histochemical observations. (A) Brush border of renal proximal convoluted tubules exhibit strong activity for alkaline phosphatase in control rat. ×400. (B) Brush border of the proximal convoluted tubules shows a poor activity for alkaline phosphatase in the kidney of CdS treated rat. ×400. (C) Alkaline phosphatase activity is absent in the brush border of proximal convoluted tubules in kidney of CdSNPs treated rat. Tubules below the capsule only exhibited moderate alkaline phosphatase activity. ×400.

Earlier studies by Coccini et al. (2013) on cadmium-doped silica nano particles given intratracheally to rats have also made similar observations. Histopathological changes caused by bulk cadmium in the kidney had been studied earlier also (Brzoska et al., 2003; Wang et al., 2016). A comparison of these results with other reports on other nanoparticles is exhibited by Table 3. Additionally, ultrastructural studies made on the kidney of CdSNPs treated rats showed NPs accumulation in vesicles. Other changes included marginalization of chromatin, proliferation of ER and mitochondrial injury. CdS also caused these changes but there was no proliferation of ER. Literature shows that nanoparticles accumulate in renal cells and cause ultrastructural changes (Pujalte et al., 2011).

Another battery of tests on kidney function i.e. creatinine and uric acid showed contradictory results. Creatinine was found to be higher in the urine samples of CdS treated rats. Earlier reports on kidney function in Cd^{2+} treated rats (ATSDR, 1999; Waalkes, 2003; Brzoska et al., 2003) support present observations. However, biodegradability of CdSNPs, their retention and oxidative stress contributed to renal malfunctions (Pujalte et al., 2011; Sarkar et al., 2014). Though it seems to be a stand-alone study as no comparative

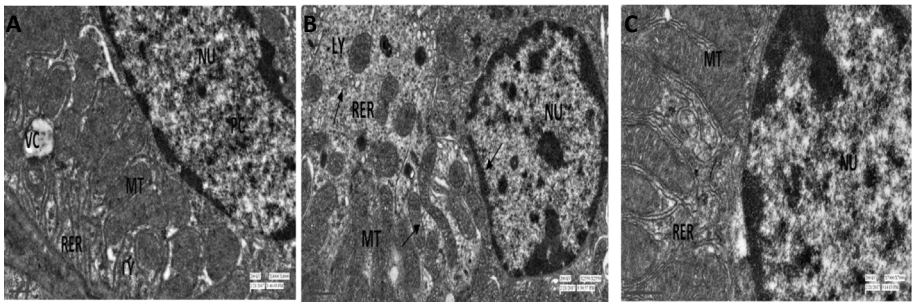


Fig. 7. Electron micrographs of kidney (A) Renal tubule of CdSNPs treated rat shows presence of perichromatin granules in the nucleus and lysosomes and vacuoles formation. RER proliferation, mitochondria of different shapes and sizes are also noticed. ×2550. (B) Renal tubule of a CdS treated rat shows marginalization of chromatin and proximal proliferation. Irregular shaped mitochondria are also observed. ×4000. (C) Renal tubule of a control rat shows no proximal or lysosomal proliferation, no change in shape and size of mitochondria are observed. ×7000.

Table 3
Comparison of results with other published reports.

Effect	Nanoparticles	References
Proximal tubular damage	Copper nanoparticles	Chen et al., 2006
Glomerulonephritis and degeneration of proximal tubular cells	Titanium dioxide nanoparticles	Wang et al., 2008
Morphological changes	Cadmium Doped Silica nanoparticles	Coccini et al., 2013
Glomerulonephritis, glomerular degeneration, atrophic brush border, medullary changes and loss of alkaline phosphatase activity	CdSNPs	Present study

statistics are available on parameters selected for present study on the nephrotoxicity of CdS nanoparticles, it is concluded that CdSNPs are more toxic than bulk Cd. The use of these particles in industry or medicine should be dealt with great care and time. More detailed investigations are underway and future work is expected to unfold the mechanism of CdSNPs toxicity.

In conclusion, it is confirmed that kidney is a target organ of toxicity for nanoparticles. Since nanoparticles differ greatly in the physicochemical properties, generalities with regard to their toxicity can -not be drawn. However, it is most likely that nanoscale particle of a agent will be toxic. Present study offers an understanding of the potential mechanisms of their toxicity. Comparison of observations between CdS and CdSNPs treated rats shows that they can induce greater amount of metallothionein even at a low bioaccumulation. Their ability to generate ROS is higher than CdS. They can induce oxidative stress and cause much severe morphological and functional changes in kidney. Thus toxicity associated with nanoparticles of Cd is different from the bulk cadmium. Further studies on the potential mechanism of nanoparticles toxicity are needed for health risk assessment and identification of suitable markers of exposure. More detailed investigations are underway and future work is expected to unfold the mystery of CdSNPs toxicity.

Conflicts of interest

The authors have no conflict of interest.

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