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A Brief Review of Synthesis Methods, Biological Activities and Cytotoxicity of Cerium Oxide Nanoparticles

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Abstract

Cerium oxide nanoparticles (CeO_2-NPs, or nanoceria) are among the most unique and promising lanthanide nanomaterials, with unique properties such as their redox activity, oxygen storage capacity, and free radical scavenging ability. Their ability to self-regenerate their surface makes them a potential candidate in different fields, especially the biomedical division. The mechanisms by which nanoceria protect against oxidative stress include the direct scavenging of radicals by mimicking the catalytic activity of redox enzymes. Nanoceria can act as a double-edged sword and display both oxidant and antioxidant properties, depending on surface characteristics and the environment. In spite of their potential for clinical applications, there are also contradictory studies reporting the potential toxicity of CNPs. This review describes: (1) the methods of synthesis for CeO_2-NPs; (2) the applications of nanoceria for antioxidant, anti-inflammatory, and anticancer activities, including the most recent studies carried out in vivo and in vitro; and (3) their cytotoxic activity.

Keywords: Cerium oxide nanoparticles, Nanoceria, Antioxidant, Synthesis, Cytotoxic activity

Introduction

Nanotechnology is the manipulation of matter on an atomic and molecular scale, ranging from 1 to 100 nanometers, and is known to be a rapidly developing field with the potential to revolutionize many industries (Peidaei et al., 2021). Nanotechnology has already been applied in various fields of science, including physics, chemistry, biology, and material sciences, as well as medicine (Hossieni et al., 2024; Rajeshkumar & Naik, 2018; Dhall & Self, 2018). Due to their small size, nanoparticles have distinctive characteristics that set them apart from bulk materials, such as electrical, optical, magnetic, and mechanical properties (Karimi et al., 2024; Singh et al., 2020;). Cerium, a rare earth metal with an atomic number of 58, is a member of the lanthanide series, which exists in both +3 and +4 oxidation states and, can be found in CeO_2 (dioxide or ceria) and Ce_2O_3 (dicerium trioxide or sesquioxide) forms (Rajeshkumar & Naik, 2018; Dhall & Self, 2018). Cerium is highly reactive and is believed to have oxidizing properties. Therefore, cerium reacts with oxygen molecules and finally forms cerium oxide, which is also known as nanoceria (Abuid et al., 2020). Cerium oxide is utilized as a UV absorber, polishing agent, gas sensor, fertilizer, an active component for heterogeneous catalysis, and therapeutic agent (Singh et al., 2020; Rajeshkumar & Naik, 2018). The most pivotal and extensively studied characteristic

of CeO_2 is undeniably their inherent ability to act as antioxidants. The oxygen vacancies on the surface of nanoceria, which provide binding sites to speed up electron transfer processes, are widely acknowledged to have a major influence on CeO2's antioxidant activity. Interestingly, CeO_2-NPs have received huge consideration in nanotechnology due to their valuable applications as reducing agents rather than catalysts (Saifi et al., 2021). Interestingly, soluble Ce^{3+} salts (nitrate, acetate, and chloride) have been described to possess antiemetic, bacteriostatic, bactericidal, immunomodulating, and antitumor activities and thus justify their use in *traditional* medicinal therapies (Nelson et al., 2016). CeO_2 nanoparticles have been shown to display an antioxidant activity expressed as oxidase, catalase and SOD-like activity, which can scavenge reactive oxygen and nitrogen species. CeO_2-NPs are also suitable for use as possible pharmacological agents due to their bio-relevant properties (Dhall & Self, 2018). Moreover, the redox property and Lewis acid and base sites of cerium catalyze a broad spectrum of agents, including reactive oxygen species (ROS) and reactive nitrogen species (RNS), in cells and animals (Abuid et al., 2020). The specific oxygen adsorption and release capacity of CeO_2-NPs as well as the Ce^{3+}/Ce^{4+} redox potentials on the CeO_2-NPs surface lead to both the enzyme-mimetic and ROS/RNS scavenging capacities of CeO_2-NPs (Nelson et al., 2016). Because of the high surface-to-volume ratio of nanoceria that results in enhanced surface oxygen vacancies, their reactivity is

