RESEARCH ARTICLE

Editorial Process: Submission:08/16/2024 Acceptance:02/13/2025

Investigating the Anticancer Activity of Cerium Nanoparticle Decorated on GO Produced by Green Methods against Cancerous Cell Lines

Huda Mahmood Al-attar[1], Maeda H. Mohammad[2]*, Ayeda Mamdooh Majeed[3], Haitham T. Hussein[4], Aysar A. Ahmed[2]

Abstract

Introduction: Green synthesis of nanoparticles is a promising technique for creating functionalized nanoparticles (NPs) without generating byproducts, unlike chemical synthesis methods compared to traditional cancer treatments. Aim: To determine the potential impact of Graphene-Oxide/Cerium Oxide nanoparticles GO/CeO2 NPs as an anti-tumor agent in biomedical field, an in vitro study. **Methods:** In the current study, CeO2 was synthesized using co-precipitation method. The method of preparation used is considered green due the usage of Allium Sativum extract in the synthesis of CeO2 nanoparticles. Graphene -Oxide (GO) was prepared by pulsed laser ablation in water (PLAL) which is also considered as a green method. The decoration of CeO2 on GO was achieved by mixing the GO with CeO2 nanocolloid, then characterized. For the in vitro study, CeO2 concentrations were used (0.98 – 250 µg/ml) as duplicate against human breast cancer cell line AMJ13, brain cancer cell line AMGM5, human esophagus cancer cell line SK-GT-4 and compared with normal cell line REF. **Results:** The FESEM results show that the CeO2 NPs are homogeneous and nearly spherical in its shape with an average dimension of 55 nm. The in vitro results showed that the GO/CeO2 had significant potential impact in the high concentration 250 µg/ml at p> 0.05 against breast cancer cell line AMJ13 (with growth inhibition 51.04%) compared with other cell lines that showed less significant effect at p> 0.05 on brain cancer cell line AMGM5 (with growth inhibition 37.12%) and increased the proliferation rate on pp cancer cell line SK-GT-4 compared with no effects on control normal cell line REF during 48 h. of time exposure. **Conclusions:** Based on the results, we concluded that the synthesized CeO2 is a promising tool for use as a drug delivery agent against breast cancer, demonstrating high safety for the normal REF cell line.

Keywords: AMJ13- AMGM5- CeO2- In vitro- SK-GT-4

Asian Pac J Cancer Prev, **26 (2)**, 541-549

Introduction

Cerium oxide nanoparticles (CeO_2 NPs) are highly captivating due to their exceptional electronic and optical properties and their wide range of applications. These include biomedical uses such as antibacterial, antifungal, and anticancer treatments, as well as catalytic applications and technologies in optics and sensors. Various synthetic methodologies have been reported for the synthesis of CeO_2 NPs, including chemical, photochemical, electrochemical, microemulsion, and microwave techniques. However, these methods often involve the use of carcinogenic chemical compounds, which can pose health risks and require stringent reaction conditions, leading to potential environmental pollution. Among these methods, green synthesis stands out as a rapid and eco-friendly approach to produce CeO_2 nanoparticles [1].

Cerium dioxide is a versatile compound utilized in a wide range of technological applications, including catalysis, oxygen sensors, synthetic membranes, and biomedicine. The use of a capping agent is crucial in nanoparticle synthesis to prevent agglomeration, which can affect the nanoparticles' size and functionality [2, 3].

Allium species are fascinating plants with numerous active components used in medical applications. These plants help reduce the risk of cardiovascular diseases and diabetes, boost the immune system to protect against infections, and have antifungal, antimicrobial, and anti-

*[1]Environmental Engineering Department, College of Engineering/University of Baghdad, Baghdad, Iraq. [2]Experimental Therapy Department, Iraqi Center for Cancer and Cancer and Medical Genetics Research, Mustansiriyah University, Baghdad, Iraq. [3]Cancer Research Department, Iraqi Center for Cancer and Cancer and Medical Genetics Research, Mustansiriyah University, Baghdad, Iraq. [4]Applied Science Department, University of Technology-Iraq, Baghdad, Iraq. *For Correspondence: maeda.hm@uomustansiriyah.edu.iq*

aging properties. Notably, they also serve as anti-cancer agents for various cancer types, a fact confirmed by epidemiological data from human clinical studies [4]. Garlic is rich in various mineral components such as sulfur, potassium, phosphorus, selenium, germanium, as well as adequate amounts of vitamins A and C, and essential amino acids. There is a significant demand for biocompatible and environmentally friendly nano products. Due to its importance, we utilized garlic in our study to prepare CeO_2 nanoparticles through an eco-friendly synthesis method [5].

The main objective of this study is to evaluate the potential of CeO_2 nanoparticles as an anticancer agent against a panel of human cell lines, including the breast cancer cell line AMJ13, brain cancer cell line AMGM5, and esophagus cancer cell line SK-GT-4, in comparison to the normal cell line REF.

Materials and Methods

Materials

Phosphate Buffered Saline (PBS) (Reidel de Haen, Germeny),, Distilled water (D.W.), 96 microtiter well plate (Cel-Cult, U.K), millipore filter 0.45 (Sigma, U.S.A), tissue culture flasks (Falcon, U.S.A), crystal violate (Sigma Aldrich, USA), MEM medium (US biological, USA), RPMI- 1640 medium (US biological, USA), Fetal bovine serum FBS (Capricorn- Scientific, Germany), penicillin and streptomycin (Capricorn- Scientific, Germany), trypsin-EDTA (T.V.) (Capricorn-Scientific, Germany), Ce(NO3)3.6H2O from Sigma Aldrich (India), CAS No. 10294-41-4, purity 99.9%, NaOH from AVONCHEM (UK), CAS NO. 1310-73-2, purity 9.9%, Allium Sativum from the local market, absolute Ethanol, Ø 45 filter paper, graphite pellet with dimensions of Poly vinyl alcohol (C2H4O)n (PVA) from Didactic (Spain), CAS NO. 9002-89-5. All the materials were used in the study without further purification.

