RESEARCH ARTICLE

Exploring the cytotoxicity of CeO_2 nanoparticles: A compendious approach

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ARTICLE INFO

Article History:
Received 2019-09-22
Accepted 2020-01-10
Published 2020-05-01

Keywords:
Ceria nanoparticles
method of synthesis
enzyme mimicking activity
toxicity
biological application

ABSTRACT

Metal oxide nanoparticles due to their antioxidant properties have attracted significant attention and exhibited good potential for use in cancer theranostics. Owing to the poor absorption in the physiological environment, they are an ideal candidate to act as nanocarriers in targeted drug delivery and bioimaging. This feature can be successfully implemented in live monitoring and imaging applications, which offer the possibilities and scope for optical, magnetic resonance, and nuclear imaging. The environment of malignant cells like the rapid proliferation of cells, specific antigen expressions, and leaky tumor vasculature can be used by the modifications in their morphology and surface functionalization. Ceria (CeO_2) nanoparticles have been fascinating in this regard. Different properties such as size, agglomeration behavior, and surface charge density facilitate the interaction of nanoparticles with cancer cells. Compared to other nanoparticles, CeO_2 nanoparticles have a potential for pharmaceutical use since they can act as a therapeutic agent in different disorders such as cancer, inflammation, and neurodegeneration, due to the ability to exhibit variable oxidation state at the nanoparticle surface. Recent literature reports the eco-friendly or 'green' synthesis of CeO_2 nanoparticles in which the biological agent acts as stabilizers for a cost-effective and feasible mode of preparation. In this review, we focus on recent literature on CeO_2 nanoparticles with an emphasis on the methods of fabrication and biomedical applications.

How to cite this article
Yaghoobi Z., Rahdar A.,Sankar S V., Amini N. Exploring the cytotoxicity of CeO_2 nanoparticles: A compendious approach. J. Nanoanalysis., 2020; 7(2): 83-95. DOI: 10.22034/JNA.2020.1879379.1160.

INTRODUCTION

Nanotechnology has opened the doors of exciting possibilities for biomedical research. The emergence of new classes of nanomaterials has built an excellent platform of the vast opportunities by overcoming the challenges faced by conventional research methods. Owing to the exciting and unique properties of nanomaterials, they are widely employed for applications in medicine, biology, and other life sciences [1-3]. Metal oxide nanoparticles are a family of nanoparticles that gained exciting research attention, especially regarding cancer treatment [4]. They can be successfully applied when traditional therapies, including surgery, radiotherapy, and chemotherapy, are not efficient for treating cancer.

Cerium (Ce; atomic number =58) is a rare earth element belonging to the lanthanide series of the periodic table. Among other rare elements, it is one of the most abundant oxidizing agent found in oxide form (two oxidation states; +3 and +4). Ce(III) shows an absorption maximum at 230–260 nm, while Ce(IV) shows an absorption maximum at 300–400 nm. This difference arises due to their difference in chemical or electronic environments

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of Ce(III) and Ce(IV) systems. Fig.1S shows the crystal structure of CeO_2.

CeO_2 nanoparticles have attracted the attention of many scientists due to various applications such as ultraviolet absorption, catalysts, gas sensors, cosmetic products, biomedical applications, including pro-oxidant and antioxidant [5-8].

Considering the unique properties of CeO_2 nanoparticles, researchers have designed and fabricated functionalized CeO_2 nanoparticles with properties tuned for specific applications. There is evidence which shows CeO_2 nanoparticles are toxic to malignant cells and are used to inhibit invasion and to enhance the impact of radiation therapy and chemotherapy to cancer cells [9]. However, CeO_2 nanoparticles are not cytotoxic to healthy tissues and cells and provide conditions to protect the generation of reactive oxygen species (ROS) of various forms [10-12].

PHYSICOCHEMICAL PROPERTIES OF CEO_2 NANOPARTICLES

The variations in chemical or physical properties of the CeO_2 nanoparticles depend on their method of synthesis. The physicochemical properties affect their biological activities, i.e., whether they are inactive, antioxidant, or pro-oxidant [13]. In nanoscale, both oxidation states, Ce(III) and Ce(IV), are apparent at the surface. The presence of lattice defects created by Ce(III) and their compensation by oxygen reactions on the surface cause a change of CeO_2 to CeO_{2-x}. CeO_2 nanoparticles serve as a remarkable catalyst to mimic the action of natural antioxidant enzymes such as superoxide dismutase (SOD) and catalase. The oxygen defects act as catalytic sites, and by decreasing the size of the CeO_2 nanoparticles, the extent of oxygen deficits decreases [14, 15].

