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ORIGINAL ARTICLE

Synthesis of water soluble CdS nanoparticles and study of their DNA damage activity

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Abstract This study reports a novel method for preparation of water soluble CdS nanoparticles using leaf extract of a plant, *Asparagus racemosus*. The extract of the leaf tissue which worked as a stabilizing and capping agent, assisted the formation of nanoparticles. Nanoparticles were characterized using a UV–vis spectrophotometer, Photoluminescence, TEM, EDAX, XRD and FT-IR. Transmission electron microscopy followed by selected area electron diffraction pattern analysis indicated the formation of spherical, polydispersed, crystalline, CdS of diameter ranging from 2 to 8 nm. X-ray diffraction studies showed the formation of 1 1 1, 2 2 0 and 3 1 1 planes of face-centered cubic (fcc) CdS. EDAX analysis confirmed the presence of Cd and S in nanosphere. The cytotoxicity test using MTT assay as well as DNA damage analysis using comet assay revealed that synthesized nano CdS quantum dots (QDs) caused less DNA damage and cell death of lymphocytes than pure CdS nanoparticles.

1. Introduction

Semiconductor nanoparticles have diverse applications such as fluorophore for biomedical imaging (Arya et al., 2005; Yu et al., 2006) and optoelectronic transistor components (Brown et al., 2001). Quantum dots (QDs)-CdS, a typical II–VI semiconductor, owing to their unique optical properties, photocatalytic and tunable photoluminescence, have received much interest (Niemeyer 2003). Fluorescent quantum dots can be conjugated with bioactive moieties (e.g., antibodies, receptor ligands) to target specific biological events and cellular structures such as labeling neoplastic cell (Gao et al., 2004), peroxisomes (Colton et al., 2004), DNA (Dubertret et al.,

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2002) and cell membrane receptor (Lidke et al., 2004). Bioconjugated QDs are also being explored as a tool for site specific gene and drug delivery (Scherer et al., 2001). The property of quantum dots as photosensitizer in treatment of cancer has been studied (Juzenas et al., 2008). The functionalized water soluble CdS nanoparticles were used as fluorescence probe in the determination of bovine serum albumin (BSA), uracil and thymine (Wang et al., 2002; Lu et al., 2012) and as fluorescence labeling material for live cell imaging (Wei et al., 2012). Glucose oxidase (GOD) was immobilized onto the nano CdS through crosslinking with chitosan (CS), acted as glucose biosensor (Qian et al., 2012). The CdS QDs/chitosan composite films exhibited a highly efficient photocatalytic activity for decolorization of methyl orange (MO) solution under visible light irradiation (Jiang et al., 2012). An activity of H_2 evolution was greatly improved when nano CdS photocatalysts were modified by polypyrrole (Zhang et al., 2012). Other applications of CdS include its use in light emitting diode (Murai et al., 2005), photo detector (Wang et al., 2006), sensor (Ponzoni et al., 2005) and address decoder (Zhong et al., 2003). There has been increasing interest in exploiting living organisms and biological molecules for synthesizing inorganic materials (Prasad et al., 2011, 2012, 2014; Prasad and Selvaraj, 2014). The chitosan, from ascospore wall of *Saccharomyces cerevisiae* (Li and Du 2003), a yeasts like *Saccharomyces pombe* (Kowshik et al., 2002), immobilized fungus *Coriolus versicolor* (Sanghi and Verma, 2009), and extracellular enzyme secreted by the fungus *Fusarium oxysporum* have been employed in the synthesis of CdS nanoparticles (Basavaraja et al., 2008). The study involving bacterial synthesis of CdS nanocrystals revealed that *Escherichia coli* could form CdS nanocrystals intracellularly (Sweeney et al., 2004). In another report, synthesis of cadmium sulfide nanoparticles by photosynthetic bacteria *Rhodopseudomonas palustris*, immobilized *Rhodobacter sphaeroides* (Hong et al., 2009a,b) and C-phycoerythrin from the marine cyanobacteria (Ali et al., 2012) has been demonstrated. Cadmium is classified as a carcinogen and is known to affect genome stability via inhibition of DNA repair and generation of free radical-induced DNA damage (Rzigalinski and Strobl 2009). Free radical formations due to the exposure of quantum dots have been considered as a primary mechanism for cytotoxicity (Ipe et al., 2005). Comet assays detect DNA damage which occurs within live mammalian cells. Hoshino et al. (2004) have reported acute genotoxicity of QD in mammalian cells using the comet assay. The present work aims to synthesize relatively benign CdS quantum dots, using biological precursors.

