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Nickel oxide nanoparticles induce cytotoxicity, oxidative stress and apoptosis in cultured human cells that is abrogated by the dietary antioxidant curcumin

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ABSTRACT

Nickel oxide nanoparticles (NiO NPs) are increasingly utilized in a number of applications. However, little is known about the toxicity of NiO NPs following exposure to human cells. This study was designed to investigate NiO NPs induced cytotoxicity, oxidative stress and apoptosis in cultured human airway epithelial (HEp-2) and human breast cancer (MCF-7) cells. The results show that cell viability was reduced by NiO NPs and degree of reduction was dose-dependent. NiO NPs were also found to induce oxidative stress in dose-dependent manner indicated by depletion of glutathione and induction of reactive oxygen species and lipid peroxidation. Induction of caspase-3 enzyme activity and DNA fragmentation, biomarkers of apoptosis were also observed in NiO NPs exposed cells. Preventive potential of a dietary antioxidant curcumin against NiO NPs induced toxicity in HEp-2 MCF-7 cells was further examined. We found that co-exposure of curcumin significantly attenuated the cytotoxicity and oxidative stress induced by NiO NPs in both types of cells. This is the first report showing that NiO NPs induced ROS mediated cytotoxicity and apoptosis that is abrogated by curcumin. The pharmacological potential of curcumin against NiO NPs induced toxicity warrants further investigation.

1. Introduction

Nanoparticles have received much attention due to not only their wide-spread applications, but also their adverse effects to the environmental and human health (Singh et al., 2009). Nickel oxide nanoparticles (NiO NPs) are increasingly used in various applications such as catalyst, gas sensor, alkaline battery cathode, electro-chromic film, magnetic material and fuel cell (Rao and Sunandana, 2008; Rani et al., 2010; Mu et al., 2011). Despite the many applications of NiO NPs, there are limited information concerning the toxicity of NiO NPs at the cellular and molecular level. Recent studies reported the toxic effects of NiO NPs in bacteria and microalgae (Baek and An, 2011; Gong et al., 2011). Horie et al. (2011) showed that NiO NPs have potential to induce oxidative stress related lung injury. However, Morimoto et al. (2011) reported that inhalation of NiO NPs did not induce the expressions of matrix metalloproteinase (MMP-2) and tissue inhibitor matrix proteinase (TIMP-2) mRNA levels in rat lungs.

Recent studies suggest that excessive production of reactive oxygen species (ROS) and oxidative stress could be one of the possible mechanisms of nanoparticle toxicity (Nel et al., 2006; Asharani et al., 2009; Ahamed et al., 2011a). ROS in general cause DNA damage, including a multitude of oxidized base lesions, abasic sites, single and double-strand breaks; all of these can be cytotoxic, genotoxic or mutagenic (Ahamed et al., 2008; Zhan et al., 2010). Earlier, we also observed that inorganic nanoparticles induce ROS mediated cytotoxicity, DNA damage and apoptosis in cultured mammalian cells and *Drosophila melanogaster* (Ahamed et al., 2008, 2010a–c, 2011a; Ahamed, 2011b; Ahamed et al., 2011c).

Curcumin is a widely used spice and coloring agent in food. It is extracted from the powdered dry rhizome of turmeric (*Curcuma longa L.*), a perennial herb widely cultivated in tropical regions of Asia. Curcumin is known to have multiple pharmacological properties such as anti-carcinogenic, anti-inflammatory and antioxidant (Dai et al., 2009; Ghosh et al., 2009; Prakobwong et al., 2011). However, the preventive potential of curcumin against nanoparticles toxicity has not been explored. This study was designed to investigate NiO NPs induced cytotoxicity, oxidative stress and apoptosis in human airway epithelial (HEp-2) and human breast cancer (MCF-7) cells. The preventive potential of curcumin against NiO NPs induced toxicity was further examined. In this study we choosen two distinct cell types; human airway epithelial (HEp-2) and human breast cancer (MCF-7) cells. HEp-2 cells derived from laryngeal epithelium and represent the first level of nanoparticles/pollutants exposure. To avoid the cell type specific response, we also

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used MCF-7 cells to compare the toxic response of NiO NPs from HEp-2 cells. Both HEp-2 and MCF-7 cell lines have been widely used in toxicological studies (Fahmy and Cormier, 2009; Wei et al., 2010).

