Silica nanoparticles-induced cytotoxicity, oxidative stress and apoptosis in cultured A431 and A549 cells

Maqusood Ahamed

Hum Exp Toxicol 2013 32: 186
DOI: 10.1177/0960327112459206

Original Article

Human and Experimental Toxicology
32(2) 186–195

DOI: 10.1177/0960327112459206
het.sagepub.com

Silica nanoparticles-induced cytotoxicity, oxidative stress and apoptosis in cultured A431 and A549 cells

Maqusood Ahamed

Abstract

In medicine, the use of silica nanoparticles (SiO_2 NPs) offers new perspectives in biosensor, drug delivery and cancer therapy. However, questions about potential toxic and deleterious effects of SiO_2 NPs have also been raised. The aim of this study was to investigate the induction of cytotoxicity, oxidative stress and apoptosis by SiO_2 NPs (size 15 nm) in human skin epithelial (A431) and human lung epithelial (A549) cells. SiO_2 NPs (concentration range 25–200 μg/ml) induced dose-dependent cytotoxicity in both types of cells, which was demonstrated by cell viability (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide) and lactate dehydrogenase leakage assays. SiO_2 NPs were also found to induce oxidative stress in a dose-dependent manner, indicated by depletion of glutathione and induction of reactive oxygen species (ROS) generation and lipid peroxidation. Quantitative real-time polymerase chain reaction analysis showed that following the exposure of cells to SiO_2 NPs, the messenger RNA level of apoptotic genes (caspase-3 and caspase-9) were upregulated in a dose-dependent manner. Moreover, activities of caspase-3 and caspase-9 enzymes were also significantly higher in both kinds of cells exposed to SiO_2 NPs. This study suggested that SiO_2 NPs induce cytotoxicity and apoptosis in A431 and A549 cells, which is likely to be mediated through ROS generation and oxidative stress.

Keywords

SiO_2 NPs, A549 cells, A431 cells, apoptosis, ROS

Introduction

Nano-biotechnology provides opportunity for the development of new unique materials in the nanometer size range (1–100 nm) that can be applied to many potential applications in biological science. Silica nanoparticles (SiO_2 NPs) has been considered an ideal material for biomedical applications and is being widely explored as biosensor,[1] biomarker,[2] cancer therapy,[3] DNA/drug delivery[4,5] and enzyme immobilization.[6] Therefore, understanding the toxicity of SiO_2 NPs at the cellular level is crucial for rational design of this material for biomedical applications.

Nanoparticles could easily enter into human body via different routes such as inhalation, ingestion and dermal contact. Inhalation and dermal contact are very common exposure routes by which nanoparticles deposit in lung and skin inducing inflammation, fibrosis and cytotoxicity.[7] To date, several studies provided ample evidence that SiO_2 NPs distributed in blood, lungs, kidneys, spleen, pancreas or other organs.[8–10] In vitro studies have shown decreased cell viability and oxidative stress after exposure to SiO_2 NPs in human lung epithelial (A549) cells, human endothelial (EAHY926) cells, murine macrophages (RAW 264.7), human embryonic kidney (HEK293) cells, rat neuronal (PC12) cells and human liver (HepG2) cells.[11–15] However, little is known about the toxic effects of SiO_2 NPs on human skin cells that represents the first level of exposure to the nanoparticles.

In this study, we investigated the induction of cytotoxicity, oxidative stress and apoptosis by SiO_2

King Abdullah Institute for Nanotechnology, King Saud University, Riyadh, Saudi Arabia

Corresponding author:
Maqusood Ahamed, King Abdullah Institute for Nanotechnology, King Saud University, Riyadh 11451, Saudi Arabia.
Email: maqusood@gmail.com

NPs in human skin epithelial (A431) cells. To avoid cell type-specific response, we also used human lung epithelial (A549) cells for SiO_2 NPs toxicity studies. To achieve this objective, we determined the cytotoxicity by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT; cell viability) and lactate dehydrogenase (LDH) leakage assays in A431 and A549 cells exposed to different concentrations of SiO_2 NPs. We further measured the oxidative stress markers such as reactive oxygen species (ROS), glutathione (GSH) and lipid peroxidation (LPO) in response to SiO_2 NPs exposure. Quantitative real-time polymerase chain reaction (PCR) was employed to see the effects of SiO_2 NPs on the messenger RNA (mRNA) level of apoptosis genes (caspase-3 and caspase-9). Moreover, activities of caspase-3 and caspase-9 enzymes were also examined in both the types of cells against SiO_2 NPs exposure. Both A431, derived from human epidermoid carcinoma, and A549 cells, derived from human lung carcinoma, have been widely utilized in toxicological studies.[16–20]

Materials and methods

Silica nanoparticles

Silica (SiO_2) nanopowder (product no. 4850MR) was purchased from Nanostructured and Amorphous Materials, Inc. (Houston, Texas, USA). Physicochemical characterization of SiO_2 NPs utilized in the present study was reported in our previous publication.[15]

Chemicals

Fetal bovine serum (FBS), Dulbecco's modified Eagle's medium (DMEM), Hank's balanced salt solution (HBSS), penicillin–streptomycin and trypsin were bought from Invitrogen Co. (Carlsbad, California, USA). GSH, MTT, 5,5-dithio-bis-(2-nitrobenzoic acid) (DTNB), 2,7-dichlorofluorescin diacetate (DCFH-DA), thiobarbituric acid (TBA) and trichloroacetic acid (TCA) were purchased from Sigma-Aldrich (St Louis, Missouri, USA). LDH, caspase-3 and caspase-9 enzymes assay kits were obtained from Bio-Vision Inc. (Milpitas, California, USA). All other chemicals used in this study were of the highest purity available from commercial sources.

Cell culture and nanoparticles exposure

Both A431 and A549 cells were obtained from American Type Culture Collection (ATCC) (Manassas, Virginia, USA). Both types of cells were used between passages 10 and 20. Cells were cultured in DMEM supplemented with 10% FBS and 100 U/ml penicillin–streptomycin at 5% CO_2 and 37°C. At 85% confluence, cells were harvested using 0.25% trypsin and were subcultured into 75 cm^2 flasks, six-well plates or 96-well plates according to selection of experiments. Cells were allowed to attach the surface for 24 h prior to nanoparticles treatment. SiO_2 NPs were suspended in cell culture medium and diluted to appropriate concentrations (25–200 μg/ml). The dilutions of SiO_2 NPs were then sonicated using a sonicator bath at room temperature for 10 min at 40 W to avoid SiO_2 NPs agglomeration prior to cells exposure. Selection of SiO_2 NPs dosage (25–200 μg/ml) and exposure time (72 h) was based on a preliminary dose–response study (data not shown).