particularly efficient at the nanoscale. As a result, the smaller the particle, the greater the redox activity per unit volume (Ma et al., 2018). Furthermore, a mixture of Ce^{4+} and Ce^{3+} can exist on the surface of CeO_2-NPs at the nanoscale, in contrast to the bulk size of nanoceria, which only exists in the pure form of CeO_2 (Ce^{4+}) or Ce2O3 (Ce^{3+}) (Nelson et al., 2016).

This review begins with a brief overview of the methods that are commonly used to prepare CeO_2-NPs. Then, we have presented significant biological activity of CeO_2-NPs, like antioxidant and *pro-oxidant*, anti-inflammatory, and anticancer effects, based on preclinical evidence from in vivo and in vitro studies. Lastly, we have discussed the cytotoxicity of nanoceria.

Synthesis of nanoceria

Researchers have described a variety of methods, including hydrothermal, solvothermal, precipitation, spray pyrolysis, and microwave for the synthesis of nanoceria as shown in Table 1 (Rajeshkumar & Naik, 2018). The parameters of the technique utilized, such as the amount of aging, temperature, and pH, are used to alter the properties of the resulting nanoparticles. By adjusting these factors, it is possible to produce nanoparticles with a specific size, shape, stability, and oxidative ratio of $3^+/4^+$ (Karakoti et al., 2012). CeO_2-NPs are typically produced by stirring a cerium precursor, such as cerium (III) nitrate, cerium (III) sulfate, or cerium (II)

chloride, with an oxidant, such as ammonia or hydrogen peroxide. It is also possible to change the resulting oxidation state of CeO_2-NPs by adjusting the concentration of reagents and the proportion of precursor to oxidizer (Abuid et al., 2020). The precipitation method is believed to be the most convenient technique that can be performed at both room temperature and higher temperatures. The precipitation method is also derived from two approaches. Hydrothermal synthesis refers to the synthesis through chemical reactions, in which water plays a significant role as a solvent (Thakur et al., 2019). The first use of the hydrothermal crystallization method was made known by Masui et al. (2002). Based on their experiment, cerium chloride hexahydrate, citric acid, and ammonia water were used as precursors. Subsequently, the obtained nanoceria particles were spherical with an average diameter of 5 nm. Citric acid was used here due to its inhibition impacts on particle growth. Solvothermal synthesis uses organic solvents in a high-pressure and high-temperature chamber to create nanomaterials of various sizes (Nyoka et al., 2020). Many of the traditional synthesis methods, as expected, have low biocompatibility. Green synthesis is recognized as an eco-friendly, sustainable, and cost-effective method. In the case of nanoceria, the green synthesis method presents a variety of approaches, such as plant-mediated synthesis, microorganism-mediated synthesis, and green chemistry-based techniques. It is also indicated that all of the green techniques have the same spherical nanoparticle form, but

the size of the particles varies depending on the technique, roughly ranging from 2 to 36 nm (Anvar et al 2023; Sharmila et al., 2019). Additionally, the nanoparticles are coated by several major active proteins in egg whites, like ovalbumin and lysozyme. These proteins have the ability to bind simultaneously to cationic and anionic metal complexes. The electrostatic interaction between cerium ions (Ce^{3+}) and proteins that have opposite charges promotes development and the formation of small, stable, and isotropic nanoparticles (Rajeshkumar & Naik, 2018).

