RESEARCH ARTICLE

Evaluation and comparison of physic-chemical properties and cytotoxic -performance of cerium oxide and zinc-doped cerium oxide nanoparticles

Faezeh khoshi[1], Foad Tosan[2], Sepehr Sadeghpour Tabaei[3], Negin Rahnama[4], Hesamoddin Arabnozari[5*]

[1] School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran,Iran

[2] Student Research Committee, Semnan University of Medical Sciences, Semnan, Iran

[3] School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran

[4] Department of Internal Medicine and Health Services, Semnan University of Medical Sciences, Semnan, Iran

[5] School of Medicine, Babol University of Medical Sciences, Babol, Iran

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ABSTRACT

This paper presented the green synthesis of pure and 5% zinc doped cerium oxide nanoparticles (Zn-CeO_2 NPs) by using the root extract of *Prosopis farcta*. Green synthesis is a method of synthesizing nanoparticles without the use of chemical solvents and is environmentally friendly and safe.The physic-chemical properties of the obtained nanoparticles were studied through the results of PXRD, UV-Vis, DRS, FESEM, EDX, and Raman techniques. The outcomes of EDX and PXRD confirmed the presence of doped zinc in the structure of cerium oxide. The particle sizes of pure and Zn-CeO_2 NPs were 20 and 8 nm. Additionally, we evaluated the cytotoxic performance of synthesized nanoparticles on brain glioblastoma cells (U87), which resulted in displaying the stronger toxic effect of zinc doped nanoparticle when compared to pure cerium oxide nanoparticles. Therefore, it is indicated that zinc doped cerium oxide nanoparticles are capable of exhibiting a superior anti-cancer impact on U87 cells.

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INTRODUCTION

The disease of cancer implicates the uncontrollable proliferation of abnormal cells that can infect nearby tissues [1-3]. It is commonly induced by a number of genetic phenomena including inactivation of tumor suppressor genes and activation of oncogenes. The nature of this disorder involves a variety of mutations, lack of heterozygosity, and epigenetic extinction of gene transcripts by promoter hypermethylation, gene replication, and the occurrence of performance mutations [1, 4].

Glioblastoma is most common and acute type of malignant brain tumor in adults. It is considered as a kind of astrocytic cell tumor with histological signs such as increased mitosis, polymorphism, endothelial proliferation, and rapid necrosis. The poor treatment of glioblastoma is due to the presence of a blood-brain barrier with the ability to prevent the entry of chemical drugs into the brain, while the recurrence of its tumor is caused by the self-renewal of glioma stem cells. Considering how these factors reduced the average survival of patients, other disadvantages such as the side effects of drugs, as well as time consuming, costly, and ineffective treatments, justify the need for discovering new methods and strategies to combat this disease [5, 6].

Today, cancer is treated through a variety of

* Corresponding Author Email: *Hesamarabnozari@yahoo.com*

methods including surgery, chemotherapy, and radiation therapy, which implicate the destruction of healthy cells as a disadvantageous side effect [2]. Therefore, researchers attempted to focus on new treatments with reduced side effects that also posed the threat of damaging healthy tissues or reversing cancerous findings. Considering their deficiencies, scientists were forced to look for new ways of diagnosis and treatment of cancer that would cause fewer side effects [2]. Nanotechnology can provide efficient tools for doctors to achieve direct contact with their target, selectively select cancer cells, and increase their effectiveness. Nanoparticles are exerted in a variety of applications such as drug delivery to cancer cells, as well as imaging and closely monitoring these cells, while having a great potential for the diagnosis and treatment of cancer [2, 7-10].

Among the available nanoparticles, recent studies pointed out the cytotoxic effect of cerium oxide nanoparticles on cancer cells, which highlights the importance of further assessments to determine the side effects of its usage in the treatment of cancer [11-14]. Cerium oxide nanoparticles are composed of a cerium surrounded by oxygen networks. These nanoparticles can display antioxidant behaviors including superoxide dismutase, catalytic enzyme activity, nitric oxide radical inhibition, and hydroxyl radical inhibition, as well as oxidative behaviors that determine the type of activity of these nanoparticles under various conditions such as ambient pH [12, 14].