Cell viability assay

Viability of A431 and A549 cells after exposure to SiO_2 NPs was assessed by MTT assay as described by Ahamed et al.[18] In brief, 10,000 cells/well were seeded in 96-well plates and exposed to different concentrations (25–200 μg/ml) of SiO_2 NPs for 72 h. At the end of exposure, culture medium was removed from each well to avoid interference of SiO_2 NPs and replaced with new medium containing MTT solution in an amount equal to 10% of culture volume and incubated for 3 h at 37°C until a purple colored formazan product developed. The resulting formazan product was dissolved in acidified isopropanol. Furthermore, 96-well plate was centrifuged at 2300*g* for 5 min to settle down the remaining SiO_2 NPs. Then, a 100-μl supernatant was transferred to other fresh well of 96-well plate and absorbance was measured at 570 nm using a microplate reader (Synergy-HT, BioTek, Winooski, VT, USA).

LDH leakage assay

LDH is an enzyme widely present in cytosol, which converts lactate to pyruvate. When plasma membrane integrity is disrupted, LDH leaks into culture media and its extracellular level is elevated.[21] LDH assay was carried out by the LDH-cytotoxicity assay kit (Bio-Vision) according to the manufacturer's protocol. In brief, 10,000 cells/well were seeded in 96-well plates and exposed to different concentrations (25–200 μg/ml) of SiO_2 NPs for 72 h. At the end of exposure, 96-well plate was centrifuged at 2300*g* for 5 min to settle down the SiO_2 NPs present in the

solution. Then, a 100-µl supernatant was transferred to a fresh well of 96-well plate that already contains 100 µl of reaction mixture from Bio-Vision kit and incubated for 30 min at room temperature. After incubation, the absorbance of solution was measured at 340 nm using a microplate reader (Synergy-HT, BioTek). LDH levels in the media versus the cells were quantified and compared with the control values according to the instruction of kit.

Determination of ROS

ROS level was determined in A431 and A549 cells exposed to different concentrations (25–200 µg/ml) of SiO_2 NPs for 72 h. The production of intracellular ROS was measured using DCFH-DA.[22] The DCFH-DA passively enters the cell, where it reacts with ROS to form the highly fluorescent compound dichlorofluorescein. Briefly, 10 mM of DCFH-DA stock solution (in methanol) was diluted in culture medium without serum or other additive to yield a 100 µM working solution. At the end of exposure, cells were washed twice with HBSS. Then cells were incubated in 1 ml working solution of DCFH-DA at 37°C for 30 min. Cells were lysed in alkaline solution and centrifuged at 2300*g* for 5 min. A 200 µl supernatant was transferred to 96-well plate and fluorescence was measured at 485 nm excitation and 520 nm emission using a microplate reader (Synergy-HT, BioTek). The values were expressed as percentage of fluorescence intensity relative to control wells.

Preparation of crude cell extract

For the measurement of LPO, GSH and caspase enzymes, A431 and A549 cells were cultured in 75 cm^2 culture flask and exposed to different concentrations (25–200 µg/ml) of SiO_2 NPs for 72 h. After the treatment, cells were harvested in ice-cold phosphate buffer saline by scraping and washed with phosphate buffer saline at 4°C. The cell pellets were then lysed in cell lysis buffer (1× 20 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1 mM Na_2EDTA, 1% Triton and 2.5 mM sodium pyrophosphate) as described in our previous publication.[15] Following centrifugation (15,000*g* for 10 min at 4°C), the supernatant (cell extract) was maintained on ice.

LPO assay

LPO was estimated by measuring the formation of malondialdehyde (MDA) using the method of Ohkawa et al.[23] MDA is one of the end products of membrane LPO. Briefly, a mixture of 0.1 ml cell extract and 1.9 ml of 0.1 M sodium phosphate buffer (pH 7.4) was incubated at 37°C for 1 h. After incubation, the mixture was precipitated with 5% TCA and centrifuged (2300*g* for 15 min at room temperature) to collect supernatant. Then 1.0 ml of 1% TBA was added to the supernatant and placed in the boiling water for 15 min. After cooling to room temperature, absorbance of the mixture was taken at 532 nm and was converted to MDA and expressed as nanomole per milligram of protein using molar extinction coefficient of $1.56 \times 10^5\ M^{-1}\ cm^{-1}$.

GSH assay

GSH level was quantified using Ellman's method.[24] Briefly, a mixture of 0.1 ml of cell extract and 0.9 ml of 5% TCA was centrifuged (2300*g* for 15 min at 4°C). Then 0.5 ml of supernatant was added to 1.5 ml of 0.01% DTNB and the reaction was monitored at 412 nm. The amount of GSH was expressed as nanomole per milligram protein.

Total RNA isolation and quantitative real-time PCR analysis

Both A431 and A549 cells were cultured in six-well plates and exposed to different concentrations (25–200 µg/ml) of SiO_2 NPs for 72 h. At the end of exposure, total RNA was extracted using Qiagen RNeasy mini Kit (Valencia, California, USA) according to the manufacturer's instructions. Concentration of the extracted RNA was determined using Nanodrop 8000 spectrophotometer (Thermo-Scientific, Wilmington, Delaware, USA), and the integrity of RNA was visualized on a 1% agarose gel using a gel documentation system (Universal Hood II, BioRad, Hercules, California, USA). The first strand of complementary DNA (cDNA) was synthesized from 1 µg of total RNA by reverse transcriptase using M-MLV (Promega, Madison, Wisconsin, USA) and oligo (dT) primers (Promega) according to the manufacturer's protocol. Quantitative real-time PCR was performed by QuantiTect SYBR Green PCR kit (Qiagen) using an ABI PRISM 7900HT Sequence Detection System (Applied Biosystems, Foster City, California, USA). Overall, 2 ml of template cDNA was added to the final volume of 20 µL of reaction mixture. Real-time PCR cycle parameters included 10 min at 95°C followed by 40 cycles involving denaturation at