Most oxygen species and nitrogen are self-replicating by the biological system and CeO_2 nanoparticles. The minimum dosage can have catalytic activity for a long time [14]. Therefore, CeO_2 nanoparticles exhibit redox properties better than their bulk counterparts. Furthermore, a mixed valance state helps to scavenge reactive oxygen and nitrogen species and is also useful in chronic oxidative stress and inflammation [16].

When cerium combines with oxygen, it maintains the structure of its fluorite lattice, and this feature is common for both the bulk and the nanoscale. Cerium retains a fluorite lattice by creating a space by losing oxygen on the lattice due to the presence of Ce(III) ions [17-19]. Eight oxygen atoms surround the cerium atoms in CeO2 in the tetrahedral position of the lattice structure. The Ce(III) ions in the nano form cause intrinsic oxygen defects due to charge deficiency [20].

The surface Ce(III)/Ce(IV) ratio of the nanoparticle depends on the method of synthesis [14]. The SOD mimetic activity protects nanoparticles with a high ratio of Ce(III)/Ce(IV) against the diseases related to oxidative stress or inflammation. Besides, CeO_2 nanoparticles with a lower ratio show anticancer and antibacterial effects due to catalase enzyme mimetic activity [21]. Many other inner transition metal ions have been employed to adjust the oxygen concentration and the ratio of Ce(III)/Ce(IV) at the surface of the nanoparticles. The results also showed that the presence of metal with a higher radius (La) causes a higher rate of Ce(III)/Ce(IV) ratio to be compared to smaller metal atoms (Nd) [14].

Under *in vivo* and *in vitro* experimental models, CeO_2 nanoparticles exhibit antioxidant properties due to the self-regeneration of the surface. This is originated from a change in the cerium oxidation state from +3 to +4. Also, the oxygen-free space will alternately change the state of the CeO_2 to Ce_2O_3 [22]. On the other hand, the results indicate that CeO_2 nanoparticles cause cell death [22]. They create a pro-oxidative effect due to ROS to bring about damage to the cell and ultimately lead to apoptosis [22].

A nanosystem should be more hydrophilic and biocompatible in order to obtain good activity in a biological environment. Due to the low solubility of nanoparticles, they are often modified by encapsulating with a hydrophilic and biocompatible polymer material like Dextran. Dextran has the excellent feature like hydrophilicity, biocompatibility, and biodegradability which makes them good candidates for coating, low water soluble nanomaterials for biological applications [22-23].

METHODS OF SYNTHESIS OF CEO_2 NANOPARTICLES

The methods of synthesis play a crucial role in both the physical and chemical properties of nanoparticles. For nanoparticles with biological applications, all of the parameters in the method of preparation should be carefully controlled to maintain their physical and chemical properties in *in vivo* applications[24].

A good number of methods have already been developed for the synthesis of CeO_2 nanoparticles which include: precipitation [25-27] co-precipitation [28], microwave, [29], solvothermal [30], ball milling [31], thermal decomposition [32], spray pyrolysis [33], thermal hydrolysis [34], sol-gel [35-37], sonochemical [38, 39], and hydrothermal [40] methods. Other recent methods include green synthesis by using plant extracts and other natural products such as honey [41-44]. CeO_2 nanoparticles with outstanding physical and chemical properties can be obtained by an appropriate method of synthesis. Wet-chemical and microemulsion methods, for example, produce small-size CeO_2 nanoparticles without surface contamination [45]. The methods involving high-temperature operations produce smaller CeO_2 nanoparticles avoiding agglomeration in spherical morphology [46]. However, conventional methods of preparation have several disadvantages since toxic solvents and reagents need to be used, and they require high temperature and pressure and external additives as stabilizing or capping agents during the reaction [16].