Method

This study was carried out at the experimental therapy department, Iraqi Center of Cancer and Medical Genetics Research (ICCMGR), Mustansiriyah University, Baghdad, Iraq. And the protocols of this work were approved by ethics committee of scientific committee of ICCMGR.

Green Synthesis of CeO_2 nanoparticle
Allium Sativum Extract

Thirty-two grams of Allium sativum cloves (collected from local Iraqi markets and authenticated according to Iraqi plant ethics for this study) were washed with tap water, followed by distilled water, and allowed to dry. The cloves were then peeled and crushed using a mortar and pestle. The crushed cloves were placed in 45 mm filter paper, and 1 liter of distilled water was poured over them to obtain Allium sativum extract. This extract was stored in a glass bottle at room temperature and covered to protect it from light exposure.

CeO_2 nanoparticles synthesis

CeO_2 nanoparticles were prepared using a co-precipitation method. In this process, 1 M of Ce(NO3)3.6H2O was dissolved in 70 mL of distilled water (D.W.). Then, 10 mL of Allium sativum extract was added to the solution. A 1 M NaOH solution was slowly added from a 50 mL burette until the color change indicated the formation of CeO_2 precipitate. The precipitate was washed several times with distilled water, followed by absolute ethanol. The CeO_2 precipitate was then dried in an oven at 80 °C for 1 hour and subsequently calcined in a furnace at 850 °C for 1 hour.

GO preparation

A graphite pellet with dimensions of (Ø 25 mm × 5 mm) was used as a target. The graphite target was immersed in 7 mL of distilled water (D.W.) inside a 40 mL beaker, which was placed in a 50 °C water bath. An x-y galvanometer with a 1064 μm fiber laser, operating at 5 W, 20 kHz, and a speed of 100 mm/s, was used to ablate the target [6, 7]. The synthesized graphene oxide (GO) was dried using a heating plate and has a final weight of 7 mg.

PVA/GO/CeO_2

0.01 g of polyvinyl alcohol (PVA) was dissolved in 50 mL of distilled water (D.W.) at 80 °C using a magnetic stirrer. Next, 5 mg of the synthesized CeO_2 was added to the PVA solution and stirred vigorously for 3-4 hours. The PVA/CeO_2 mixture was then ultracentrifuged at 20,000 rpm and -20 °C for 30 minutes. The precipitate at the bottom of the tube was placed in a beaker, and 20 mL of PBS was added. This mixture was then subjected to ultrasonication for 20 minutes until a homogeneous solution was obtained. The dried GO was then added to the sonicated solution with vigorous stirring until a homogeneous solution was achieved. The ratio of CeO_2 to GO was 1:3.333 (w/w). The GO/CeO_2 in PBS was then used on normal and cancerous cell lines after filtration through a sterile filter.

In Vitro study
Cell Lines

Three human cancer cell lines were used as AMJ13, AMGM5, SK-GT-4, and one normal cell line REF that were provided and authenticated by the cell bank unit/ experimental therapy department/ ICCMGR, Mustansiriyah University.

AMJ13. (as a locally Iraqi established cell line), [8] that cultured in a RPMI-1640 medium, supplemented with 10% fetal bovine serum (FBS), 100 units/mL penicillin, and 100 μg/mL streptomycin.

AMGM5. Ahmed-Majeed-Glioblastoma-Multiforme-2005 the Iraqi locally brain cell line that stablished in the experimental therapy department/ ICCMGR, Mustansiriyah University. It's a human brain Glioblastoma GBM cell line which was isolated from a 72-year-old Iraqi man who underwent a surgery for an intracranial tumor. This cell line was cultured and maintained on RPMI-1640 with 10% FBS, with 100 units/mL penicillin, and 100 μg/mL streptomycin [9].

SK-GT-4. Esophageal carcinoma cell line was created from a primary human patient who had dysphagia as a result of a well-differentiated adenocarcinoma that

originated in the distal esophageal Barrett epithelium. It was grown and maintained in MEM growth medium with 10% FBS, 100 units/mL penicillin, and 100 µg/mL streptomycin [10].

REF. The rat embryonic fibroblast cell line as normal cells, was established in the experimental therapy department/ ICCMGR for used as control. This cell line was grown in RPMI-1640 medium with 10% FBS, with 100 units/mL penicillin, and 100 µg/mL streptomycin.

Cell culture and maintenances

All cell lines used in this study were cultured at 37 ºC in a 5% CO_2 incubator, authenticated, and tested for mycoplasma by the cell bank unit of the Experimental Therapy Department at ICCMGR. The AMGM5, AMJ13, and REF cell lines were grown and maintained in RPMI-1640 growth medium, while the SK-GT-4 cell line was cultured and maintained in MEM medium at 37 °C in a humidified atmosphere of 95% air and 5% CO2 [11].

Cells were harvested after being trypsinized with trypsin-EDTA (T.V.) for use in this study. Once cellular growth reached an adherent confluent monolayer, the RPMI media was decanted, and T.V. (2-3 ml) was added to the flask, which was gently rocked. After 30 seconds, most of the T.V. was poured off, and the cells were incubated at 37 ºC until they detached. The cells were then further dispersed by pipetting them in the RPMI medium, making them ready for the next experiments [12, 13].

Cytotoxicity assay

Under sterile conditions, the GO/CeO_2 nanocolloid was filtered using a sterile syringe filter with a pore size of 0.45 µm to prepare a stock solution for the cytotoxicity assay. In this assay, three cancer cell lines (AMJ13, AMGM5, and SK-GT-4) and a normal cell line (REF) were used. The study involved nine different concentrations (250, 125, 62.5, 31.25, 15.6, 7.8, 3.9, 1.9, and 0.98 µg/ml) along with a control. Each concentration was tested in five replicates.

