Fucoidan functionalized cerium oxide nanoparticles (Fu/CeO2NPs): A multidimensional investigation

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Research Article

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**Abstract**

Nanotechnology is an expanded branch of current research that involves the production and customization of particle structures with typical sizes of less than 100nm for various uses. A large area of surface to volume ratio was related with minimum surface functionalization, resulting in nanostructured metal oxide nanoparticles with outstanding biomedical characteristics. Cerium oxide (CeO\(_2\)) is the most approachable rare earth metal oxide due to its selective nature. In this first time reported that the nature of fucoidan functionalized Cerium oxide nanoparticles were determined by using UV-Vis, FE-SEM, HR-TEM, DLS, Zeta potential, FT-IR, XRD and XPS spectroscopic examination. The CeO\(_2\)NPs and Fu/CeO\(_2\)NPs have been effectively cause cell wall damage of pathogenic bacteria *E.coli* and *S.aureus*. In-vitro antioxidant activities were showed significant results were obtained CeO\(_2\)NPs and Fu/CeO\(_2\)NPs. Dose-dependent cytotoxicity assay of CeO\(_2\)NPs and Fu/CeO\(_2\)NPs have been treated against A549 (lung) and Hela (cervical) cancer cells. Furthermore, cell morphological changes have analysed by using fluorescent microscopic staining techniques like AO/EB, Hoechst and JC-1. Fu/CeO\(_2\)NPs have more effectively degraded the carcinogenic dye Congo red under sunlight. The present investigation of CeO\(_2\)NPs and Fu/CeO\(_2\)NPs as a novel theranostic platform for bacterial/cancer treatment.

**Introduction**

Nanotechnology and nanoscience are among the most intriguing research areas in current material science because to its distinct chemical, structural, optical, electrical, mechanical, morphological, and biological properties (Nithya and Sundrarajan 2020) Nanotechnology is concerned with the manufacture and application of metallic particles with sizes ranging from 1 to 100 nm or less. Because of their unique size, high distribution, and architecture, nanoparticles (NPs) have innovative and improved properties (Liang et al. 2015). Cerium oxide (CeO\(_2\)) is a rare earth element found in the periodic table’s lanthanide family. Cerium oxide nanostructures are becoming more popular among researchers because to the lower (Ce\(^{3+}\)) and greater (Ce\(^{4+}\)) energy differences in ceria, as well as their superior oxygen mobility and storage capacity (Magdalane et al. 2018; Magdalane et al. 2017). Furthermore, cerium oxide is a low-cost substance that is significant in catalysis, environmental chemistry, pharmaceutical, biomedical, and industrial applications. CeO\(_2\) NPs are employed in pharmacology as antibacterial, antioxidant, anticancer, drug carrier, bone implant material and also involve in as photocatalyst for degradation of environmental pollutants dye(Magdalane et al. 2017; Dhall and Self 2018).

In fucoidan *functionalized* cerium oxide nanoparticles with sufficient dispersibility for enhanced antibacterial behaviour, the paired oxidation state Ce\(^{3+}\) and Ce\(^{4+}\) surface made available a catalytic site for the attach and hydrolysis of long-chain *phospholipids* observed on the bacterial membranes (Khulbe et al 2020). Cells produce ROS as a by-product of their aerobic metabolism, and they are essential for cell homeostasis to be maintained (Schieber and Chandel 2014). CeO\(_2\)NPs is found to serve as cellular antioxidants with co-localizations within mitochondria, lysosomes, endoplasmic reticulum, nucleus, and cytoplasm. CeO\(_2\)NPs have been shown to shield cells from ROS, due to their intrinsic antioxidant
properties (Datta et al. 2020) which is innately cytotoxic and genotoxic to tumour cells that the investigation at the anticancer effects. CeO$_2$NPs has been shown to cause significant cytotoxicity to lung adenocarcinoma A549 cells and Hela cells, resulting in a reduction in cell antioxidant levels and induce apoptotic cell death (Nithya and Sundrarajan 2020). Nanoparticle-based anticancer therapy in more effective and reduce side effects, and improve pharmacokinetics (Farokhzad and Langer 2009). Polymeric functionalized nanoparticles with a high potential for diagnostic and therapeutic uses, as well as the ability to provide site-specific drug delivery (Lira et al. 2011). The combining polysaccharide on the surface of a polymer nanoparticles have been stated to cause drastic changes in the biological reaction, thus contributing to the difficulty (Li et al. 2008) many other polysaccharides with biological activity might be of concern for use. Fucoidan (Fu) seems to be an outstanding candidate among them. Fucoidan, also known as array of digital or sulfated fucan, is a sulfated polysaccharide. It is derived from brown algae and exhibits a wide range of biological characteristics (Pinna et al. 2020).

In this present study, first time reported that the CeO$_2$NPs functionalized with fucoidan were characterized using different techniques such as UV-Vis, FE-SEM, HR-TEM, FT-IR, DLS, Zeta potential, XRD and XPS. The in-vitro biological application of Fu/CeO$_2$NPs were studied using pathogenic microorganisms like Staphylococcus aureus and Escherichia coli, in-vitro antioxidant potential of Fu/CeO$_2$NPs by using DPPH and ABTS, cytotoxicity potential against lung cancer cells (A549) and cervical cancer cells (Hela) and Photocatalytically, the carcinogenic Congo red dye has deteriorated.

Materials And Methods

Functionalization of cerium oxide nanoparticle with Fucoidan (Fu/Ceria)

Sigma Chemical Co. Ltd. (St. Louis, MO, USA) made available the cerium oxide nanoparticles; fucoidan functionalization was accomplished with minor modifications based on the work of (Pinna et al. 2020). In briefly 375µL of deionized water, 10 mg of Fucoidan (Fu) was dissolved. At room temperature, the dispersion was sonicated until full dissolution. 25 mg of nanoceria was dispersed in 225 µL of deionized water, as a suspension stock, was disseminated in 100 µL of deionized water in a separate vial and juxtaposed with a Fu solution that was sonicated at room temperature for 8 hours in an ultrasound bath. Following that, the Fu/ceeria nanoparticles were washed three times with water and resuspended at 5000 rpm. The supernatant was fully transparent after the third washing step, indicating the elimination of non-reacted Fu molecules. As a result, the supernatant was discarded, and 1 mL of water added with pellet.