These nanoparticles can find entry into mammalian cells through multiple pathways such as receptor-mediated endocytosis [15-17]. Cerium oxide nanoparticles are capable of activating the factors that are involved throughout the process of cellular apoptosis by increasing the production of ROS, which is non-enzymatically and deliberately induced in mitochondria, especially in mitochondrial electron transfer complexes I and III [18]. Moreover, high levels of ROS can lead to the destruction of outer and inner membranes of mitochondria, the release of cytochrome c, and the activation of apoptotic cascade [18]. Therefore, this study attempted to synthesize pure and zinc doped cerium oxide nanoparticles through a simple and fast method by using the root extract of *Prosopis farcta* and also evaluated their cytotoxic activity on brain glioblastoma cell (U87) line.

MATERIALS AND METHOD

Extraction of P. farcta

In this section, the root of *P. farcta* was used to perform the synthesis of nanoparticles. For this purpose, the roots were washed, dried in room temperature, and crushed. Then, 20 mL of solvent (distilled water) was added to 2 gr of roots powder to be shaken at 150 rpm for 10 hours. The obtained mixture was filtered and exerted in the synthesizing process of nanoparticles.

Synthesis of CeO_2 NPs

In order to prepare pure CeO_2 NPs, 45 mL of $Ce(NO_3)_2.6H_2O$ (0.025 M, Merk) solution was added to 5 mL of the extract to be stirred at 70 °C for 2 h. Once the solution was dried at 90 °C for 18 hours, the dried powder was calcined at 500 °C for 2 hours. The obtained yellow powder was confirmed to be pure CeO_2 NPs.

Synthesis of Zn-CeO_2 NPs

To prepare Zn-CeO_2 NPs, 45 mL of $Ce(NO_3)_2.6H_2O$ (0.025 M, Merk) and $Zn(NO_3)_2.6H_2O$ (5% W/W, Merk) solution was added to 5 mL of the extract to be stirred at 70 °C for 2 h. Once the solution was dried at 90 °C for 18 hours, the achieved powder was calcined at 500 °C for 2 hours. The obtained powder was affirmed to be Zn doped CeO_2 NPs.

Cytotoxic test

Cell culture

Our study exerted brain glioblastoma cells (U87) to evaluate the cytotoxicity of pure and Zn-CeO_2 NPs. U87 cells were procured from the Pasteur Institute of Iran and thawed in prior to being cultured. The cells were transferred to Falcon tubes and centrifuged at 833 rpm for 9 minutes. Once the supernatant was removed, a complete culture medium was added to the cells to pour the prepared suspensions into flasks. DMEM culture medium was utilized for the process of cell culturing, while the addition of 10% fetal bovine serum (FBS), 100 µg/mL of streptomycin, and 100 international units/mL of penicillin to each culture medium were required to prevent microbial growth. In order to proliferate and grow the cells, the culture medium was incubated under 5% CO_2 at 37 °C.

MTT assay

MTT test was conducted to investigate the

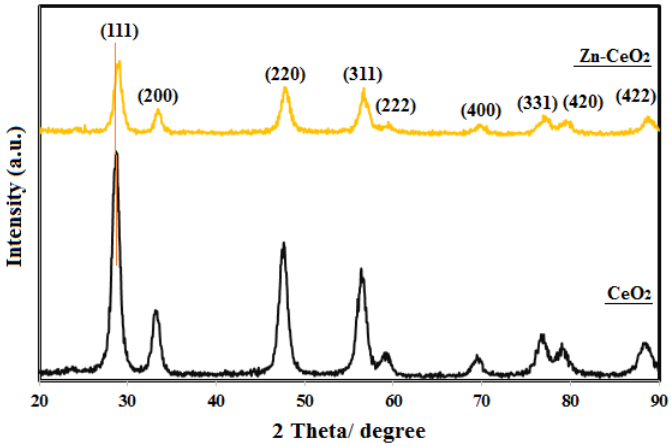

Fig. 1. PXRD spectra of synthesized pure and 5% Zn-CeO_2 NPs using *P. farcta* extract.

cytotoxicity performance of pure and Zn-CeO_2 NPs on U87 cells. In addition, the cytotoxicity of each sample was examined at the serial concentrations of 1-500 µg/mL, while cell culture medium was set as the control. At first, a cell suspension of 10^4 cells was added to every well of the 96-well plate to be incubated for 24 hours. Subsequent to ensuring the adherence of cells to the floor of plate, the culture medium was drained from each well of the 96-wells plate. Then, 100 µL of nanoparticles was added to the relevant wells to perform incubation for 24 hours under 5% CO_2 at 37 ˚C. Once the plate was removed, 10 µL of MT solution was added to each well of the 96-well plate to be re-incubated for 4 hours. Finally, the adsorption of each sample was recorded by the usage of ELISA reader at the wavelength of 490 nm. In addition, the percentage of cell viability (survival) was calculated through the following formula:

(%) = [100 × (sample abs)/ (control abs)].