Under sterile conditions, $1x10^4$ cells per well in 200 µl of growth media with 10% FBS were added to each well of a sterile 96-well microtitration plate. The plate was covered with a lid and incubated overnight at 37 ºC in a CO2 incubator. The next day, the medium in each well was removed and serial dilutions of the GO/CeO_2 nanoparticle colloid in serum-free media (SFM) were added. The SFM, prepared without FBS, consisted of RPMI-640 medium supplemented with 100 µg/ml streptomycin and 100 units/ml penicillin. The plates were then sealed with a self-adhesive film, covered, and incubated for 72 hours to allow for exposure.

Finally, after the exposure period, the cell culture supernatants were discarded from the wells. Then, 100 µl of crystal violet stain was added to each well, and the plates were covered and incubated for 20 minutes at 37 ºC in a CO_2 incubator. After incubation, the cells were washed with tap water and allowed to dry. The optical density (OD) was then determined at a wavelength of 584 nm using a fluorometer (BMG LABTECH, Germany), [12, 14]. Then, microscope images for both treated and control cells were taken using an inverted light microscope (Leica, Karl Klob, Scientific Technical Supplier, Driesch Germany).

The percentage of Inhibition rate (I.R.) and cell viability were calculated according to the following equations:

$$I.R.\%=(C-C\_t)/C*100\% \quad (1)$$

$$\text{Cell Viability } \%=100-I.R\% \quad (2)$$

Where: I.R.% is the percentage of inhibition rate of cells, C= O.D of the control, Ct is the O.D. of the treated cells with the as-prepared material., Cell viability % is the percentage of the cell viability.

Statistical Analysis

Experiments data of variance were used for data comparison between studied groups (control and samples) using five replicates for each experiment. It was determined using one-way ANOVA and one sample t- test. A P value ≤ 0.05 was considered statistically significant. For this analysis, we used the GraphPad Prism 8 software (GraphPad Software, Inc. San Diego, California).

Results

XRD Analysis

Figure 1 (a) and (b) shows the XRD characteristics peaks of the as-prepared CeO_2 and GO nanosheet respectively. From examining Figure 1 (a) we can see that all the peaks are related to CeO_2 and there are no peaks for any other phase, which indicates the high purity of the as-synthesized CeO_2. The characteristic peaks are narrow and indicates the multi-crystallinity of the as-prepared material. The prominent peak corresponds to [111] located at $2\theta=28.757^o$. while the other peaks correspond to [200], [220], [311], [222], [400], [331], [420] and [422] located at 2θ = 33.256, 47.743, 56.620, 59.733, 77.091, 79.544, 88.762 and 95.824 [15]

The peaks are in good agreement with JCPDS card no: 34-0394. The CeO_2 nanoparticles have face centered cubic structure with lattice parameters a=b=c=5.4100 Aº and $\alpha=\beta=\gamma = 90^o$ [16].

The crystal size D can be calculated using Debye-Sherer' equation where:

$$D=D=(0.9\,\lambda)/(\beta\cos(\theta)) \quad (1)$$

Where D is the crystal size in nm, λ is the wavelength of x-ray 1. 5406 Aº, θ is the angle of diffraction, β is the FWHM of the diffraction peak. The crystal size was found to be 27 nm [17].

Figure 1 (b) illustrates the XRD pattern of GO nanosheet. The sharp diffraction peak at $2\theta=25.545^o$ corresponds to 002 diffraction plane which indicates the graphene layer distance, this coincides the results obtained by [18] and [19]. The d-spacing according to equation 1 has a value of 0.927 nm. The position of 002 peak indicates that the aromatic carbon sp2 lattice spacing was closely restored or completely [20]. The high value of the interlayer d-spacing of GO is proportional to the content of oxygen [21].

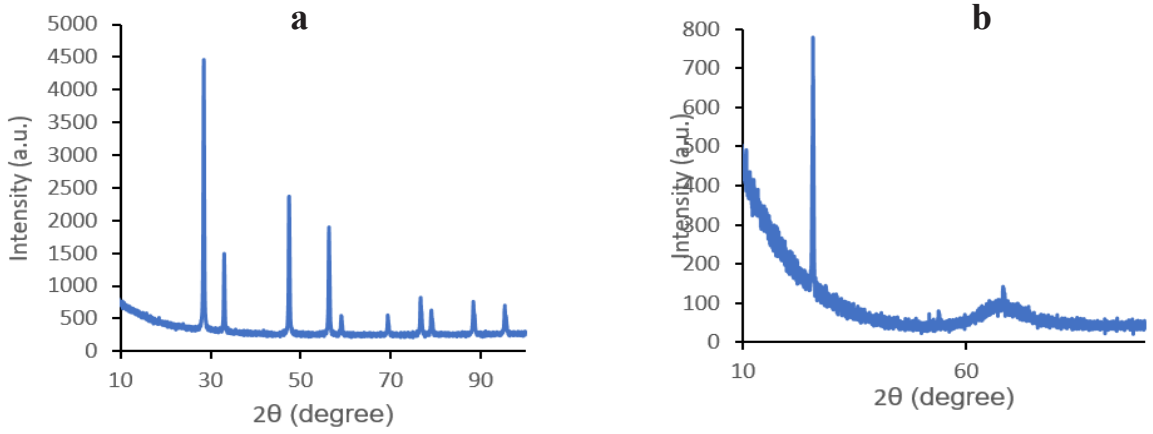

Figure 1. The XRD Pattern of (a) The as- Prepared CeO2 nanoparticles, (b) The as-prepared GO nanosheet.

