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Dose- and duration-dependent cytotoxicity and genotoxicity in human hepato carcinoma cells due to CdTe QDs exposure

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Abstract

Nanotechnology has achieved more commercial attention over recent years, and its application has increased concerns about its discharge in the environment. In this study, we have chosen human hepatic carcinoma (HuH-7) cells because liver tissue has played an important role in human metabolism. Therefore, the objective of this study was to determine DNA damaging and apoptotic potential of cadmium telluride quantum dots (CdTe QDs; average particle size (APS) 10 nm, 1–25 μg/ml) on HuH-7 cells and the basic molecular mechanism of its cellular toxicity. Cytotoxicity of different concentrations of CdTe QDs on HuH-7 cells was determined by 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) and lactate dehydrogenase (LDH) tests. Moreover, reactive oxygen species (ROS) generation, mitochondrial membrane potential, DNA damage, and Hoechst 33342 fluorescent staining morphological analysis of necrotic/apoptotic cells were detected; cellular impairment in mitochondria and DNA was confirmed by JC-1 and comet assay, respectively. A dose- and time-dependent cytotoxicity effect of CdTe QDs exposure was observed HuH-7 cells; the significant ($p < 0.05$) cytotoxicity was found at 25 μg/ml of CdTe QDs exposure. The percentage of cytotoxicity of CdTe QDs (25 μg/ml) in HuH-7 cells reached 62% in 48 h. CdTe QDs elicited intracellular ROS generation and mitochondrial depolarization, and DNA integrity cells collectively advocated the apoptotic cell death at higher concentration. DNA damage was observed in cells due to CdTe QDs exposure, which was mediated by oxidative stress. This study exploring the effects of CdTe QDs in HuH-7 cells has provided valuable insights into the mechanism of toxicity induced by CdTe QDs.

Keywords

CdTe QDs, HuH-7 cells, apoptosis, ROS, DNA damage, comet assay

Introduction

The large-scale application of man-made nanoparticle in industry raises concerns about the accidental release of nanomaterials in the environment. Nanocrystalline quantum dots (QDs) are developing as an alternative to organic fluorescent dyes besides its semiconductor properties.[1] The scope of usages is miscellaneously ranging from chemical sensors, bio imagery[2] to diverse optoelectronic application.[3] Cadmium telluride (CdTe) is a well-known semiconductor, having an enormous potential in the area of solar cells, electro-optics, photorefractive devices and room temperature radiation detectors.[4] QDs are a very useful class of nanomaterials with significant imaging applications in biological and health sciences[5] such as drug delivery[6] and cancer targeting and diagnosis.[7] Cadmium telluride quantum dots (CdTe QDs) induce apoptosis in human breast cancer cell lines.[8] Toxicity of QDs has been widely reported at the various levels,

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such as protozoa,[9] cell,[10] subcellular organelles,[11] biological molecules[12] and others.[13] However, it was recognized that more application of CdTe QDs poses serious concerns about toxicity and safety because it contains cadmium.[14] Lim et al.[15] has documented that CdTe QDs have more useful in biological activity due to their high luminescence efficiency, photostability and broad absorption and narrow emission spectra. The potential biological activity of CdTe QDs has been extensively involved in new-type drug design because of their more specific properties.

3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide test is a popular assay to find out the toxicity of different materials. AshaRani et al.[16] and Zhang et al.[17] have reported nanomaterials toxicity on human cells. Apoptosis is an important biological process in living cells and may be initiated by a variety of stimuli received by the cells.[18] It is well-known that apoptosis may be initiated by two main pathways: the death receptor-mediated extrinsic apoptotic pathway and the mitochondrion-mediated (cytochrome c, caspase-9) intrinsic apoptotic pathway.[19]

Human hepatic cancer is one of the most common tumours worldwide and a primary malignancy of the liver. Therefore, we choose a typical HuH-7 cell, which has been widely used as the human hepatoma model cells in the development of new anti-tumour medicines, which is important for us to understand the mechanistic effect of the nanoscale material on cancerous cells. In this work, we investigate the toxicity and cell death in human HuH-7 cells due to CdTe QDs.

Materials and methods

The cell culture medium (Dulbecco's Modified Eagle Medium (DMEM)) was obtained from Gibco BRL (Grand Island, New York, USA). CdTe-QDs (product number: MKN-CdTe-510) were purchased from Nano Impex Canada (Mississauga, Ontario). N-acetyl cysteine, penicillin, streptomycin, neutral red dye, Hoechst 33342, ethidium bromide, penicillin, streptomycin, propidium iodide (PI), bovine serum albumin, NaCl, KCl, EDTA, Triton® X-100 and dimethyl sulphoxide (DMSO) were obtained from Sigma-Aldrich (Saint-Quentin Fallavier, France). Normal and low melting point agarose (NMPA and LMPA) were purchased from Biorad (Marnes-la-Coquette, France) and L-glutamine and NaOH from Merck (Darmstadt, Germany). MilliQ185 water (Waters, Saint-Quentin-en-Yvelines, France) was used in all experiments. Culture reagents were obtained from Sigma (St Louis, Missouri, USA) and Lonza (Verviers, Belgium). All other reagents were of analytical grade.

Characterization of CdTe QDs

CdTe QDs were suspended in culture medium (1 mg/ml) and sonicated at 40 W for 15 min before exposure. The suspended CdTe QDs were characterized by transmission electron microscopy (TEM; JEM-2100, JEOL Ltd, Musashino, Akishima, Tokyo 196-8558, Japan). Hydrodynamic size of CdTe QDs was determined by dynamic light scattering (DLS; ELS-8000 L, Otsuka Electronics Co. Ltd, Osaka, Japan).

Cell morphology

Morphology of HuH-7 cells was seen after the treatment of CdTe QDs (1, 5, 10 and 25 μg/ml) for 24 and 48 h using an inverted microscope (Leica DM IL LED, Wetzlar, Germany).

Cytotoxicity assays

MTS assay. HuH-7 was seeded in a culture plate (96 well) in 100 μl of DMEM supplemented with 10% FBS and antibiotics at a density of 2×10^4 cells per well. CdTe QDs diluted in 100 μl of supplemented medium were then added. After 24 h of treatment, supernatants were discarded and cells were washed twice with PBS. The MTS assay is a formazan dye-based assay (CellTiter 96® AQueous One Solution from Promega, Woods Hollow Road, Madison, USA). Briefly, 120 μl of 1:5 MTS in the supplemented medium was added to the cells. After 3 h, the optical density was read at 490 nm using a Benchmark™ Plus microplate spectrophotometer (BioRad, Hercules, California, USA).

$$\text{Viability of cells (\% of control cells)} = (\text{OD}_{\text{expm}} - \text{OD}_{\text{blank}})/(\text{OD}_{\text{untreated}} - \text{OD}_{\text{blank}}),$$

where OD_{expm} and OD_{blank} were the optical density of the cells exposed to CdTe QDs dilution and to the supplemented medium alone, respectively, and OD_{blank} is the optical density of the wells without any cells.

