Evaluation of Cytotoxicity and Apoptotic behavior of Cerium Oxide/Zinc Oxide/ Graphene Oxide in HeLa and VERO cell Lines

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Abstract

Using the ultrasonic approach, we produced a morphology involving cerium oxide/ Zinc oxide/graphene oxide ($\text{CeO}_2/\text{ZnO}/\text{GO}$) nanocomposite-based system. The developed nanocomposite was examined using X-Ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), and field emission scanning electron microscopy (FESEM). The average crystallite size was found to be 11.44 nm, as determined by XRD. FTIR analysis was used to confirm the existence of functional groups. FESEM was used to verify the morphological properties of $\text{CeO}_2/\text{ZnO}/\text{GO}$. The micromorphology of $\text{CeO}_2/\text{ZnO}/\text{GO}$ nanocomposite reveals a smoother sheet-like structure. In addition, using an antiproliferative assay test, the developed nanosystem was evaluated for its scavenging anti-cancer capability against HeLa cell lines at various doses and incubation intervals. In our investigation, the effective IC_{50} concentration was reported to be 62.5 $\mu\text{g}/\text{ml}$ at 72 h. Further, the developed nanosystem was evaluated for its killing efficacy against normal cell line. To identify apoptosis-associated alterations of cell membranes throughout the apoptosis process, a dual acridine orange/ethidium bromide (AO/EB) fluorescent staining was done using $\text{CeO}_2/\text{ZnO}/\text{GO}$ nanocomposite at three specific concentrations. The quantitative analysis was carried out using flow cytometry (FACS study) to determine the cell cycle during which the greatest number of HeLa cells were destroyed. According to the results of the FACS investigation, maximum cell cycle has taken place in P2, P4. As a result, the newly designed $\text{CeO}_2/\text{ZnO}/\text{GO}$ hybrid has demonstrated improved anti-cancer efficacy against the HeLa cell line, making it a better therapeutic agent for cervical cancer detection.

1 Introduction

Cervical cancer has a global incidence rate of 13.1/105 and a death rate of 6.9/105, respectively. In India, cervical cancer is the second most frequent cancer among women, with an estimated 96,922 new cases and 60,078 deaths. Although the incidence and death rates vary among the Indian population, they are 14.7/105 and 9.2/105, respectively. The majority of these cervical patients appear at a late stage of disease in a health-care facility due to a lack of knowledge (1). As a result, research into the toxicity of various types of nanomaterials has confirmed the feasibility of diagnosing cancer. Various nanoparticle formulations for diagnostic and therapeutic purposes have been developed as a result of recent breakthroughs in nanotechnology. Diagnostic nanoparticles are designed to aid in the visualization of pathologies and the better understanding of important (patho-) physiological principles underlying a variety of diseases and treatments. However, due to the complicated demands on their pharmacokinetic characteristics and elimination, nano-diagnostics are only helpful in a restricted number of clinical settings. As a result, the vast majority of nanoparticle formulations employed in clinics today are for therapeutic purposes (2). Particle size and charge, core and surface properties, shape and flexibility, multivalency and controlled synthesis, as well as multivalency and controlled synthesis, are the most important properties of nanoparticle formulations, as they determine the nanoparticle's in vivo distribution, targeting ability, and toxicity. Furthermore, these characteristics have a significant impact on drug loading capacity and release, as well as nanoparticle stability (3,4,5). Metal oxide NPs (MONPs), in