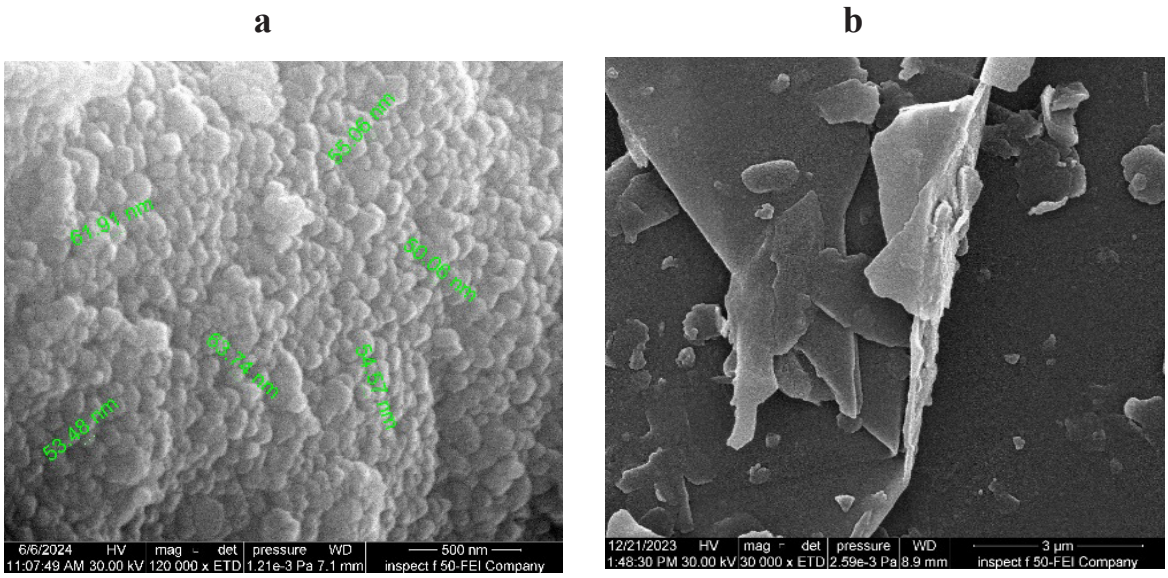

Figure 2. FESEM of the as Prepared (a) CeO_2, (b) Graphene Oxide Nano Sheet.

Morphology

Figure 2. (a) and (b) shows the morphology of the as-prepared CeO_2 and graphite oxide nanosheet respectively. Figure. 2 (a) shows the FESEM image of the CeO_2 which are nearly spherical in its shape and homogeneous. The average particle size is 55 nm. The obtained results are in good agreement with the results obtained by.[20]

The EDS analysis illustrates that CeO_2 consists mainly of Ce with a percentage of 65% and the rest are divided between Oxygen and Carbon with a percentage of 25% and 8% respectively (Figure 3 & 4).

The Table 1. illustrates the elemental analysis of CeO_2

Table 1. The Elemental Analysis of CeO_2 Used in This Study which Consists of 65.7% Ce, 25.9% O, and 8.5% C.

Weight % Error	Weight %	Atomic % Error	Atomic %	Element
0.2	8.5	0.7	25.3	C
0.4	25.9	0.9	57.9	O
0.6	65.7	0.2	16.8	Ce

and Table 2. illustrates the percentage elemental content of GO, which consists of 40% C and 28.3% O.

Uv-Visible Analysis

Figure 5. illustrates the optical properties of the as-prepared CeO_2, which are depicted by Figure 5. (a & b) that show the Uv-visible spectrum and the Tauc plot respectively. Figure 5 (a) clearly shows an absorption peak located at 287 nm. The absorption peak is sharp and strong indicates that the as-prepared CeO_2 has a narrow

Table 2. The Percentage Elemental Content of GO Nanosheet Used in This Study, that Consists of 40% C and 28.3% O.

Weight % Error	Weight %	Atomic % Error	Atomic %	Element
0.4	40	0.5	53.5	C
0.3	28.3	0.3	28.4	O
0.1	31.3	0.1	17.9	Si
0	0.3	0	0.1	S
0	0.1	0	0.1	Cl