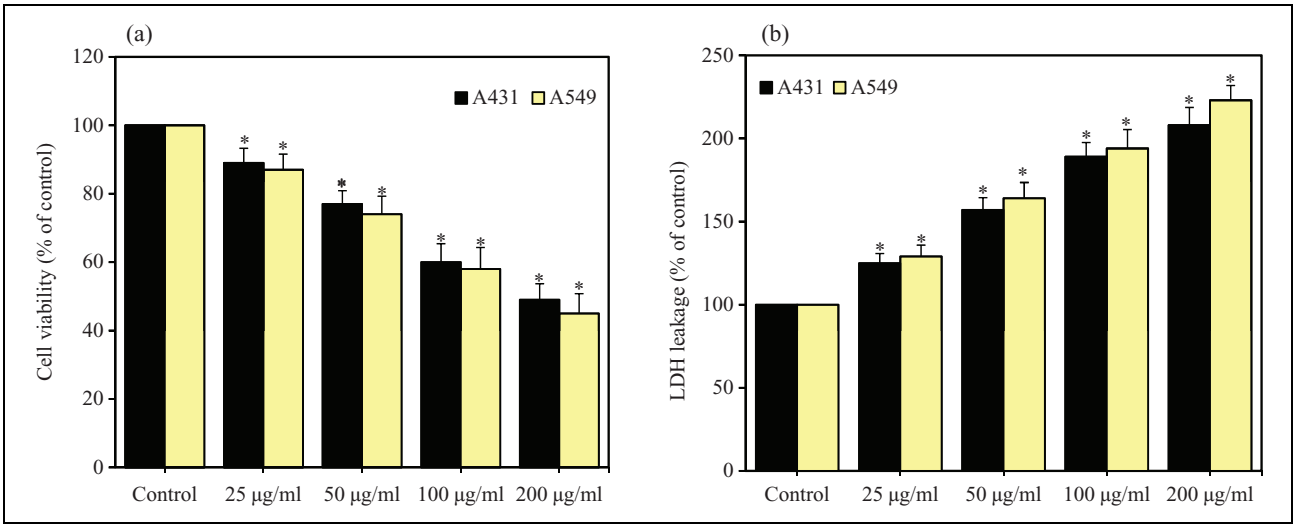

Figure 1. SiO_2 NPs induced cytotoxicity in A431 and A549 cells. Cells were exposed to different concentrations (25–200 µg/ml) of SiO_2 NPs for 72 h. At the end of exposure, cytotoxicity was determined as described in the Materials and Methods section. (a) MTT assay and (b) LDH assay. Data represented are mean ± SD of three identical experiments made in three replicate. *Significant difference compared with the controls ($p < 0.05$ for each). LDH: lactate dehydrogenase; MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide; SiO_2 NPs: silica nanoparticles.

95°C for 15 s, annealing at 60°C for 20 s and elongation at 72°C for 20 s. The sequences of the specific sets of primer for caspase-3, caspase-9 and β-actin used in this study are given in our previous publication.[18] Expressions of selected genes were normalized to the β-actin gene, which was used as an internal housekeeping control. All the real-time PCR experiments were performed in triplicate, and data were expressed as the mean of at least three independent experiments.

Caspase-3 and caspase-9 enzymes assay

Activities of caspase-3 and caspase-9 enzymes were measured using Bio-Vision assay kit. Crude cell extract was prepared as described above. This assay is based on the principle that activated caspases in apoptotic cells cleave the synthetic substrates to release free chromophore *p*-nitroanilide (pNA), which is measured at 405 nm. The pNA was generated after specific action of caspase-3 and caspase-9 on tertrapeptide substrates DEVD-pNA and LEHD-pNA, respectively.[18,25] The reaction mixture consisted of 50 µl of cell extract protein (50 µg), 50 µl of 2× reaction buffer (containing 10 mM dithiothreitol) and 5 µl of 4 mM DEVD-pNA (for caspase-3) or LEHD-pNA (for caspase-9) substrate in a total volume of 105 µl. The reaction mixture was incubated at 37°C for 1 h and absorbance of the product was measured using microplate reader (Synergy-HT, BioTek) at 405 nm according to manufacturer's protocol.

Protein estimation

Protein content was estimated using a bicinchoninic acid protein assay kit (Pierce Biotechnology, Inc., Rockford, Illinois, USA).

Statistical analysis

Data presented in this study are mean ± SD of three identical experiments made in three replicate. Statistical significance was determined by one-way analysis of variance followed by Dunnett's multiple comparison test. Significance was ascribed at $p < 0.05$. All analyses were conducted using the Prism software package (GraphPad Software, Version 5.0, GraphPad Software Inc., San Diego, California, USA).

Results

SiO_2 NP-induced cytotoxicity

Cytotoxicity of SiO_2 NPs in A431 and A549 cells was evaluated by MTT and LDH assays. MTT results showed that cell viability was reduced by SiO_2 NPs and degree of reduction was dose-dependent. Viability of A431 cell was decreased to 89%, 77%, 60% and 49%, while A549 cell viability reduction was 87%, 74%, 58% and 45% at the concentrations of 25, 50, 100 and 200 µg/ml, respectively ($p < 0.05$ for each; Figure 1(a)).

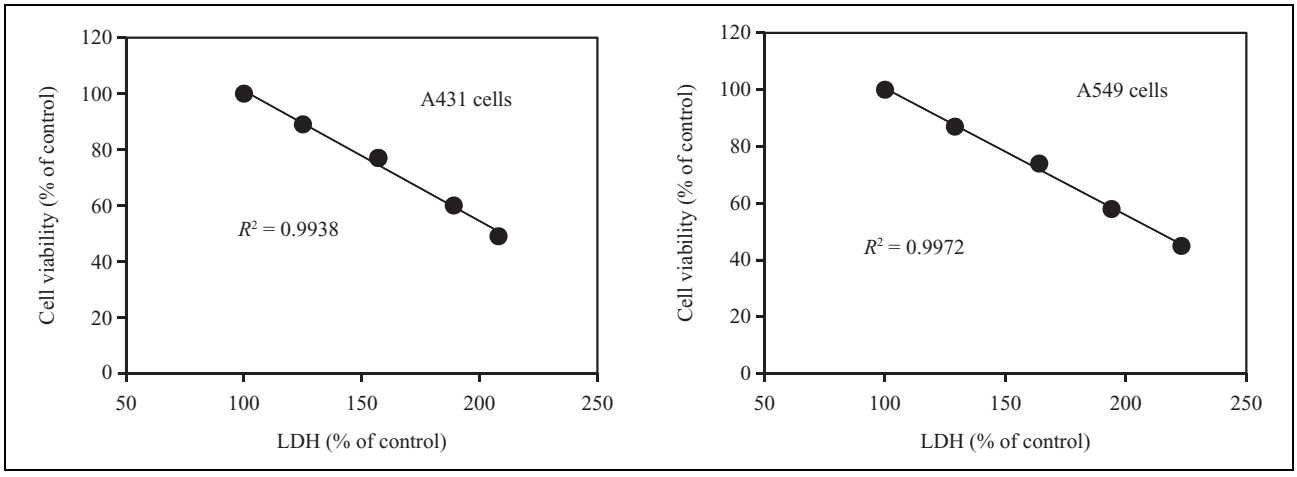

Figure 2. Correlation between cell viability and LDH leakage in A431 and A549 cells after 72 h exposure to 25, 50, 100 and 200 μg/ml of SiO_2 NPs. LDH: lactate dehydrogenase; SiO_2 NPs: silica nanoparticles.