particular, have several advantages, including high stability, simple preparation processes, easy engineering to the desired size, shape, and porosity, no swelling variations, easy incorporation into hydrophobic and hydrophilic systems, and easy functionalization by various molecules due to the negative charge of the surface, all of which make them a promising tool for biomedical applications (6). Different cell target specificity exhibits varying metabolic activity and, as a result, varying cell death processes and sensitivity to MONP exposure. When nano SiO₂ NPs were compared to the toxic effects of human monocytes (THP-1) and human lung epithelial cells (L-132), it was discovered that THP-1 cells were more cytotoxic than L-132 cells (7). Metal oxide nanosystems with semiconducting characteristics, such as titanium dioxide (TiO₂), zirconium dioxide (ZrO₂), copper-II oxide (CuO), zinc oxide (ZnO), and cerium oxide (CeO₂) in various morphologies, help to provide biological potencies (8,9). Because of its large band gap (3.19 eV), cerium oxide is an outstanding semiconductor oxide. Because of its antioxidative and anti-inflammatory capabilities, CeO₂ nanoparticles (NPs) have sparked interest in the biomedical area [13, 14]. Researchers are currently working on GO/RGO-based nanocomposites that have better optoelectronic properties than pure GO or RGO. Also, Graphene is a two-dimensional sheet of sp² hybridized carbon atoms that has a variety of features, including elasticity, flexibility, high conductivity, and ease of functionalization [8, 9]. Graphene has sparked a lot of interest in synthetic biology and nanomedicine, in addition to its uses in photonics, optoelectronics, and environmental remediation [10–12]. Furthermore, because of its anti-inflammatory, antibacterial, and antifungal properties, the use of ZnO in medicine is particularly promising, as evidenced by the development of special textiles for patients with chronic inflammatory skin illnesses [15]. Also, in vitro, ZnO-NP coupled with chemotherapeutic medicines shown specific anti-cancer efficacy [16]. However, studies on combining the properties of cerium oxide, zinc oxide and graphene oxide nanoparticles has not yet been explored. This prompted us to study anticancer and apoptotic activity of CeO₂/ZnO/GO nanocomposite. In addition, we have also experimented anticancer effectiveness upon loading cisplatin (anticancer drug) in the developed CeO₂/ZnO/GO platform. X-Ray diffraction (XRD) was used to examine their physio-chemico characteristics, field emission scanning electron microscopy (FESEM) was used to examine their morphological properties, and FTIR was used to validate the presence of functional groups. Their crystalline, morphological, and functional existences gave a great track record in anticancer experiments against HeLa cell lines, and they are addressed in more detail below.

2 Materials And Methods

All chemicals and solvents were of analytical grade and were utilized without being purified further. Fischer Scientific in India provided cerous nitrate hexahydrate (CeN₃O₉·6H₂O), graphene oxide (GO) and sodium hydroxide (NaOH). Zinc acetate dihydrate Zn(CH₃COO)₂·2H₂O (99.5%), Acetone CH₃CHO (99%) was acquired from Sisco Research Laboratories Pvt. Ltd (SRL)-India. The CeO₂/GO hybrid nanosystem's X-Ray diffraction (XRD) spectrum was captured using a RIGAKU mini fux 2C, Japan. The functional groups found in the product were analyzed using the spectrum of Fourier infrared spectroscopy (JASCO FT-IR-6300, Japan)..Surface morphology was obtained using a Carl Zeiss NTS GMBH FESEM model

SUPRA 55. A spectrophotometer (Labman Scientific Instruments, India) was used to collect absorption spectra. Metzer Inverted Confocal Microscope, India, was used to obtain confocal pictures of HeLa cells.

2.1 In vitro investigation on anticancer efficacy using $\text{CeO}_2/\text{ZnO}/\text{GO}$ & $\text{CeO}_2/\text{CDDP-ZnO}/\text{GO}$ system

The cellular toxicity of $\text{CeO}_2/\text{ZnO}/\text{GO}$ hybrid (blank) and $\text{CeO}_2/\text{CDDP-ZnO}/\text{GO}$ system (cisplatin loaded porous hybrid nanosystem) was investigated in cancer cell lines (HeLa). The cells were kept in a humidified atmosphere of 50 $\mu\text{g}/\text{ml}$ CO_2 at 37 °C for 24 hours in Minimal Essential Medium supplemented with 10% FBS, penicillin (100 U/ml), and streptomycin (100 $\mu\text{g}/\text{ml}$). The cells were removed from the cell culture flasks using trypsin-EDTA when they reached 95 percent confluence. The cell solution was centrifuged for 5 minutes at 900 rpm before being resuspended in culture media at a concentration of 1×10^5 cells/mL. In each well of a transparent flat bottom 96-well plate (Costar), 200 μL of the cell suspension was transferred and incubated for 24 hours. After that, the medium was withdrawn and replaced with fresh medium containing 2-fold serial dilutions. A methylthiazolyldiphenyltetrazolium bromide (MTT) viability experiment was used to assess the toxicity of the porous hybrid nanosystem. A total of 5 mg of MTT was mixed in 1 mL PBS solution and then diluted 10 times in culture medium. After removing the supernatants, 100 μL of MTT solution was added to each well, which was then incubated for 4 hours before adding 100 μL of the solubilization solution (anhydrous isopropanol containing 0.1 N HCl and 10 percent Triton X-100). After that, the plates were incubated overnight and the absorbance was measured on a microplate reader at 570 nm (BMG FLUO star OPTIMA). Non-treated cells (M) in culture medium were assumed to be 100 percent viable (live cells). The following formula was used to compute the percent cell inhibition:

$$\% \text{ Cell inhibition} = \frac{\text{O.D value of treated cells using hybrid nanosystem}}{\text{O.D value of non-treated cells}} \times 100 \quad (1)$$