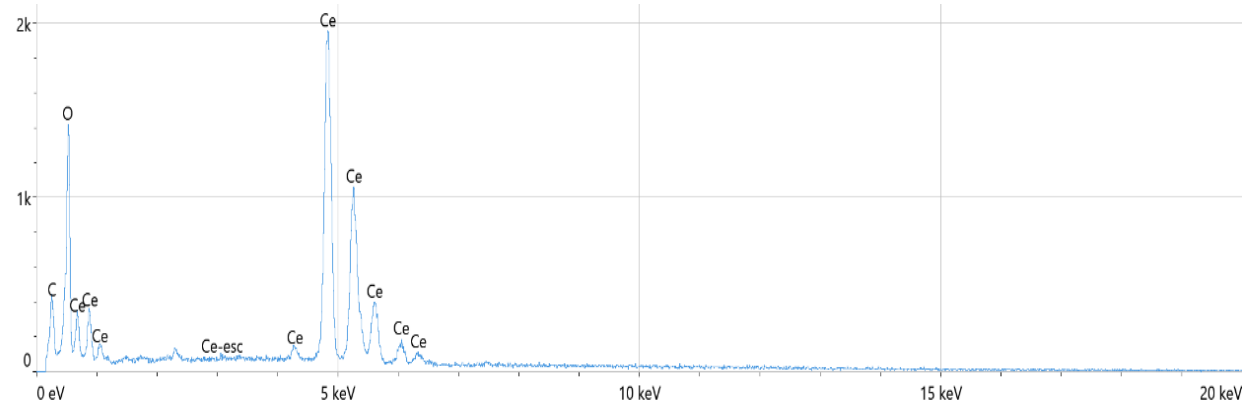

Figure 3. The EDS Analysis of the as-Prepared CeO_2 Nanomaterial

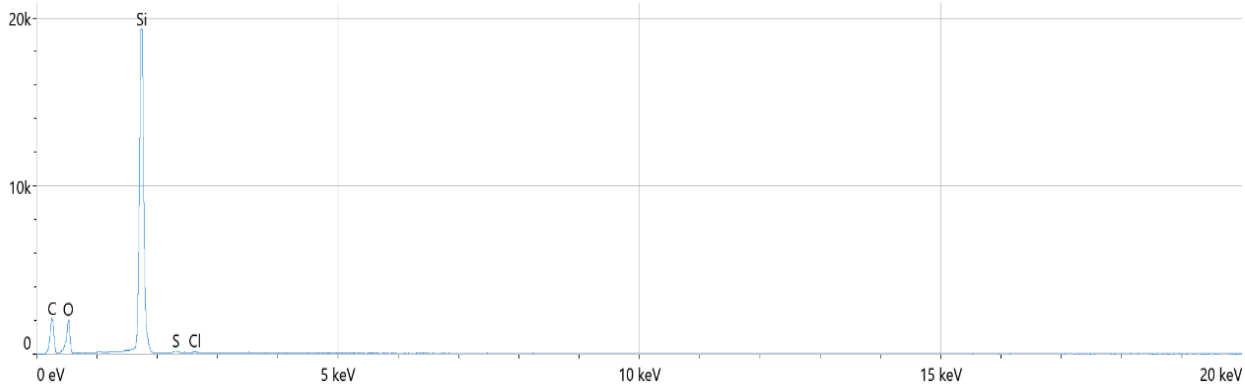

Figure 4. The EDS Analysis of the as-Prepared GO Nanosheet

particle size distribution [22] Comparing this peak with those reported in the literature, we can see that there is a blue shift towards lower wavelengths. There are no other peaks related to the impurities or defects observed in the spectrum, which assures that the as-prepared CeO_2 is pure. [16] Figure 5 (b) illustrates Tauc's plot for direct allowed transition. The value of the Eg is 3.55 eV which was found to be higher than that for the bulk. The increase in the band gap energy is due to the quantum confinement effect which explains the blue shift in the absorption peak [22]

In Vitro Study

The cytotoxicity of the GO/CeO_2 NPs on the breast cancer cell line AMJ13 was concentration dependent, the cells viability was decreased significantly (at P value ≤ 0.05) with the highest effect at a concentration of 250 µg/ml (51%) as shown in Figure (6- a) and Figure (7- a &b) compared with control. The brain cancer AMGM5 showed cytotoxic effects (37%) significantly at P value ≤ 0.05 with the highest effects at 250 µg/ml as shown in Figure (6- b) and Figure (7- c &d) compared with control. The esophagus cancer SK-GT-4 showed increased

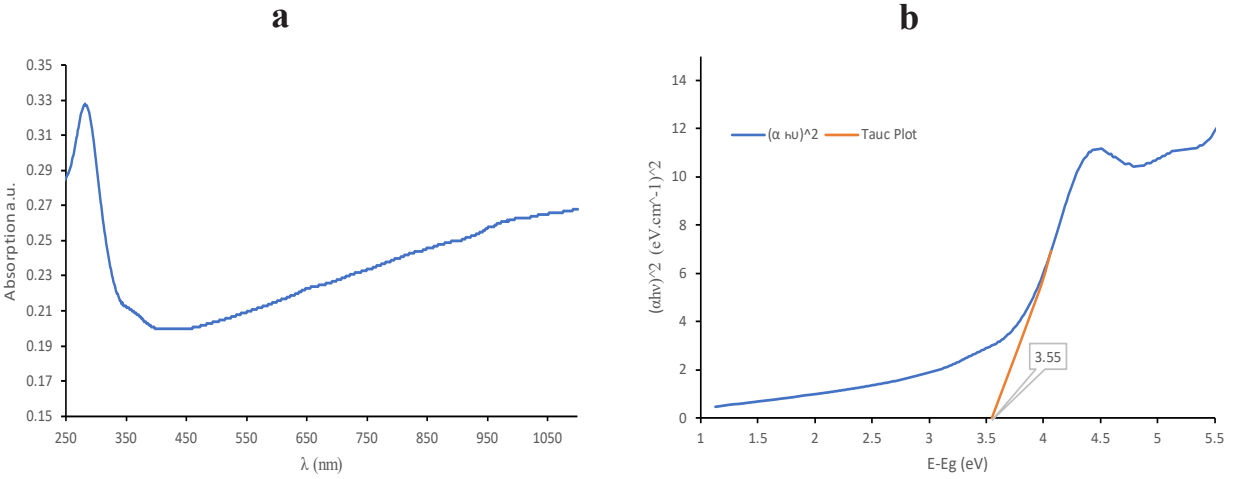

Figure 5. (a) The Uv-visible spectrum of the as-prepared CeO_2, (b) The Tauc-plot

