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Original Article

Biochemical and histopathological evaluation of Al_2O_3 nanomaterials in kidney of Wistar rats

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ABSTRACT

The present study was conducted to evaluate the response of kidneys in Wistar rats following long-term exposure to Al_2O_3 nanomaterials (NMs). To achieve this objective, Al_2O_3 of three different sizes (30 nm, 40 nm and bulk) was orally administered for 28 days to 9 groups of 10 Wistar rats each at the dose of 500, 1000 and 2000 mg/kg/rat. A tenth group of 10 rats received distilled water and served as control. After 28 days of exposure the animals were sacrificed and the serum was collected and tested for the activity levels of creatinine and urea following standard methods. Induction of oxidative stress was also investigated by assessing thiobarbituric acid reactive substances (TBARS) (MDA), protein carbonyl, reduced glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT) activities. A histopathological evaluation was also performed to determine the extent of kidney damage. The results showed that both serum creatinine and serum urea levels increased significantly in the treated rats compared to control animals. The increase was found to be more in Al_2O_3-30 nm treated rats followed by Al_2O_3-40 nm and Al_2O_3-bulk treated rats in a dose-dependent manner. Further administration of Al_2O_3 significantly increased the activities of TBARS, protein carbonyl, catalase and decreased the activities of GSH and SOD in a dose-dependent manner in the kidney of rats compared with the control group. Histopathological evaluation showed significant morphological alterations in kidney tissues of treated rats in accordance with biochemical parameters. Taken together, the results of this study demonstrate that Al_2O_3 is nephrotoxic and its toxicity may be mediated through oxidative stress. Further, the results suggest that prolonged oral exposure to Al_2O_3 NMs has the potential to cause biochemical and histological alterations in kidney of rats at high concentration.

KEYWORDS: Al_2O_3, serum creatinine, serum urea, TBARS, protein carbonyl, GSH, SOD, CAT, histopathology, Wistar rats.

INTRODUCTION

Aluminium (Al) is ubiquitous in the environment. It makes up close to 8% of the Earth's crust by weight. It is released into the environment either naturally through weathering and erosion processes or from various anthropogenic sources. This element has a myriad of uses in daily life throughout the world and in particular in developing countries. It is alloyed with other metals and used in many industries including ship building, electrical building and motor vehicle industries. Exposure to aluminium is almost inevitable. Humans are frequently exposed to aluminium by the inhalation of ambient air and the ingestion of food and water [1]. Consumption of processed food and water purified using Al-containing additives is the main route for this metal to enter the human body [2]. Another source of exposure is the use of aluminium-containing

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compounds such as antiperspirants, cosmetics, internal analgesics, anti-ulcerative medications, astringents and antacids [3]. Industrial waste water and particulate matters emitted from cement-producing factories also contain high amount of Al which results in environmental pollution [4].

Aluminium has the potential to be toxic for both humans and animals. It was included in the priority list of hazardous substances identified by the Agency for Toxic substances and Disease Registry [5]. It accumulates in various mammalian tissues such as the kidneys, liver, heart, brain and is related to cardiotoxicity, nephrotoxicity, neurotoxicity and hepatic dysfuntions [6].

Nanotechnology involves the creation and manipulation of materials at nanoscale levels to create products that exhibit novel properties. Nanotechnology uses engineered materials or devices at the nanometer scale typically ranging from 1 to 100 nm. Various nanotechnology applications have been used for treatment, diagnosis, monitoring and controlling of biological systems. Their small size facilitate cellular uptake and transcytosis across epithelial and endothelial cells into the blood and lymph circulations to reach potentially sensitive target sites such as brain, bone marrow, lymph nodes, spleen and heart. Humans may be exposed to nanoparticles via several possible routes including inhalation, dermal absorption and gastrointestinal absorption [7]. Due to their unique properties such as small size and corresponding large specific area, nanomaterials may impose biological effects different from their micro-scale material counterparts. Although nanomaterials are currently being used in modern technology, there is a serious lack of information concerning the human health and environmental implications of manufactured nanomaterials. The major toxicological concern is the fact that some of the manufactured nanomaterials are redox active and some particles transport across cell membranes, especially into mitochondria.

Alumina is among the most abundantly produced chemical in nanosized particles, estimated to account for approximately 20% of the 2005 world market of nanoparticles. Aluminium oxide (Al_2O_3) nanoparticles have been applied in catalysis, structural ceramics for reinforcements, polymer modification, functionalization of textiles, heat transfer fluids and wastewater treatment. In addition Al_2O_3 nanoparticles have shown wide biological applications in biosensors, biofiltration, drug delivery and antigen delivery for immunization purposes.