RESULTS AND DISCUSSION

PXRD analysis

Fig. 1 depicts the PXRD patterns of pure and 5% Zn-CeO_2 NPs. The PXRD of pure CeO_2 NPs exhibited diffraction peaks at 2θ= 28.73, 33.06, 47.74, 56.45, 59.19, 69.57, 76.59, 79.19, and 88.30˚, which attributed to (111), (200), (220), (311), (222), (400), (331), (420), and (422) planes, and illustrated the cubic phase of CeO_2 NPs (JCPDS code No: 81–0792) [14]. According to Fig. 1, the doping of zinc into cerium oxide structure caused a reduction in peak intensity. One can notice a little shift in the position peak of (101) throughout the graph of doped nanoparticles, caused by the partial replacement of zinc ion in the lattice of cerium oxide. The crystallite size of NPs was estimated through the Scherrer's equation [19] and reported to be 22.58 and 9.15 nm for pure and 5% Zn-CeO_2 NPs, respectively. Therefore, it can be stated that the lower ionic radius of zinc (0.74 Å) than that of cerium (1.034 Å) resulted in decreasing the particle size of doped nanoparticles.

ESEM and EDX analysis

FESEM analysis can provide data on the particle size and morphology of pure and 5% Zn-CeO_2 NPs. According to Fig. 2, the particle size of pure CeO_2 NPs was proposed to be about 20-30 nm. Moreover, it is assumed that the size of doped particles was decreased as a result of their addition into the crystalline lattice of pure cerium oxide. In conformity to the PXRD section, this case was happed due to the lower ionic radius of zinc atom than that of cerium atom. The EDX results of synthesized NPs supported the satisfying incorporation of zinc atoms into the crystalline structure of cerium oxide. Based on Fig. 3, the percentage of zinc in pure and 5% Zn-CeO_2 NPs was 0 and 4.34%, respectively, which confirmed the absence of any impurity in the construction of synthesized nanoparticles.

UV-Vis/DRS analysis

Fig. 4 presents the electronic absorption spectra of synthesized pure and 5% Zn-CeO_2 NPs obtained by the usage of *P. farcta* extract. The UV-Vis spectra exhibits the changes caused by zinc doping in the energy band structure of cerium oxide. As it is

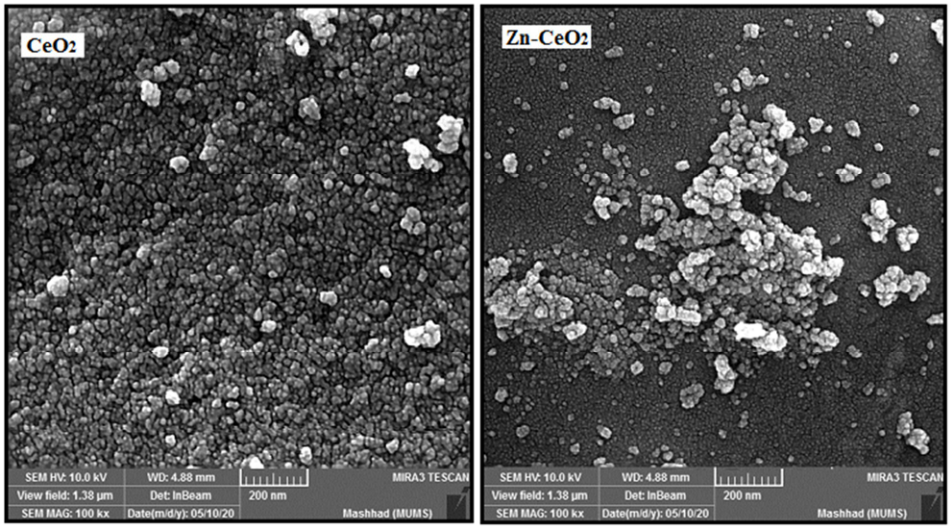

Fig. 2. FESEM images synthesized pure and 5% Zn-CeO_2 NPs using *P. farcta* extract.