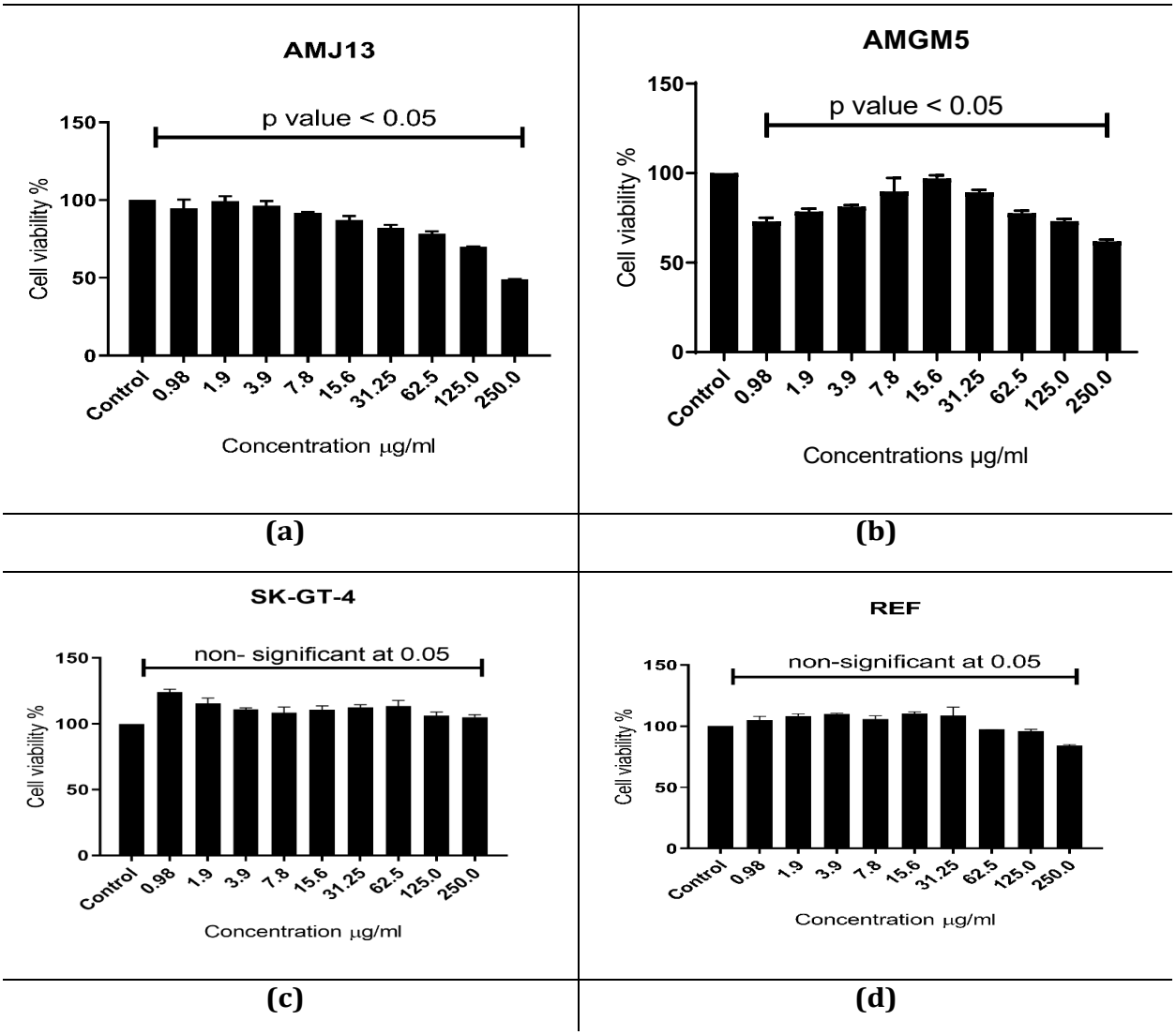

Figure 6. The Percentage of Cell Viability of Cells Lines (AMJ13, AMGM5, SK-GT-4, REF) (a-d) after being exposed to CeO2 for 72 h. incubation time that showed a significant effect with highly effect on AMGM5 (a) and AMJ13 (b) at P value ≤ 0.05 while with no cytotoxic effects on both SK-GT-4 (c) and REF (d).

proliferation rate (with non-significant effects compared with control) as shown in Figure (6- c) and Figure (7- e &f) compared with control. While, the results showed that the GO/CeO_2 nanoparticles was safe on the normal cell in culture (REF cell line) as shown in Figure (6-d) and Figure (g &h).

According to the above results, GO/CeO_2 NPs have the best effect on breast cancer AMJ13 and less than in brain cancer cell line AMGM5, therefore it's indicated that this drug may be used promising tool in drug delivery against breast cancer treatment, especially with its safety on normal cells.

Discussion

According to the literature, CeO_2 nanoparticles are highly biocompatible, easily eliminated from the body, and exhibit low environmental toxicity [23]. CeO_2 nanoparticles exhibit antioxidant properties at a neutral pH, while in an acidic tumoral environment, they display prooxidant abilities [24]. Acidic cancer cells, being sensitive, promote the prooxidant effect of nanoparticles. In contrast, multidrug-resistant cells and normal cells exhibit a more alkaline pH [25]. In this study, we reported the biocompatibility of GO/CeO_2 on a normal cell line. The cytotoxicity study clearly showed that as the concentration of GO/CeO_2 increased, the viability of the AMJ13 cancer cell line decreased. The changes in cell morphology, particularly cell shrinkage, indicated apoptosis. This apoptosis is a result of damage induced by reactive oxygen species (ROS) through the oxidation of intracellular macromolecules such as DNA, lipids, and proteins [26]. Several studies have reported the concentration-dependent cytotoxic effects of CeO_2 nanoparticles against various cell lines, including MCF7 (breast cancer), A549 (lung cancer), and HCT116 (colorectal cancer) [27]. The cytotoxic activity of CeO_2 nanoparticles is attributed to their ability to penetrate cancer cells through endocytic pathways. Recent studies suggest that nanoparticles prepared using plant derivatives have a higher potential to inhibit cancer cells. This enhanced effectiveness is likely due to the plant secondary metabolites involved in the