2. Material and methods

2.1. Preparation of leaf extract

Fresh and young leaves (5 g) of *Asparagus racemosus* plant were macerated in 20 ml of Tris–Cl (20 mM, pH 7.5) with the help of mortar and pestle. Thick slurry of leaf thus recovered was subjected to centrifugation at 10,000 rpm for 5 min at 4 °C. The supernatant was transferred into fresh sterile centrifuge tubes followed by its preservation in refrigerated condition. The aqueous extract of *A. racemosus* leaf obtained in this manner was used as a capping agent in the synthesis of cadmium sulfide nanoparticles. All the chemicals used in this experiment were procured from Sigma Chemicals Co. (St. Louis, USA) unless mentioned otherwise.

2.2. Synthesis of CdS nanoparticles and photoluminescence studies

In a typical synthesis of cadmium sulfide nanoparticles, leaf extract (5 ml) was added into 50 ml cadmium chloride (2 mM) solution. Sodium sulfide (50 ml, 2 mM) dissolved in deionized water was added drop wise into the solution of cadmium chloride under magnetic stirring. The content was later on placed on to a rotatory orbital shaker operating at 200 rpm, 30 °C for 12 h in dark condition. The formation of the particle was monitored by sampling an aliquot (3 ml) of the mixture after 12 h, followed by measurement of the UV–vis spectra using a spectrophotometer (OPTIZEN 3220, Korea). In order to find the absorption maximum or λ_{max}, a spectral scanning analysis was carried out by measuring optical density of the content from wavelength, 250–700 nm. Photoluminescence spectra were recorded in a Varian (Cary Eclipse) spectrofluorimeter using 90° illumination. Initially, prescan was performed to find out the excitation and emission maxima for the CdS nanoparticles. The excitation and emission slit widths were kept at 5 and 10.0 nm, respectively. The entire scanning was performed at the speed of 600 nm/min. Data were analyzed using the WINFLR software.

2.3. Sample preparation for transmission electron microscopy (TEM), energy dispersive X-ray spectroscopy (EDAX), X-ray diffraction (XRD) and Fourier transformed infra red spectroscopy (FT-IR) study

Transmission electron microscopic (TEM) analysis was performed with Techni 20 (Philips, Holland). A thin film of the sample was prepared on a carbon coated copper grid by dropping a very small amount of the sample on to the grid. The aqueous solution of QDs was centrifuged at 13,000 rpm for 10 min. The pellet thus recovered was subjected to washing by its re-suspension in deionized water followed by centrifugation at 13,000 rpm for 10 min, to remove possible organic contamination present in nanoparticles. Finally, pellet was freeze dried using a lyophilizer (Labconco, Kansas, Missouri). The powder of synthesized nanoparticles obtained in this manner was subjected to elemental analysis using a scanning electron microscope (Philips, Netherlands) equipped with energy dispersive X-ray spectroscopic system (EDAX-XL-30) operating at 15–25 kV and X-ray powder diffraction study. XRD data were collected using a Phillips PW1710 diffractometer with Cu-Kα radiation operating at 40 kV, 35 mA in step scan mode, between 5° and 75° and with a counting time of 8 s per step. Disk of 100 mg KBr containing 1% freeze dried CdS powder served as material for recording transmission spectra. Spectral scan analysis was carried out at wave number ranging from 400 to 4000 cm^{-1} by using a FT-IR spectrometer (Perkin Elmer, Spectrum GX, USA) with a resolution of 0.15 cm^{-1} to evaluate functional groups of the leaf extract that might be involved in particle formation process.

2.4. Isolation of lymphocytes

Blood sample (2 ml) from a healthy human donor was collected in a conical centrifuge tube. The tube was centrifuged at 2000 rpm for 15 min. The buffy coat was collected in a fresh micro-centrifuge tube and washed with NH_4Cl (1 ml, 0.85%)

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followed by centrifugation at 2000 rpm for 15 min to remove contaminant RBC. Lymphocytes became visible in the form of white pellet, which was subsequently resuspended in PBS (Phosphate buffer saline) and stored at 4 °C.