Characterization

UV-visible spectroscopy measurement was performed for chemically synthesized cerium oxide nanoparticle (CeO$_2$NPs) and fucoidan (Fu) functionalised cerium oxide nanoparticle Fu/CeO$_2$NPs using Spectrophotometer UV – 2450 (shimadzu) at room temperature observed in the range of 200 nm-800 nm at different time intervals 0 hour to 30 days at room temperature for observing stability of CeO$_2$NPs. Determination of hydrodynamic size of NPs and the zeta ($\zeta$) potential was determined using a Zetasizer.
Nano ZS (Malvern Instruments Ltd. Malvern, UK). Fourier transform infrared spectroscopy (FT-IR) evaluation has been performed in the 4000-400 cm\(^{-1}\) range (Perkin Elmer, USA). The crystalline morphology of the CeO\(_2\)NPs had been examined using X-ray diffractometer (XRD) (SmartLab, Rigaku Corporation, Japan). The topographical architecture of the CeO\(_2\)NPs and after Fu/CeO\(_2\)NPs has been investigated by field emission scanning electron microscopy (FE-SEM) (JSM-6480 LV). The crystal facets in individual CeO\(_2\)NPs and Fu/CeO\(_2\)NPs were determined using high resolution transmission electron microscopy (HR-TEM). X-ray Photoelectron Spectroscopy was used to investigate the surface chemistry of cerium oxide nanoparticles (XPS).

**In vitro assessment of antibacterial activity**

**Diffusion method in agar well**

Antibacterial efficacy of CeO\(_2\)NPs and Fu/CeO\(_2\)NPs has been evaluated by the agar well diffusion method against gram-positive and gram-negative bacterial strains such as *E. coli* and *S. aureus*. Cotton swabs were used to cover the freshly prepared nutrient agar (HiMedia, India) plates with two strains. Nutrient agar plate wells with a diameter of 6 mm are performed using Gel puncture. On each well, different concentrations of CeO\(_2\)NPs and Fu/CeO\(_2\)NPs (25 \(\mu\)g/mL, 50 \(\mu\)g/mL, 75 \(\mu\)g/mL, and 100 \(\mu\)g/mL) and Streptomycin (10 \(\mu\)g/mL) were poured. The cultures were incubated at 37°C for 24 hours, and the zone of inhibition was measured by measuring the diameter (mm) (Pop et al. 2020).

**Bacterial Growth curve analysis**

The growth of *E-coli* and *Staphylococcus aureus* in the appearance and absence of CeO\(_2\)NPs and Fu/CeO\(_2\)NPs at various concentrations of 25\(\mu\)g/mL, 50\(\mu\)g/mL, 75\(\mu\)g/mL, 100\(\mu\)g/mL in compare to a control samples has been evaluated and further investigated for antimicrobial potential. In brief, 20\(\mu\)L of test suspension inoculums (10\(^7\) CFU/mL) were added to a 96-well microliter plate containing nanoparticles in 250 \(\mu\)L of LB and incubated at 37°C. The growth rate was measured every 3 hours for 24 hours at 580 nm using a UV–Vis spectrophotometer. The investigations were performed out in threes times (Deepika et al 2019).

**Investigation of Antioxidant activity in in vitro**

**DPPH Radical scavenging activity**

The free radical scavenging activity of CeO\(_2\)NPs and Fu/CeO\(_2\)NPs against 2, 2-diphenyl-1-picrylhydrazile (DPPH) was calculated using the process of (Brand-Williams et al 1995). In 96 well plates, 100 \(\mu\)l of CeO\(_2\)NPs and Fu/CeO\(_2\)NPs at increasing doses (20\(\mu\)g/ml, 40\(\mu\)g/ml, 60\(\mu\)g/ml, 80 \(\mu\)g/ml and 100\(\mu\)g/ml) were prepared. The benchmark was ascorbic acid (Vitamin C). 100 \(\mu\)l of solution was prepared DPPH (1 mM) solution was added to each well, and the samples were incubated at room temperature in the dark for 30 minutes. The solution's violet to yellow colour implied that reactive oxygen species had been
scavenged, and it has been evaluated at 517 nm with a Synergy HT Multimode Reader (Biotek, Winooski, USA). Finally, the following equation was used to calculate the scavenging capability.

\[
\text{% inhibition} = \frac{A_c - A_s}{A_c} \times 100\%
\]

Whereas, \(A_c\) – OD value of blank, \(A_s\) – OD value of CeO\(_2\)NPs and Fu/CeO\(_2\)NPs treated.

**ABTS Radical scavenging activity**

The scavenging properties of CeO\(_2\)NPs and Fu/CeO\(_2\)NPs at various concentrations (20\(\mu\)g/ml, 40\(\mu\)g/ml, 60\(\mu\)g/ml, 80\(\mu\)g/ml and 100\(\mu\)g/ml) against radical cations ABTS\(^+\) (2,2’-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)) have been evaluated using a procedure previously described. The ability of CeO\(_2\)NPs and Fu/CeO\(_2\)NPs to scavenge the ABTS radical (ABTS.ABTS\(^+\)) is compared to the ascorbic acid as corresponding antioxidant in the ABTS assay\(^+\). The ABTS\(^+\) solution was prepared for use by simply responding 7 mM of aqueous ABTS solution with 2.45 mM of potassium persulfate in a dark medium at room temperature for 12 hours. After diluting this same standard solution with ethanol to make an ABTS\(^+\) reaction mixture, the spectrophotometrically of CeO\(_2\)NPs and Fu/CeO\(_2\)NPs and ABTS mixture has been observed at 734 nm after 10 minutes of the dark medium (Pop et al. 2020).

**Cell culture**

The lung carcinoma cells (A549) and cervical carcinoma cells (Hela) has been cultivated in DMEM with 1000 mg/L glucose which was augmented with 10% fetal bovine serum, streptomycin sulfate (0.1 mg/mL) and penicillin G (100 units/mL) in the kept moist environment consisting of 5% CO\(_2\) at 37°C.

