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Curcumin and β-caryophellene attenuate cadmium quantum dots induced oxidative stress and lethality in *Caenorhabditis elegans* model system

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ABSTRACT

Curcumin (CUR) and β-caryophellene (BCP) are well known bioactive phytomolecules which are known to reduce oxidative stress in living organisms. Therefore, the present study was envisaged to explore the possible effects of CUR and BCP in suppression of cadmium quantum dots (CdTe QDs) induced toxicity in *Caenorhabditis elegans*. CdTe QD are luminescent nanoparticles extensively exploited for *in vivo* imaging, but long term bioaccumulation confer deleterious effects on living organisms. The 24-h LC_{50} and LC_{100} of CdTe QD were found to be 18.40 μg/ml and 100 μg/ml respectively. The CdTe QD exposure elevated HSP-16.2 expression mediating induction of the stress response. The CdTe QD lethality was due to increment in ROS and decline in SOD and GST expression. The present study demonstrates improved survival in BCP (50 μM) and CUR (20 μM) treated worms by over 60% ($P < 0.01$) and 50% ($P < 0.029$) in CdTe QD (100 μg/ml) exposed worms. Furthermore, BCP and CUR attenuate oxidative stress triggered by QD. The present study for the first time demonstrates CdTe QD toxicity remediation *via* BCP and CUR. The future investigations can unravel underlying protective effects of phytomolceules for remediating cyotoxicolgical effects of QDs.

1. Introduction

The development in nanotechnology in the past decade has changed the scenario of biomedical research. The engineered nanoparticles have emerged as a magnificent tool in biomedical diagnostics and research (Nune et al., 2009). The luminescent nanocrystals "quantum dots" (QD) possess unique optical properties, making them a promising candidate for *in vivo* imaging in biomedical sciences (Weng et al., 2006). QDs containing cadmium telluride (CdTe QDs) offer great potential in medical and molecular imaging (Medintz et al., 2005). Despite various advantages of CdTe QD over fluorescent dyes the toxic effects are still not well elucidated. Therefore, concern has been raised regarding risk assessment of CdTe QD as cadmium is known to be highly toxic to living systems (Hamilton, 2004). The ecotoxicological and *in vivo* toxic effects are yet to be explored extensively. The intracellular enzymes and microenvironment can destabilize the QDs which lead to the generation of cytotoxic nanoparticles (Soenen et al., 2011). Although QDs are non-toxic, but long term bioaccumulation yield "naked" QDs which confer deleterious effects in living organisms (Prasad et al., 2012). The destabilized CdTe QD yield significant amount of reactive oxygen species *in vitro* (Lovric et al., 2005). The toxicity of CdTe QD has been proposed to be associated with the oxidation reaction of the metal core (Samia et al., 2006). This reaction generates reactive oxygen species (ROS) and cadmium ions (Cd^{2+}) through photo-oxidation reaction, which contribute to QD-induced cellular damage (Yildirimer et al., 2011). Earlier studies have also suggested that the toxicity of CdTe QDs could arise from several intrinsic properties such as size, chemical composition, reactivity of the inner core metals and surface-coating components (Hardman, 2006). Oxidative stress can induce apoptosis, necrosis, membrane lipid peroxidation and ultimately cell death (Nguyen et al., 2013). To this end, we evaluated CdTe QDs toxicity using *C. elegans* as biosensor and effect of two bioactive phytomolecules, beta-caryophyllene (BCP) and curcumin (CUR) on CdTe QDs induced toxicity. A number of earlier reports have

Abbreviations: QD, quantum dots; CdTe QD, cadmium quantum dots; CUR, curcumin; BCP, β-caryophellene; HSP, heat shock protein; GST, glutathione-S-transferase; SOD, superoxide dismutase; ROS, reactive oxygen species; NGM, nematode growth medium; DMSO, dimethy sulphoxide.

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shown that the active constituents of different plant selections are effective in improving stress tolerance against ROS production (Barrera, 2012; Pandey et al., 2013). BCP possesses several biological activities such as anti-inflammatory, antibiotic, antioxidant, anti-carcinogenic and local anaesthetic activities (Wiegant et al., 2009; Calleja et al., 2013). On the other hand curcumin is a potent antioxidant and possesses the capacity to mitigate age-associated cellular damage induced by the production of ROS (Pant et al., 2014; Bengmark, 2006; Nishino et al., 2004). The present study highlights the use of *C. elegans* model system to evaluate the systematic dose and time dependent exposures of manufactured QDs. This study for the first time demonstrates toxic effects of CdTe QDs and its remediation with antioxidant molecules (BCP and CUR). The study has future implication in risk assessment of nanotized products in quick and inexpensive manner and their remediation by phytomolecules.

2. Materials and methods

2.1. C. elegans *strains, culture and maintenance*

C. elegans strains *viz.* N2-Bristol (wild type); CF1553, muIs84 [pAD76 (*sod-3*::gfp)]; CL2166 *gst-4*::gfp (dvls19), CL2070, dvIs70 (*hsp-16.2*::gfp) and *Escherichia coli* OP50 were procured from the *Caenorhabditis* Genetics Centre (CGC), University of Minnesota (Minneapolis, MN, USA). The strains were cultured and maintained at 20 °C on nematode growth medium (NGM) seeded with *E. coli* OP50 bacteria using the standard protocol (Maheshwari et al., 2006). The age synchronized culture was obtained by sodium hypochlorite treatment, which kills adult worms, but not their eggs (Brenner, 1974).