2.5. CdS treatment and MTT assay

The cell viability assay as well as CdS treatment was carried out in a 96 well micro titer plate. Ten thousand cells (~20 µl WBCs, White Blood Cells) were treated with 0.2 µg of pure CdS (~2.9 nm) followed by incubation at RT for 1 h. In next set up, white blood cells suspended in PBS were mixed with synthesized CdS nanoparticles (final conc. 0.01 µg/µl). Incubation of this mixture was performed for similar duration at RT. Solution of 3-(4,5-dimethyl thiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT, 5 mg/ml) was prepared by dissolving the reagent to phosphate buffer saline (PBS) followed by filter sterilization using a 0.2 µm filter paper. Solution of MTT (20 µl) was added to each sample including untreated lymphocytes which served as control. Furthermore, 20 µl of MTT mixed with PBS acted as a blank in this study. The plate was incubated at 37 °C for 5 h. DMSO (200 µl) was added to each well followed by thorough pipetting for complete dissolution of formed crystals. Finally, the plate was subjected to ELISA micro plate reader, Victor X3 (Perkin Elmer, USA) for measurement of absorbance at 550 nm.

2.6. Comet assay

The procedure of comet assay was adopted from a published article of Dhawan et al., 2002. Briefly, two different kinds of agarose, low melting point agarose (LMPA, 0.5% in PBS) and normal melting agarose (NMA, 1% in PBS) were prepared separately. A conventional pathological slide was dipped up to one-third into NMA agarose and gently removed. LMPA (25 µl) and WBC cells (25 µl) were mixed together and layered over the slide followed by gentle pressing of the mixture using a cover slip. Slide was further placed in freshly prepared cold lysis solution (2.5 M NaCl + 100 mM, EDTA + 10 mM, Trizma base + 1%, Triton X-100 and 10% DMSO) for 2 h, at 4 °C. After removing slide from the lysis solution, it was placed in a horizontal electrophoresis tank filled with freshly made electrophoresis buffer, pH > 13 (300 mM NaOH + 1 mM EDTA). Power supply was turned on to 24 volts (~0.74 V/cm) and the current was adjusted to 300 mA by raising or lowering the buffer level. Slides were electrophoresed for 30 min followed by their neutralization using neutralization buffer (0.4 M Tris–Cl, pH 7.5). Slides were stained with 80 µl 1X ethidium bromide and rinsed with chilled distilled water to remove excess stain. The DNA damage analysis was carried out using an inverted styled fluorescent microscope equipped with epiilluminator (Olympus, IX70, USA).

3. Results and discussion

3.1. UV–vis spectroscopy and photoluminescence of CdS nanoparticles

Mixing the solution of $CdCl_2$ and Na_2S resulted in the formation of CdS nanoparticles. Initially, at 0 h mixture was light green (Fig. 1a) which ultimately turned into dark yellow after

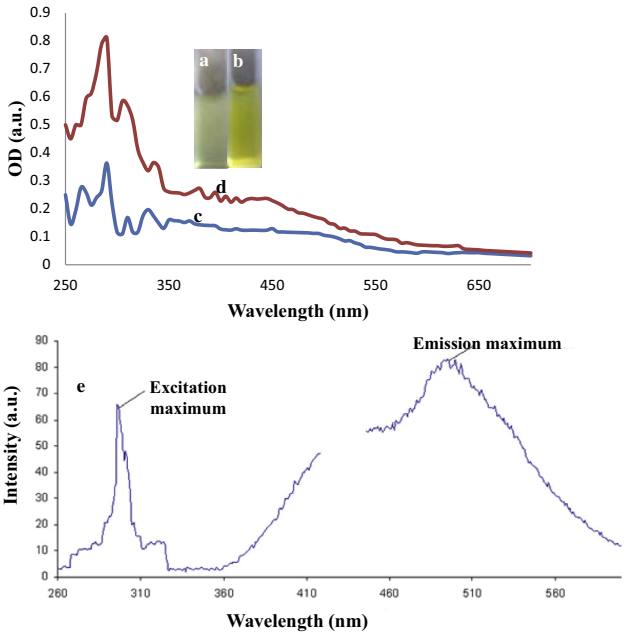

Figure 1 A visible observation of change in color during the CdS nanoparticle formation. A cuvette containing 2 mM $CdCl_2$ and *Asparagus racemosus* plant leaf extract, immediately after addition of 2 mM of Na_2S (final con. 1 mM) (a). A yellow color appeared when mixture was incubated for 12 h. UV–vis spectra of QD nanoparticles synthesized using plant leaf extract (c) at 0 h, and (d) at 12 h. Photoluminescence spectra of QD nanoparticles synthesized using leaf extract (g).