Novel strategies have been developed in which natural products and biomolecules are used as stabilizing agents in the synthesis of CeO_2 nanoparticles. These 'green synthesis' methods gained research interest due to environmental compatibility, safer, reliable, and eco-friendly purpose. Further, this method is an alternative, inexpensive, and simple alternative to the traditional method. The main factors rendering green synthesis as a promising means are plant-, fungus-, polymer-, and nutrient-mediated syntheses [24].

Another interesting green method is used to synthesize nanoparticles using biological molecules such as apoferritin (cage-shaped protein). In this synthesis, apoferritin is considered as a bio-template. The oxidation of cerium ions is enhanced and eventually causes the formation of CeO_2 nanoparticles [47]. Other than these, several studies are reported using different natural materials and nutrients, such as egg white protein and honey for green synthesis of CeO_2 nanoparticles [48].

The phytosynthesis uses plant extracts as a stabilizing medium for the synthesis of CeO_2, but it is not suitable for biomedical applications in the present scenario [49]. The methods of producing small nanoparticles of CeO_2 using a fungus-mediation have solved this problem, which results in more stable, well dispersed, and highly fluorescent nanoparticles. CeO_2 nanoparticles could also be prepared using the culture filtrate of *Curvularia lunata* [42]. It has recently been noted that the use of leaf extract of *Acalypha indica* and *Aloe vera* is used to synthesize CeO_2 nanoparticles [50]. The extract of *Hibiscus sabdariffa* flower also served as a chelating agent for the synthesis of CeO_2 nanoparticles [51]. The extract of these plants has been introduced as a stabilizing and a synthesizing agent in the synthesis of CeO_2 nanoparticles. Kargar et al. reported the green synthesis of CeO_2 nanoparticles stabilized by agarose polymers via the sol-gel method [52].

Factors affecting the synthesis of CeO_2 nanoparticles

Even though CeO2 nanoparticles contain identical chemical constituents, their biological effects vary depending on the synthetic method and factors employed during the fabrication step**.** The factors of synthesis in both traditional and green synthesis methods dictate the physical parameters of CeO_2 nanoparticles, including particle size, shape, dispersion level, and surface charge. Residual surfactant contamination, nature of stabilizing agent, and the Ce(III)/Ce(IV) surface ratio can influence the interactions at CeO_2 nanoparticles with the biological environment [53]. Efficient manipulation of the ratio of Ce(III)/Ce(IV) and the free space of the oxygen causes the change in biological interactions.

The counter anions in cerium salts greatly influence the size and morphology of particles. For example, anions such as SO_4^{2-}, or halides (Cl^-, Br^-, and I^-) form nanorods of CeO_2, while NO_3^- anion forms the nanocube structure [54]. The dependence of the morphology of CeO_2 nanoparticles on temperature is not straightforward. CeO_2 nanoparticles with different morphology can be fabricated with a single reaction at the different temperatures as well as a combination of various synthetic methods. For instance, in the hydrothermal method, at room temperature, nanorods were synthesized and converted to nanotubes at 100 °C. Similarly, CeO_2 nanoparticles could be prepared as nanowires at a temperature of 110 °C, nanospheres at 120 °C, nanospheres and nanocubes at 140 °C, and nanocubes at both 160 and 180 °C [55].

Another important factor in the synthesis of CeO_2 nanoparticles is the amount of dispersion. This determines the extent of agglomeration in aqueous solutions and other biological environments. The amount of dispersion enhances stability, along with