Table 1. Commonly used methods for synthesis of cerium oxide nanoparticles

Method of Preparation	Capping Agent	Particle Size (nm)	Morphology	Reference
Precipitation	Dextran	3–5	Spherical	(Nyoka et al., 2020)
	Sarium	10–13	Spherical	(Alpaslan et al., 2017)
	Ethylene glycol	5-10	Square	(Ramachandran et al., 2019)

Hydrothermal	Citric acid	<5	Spherical	(López et al., 2015)
	Trisodium phosphate	5-60	Rods	(Tok, 2007)
Solvothermal	Ethylene glycol	-	Plate; Spherical	(Nyoka et al., 2020)
Spray Pyrolysis	-	17	Cubic	(Dhall & Self, 2018)
Green synthesis	Acalypha indica	8–54	Spherical	(Kannan & Sundrarajan 2014)
	Fructose; Glucose	2–6	Spherical	(Nyoka et al., 2020)
	Egg White	25	Spherical	(Nyoka et al., 2020)

Biological properties of nanoceria

CeO2 nanoparticles are well known for their powerful and broad-spectrum antioxidant, anti-inflammatory, and anticancer activities, as shown in Figure. 1 A list of some of the functions of nanoceria in biology or medicine is given below:

Antioxidant and pro-oxidant activities

ROS are generated during aerobic metabolism as byproducts, and elevated levels of them are frequently associated with oxidative stress (Miakhil et al., 2024). In this context, antioxidants are exogenous or endogenous substances that either neutralize ROS or prevent their formation (Karimi et al 2019; Schieber & Chandel, 2014). It has been demonstrated that metal and metal-based nanoparticles could have the potential to be applied as pro-oxidants or as an antioxidant strategy (Hussain AlDulaimi, 2024; Dhall & Self, 2018). The Ce ions can transform between trivalent and tetravalent oxidation states on the surface of CeO2-NPs under an aqueous solution, which is considered to be the main reason for their unique regenerative antioxidant activity. Generally, synthesis procedures, physicochemical properties, and the chemical environment are all relevant issues that impact the catalytic antioxidant activities of CeO2-NPs (Nelson et al., 2016). Nanoceria has been shown to act as a mimetic of superoxide dismutase (SOD) and catalase (CAT), effectively degrading superoxide ($O2^{\bullet-}$) and hydrogen peroxide (H_2O_2). (Figure 1).

