INTRODUCTION

Zinc oxide nanoparticles (ZnO NPs) are well recognized as a biocompatible multifunctional material with exceptional semiconducting, optical, and piezoelectric properties [1]. These materials are used in different potential application such as light-emitting diodes, lasers, sensors, actuators, transducers, and nanogenerators [1-6]. ZnO is one of the important semiconductor materials for wide band gap 3.36 eV and large exciton binding energy 60 meV at room temperature. This makes us interesting for its electro-optical applications [7].

The metal oxide nanomaterials are attractive for use in biomedical applications. It has been proposed that the high surface area of metal oxide nanoparticles significantly enhances their ability to produce reactive oxygen species (ROS) [8,9]. Toxicity of ZnO NPs is reported due to the generation of intracellular ROS and dissolved Zn ions [10]. ROS is generated through various mechanisms such as illumination of nanomaterials by ultraviolet (UV) light, disturbance of intracellular metabolic activities, and antioxidant system result, in the generation of oxidative stress in the cells. ROS can damage DNA, cell membrane, and proteins which may lead to cell death [11,12].

To traverse new strategies to discuss and develop the next generation of drugs or agents to control bacterial infections and cytotoxic effects, the antibacterial and anticancer properties of ZnO and Ce-doped ZnO NPs are examined with the support of the structural and optical characterization studies.

METHODS

Synthesis

Zinc (II) nitrate hexahydrate (AR), cerium (III) nitrate hexahydrate (AR), and NaOH (AR) are used as precursor without further purification. The details of experimental procedure for the preparation of pure ZnO and Zn$_{1-x}$Ce$_x$O ($x=0.05$ and 0.15) samples have been reported in our previous paper [7].

Antibacterial activity

The antibacterial activity of ZnO and Ce-doped ZnO NPs was investigated by disc diffusion method against the test Gram-positive bacteria (Streptococcus aureus and Streptococcus pneumonia) and Gram-negative bacteria (Escherichia coli, Pseudomonas aeruginosa, Proteus vulgaris, Klebsiella pneumonia, and Shigella dysenteriae) on Mueller-Hinton agar (MHA) according to the Clinical and Laboratory Standards Institute [13]. The media plates (MHA) were streaked with bacteria vulgaris, Pseudomonas, Proteus, and Shigella to prepare the stock solution and kept in an incubator (Thermo Scientific, USA). All experiments were performed using discs (6 mm Hi-Media) loaded with 1 mg of the test samples were placed on the bacteria-seeded disc plates using sterile forceps. The plates were then incubated at 37°C for a day. The inhibition zone around the disc was measured and recorded. Amoxicillin (Hi-Media) was used as the positive control against Gram-positive bacteria and Gram-negative bacteria, respectively, to compare the efficacy of the test samples.

Cell culture

The A549 human lung cancer cell line was obtained from the National Center for Cell Science, Pune, India. The cells were cultured in DMEM high glucose medium (Sigma-Aldrich, USA), supplemented with 10% fetal bovine serum (Gibco), and 20 ml of penicillin/streptomycin as antibiotics (Gibco). In 96 -well culture plates, at 37°C in a humidified atmosphere of 5% CO$_2$ in a CO$_2$ incubator (Thermo Scientific, USA). All experiments were performed using cells from passage 1.5 or less.

Cell viability assay

The ZnO and Ce-doped ZnO NPs were suspended in dimethyl sulfoxide (DMSO) to make stock solution. These stock solutions were used to prepare different concentrations of ZnO and Ce-doped ZnO NPs at the test samples.

Research Article

EFFECT OF CE$^{3+}$ METAL IONS ON THE ANTIBACTERIAL AND ANTICANCER ACTIVITY OF ZINC OXIDE NANOPARTICLES PREPARED BY COPRECIPITATION METHOD

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ABSTRACT

Objective: This study was undertaken to know about the antibacterial and anticancer activity of synthesized zinc oxide (ZnO) nanoparticles (NPs).