**MTT assay**

MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide) bromide method was used to assess the cytotoxicity of CeO\(_2\)NPs and Fu/CeO\(_2\)NPs against lung carcinoma cells (A549) and cervical carcinoma cells (Hela). A total of \(5 \times 10^3\) cells were cultured in a 96-well plate in CO\(_2\) chamber at 37°C. To treat the cells, different doses of CeO\(_2\)NPs and Fu/CeO\(_2\)NPs (0 to 200 \(\mu\)g/ml) were prepared and incubated for 24 hours. The control cells were not treated with nanoparticles. To achieve concordance, the assay was performed in triplicate. Following the exposure to CeO\(_2\)NPs and Fu/CeO\(_2\)NPs, 20\(\mu\)l of MTT reagent (5 mg/ml stock PBS) was transferred into a 100\(\mu\)l of cell suspension in every well, and the plate was incubated in the dark for 4 hours. After discarding the medium, 200\(\mu\)l of DMSO was added to each well to dissolve the MTT formazan crystals. In a multi-well micro plate reader, the outcomes had been read at 570 nm absorbance (Bio-Rad iMark reader). The percentage inhibition was calculated by the formula below (Dhivya et al 2015).

\[
\text{% of inhibition} = \frac{\text{OD of untreated cells (control)} - \text{OD of treated cells}}{\text{OD of untreated cells (control)}} \times 100
\]
AO/EB and Hoechst 33528 staining for cell death analysis

The apoptotic structure of lung cancer cell lines (A549) and cervical cancer cell lines (Hela) treated with CeO$_2$NPs and Fu/CeO$_2$NPs was examined using the dual staining acridine orange (AO) and ethidium bromide (EB) method. In a 96-well plate ($5 \times 10^5$ cells/well), viable cells were incubated for 24 hours with the $IC_{50}$ concentrations CeO$_2$NPs and Fu/CeO$_2$NPs. After removing the media, the cells were trypsinized and rinsed with phosphate buffered saline. Following washing, the cells were stained with AO/EB (10 mg/ml), positioned on a microscopic glass slide, covered with a cover slip, and examined under a fluorescent microscope (Carl Zeiss, Axio scope2 plus) with absorption and emission wavelengths of 450 nm and 490 nm, respectively. (Manikandan et al. 2021).

Evaluation of mitochondrial membrane potential (JC1 staining)

The fluorescent stain JC-1 has been used to examine the mitochondrial membrane potential ($\Delta \psi_m$), that also generates orange-red fluorescence once gathered in the mitochondria of healthy cells and yet fluoresces green while seeped out into the cytosol because of ($\Delta \psi_m$), loss, leading to a negative intrinsic prospects. A549 and Hela cultured in cover glass slips (22×22 mm) positioned in 6-well plates and allowed to treat with the CeO$_2$NPs and Fu/CeO$_2$NPs at the 24 h $IC_{50}$ dosage, with 0.03 % DMSO used it as a control. After 24 hours, the cells were stained with JC-1 dye. The fluorescent microscope was used to monitor the mitochondrial depolarization shapes of the cells, as well as the abnormal changes in the cells.

Photocatalytic degradation of Congo red using CeO$_2$NPs and Fu/CeO$_2$NPs

Fucoidan has been functionalized with Cerium oxide nanoparticles (Fu/ CeO$_2$NPs) were tested for their potential to catalyse the decrease of carcinogenic dyes like Congo red at 37°C. The test sample was made up of dye (0.5mg/mL), CeO$_2$NPs and Fu/CeO$_2$NPs (5mg/mL), while the control was dye alone. In brief, 100μl of dye and 50μl of Fu/ CeO$_2$NPs have been mixed and separately exposed to sunlight and UV irradiation at 365nm. A microplate reader (Biotek Instrument Inc.) was used to monitor the photocatalytic reaction at 15-minutes durations between the wavelength of 200-800nm. The following formulae were used to calculate the percentage of photocatalytic adequacy (Manikandan et al. 2021).

$$\text{Photocatalytic degradation (\%) } = \left( \frac{C_0-C_t}{C_0} \right) \times 100$$

Whereas, $C_0$-initial OD value of dye without Fu/ CeO$_2$NPs, $C_t$-OD value of Fu/ CeO$_2$NPs treated.

Statistical analyses

The statistical analyses were performed with SPSS 16.0 software and expressed as mean standard error of the mean (SEM) (SPSS, Chicago, IL, USA). Graphs were created with Origin Pro 9.0 (Northampton, US) and visualised with Graph Pad prism (Graph Pad Software, San Diego, California USA).
Results

Characterization of CeO$_2$NPs and Fu/CeO$_2$NPs

UV-Vis absorption investigations might be helpful in determining the structural integrity, changes, and to keep track of the creation of aggregations as a result of the interaction between nanoparticles and polysaccharide (Deepika et al. 2019). The absorption spectra of CeO$_2$NPs, fucoidan and fu/CeO$_2$NPs surface plasmon resonance effect were observed at 300 nm. The UV-Vis spectra of fu/CeO$_2$NPs revealed a hypochromic effect according to Fig. 2, due to the strong chemical bonding interaction together fucoidan and CeO$_2$NPs (Pandey et al. 2021).

The stability of the CeO$_2$NPs was investigated from 0 to 60 days and the presence of a hypochromic shift indicates that as the incubation time increases in CeO$_2$NPs Fig. 3a (Badi’ah et al. 2019). According to Fig.3b Fu/CeO$_2$NPs was more stable than CeO$_2$NPs because any shift did not occur in the UV-Vis spectrum of Fu/CeO$_2$NPs after 60 days. The absorption spectra portrayed a strong absorption peaks at 300 nm, which is the specific characteristic reported on previous studies (Parimi et al. 2019) Absorption peak of CeO$_2$NPs containing the Ce$^{3+}$ and Ce$^{4+}$ ions at a wavelength of 300 nm. The interaction of Ce$^{3+}$ ions is important in the anticancer and antibacterial activity of CeO$_2$NPs, which was previously reported (Nurhasanah et al. 2018).

The surface morphology of spherical shaped CeO$_2$NPs (positive charge) and fu/CeO$_2$NPs (negatively charge) were analysed using FE-SEM (Fig. 4 a-d). The CeO$_2$NPs had been showed sphere-shaped in morphologically, with narrow particle suggesting substantial homogeneity of particle distributions and the creation of a huge mass of spherical agglomerates. According to a previous report, the abrupt appearance of this type of behaviour could be attributed to the synthesis process used or other critical reaction parameters such as synthesis time and temperature, solvent, and calcination temperature (Habib et al. 2018). The phenotypic characteristics of CeO$_2$NPs indicate that the material is made of spherical nanocrystals with smooth surfaces. The surface shape of fu/CeO$_2$NPs exhibits mild deformation due to the presence of stabilising agents as the fucoidan functionalized particles increase from early study. Fucoidan functionalization was indicated by cauliflower-shaped nanoparticles, which may be due to the formation of layered on the surface of CeO$_2$NPs, as shown in Fig. 4d. The surface topology and microstructure of the CeO$_2$NPs and fu/CeO$_2$NPs analyzed using HR-TEM and SAED pattern Fig. 5a-5e. The plots in Fig. 5c suggest excellently spotty rings, implying that the polycrystalline nature (Venkatesh et al. 2016). The results show that fucoidan was successfully coated on CeO$_2$NPs due to the formation of a stratum surface Fig. 5 d and e (Khadar et al. 2019).