All results represent the mean ± SEM (standard error of the mean) from three independent experiments. To confirm the role of reactive oxygen species (ROS) in cytotoxicity induced by CdTe QDs, we used NAC (10 mM) against MTS assay.

Measurement of membrane leakage by lactate dehydrogenase release assay. Cell toxicity induced by

CdTe QDs was assessed by lactate dehydrogenase (LDH) assay. The leakage of LDH was measured using culture supernatant (50 µl) with LDH substrate (100 µl) including ferric alum (and 5 µl, 5 mg/ml), at 37°C for 3 min. Then, nicotinamide adenine dinucleotide (NAD) solution (100 µl) and phenazine methosulphate (PMS) were mixed to the mixture and incubated at 37°C for 5 min (Labrax Commercial Kit, Clontech, Palo Alto, California, USA). This enzyme is an oxidoreductase that catalyzes the interconversion of lactate and pyruvate and, for being a stable cytosolic enzyme, after damage to cell membrane, it is released in the cellular environment and because of this LDH is an indirect indicator of cytotoxicity. The concentration of LDH was measured at 492 nm by colorimetry. The amount of LDH leakage was calculated as the percentage of the controls.[20] To confirm the role of ROS in cytotoxicity induced by CdTe QDs, we used N-acetylcysteine (NAC) (10 mM) against LDH assay.

Measurement of ROS

Assessment of intracellular ROS in HuH-7 cells after treatment of CdTe QDs (1, 5, 10 and 25 µg/ml) was done by 2,7-dichloro dihydro fluorescein diacetate (H2-DCFH-DA) dye as a detection reagent.

In short, H2-DCFH-DA (10 mM) solution was diluted in DMEM/F as a working solution (5 mM). CdTe QDs-exposed HuH-7 cells were kept with H2-DCFH-DA (5 mM) at normal room temperature, after 1-h incubation, the cells were cleaned with low warm PBS and fixed with paraformaldehyde (4%). Fluorescence of HuH-7 cells was observed by upright microscope fluorescence (Nikon Eclipse 80i, Japan).

HuH-7 cells (10^4/well) were cultured in black bottom culture plate (96 well) and treated with CdTe QDs over 48 h. After treatment, HuH-7 cells were kept with H2-DCFH-DA (5 Mm) for 1 h. Then, it washed with pre-warmed Phosphate-buffered saline (PBS). To evaluate the protective effect of NAC, HuH-7 cells were pretreated with NAC (10 mM) at 37°C for 2 h and then incubated with CdTe QDs at 37°C for 24 h and 48 h. The intensity of fluorescence was quantified at the excitation wavelength (485 nm) and emission (528 nm). The results are presented as % of fluorescence intensity relative to control wells.

Mitochondrial membrane potential

To measure changes in mitochondrial membrane potential (MMP), we have used JC-1 probe. Lipophilic cationic probe JC-1 shows a potential-dependent accumulation into the mitochondrial matrix. When MMP decreases, the maximum emission wavelength of JC-1 shifts from approximately 590 to 525 nm. Therefore, the loss of $\Delta\Psi$ m was examined using fluorescence spectrophotometer (Shimadzu Corporation, Japan) and microscope fluorescence (Nikon Eclipse 80i, Japan).

Preparation of cell extract and oxidative stress

After treatment of CdTe QDs (1, 5, 10 and 25 µg/ml) for 24 and 48 h, washed with chilled PBS and lysed chilled RIPA buffer (phenylmethylsulphonyl fluoride and phosphatase inhibitor) for 30 min. Lysate of the cell was centrifuged at 12,000 r/min for 10 min at 4°C, and supernatant was collected for determination of malondialdehyde (MDA) production, glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT) activity. The concentration of protein of cell lysate was quantified using Bradford method.[21]

GSH test. The content of GSH was evaluated according to Ellman's method.[22] One hundred microlitres of cell lysate were added to 2,2,2-trichloroacetic acid (TCA) (5%, TCA 900 µl) and centrifuged at 3000 × *g* for 10 min at 4°C. Again 500 µl supernatant was added with 5,5'-dithio-bis(2-nitrobenzoic acid) (DTNB) (0.01%, 1.5 ml) and OD of the mixture was observed at 412 nm. The quantity of glutathione was represented in nM GSH/mg protein.

Lipid peroxide test. Lipid peroxide (LPO) was measured according Ohkawa et al. method.[23] Cell lysate (100 µl) was mixed with 1.9 ml sodium phosphate buffer (0.1 M, pH 7.4) and incubated for 60 min at 37°C. After incubation, 5% TCA was added and centrifuged at 3000 × *g* for 10 min at room temperature) to obtain a supernatant. The supernatant was mixed with 1 ml TBA (1%) and put in a water bath at 100°C for 30 min. The optical density of the cooled mixture was examined at 532 nm and expressed in nanomolar of MDA per milligram of protein.

SOD and CAT. All observations were done using Cayman chemical kit for SOD (item no 706002) and CAT (item no 707002; Ann Arbor, Michigan, USA) according to manufacturer's instructions.

Apoptosis

Chromosome condensation. Chromosome condensation in HuH-7 cells due to CdTe QDs (1, 5, 10 and 25 µg/

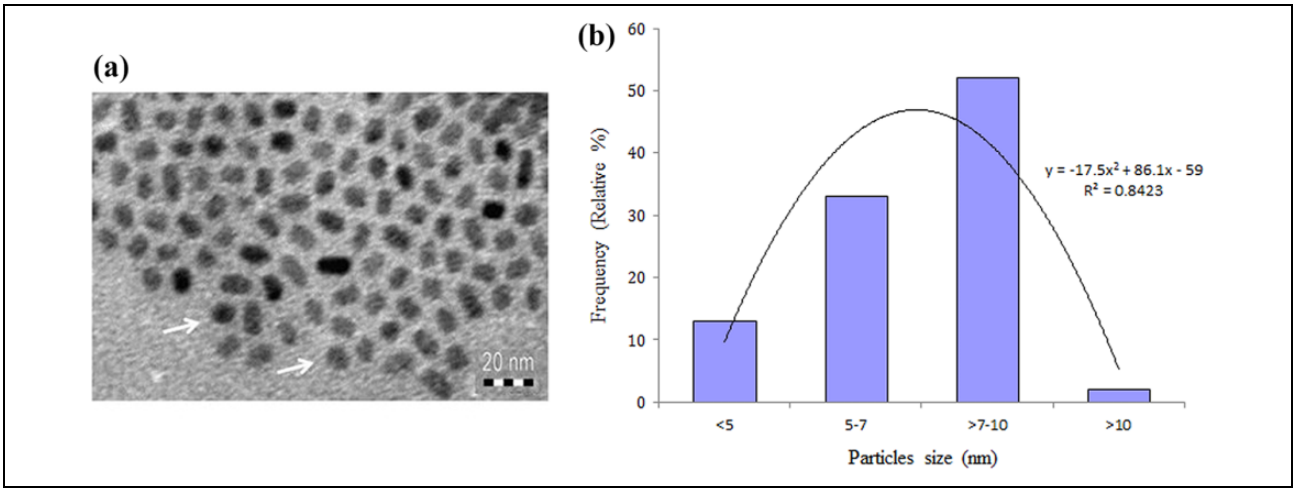

Figure 1. Characterization of CdTe QDs. (a) TEM image and (b) size distribution (%) of CdTe QDs generated by TEM image. TEM: transmission electron microscopy; CdTe QDs: cadmium telluride quantum dots.

ml) exposure was observed by Hoechst 33342 fluorescent stain. Hoechst 33342 solution was used to stain the exposed cells in six-well slides and the slides were incubated for 10 min in the dark at 37°C. Images of the nucleus were captured using a fluorescence microscope (Nikon Eclipse 80i, Japan).