Methods: The ZnO NPs and different concentration of Ce$^{3+}$ (0.05M, 0.10M, and 0.15M)-doped ZnO NPs were synthesized by coprecipitation method. The synthesized nanoparticles were analyzed by X-ray diffraction (XRD) and HRSEM. The antibacterial studies were performed against a set of bacterial strains as Gram-positive bacteria (Streptococcus aureus and Streptococcus pneumonia) and Gram-negative (Escherichia coli, Pseudomonas aeruginosa, Proteus vulgaris, Klebsiella pneumonia, and Shigella dysenteriae) bacteria. The cytotoxic effect of ZnO and Ce-doped ZnO was analyzed in cultured (A549) human lung cancer cell line.

Result: The XRD studies showed the wurtzite structure of nanoparticles. HRSEM analysis showed the spherical shape of ZnO and Ce-doped ZnO. The Zn$_{1-x}$Ce$_x$O NPs possessed more antibacterial effect as compared to the other ZnO and Ce-doped ZnO NPs. The Zn$_{1-x}$Ce$_x$O NPs created the highest cytotoxicity activity. With respect to cell death, as low a concentration of 68±0.05 μg/ml of Zn$_{1-x}$Ce$_x$O NPs was good enough to cause loss of viability of 50% of the cell as compared to ZnO and Zn$_{1-x}$Ce$_x$O ($x=0.05$ and 0.15) NPs.

Conclusion: Results from this work concluded that Zn$_{1-x}$Ce$_x$O and Zn$_{1-x}$Ce$_x$O NPs possess antibacterial and anticancer activity, respectively.

Keywords: Zinc oxide nanoparticles, Coprecipitation method, Antibacterial activity and anticancer activity, Human lung cancer cell line.

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diluted separately with media to get various concentrations of the complex. Two hundred million cells were added to wells containing 5×10⁴ AS549 cells per well. DMSO solution was used as the solvent control. After 24 hr, 20 µl of 3-(4, 5-di-methylthiazol-2-yl)-2, 5-diphenyl-2H-tetrazolium bromide solution (5 mg/ml in phosphate-buffered saline [PBS]) was added to each well, and the plate was covered with aluminum foil and incubated for 4 hr at 37°C [14]. The purple formazan outcome was disposed by adding 100 µl of DMSO to each well. The absorbance was recorded at 570 nm (measurement) and 630 nm (reference) using a 96-well plate reader (Bio-Rad, iMark, USA). Data were possessed for three replicates each and were used to calculate the respective mean. The percentage inhibition was calculated from the data using the formula:

\[
\text{Mean OD of untreated cells (control)} = \frac{\text{Mean OD of treated cells}}{\text{Mean OD of untreated cells (control)}} \times 100
\]

Acridine orange (AO) and ethidium bromide (EB) staining
Apoptotic morphology was investigated by AO/EB double-staining method as described by Spector et al. with some modifications [15]. Briefly, the cells were treated with IC₅₀ concentration of ZnO and Ce-doped ZnO for a day. After incubation, the cells were harvested and washed with cold PBS. Cell pellets were diluted with PBS to a concentration of 5×10⁴ cells/ml and mixed with 25 µl of AO/EB solution (3.8 µM of AO and 2.5 µM of EB in PBS) on clean microscope slide and immediately examined under fluorescent microscope (Carl Zeiss, Axioscope2 plus) with UV filter (450-490 nm).

The X-ray diffraction (XRD) patterns were recorded using (PANalytical X'Pert Pro). The surface morphology was studied through FEI QUANTA 250 scanning electron microscope (SEM).