When the $\text{CeO}_2/\text{CDDP-ZnO}/\text{GO}$ system was interacted with the HeLa cell line under in – vitro conditions, the same approach was used to determine the percent Cell inhibition for the purpose of estimation of its synergetic effect with the anticancer drug. Finally, based on the data collected, the sample concentration required to achieve 50% cell inhibition was calculated and recorded as the IC_{50} value. This IC_{50} value will be used as a benchmark for further research into the anti-cancer potential of our $\text{CeO}_2/\text{ZnO}/\text{GO}$ hybrid system.

2.2 Apoptosis Study using Direct Fluorescence Microscopic Analysis

The morphological induction of apoptosis on the $\text{CeO}_2/\text{ZnO}/\text{GO}$ hybrid nanocomposite treated cells was detected by acridine orange/ethidium bromide (AO/EB) staining. The cells were fixed for 1 hour at room temperature in a 3:1 mixture of ethanol and phosphate buffer solution (PBS). The cells treated with hybrid nanocomposite were incubated after being labeled with a 1:1 ratio of AO and EB in PBS. After washing away the excess unbinding dye with PBS, the stained cells were examined under a fluorescence microscope.

2.3 Cell cycle Analysis

The cell cycle arrest was tested using procedure [40], in which (1×10^6) cells were cultivated in a tissue culture dish and allowed to mature for the entire night. The cells were preserved as controls after being treated with $\text{CeO}_2/\text{ZnO}/\text{GO}$ complexes. The cells were trypsinized and collected in appropriate centrifugal tubes after reaching 75 percent confluence. The cells in the pellet were then re-suspended in 300 mL of phosphate buffer solution–ethylenediamine tetra acetic acid (PBS–EDTA) to which 700 mL of cold 70% ethanol was added drop-wise with gradual mixing after centrifugation at 2500 rpm for 5 minutes at room temperature (RT). The solution was added to guarantee 100% ethanol combination, and the samples were stored overnight at 0 °C. Following that, 100 I RNase A was added to the cell suspension and incubated for 1 hour at 37 °C. 400 I propidium iodide was added to the stain and let to sit at room temperature for 10–20 minutes in the dark. A flow cytometer was used to examine the labeled cells for cell phase distribution.

2.4 Preparation of $\text{CeO}_2/\text{ZnO}/\text{GO}$ hybrid nanocomposite

In a recent article, we described the procedure for making CeO_2 nano-particles and CeO_2/GO hybrid nanocomposite (28). ZnO nanoparticle was prepared by using simple wet chemical route in which 1.2 g of Zinc acetate dihydrate was stirred along with 0.4 g of sodium hydroxide (41). Further, 1 g of CeO_2 NPs powder was diluted in 100 ml distilled water and ultrasonically processed for 30 minutes. 1 g of GO NPs powder was diluted in 100 ml distilled water and ultrasonically processed for 2 hours. 0.5 g of ZnO nanoparticles was diluted in 50 ml distilled water and ultrasonically processed for 30 minutes. Finally, the three mixture-containing solutions are blended to create a nanocomposite solution. Furthermore, after combining the mixtures, they were stirred for 2 hours to achieve a homogenous solution. After allowing the materials to settle, they were cleaned three times with distilled water as shown in fig 1.