Caspase-3 assay. The activity of caspase-3 was determined from the cleavage of the caspase-3 substrate I (*N*-acetyl-DEVD-*p*-nitroaniline). The *p*-nitroaniline was used as the standard. Cleavage of the substrate was monitored at a wavelength of 405 nm, and the specific activity was expressed in picomoles of the product (nitroaniline) per minute per milligram of protein.

Apoptosis through Annexin V-FITC/PI staining. Annexin Fluorescein isothiocyanate (V-FITC)/PI staining was used to detect apoptosis in HuH-7 cells after the exposure of respective test compound for 24 and 48 h. The cells were collected by trypsinization and washed with PBS and resuspended in binding buffer (10 mM HEPES/NaOH, pH 7.5 containing 140 mM NaCl and 2.5 mM $CaCl_2$) at a concentration of 10^6 cells/ml. Annexin V-FITC (5 µl) and PI (10 µl) were mixed to cell suspension (500 µl) and incubated for 30 min in the dark at room temperature. Then, cells were screened by flow cytometry (FACS Canto TM II, BD BioSciences, San Jose, California, USA). Fluorescence emitted by Annexin-V-bound FITC and DNA-bound PI in each event was detected as green and red fluorescence, respectively. Results were analysed by FACSDiva 6.1.2 software.

Comet test

DNA damage was measured by Comet test.[24] Briefly, cells (5.10^5)/well were placed in a six-well plate. Cells were treated with CdTe QDs (1, 5, 10 and 25 µg/ml) over 48 h. Cell viability was determined by the Trypan blue exclusion method,[25] and specimen having cell viability more than 85% was further processed for comet assay. Cell suspension (15 µl) was added with 0.5% LMPA (85 µl) and layered on plain glass slide, before being coated with a layer of 200 µl normal agarose (1%). Then, a third layer of LMPA (100 µl) was made. After solidification of the gel, the slides were dipped in lysis buffer (2.5 M NaCl, 100 mM Na_2 EDTA, 10 mM Tris pH 10 with 10% DMSO and 1% Triton X-100 added fresh) overnight at 4°C. The slides were then placed in a horizontal gel electrophoresis unit, immersed in fresh cold alkaline electrophoresis buffer (300 mM NaOH, 1 Mm Na_2EDTA and 0.2% DMSO, pH > 13.5) and left in the solution for 20 min at 4°C for DNA unwinding and conversion of alkali-labile sites to single-strand breaks. Electrophoresis was carried out using the same solution at 4°C for 20 min, using 16 V (0.8 V/cm) and 300 mA. The slides were washed three times with 0.4 M Tris buffer at (pH 7.5) to remove excess alkali and for observation of DNA damage, slide stained with 75 µl ethidium bromide (20 µg/ml) for 10 min. For positive control, the cells were treated ex vivo with 100 µM H_2O_2 for 10 min at 4°C. Two slides per concentrations prepared and 50 cells per slide (100 cells per concentration), and the slides were randomly scored using an image analysis system (Komet-5.5, Kinetic Imaging, Springvale Business Park, Belfast, Northern Ireland) attached to a fluorescent microscope

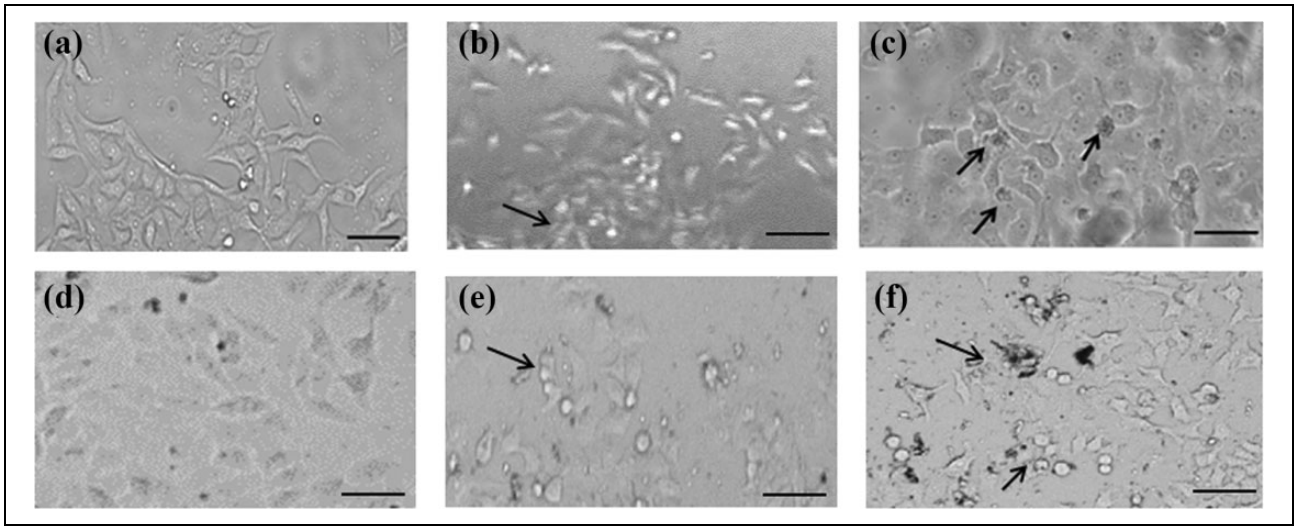

Figure 2. Morphology of HuH-7 cells: (a) HuH-7 cells without CdTe QDs exposure for 24 h; (b) HuH-7 cells after 10 μg/ml CdTe QDs exposure for 24 h; (c) HuH-7 cells after 25 μg/ml CdTe QDs exposure for 24 h; (d) HuH-7 cells without CdTe QDs exposure for 48 h; (e) HuH-7 cells after 10 μg/ml CdTe QDs exposure for 48 h; (f) HuH-7 cells after 25 μg/ml CdTe QDs exposure for 48 h. Arrow (→): shrunk and damaged HuH-7 cells. Scale bar, 400 μm. CdTe QDs: cadmium telluride quantum dots.