The kidneys are responsible for the removal of metabolic waste such as urea and ammonia. However, it is also believed that the other waste products and toxic substances such as NPs (Nanoparticles) could also be excreted through urine [8]. Despite their relatively small size, the kidneys receive approximately 20% of the entire cardiac output making the organ highly susceptible to xenobiotics such as NPs [9]. Since the major function of the kidneys is to eliminate a variety of potentially harmful substances including the excretion of NPs, this organ is an extremely important target for investigation with regard to nanoparticle exposure and hazard [10]. Further, the kidney tissue happens to be vulnerable to the nephrotoxic effects of aluminium [11, 12]. Exposure to aluminium causes changes in renal function [13-16]. The assessment of harmful effects of aluminium is realized by measuring biochemical parameters [17]. Further, creatinine and urea are significant indicators of renal function [18, 19]. Kidneys have a very active oxidative metabolism that results in reactive oxygen species (ROS) generation which can damage major cellular components. Aluminium is known to induce toxic effect due to its ability to transfer electrons producing free radicals in the body. The free radicals affect the cell integrity producing the peroxidation of the lipids in the intracellular membranes and cross linking with the macromolecules in the membranes. Aluminium is also known to affect the permeability of cellular membranes of subcellular organelles, the structure and functions of proteins and the structure of nucleic acids [20]. Hence the present study was undertaken to assess the function of kidney of Wistar rats administered with Al_2O_3 nanoparticles by measuring creatinine & urea levels in the serum sample. The induction of oxidative stress was also investigated by assessing reduced glutathione, lipid peroxidation, protein carbonyl, superoxide dismutase and catalase activities followed by histopathological study of the kidney tissue.

MATERIALS AND METHODS

Al_2O_3-30 nm and Al_2O_3-40 nm both with a purity of over 90% were purchased from Nano technologies, Austin, Texas, USA. Al_2O_3 bulk with a purity of 90%

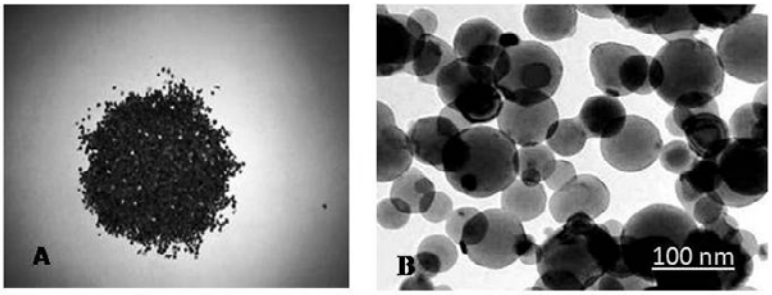

Figure 1. A: Bulk aluminium oxide crystals **B**: Transmission electron microscope (TEM) image of aluminium oxide nanoparticles (Al_2O_3).

was purchased from Sigma Aldrich, St. Louis, MO (Figure 1). All the other chemicals used in the present study were of analytical grade and were purchased from Sigma Aldrich (St. Louis. MO, USA).

Treatment of animals

Six to eight weeks old male Wistar (Albino) rats weighing about 100–120 g were purchased from National Institute of Nutrition, (Hyderabad, India). The animals were fed on commercial pellet diet and water *ad-libitum* and allowed to acclimate to laboratory conditions for a week prior to experimentation. The animals were divided into 10 groups of 10 animals each (9 experimental and a control group). Al_2O_3 NMs were diluted with de-ionized water and administered to the rats orally by gastric intubation. The groups (1-9) considered as treatment groups were administered Al_2O_3-30 nm, Al_2O_3-40 nm and Al_2O_3-bulk (101.96) and the tenth group (10) animals considered as controls were given de-ionized water. The groups (1-9) were given 500, 1000 and 2000 mg/kg bodyweight/day of Al_2O_3-30 nm, Al_2O_3-40 nm and Al_2O_3-bulk for 28 days following the Organization for Economic co-operation and Development (OECD) guidelines – 407 [21]. The doses were selected based on the preliminary acute oral toxicity of Al_2O_3-30 nm, Al_2O_3-40 nm and Al_2O_3- bulk. The LD_{50} of these compounds was > 2000 mg/kg bw (unpublished data). The treated and control rats were maintained at 22–25 °C with a relative humidity of 30–70% and 12 hours light and 12 hours darkness. The experiments were conducted in strict accordance with directive 86/609/EEC on the protection of laboratory animals. Institutional animal ethics committee approved the study. The treated animals were observed daily for any behavioral patterns whereas the feed consumption and body weight were monitored weekly for 4 weeks. All the treated rats were sacrificed by cervical dislocation after 24 h of last administration of a dose. Blood samples were collected immediately from the ventricle of the sacrificed animals by using a disposable syringe. Serum was separated and used for the estimation of creatinine and urea. Simultaneously the kidney tissue was removed and rinsed with saline solution (0.9% NaCl). For evaluation of oxidative stress the kidney tissue was homogenized in suitable buffers: in 0.1 M phosphate buffer (pH 7.1) for superoxide dismutase (SOD), catalase (CAT) and reduced glutathione (GSH); in phosphate buffer saline (pH 7.4) for protein carbonyl and in 1.15% Kcl for thiobarbituric acid reactive substances (TBARS). Some portions of the kidney were fixed in 10% formalin for histological study.

Biochemical parameters

Serum samples collected from both control and treated rats were used for the estimation of creatinine and urea levels. Superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH) thiobarbituric acid (TBARS) and protein carbonyl levels were analysed in the kidney tissue.