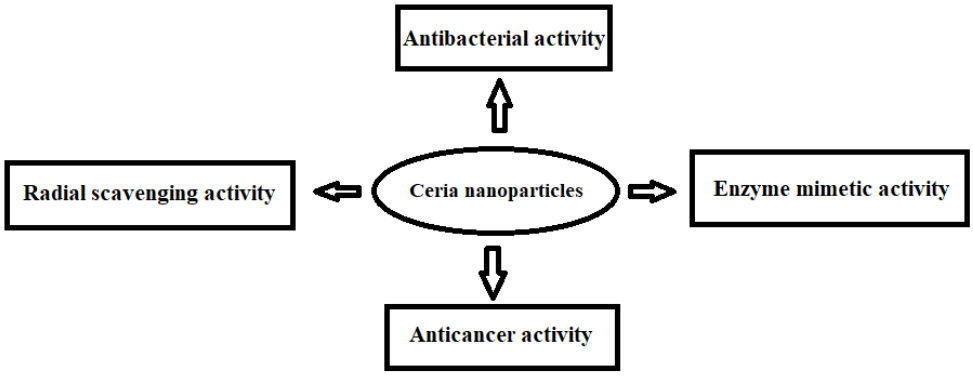

Fig. 1: Major biological applications of CeO_2 nanoparticles

the reduction of unnecessary interactions with other cells or proteins present in the vicinity. It also increases the survival time in the circulation, reduces the toxicity of nanoparticles, and provides an effective dose to minimize side effects. The dispersion rate is effectively improved by coating CeO_2 nanoparticles with biocompatible polymers, composite materials, surfactants, stabilizers, or biomacromolecules during synthesis or even after synthesis [14].

Many traditional methods are non-ecofriendly and not feasible. Generally, biocompatible coatings increase sustainability, longer retention times, and reduce the toxicity of CeO_2 NP. These coatings include polyacrylic acid [56], polyethylene glycol (PEG) [57], polyethylene mine [58], cyclodextrin [59], glucose [60], and folic acid [61]. Literature suggests that the coatings can increase the stability, influence mechanism of uptake, and intracellular localization of CeO_2 nanoparticles in different cell compartments and cytotoxicity in biological applications [62].

BIOLOGICAL APPLICATIONS OF CEO_2 NANOPARTICLES

CeO_2 nanoparticles have many uses in the biological fields, but there are many concerns about their consequences on health and the environment. The impact of CeO_2 nanoparticles on human health has attracted scientists and researchers. The inhalation of CeO_2 nanoparticles will affect the lungs and lymph nodes. When it enters the circulatory system, it may be distributed in other organs like the liver, kidney, and spleen. Eventually, the excretion of cerium in the feces takes place [22]. For this, it is essential to study their interaction with physiological activity and toxicity in the human body. The main areas of biological activities reported for CeO_2 nanoparticles are represented in Fig.1.

Antibacterial activity

CeO_2 nanoparticles have antibacterial activity against *P. pseudomonas aeruginosa*. Research confirms that by increasing the concentration of CeO_2 nanoparticles, the growth of *P. aeruginosa* (NCIM-2242) is inhibited. Hydrothermally synthesized CeO2-ZnO nanocomposite were studied for activity against Streptococcus mutans. The authors observed that minimum inhibitory concentration (MIC) against the microorganisms was 0.22 mg / mL[63].

Genotoxicity

The literature shows the investigation of the mechanism of molecular toxicity of CeO_2 nanoparticles on lung adenocarcinoma cells (A549). These nanoparticles have changed the morphology of cancer cells A549. Due to the presence of CeO_2 nanoparticles and ROS, damage to the DNA and cell cycle stopped causing the death of the A549 cell [64]. Another genotoxicity study on female albino Wistar rats was performed by comet and chromosomal aberration and micronucleus tests. The final results indicated that in high doses of CeO_2, nanoparticles would damage DNA in the liver and peripheral blood leukocytes (PBLs)[43]. Cytotoxic and genotoxic study of CeO_2 nanoparticles in a human neuroblastoma cell line (IMR32) were also reported. CeO_2 nanoparticles caused cytotoxicity, which was confirmed by lactate dehydrogenase assays and 3-[4,5-dimethylthiazol-

2-yl-2, 5-diphenyl tetrazolium bromide. The results showed that ROS was involved in the toxicity of CeO_2 nanoparticles [65].

Neurotoxicity

Because of the presence of the blood-brain barrier (BBB), the targeted drug delivery is the hardest task in neural drugs since the BBB acts as a selective filter for these molecules. Investigation of the responsive action of the brain was performed that instructs the administration of suramin into the intracerebral region. The results showed that CeO_2 nanoparticles labeled as fluorescence deposited into the liver and spleen when they entered the body of the mice [22].