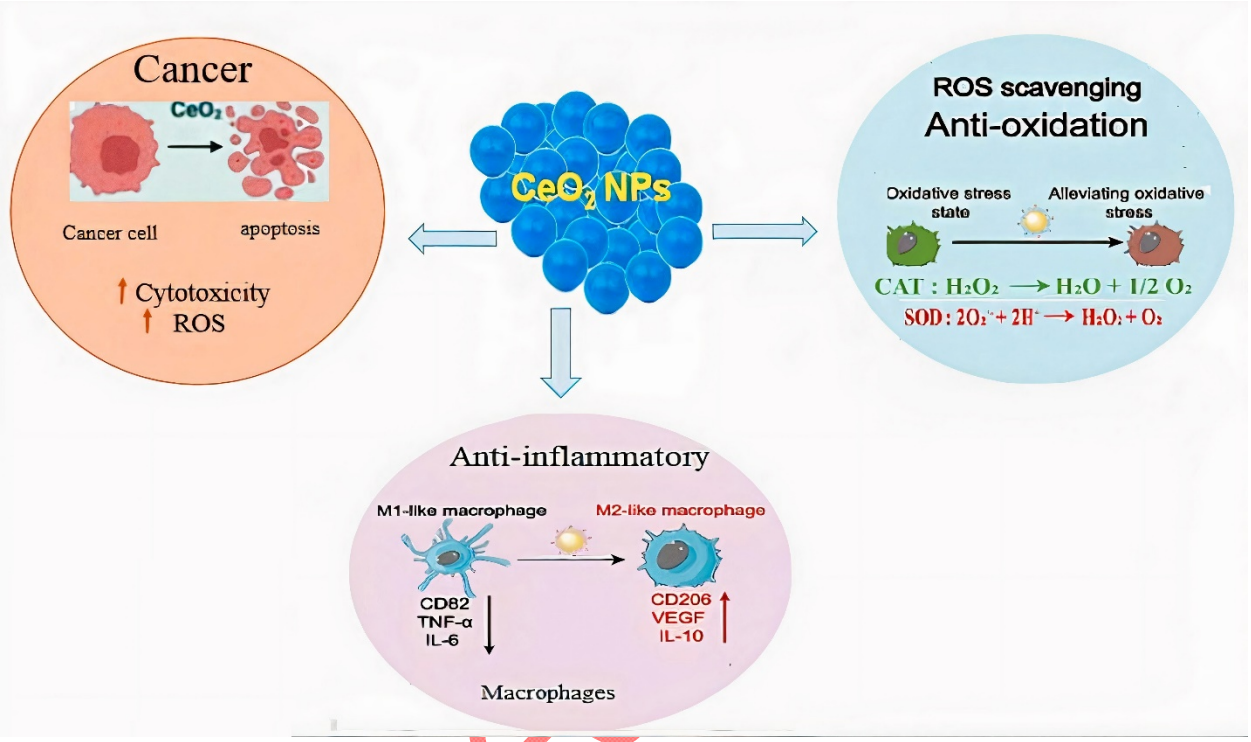

Figure 1. Graphical depiction of some of the functions and benefits of cerium oxide nanoparticles in the field of biomedicine. Applications of CeONPs in cancer treatment can be explained by the loss of the antioxidant ability of CeONPs in acidic conditions, which act as strong oxidants in cancer cells and influence the oxidation of intracellular and extracellular components, leading to apoptosis. The anti-inflammatory properties of cerium oxide nanoparticles are linked to the shifting of macrophage polarization from M1, which can exacerbate inflammatory responses, to M2, which possesses anti-inflammatory properties. The excellent efficiency of scavenging ROS by cerium oxide nanoparticles is attributed to their SOD- and CAT-mimetic activities.

Furthermore, CNP has an oxidizing property that depends on the pH of the particular solution. CNP seems to have more SOD-mimetic than CAT-mimetic characteristics at acidic pH levels and possesses oxidase-like activity (peaking at pH 4). Depending on the particle's features and destination, CNP can be designed to behave as an oxidant or a scavenger of various radicals (Abuid et al., 2020). It has been shown that lowering the pH of the CNP environment can convert the antioxidant properties of CNP into pro-oxidants (Nelson et al., 2016). Perez et al. (2008) revealed the dependence of the antioxidant properties of dextran-coated iron oxide nanoparticles on the pH medium of solutions. The authors demonstrated that under neutral and alkaline conditions, dextran-coated CeO2-NPs exhibited CAT-mimetic activity and were inactivated in acidic (pH 4) solutions. Thakur et al. (2019) documented that the pro-oxidant effect of CeO_2-NPs within the cancer cell can be explained by the acidic intracellular pH of them, which increases the SOD mimetic activity of CeO_2-NPs, and inhibits their CAT mimetic activity, resulting in the accumulation of a huge amount of H2O2 and apoptosis of the cancer cell. However, in normal cells which have a physiological pH, CeO_2-NPs exert a protective effect by scavenging both of $O2^{\bullet-}$ or H_2O_2 due to SOD and CAT mimetic activities (Thakur et al., 2019). The mechanism of the pro-oxidant and antioxidant effects of nanoceria is shown in figure 2.

Furthermore, the concentration, size, and shape of nanoceria have been suggested as factors that could lead to a conversion between their antioxidant and oxidant activities. For instance, CeO_2-NPs exhibited antioxidant activity at low concentrations, while higher levels of nanoceria could promote the production of $OH^{\cdot}$ and exhibit oxidant activity. In addition, the smaller particles have a stronger antioxidant effect and can scavenge a greater amount of $OH^{\cdot}$ (Lu et al., 2016). It is indicated that high CeO2-NPs doses (≥750 μmol/L) can induce pro-oxidant activities (Nelson et al., 2016).