Serum creatinine

Serum creatinine was determined using the alkaline picrate method (Jaffe's Method) [22] where creatinine reacts with picric acid in an alkaline medium to give an orange coloured complex which shows maximum absorbance at 520 nm. The intensity of the color is proportional to the concentration of the creatinine present in the serum sample and the results are expressed as mg/dl.

$$\text{Creatinine} + \text{picric acid} \xrightarrow[\textit{alkaline medium}]{\textit{NaOH}} \underset{\text{orange coloured complex}}{\text{creatinine picrate}}$$

In brief, to 0.5 ml of serum sample taken in a centrifuge tube, 1.5 ml of water, 1 ml of 10% sodium tungstate and 1 ml of 2/3 N sulfuric acid were added, mixed and centrifuged at 2500 rpm for 10 min to obtain a clear supernatant. This process precipitates the proteins from the sample. 1 ml of the supernatant was transferred into another set of labelled test tubes containing equal quantity of (0.75 N) sodium hydroxide and 1% picric acid. The contents were mixed well and incubated at room temperature for 15 minutes for the development of color. The intensity of the orange colour developed was measured at 520 nm using the Hitachi spectrophotometer against the reagent blank prepared by adding 3 ml of distilled water to 1 ml of sodium hydroxide and 1 ml of picric acid. The concentration of creatinine in the samples was determined using the standard curve prepared by taking known concentrations of creatinine solution ranging from 0.01–2 mg/dl and processed simultaneously in the same way as for the serum sample.

Serum urea

Serum urea was determined using the diacetylmonoxime method [23], wherein urea reacts with hot acidic diacetylmonoxime in the presence of thiosemicarbazide to produce red colored complex with maxima at 525 nm. The intensity of the colour is proportional to the concentration of urea in the sample and the results were expressed as mg/dl.

$$\text{Urea} + \text{Diacetylmonoxime} \xrightarrow[\text{Thiosemicarbazide}]{100^{O}\text{C}} \xrightarrow{\text{red coloured complex}}$$

In brief, to 0.2 ml of serum sample taken in a centrifuge tube, 0.8 ml of water and 1 ml of 10% trichloroacetic acid (TCA) were added, mixed and centrifuged at 2500 rpm for 10 min to obtain a clear supernatant. This process precipitates the proteins from the sample. 0.1 ml of the supernatant was transferred into another set of labeled test tubes containing 3 ml of chromogenic reagent prepared by mixing two parts of reagent I and one part of reagent II immediately before use (Reagent I is acid ferric solution containing 100 ml of 85% conc. phosphoric acid, 300 ml of 95% conc. sulphuric acid, 100 mg of ferric chloride and 600 ml of distilled water. Reagent II is Diacetylmonoxime (DAMO) – thiosemicarbazide (TSC) solution containing 500 mg of DAMO and 10 mg of TSC in 100 ml of distilled water). The contents were mixed well and boiled in a water bath for 5 minutes. After boiling the contents were cooled to room temperature and the intensity of the colour developed was measured at 525 nm against a reagent blank composed of distilled water and chromogenic reagent using a Hitachi spectrophotometer. The concentration of urea in the samples was determined using the standard curve prepared by taking known concentrations of urea solution ranging from 0–150 mg/dl and processed simultaneously in the same way as for the serum sample.

Lipid peroxidation (TBARS)

TBARS in the kidney tissue was estimated following the method of Ohkawa *et al*. [24]. The principle depends on the reaction between thiobarbituric acid and malondialdehyde (MDA), a secondary product of lipid peroxidation at pH 4. A reddish pink colour developed was estimated at 532 nm which indicates the extent of peroxidation. Briefly, to 0.2 ml of tissue homogenate, 1.5 ml of 20% acetic acid, 0.2 ml of 8.1% sodium dodecyl sulphate (SDS) and 1.5 ml of 8% thiobarbituric acid were added. The volume of the mixture was made up to 4 ml with distilled water and then heated at 95 °C in a water bath for 60 minutes. The tubes were cooled to room temperature under running water and the final volume was made to 5 ml in each tube. 5 ml of butanol:pyridine (15:1 ratio) mixture was added and the contents were vortexed thoroughly for 2 min. After centrifugation at 3000 rpm for 10 min, the upper organic layer was taken and its absorbance was measured at 532 nm against an appropriate reagent blank without the sample using an extinction coefficient of 1.56×10^5 $MLcm^{-1}$. The extent of lipid peroxidation was expressed as n mol/gm protein.

Protein carbonyl

The protein carbonyl levels were determined following the method of Levine *et al*. [25]. In brief the tissue homogenate prepared in phosphate buffered saline

containing 10 mM sodium phosphate, pH 7.4, and 0.14 M NaCl was centrifuged twice for 10 min at 14000 rpm to eliminate all the particulate matter. The diluted proteins were then precipitated with 20% TCA and collected by centrifugation for 5 min. A solution of 10 mM 2,4-Dinitrophenylhydrazine (DNPH) in 2N HCl was added to the protein pellet of the sample to give a final protein concentration of 1-2 mg/ml with 2N HCl added to the reagent blank. Samples were incubated in the dark at room temperature for 1 hr with vortexing for every 10 min intervals. They were then precipitated with 20% TCA and centrifuged again for 5 min. The supernatant was discarded and the protein pellets were washed once more in 20% TCA and 3 times with 1 ml of ethanol/ethyl acetate (1:1 ratio) to remove any free DNPH. Samples were then suspended in 6 M guanidine hydrochloride (dissolved in 2N HCl or in 20 mM phosphate buffer, pH 2.3) at 37 °C for 15 min with vortex mixing. Carbonyl content was measured at 366 nm using a molar extinction coefficient of 22,000 $M^{-1}cm^{-1}$. The activity of protein carbonyl was expressed as n moles/mg protein.