2. Materials and methods

2.1. Nickel oxide nanoparticles and reagents

Nickel (II) oxide (NiO) nanopowder [Product No.: 637130, APS:<50 nm (BET) and purity: 99.8% trace metal basis] was purchased from Sigma–Aldrich (St. Louis, Missouri). Fetal bovine serum (FBS), penicillin–streptomycin, DMEM/F-12 medium, HBSS was bought from Invitrogen Co. (Carlsbad, California). MTT [3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide], curcumin, reduced glutathione (GSH), 5,5-dithio-bis-(2-nitrobenzoic acid) (DTNB) and thiobarbituric acid (TBA) were obtained from Sigma–Aldrich (St. Louis, Missouri). All other chemicals used were of the highest purity available from commercial sources.

2.2. Nickel oxide nanoparticles characterization

Crystalline nature of NiO NPs was examined by X-ray diffraction (XRD) (PANalytical, ALMELO, Netherlands). Morphology and size of NiO NPs was examined by field emission transmission electron microscopy (FETEM, JEM-2100F, JEOL Inc., Japan) at an accelerating voltage of 200 kV. Dynamic light scattering (DLS) (ZetaSizer-HT, Malvern Instruments Ltd, UK) was used to determine the hydrodynamic size of NiO NPs in cell culture medium.

2.3. Cell culture and treatment with NiO nanoparticles

HEp-2 and MCF-7 cells were used between passages 10 to 20. Cells were cultured in DMEM/MEM medium 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 sub-cultured into 25 cm^2 flasks, 6-well plates or 96-well plates according to selection of experiments. Cells were allowed to attach the surface for 24 h prior to treatment. NiO NPs were suspended in cell culture medium and diluted to appropriate concentrations. The appropriate dilutions of NiO NPs were then sonicated using a sonicator bath at room temperature for 10 min at 40 W to avoid nanoparticles agglomeration prior to administration to the cells. Curcumin was co-exposed with NiO NPs to evaluate its protective effect against NiO NPs induced toxicity. To identify the biologically safe dosages of curcumin, HEp-2 and MCF-7 cells were exposed to various concentrations of curcumin (5–30 μM) for 24 h. Curcumin dosages used in this study were according to previous publications (Motterlini et al., 2000; Watson et al., 2008; Hosseinzadeh et al., 2011).

2.4. MTT assay

The viability of HEp-2 and MCF-7 cells was measured by MTT assay as described by Mossman (1983) with some specific modifications (Ahamed et al., 2011a).

2.5. NRU assay

Neutral red uptake (NRU) assay was performed following the procedure as described by Borenfreund and Puerner (1984) with some specific modifications (Ahamed et al., 2011a).