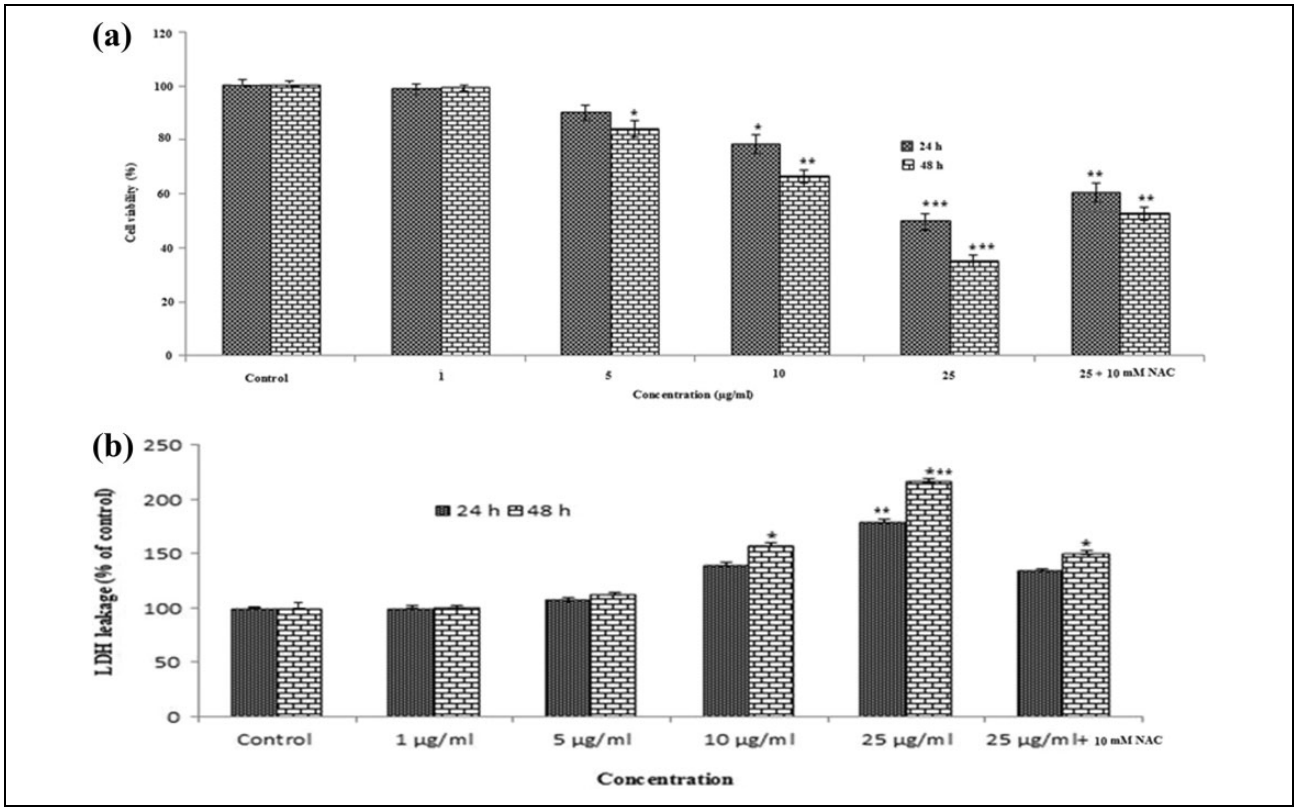

Figure 3. Cytotoxicity of CdTe QDs in HuH-7 cells for 24 and 48 h as assessed by (a) MTS and (b) LDH assays. Each value represents the mean ± SEM of three experiment. *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$ versus control. CdTe QDs: cadmium telluride quantum dots; SEM: standard error of the mean.

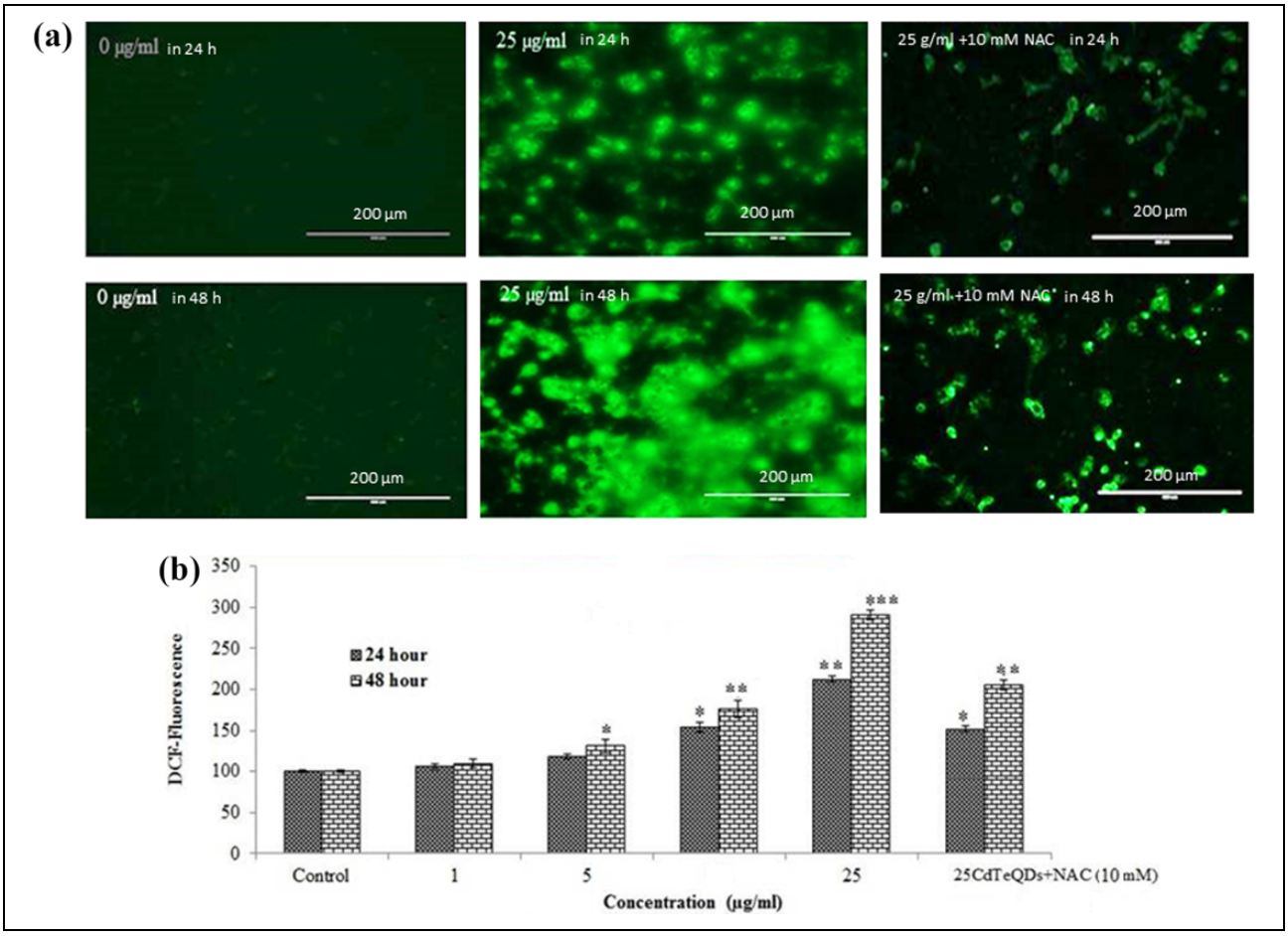

Figure 4. Generation of ROS induced by CdTe QDs. (a) The florescence image of HuH-7 cells treated with 25 µg/ml of CdTe QDs for 24–48 h and stained with DCFHDA. Bar = 200 µm. (b) % ROS production due to CdTe QDs in cells. HuH-7 cells. HuH-7 cells were pretreated with or without NAC (1.0 mM) for 1 h and then exposed to CdTe QDs (25 µg/ml) for 24 and 48 h. Images were snapped in phase contrast cum fluorescence microscope (Nikon. model 80i). Each value represents the mean ± SEM of three experiments. *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$ versus control. CdTe QDs: cadmium telluride quantum dots; SEM: standard error of the mean; ROS: reactive oxygen species.