When negatively charged fu/CeO2NPs was compared to uncoated positively charged CeO$_2$NPs, the fu/CeO$_2$NPs morphologically changed. CeO$_2$NPs were found to be significantly smaller in size when compared to as-prepared fu/CeO$_2$NPs, which could be attributed to the sulfated polysaccharide from the as layer on CeO$_2$NPs ((Parimi et al. 2019). The HR-TEM displays the self-assembly of polysaccharides
into structured masses when treated with CeO₂ NPs in aqueous (Pop et al. 2020). To determine the hydrodynamic size distribution of CeO₂ NPs and fu/CeO₂ NPs were determined using dynamic light scattering (DLS) analysis. Average dispersion of size of the synthesized CeO₂ NPs 35 ±0.4 nm Fig. 6a and fu/CeO₂ NPs 44±0.9 nm Fig. 6b. Zeta potential analysis of CeO₂ NPs and fu/CeO₂ NPs were showed + 24.3 mV and -19.9 mV respectively Fig. 7a and 7b.

Fucoidan has been functionalized on surface of the CeO₂ NPs, after fu/CeO₂ NPs charge could be moved to positive to negative. This could be strongly suggested that the higher value of fu/CeO₂ NPs indicates high colloidal stability in aqueous medium. This study to confirm their surface charge kinetics of the fu/CeO₂ NPs, therefore fucoidan has been improved the colloidal good stability in fu/CeO₂ NPs. Fourier transform infrared spectroscopy (FT-IR) technique has been found to be useful for determining the functional groups of CeO₂ NPs, fucoidan, and fu/CeO₂ NPs. The broad band in the higher region spectrum reveals a strong absorption at 3435 cm⁻¹, which equates to residual water and O-H stretching vibration/physical absorbed H₂O/surface OH group. A weak shoulder peak at 2073 cm⁻¹ assigned bending vibration of related water (H-O-H). The sharp peaks at 1633 cm⁻¹ were contributed to O–C–O symmetric extending, and the 666 cm⁻¹ are directly assigned to frequency of CeO stretching (Girija et al. 2011; Ketzial and Nesaraj 2011; Zayed et al. 2016). The FT-IR spectra further verified the fucoidan, with a large peak at 1384 cm⁻¹ for the sulphate group's non-symmetric bending vibrations of S=O and C-H bending carboxylic group, and a broad spectra at 1270 cm⁻¹ for the ether bond C–O bending. C-OH stretch was represented at 1121 cm⁻¹ according to previous report, in the spectrum of fu/CeO₂ NPs, 691 cm⁻¹ indicate C-O-S stretching of the sulfate groups (Manivasagan et al. 2017). As a result, FT-IR spectrum of fu/CeO₂ NPs investigations indicate the interplay of fucoidan and CeO₂ NPs, based on these absorption characteristics, which is arbitrated by hydrogen bonds between the hydroxyl groups of the CeO₂ NPs as well as the sulfated functional group of fucoidan. According to adsorption capacities, the biomolecules interaction of fucoidan and CeO₂ NPs and fu/CeO₂ NPs was illustrated in Fig. 8a (Deepika et al. 2019).

Fig.8b depicts the X-ray diffraction (XRD) pattern of CeO₂ NPs produced through precipitation. The XRD pattern demonstrates that the synthesized nanoparticles have a cubic fluorite CeO₂ structure. Preferred diffraction peaks are found the values of approximately 28.41°, 32.87°, 47.54°, 56.38°, and 60.18°, corresponding to the (111), (200), (220), (311) and (222) planes, correspondingly. These deflection peaks match the Joint Committee on Powder X-ray diffraction Standard (JCPDS) No. 34-0394 well. In the XRD pattern, no other peaks associated to impurities or other phases were observed, confirming that the produced CeO₂ NPs are single phase crystalline of CeO₂ (Nurhasanah et al. 2018).

X-ray photoelectron spectroscopy (XPS) analysis has been showed to distinguish between consequently oxidation states of fu/CeO₂ NPs. Determine the fraction of Ce³⁺ and Ce⁴⁺ were depicted in Fig. S5. The inherent feature of fu/CeO₂ NPs surface with double oxidation states Ce³⁺ and Ce⁴⁺ sites that is active. To
counteract radical activity because of the presence of Ce$^{3+}$ and Ce$^{4+}$ ions on the NPs surface. The XPS result of Ce3d spectra showed two satellite peaks were appeared at (883.23 eV and 882.27 eV), O1s spectra of (530.26 eV, and 529.21 eV), and C1s spectra of (285.37 and 284.74 eV). The elemental composition of synthesized fu/CeO$_2$NPs strongly suggest that the high purity of material. The chemical composition of the medium is found to have vital role on the electrostatic surface charge of the NPs, influencing the rate at which these NPs agglomerate/aggregate and affecting the stability of the NPs. Most of the synthesized NPs surface were decorated / coated with surfactants to increase the stability of the suspension. The presence of a surface coating on synthesized NPs might be significantly change their surface chemistry and compared with the uncoated materials. The blended oxidation state of cerium (Ce$^{3+}$ and Ce$^{4+}$) and the reductive having switched characteristics of CeO$_2$NPs are the primary reasons for the diverse biological activity (Nyoka et al. 2020; Szymanski et al. 2015).