Reduced glutathione (GSH)

GSH activity was estimated by the method of Ellman [26]. The tissue homogenate prepared in 0.1 M phosphate buffer, pH 7.4, was taken and added with equal volume of 20% trichloroacetic acid (TCA) containing 1 mM Ethylenediamine tetraacetic acid (EDTA to precipitate the tissue proteins. The mixture was allowed to stand for 5 min and centrifuged at 2000 rpm for 10 min. An aliquot of 50 µl tissue supernatant was mixed with 1.7 ml of disodium hydrogen phosphate solution (0.3 M). The final volume of 2 ml was made by adding 250 µl of Ellman's reagent (DTNB reagent – 4 mg of 5, 5-dithiobis (2-nitro benzoic acid) in 10 ml of 1% (w/v) sodium citrate. The absorbance of the sample was measured at 412 nm against a reagent blank. GSH solution of known concentrations (10–50 µg) was processed simultaneously to prepare a standard curve. The amount of GSH in the sample was compared with the standard curve and was expressed as µ mole/mg protein.

Superoxide dismutase (SOD)

The activity of SOD was assayed by the method of Kakkar *et al.* [27] based on the oxidation of epinephrine-adrenochrome transition by enzyme. Briefly, in a test tube about 0.5 ml of tissue supernatant was taken and to this 1.5 ml of carbonate buffer (pH 10.2), 0.5 ml of EDTA and 0.4 µl of epinephrine were added. The contents were mixed well and the absorbance was measured at 480 nm. Epinephrine was added just before taking the optical density (O.D). Change in O.D. (per minute) at 50% inhibition to adrenochrome transition by the enzyme was taken as one unit. The enzyme activity was expressed in terms of units/min/mg protein.

Catalase (CAT)

The catalase activity was estimated by the method of Sinha [28]. In brief, the reaction mixture of 1.5 ml taken in a test tube containing 1 ml of phosphate buffer (0.01 M, pH 7.0), 0.1ml of tissue supernatant and 0.4 ml of $2MH_2O_2$ was incubated at 37 °C for 10 minutes. After incubation the reaction was stopped by the addition of 2.0 ml of dichromate-acetic acid reagent (5% potassium dichromate and glacial acetic acid mixed in 1:3 ratio). The control was carried out without the addition of H_2O_2 and the absorbance was read at 620 nm against the control. The enzyme activity was expressed as µ moles/ min/ mg protein.

Histopathological study

Fresh portions of both the kidneys from each rat were cut out rapidly, washed in 1% ice-cold saline solution and fixed in 10% neutral buffered formalin. The fixed tissues were then dehydrated with different grades of ethanol (70%, 80%, 90%, 95% and 100%). Dehydration was then followed by clearing the samples in two changes of xylene. Tissue samples were then impregnated with three changes of molten paraffin wax, then embedded and blocked out. The paraffin blocks were sliced into ribbons of 4 µm thick sections using a Microm HM 360 microtome and mounted on a glass microscope slide. The slides were then stained in Haematoxylin and eosin (H&E) using a Microm HMS-70 stainer and examined under Nikon eclipse E 800 microscope at X400 magnification. A minimum of four random sections per slide and at least four different fields were assessed for histopathological study.

Statistical analysis

The statistical significant changes between treated and control groups were analysed by one-way analysis of variance. All the results were expressed as mean ± S.D. The value of $p < 0.05$ was considered to be statistically significant.

RESULTS

Clinical signs, food intake, body weight and organ weight

Mortality was not observed in rats throughout the experimental period. However towards the end of the experimental period, treated rats showed abnormal behavioral patterns such as sluggishness, dullness, lethargy and irritation. Further the rats showed insignificant decrease in food intake and loss in body weight and organ weight.

Serum creatinine and serum urea

The mean values of creatinine and urea are presented in Figure 2 and 3, respectively. Both creatinine and urea levels increased significantly in the serum

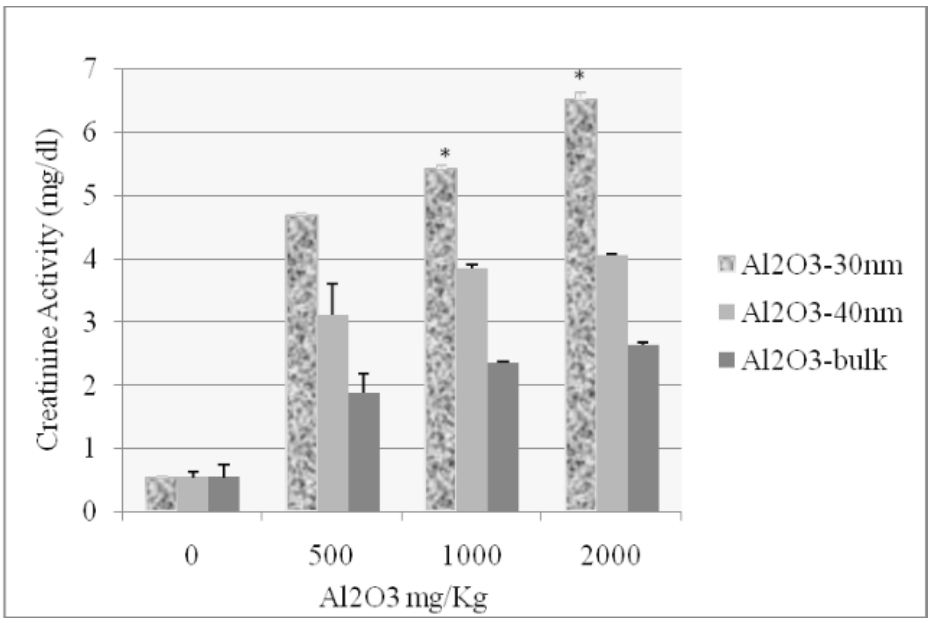

Figure 2. Effect of Al_2O_3 NMs and bulk on creatinine activity in serum of male Wistar rats.