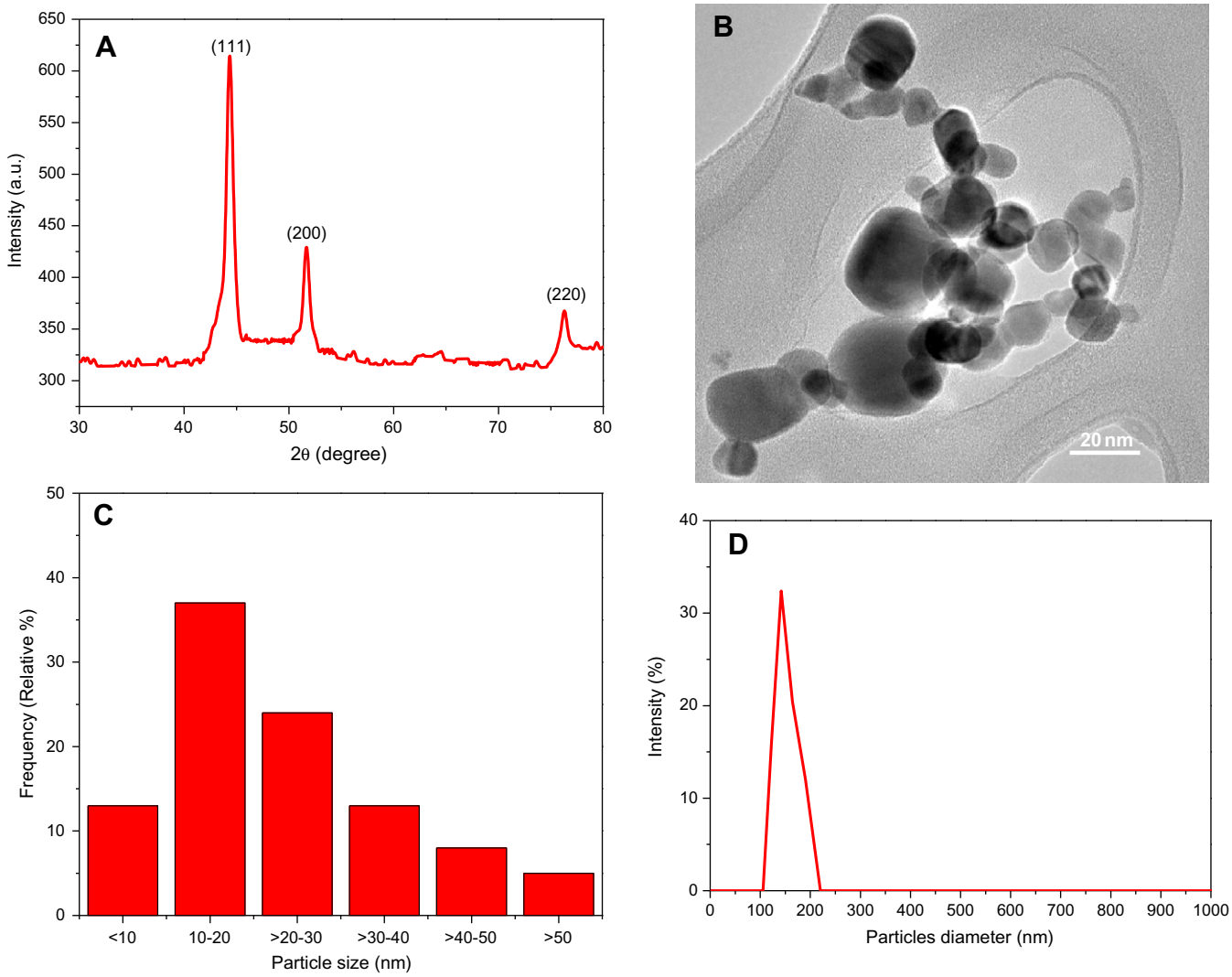

Fig. 1. Characterization of NiO NPs. (A) X-ray diffraction pattern, (B) field emission transmission electron microscopy image, (C) frequency of size distribution and (D) average hydrodynamic size in cell culture medium.

2.6. Measurement of reactive oxygen species

The production of intracellular ROS was measured using 2,7-dichlorofluorescin diacetate (DCFH-DA) as described by Wang and Joseph (1999).

2.7. Assay of lipid peroxidation and glutathione

The extent of membrane lipid peroxidation (LPO) was estimated by measuring the formation of malondialdehyde (MDA). MDA is one of the end products of membrane LPO. MDA formation was measured using thiobarbituric acid reactive substances (TBARS) protocol (Buege and Aust, 1978). Intracellular GSH level was determined as described by Chandra et al. (2002) with some specific modifications (Ahamed et al., 2011a)

2.8. Casapase-3, activity assay

Activity of caspase-3 enzyme was measured in treated and control cells using standard assay kit (BioVision, Inc.). 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 (Walsh et al., 2008). The pNA was generated after specific action of caspase-3 on tertrapeptide substrates DEVD-pNA.

2.9. Apoptotic DNA ladder assay

Apoptotic DNA ladder assay was performed in HEp-2 and MCF-7 cells exposed to 25 μg/ml NiO NPs for 24 h. At the end of exposure DNA was extracted using an apoptotic DNA Ladder Kit (Roche, USA Cat # 11835246001). The extracted DNA was then evaluated on a 1% agarose gel using ethidium bromide. DNA fragmentation pattern was documented by a gel documentation system.

2.10. Protein estimation

Protein content was estimated using a BCA protein assay kit (Pierce Biotechnology, Inc.).

2.11. Statistical analysis

Statistical significance was measured by one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test. Significance was ascribed at $p < 0.05$. All statistical analyzes were conducted using the GraphPad Prism Software.

3. Results

3.1. Characterization of nickel oxide nanoparticles

We utilized XRD, FETEM and DLS techniques to characterize NiO NPs. The crystalline nature of NiO NPs was confirmed by XRD (Fig. 1A). Fig. 1B shows the typical TEM image of NiO NPs. The average particle size of NiO NPs calculated by TEM and XRD was around 22 nm. Fig. 1C represents frequency of size distribution of NiO NPs. The average hydrodynamic size of NiO NPs in cell culture medium determined by DLS was around 151 nm (Fig. 1D). The higher size of nanoparticles in hydrodynamic state as compared to sizes of TEM and XRD might be due to the tendency of nanoparticles to aggregate in aqueous state.