**Antimicrobial activity Study**

The antibacterial activity of CeO$_2$NPs and fu/CeO$_2$NPs was determined against gram positive and gram negative pathogenic bacteria like as *E. coli* and *S. aureus*. According to images (Fig. 10a&b) fu/CeO$_2$NPs exhibits a higher zone of inhibition (ZOI) as compared with unfunctionalized CeO$_2$NPs. These observations indicate that the interaction between fu/CeO$_2$NPs bacteria cell membrane effectively induces the toxicity to bacteria and caused cell death compared with CeO$_2$NPs alone. Among the four dosages (25µg/mL, 50 µg/mL, 75 µg/mL and 100 µg/mL) of fu/CeO$_2$NPs 100 µg/ml showed higher zone of inhibition against gram positive *S. aureus* (17 mm) and gram-negative *E.coli* (19 mm). This is attributable to the fact that the Gram-positive bacterial cell membrane comprises a dense layer of peptidoglycan linked to teichoic acids, which may explain the interaction with CeO$_2$ NPs in antibacterial activity. The diameter of inhibition zone of antibacterial activity is determined by the concentration of CeO$_2$ NPs. The obtained results could be attributed to ionic interactions with both negatively charged organisms and positively charged nanoparticles, which caused metal and metal oxide nanoparticles to bind to the bacterial cell wall (Magdalane et al. 2018). The molecular mechanism of antibacterial activity of CeO$_2$NPs and fu/CeO$_2$NPs can be interaction with bacterial cell membrane and binding to chondrioids, which could be disturb the chondrioids functions of cell division, DNA replication, cellular respiration, and directly involved cell death, especially glycoprotein receptors found on surface of fucoidan are interact with bacterial cell wall to responsible for significantly increase antibacterial efficacy of fu/CeO$_2$NPs compared with CeO$_2$NPs and (Gopinath et al. 2015). The observed antibacterial potential results of CeO$_2$NPs and fu/CeO$_2$NPs of 100 µg/ml concentration shows that the significant efficacy against Gram-positive and Gram-negative bacteria, because of the strong electrostatic forces needed to attach the cell membrane to inhibit the growth of bacteria.

**Effect of CeO$_2$NPs and Fu/ CeO$_2$NPs on bacterial Growth (*E.coli* and *S.aureus*)**

The impact of CeO$_2$NPs and fu/ CeO$_2$NPs on bacterial growth was shown in a series of investigations of bacteria growth under untreated and under the impact of CeO$_2$NPs and fu/ CeO$_2$NPs. The growth curves
of *E. coli* Fig 11a and 11b and *S. aureus* Fig 11c and 11d respectively were clearly depicted the lag, log, stationary, and death phase. Although under the effect of various concentrations of CeO$_2$NPs and fu/CeO$_2$NPs 25 µg/mL to 100 µg/mL the incremental constriction of log phase was evident indicating the microbiostatic effect of CeO$_2$NPs and fu/CeO$_2$NPs on *E. coli* and *S. aureus* in a concentration dependant manner.

The results could show that the binding fu/CeO$_2$NPs with the bacterial cell membrane surface, because of the backbone of glycoprotein receptors found on the cell membrane of fucoidan has been to attach and interact with substances present in the bacteria like cytoplasmic membrane, and DNA so that to be responsible for inhibition of the bacterial growth (Chatterjee et al. 2011). In a time dependent manner representative that fu/CeO$_2$NPs interacts with the treated pathogens result in cell membrane damage and reduces biomass. It can be observed that the bacterial growth inhibition is significantly high fu/CeO$_2$NPs compare with CeO$_2$NPs, the morphological changes of fu/CeO$_2$NPs treated pathogens (*E. coli* and *S. aureus*) shown in Fig. 12&13.

The SEM images revealed that the bacterial cell *E. coli* membrane was damaged by treated fu/CeO$_2$NPs, whereas untreated cells revealed no membrane damage. The fu/CeO$_2$NPs treated cells formed with cytoplasmic leakage, as indicated by the yellow arrow Fig. 12b. The cell walls of fu/CeO$_2$NPs-treated *S. aureus* cells were disrupted, as shown by the yellow arrow, and following treatment, some holes were discovered on the cell wall surface. The cell wall was smooth in the control group, and no cell integrity disruption was observed Fig. 13b Under SEM image.

### 3.4 DPPH Radical Scavenging activity

The antioxidant activity of CeO$_2$NPs and fu/CeO$_2$NPs was estimated using 2, 2-diphenyl-1-picrylhydrazyl (DPPH). The DPPH reacts with an antioxidant; it generates a stable free radical that can be converted to a non-radical form. When an antioxidant reduced the DPPH radical, the colour changed to yellow, which would be the colour of the non-radical form. Free radical scavenging (DPPH) activity of CeO$_2$NPs and fu/CeO$_2$NPs 48.56 % were showed significant antioxidant potential as compared with standard (Vitamin-C) in Fig.14a. Past study reported that the levan coated CeO$_2$NPs has better antioxidant capacity, because of polysaccharide contains many hydroxyl group can be react with free radicals and reduced radical chain reactions are related to antioxidant (Pitchumani Krishnaveni and Annadurai 2019; Leung et al. 2015; Pozharitskaya et al. 2020). This study also suggests that fucoidan, a natural polysaccharide that can be functionalized with CeO$_2$, may influence the antioxidant property of fu/CeO$_2$NPs.

### ABTS Radical Scavenging activity

Antioxidant activity of CeO$_2$NPs and fu/CeO$_2$NPs was evaluated by the ABTS method, the decrease of freeradicals by the CeO$_2$NPs and fu/CeO$_2$NPs using ABTS was observed at 734 nm. The free radicals have been scavenged by CeO$_2$NPs and fu/CeO$_2$NPs in a dose-related, with the optimum scavenging
property was observed in Fig.14b. The results indicate that the function of CeO$_2$NPs and fu/CeO$_2$NPs inhibit the ABTS radicals formation in dose dependent manner. It's indeed easy to assume that antioxidant properties rise in direct proportion to concentration of nanoparticles (Pop et al. 2020). The fucoidan functionalised cerium oxide nanoaprticles is significantly antioxidant ability as compared with CeO$_2$NPs.

**In-vitro cytotoxicity**

To assess the *in vitro* cytotoxicity of CeO$_2$NPs and fu/CeO$_2$NPs treated against lung cancer cells (A549) and cervical cancer cells (HeLa) using MTT assay shown in A549 Fig.15a and Hela Fig.15b cancer cell lines with their IC$_{50}$ values of 176±0.5µg/ml, 97±0.5µg/ml, and 78±0.5µg/ml, 56±0.5µg/ml respectively. The results indicated that CeO$_2$NPs and fu/CeO$_2$NPs will be increased the production of ROS in A549 and Hela cells in a dependent on dosage when compared to control cells. This oxidative stress to cells may result in significantly reduced cell growth or even cell death via an apoptotic or necrotic pathway (Lin et al. 2006) Cell-free positive-control studies revealed that no ROS were generated by direct communication with Fu/ CeO$_2$NPs, implying that the OS levels were from the cells following exposure to Fu/ CeO$_2$NPs (Mittal and Pandey 2014). Also discovered that CeO$_2$NPs scavenged by generated an increased quantity of ROS in cancer cells, ROS linked to severe DNA damage and cell cycle disruption, with an increase in cells undergoing apoptosis in the sub-G1 phase. Furthermore, when a certain ROS is present and apoptosis inhibitor, both processes were fully enervated, demonstrating that the source of CeO$_2$NPs toxicity is ROS-mediated DNA damage leading to apoptosis (Koyanagi, et al. 2003).