(Leica, Germany) equipped with appropriate filters. The parameter selected for quantification of DNA damage was percent tail DNA (i.e. % tail DNA = 100 − % head DNA) as determined by the software.

Statistical analysis

Data were statistically analysed using one-way analysis of variance (SPSS 13.0) to find out the significant variation (*$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$) between concentrations and control.

Results

CdTe QDs characterization

CdTe QDs (1 mg/ml) was mixed in culture media. The average size of CdTe QDs was the 5 ± 1.04 and spherical shape as indicated in Figure 1. Hydrodynamic size and zeta potential were measured by DLS, and it was 25 nm and −10 mV, respectively.

Alteration in the morphology of HuH-7 cells

After exposure to CdTe QDs, the morphology of HuH-7 cells did not change at 1 µg/ml, but the shape of cells was slightly changed at 5 and 10 µg/ml (Figure 2(b) and (e)) for 24 and 48 h exposure. HuH-7 cells-exposed CdTe QDs (25 µg/ml) become shrunken, round shaped, deformed and detached from the surface in 24 and 48 h exposure (Figure 2(c) and (f)).

Cytotoxicity

Cytotoxicity of HuH-7cells due to exposure of CdTe QDs (0, 1, 5, 10 and 25 µg/ml) was observed in 24 and

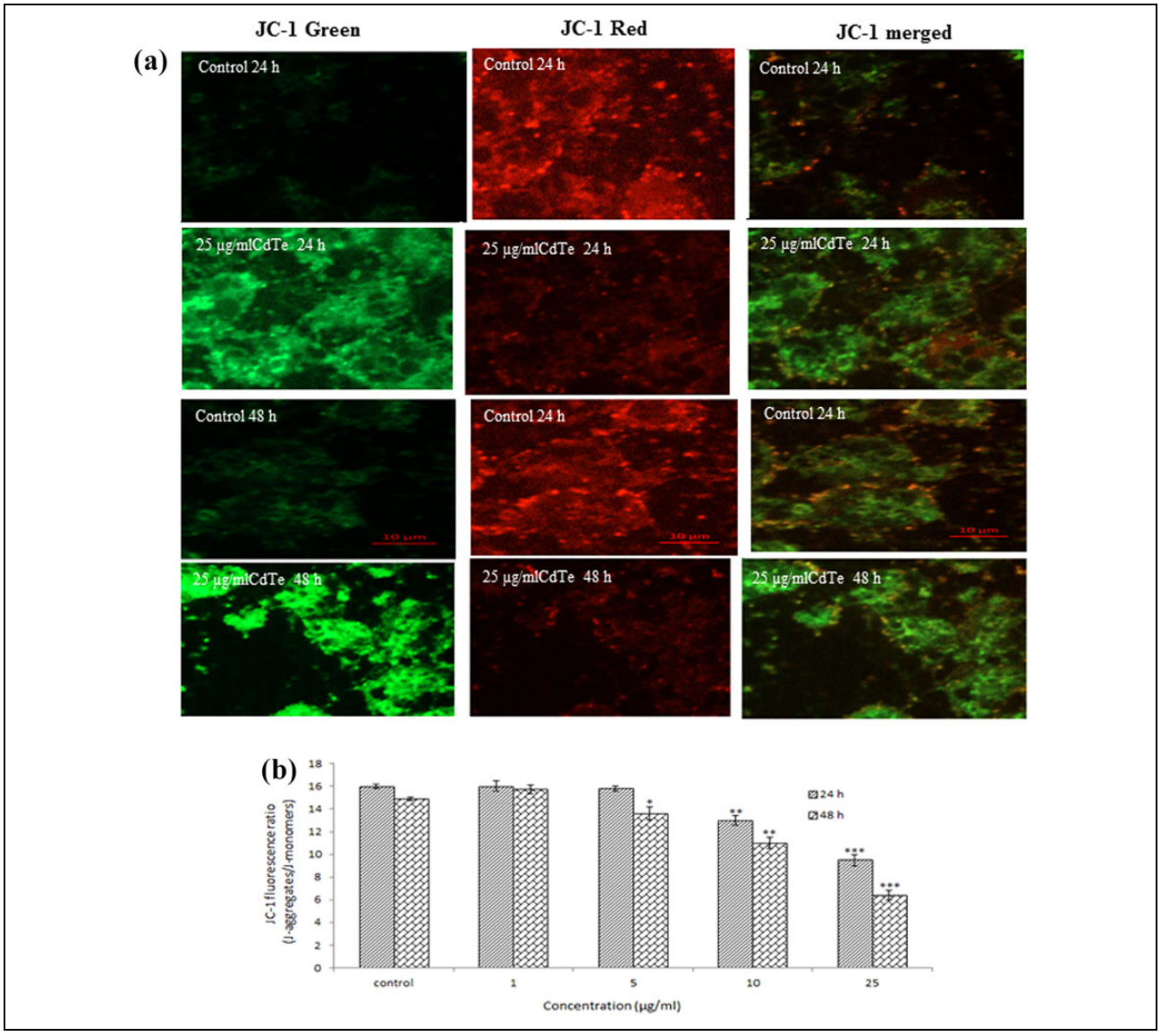

Figure 5. (a) Images representing NEVIP loss in HuH-7 cells after CdTe QDs exposure at concentrations of 25 µg/ml for 24 and 48 h. (b) Change in MMP in HuH-7 cells. Each value represents the mean ± SEM of three experiments. *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$ versus control. CdTe QDs: cadmium telluride quantum dots; SEM: standard error of the mean; MMP: mitochondrial membrane potential.

48 h (Figure 3). The results showed that QDs induced a significant dose- and time-dependent decrease in cell viability demonstrating that CdTe QDs had cytotoxicity. After exposure to CdTe QDs, the level of cytoplasmic LDH was increased indicating plasma membrane damage. After 2-h pre-incubation with 10 mM NAC, there was a significant decrease in toxicity compared to only CdTe QDs (25 µg/ml) exposed cells. These results demonstrated that NAC is effective on CdTe QDs induced toxicity in HuH-7 cells and ROS may play an important role in CdTe QDs-induced HuH-7 cell toxicity. Thus, cytotoxic effects of CdTe QDs in HuH-7 cells were dose- and time-dependent manner in both assays (Figure 3(a) and (b)).