3.2. Nickel oxide nanoparticle-induced cytotoxicity

HEp-2 and MCF-7 cells were exposed to NiO NPs (1–100 μg/ml) for 24 h and cytotoxicity was measured by MTT and NRU assays. Results show that cell viability was reduced by NiO NPs and degree of reduction was dose-dependent. In MTT assay, HEp-2 cell viability was decreased to 81%, 79%, 59%, 43%, 20% and 11% while MCF-7 cell viability reduction was 75%, 71%, 42%, 33%, 16% and 9% for the concentrations of 2, 5, 10, 25, 50 and 100 μg/ml respectively ($p < 0.05$ for each) (Fig. 2A). Fig. 2B shows the results of cell viability obtained by NRU assay. NRU results were consistent with MTT data.

3.3. Nickel oxide nanoparticle-induced oxidative stress

The potential of NiO NPs to induce oxidative stress was examined by measuring the ROS, LPO and GSH in HEp-2 and MCF-7 cells. We found that NiO NPs induce intracellular production of ROS in dose-dependent manner in both types of cells ($p < 0.05$ for each) (Fig. 3A). MDA level was significantly higher while GSH level was significantly lower in both types of cells exposed to NiO NPs in the concentration range of 2–100 μg/ml (Fig. 3B and C).

3.4. Nickel oxide nanoparticle-induced apoptosis

We observed that activity of caspase-3 enzyme, a marker of apoptosis was induced by NiO NPs and degree of induction was dose-dependent (Fig. 4A). We further analyzed the DNA profiles of the cells treated with 25 μg/ml of NiO NPs for 24 h. In control cells the DNA was not fragmented, whereas the cells treated with NiO NPs had started the apoptotic process, as evident by DNA fragmentation (Fig. 4B).

3.5. Effect of curcumin on cell viability

Fig. 5 show the viability (MTT assay) of HEp-2 and MCF-7 cells exposed to curcumin (5–30 μM) for 24 h. Results showed that cur-

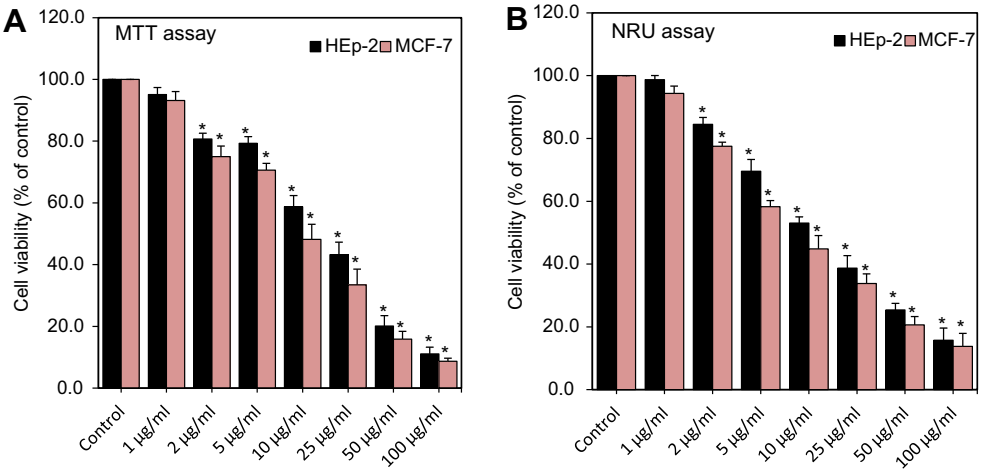

Fig. 2. NiO NPs induced cytotoxicity in HEp-2 and MCF-7 cells. Cells were exposed to different concentrations of NiO NPs for 24 h. At the end of exposure cell viability was determined as described in the materials and methods. (A) MTT and (B) NRU assays. Data represented are mean ± SD of three identical experiments made in three replicate. *Significant difference as compared to the controls ($p < 0.05$ for each).