Interestingly, metal oxide nanoparticles produce more intracellular ROS, and this is one of the paradigms of high cytotoxicity (Manikandan et al. 2021). The sulphate groups in fucoidan play a significant role in repressing cancer cell growth by communicating with cationic proteins on the cell membrane, and it's also been disclosed that the structure of this instinctual polysaccharide enables it to be crosslinked with anticancer medicines derived from previous reports (Deepika M. S et al 2019; Qiu et al. 2006).

**Cell apoptotic morphology investigated by AO/EB staining and Hoechst 33342 staining.**

The cell morphological abnormalities caused by the treatment with CeO$_2$NPs and fu/CeO$_2$NPs were evaluated using a fluorescence microscope upon staining with AO/EB and Hoechst. Acidin Orange is an essential dye that could penetrate cell nuclei that are both alive and dead. On the other hand, EB will only stain cells that have managed to lose their membrane permeability to red. (Yang et al. 2013). The AO/EB staining in Fig. 16 indicated that A549 and Hela cells had congenital defects such as cell shrinkage, chromosome segregation, nucleus segmentation, blebbing, as well as the induction of apoptosis. As a result, living cells will be consistently green, whereas apoptotic cells will have compressed or fragmentary nuclei that are bright green. Late apoptotic cells will have condensed and fragmented orange chromatin (Venkatesan, R et al. 2013 and Arumugam et al. 2021). The findings indicate that provoked apoptotic morphologies in a large population of cells, but instead of necrotic cell death and progressive rises in orange and red staining associated by decreases in green staining of nuclei, showing apoptosis and cell
damage in fu/ CeO$_2$NPs compared to CeO$_2$NPs due to sulfated polysaccharide fucoidan exhibits anticancer efficacy which may have been the source of apoptotic stimulation observed on both cancer cell lines A549 and Hela. Hoechst-stained A549 and Hela cells were incubated for 24 hours with CeO$_2$NPs, Fu/ CeO$_2$NPs, and a control Fig.17. Untreated control cells had uniformly stained DNA with normal blue fluorescence and a dark blue nucleus. Cells treated with CeO$_2$NPs and Fu/ CeO$_2$NPs, on the other hand, had cellular morphology in both particles. To test and evaluate the apoptotic characteristic of both Fu/CeO$_2$NPs treated cells had blue nuclei had chromatin separation, binucleation, cytosolic vacuolation, cell wall blebbing, and late cell death are all signs of a chromatin that looks like dots nucleus with blue fluorescence has been indicated in fig compares favourably with CeO$_2$NPs treated cells (Gnanakani, P.E et al. 2019).

The preponderance of the supersaturation and segmentation of apoptotic bodies has been identified mechanism of cell death, which was triggered by oxidative stress-induced Reactive Oxygen Species increase. This oxidative stress has the potential to harm the DNA of malignant cells in a variety of ways. The findings of the hoechst 33342 staining appear to be consistent with the results of the AO/EB staining. These data revealed that the vast majority of cells died as a result of apoptotic cell death.

**Identification of mitochondrial membrane potential loss by JC1 stain**

The treatment of CeO$_2$NPs and Fu/ CeO$_2$NPs leads to the loss of mitochondrial membrane potential ($\Delta \psi_m$), the fluorescent positive charge JC-1, which produce red fluorescence when sequestered through into mitochondria of wholesome cells with high level of ($\Delta \psi_m$), is often used in an experiment to identify patterns in mitochondrial dysfunction. Leading to the decrease of ($\Delta \psi_m$), cells undergoing apoptotic cell death are no longer allowed to disturb the JC-1 cation further into mitochondria, having caused each other to fluoresce green. Fig 18 depicts the JC-1-staining outcomes of lung cancer and cervical cancer allowed to treat with CeO$_2$NPs and Fu/ CeO$_2$NPs at their 24 h IC$_{50}$ concentrations. In both A549 and Hela cells, the rehabilitation resulted in a high terms of percentage of apoptotic cells. In healthy cells, the JC-1 dye collected in the mitochondria as aggregates redishorange fluorescing in cells treated with CeO$_2$NPs and Fu/CeO$_2$NPs for 24 h, the JC-1 dye persisted in the cytoplasm in its dispersed state, which fluorescence green, due to breakdown of mitochondrial membrane potential (Dhivya, R. et al 2015).

**Photocatalytic degradation**

The existence of aromatic amines in the structure of Congo red (CR) dye, It is used in the industrial production of cosmetic products, notebook, medical products, chemical products, and textile industries and a significant percentage of dye-containing untreated sewage ends up in natural water sources which have been lead to cause carcinogenesis (Bhat et al. 2020), poses a serious threat to aquatic life and humans. Many studies used different photocatalysts, including nanoparticles such as CeO$_2$NPs to investigate the photodegradation of Congo red azo-dye from aqueous phase (Al-Onazi et al. 2021). The degradation of the Congo red dye experiment has been carried out with CeO$_2$NPs and Fu/CeO$_2$NPs treated at presence of sunlight. As shown in Fig. 19a, the deterioration of dye in the absence of fucoidan
is indeed very low. The absorbance of dye degradation using CeO$_2$NPs has been low in 15-minutes interval till 135 minutes of reaction time. After that the decomposition of dye was increased with Fu/ CeO$_2$NPs treated, it was discovered that the degradation was greater than the dye degradation in the utter lack of fucoidan. Dye degradation was observed and monitored using a microplate reader. The absorbance of dye degradation results show that the dye's absorption peak gradually shifts in CeO$_2$NPs treated. However, depending on the dye peak's deterioration and reduced height with hypochromism shift and less absorbance was observed in the Fu/ CeO$_2$NPs treated dye degradation shown in Fig.19b.