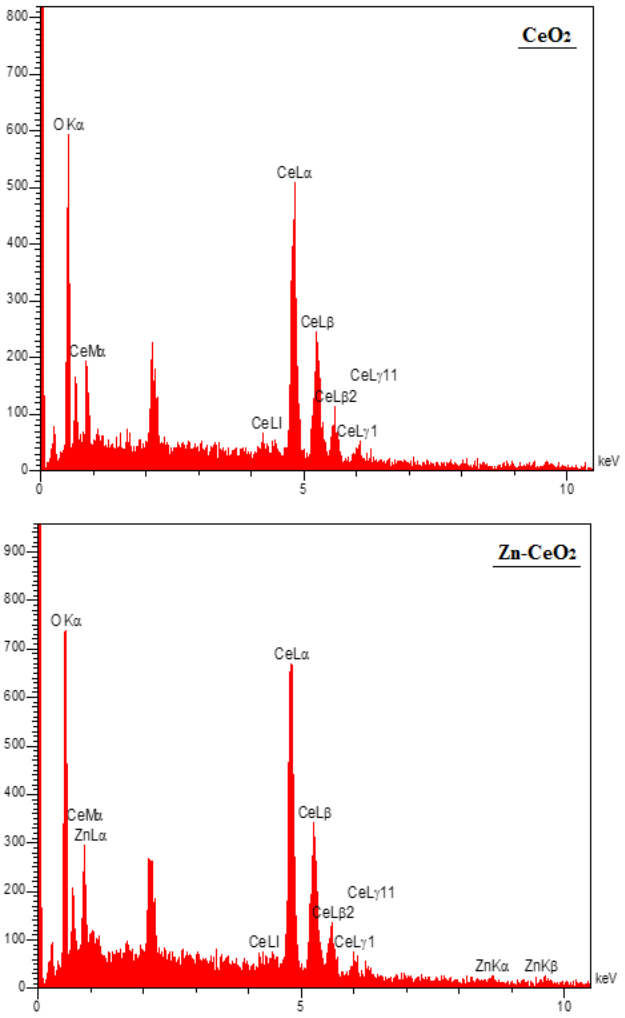

Fig. 3. EDX spectra of synthesized pure and 5% Zn-CeO_2 NPs using *P. farcta* extract.

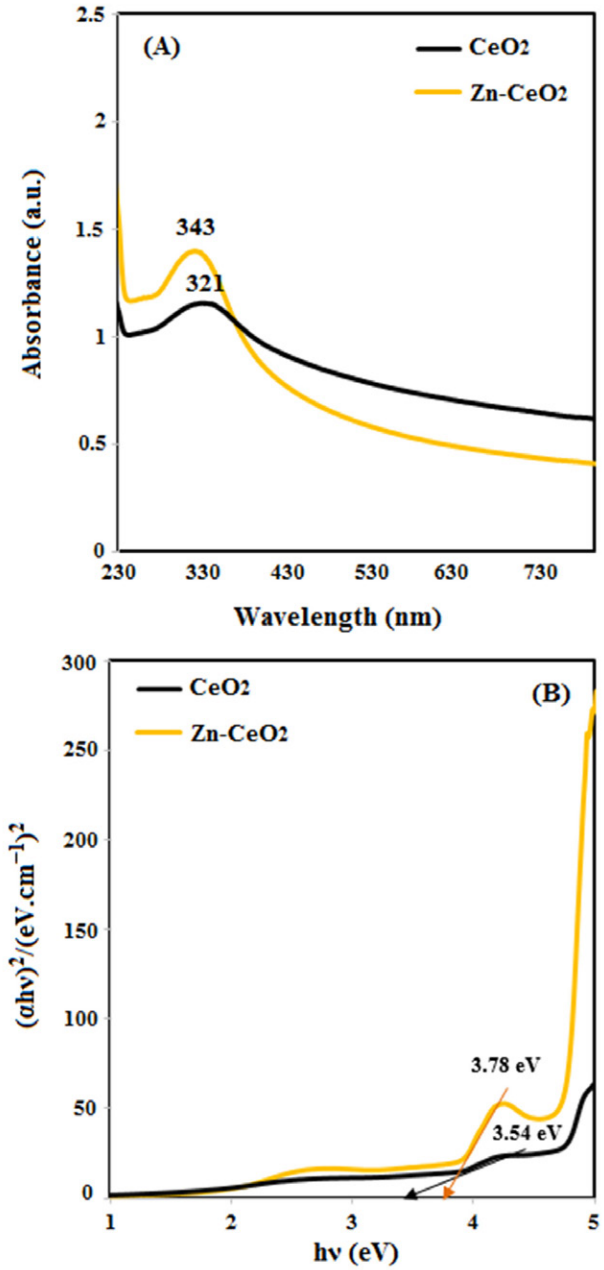

Fig. 4. (A) UV-Vis absorption spectra and (B) DRS of pure and 5% Zn-CeO_2 NPs using *P. farcta* extract.