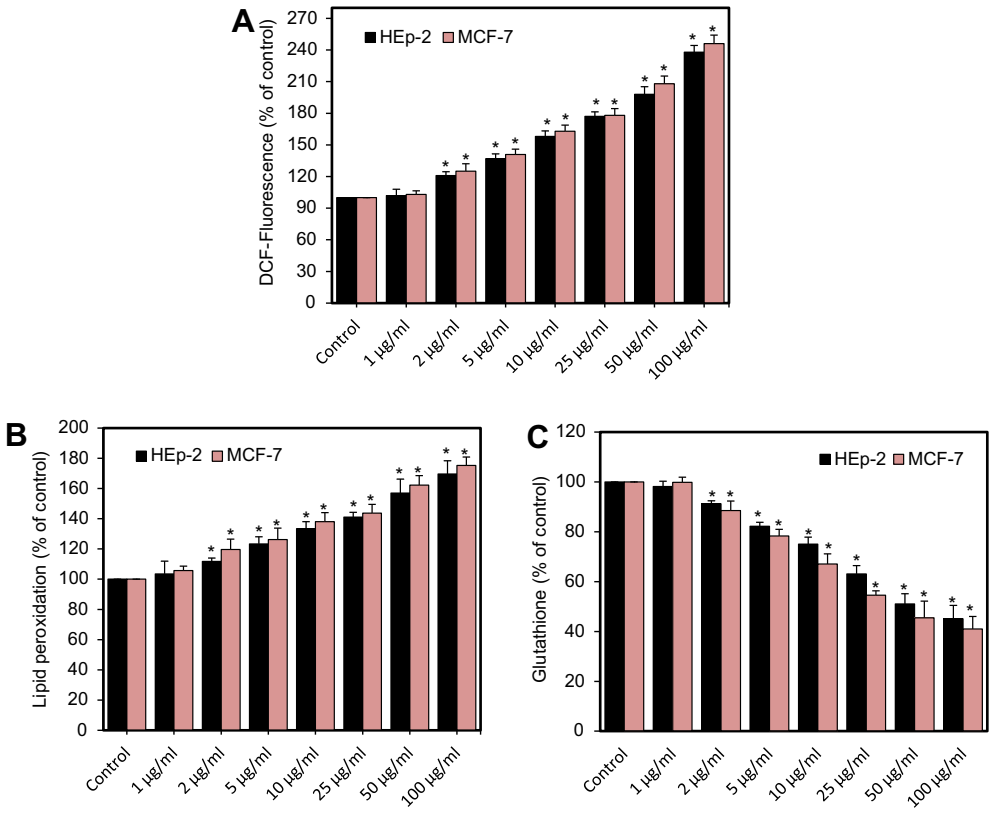

Fig. 3. NiO NPs induced oxidative stress in HEp-2 and MCF-7 cells. Cells were exposed to different concentrations of NiO NPs for 24 h. At the end of exposure oxidative stress parameters were determined as described in the materials and methods. (A) ROS, (B) LPO and (C) GSH. Data represented are mean ± SD of three identical experiments made in three replicate. *Significant difference as compared to the controls ($p < 0.05$ for each).