12 h (Fig. 1b). The synthesis of CdS leading to change in color can be attributed to surface plasmon resonance of CdS nanoparticles (Ali et al., 2012). In case of control experiment where leaf extract was not added to $CdCl_2$, insoluble yellowish precipitate of CdS was observed. The spectral analysis of CdS nanoparticles was performed at 0 h (Fig. 1c) and 12 h (Fig. 1d). The 0 h spectra however showed an absorbance maximum but there was significant increase in the absorbance at 290 nm after 12 h of incubation period. A small absorption peak was observed at 320 nm which could be due to heterogeneity in size during the synthesis of nanoparticles. The optical property synthesized CdS nanoparticles were evaluated by recording photoluminescence spectra. Fig. 1e, shows photoluminescence spectra of CdS nanoparticles synthesized using leaf extract. The excitation peak was found at 295 nm while emission peak was observed at 495 nm (Wang et al., 2002; Wei et al., 2012). The excitation peak at 295 nm, very well correlated with absorption maxima recorded with a UV–vis spectrophotometer (290 nm).

3.2. TEM, EDAX, XRD and FT-IR study

The TEM analysis of solution indicated the formation of CdS nanoparticles. Fig. 2(a) showed that size of generated particles using leaf extract ranged from ~2 to 8 nm. The formation of variable size of particles suggested that leaf extract could form polydispersed nanoparticles. Fig. 2(b) shows selected area electron diffraction (SAED) pattern of CdS nanoparticles. Results indicated that particles were crystalline in nature as diffraction

ring appeared which correspond to diffraction angle of 111, 220 and 311, respectively. The X-ray diffraction patterns obtained for CdS nanoparticles synthesized using leaf extract is shown in Fig. 3(a). The XRD diffractogram contained three prominent peaks that were clearly distinguishable. All of them can be perfectly indexed to crystalline CdS not only in peak position, but also in their relative intensity. The peaks with 2θ values of 27.5, 43.4 and 52.6 corresponded to the crystal planes of 111, 220 and 311 crystalline CdS, respectively (Li and Du 2003). Sizes of crystallite were estimated using Scherrer's formula, $D = K\lambda/\beta\cos\theta$, where the constant K is taken to be 0.94, λ is the wavelength of X-ray, and β and θ are the half width of the peak and half of the Bragg angle, respectively. Using the equation, the crystallite sizes were found to be in the range of 6–10 nm. EDAX analysis gives qualitative as well as quantitative status of elements that may be involved in the formation of nanoparticles. Fig. 3(b) shows the elemental profile of synthesized nanoparticles using the leaf extract.

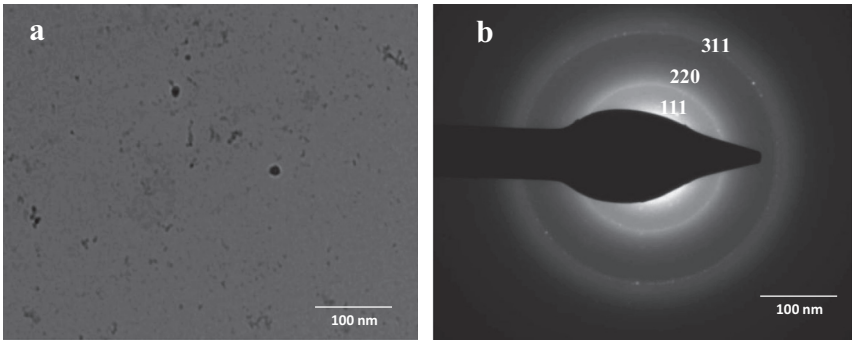

Figure 2 TEM micrographs recorded from drop-cast films of CdS nanoparticle solution formed by mixing and incubation of $CdCl_2$ and Na_2S solution with *Asparagus racemosus* leaf extract (a). Selected area electron diffraction (SAED) pattern recorded from the CdS nanoparticles (b). The bar scale in photomicrograph shows 100 nm.