depicted in Fig. 4(A), the absorption peaks of pure and 5% Zn-CeO_2 NPs were observed in the regions of 345 and 326 nm, respectively. The doping of Zn forced the absorption peak of CeO_2 NPs to take a blue shift towards the lower wavelengths. The DRS spectra of pure and 5% Zn-CeO_2 NPs are demonstrated in Fig. 4(B). The energy gap (Eg) of nanoparticles was estimated by using the absorption data and the following equation [20]:

$$(\alpha h\nu)^2 = A(h\nu - Eg) \quad (2)$$

Where α is the absorption coefficient, hν refers to the photon energy, and A stands for a constant that does not depend on photon energy. The band gap of CeO_2 NPs was observed to be 3.51 eV, which was increased as a result of Zn doping and reported to be 3.78 eV for 5% Zn-CeO_2 NPs. This value is higher than that of bulk CeO_2 (Eg ~

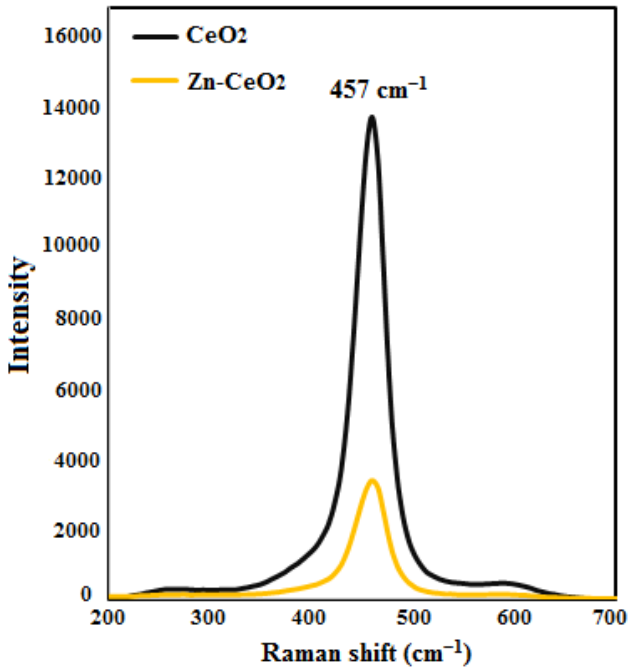

Fig. 5. Raman spectra of synthesized pure and 5% Zn-CeO_2 NPs using *P. farcta* extract.

3.19 eV) [10] due to the quantum confinement effect. Since similar outcomes were provided in the report of Hamidian *et al* [10], it can be stated that by reducing the size of doped particles, the value of band gap increases; this fact was also confirmed by the FESEM and PXRD results.

Raman analysis

As an accurate and practical method for identifying molecular structures, Raman spectroscopy can provide data on determining the rotational and vibrational frequencies of a molecule, geometric evaluation, and even the symmetry of molecules [19]. This technique can detect the significant reforms of band frequencies upon the occurrence of any changes in lattice parameters and chemical environment. Fig. 5 depicts the Raman spectra of pure and 5% Zn-CeO_2 NPs. Accordingly, a Raman active band F2g was perceived at 457 cm^{-1} in CeO_2 graph that may be associated with the fluorite type structure of CeO_2. This observation once again validated the fcc crystalline structure of nanoparticles and hence, the vibrational mode can be assumed to be nearly independent of CeO_2 ionic mass by the movement of O atoms. In conformity to Fig. 5, the peak intensity of 457 cm^{-1} was reduced as a result of doping Zn into the structure of CeO_2.

Cytotoxic performance

Nowadays, cancer is one of the most common causes of death worldwide. There are disadvantages to the application of current cancer treatments, which include chemotherapy and radiotherapy, such as damaging the healthy cells that surround the tumor [1]. Therefore, it is an innovative technological performance to solve this problem by the usage of nanoparticles [1]. Cerium oxide nanoparticles proved to be cytotoxic to cancer cells, as well as capable of inhibiting cell invasion and sensitization to radiation therapy and chemotherapy, while protecting the reactive oxygen species (ROS) and increasing the rate of apoptosis in cells [10]. This study attempted to evaluate the cytotoxic performance of synthesized pure and 5% Zn-CeO_2 NPs against brain glioblastoma (U87) cell line by the conduction of MTT test. As it is displayed in Fig. 6, the cytotoxic effect of doped nanoparticles was stronger than the pure CeO_2 nanoparticles, which seemed to be heightened by increasing the applied concentration of samples. In this regard, the cytotoxic performance of nanoparticles against U87 cells was assumed to be concentration dependent.