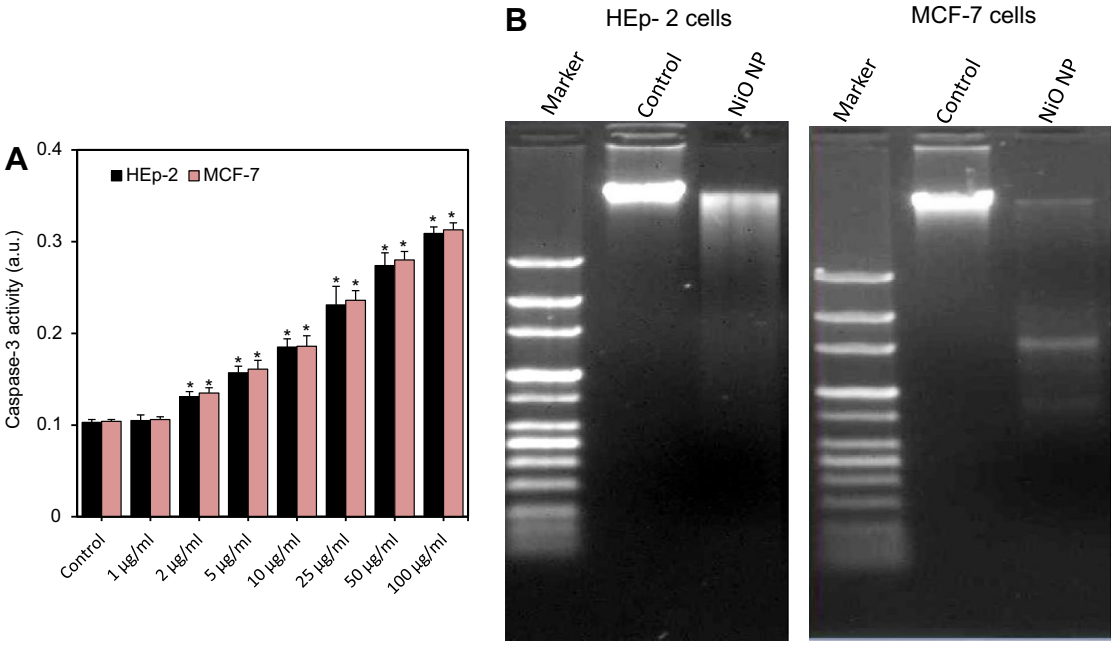

Fig. 4. NiO NPs induced apoptotis in HEp-2 and MCF-7 cells. (A) Higher activity of caspase-3 enzyme in treated cells. Cells were exposed to different concentrations of NiO NPs for 24 h. At the end of exposure activity of caspase-3 enzyme was determined as described in the materials and methods. Data represented are mean ± SD of three identical experiments made in three replicate. *Significant difference as compared to the controls ($p < 0.05$ for each). (B) Representative image of DNA ladder analysis of HEp-2 and MCF-7 cells treated with NiO NPs at the concentration of 25 μg/ml for 24 h.

cumin in the concentration range 5–25 μM did not decrease significant number of viable cells ($p > 0.005$). However, a higher concentration of curcumin (30 μM) induce 16% reduction in cell viability ($p < 0.05$). The safe doses of curcumin (5–25 μM) were further utilized to examine its preventive potential against NiO NPs induced cytotoxicity and oxidative stress in HEp-2 and MCF-7 cells.

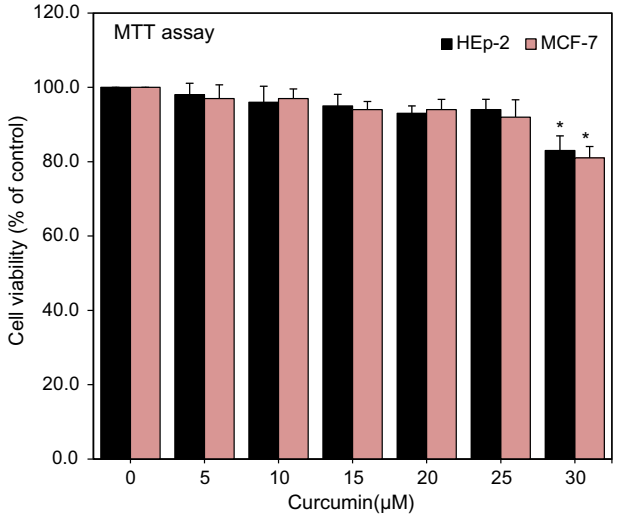

Fig. 5. Effect of curcumin on the viability of HEp-2 and MCF-7 cells. Cells were exposed to different concentrations of curcumin for 24 h. At the end of exposure cell viability was determined by MTT assay. Data represented are mean ± SD of three identical experiments made in three replicate. *Significant difference as compared to the controls ($p < 0.05$).

3.6. Preventive potential of curcumin against nickel oxide nanoparticle-induced cytotoxicity and oxidative stress

The potential of curcumin to prevent the cytotoxicity and oxidative stress induced by NiO NPs in HEp-2 and MCF-7 cells was examined. Cells were exposed to NiO NPs at the concentration of 25 μg/ml for 24 h in the presence or absence of curcumin (15–25 μM). We observed that co-exposure curcumin (15–25 μM) significantly prevented the loss of cell viability induced by NiO NPs (Fig. 6A and B). Curcumin also attenuated the oxidants (ROS and LPO) induction and antioxidant (GSH) reduction in HEp-2 and MCF-7 cells exposed to NiO NPs (Fig. 7A–C).

4. Discussion

In the present study, NiO NPs induced cytotoxicity in dose-dependent manner in cultured human airway epithelial (HEp-2) and human breast cancer (MCF-7) cells. ROS generation and oxidative stress has been cited to be one of the possible mechanisms of toxicity related to nanoparticle exposure (Nel et al., 2006; Wise et al., 2010). We also observed that NiO NPs induce oxidative stress in HEp-2 and MCF-7 cells. ROS and LPO levels were significantly higher while GSH level was significantly lower in NiO NPs treated cells. The depletion of GSH in NiO NPs exposed cells combined with the increased level of ROS and LPO suggests that oxidative stress is the primary mechanism for toxicity of NiO NPs in HEp-2 and MCF-7 cells. Our results are consistent with previous studies suggesting that cytotoxicity of NiO NPs is mediated through the ROS generation and oxidative stress (Horie et al., 2011; Morimoto et al., 2011). Nanoparticle induced oxidative stress leads to DNA damage and apoptosis (Ahamed et al., 2011a). We provided evidence that NiO NPs induced apoptosis in HEp-2 and MCF-7 cells. Activity of caspase-3 enzyme was higher along with the fragmentation of DNA in NiO NPs exposed cells. These results are in agreement with our previous reports where nickel nanoparticles and nickel ferrite nanoparticles induced ROS mediated apoptosis in human lung epithelial cells (Ahamed et al., 2011a; Ahamed, 2011b).