3 Results & Discussions

3.1 X-ray Diffraction Study

Figure 2 shows the XRD pattern of the $\text{CeO}_2/\text{ZnO}/\text{GO}$ nanocomposite samples. The X-Ray diffraction pattern of the $\text{CeO}_2/\text{ZnO}/\text{GO}$ composite shows the characteristic peaks of at $2\theta = 27.01, 32.22, 45.27, 54.7, 60.0, 69.52$ and 76° corresponding to the lattice planes (111), (200), (220), (311), (222), (400) and (311) respectively which were well matched with the JCPDS card no. 65-5923 associated to the CeO_2 cubic face centered crystal system (18). The observed XRD pattern shows that CeO_2 is in high concentration compared to other nanoparticles. Crystallite size of the prepared sample was calculated by Scherrer equation (Eq. 1) and the average crystallite size is around 11.44 nm (19)(20). The d spacing values of the important peaks were calculated by Bragg's equation (Eq. 2) and the obtained values are 0.33, 0.22, 0.20, 0.17 and 0.15 nm for the corresponding lattice planes of (111), (200), (220), (311) and (222) respectively. The observed result confirms that the formation of nanocomposite.

3.2 FTIR study

In order to identify the functional groups, transmittance in the infrared (IR) region takes place ($4000\text{--}500\text{ cm}^{-1}$) due to the vibrational and rotational movement of the chemical bond and molecular groups of the prepared sample. The observed FTIR result of the prepared sample was given in figure 3 which shows that various functional groups were present in the sample. The observed various functional group can be due to the presence of CeO_2 , ZnO and GO particles. The band at 3373.71 cm^{-1} corresponds to the --OH stretching vibrations of the hydroxyl groups which may be observed due to the surface observed water molecules (21). Moreover, other bands observed at 2919.42 , 1454.28 , and 1097.65 cm^{-1} , corresponding to the band reflection of C--H , C=O , and C--O stretching vibrations, respectively which confirms the presence of GO in the composite sample (22)(23)(24). Similarly, the band observed 1631 cm^{-1} corresponds to hydrogen-bonded O--H groups of water molecules (25). The observed bands at 608.18 cm^{-1} and 837.75 cm^{-1} could be owed to the envelope of phonon band of the metal oxygen stretching vibrations (26). Hence the FTIR results clearly confirm the formation of GO with $\text{M--O}(\text{CeO}_2/\text{ZnO})$.

3.3 Surface Morphology Study

Figure 4 shows the representative morphological structure of the $\text{CeO}_2/\text{ZnO}/\text{GO}$ nanocomposite revealed by the FE-SEM images. In figure 3 the micro morphology of $\text{CeO}_2/\text{ZnO}/\text{GO}$ nanocomposite shows a smoother sheet-like structure. Particularly, the existence of a GO sheet comprised of folded and wrinkled sheets like structure. In figure 3 (b) the appearance of a minor rough structure on the wrinkle surface shows that the CeO_2/ZnO nanoparticles have been effectively attached on the surface of GO to the residual oxygen-containing functional groups of the GO (27). From this observed FE-SEM image the growth of CeO_2/ZnO nanoparticles can be seen on the surface of GO sheets which eventually increase the surface area of the as prepared nanocomposites.

3.4 In-vitro cytotoxicity evaluation of $\text{CeO}_2/\text{ZnO}/\text{GO}$ and $\text{CeO}_2/\text{CDDP--ZnO}/\text{GO}$ systems on HeLa cells and normal cells

HeLa cells were treated with $\text{CeO}_2/\text{ZnO}/\text{GO}$ nanocomposite and % cell viability was recorded after day 1 & day 3 (24 hrs and 72 hrs) as shown in figure 5. Further, developed nanocomposite was subject to non-cancerous cell line (VERO) and its killing effect on normal cells were recorded after 24 hrs. Here cell viability refers to the number of HeLa cancer cells that stay alive after interacting with $\text{CeO}_2/\text{ZnO}/\text{GO}$ hybrid that has been developed. These cytotoxicity assays were often carried out at various concentration levels (1000 , 500 , 250 , 125 , 62.5 , 31.2 , 15.6 and $7.8\text{ }\mu\text{g/ml}$) of materials at varying incubation intervals (24 , 48 , and 72 hours) in order to obtain an accurate IC_{50} value for which nearly 50% of malignant cells were destroyed. IC_{50} refers to the lowest concentration of developed nanomaterial at which almost half of the HeLa cell line remains alive while the rest dies. In our investigation, $500\text{ }\mu\text{g/ml}$ was determined to be the IC_{50} value, indicating that cell viability was nearing 50% for 24 hours and it is $62.5\text{ }\mu\text{g/ml}$ for 72 hours. The MTT results show that as the concentration of our produced $\text{CeO}_2/\text{ZnO}/\text{GO}$ hybrid increases,