ROS generation

To determine the production of ROS in HuH-7 cells exposed with CdTe QDs (1, 5, 10 and 25 µg/ml), DCFH-DA fluorescence dye was used. A significant increase of dichlorofluorescein (DCF) fluorescence intensity was seen in cells exposed to CdTe QDs at the concentrations of 5, 10 and 25 µg/ml. The cells were pre-exposed with NAC for 2 h, and the DCF

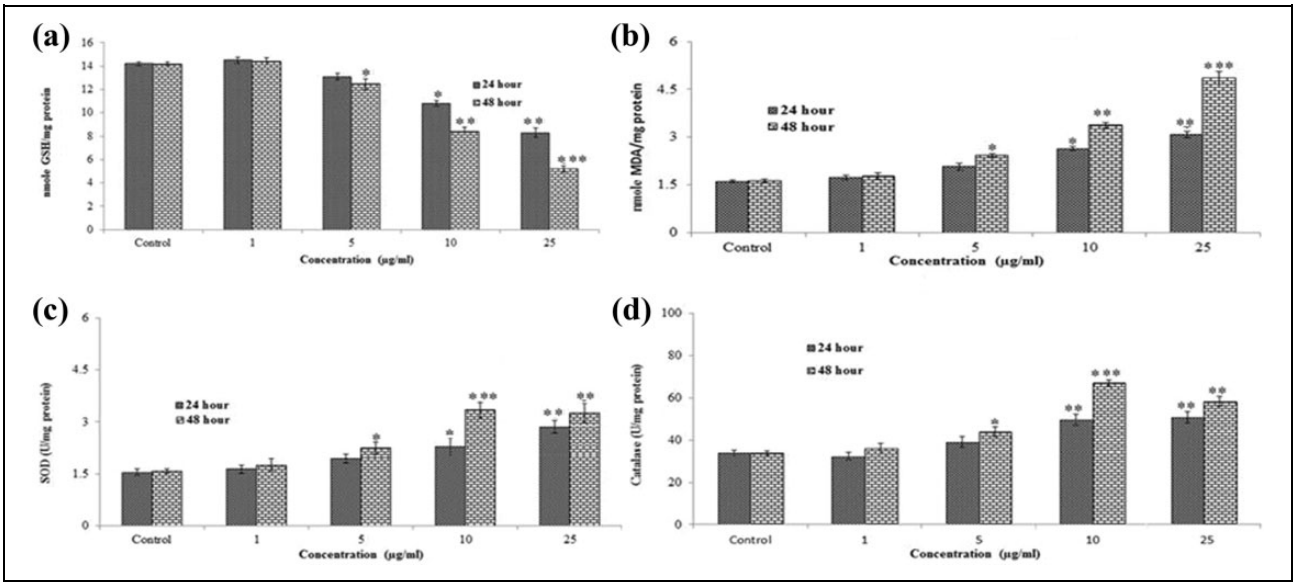

Figure 6. CdTe nanocrystals induced oxidative stress biomarkers. (a) GSH, (b) LPO, (c) SOD and (d) CAT in HuH-7 cells. Each value represents the mean $\pm$ SEM of three experiments. *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$ versus control. CdTe: cadmium telluride; GSH: glutathione; LPO: lipid peroxide; SOD: superoxide dismutase; CAT: catalase.

fluorescence intensity in cells was reduced as compared to CdTe QDs (25 µg/ml) exposed cells only (Figure 4). This result showed that CdTe QDs exposure induced more ROS production (0.21–0.29 fold) of over control cells.

Effects of CdTe QDs on MMP

JC-1 is extensively used to measure mitochondrial depolarization. The healthy mitochondria are stained with JC-1 probes and show bright and red emission. MMP downfalls in apoptotic cells, and JC-1 dye emits green fluoresce (Figure 5(a)). As a result of mitochondrial depolarization is indicated in the ratio of red/green emission intensity (Figure 5(b)). Reduction of intensity of red fluorescence demonstrates the loss of MMP (Figure 5(a)).

Oxidative stress

Glutathione is the best thiol complex, which plays various activities including protection against ROS and upkeep of protein thiol components in animal cells. In addition, GSH plays an important role in the antioxidant defence system of cells,[26] and its reductions indicate the oxidative stress. In this study, exposure of CdTe QDs significantly decreased GSH level in HuH-7cells over 48 h (Figure 6(a)). CdTe QDs-induced oxidative stress was further evidenced by depletion of GSH (Figure 6(a)) and elevation of LPO, SOD and CAT with dose- and time-dependent effect of CdTe QDs exposure (Figure 6(b)). Whereas the level of SOD and CAT was increased at lower concentration, but it slightly decreased at higher concentration of CdTe QDs (Figure 6(c) and (d)).

Apoptosis

HuH-7cells were exposed to CdTe QDs (1, 10 and 25 µg/ml) for 24 and 48 h. Apoptosis was determined using Hoechst 33342 staining. The stained unexposed cells and CdTe QDs-exposed cells were observed for chromosome condensation in HuH-7 cells (Figure 7(a)). The activity of caspase-3 was increased as the dose- and time-dependent manner (Figure 7(b)).

Flow cytometry analysis was carried out using Annexin V-FITC/PI staining to confirm the process of cell death due to CdTe QDs exposure. HuH-7 cells was exposed to CdTe QDs (25 µg/ml) for 24 and 48 h. The control cells were not stained with Annexin V-FITC and PI. However, the cells exposed with 25 µg/ml CdTe QDs for 24 and 48 h showed 29.39% and 36.9% apoptotic cells, respectively (Figure 8).

DNA single-strand breakage

As represented in Figure 9(a) and (b), upturns in DNA breakage were found at 5 µg/ml, 10 µg/ml and 25 µg/ml of CdTe QDs exposure. There was no significant difference in the amount of DNA breakage at 1 µg/ml