Importance of curcumin has increased due to new discoveries about its therapeutic applications (Bishnoi et al., 2008). It has been demonstrated that curcumin is able to protect cells against some hazardous agents such as gamma radiation, haloperidol and 6-hydroxydopamine (Srinivasan et al., 2006; Wang et al., 2009). We also demonstrated that curcumin significantly attenuated the cytotoxicity induced by NiO NPs in both HEp-2 and MCF-7 cells. However, curcumin itself was nontoxic to both types of cells in the range between 5–25 μM. Other investigators also reported that curcumin in the range of 5–25 μM did not produce cytotoxicity in mammalian cells (Watson et al., 2008; Hosseinzadeh et al., 2011).

Antioxidant potential of curcumin has been reported in scientific literature (Reuter et al., 2010; Wang et al., 2009). Elevated levels of ROS are well-known etiological factors associated with oxidative stress leading to cell death via apoptosis in variety of cell types (Sompol et al., 2008; Reuter et al., 2010), and such effects can be blocked or delayed by a wide variety of antioxidants like curcumin (Hosseinzadeh et al., 2011). In order to understand the mechanisms through which curcumin protect the cells from NiO NPs, we determined the endpoints of oxidative stress in HEp-2 and MCF-7 cells. We found that curcumin significantly attenuated the induction of ROS and LPO along with the reduction of GSH caused by NiO NPs. These experiments suggest that curcumin acts as an anti-

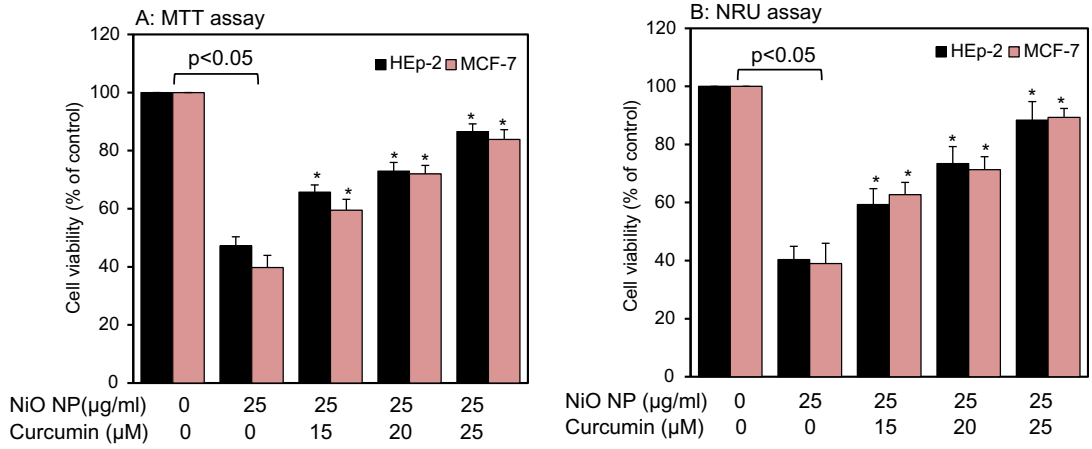

Fig. 6. Preventive potential of curcumin against NiO NPs induced cytotoxicity in HEp-2 and MCF-7 cells. Cells were exposed to NiO NPs (25 μg/ml) in the presence or absence of curcumin (15–25 μM) for 24 h. At the end of exposure cell viability was determined as described in the materials and methods. (A) MTT and (B) NRU assays. Data represented are mean ± SD of three identical experiments made in three replicate. *Significant inhibitory effect of different concentrations of curcumin on the reduction of cell viability caused by NiO NPs ($p < 0.05$).