We further observed that SiO_2 NPs-induced a dose-dependent LDH leakage in both the types of cells. LDH leakage in A431 cells was increased to 125%, 157%, 189% and 208%, while in A549 cells LDH leakage was increased to 129%, 164%, 194% and 223% for the concentrations of 25, 50, 100 and 200 μg/ml, respectively ($p < 0.05$ for each; Figure 1(b)). Moreover, a reverse linear correlation was observed between LDH leakage and cell viability in A431 ($R^2 = 0.9938$) and A549 ($R^2 = 0.9972$) cells (Figure 2).

SiO_2 NP-induced oxidative stress

The potential of SiO_2 NPs to induce oxidative stress was examined by measuring the ROS, LPO and GSH levels in A431 and A549 cells. We found that SiO_2 NPs induced intracellular production of ROS in dose-dependent manner ($p < 0.05$ for each; Figure 3(a)). The MDA level, an end product of LPO was significantly higher, while antioxidant GSH level was significantly lower in cells exposed to SiO_2 NPs in the concentration range of 25–200 μg/ml for 72 h (Figure 3(b) and (c)). Furthermore, an inverse linear correlation was observed between ROS and GSH (Figure 4) and a similar negative correlation was observed between ROS and cell viability (Figure 5).

SiO_2 NP-induced apoptosis

It is well known that ROS generation leads to apoptotic cell death. We utilized quantitative real-time PCR to analyze the mRNA levels of two apoptotic genes (caspase-3 and caspase-9) in A431 and A549 cells exposed SiO_2 NPs at concentrations of 25, 50, 100 and 200 μg/ml for 72 h. Results showed that mRNA levels of caspase-3 and caspase-9 genes was upregulated in dose-dependent manner in both the types of cells due to SiO_2 NPs exposure ($p < 0.005$ for each; Figure 6). To support our hypothesis of apoptosis induction by SiO_2 NPs, we further examined the activities of caspase-3 and caspase-9 enzymes at the concentrations of 25, 50, 100 and 200 μg/ml. These results also demonstrated that SiO_2 NPs induced the activities of both the apoptotic enzymes in dose-dependent manner ($p < 0.05$ for each; Figure 7).

Discussion

Physicochemical characterization of nanoparticles is an important aspect of nanotoxicity research. Characterization of present SiO_2 NPs was reported in our previous article.[15] Briefly, SiO_2 NPs were highly pure, amorphous, smooth surface and spherical with an average diameter of 15 nm (transmission electron microscopy (TEM) size). The average hydrodynamic diameter of SiO_2 NPs in complete cell culture medium as determined by dynamic light scattering was around 96 nm. The higher size of SiO_2 NPs in aqueous suspension compared with TEM size might be due to the tendency of particles to agglomerate in aqueous state.[15]

SiO_2 NPs are being considered a potential candidate for biomedical applications because of their unique physicochemical properties. However, recent studies reported that SiO_2 NPs have a potential to induce toxicological effects to human health.[10,26–29] These studies suggested that hazards have not been defined yet and more safety research is necessary.