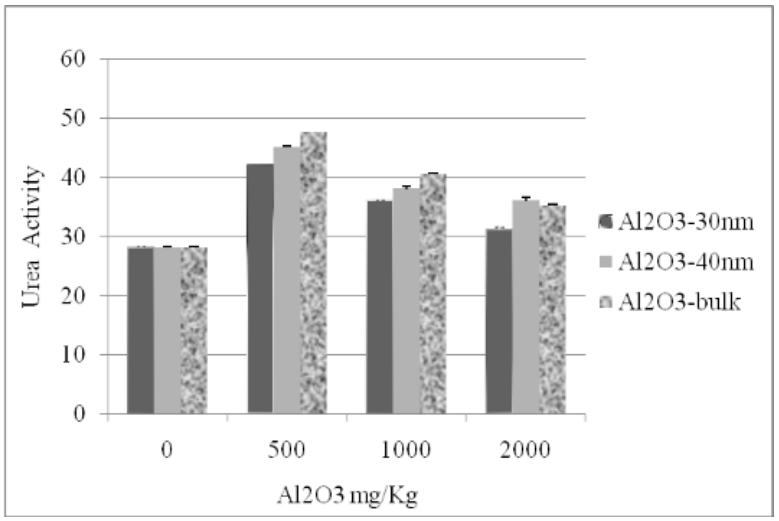

Figure 3. Effect of Al_2O_3 NMs and bulk on urea activity in serum of male Wistar rats.

of Al_2O_3-30 nm, Al_2O_3-40 nm and Al_2O_3-bulk-treated rats when compared to the control group in a dose-dependent manner. It was observed that both creatinine and urea levels increased significantly at 1000 and 2000 mg/kg. However the increase was found to be more in Al_2O_3-30 nm followed by Al_2O_3-40 nm and Al_2O_3-bulk-treated rats.

Lipid peroxidation (TBARS)

Lipid peroxidation assay was performed to determine the TBARS (MDA) levels in the kidney tissue of Al_2O_3-30 nm, Al_2O_3-40 nm and Al_2O_3-bulk-treated rats and results are presented in Figure 4. From the data it is revealed that TBARS levels were elevated significantly in all the treated rats when compared to the control group in a dose-dependent manner with maximum elevation observed at 2000 mg/kg. However significantly higher TBARS levels were seen in Al_2O_3-30 nm and Al_2O_3-40 nm-treated rats when compared with control and bulk.

Protein carbonyl

Figure 5 summarizes the changes in the protein carbonyl. The protein carbonyl was found to be elevated in the kidney tissue of Al_2O_3-30 nm, Al_2O_3-40 nm and Al_2O_3-bulk-treated rats when compared with control group in a dose-dependent manner. However maximum elevation was observed at 2000 mg/kg. Further significant elevation was observed in Al_2O_3-30 nm and Al_2O_3-40 nm-treated rats when compared with control and bulk.

Reduced glutathione (GSH)

Figure 6 summarizes the changes in the reduced glutathione content in the kidney tissue of rats following repeated oral treatment with Al_2O_3-30 nm, Al_2O_3-40 nm and Al_2O_3-bulk. Al_2O_3-NM treatment caused a significant decrease in the GSH content in all the treated rats when compared with control rats in a dose-dependent manner. However Al_2O_3-30 nm and Al_2O_3-40 nm treatment at 2000 mg/kg caused greater depletion in GSH content than the bulk.

Superoxide dismutase (SOD)

The effect of Al_2O_3-30 nm, Al_2O_3-40 nm and Al_2O_3-bulk on SOD activity is presented in Figure 7. The SOD activity was found to be decreased in all the treated animals when compared to control in a dose-dependent manner with significant inhibition being observed at 1000 and 2000 mg/kg. However Al_2O_3 NM treatment caused significant inhibition in the SOD activity than the bulk.

Catalase (CAT)

Figure 8 summarizes the changes in CAT activity in the kidney tissue of Al_2O_3-30 nm, -40 nm and –bulk-treated rats. The CAT activity was found to be elevated in all the treated animals when compared

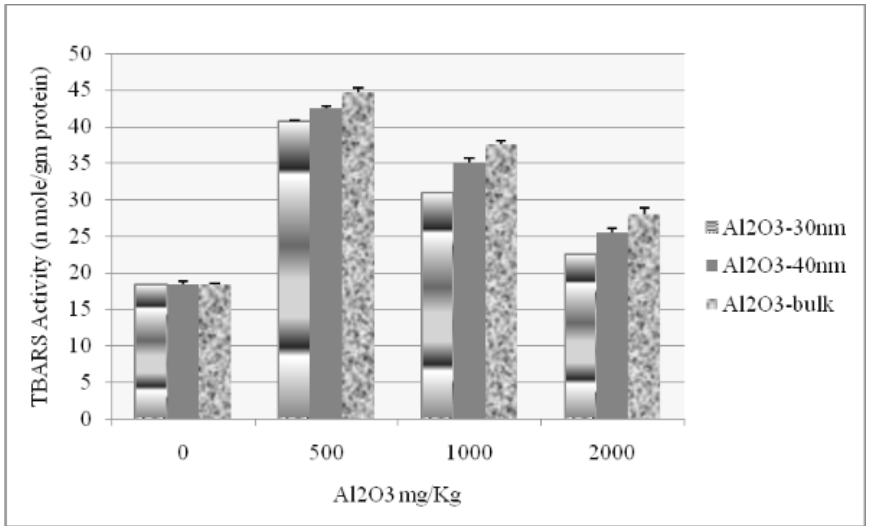

Figure 4. Effect of Al_2O_3 NMs and bulk on TBARS (MDA) activity in the kidney of male Wistar rats.