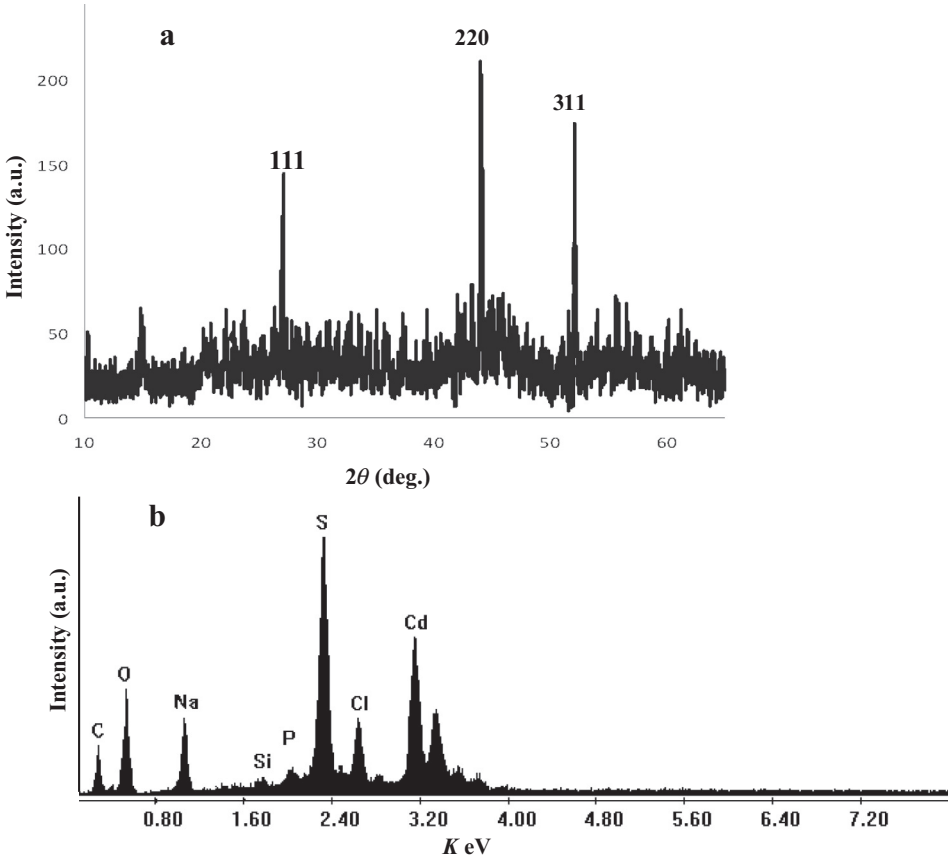

Figure 3 XRD diffractogram of CdS nanoparticles prepared using *Asparagus racemosus* leaf extract (a), EDAX spectrum of synthesized CdS nanoparticles (b).

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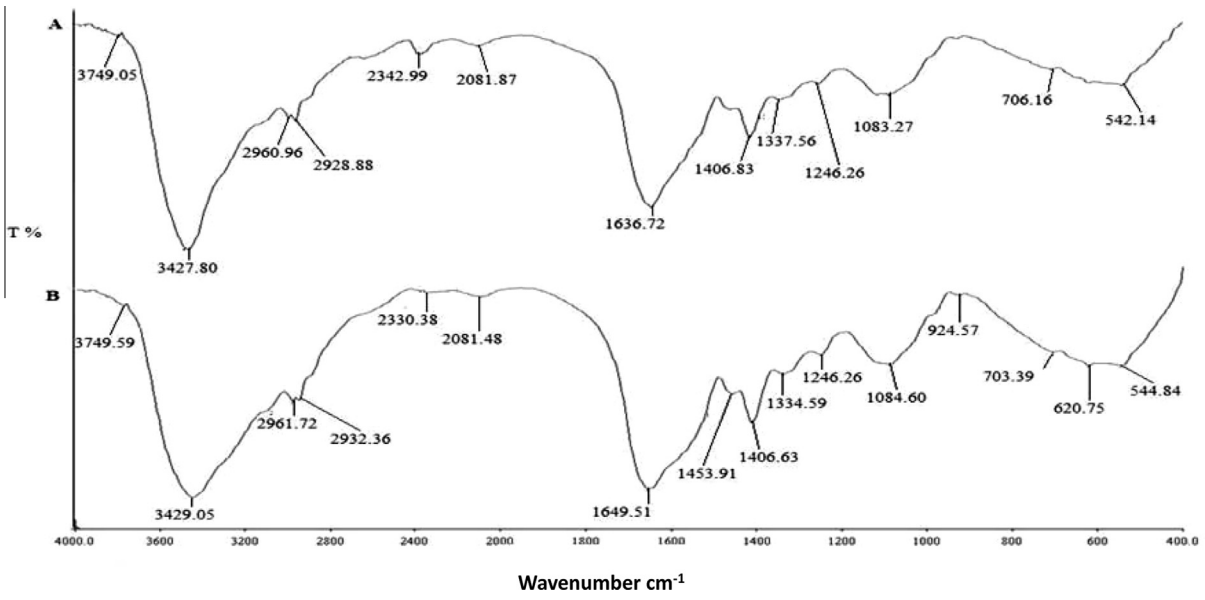