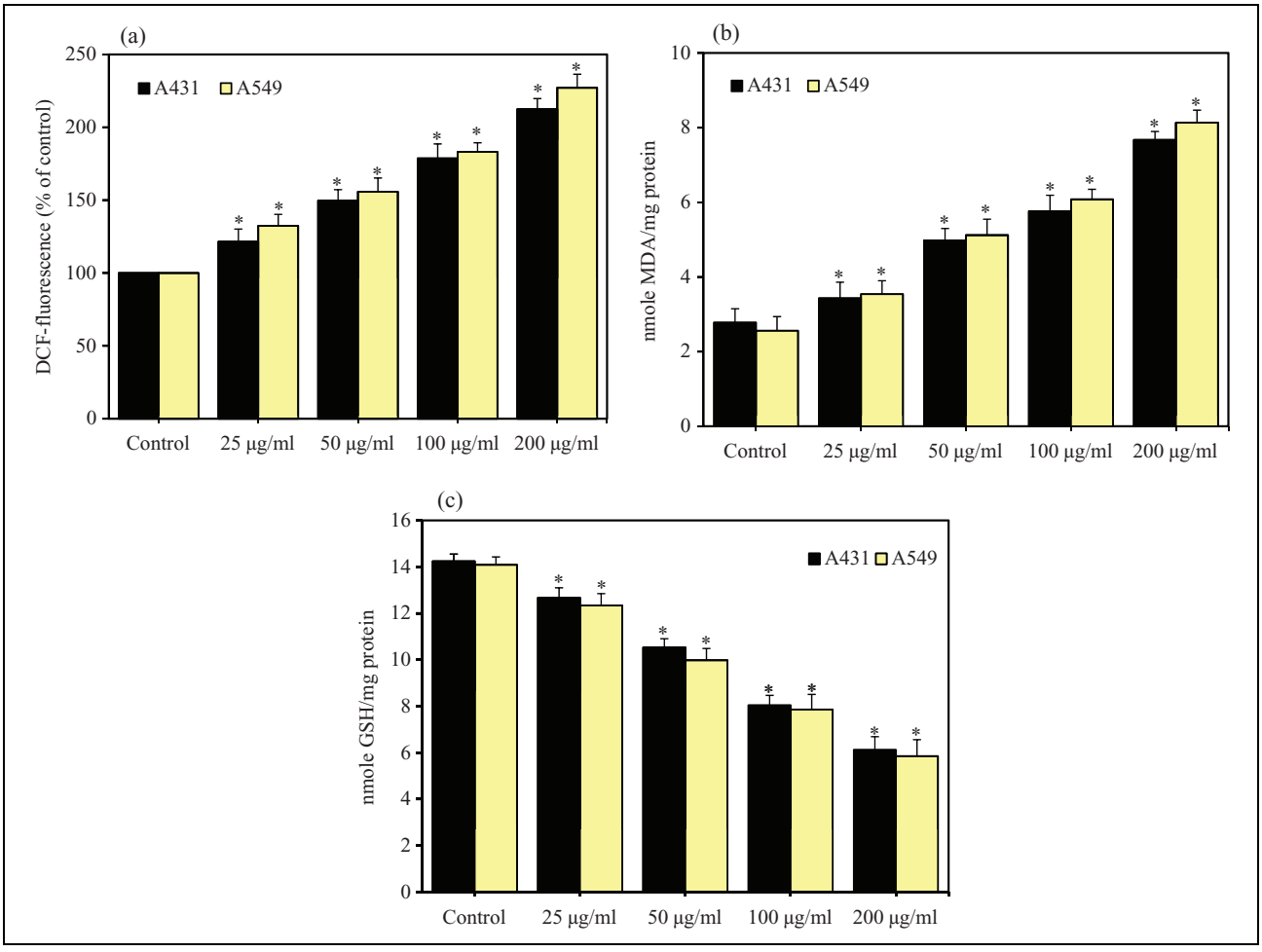

Figure 3. SiO_2 NPs induced oxidative stress in A431 and A549 cells. Cells were exposed to different concentrations (25–200 µg/ml) of SiO_2 NPs for 72 h. At the end of exposure, oxidative stress parameters were determined as described in the Materials and Methods section. (a) ROS, (b) LPO and (c) GSH. Data represented are mean ± SD of three identical experiments made in three replicate. *Significant difference compared with the controls ($p < 0.05$ for each). GSH: glutathione; LPO: lipid peroxidation; ROS: reactive oxygen species; SiO_2 NPs: silica nanoparticles.

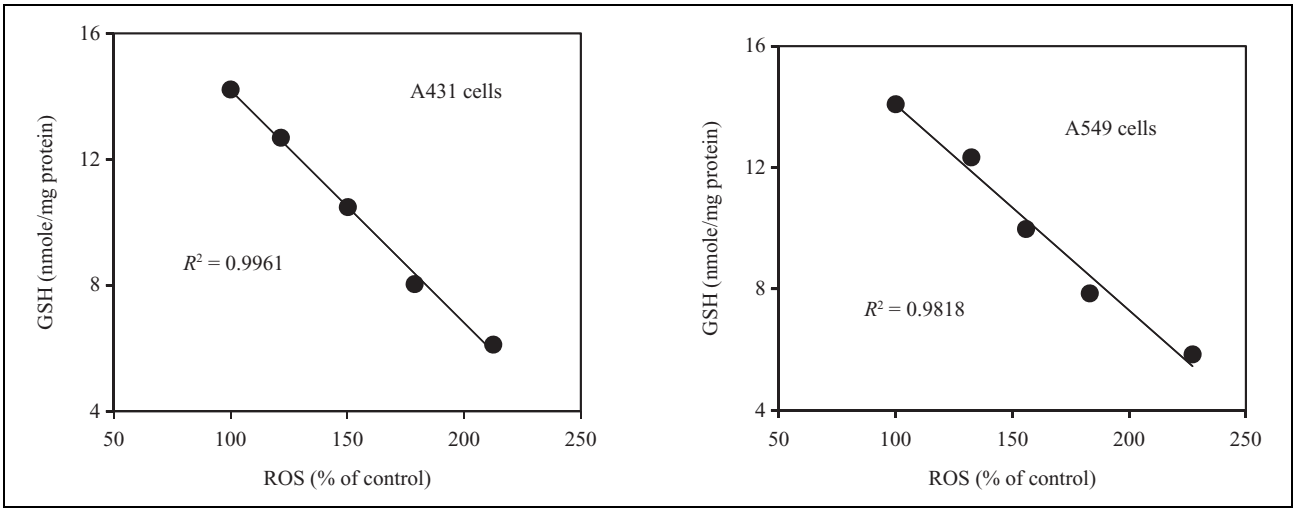

Figure 4. Correlation between ROS level and GSH level in A431 and A549 cells after 72 h exposure to 25, 50, 100 and 200 µg/ml of SiO_2 NPs. GSH: glutathione; ROS: reactive oxygen species; SiO_2 NPs: silica nanoparticles.

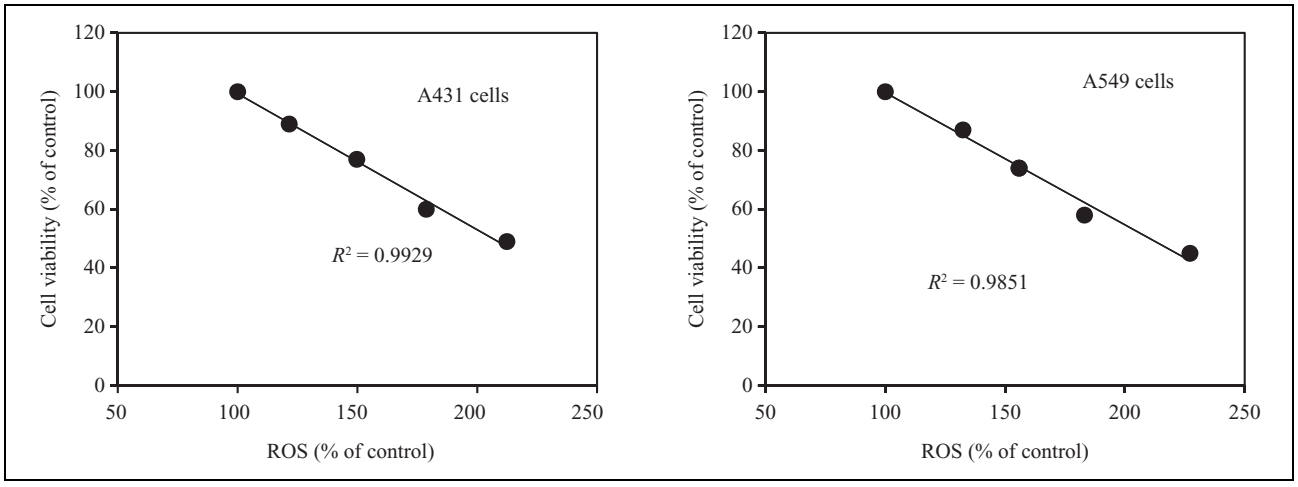

Figure 5. Correlation between cell viability and ROS level in A431 and A549 cells after 72 h exposure to 25, 50, 100 and 200 μg/ml of SiO_2 NPs. ROS: reactive oxygen species; SiO_2 NPs: silica nanoparticles.