RESULTS AND DISCUSSION

XRD studies
From Fig. 1, the XRD pattern confirmed the synthesis of ZnO and Ce-doped ZnO NPs is in hexagonal wurtzite structure. The Ce-doped ZnO NPs have no impurity peaks and are detected as Ce³⁺/Ce⁴⁺ ions with cerium oxide between CeO₂ and Ce₂O₃. The lattice constant “a” and “c” values at 3.2521 Å and 5.2111 Å for pure ZnO NPs. The substitution of Ce³⁺ ion instead of Zn²⁺ ion at their lattice site increases. The changes in lattice parameter values are due to the broadness of ZnO lattice by the substitution of Ce³⁺ (1.02 Å) ion to Zn²⁺ (0.74 Å) sites, with a greater ionic radius compared to Zn²⁺ in their tetrahedral coordinates. The lattice constant “a” and “c” values are (3.2540 Å, 3.2539 Å, and 3.2543 Å) and (5.2138 Å, 5.2150 Å, and 5.2113 Å) for Znₙ₀CeₓOₙ, Znₙ₀CeₓOₙ, and Znₙ₀CeₓOₙ NPs, respectively. The average crystalline size was 39, 32, 30, and 27 nm for ZnO and Ce-doped ZnO NPs, respectively. The decrease in D is mainly because of the distortion in the host ZnO lattice by the foreign impurity, i.e., Ce³⁺ [7].

Field emission SEM analysis
Fig. 2 shows the HRSEM Image of ZnO and Ce-doped ZnO NPs. The unadulterated ZnO NPs and Ce-doped ZnO NPs are found to be in spherical-shaped morphology. Different concentration of Ce-doped ZnO NPs agglomerates with each other due to the increasing nucleation of Ce³⁺ ions and subsequent growth of ZnO NPs [7].

Antibacterial activity
Antibacterial activity of (Zn) ZnO and Ce-doped ZnO as Ce₁, Ce₂, and Ce₃ is investigated against Gram-positive bacteria (S. aureus and S. pneumonia) and Gram-negative bacteria (E. coli, P. aeruginosa, P. vulgaris, K. pneumonia, and S. dysenteriae) are studied by disc diffusion method as shown in Fig. 3. Fig. 4 communicates the area of zone inhibition, and the activity as antibacterial which surrounds each ZnO and Ce-doped ZnO NPs filled with test specimen.

The antibacterial efficacy of ZnO NPs is commonly influenced by ROS, which is mainly related to the size, larger surface area, an increase in oxygen vacancies, the diffusion capacity of the reactant, and the release of Zn²⁺ [16].

From antibacterial activity, ZnO and Ce-doped ZnO NPs; Znₙ₀CeₓOₙ NPs possessed more antibacterial effect as compared to the other ZnO, Znₙ₀CeₓOₙ, and Znₙ₀CeₓOₙ NPs. Increasing the concentration of Ce³⁺ increases the zone of inhibition.

The smaller sized NPs indeed have higher activity as antibacterial [17,18]. The XRD pattern shows the particles size of ZnO and Ce-doped ZnO NPs as 39, 32, 30, and 27 nm, respectively. Znₙ₀CeₓOₙ NPs particles’ size is lesser as compared to the other ZnO, Znₙ₀CeₓOₙ, and Znₙ₀CeₓOₙ.
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Fig. 3: The antibacterial activity of the zinc oxide and Zn$_{1-x}$Ce$_x$O (x=0.05, 0.10, and 0.15) against Gram-positive (Streptococcus aureus (2) and Streptococcus pneumonia (6)) and Gram-negative (Escherichia coli (4), Pseudomonas aeruginosa (5), Proteus vulgaris (7), Klebsiella pneumonia (1), and Shigella dysenteriae (3)) NPs [7]. The particle with a small size can easily penetrate into bacterial membranes due to their large interfacial area, thus enhancing their antibacterial efficiency.

From the photoluminescence spectra, wavelengths of the blue emissions values are at 440, 439, 439, and 446 nm for ZnO and Ce-doped ZnO NPs. This illustrates the larger count defects such as zinc and oxygen vacancies in the Zn$_{1-x}$Ce$_x$O NPs. Thus, there are increased count of ROS as compared to ZnO and Zn$_{1-x}$Ce$_x$O (x=0.05 and 0.10) NPs.