Figure 4 FT-IR spectrum of CdS treated *Asparagus racemosus* leaf extract (B), and untreated leaf extract (A).

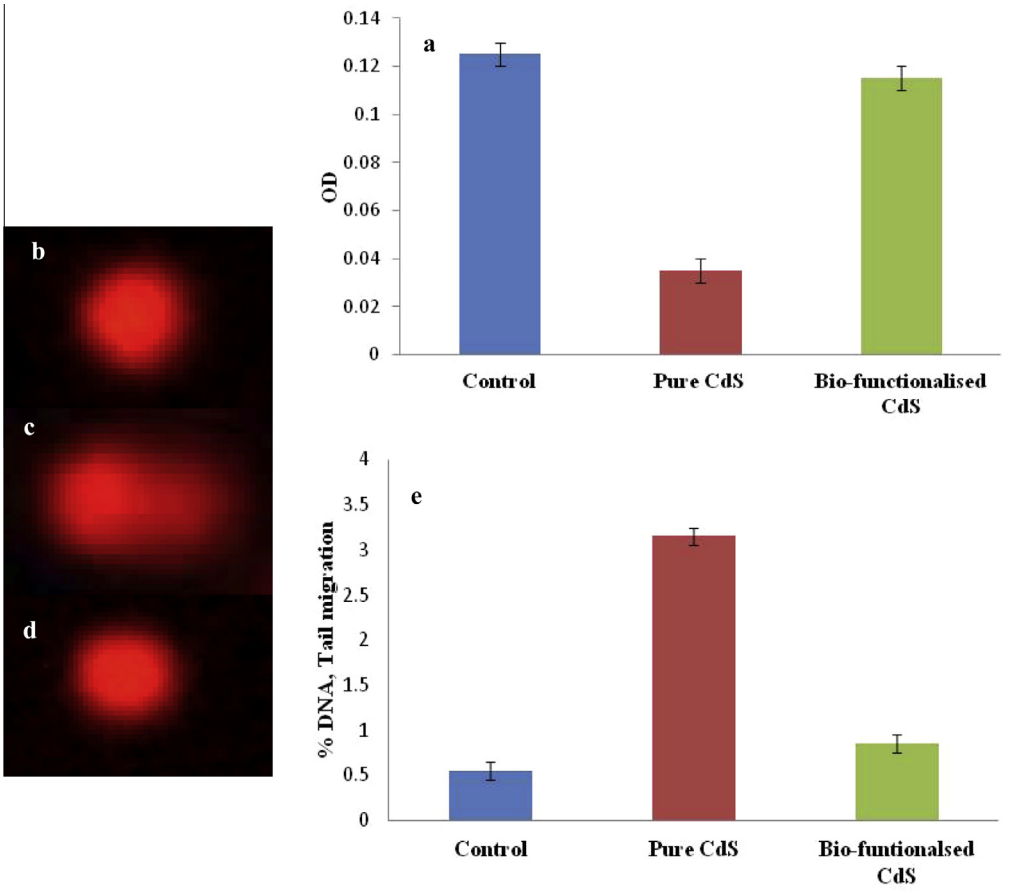

Figure 5 MTT assay of isolated lymphocytes in three different treatment conditions (a). Comet assay: Intact cells of lymphocytes where DNA tail formation was not noticed (b); DNA comet formation in lymphocyte cell after treatment of cell with pure CdS nanoparticles (c); minimization of DNA tail formation, when cells were treated with biogenic nanoparticles (d). A comparative migration of damaged DNA, in three different treatment conditions (e). The data is expressed as mean ± SD of three independent experiments.