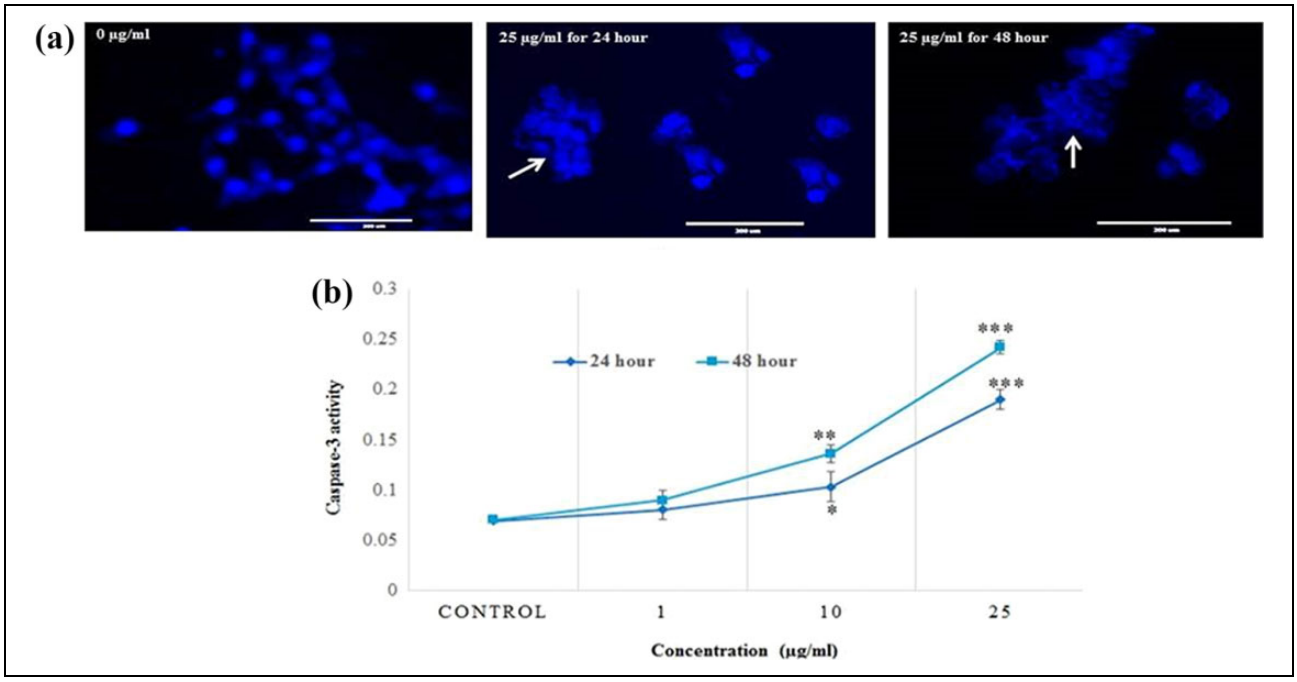

Figure 7. (a) Chromosomal condensation at 25 µg/ml for 24 and 48 h and (b) induction of caspase-3 activity in HuH-7 cells after exposure to CdTe QDs for 24 and 48 h. Each value represents the mean ± SEM of three experiments. *p < 0.05, **p < 0.01 and ***p < 0.001 versus control. Arrow (→) indicates fragmented chromosome. CdTe QDs: cadmium telluride quantum dots; SEM: standard error of the mean.

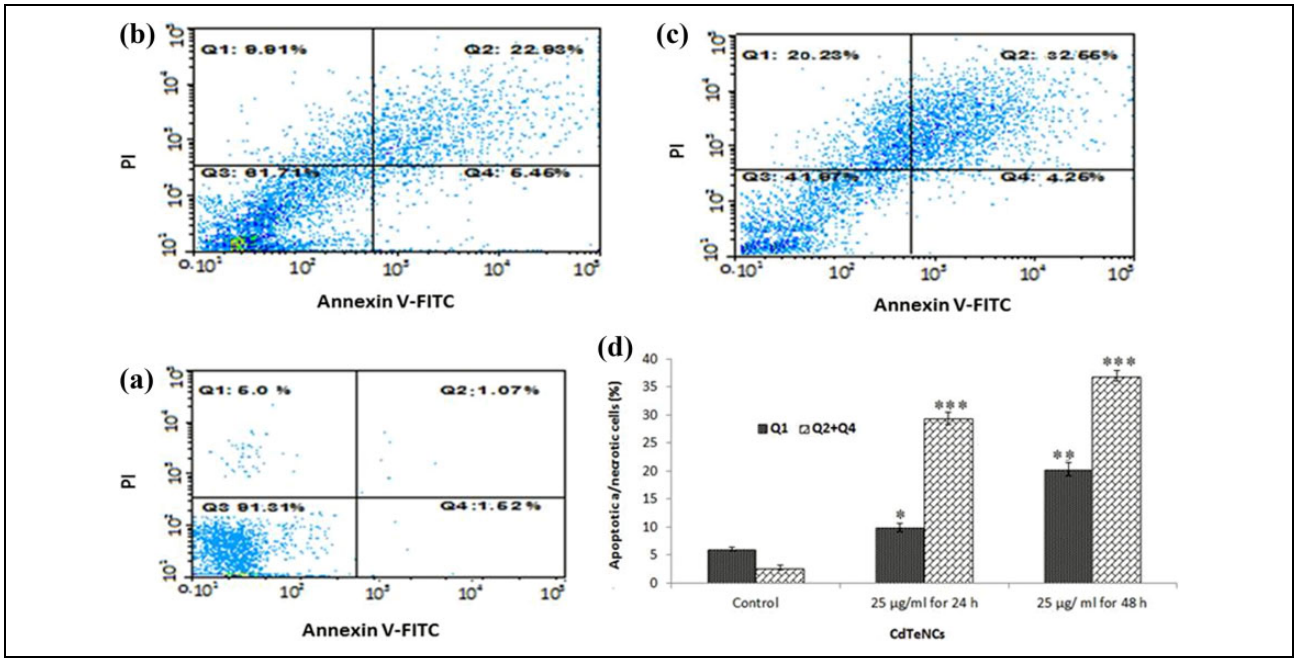

Figure 8. CdTe nanocrystals induced apoptosis in HuH-7 cells: (a) to (d) Flow cytometric analysis of Annexin V-FITC/PI-stained cells. (a) Control, (b) 25 µg/ml for 24 h, (c) 25 µg/ml for 48 h and (d) % of apoptotic and necrotic cells. Data represent mean ± SEM of three experiments. *p < 0.05, **p < 0.01 and ***p < 0.001 versus control. Representative dot plots of three independent experiments are presented. SEM: standard error of the mean.