Antioxidant activity

Anti-oxidant properties appear when CeO_2 nanoparticles are combined with levan. Levan acted as a reducing and stabilizing agent. Levan combined with nanoparticles were favorable towards the disease related to ROS [66]. Recent work has shown that CeO_2 nanoparticles with a size of 20 nm increase longevity and maintains function expression in brain cell cultures. In a size of 10 nm, cell death is reduced with CeO_2 nanoparticles and UV and H_2O_2. Therefore, the results indicate that the antioxidant activity of the CeO_2 nanoparticles is dependent on the size [67].

In vitro studies

A large number of *in vitro* studies were available, which reports the cytotoxic action of CeO_2 nanoparticles in various cancer cell lines. A study found that CeO_2 nanoparticles are toxic to lung cancer cell lines. Sulforhodamine B was used to examine the cell viability at different concentrations and times. The significant cellular loss was observed with regard to the dose of nanoparticles and the incubation time with CeO_2 nanoparticles. Cytotoxicity assay of CeO_2 nanoparticles was carried on human fibrosarcoma (HT- 1080) cells and breast cancer (MCF-7). There were no cell deaths in the concentration range of 20-200 μg mL^{-1} when CeO_2 nanoparticles were incubated with cancer cells [68].

There has been an investigation on the protective effects of CeO_2 nanoparticles on primary human skin fibroblasts [69]. The CeO_2 nanoparticles were found to be internalized and showed strong ROS scavenging activity, and the viability of the fibroblasts was not affected. CeO_2 nanoparticles affect the mitochondrial activity and lead to more productive ATP. Sack et al. reviewed cancer treatment using the redox activity of CeO_2 nanoparticles in combination with doxorubicin. A comparison of the antitumor activity of CeO_2 nanoparticles with doxorubicin shows that doxorubicin has antitumor activity and ROS formation and oxidative damage against human melanoma cells (A375 cells) are noticeable. The use of the combination of doxorubicin and nanostera exhibits better anti-tumor activity than when it is used alone [70]. In another study, Sack-Zschauer et al. have shown that CeO_2 nanoparticles have destroyed the cancer cells of the glioma and protected healthy cells. CeO_2 nanoparticles have cytotoxicity activity on astrocytoma (grade III glioma), while on microvascular endothelial cells, no influence could be marked [71]. One of the major features of CeO2 nanoparticles is the activity in cancer cells. Enough evidences were available in literature showing the potent activity of CeO2 in various types of malignant cells. For example, the viability of lung cancer cells got decreased with increasing the concentration and exposure time of CeO2 NPs. The mechanism was reported to be oxidative induced free radical formation[110]. Lack of toxicity in normal cell lines(L929) with significant toxicity against prostate cancer cells (PC-3) were also an interesting result confirmed using MTT assay[111]. Apart from direct activity against cancer cells, CeO2 NPs have found to enhance the effectiveness of cancer treatment strategies. A good example of this was reported in pancreatic cell lines in which the authors confirmed that CeO2 NPs can act as sensitizer in radiation treatment associated with pancreatic cancer which increases the therapeutic outcome [112].The role of CeO2 NPs have been thoroughly reviewed in literature which further discuss the mode of action and possibilities for cancer therapeutics. These studies indicate that CeO2 NPs have the ability to selectively attack malignant cells through increasing the sensitivity to other treatments and also inducing toxicity with differentiating them from healthy cells [113]. This differential cytotoxcity of CeO2 in malignant cells gives future possibilities to use as a nanotherapeutic agent in cancer treatment [114]. Similar results were obtained by coupling conventional Chemotherapeutic drugs such as Doxorubicin (DOX) with CeO2 NPs for enhanced synergistic effect [115]. Recently the efficiency of CeO2 as an agent in near-infrared light controlled

Photo dynamic therapy (PDT) against hypoxia cancer is also reported which gives further scope in this area[116].

In vivo studies

Literature shows that CeO_2 nanoparticles can affect *in vivo* models such as the rat, mice, and non-rodent models in different ways. There are a good number of reports on the liver, lungs, spleen, kidneys, and brain to comprehend the activity of CeO_2 nanoparticles [72-74]. Also, there have been studies on plant crops like rice [75], wheat, sunflower, pumpkin [76], tomato [77], kidney bean [78], radish [79], cucumber [80], and Rubia cordifolia [81] to determine the absorption rate of CeO_2 nanoparticles. The absorption rate in the roots is higher than in other parts of the plant. This is due to various factors, such as the size of nanoparticle [80], the extent of agglomeration [76, 80], and concentration [77, 78].