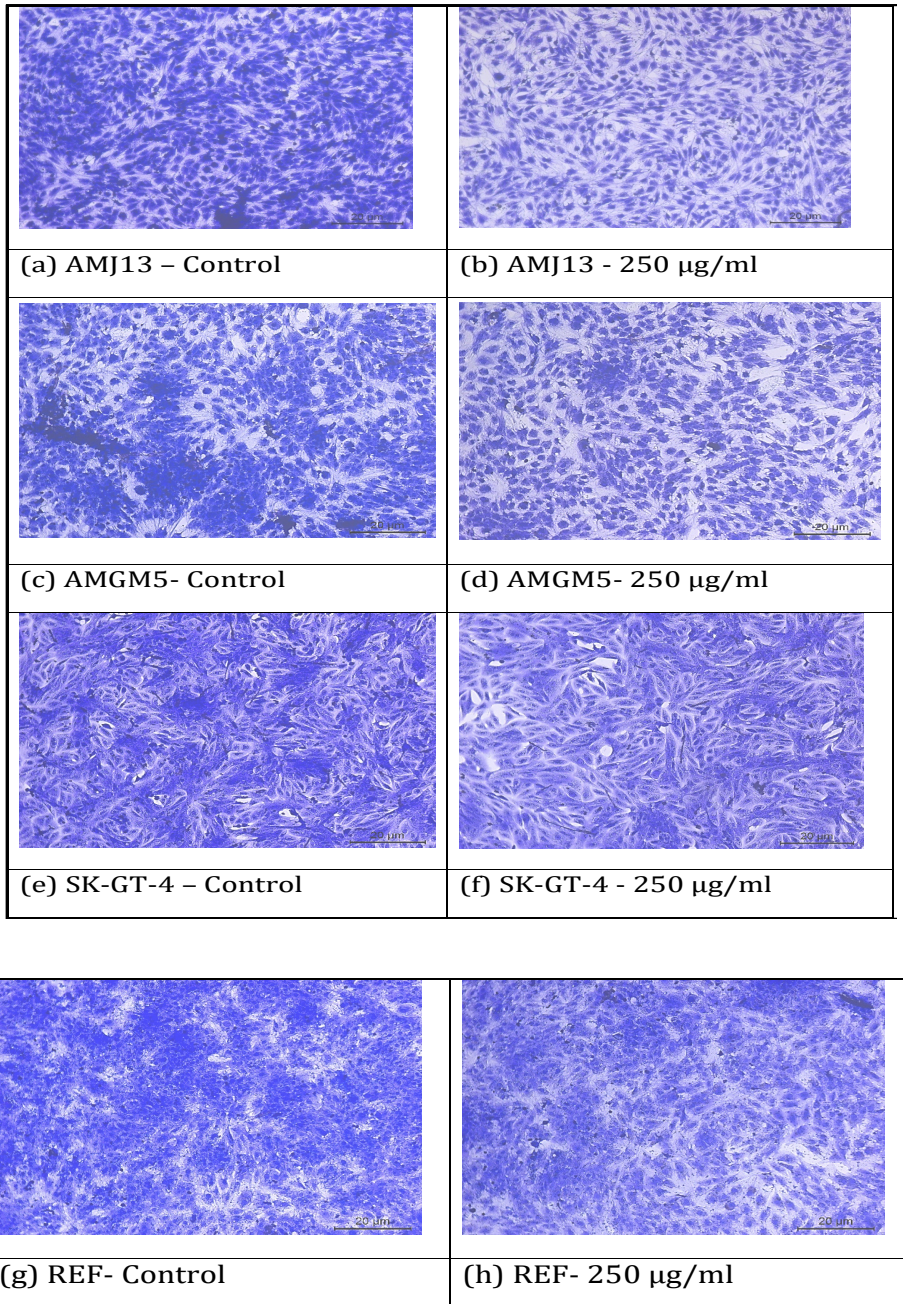

Figure 7. Microscope Images of Cytotoxicity of Cell Line AMGM5, AMJ13, SK-GT-4, REF before and after been exposed to CeO_2 (for 48 h.). (a, c, e, g) control and (b, d, f, h) 250 µg/ml that showed a highly cytotoxic effect on AMJ13 compared with other cell lines as shown by the inverted microscope at 10X.

green synthesis of the nanoparticles.

In conclusions, from the results of the in vitro study, we can conclude that GO/CeO_2 nanoparticles were found to be safe on normal cell lines and show great potential as a drug delivery tool against the breast cancer cell line AMJ13.

Author Contribution Statement

Maeda Hussain Mohammad, Huda Mahmood Al-attar, and Ayeda Mammduh Ahmed, Haitham T. Hussein performed the experimental tests. Cell culture was carried out by Ayser Ayed Ahmed. Maeda Hussain Mohammad, and Huda Mahmood Al-attar, performed the statistical analysis. Maeda Hussain Mohammad, Huda Mahmood Al-attar, Ayeda Mammdooh Ahmed, Haitham T. Hussein wrote the article.

Acknowledgements

The authors would like to thank Iraqi Center for cancer and Medical Genetics Research, Mustansiriyah University (www.uomustansiriyah.edu.iq) Baghdad, Iraq for their support in the present work.

Approved by any scientific Body

Not applicable as the manuscript is not a part of any student thesis or study.

Consent for publication

All authors have given consent for publication.

Approval

This work approved by the scientific committee of Iraqi Center for cancer and Medical Genetics Research, Mustansiriyah University.

Conflict of interest

The authors declare no potential conflict of interest.

References

1. Renganathan S, Vijayakumar P, Rajesh S. Bio-fabrication of cerium oxide nanoparticles using azadirachta indica and their antibacterial activity. Int J Adv Sci Eng. 2020;06:1462-8. https://doi.org/10.29294/IJASE.6.3.2020.1462-1468.
2. Bruix a, neyman km. How to design models for ceria nanoparticles: Challenges and strategies for describing nanostructured reducible oxides. Infrontiers of nanoscience. Vol. 12. Elsevier ltd.;2019.
3. Rath K, Sen S. Garlic extract based preparation of size controlled superparamagnetic hematite nanoparticles and their cytotoxic applications. Indian J Biotechnol. 2019;18:108-18.
4. El-Saber Batiha G, Magdy Beshbishy A, G Wasef L, Elewa YHA, A Al-Sagan A, Abd El-Hack ME, et al. Chemical constituents and pharmacological activities of garlic (allium sativum l.): A review. Nutrients. 2020;12(3). https://doi.org/10.3390/nu12030872.
5. Krishna sb. Emergent roles of garlic-based nanoparticles for bio-medical applications-a review. Curr Trends Biotechnol Pharm. 2021;15(3):349-60.
6. Al-attar hm, mohammad mh, alwan ah. Laser ablation of asphalt and coal in different solvents an in vitro study. Lasers Med Sci. 2023;38(1):135. Https://doi.Org/10.1007/s10103-023-03796-0.
7. Al-attar HM, Azzawi MM. The influence of ablation speed on the synthesis of carbon nanostructures via pulsed laser ablation of asphalt in ethanol. Baghdad Sci J. 2023;20(5):1763-71.
8. Al-Shammari AM, Alshami MA, Umran MA, Almukhtar AA, Yaseen NY, Raad K, et al. Establishment and characterization of a receptor-negative, hormone-nonresponsive breast cancer cell line from an iraqi patient. Breast Cancer (Dove Med Press). 2015;7:223-30. https://doi.org/10.2147/bctt.S74509.
9. Al-Shammari A, Hassani H, Jaleel O. Newcastle disease virus (ndv) iraqi strain ad2141 induces DNA damage and fasl in cancer cell lines. J Biol Life Sci. 2014;5:1-11. https://doi.org/10.5296/jbls.v5i1.4081.
10. Altorki N, Schwartz GK, Blundell M, Davis BM, Kelsen DP, Albino AP. Characterization of cell lines established from human gastric-esophageal adenocarcinomas. Biologic phenotype and invasion potential. Cancer. 1993;72(3):649-57. https://doi.org/10.1002/1097-0142(19930801)72:3<649::aid-cncr2820720305>3.0.co;2-l.
11. Capes-Davis A, Freshney RI. Freshney's culture of animal cells: A manual of basic technique and specialized applications. John Wiley & Sons; 2021 Feb 17.