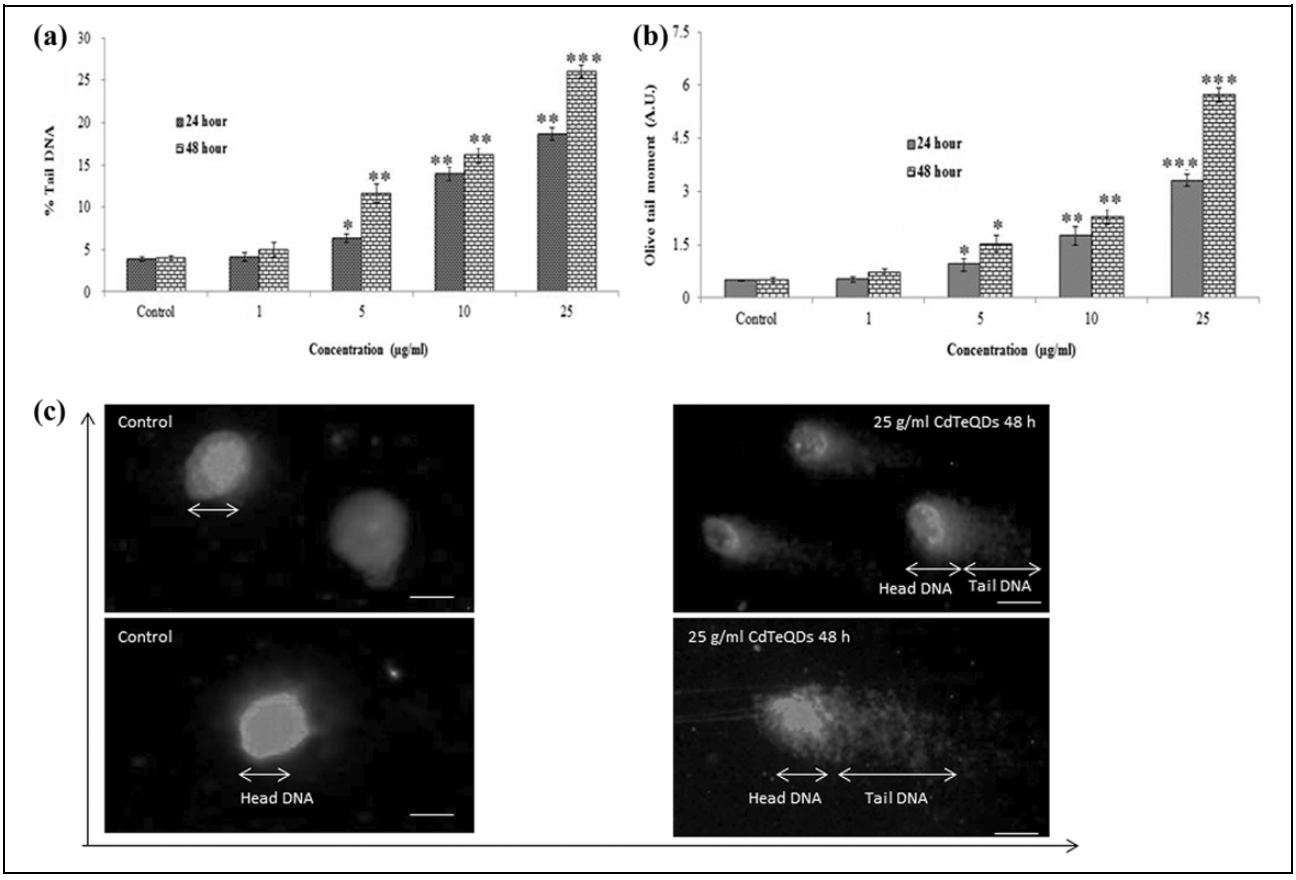

Figure 9. DNA strand breakage in HuH-7 cells due to CdTe QDs. (a) % Tail DNA, (b) olive tail moment and (c) photomicrograph of control and damaged DNA cell at CdTe QD 25 µg/ml for 24 and 48 h. Each value represents the mean ± SEM of three experiments. *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$ versus control. CdTe QDs: cadmium telluride quantum dots; SEM: standard error of the mean.

of CdTe QDs. Figure 9(c) is fluorescence photographs of comets electrophoresis of HuH-7 cells.

Discussion

In the present study, we found that CdTe QDs are cytotoxic to HuH-7 cells, and it has shown more toxicity with increased exposure to concentration and duration. ROS generation and oxidative stress induce the cytotoxic effect of CdTe QDs, and NAC defends HuH-7 cells from its damage. The size of CdTe QDs was observed using TEM, and it was spherical shaped with an average size 5 nm. Nanosized particles have a greater surface area as compared with larger particles of the same chemical composition could upturn more generation of ROS due to particle surface-dependent reaction.[27] CdTe QDs may increase ROS level through different pathways, first upon excitation, QDs can form electron–hole pairs to transfer an electron to oxygen[28]; second, the intracellular antioxidant system may be directly damaged via interaction with QDs; and third, cadmium ion released from QDs causes intracellular ROS elevation.[29] The production of intracellular ROS in HuH-7 cells in the current study is in accordance with the finding of Nguyen et al.,[30,31] in mammalian hepatocyte cells ($HepG_2$) for CdTe QDs. Mitochondria are a significant organelle in QDs-induced toxicity.[28] In this study, we have found that MMP was decreased at 25 µg/ml CdTe exposure, and toxicity shows a dose-dependent response.

The surplus ROS may destruct proteins, membrane lipids and DNA in cells.[32] Lovric et al.[33] has reported that QDs produced ROS in both the absence and presence of light. The CdTe QDs induced ROS generation as compared to control cells (Figure 4). Osseni et al.[34] reported that intracellular GSH depletion has been reported important to induce cellular oxidative stress, which is considered to be one main mechanism of cytotoxicity. GSH is widely distributed in many animal cells and is involved in ROS scavenge, protecting

against toxins, protein and DNA synthesis, maintenance of membrane integrity and regulation of enzyme activities.[35] In the GSH measurement results, we have found that GSH depletion in HuH-7 cells signalled the impairment of cellular antioxidant system. Besides, we found a rise in LPO, SOD and CAT while the drop in GSH level in HuH-7 cells after exposure to CdTe QDs, which shows a marker of oxidative stress. LPO can give rise to more radicals and fragments biomolecules with ROS. As we have observed in MTS assay, CdTe QDs showed more toxicity when their concentrations were high (>10 μg/ml), which suggests that the concentration plays a leading role in the cytotoxicity. Also, Liu et al.[36] has reported that CdTe QDs (below 10 μg/ml) were non-toxic for L929 cells, and on the other hand, it was highly toxic at above 10 μg/ml concentration. NAC was a well-known effective antioxidant and precursor of GSH.[37] It could protect cell metabolic activity from CdTe QDs toxicity in several aspects such as directly scavenge the ROS and promote the synthesis of GSH.[33] The protection of NAC to low concentration of CdTe QDs-treated cells reflected that NAC reduced oxidative stress in cells. CdTe QDs induce injury to the cell membrane as indicated by LDH assay. Apoptosis is a programmed process that can be triggered by different stimuli and is mediated by a cascade of enzymes.[38] To further understand the molecular mechanisms underlying the CdTe QDs treatment-mediated apoptosis in HuH-7 cells, we investigated apoptosis-related chromosome condensation in HuH-7 cells. CdTe QDs treatment induces cytochrome c release, causing caspase-3 activation. Subsequently, DNA fragmentation is induced during the cells apoptosis. Some studies have demonstrated that the presence of smaller DNA fragments is believed to reflect the release of nucleosomes from apoptotic cells and higher molecular weight DNA molecules are believed to reflect release from necrotic cells.[39] When free radicals come in close with the cellular organelles, they may oxidize and reduce macromolecules (DNA, lipids, proteins) resulting in significant oxidative damage to the cell.

In conclusion, the data confirm that CdTe QDs have the capacity to induce apoptosis and DNA fragmentation HuH-7 cells. The ROS production, GSH depletion and oxidative stress are probably its toxicity mechanisms. Future research will focus on the in vivo studies assessing the effects of CdTe QDs in hepatoma cells and explaining its toxicity mechanism.

Declaration of conflicting interests

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