the number of dead cells increases while the number of living cells decreases. When a maximum concentration of about 1000 µg/ml of CeO₂/ZnO/GO hybrid was targeted on HeLa cell line for 72 hours, %cell death was recorded to 81.82 percent, while the least concentration of about 7.8 µg/ml of CeO₂/ZnO/GO hybrid were applied to the HeLa cell line, it resulted in decreased cell death by about 16.89 percent. The antiproliferative experimentation was carried out in this work for two different incubation periods (24 hrs and 72 hrs), and the effective IC₅₀ concentration was reported to be 62.5 µg/ml at 72 h (Fig. 5). In addition, when a maximum concentration of 1000 µg/ml of CeO₂/ZnO/GO hybrid were targeted on normal cells % live cells was 67.74 and for lowest concentration of 7.8 µg/ml it was 96.77% as shown in fig 6. These findings show that developed CeO₂/ZnO/GO hybrid platform is potent to kill HeLa cells and possess less toxicity on normal cells. Previous studies (28) on HeLa cells using CeO₂/GO hybrid nanoparticles in various concentrations and incubation durations found similar effects. The inhibition rate was found to be 88.89%. Also, (29) demonstrated % cell viability of CeO₂/ZnO hybrid with maximum attainment of 95.44% and ZnO NPs with 90.48% against HeLa cells. Based on these findings, CeO₂/ZnO/GO hybrid were combined with cisplatin and was subject to antiproliferative assay to record their synergetic biomolecular interactions. Fig 5 reveals that, when a maximum concentration of 1000 µg/ml of CeO₂/CDDP-ZnO/GO were interacted with HeLa cell line, 95.47% cells were killed and for 7.8 µg/ml it was 59.35%. This further affirms the ability of CeO₂/ZnO/GO hybrid to act as drug delivery platform. The electrostatic interaction between the nanocomposite and the cell lines may have caused a reduction in the cytotoxicity effects, resulting in a lower rate of death with extended incubation time (32). The nonspecific surface reactivity had a hatching rate that was proportional to the concentration. The CeO₂/ZnO/GO hybrid nanocomposite demonstrated stronger cytotoxicity effects against HeLa cells than previously reported CeO₂ nanoparticles (33, 34) when tested for anticancer activities. The %cell inhibition rate induced by interacting cerium oxide nanoparticles, zinc oxide NPs and the hybrids against various cancer cell lines was compared to previously published results and is summarized in Table 1.

Table 1: Comparative analysis of different NPs & hybrids against different cell lines

S. No	Materials Developed	Types of cell line used	% Cell Inhibition	References
1.	CeO₂/ZnO	HeLa	95.44	12
2.	CeO₂/GO	HeLa	88.89	11
3.	ZnO NPs	HeLa	90.48	12
4.	CeO₂NPs	A549	~15	13
5.	DOX-loaded nanoceria (CeO₂/DOX)	A2780	63.32	14

The induced % cell death upon interaction of CeO₂/ZnO/GO hybrid with HeLa cell line was further subject to qualitative analysis using AO/EB Dual Staining study which was performed at different concentrations such as 1000µg/ml, 500µg/ml and 250µg/ml.

3.5 Apoptotic study on CeO₂/ZnO/GO nanocomposite using AO/EB Dual Staining

The morphological and synergetic biochemical alterations when CeO₂/ZnO/GO nanocomposite was interacted with HeLa cells were explored further. The AO stain can enter both living and apoptotic cells, emitting green fluorescence, while the EB stain can only enter necrotic cells, emitting red fluorescence (36-37). Red fluorescence was generated by apoptotic cells which includes only dead cells (35). The morphological changes due to interaction of CeO₂/ZnO/GO hybrid with HeLa cells were recorded and is shown in Fig 7. Fig 7a shows untreated HeLa cells which emits green fluorescence due to absorption of AO by live cancer cells. Fig 7b shows red fluorescence due to absorption of EB by dead cancer cells and this happened due to interaction of maximum concentration of 1000µg/ml of CeO₂/ZnO/GO with HeLa cells. Fig 7(c,d) shows presence of few green fluorescence amidst large red fluorescence and this happened due to interaction of 500µg/ml and 250µg/ml CeO₂/ZnO/GO nanocomposite with HeLa cells. All these findings affirms that the developed CeO₂/ZnO/GO hybrid possess good anti-cancer properties against HeLa cells. Further, quantitative analysis was performed on developed system to evaluate apoptotic behavior using flow cytometry study.