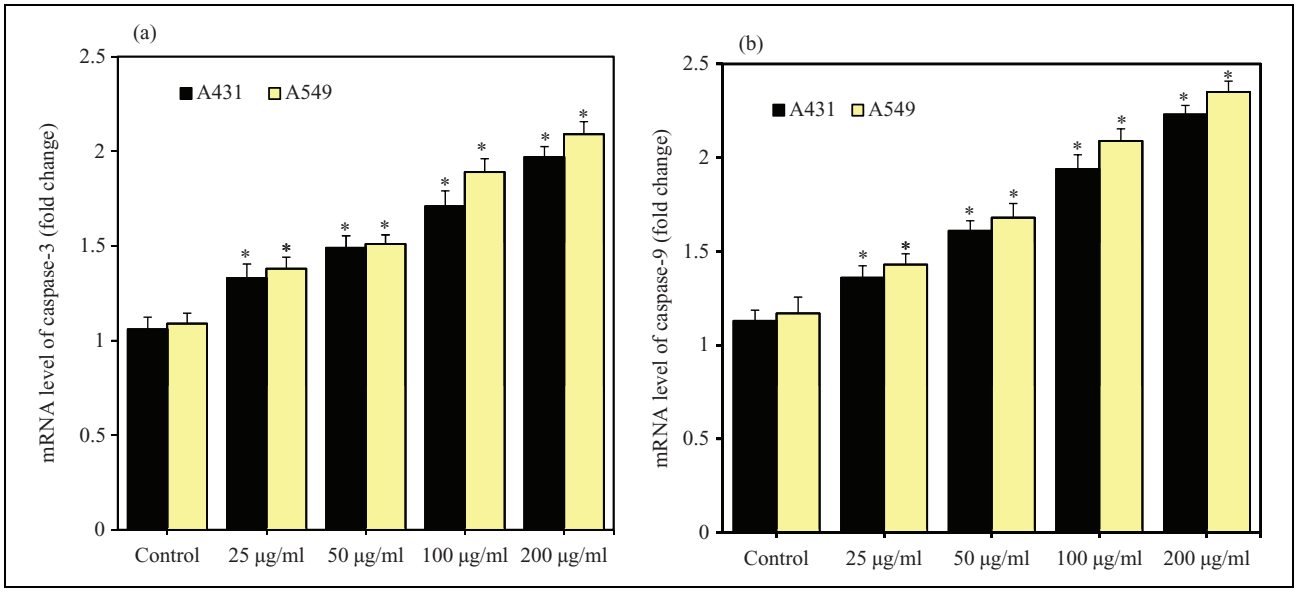

Figure 6. SiO_2 NPs upregulated the mRNA levels of caspase-3 and caspase-9 genes in A431 and A549 cells. Cells were treated with different concentrations (25–200 μg/ml) of SiO_2 NPs for 72 h. Quantitative RT-PCR was performed by QuantiTect SYBR Green PCR kit using ABI PRISM 7900HT Sequence Detection System. The β-actin was used as the internal control to normalize the data. SiO_2 NPs-induced alterations in mRNA expression levels are expressed in relative quantity compared with those for the respective unexposed control cells. Data represented are mean ± SD of three identical experiments made in three replicate. *Statistically significant difference compared with the controls ($p < 0.05$ for each). RT-PCR: real-time polymerase chain reaction; SiO_2 NPs: silica nanoparticles; mRNA: messenger RNA.

Therefore, we investigated the toxic response of SiO_2 NPs in human skin epithelial (A431) and lung epithelial (A549) cells. We found that SiO_2 NPs have a potential to induce cytotoxicity, oxidative stress and apoptosis in both the types of human cells.

Our MTT data showed that SiO_2 NPs-induced reduction in the viability of A431 and A549 cells in dose-dependent manner. LDH leakage from cells is another evidence for the penetration of nanoparticles into the cells and cell membrane damage.[14,30] It has been well documented that LDH levels in the cell medium elevated after the cells exposed to nanoparticles.[18,19,31–33] We also observed that LDH leakage was higher in a dose-dependent manner in both the types of cells treated with SiO_2 NPs. A significant reverse correlation between MTT and LDH was also

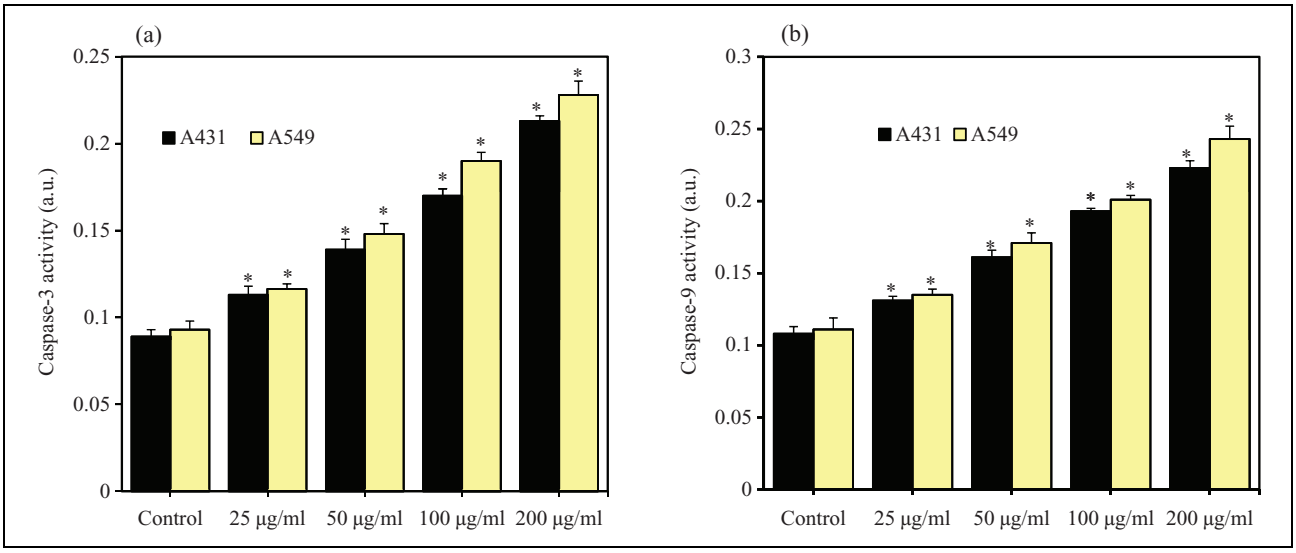

Figure 7. SiO_2 NPs induced the activities of caspase-3 and caspase-9 enzymes in A431 and A549 cells. Cells were treated with different concentrations (25–200 μg/ml) of SiO_2 NPs for 72 h. At the end of exposure, activities of caspase-3 and caspase-9 enzymes were determined using BioVision assay kit as described in the materials and methods section. (a) Caspase-3 and (b) caspase-9. Data represented are mean ± SD of three identical experiments made in three replicate. *Statistically significant difference compared with the controls ($p < 0.05$ for each).