Available from: https://www.wiley.com/en-hk/exportProduct/pdf/9781119513049

12. Alsaraf KM, Mohammad MH, Al-Shammari AM, Abbas IS. Selective cytotoxic effect of plantago lanceolata l. Against breast cancer cells. J Egypt Natl Canc Inst. 2019;31(1):10. https://doi.org/10.1186/s43046-019-0010-3.
13. Alsaraf K, Mohammad M, Al-Shammari A, Abbas I. Investigating the anti-proliferation activity of conocarpus erectus against breast tumor cells in vitro. Sys Rev Pharm. 2020;11:1901-8. https://doi.org/10.31838/srp.2020.12.289.
14. Alsaraf K, Mohammad M, Al-Shammari A, Abbas I. Broad-spectrum cytotoxic effect of calendula officinalis l against breast cancer cells. Indian J Forensic Med Toxicol. 2020;14:803-9. https://doi.org/10.37506/v14/i1/2020/ijfmt/193002.
15. Liu YH, Zuo JC, Ren XF, Yong L. Synthesis and character of cerium oxide (ceo2) nanoparticles by the precipitation method. Metalurgija. 2014;53:463-5.
16. Jayakumar G, Irudayaraj AA, Raj AD. Particle size effect on the properties of cerium oxide (CeO_2) nanoparticles synthesized by hydrothermal method. Mechanics. Mech Mater Sci Eng. 2017;9(1).
17. Batista A, Miranda M, Martins F, Morilla-Santos C, Sasaki J. Synthesis of cerium oxide (ceo2) by co-precipitation for application as a reference material for x-ray powder diffraction peak widths. Powder Diffr. 2018;33:1-5. https://doi.org/10.1017/S0885715617001208.
18. Huang H, Sun G, Hu J, Jiao T. Low temperature synthesis of mno 2 /graphene nanocomposites for supercapacitors. J Chem. 2015;2015:1-8. https://doi.org/10.1155/2015/629362.
19. Kottegoda I, Idris N, Lu L, Wang J, Liu H-K. Synthesis and characterization of graphene–nickel oxide nanostructures for fast charge–discharge application. Electrochimica Acta. 2011;56:5815-22. https://doi.org/10.1016/j.electacta.2011.03.143.
20. Hayes W, Joseph P, Mughal M, Papakonstantinou P. Production of reduced graphene oxide via hydrothermal reduction in an aqueous sulphuric acid suspension and its electrochemical behaviour. J Solid State Electrochem. 2014;In Press. https://doi.org/10.1007/s10008-014-2560-6.
21. Drewniak S, Muzyka R, Stolarczyk A, Pustelny T, Kotyczka-Morańska M, Setkiewicz M. Studies of reduced graphene oxide and graphite oxide in the aspect of their possible application in gas sensors. Sensors (Basel). 2016;16(1). https://doi.org/10.3390/s16010103.
22. E K, Selvarajan P, Muthuraj D. Synthesis and characterization of ceo2 nanocrystals by solvothermal route. Mater Res. 2013;16:269-76. https://doi.org/10.1590/S1516-14392013005000021.
23. Ahmed HE, Iqbal Y, Aziz MH, Atif M, Batool Z, Hanif A, et al. Green synthesis of ceo(2) nanoparticles from the abelmoschus esculentus extract: Evaluation of antioxidant, anticancer, antibacterial, and wound-healing activities. Molecules. 2021;26(15). https://doi.org/10.3390/molecules26154659.
24. Pešić M, Podolski-Renić A, Stojković S, Matović B, Zmejkoski D, Kojić V, et al. Anti-cancer effects of cerium oxide nanoparticles and its intracellular redox activity. Chem Biol Interact. 2015;232:85-93. https://doi.org/10.1016/j.cbi.2015.03.013.
25. Saranya J, Saminathan P, Ankireddy S, Shaik MR, Khan M, Khan M, et al. Cerium oxide/graphene oxide hybrid: Synthesis, characterization, and evaluation of anticancer activity in a breast cancer cell line (mcf-7). Biomedicines. 2023;11:531. https://doi.org/10.3390/biomedicines11020531.
26. Yaghoobi z, rahdar a, sankar v, amini n. Exploring the cytotoxicity of ceo 2 nanoparticles : A compendious approach. J nanoanalysis. 2020;7(2):83–95. https://doi.org/10.22034/jna.2020.1879379.1160.
27. Taherzadeh D, Hosseinpour H, Ebrahimi S, Ghafarpour A, Samandarinejad N, Darroudi M, et al. Green synthesis of cerium oxide nanoparticles using falcaria vulgaris leaf

extract and its anti-tumoral effects in prostate cancer. 2023.