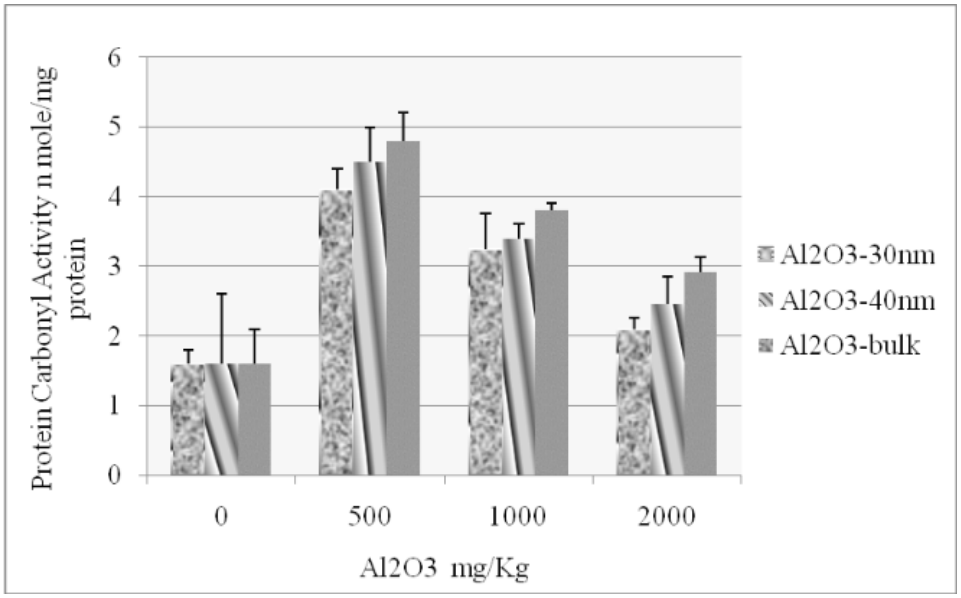

Figure 5. Effect of Al_2O_3 NMs and bulk on protein carbonyl activity in the kidney of male Wistar rats.

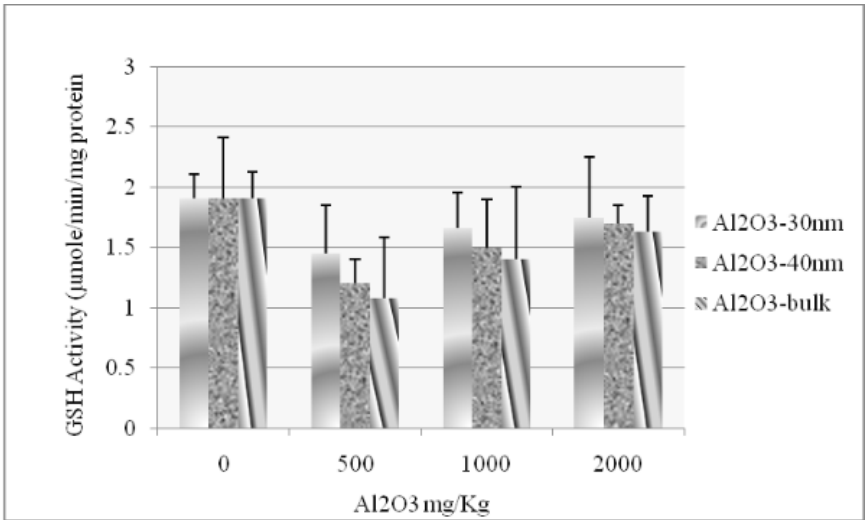

Figure 6. Effect of Al_2O_3 NMs and bulk on reduced glutathione (GSH) activity in the kidney of male Wistar rats.

with control group in a dose-dependent manner showing significant elevation at 1000 and 2000 mg/kg. The increase in CAT activity with Al_2O_3-NMs was higher than the bulk.

Histopathological evaluation

The renal cortex and medulla from all the experimental and control rats were examined with the light microscope. At least 4 fields from each section of cortex and medulla were assessed for the appearance of glomerulus, proximal and distal tubules, interstitial tissue and the collecting tubules and were compared with the control sections taken at the same time and processed by exactly the same method.