observed. These results are in agreement with the results from other investigators showing a dose-dependent cytotoxicity induced by SiO_2 NPs in human embryonic kidney cells, human neuronal cells and human liver cells.[13–15]

Concomitant cellular oxidative stress was manifested by elevated oxidant levels and depleted antioxidant levels. In the present study, the oxidant (ROS and LPO) levels were higher, while the antioxidant (GSH) level was lower in A431 and A549 cells due to SiO_2 NPs exposure. Furthermore, negative linear correlation between the ROS and GSH indicated that free oxygen radical species were generated by exposure to SiO_2 NPs, which reduced intracellular antioxidant GSH levels. There was also a strong inverse correlation between decreased cell viability and increased ROS level. The inverse correlation between the decreased cell viability and the increased ROS level suggested that cell death could be the primary cause of ROS generation. Consequently, higher production of ROS leads to increased membrane LPO with a concomitant decrease in antioxidants such as GSH to scavenge these free oxygen radicals appears to be the underlying mechanisms of SiO_2 NPs toxicity.

ROS production and oxidative stress have received much attention in nanoparticles-induced apoptosis.[18,34–36] ROS has been suggested to be signaling molecule for the initiation and execution of the apoptotic death program.[37,38] We analyzed the mRNA expression of two apoptotic genes (caspase-3 and caspase-9) in response to SiO_2 NPs in A431 and A549 cells, because apoptosis is controlled through these pathways. Our quantitative real-time PCR results showed that SiO_2 NPs upregulated mRNA level of these genes. Furthermore, activities of caspase-3 and caspase-9 enzymes were also higher in SiO_2 NPs-treated cells. Taken together, ROS induces permeabilization of the outer mitochondrial membrane, which releases soluble proteins from the intermembrane space into the cytosol, where they promote caspase activation.[39,40] The best studied of these proteins is cytochrome c, which binds to apoptosis protease activating factor-1 (Apaf-1) and leads to the assembly of an apoptosome complex. This apoptosome binds to procaspase-9 and causes its auto-activation through a conformational change. Once initiated, caspase-9 goes on to activate caspase-3 (effector caspase), which cleaves substrates at aspartate residues and activation of this proteolytic activity appears to be an event in nanoparticle-induced apoptosis.[18,41] Apoptotic response of SiO_2 NPs in A431 and A549 cells are supported by our previous investigation, where SiO_2 NPs induced apoptosis in human liver cells (HepG2).[15]

In conclusion, we have shown that SiO_2 NPs produce significant cytotoxicity to A431 and A549 cells in dose-dependent manner in the concentration

range of 25–200 μg/ml. This nanoparticle was also found to induce oxidative stress by inducing oxidants (ROS and LPO) and depleting antioxidant (GSH). Furthermore, mRNA levels and activities of casapase-3 and capase-9 enzymes were upregulated by SiO_2 NPs. It is also worth mentioning that A549 cells seem to be marginally more susceptible to SiO_2 NPs exposure than A431 cells. Taken together, this study suggesting that SiO_2 NPs may induce cytotoxicity and apoptosis in A431 and A549 cells, which is likely to be mediated through ROS generation and oxidative stress. Further studies are underway to examine the molecular mechanism of toxicity induced by SiO_2 NPs.

Conflict of interest

The authors declared no conflicts of interest.

Funding

This work was supported by King Abdulaziz City for Science and Technology under the National Plan for Science and Technology (Grant No.: 10-NAN1201-02).

References

1. Zhang FF, Wan Q, Li CX, Wang XL, Zhu ZQ, Xian YZ, et al. Simultaneous assay of glucose, lactate, L-glutamate and hypoxanthine levels in a rat striatum using enzyme electrodes based on neutral red-doped silica nanoparticles. *Anal Bioanal Chem* 2004; 380: 637–642.
2. Santra S, Zhang P, Wang K, Tapec R and Tan W. Conjugation of biomolecules with luminophore-doped silica nanoparticles for photostable biomarkers. *Anal Biochem* 2001; 73: 4988–4993.
3. Hirsch LR, Stafford RJ, Bankson JA, Sershen SR, Rivera B, Price RE, et al. Nanoshell-mediated near infrared thermal therapy of tumors under magnetic resonance guidance. *Proc Natl Acad Sci USA* 2003; 100: 13549–13554.
4. Bharali DJ, Klejbor I, Stachowiak EK, Dutta P, Roy I, Kaur N, et al. Organically modified silica nanoparticles: a nonviral vector for *in vivo* gene delivery and expression in the brain. *Proc Natl Acad Sci USA* 2005; 102: 11539–11544.
5. Slowing II, Vivero-Escoto JL, Wu CW and Lin VSY. Mesoporous silica nanoparticles as controlled release drug delivery and gene transfection carriers. *Adv Drug Deliv Rev* 2008; 60: 1278–1288.
6. Barik TK, Sahu B and Swain V. Nanosilica-from medicine to pest control. *Parasitol Res* 2008; 103: 253–258.
7. Oberdorster G, Oberdorster E and Oberdorster J. Nanotoxicology: an emerging discipline evolving from studies of ultrafine particles. *Environ Health Perspect* 2005; 113: 823–839.
8. Kaewamatawong T, Shimada A, Okajima M, Inoue H, Morita T, Inoue K, et al. Acute and subacute pulmonary toxicity of low dose of ultrafine colloidal silica particles in mice after intratracheal instillation. *Toxicol Pathol* 2006; 34: 958–965.
9. Park EJ and Park K. Oxidative stress, pro-inflammatory responses induced by silica nanoparticles *in vivo* and *in vitro*. *Toxicol Lett* 2009; 184: 18–25.
10. Liu T, Li L, Teng X, Huang X, Liu H, Chen D, et al. Single and repeated dose toxicity of mesoporous hollow silica nanoparticles in intravenously exposed mice. *Biomaterials* 2011; 32: 1657–1668.
11. Lison D, Thomassen LCJ, Rabolli V, Gonzalez L, Napierska D, Seo JW, et al. Nominal and effective dosimetry of silica nanoparticles in cytotoxicity assays. *Toxicol Sci* 2008; 104: 155–162.
12. Napierska D, Thomassen LC, Rabolli V, Lison D, Gonzalez L, Kirsch-Volders M, et al. Size-dependent cytotoxicity of monodisperse silica nanoparticles in human endothelial cells. *Small* 2009; 5: 846–853.
13. Wang F, Gao F, Lan M, Yuan H, Huang Y and Liu J. Oxidative stress contributes to silica nanoparticle-induced cytotoxicity in human embryonic kidney cells. *Toxicol In Vitro* 2009; 23: 808–815.
14. Wang F, Jiao C, Liu J, Yuan H, Lan M and Gao F. Oxidative mechanisms contribute to nanosize silican dioxide-induced developmental neurotoxicity in PC12 cells. *Toxicol In Vitro* 2011; 25: 1548–1556.
15. Ahmad J, Ahamed M, Akhtar MJ, Alrokayan SA, Siddiqui MA, Musarrat J, et al. Apoptosis induction by amorphous silica nanoparticles mediated through reactive oxygen species generation in human liver cell line HepG2. *Toxicol Appl Pharmacol* 2012; 259: 160–168.
16. Arora S, Jain J, Rajwade JM and Paknikar KM. Cellular responses induced by silver nanoparticles: in vitro studies. *Toxicol Lett* 2008; 179: 93–100.
17. Ahamed M, Siddiqui MA, Akhtar MJ, Ahmad I, Pant AB and Alhadlaq HA. Genotoxic potential of copper oxide nanoparticles in human lung epithelial cells. *Biochem Biophys Res Commun* 2010; 396: 578–583.
18. Ahamed M, Akhtar MJ, Siddiqui MA, Ahmad J, Musarrat J, Al-Khedhairy AA, et al. Oxidative stress mediated apoptosis induced by nickel ferrite nanoparticles in cultured A549 cells. *Toxicology* 2011; 283: 101–108.