3.6 Flow Cytometry Analysis

The cell cycle at which the most cancer cells were killed was estimated using CeO₂/ZnO/GO hybrid formulations at various concentrations (125 µg/ml, 31.2 µg/ml, and 62.5 µg/ml) using flow cytometry as shown in figure 8(a-d). Their anticancer efficacy was estimated using cellular uptake study using monolayer cell culture model. Fig 8(a) reveals flow cytometry of untreated HeLa cells with p1 highly populated. Fig 8 (b-d) shows the flow cytometry upon interaction of 125 µg/ml, 62.5 µg/ml and 31.2 µg/ml concentration of CeO₂/ZnO/GO hybrid on HeLa cells. These findings affirms that maximum cell cycle has taken place in P2, P4 events. In addition, among all these concentrations, IC50 concentration of 62.5 µg/ml was able to arrest maximum cancer cells across 4 phase cycles (p1,p2,p3,p4). significant cell cycle arrest was generated as a result of increased cytotoxicity, which then caused apoptotic behavior at an early stage (Child phase). Furthermore, as the IC50 concentration was increased, the peak intensity in the p3 cycle decreased, indicating better cell death. The intensity changes are related to the surface charged characteristics, and we saw a similar impact in our previous CeO₂/GO hybrid work (28). Culcasi et al. demonstrated that pretreatment of human dermal and murine 3T3 fibroblasts with the CONP dosedependently triggered the release of superoxide dismutase and catalase-inhibitable 5-(diethoxyphosphoryl)-5-methyl-1-pyrroline-N-oxide/hydroxyl radical adducts (DEPMPO-OH) and ascorbyl radical in the culture medium using DEPMPO spin trapping of hydroxyl radical in an EPR study. This reaction is thought to be mediated by both mitochondrial activation and the NADPH oxidase complex, according to the researchers. The findings backed with a twofold mechanistic explanation for oxidative

stress caused by CONP exposure, with an early signaling at micromolar concentrations modulated by thiol-containing molecules (38). Also, the prooxidant activity of the nanoparticles is partly responsible for the alterations in intracellular redox state generated by CONP. Furthermore, cell damage caused by ROS may finally result in cell death (39). Aside from these porous and crystalline properties, our hybrid system has paved the road for it to operate as an ideal hydrophilic surface, allowing it to collect the maximum cancer cells and enhance %cell inhibition.

4 Conclusion

In conclusion, an ultrasonic approach was used to create CeO₂/ZnO/GO nanocomposite. The average size of crystallites was discovered to be 11.44nm. XRD and FTIR measurements were used to establish the physiochemical properties. FESEM was used to investigate the surface morphology of the produced nanocomposite. The anticancer properties of the developed CeO₂/ZnO/GO platform were later validated utilizing cytotoxicity, apoptosis, and flow cytometry. Antiproliferative assay was also used to test the synergetic impact of the synthesized hybrid and cisplatin, an anticancer medication. These findings support the use of the CeO₂/ZnO/GO hybrid system in cervical cancer screening and diagnosis.

Declarations

Declaration of interests

☒√The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐xThe authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

J. Saranya

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Figures

Figure 1

Schematic view of the synthesis steps in the development of a $\text{CeO}_2/\text{ZnO}/\text{GO}$ nanocomposite

Figure 2

XRD pattern of CeO₂/ZnO/GO nanocomposite

Figure 3

FTIR spectra of CeO₂/ZnO/GO nanocomposite

Figure 4

Figure 5

% Cell Viability of CeO2/ZnO/GO & CeO2/CDDP-ZnO/GO nanocomposite against HeLa Cell Line

Figure 6

% Cell Viability of CeO₂/ZnO/GO nanocomposite against normal Cell Line.

Figure 7

Microscopic Fluorescence images (a) untreated HeLa cells; (b), (c), (d) treated HeLa cells using 1000µg/ml, 500µg/ml and 250µg/ml CeO₂/ZnO/GO nanocomposite.

Figure 8

Flow cytometry histograms of (a) untreated HeLa cells; (b), (c), (d) 125, 62.5 & 31.2 $\mu\text{g/ml}$ of $\text{CeO}_2/\text{ZnO}/\text{GO}$ nanocomposite treated with HeLa cells.