Caenorhabditis elegans have been exposed to various levels of nanoscale coated surfaces in an *in vivo* model. The different surfaces coated with CeO_2 nanoparticles showed a difference in absorption [82]. Kwon et al. showed that the introduction of CeO_2 nanoparticles within mitochondria is a successful treatment for neurodegenerative diseases [83]. The synthesis of triphenylphosphonium-conjugated CeO_2 nanoparticles has also been reported, which is located in the mitochondria of different cells. This can be used to treat diseases associated with mitochondria, Alzheimer's, and other neurodegenerative diseases. Hijaz et al. reported that combining folic acid-CaPs with cisplatin results in the death of ovarian cancer xenograft nude cells [84]. Also, folic acid- CeO_2 nanoparticles reduce vimentin, which indicates the break of the metastasis of ovarian cancer. Reports also demonstrate the cellular mechanism and the catalytic activity of CeO_2 nanoparticles to prevent the reduction of photoreceptor cells. The results showed that CeO_2 nanoparticles led to a reduction in apoptotic cells and lipid peroxidation in the retina [85].

Physiological studies

The co-precipitation method was used for the synthesis of CeO_2 nanoparticles, and the biological effects of synthesized particles were studied using the intraperitoneal technique, where nanoparticles spread throughout all tissues. The results showed that the antioxidant effects of CeO_2 nanoparticles depend strongly on the dosage [86]. The antioxidant effects of CeO_2 nanoparticles were also reported wherein it was demonstrated that CeO_2 nanoparticles help reducing weight gain and lower the plasma levels of leptin, insulin, glucose, and triglycerides [87].

CeO_2 nanoparticles were used for prophylactic treatment of hepatic ischemia reperfusion injury in Sprague Dawley rats. The CeO_2 nanoparticles caused a decrease in hepatocyte necrosis, alanine aminotransaminase, macrophage-derived chemokine, lactate dehydrogenase, myoglobin, macrophage inflammatory protein-2, keratinocyte chemoattractant (KC)/human growth-regulated oncogene (GRO), and plasminogen activator inhibitor-1 [88].

Enzyme Mimetic Activities

Superoxide Dismutase and Catalase Mimetic Activity

In mammalian cells, natural aerobic metabolism produces free radicals, called superoxide radicals. They are produced due to. The radicals of this kind serve as signaling molecules and have been an important factor in the oxidative response to pathogens. The extra amount of these radicals is destroyed by enzymes SOD [89]. A ratio higher than Ce(III)/Ce(IV) in CeO_2 nanoparticles shows more SOD-mimetic activity than lower values. Catalase causes H_2O_2 to be lost, and an oxidizing agent is harmful [90]. Low extent of Ce(III)/Ce(IV) ratio shows catalase-mimetic activity more compared to higher ratio of Ce(III)/Ce(IV) [91].

Phosphatase Mimetic Activity

Phosphatases are the kind of enzymes used for the hydrolysis of esters into phosphate ions to remove phosphate groups from their substrates [92]. The relatively low Ce(III)/Ce(IV) ratio in CeO_2 nanoparticles can mimic phosphatase for artificial phosphate substrates [93] and bio-relevant substrates, such as ATP [94].

Radical/ROS Scavenging Activity

Due to the redox properties, rare metal ions such as cerium ion can serve as oxygen free radical scavenger. ROS comprises free radicals and non-radicals and is more reactive than molecular oxygen. Free radicals are less stable and more reactive and can have a significant effect on the biochemistry and metabolism of living environments. ROS are produced as an ancillary

Table 1. Summary of major synthesis methods and features of CeO_2 nanoparticles

Method of Preparation	The diameter of particles (nm)	Morphology	Reference
Precipitation	15	Spherical	28
Hydrothermal	5	Octahedral	40
Solvothermal	8	Polyhedral	30
Spray Pyrolysis	17	Cubic	33
Plant- mediated	36	Spherical	115
Fungus- mediated	5	Spherical	50
Polymer- mediated	2	Spherical	116
Nutrient-mediated	25	Spherical	48

product in aerobic activity and are associated with oxidative stress. Increasing levels in the body cause many diseases. However, ROS plays the role of signaling molecules in physiological processes. Given these points, antioxidants are molecules that can destroy or inhibit the production of ROS. Excess ROS levels create oxidative stress, depletion of cellular antioxidants, and, thus, oxidation of the biomolecules like proteins or lipids of cellular membranes, carbohydrates, DNA, and ultimately causing cell damage and cell death.