19. Ahamed M. Toxic response of nickel nanoparticles in human lung epithelial A549 cells. *Toxicol in Vitro* 2011; 25: 930–936.
20. Ahamed M, Akhtar MJ, Raja M, Ahmad I, Siddiqui MKJ, AlSalhi MS, et al. ZnO nanorod-induced apoptosis in human alveolar adenocarcinoma cells via p53, survivin and bax/bcl-2 pathways: role of oxidative stress. *Nanomedicine: NBM* 2011; 7: 904–913.
21. Wroblewski F and LaDue JS. Lactate dehydrogenase activity in blood. *Proc Soc Exp Biol Med* 1955; 90: 210–213.
22. Wang H and Joseph JA. Quantifying cellular oxidative stress by dichlorofluorescin assay using microplate reader. *Free Radic Biol Med* 1999; 27: 612–616.
23. Ohkawa H, Ohishi N and Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem* 1979; 95: 351–358.
24. Ellman GI. Tissue sulfhydryl groups. *Arch Biochem Biophys* 1959; 82: 70–77.
25. Berasain C, Garcia-Trevijano ER, Castillo J, Erroba E, Santamaria M and Lee DC. Novel role for amphiregulin in protection from liver injury. *J Biol Chem* 2005; 280: 19012–19020.
26. Adams LK, Lyon DY and Alvarez PJ. Comparative eco-toxicity of nanoscale TiO2, SiO2, and ZnO water suspensions. *Water Res* 2006; 40: 3527–3532.
27. Fent K, Weisbrod CJ, Wirth-Heller A and Pieles U. Assessment of uptake and toxicity of fluorescent silica nanoparticles in zebrafish (*Danio rerio*) early life stages. *Aquat Toxicol* 2010; 100: 218–228.
28. Hoecke KV, De Schamphelaere KAC, der Meeren PV, Lucas S and Janssen CR. Ecotoxicity of silica nanoparticles to the green alga *Pseudokirchneriella subcapitata*: importance of surface area. *Environ Toxicol Chem* 2008; 27: 1948–1957.
29. Nishimori H, Kondoh M, Isoda K, Tsunoda S, Tsutsumi Y and Yagi K. Silica nanoparticles as hepatotoxicants. *Eur J Pharm Biopharm* 2009; 72: 496–501.
30. Balduzzi M, Diociaiuti M and Berardis BD. *In vitro* effects on macrophages induced by noncytotoxic doses of silica particles possibly relevant to ambient exposure. *Environ Res* 2004; 96: 62–71.
31. Hussain SM, Hess KL and Gearhart JM. In vitro toxicity of nanoparticles in BRL 3A rat liver cells. *Toxicol In Vitro* 2005; 19: 975–983.
32. Akhtar MJ, Ahamed M, Kumar S, Siddiqui H, Patil G, Ashquin M, et al. Nanotoxicity of pure silica mediated through oxidant generation rather than glutathione depletion in human lung epithelial cells. *Toxicology* 2010; 276: 95–102.
33. Akhtar MJ, Kumar S, Murthy RC, Ashquin M, Khan MI, Patil G, et al. The primary role of iron-mediated lipid peroxidation in the differential cytotoxicity caused by two varieties of talc nanoparticles on A549 cells and lipid peroxidation inhibitory effect exerted by ascorbic acid. *Toxicol In Vitro* 2010; 24: 1139–1147.
34. Nel A, Xia T, Madler L and Lin N. Toxic potential of materials at the nano level. *Science* 2006; 311: 622–627.
35. Akhtar MJ, Ahamed M, Kumar S, Ahmad J, Khan MAM and Alrokayan SA. Zinc oxide nanoparticles selectively induces apoptosis in cancer cells through reactive oxygen species. *Int J Nanomed* 2012; 7: 845–857.
36. Ahamed M, AlSalhi MS and Siddiqui MKJ. Silver nanoparticle applications and human health. *Clin Chim Acta* 2011; 411: 1841–1848.
37. Lunov O, Syrovets T, Buchele B, Jiang X, Rocker C, Tron K, et al. The effect of carboxydextran-coated superparamagnetic iron oxide nanoparticles on c-Jun N-terminal kinase-mediated apoptosis in human macrophages. *Biomaterials* 2010; 31: 5063–5071.
38. Stone V and Donaldson K. Nanotoxicology: signs of stress. *Nat Nanotech* 2006; 1: 23–24.
39. Fuentes-Prior P and Salvesen GS. The protein structures that shape caspase activity, specificity, activation and inhibition. *J Biochem* 2004; 384: 201–232.
40. Youle RJ and Strasser A. The BCL-2 protein family: opposing activities that mediate cell death. *Nat Rev Mol Cell Biol* 2008; 9: 47–59.
41. Timmer JC and Salvesen GS. Caspase substrates. *Cell Death Differ* 2007; 14: 66–72.