As a result, as shown in Fig. 19b, significantly dye degraded in the presence of Fu/ CeO$_2$NPs, the biomolecular characteristics of photocatalytic decomposition change as a feature of decomposition time. The Congo red exhibited a major peak at 490 nm, peaks at 335 nm and 235 nm from the degraded peak. The slight absorption peaks at 235 nm and 338 nm correspond to the benzene and naphthalene rings respectively, while the absorption peak at 496 nm corresponds to the Congo red azo bond Fig. 19a and 19b. During the photocatalytic decomposition process, the peaks all three reduce the absorbance in the 15-minute interval more specifically. The decreased absorption after 135 minutes had a significant impact on peak absorption caused by azo bond cleavage. The next steps are as follows: The primary components of Congo red dye degradation are photogenerated electrons from oxygen in the water and an optimistic hole on CeO$_2$NPs, which degrades the Congo red dye in and out of degraded products. Because of the sudden recombination of electron hole pairs in semiconductor nanoparticles (CeO$_2$), their photocatalytic efficiency can be reduced. Fu/CeO$_2$NPs has good photocatalysts for degrade the carcinogenic dye Congo red in time dependent manner with 81.5% of degradation but CeO$_2$NPs treated dye has been significantly with 62.6% of degradation at presence of sunlight compared with Fu/CeO$_2$NPs treated according to Fig.20.

**Discussion**

In this present study, the CeO$_2$NPs and fu/CeO$_2$NPs were successfully synthesized and characterized by various analytical techniques such as UV-Vis, FE-SEM, HR-TEM, FT-IR, XRD, and XPS. These analyses to validate their surface plasmon resonance effect, surface morphology, important functional biomolecules, crystalline structure, and electrochemical binding were studied. The fucoidan and cerium oxide nanoparticles bind together due to electrostatic interaction because of fucoidan has negative charge and cerium oxide nanoparticles has positive charge so easily interact together moreover that binds will be shown in UV-Spectra of fucoidan functionalized cerium oxide nanoparticles. There is hypochromic shift was occur compare with un functionalised cerium oxide nanoparticles which is the one of the confirmation study of fucoidan is fucntionlized with cerium oxide nanoparticles, similarly shift was observed in gold nanorod coated with fucoidan in previous study (Manivasagan, P et al 2017). The CeO$_2$NPs and fu/CeO$_2$NPs have been showed potential biological applications such as antibacterial activity. The antibacterial activity of CeO$_2$NPs and fu/CeO$_2$NPs against *E.coli* and *S. aureus* bacteria suggests that the increase in oxidative stress caused by opposite charged electromagnetic interactions.
and ROS generation has always been the driving force remains the potential antibacterial activity. Moreover fu/ CeO$_2$NPs have highly penetrate the cell wall of bacteria and cause membrane disruption/damage like cytoplasm leakage and morphologically changes of cell wall such as formation of pits observed under SEM was similar reported in earlier. Bacterial cells with and without PAA-Cnp treatment produced bacterial cell colonies that are densely packed, but the PAA-Cnp-treated bacterial cells formed few colonies with cytoplasmic leaking from punctured cells, defining bacterial cell membrane damage in a previous study (Poveda-Castillo et al. 2018). The microbial biomass stressed by nCeO$_2$-NPs was compared to a free-sample of nCeO$_2$-NPs. When compared to the microbial cells in the control sample, the integrity of the bacterial cell structure seemed to be disturbed, resulting in aggregation and lysis of microbial cells. Microbial population in the control sample was higher than in the nCeO$_2$-NPs treated samples, with a reduction in microbial biomass as the concentration of nCeO$_2$-NPs increased. SEM images further indicated a diverse microbial architecture in both nCeO$_2$-NPs treated and nCeO$_2$-NPs free samples. The microbial population was found to be dominated by rod-shaped bacteria, followed by cocci-shaped microorganisms (Dhall and Self 2018). Moreover, the physiological character of MDR-2 bacteria has been studied by Scanning Electron Microscope following treatment with 14 g/ml GO-Ag nanocomposites. Some holes were identified on the surface of the cell wall, indicating that perhaps the cell walls had been damaged as a result of the treatment of GO-Ag nanocomposites. The cell wall of the control group has been smooth, and no disruptions to cell integrity were found, however the Fu/CeO$_2$NPs caused cellular membrane and cytoplasmic damages were observed (Kamika and Tekere 2017; Chen et al. 2020). Antioxidant assay of CeO$_2$NPs and fu/CeO$_2$NPs were showed significant result obtained both DPPH and ABTS. In previous study, The ABTS radical decolorization test relies on the decolorization of ABTS$^+$ when it reacts with a hydrogen-donating antioxidant. The ABTS$^+$ scavenging capabilities of fucoidan and vitamin C (as a reference) were discovered to have dose-dependent ABTS$^+$ scavenging activity (Higuchi 2004). The cytotoxic activity of CeO$_2$NPs and fu/CeO$_2$NPs on lung cancer cells (A549) and cervical cancer cells (HeLa) revealed that these NPs induced the apoptosis for the destruction of A549 and Hela cancer cells. The loss of mitochondrial membrane potential in A549 and Hela cancer cells were observed depolarization pattern. The CeO$_2$NPs and fu/CeO$_2$NPs allowed treating the cancer cells, discharging them to apoptosis via the mitochondrial signal transduction pathway, as analysed by the transformation in mitochondrial transmembrane potential using JC-1 staining. A cysteine protease group catalyses the mitochondrial apoptotoc pathway. Furthermore the detailed study on drug delivery application in A549 and HeLa cancer cell lines as well as other cancerous cells may be endow with important information about therapeutic applications in in-vivo animal model. In previous study fucoidan have significantly induced the apoptosis in various types of cancers such as prostate cancer PC-3 via ERK1/2 MAPK signalling pathway activation with in activation of p38 MAPK & PI3K/Akt and the Wnt/β-cateinin signalling pathway inhibition (Boo et al 2013). Also fucoidan has been triggered apoptosis mediated by ROS in myeloid leukemia (Deepika, M. S et al 2019). A novel photocatalysts such as biopolymers are required to develop new approaches for photocatalytic activity fucoidan (Saha et al. 2020). Such phytochemicals degrade dyes either by minimising or acting as a capping agent or functionalization material for metal oxide or metal nanoparticles with a greater surface area (Fowsiya et
The negative charged fucoidan's plentiful OH group could cause electrostatic repulsion between negatively charged CR dyes. Fucoidan functionalized cerium oxide nanoparticles have a higher degradation efficiency than pure cerium oxide nanoparticles (Al-Onazi et al. 2021). The least percentage of dye degradation has been observed in CeO$_2$NPs treated carcinogenic dye Congo red in the existence of sunlight with compared Fu/CeO$_2$NPs treated dye was higher percentage of degradation.