The results of the histopathological study are illustrated in Figure 9. The normal histological structure of kidney cortex shows the Bowmann's

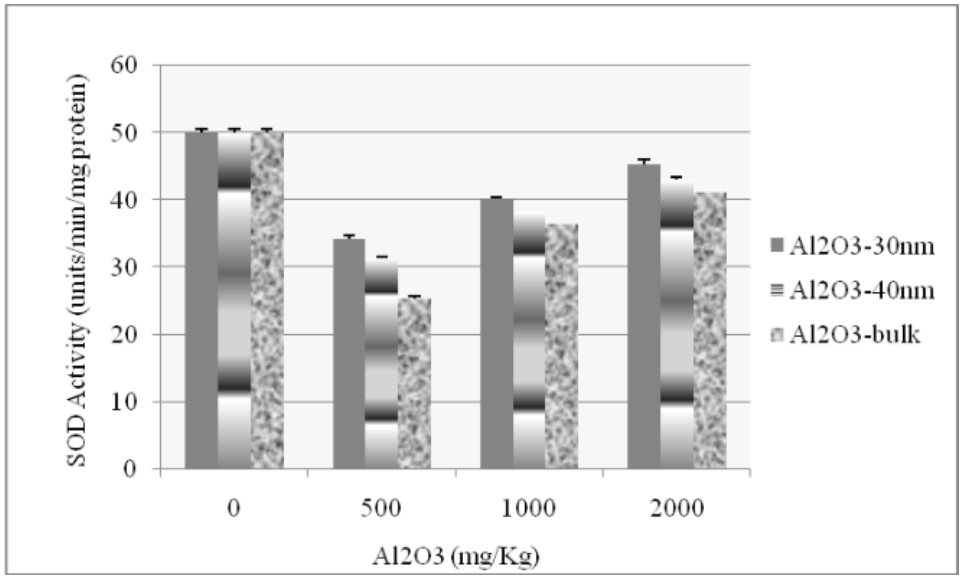

Figure 7. Effect of Al_2O_3 NMs and bulk on superoxide dismutase (SOD) activity in the kidney of male Wistar rats.

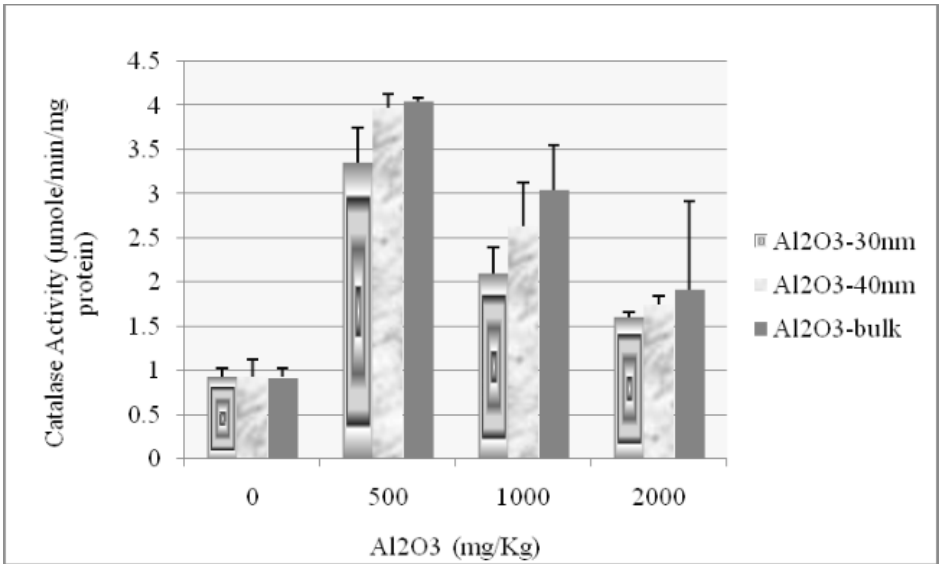

Figure 8. Effect of Al_2O_3 NMs and bulk on catalase (CAT) activity in the kidney of male Wistar rats.

capsules with normal regular glomeruli, proximal and distal tubules with basal prominent nuclei (A) and the kidney medulla shows the collecting tubules with normal distinct and well defined tubular cells (B). The control kidney showed no evidence of glomerular, tubular or interstitial injury. Kidney of Al_2O_3-30 nm, -40 nm and -bulk-treated rats revealed a number of degenerative changes at 2000 mg/kg dose when compared to the other 2 doses. However significant pathological changes were observed in Al_2O_3-30 nm and Al_2O_3-40 nm treated rats than the bulk.

The most prominent changes observed in the treated rats consisted of glomerular degeneration (C), glomerular necrosis with lymphocytic infiltration (D), tubular degeneration (E) tubular necrosis with