Anticancer properties
The cytotoxic effect of the ZnO and Zn$_{1-x}$Ce$_x$O (x=0.05, 0.10, and 0.15) NPs were examined in cultured (A549) human lung cancer cell line by exposing cells for 24 hr to culture medium containing unmixed ZnO, Zn$_{1-x}$Ce$_x$O, Zn$_{1-x}$Ce$_{1-x}$O, and Zn$_{1-x}$Ce$_{1-x}$O NPs at 280±.05, 82±.05, 68±.05, and 76±.05 µg/ml for IC$_{50}$ concentration as shown in Fig. 5a-d. In relation to cell death, a minimum concentration 68±.05 µg/ml for 24 hr treatment of Zn$_{1-x}$Ce$_{1-x}$O NPs was well enough to induce 50% cell mortality as compared to other ZnO, Zn$_{1-x}$Ce$_{1-x}$O, and Zn$_{1-x}$Ce$_{1-x}$O NPs. The Zn$_{1-x}$Ce$_{1-x}$O NPs showed a highly effective cytotoxic activity against (A549) human lung cancer cell. The cytotoxic efficiency of the ZnO NPs generally depends on the presence of ROS.

AO and EB staining
The most important characteristics of apoptosis are morphological changes during cell death. Fig. 6 represents that AO/EB double-stained A549 human lung cancer cell line treated with test substances 24 hr underwent both early apoptosis and late apoptosis. The control or viable cells show green fluorescence and normal cell features of uniform chromatin with an intact cell membrane, whereas the early apoptosis cells showed bright green region with yellowish green nuclear fragmentation and membrane bubbles and apoptotic bodies outside. The late apoptosis cells exhibited orange-yellow or red nuclei with condensed or fragmented chromatin. The results demonstrate that all substances induce the majority of cell death through apoptosis mode and very fewer in necrosis for 24 hr treatment. Chromatin condensation and fragmentation were observed in ZnO and Zn$_{1-x}$Ce$_x$O (x=0.05, 0.10, and 0.15) NPs treated cells.

The cellular toxicity mechanisms are based on ROS production, which exceeds the capacity of cellular antioxidant defense system causes cells to enter the state of oxidative stress. These oxidative stresses damage the cellular components such as lipids, proteins, and DNA [19,20]. The oxidation of fatty acids leads to the generation of lipid peroxides that initiates a chain reaction leading to disruption of plasma, organelle membranes, and subsequent cell death by induction of apoptosis. The ROS act as the critical signaling mechanism in the induction of apoptosis/cell death by many different stimuli [21,22].

CONCLUSIONS
Thus ZnO and Ce-doped ZnO NPs were prepared by co-precipitation method. From the XRD pattern, ZnO and Ce-doped ZnO NPs were revealed as wurtzite structure. The average crystal size was calculated as 39, 32, 30, and 27 nm for ZnO and Ce-doped ZnO NPs, respectively. HRSEM image showed the spherical shape of ZnO and Ce-doped ZnO NPs. From antibacterial result, Zn$_{1-x}$Ce$_x$O NPs possessed more antibacterial effect as compared to the other ZnO and Zn$_{1-x}$Ce$_x$O (x=0.05 and 0.10) NPs. The cytotoxic effect of the ZnO and Zn$_{1-x}$Ce$_x$O (x=0.05, 0.10, and 0.15) NPs were examined in cultured (A549) human lung cancer cell line, in which the Zn$_{1-x}$Ce$_x$O NPs showed the highest cytotoxic activity. With reference to cell death, a minimum concentration of 68±.05 µg/ml of the Zn$_{1-x}$Ce$_x$O NPs was well enough to induce 50% cell mortality as compared to ZnO and Zn$_{1-x}$Ce$_x$O (x=0.05 and 0.15) NPs, respectively.
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