**Conclusion**

When fucoidan is functionalized with cerium oxide nanoparticles, the multidimensional approaches of biopolymer like sulfate backbone of polysaccharide which has been a considerable combatant against environmental hazardous like microbial pathogens, cancer cell growth, carcinogenic dye are unequivocally proved. Cerium oxide is a lanthanide has a strong antioxidant property due to its paired oxidation states Ce$^{3+}$ and Ce$^{4+}$. Cerium oxide nanoparticles also play an important role in antibacterial activity. Ionic interaction with the bacterial membrane consists of a thick layer of peptidoglycan connected to teichoic acid, particularly glycol protein receptor presented fucoidan, which has interacted with the bacterial cytoplasmic membrane and DNA, causing cytoplasmic leakage and the formation of holes in the cell surface wall proved according to SEM image. The fucoidan functionalized cerium oxide nanoparticles Fu/CeO$_2$NPs developed in this research have been shown to have more enhanced anticancer activity against A549 and Hela cells through the induced intrinsic apoptosis pathway. It will be incorporated in cancerous cells A549 and Hela, having caused pathological alterations and toxic effects in a dose-dependent manner. In fu/CeO$_2$NPs exposed to A549 and Hela cells, comprehensive DNA damage-mediated molecular disturbance results in apoptotic cell death. Furthermore, Fu/CeO$_2$NPs were subjected to photodegradation of the carcinogenic Congo red dye which is caused DNA damage, cancer, and genomic instability. Overall, this research could contribute to the development of fucoidan functionalized cerium oxide nanoparticles has been breaking the factors to cause hazard for environment.

**Declarations**

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**Author Contributions**

Karthikeyan Kandhasamy: investigation, methodology, writing-original draft, review and editing. Dinesh Babu Manikandan: investigation, resources, review and editing. Kumpati Premkumar: conceptualization, project administration, supervision, validation, writing-review and editing.
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Data availability

All data generated or validated during this study had been included in the manuscript.

Ethical Approval

Not applicable.

Consent to participate

Not applicable.

Consent to publish

Not applicable.

Competing Interests

The authors declare no competing interest.

References


doi


**Figures**

![Diagram of fucoidan functionalized cerium oxide nanoparticles Fu/CeO₂NPs](image)

**Figure 1**

Diagram of fucoidan functionalized cerium oxide nanoparticles Fu/CeO₂NPs
Figure 2

UV-Vis spectra of CeO$_2$NPs, fucoidan and Fu/CeO$_2$NPs
Figure 3

UV-Vis spectra of 60 days stability study of CeO$_2$NPs (a) and UV-Vis spectra of 60 days stability study of Fu/CeO$_2$NPs (b).
Figure 4

FE-SEM image of CeO$_2$NPs (a), Fu/CeO$_2$NPs (b) and (c), Fu/CeO$_2$NPs nanocauliflower (d).
Figure 5

HR-TEM image of CeO$_2$NPs (a) and (b), selected area electron diffraction (SAED) pattern (c) and Fu/CeO$_2$NPs (d) and (e).
Figure 6

Hydrodynamic diameters of CeO$_2$NPs (a) and Fu/CeO$_2$NPs (b) for the DLS measurement.

Figure 7

Surface charge (Zeta potential) of CeO$_2$NPs (a) and Fu/CeO$_2$NPs (b)
Figure 8

FT-IR spectra of CeO$_2$NPs fucoidan and Fu/CeO$_2$NPs (a) and XRD(b)
Figure 9

XPS analysis of Ce$^{3d}$ deconvoluted spectra for CeO$_2$NPs depicting Ce in both +3 and +4 oxidation states (a), and O1s spectrum of CeO$_2$NPs oxygen vacancy (b).

Figure 10

Antibacterial activity comparison of CeO$_2$NPs and Fu/CeO$_2$NPs (a) E.coli and (b) S.aureus.

Figure 11

(a) Growth curve of E. coli under the treatment of CeO$_2$NPs and (b) under the treatment of Fu/CeO$_2$NPs. (c) Growth curve of S. aureus under the treatment of CeO$_2$NPs and figure (d) under the treatment of Fu/CeO$_2$NPs.

Figure 12

SEM image of E.coli after 24h growth in media only (a) and media containing 75µg/mL of Fu/CeO$_2$NPs (b), respectively treated reveals membrane damage and cytoplasmic leakage noted in yellow arrow.
Figure 13
SEM image of S.aureus after 24h growth in media only (a)and media containing 75µg/mL of Fu/CeO$_2$NPs (b), respectively treated shows some pits were found on the surface of the cell wall marked in yellow arrow.

![Figure 13 Image](image_url)

Figure 14
DPPH radical scavenging activity for CeO$_2$NP(a), Fu/CeO$_2$NPs and Ascorbic acid, (B) ABTS radical scavenging activity for CeO$_2$NPs, Fu/CeO$_2$NPs and Ascorbic acid.

![Figure 14 Images](image_url)

Figure 15
MTT Assay of A549 cells treated with CeO$_2$NPs and Fu/CeO$_2$NPs (a), MTT Assay of HeLa cells treated with CeO$_2$NPs and Fu/CeO$_2$NPs (b).

![Figure 15 Images](image_url)

Figure 16
Morphological changes observed for Control, CeO$_2$NPs and Fu/CeO$_2$NPs treated (24 h) A549 cells and HeLa cells stained with AO/EB.

![Figure 16 Images](image_url)
Figure 17

Morphological features of nuclei observed for control, CeO$_2$NPs and Fu/CeO$_2$NPs treated (24 h) A549 cells and HeLa cells stained with Hoechst 33258.

Figure 18

Mitochondria membrane potential using JC-1 stain on A549 and Hela cells under CeO$_2$NPs and Fu/CeO$_2$NPs treatment.
Figure 19

Photocatalytic degradation of congo red under sunlight irradiation treated with CeO$_2$NPs (a) and Fu/CeO$_2$NPs (b).
Figure 20

The percentage of photocatalytic dye degradation of congo red treated with CeO$_2$NPs and Fu/ CeO$_2$NPs.