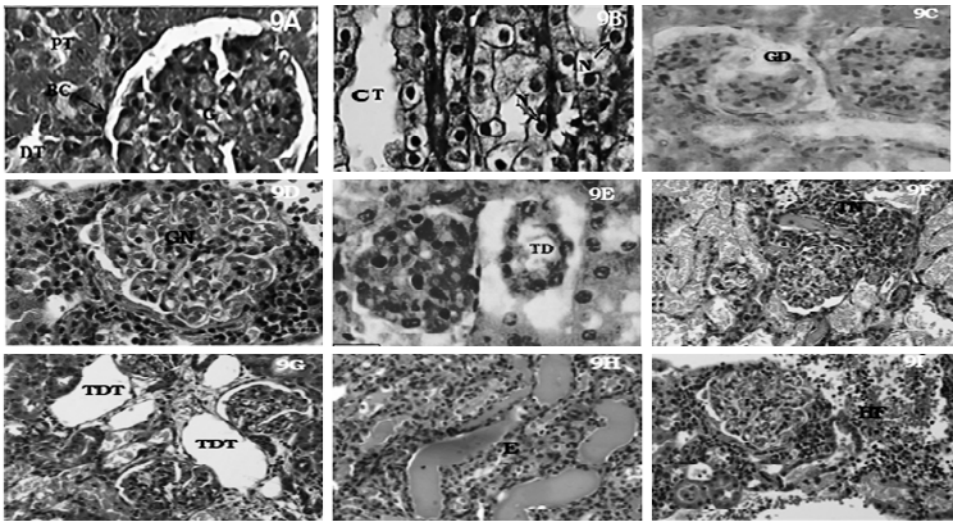

Figure 9. Photomicrographs of kidney histology: **A**: Kidney of control rat showing cortex region, **B**: Kidney of control rat showing medulla region, **C**: Kidney of treated rat showing glomerular degeneration, **D**: Kidney of treated rat showing glomerular necrosis with lymphocytic infiltration, **E**: Kidney of treated rat showing tubular degeneration, **F**: Kidney of treated rat showing tubular necrosis with lymphocytic infiltration, **G**: Kidney of treated rat showing tubular dilatation, **H**: Kidney of treated rat showing degeneration of hematopoietic tissue and eosinophilic exudates. **I**: Kidney of treated rat showing hemorrhagic foci.

lymphocytic infiltration (F), tubular dilatation (G), degeneration of haematopoietic tissue and eosinophilic exudates (H), and hemorrhagic foci (I).

DISCUSSION

Nanoparticles/nanomaterials have been known to enter systemic circulation. Therefore they have the potential to cause organ damage throughout the body. The organs with extensive blood supply such as liver, spleen and kidneys are especially vulnerable. The kidneys play an important role as they are capable of filtering NPs out of the systemic circulation. In doing so, they are frequently exposed to damage. Nanomaterials are known to affect the tissue or disrupt the physiological [29, 30] processes by generation of reactive oxygen species (ROS). The antioxidant defenses comprising the enzymatic and non-enzymatic are particularly essential because they are responsible for the direct removal of free radicals thus providing conferring protection for biological tissues including kidneys. Very few studies have discussed the potential toxic effects of NPs on the renal function. The present study demonstrated that long-term exposure of rats to Al_2O_3-NMs had adverse effects on the antioxidant status of the kidney tissue.

The increase in TBARS (MDA) levels in the kidney tissue in Al_2O_3-30 and -40 nm-treated rats suggest enhanced lipid peroxidation leading to tissue damage and failure of antioxidant mechanisms to prevent the production of excessive free radicals. Lipid peroxidation, an autocatalytic process and a common consequence of cell death may cause peroxidative tissue damage in inflammation, cancer and ageing as suggested by Bhadauria and Nirala [31]. Similarly the protein carbonyl was also found to be elevated in the kidney of NM-treated rats compared to the control group. Reactive oxygen species-mediated reactions lead to the formation of protein carbonyl derivatives which serve as a marker of protein damage. A reactive carbonyipidl compound is the result of oxidative stress and could be an active contributor to pathogenesis [32]. In the present study the reason for increased protein carbonyl level in the kidney tissue might be due to induced oxidative stress due to intoxication of NMs.

The GSH levels were also decreased significantly in the Al_2O_3-NM-treated rats. GSH is important for cell survival. It is present in majority of the cells responsible for hydrophilic conjugation of xenobiotics and probably the most important

protective mechanism for free radical scavenging, and the inhibition of xenobiotics electrophilic attack on cellular macromolecules as well [33].

When excessive ROS are produced, the levels of lipid peroxide (LPO) will rise and GSH levels will decline, which signify that the treated rats suffered severe oxidative stress conditions.

The antioxidant enzymes such as SOD and CAT play a prominent role in antioxidation and elimination of ROS [34]. SOD catalyses the dismutation of superoxide to H_2O_2 whereas CAT decomposes H_2O_2 into water and O_2. In the present study the increase in LPO, protein carbonyl and depletion in the GSH content were accompanied by increase in CAT activity and a decrease in SOD after acute oral treatment with Al_2O_3-NMs and bulk. SOD is considered as the first line of defense against oxygen toxicity owing to its inhibitory effects on oxyradical formation [35]. The inhibition in SOD activity in the present study could be due to high flux of superoxide radicals resulting in H_2O_2 production in cells [36]. High levels of H_2O_2 upregulate CAT activity and downregulate the activity of SOD as suggested by Fernandez-Umusuno *et al*. [36]. Hence the elevation of CAT activity may be related to its role in degradation of resulting H_2O_2.

The present investigation also revealed that administration of Al_2O_3-NMs elevated both serum creatinine and urea in the treated groups compared to control. High levels of serum creatinine and urea in the blood indicate that the kidneys are not functioning properly which could be due to several factors such as kidneys damage or infection, reduced blood flow to the kidneys due to shock heart failure. The results of the biochemical markers were further supported by the histopathological examination of kidney tissue.

CONCLUSION

In conclusion the present study revealed that long-term exposure of male Wistar rats to Al_2O_3-NMs elicited oxidative stress response in the kidneys. The toxic effects of Al_2O_3-NMs are associated with disruption of antioxidant enzyme activities, subsequent increase in lipid peroxidation and progressive tubular, glomerular and interstitial histological alterations in the experimental rats. The present findings thus add to the increasing evidence that exposure of NMs may lead to harmful biological responses. Therefore further studies are required to evaluate the hazards of occupational or environmental exposure to NMs.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

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