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The analysis revealed that apart from S (41%) and Cd (35%), elements like O (11%), Na (8%), Cl (6%), P (4%), and C (3%) were integral part of synthesized CdS QDs. FT-IR analysis provides a presumptive idea of interaction between the functional group of chemical constituents of leaf extract and metal ions, leading to the formation and stabilization of CdS nanoparticles. The control FT-IR spectrum is presented in Fig. 4a while Fig. 4b represents IR spectrum of the leaf extract involved in nano CdS. The shift in peak located around 3427 can be assigned to the involvement of O—H (of alcohol) and N—H group in the formation of CdS nanoparticles. Similarly, shift of peak from 2928 to 2932 in 3200–2800 wave range suggested the involvement of C—H (sp^3 hybridized) and O—H (of acid origin) group of plant extract in nanoparticle formation. The shift of peak 2342–2330 in wavenumber range from 2400 to 2100 can be assigned to the presence and involvement of triple bond molecules like —C≡C— (alkynes) and —C≡N— (nitriles) in organic mixture. The adsorption peaks located at 1636 and 1649 in wave range of 1800–1500 can be assigned as peaks due to the presence and involvement of —C═O, —C═N and —C═C—. Peaks between finger print regions i.e. 1500–400 cm^{-1} are due to all manner of bending vibrations within the molecules. The peaks around 1300 and 1200 are attributed to the stretching vibration of single bond between C—O. The peaks around 1200 and 1000 may be due to stretching and vibration between N—H.

3.3. Cell viability, MTT assay and fluorescence microscopy

MTT is reduced to purple formazan by the action of mitochondrial enzymes of viable cells. Here a comparative mortality of cells (WBC) was studied using an MTT assay. The analysis of result suggested a low viability of lymphocyte cells i.e. cell death was higher in case when cells were treated with pure CdS nanoparticles. An incidence of low cell death has been noticed when cells were mixed with biologically synthesized CdS nanoparticles. Fig. 5(a) clearly shows that addition of biologically synthesized nanoparticles prevented the death of lymphocyte cells. An important mechanism of QD toxicity is the induction of DNA damage that involves reactive oxygen species (ROS) (Rzigalinski and Strobl 2009). In the present study comet assay technique was adopted to show the comparative genotoxic property of pure CdS versus biologically synthesized CdS nanoparticles. A visual comet scoring method was followed to evaluate % DNA breakage, based on the formation of the length of DNA tail. An arbitrary scale 0–4, was attributed to this event i.e., value 0 represented a group of cells whose DNA remained intact and nucleus appeared perfectly round, while value 4 represented a group of cells with a maximum length of tail (Anderson and Plewa 1998; Prasad et al., 2013). Exposure of pure commercially procured CdS to lymphocytes caused extensive DNA damage which is evident from the formation of comet, as shown in Fig. 5(c). Biomimetically synthesized CdS nanoparticles using leaf extract exhibited a mild toxic effect and did not cause DNA damage (Fig. 5d). The figure shows a comparative DNA tail formation in three different treatment conditions (Fig. 5e). Water soluble nano CdS (QDs) synthesized using leaf extract can be a suitable candidate for use in biomedical applications, since they showed less cytotoxic as well as genotoxic effects than chemically synthesized nano CdS.

4. Conclusions

This is a first report, where a leaf extract of *A. racemosus* plant has been used in the synthesis of water soluble CdS nanoparticles. Solution containing nano CdS showed absorption maximum at 295 nm and produced an emission maximum at 495 nm, upon excitation. TEM, SEAD, XRD and EDAX results showed the formation of polydispersed, crystalline CdS nanoparticles. Bio-functionalized CdS nanoparticles did not promote cell death as well as genetic damage in human lymphocytes, whereas chemically synthesized CdS QD caused significant cell death followed by DNA fragmentation and tail formation. Application of these types of nanoparticles on cancerous cell line and elucidation of exact molecular events are being proposed. Water soluble nano CdS (QDs) synthesized using leaf extract can be a suitable candidate for use in biomedical applications, since they showed less genotoxic effect than chemically synthesized QDs.

Author's contribution

KSP was involved in initial synthesis of CdS nanoparticles, MTT and comet assay. TA, SK and MK were responsible for identification and collection of plant materials, extract preparation and UV–vis analysis, blood sample collection, WBC isolation etc. KS performed detailed characterization of nanoparticles e.g. PL, TEM, SAED, EDAX and XRD and contributed significantly in manuscript preparation.

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