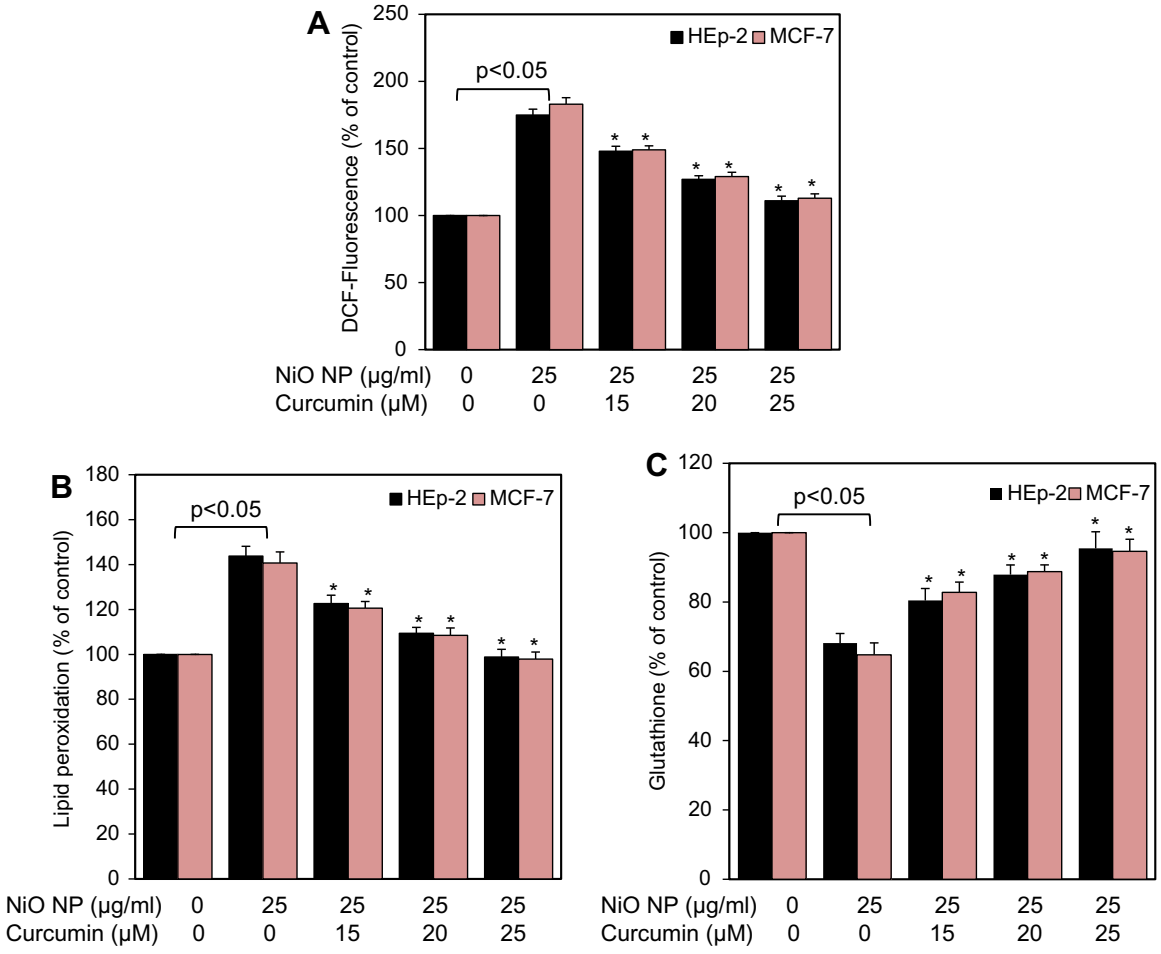

Fig. 7. Preventive potential of curcumin against NiO NPs induced oxidative stress in HEp-2 and MCF-7 cells. Cells were exposed to NiO NPs (25 μg/ml) in the presence or absence of curcumin (15–25 μM) for 24 h. At the end of exposure oxidative stress parameters were determined as described in the materials and methods. (A) ROS, (B) LPO and (C) GSH. Data represented are mean ± SD of three identical experiments made in three replicate. *Significant inhibitory effect of different concentrations of curcumin on ROS and LPO generation and GSH depletion induced by NiO NPs ($p < 0.05$).

oxidant. Antioxidant activity of curcumin in diverse pharmacological activities like anti-inflammatory, hepatoprotection and cardiac protection have also been demonstrated by other investigators (Naik et al., 2011; Prakobwong et al., 2011).

To summarize, the results of our study demonstrated that NiO NPs induce cytotoxicity and apoptosis in human airway epithelial and human breast cancer cells, which is likely to be mediated through ROS generation and oxidative stress. We also showed that NiO NPs induced toxic insult was effectively abrogated by a dietary antioxidant curcumin. This study suggests that curcumin can be a preventive agent against NiO NPs induced toxicity. The possible mechanisms through which curcumin mediates protection against toxicity exerted by NiO NPs should be further investigated.

Conflict of Interest

The authors declare that there are no conflicts of interest.

Acknowledgments

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