The smaller CeO_2 nanoparticles achieve more cellular absorption due to volumes larger than their surface and faster kinetics. Kumari et al. reported that CeO_2 nanoparticles with a size of 25 nm to the neuroblastoma cells were more toxic than particles with a size of 3 μm. [65].

Synthesis of various forms of nanoparticles, such as spheres, pillars, rods, cubes, helices, wires, polygonal, octahedral, has been reported.[95-98]. Due to the type of nanoparticle structure, they have different properties of different chemical, electrical, optical, and magnetic properties. Therefore, they can interact with different cells and biological molecules. For example, nanoparticles in polygonal, cube, or rod morphology have sharp edges and can cause mechanical damage to cells [99]. The smaller nanoparticles have a larger surface-to-large ratio for interactions with cells. The smaller nanoparticles have a higher Ce(III) content, which eliminates intracellular and extracellular ROS [100].

Studies have shown that the toxicity of CeO_2 nanoparticles to human hepatoma cells of SMMC-7721 is not related to the size and morphology of cerium oxide nanoparticles [101]. This can be due to aggregation, which reduces their toxicity. Therefore, the mechanism of interaction does not only depend on CeO_2 nanoparticles but also depends on the type of cell and the culture medium.

The hydroxyl radical is one of the most biologically active free radical [102]. CeO_2 nanoparticles are capable of removing hydroxyl radicals at certain sizes. CeO_2 nanoparticles show a neuroprotective effect on adult rat spinal cord neurons.

INFLUENCE OF MORPHOLOGY ON THE ACTIVITY CEO_2 NANOPARTICLES

Various methods of preparation have influences on the features of nanoparticles formed, such as morphology, size, etc. as shown in Table 1. Also, surface charge density on the nanoparticle plays a crucial role in many applications such as *in vitro* and *in vivo* processes.

The Influence of morphology on the cytotoxicity of CeO2 nanoparticles has been investigated and reported in literature. A good example of this exploration was carried out by fabricating CeO2 nanoparticles having a different morphology such as rod shaped and cubic nanoparticles. Toxicity was evaluated using important parameters such as LDH release, ROS production and TNF-α production in Macrophages from RAW264.7 cell line [117]. The authors were able to reach a conclusion that cytotoxicity is significantly enhanced in a dose-dependent manner by rod-like nanoparticles. But a contrary result was also reported in which rod shaped CeO2 nanoparticles were found less cytotoxic compared to Cubic shaped nanoparticles in HepG2 cells [118]. These results indicate the morphological influence may depend significantly on the nature of cell line exposed to CeO2 nanoparticles and needs further exploration to reveal more information about toxicity pathways.

The surface charge of CeO_2 nanoparticles is essential in cell targeting, cell adhesion, uptake, subcellular distribution, and cytotoxicity. The surface charge can be modified by using appropriate acid, base buffers, or coatings with polymer, biomolecule, ligand, or surfactant and stabilizer.

The uptake of nanoparticles by cells is, in general, associated with two steps: a binding step on the cell membrane and an ultimate internalization step. The first stage is influenced by the surface charge of cell membranes and CeO_2 nanoparticles. The higher the surface charge of the CeO_2 nanoparticles, the stronger is the bond through the electrostatic interaction with the membrane of the cell [103]. In general, the cell surface has a negative charge, so nanoparticles interact with a positive charge by electrostatic interaction. It has also been reported that negative CeO_2 nanoparticles can be located at specific cationic locations at the cell surface.

Research shows that Ce(III) is related to the toxicity of CeO_2 nanoparticles [104]. The higher Ce(III) levels show toxicity effects and *vice versa* in the proposed animal model. Further CeO_2 nanoparticles with higher Ce(IV) on their surface-displayed catalase mimetic activity. This destroys H_2O_2 to molecular oxygen and protects the cells against the toxic ROS. CeO_2 nanoparticles with higher Ce(III) on their surface shows the efficacy to remove radicals of superoxide and produce H_2O_2, which is toxic to the cells.