2.2. Test compound

CdTe QD (Reinste, 3–7 nm, 520 nm λ emission) was dissolved in ultrapure Milli-Q water to prepare a 100 μg/ml stock. The suspensions (CdTe QD) were prepared freshly before toxicity testing. BCP and CUR (Sigma–Aldrich) separately were dissolved in 10% dimethyl sulfoxide (DMSO) to prepare a 10 mM stock. BCP and CUR were earlier found to be effective against oxidative stress and intracellular ROS (Calleja et al., 2013). The L1 stage worms were treated with indicated CdTe QD (100 μg/ml), CdTe QD + BCP (100 μg/ml + 50 μM) and CdTe QD + CUR (100 μg/ml + 20 μM) and 0.05% DMSO was used as a vehicle control. The plates were freshly prepared 2 h before use and seeded with *Escherichia coli* OP50 one day before use (Fabian and Johnson, 1994).

2.3. Toxicity assay

Toxicity assay was performed with different concentrations of CdTe QD (1, 3, 5, 7, 10, 25, 50 and 100 μg/ml) at 20 °C (Zhang et al., 2007; Hartwig et al., 2009). The day 2 age synchronized wild type (N2) worms were transferred to 24 well plate containing respective doses of CdTe QD (1, 3, 5, 7, 10, 25, 50 and 100 μg/ml) in Milli-Q whereas 0.1% DMSO served as control. No obvious toxic effect of CdTe QD was observed at 1–7 μg/ml but at 10–100 μg/ml was found to be toxic to worms. Therefore, these doses (10–100 μg/ml) were utilized for assessing CdTe QD induced toxic effects.

2.4. Visualization of uptake of the quantum dot

The uptake of fluorescent CdTe QD by *C. elegans* was confirmed by visualizing it under a fluorescence microscope (Khare et al., 2011). The CdTe QD (100 μg/ml) exposed worms were anesthetized with 10% sodium azide (NaN3, Sigma) and mounted on the 3% agarose pad. The images were captured using a DAPI filter (with excitation at 340–380 nm and emission at 435–485 nm) under a fluorescence microscope DMI 3000 B (Leica, Wetzlar, Germany).

2.5. Fecundity assay

The age synchronized L1 worms were exposed to CdTe QD (100 μg/ml), CdTe QD + BCP (100 μg/ml + 50 μM) and CdTe QD + CUR (100 μg/ml + 20 μM) and control (without treatment) were incubated for 72 h. The N2 worms were individually transferred to a fresh plate with or without treatments each day until the reproduction ceased. The offspring of each animal was counted at the L2 and L3 stage (Calleja et al., 2013).

2.6. Oxidative stress assay

To assess the effect of CdTe QD on oxidative stress resistance in worms, juglone (5-hydroxy-1, 4-napthoquinone, Sigma–Aldrich), an intracellular ROS generator was used (Contreras et al., 2012). The age synchronized worms were raised from L1 to adult with CdTe QD (100 μg/ml), CdTe QD + BCP (100 μg/ml + 50 μM) and CdTe QD + CUR (100 μg/ml + 20 μM) and 0.05% DMSO respectively. The treated or untreated day 2 adult worms were transferred to 24-well plate containing liquid NGM and oxidative stress was induced by an acute, lethal concentration of juglone (250 μM) in a total volume of 300 μL per well. The worms were incubated at 20 °C and scored for viability for 24 h of continuous exposure.

2.7. Determination of intracellular ROS level

The age synchronized L1 larva were transferred to NGM plates seeded with *E. coli* OP50 lawn and CdTe QD (100 μg/ml), CdTe QD + BCP (100 μg/ml + 50 μM) and CdTe QD + CUR (100 μg/ml + 20 μM) and 0.05% DMSO at 20 °C respectively. The intracellular reactive oxygen species levels were quantified using 2,7-dichlorofluorescin diacetate (H2DCF-DA: Sigma) (Back et al., 2012). The day 4 of live adult worms were collected in 300 μL of PBS (phosphate buffer saline) with 0.1% Tween-20 (PBST) and washed three times with PBST. Worms were transferred to 96 well plate in the presence of 50 μM H2-DCFDA in PBS (Held, 2012). The fluorescence was recorded after every 20 min for 2 h 30 min at 37 °C using a fluorescence micro plate reader (Spectramax M2: Molecular devices) at excitation 485 nm and emission 530 nm.

2.8. GFP reporter assay

This transgenic GFP reporter driven by *hsp-16.2* heat-shock promoter (*Phsp-16.2*::gfp) can be used as an early signal for intolerability or toxicity to the organism. The increment in intracellular ROS compromises cellular redox defense mechanism, which leads to oxidative stress in organisms (Buchter et al., 2013). The antioxidant enzymes such as SOD and GST protects organism from oxidative stress (Back et al., 2012; Held, 2012; Buchter et al., 2013; Poljsak et al., 2013). The expression of HSP-16.2, SOD-3 and GST-4 was visualized and quantified using GFP tagged CL2070, CF1553 and CL2166 transgenic strains (Calleja et al., 2013). The respective transgenic strains were raised on *E. coli* OP50 spotted NGM plates containing CdTe QD + BCP(100 μg/ml + 50 μM), CdTe QD + CUR (100 μg/ml + 20 μM), CdTe QD (100 μg/ml), and 0.05% DMSO control at 20 °C. For quantifying the GFP expression, the day 2 CdTe QD exposed CL2070 (*hsp-16.2*::GFP), CF1553 (*sod-3*::gfp) CL2166 (*gst-4*::gfp) and control worms were anesthetized with 2% sodium azide and mounted on glass slides coated with 3% agarose pad. The worms were visualized under Leica fluorescence microscope DMI 3000 B (Leica, Wetzlar, Germany) using GFP filter set (with excitation at 365 nm and emission at 420 nm). The GFP fluorescence was measured by digital imaging using 20× objective