Various studies tried to investigate the cytotoxic effects of cerium oxide nanoparticles on different

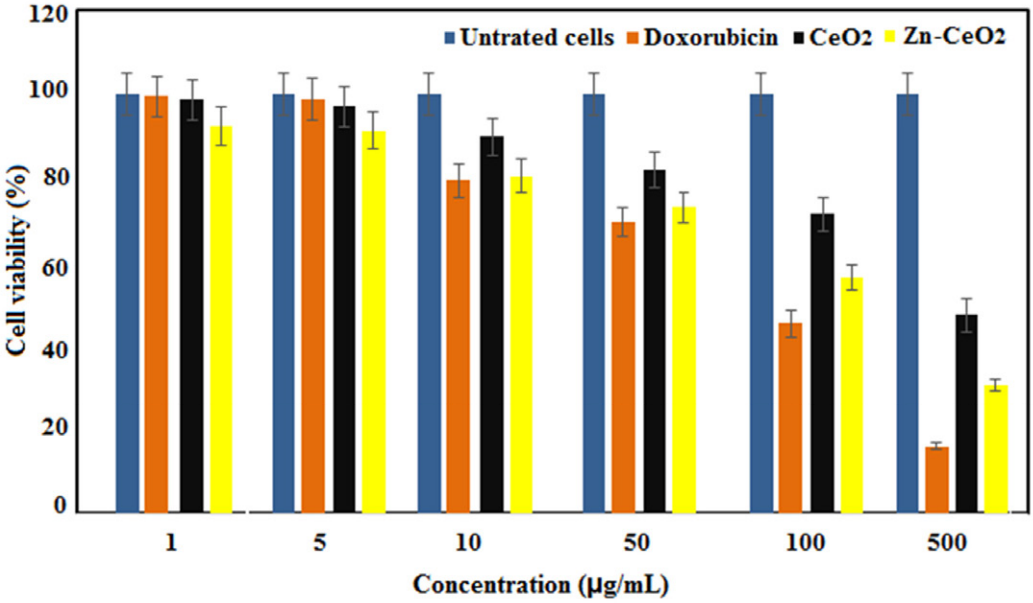

Fig. 6. Cytotoxic performance of synthesized pure and 5% Zn-CeO_2 NPs against U87 cells after 24 h treatment.

cell lines. For instance, the work of Sandeep *et al* indicated that these nanoparticles cause significant toxicity and morphological changes in lung cancer (A549) cell lines [21]. Also, Saikat Jana *et al* investigated the toxicity effect of cerium oxide nanoparticles on HCT-15 cell line [22]. In this experiment, ROS and lipid peroxidation, which stand as the indicators of oxidative stress and cytotoxicity, were significantly increased based on the applied dosage [22]. Moreover, according to the study of Lin *et al* on the effect of this product in lung cell line, their results confirmed the ability of cerium oxide nanoparticles in increasing the death of cancer cells and inducing oxidative stress in the cell [23]. Our outcomes were also suggestive of the stronger toxic effect of doped nanoparticles on U87 cells when compared to pure cerium oxide nanoparticles, which confirms the superior anti-cancer impact of zinc doped cerium oxide nanoparticles on U87 cells.

CONCLUSION

In this work, pure and 5% Zn-CeO_2 NPs were prepared by the exertion of *Prosopis farcta* root extract to perform a survey on their physic-chemical properties through various analytical techniques such as PXRD, FESEM/EDX, Raman, and UV-Vis/DRS. The results of PXRD and FESEM analyses reported the particle size of pure and doped cerium oxide nanoparticles to be 20 and 8 nm, respectively. Additionally, the cytotoxic performance of synthesized pure and 5% Zn-CeO_2 NPs against brain glioblastoma (U87) cell line was studied by the conduction of MTT test. According to the outcomes, Zn-CeO_2 NPs exhibited a higher toxic effect on U87 cells when compared to pure CeO_2 NPs and therefore, it is suggested that the doping of zinc into the structure of CeO_2 NPs increased the rate of anti-cancer activity against U87 cells.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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