CeO_2 nanoparticles act as direct antioxidants and act as radical scavenging agents as their own since the interaction of superoxide radical, hydroxyl radical, and hydrogen peroxide, and ultimately oxidative stress causes cell death. The level of a quantitative assessment of total ROS, malondialdehyde, α-tocopherol, glutathione, and lactate dehydrogenase shows a reduction in the level of glutathione and α- tocopherol indicating cytotoxicity. Free radicals are generated due to interaction with nanoparticles and enhancement in oxidative stress, yielding a high level of lactate dehydrogenase and malondialdehyde, which causes cell membrane damage and lipid peroxidation.

Ovalbumin and lysozyme are two proteins in the egg which can be effectively used as stabilizing agent in synthesis CeO_2 nanoparticles. The mechanism involves electrostatic interactions between cerium ions, and opposite- charged protein leads to controlled and stable growth of CeO_2 nanoparticles [105].

The activity of CeO_2 nanoparticles in a microbial environment has shown that nanoparticles cannot penetrate the cells of bacteria and algae. For this reason, the toxic effects of CeO_2 nanoparticles have been suggested to be caused by their direct connection to the cell wall of algae and bacteria[106]. CeO_2 nanoparticles cause a change in the nutrient transport functions of the membrane bacterial cells and algae, which results in mechanical damage and membrane disruption, or generation of ROS and oxidative stress [107].

Sadhu et al. observed that high concentrations of CeO_2 nanoparticles in BY-2 cells cause cellular toxicity and can affect metabolic activity [108]. Ce(III) and ROS concentration-dependent accumulation was observed for all CeO_2 NPs. Significant DNA damage and changes in the antioxidant defense system were observed for concentration 50g/mL and 250g/mL. Another study reports the synthesis of CeO_2 nanoparticles and a comparison of the toxicity of CeO_2 powder on nano and a bulk scale on cancer cells and normal healthy cells. MTT assay test was performed for SKBR3 (human breast cancer cell line), A431 (Human epidermis carcinoma cell line), and C2Cl2 (ATCC mouse skeletal muscle cell line). The toxicity of CeO_2 nanoparticles is significantly higher than the bulk form, and their toxicity is higher on cancer cells. This issue is further observed at higher concentrations [109].

CONCLUSION

Metal oxide nanoparticles are hotspots of nanotechnology research. They are relevant and received attention in almost all thrust areas of research like drug delivery, bioimaging, and microbiology, etc. Compounds of inner transition metals are a unique family in nanomaterials due to their unique chemical properties like spectral behavior, magnetic property, etc. In this review, we have discussed CeO_2 nanoparticle by focusing on their various biological applications and synthetic methods. Since cytotoxicity remains as a challenge of application of nanomaterials, special attention has been given to explain cytotoxicity of CeO_2 nanoparticles. This may pave new possibilities for applications of CeO_2 nanoparticles by summarizing the major outcome of research and various aspects of toxicity.

SCOPE AND FUTURE PERSPECTIVES

CeO_2 nanoparticles stand out the unique class of candidates due to their chemical properties and lack of significant cytotoxicity. Over the past years, new methods for controlling dispersion, reducing the accumulation and reduction of protein interactions, including coatings with compatible biocompatible and biodegradable compounds, are designed. Green synthesis methods are promising

for the production of CeO_2 nanoparticles and their biological applications among the various methods reported for the synthesis of CeO_2 nanoparticles. Also, studies have to be conducted to determine the influences of cell types and cellular features on the cellular toxicity of CeO_2 nanoparticles. All these developments can be effectively applied to new real-life applications that are yet to be explored. Even though the possibilities of these materials are still not explored, their cytotoxicity should be carefully analyzed in different media and systems of administration. Also, features like vitro conditions, medium pH, protein, and salt concentration should be taken into consideration. The characteristic CeO_2 nanoparticles should also be performed at each stage of their application periodically and *in vitro* / *in vivo* tests to better explain the changes in toxicity.

ACKNOWLEDGMENT

A. Rahdar would like to thank the University of Zabol for financial support for this work.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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