In the literature, CeO_2-NPs are recognized as important typical nanozymes (NZs) with different mimicking activities such as catalase, SOD, oxidase, peroxidase, and phosphatase. (Saifi et al., 2021). It was reported that some NZs are preferred compared to natural enzymes in terms of their higher stability, longer lifetimes and simple preparation (Mukherjee et al., 2023). Due to the nanoenzyme properties of CeO_2-NPs, they can convert a number of substrates, such as hydroxyl, superoxide, peroxide, peroxynitrite, and nitric oxide, into measurable reagents and exert significant antioxidant properties (Saifi et al., 2021). Furthermore, according to a previous study by Pulido-Reyes et al. (2016), CeO_2-NPs were able to scavenge the hypochlorite anion, which is involved in the inflammatory process due to the presence of different oxidation states on the surface of nanoparticles. It was discovered that the CAT-mimetic activity of CeO_2-NPs increases

as the concentration of Ce^{4+} increases. On the other hand, an elevated concentration of cerium in the 3^+ state is primarily responsible for the superoxide scavenging property (Nelson et al., 2016). Nevertheless, Dowding et al. (2012) indicated that the nitric oxide radical scavenging property was present in the CeO_2-NPs with a lower level of cerium in the 3^+ state.

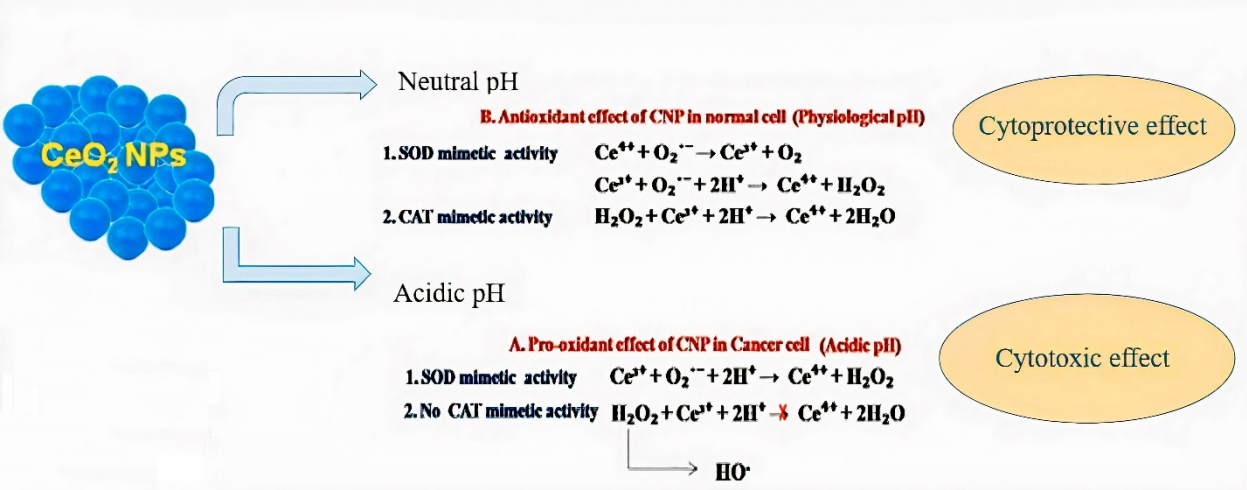

Figure 2. Mechanism of pro-oxidant and antioxidant effect of nanoceria. Cerium oxide nanoparticles (CNPs) exhibit a pro-oxidant effect on cancer cells. This effect is attributed to the acidic intracellular pH, which enhances the SOD mimetic activity of CeO_2 NPs while inhibiting their CAT mimetic activity. As a result, a significant amount of H_2O_2 accumulates within the cancer cell that triggers cancer cell death. Nanoceria mimics ROS-related enzymes that protect normal cells at physiological pH from oxidative stress.

Evidence of antioxidant activity from in Vitro and in Vivo Studies

CeO_2-NPs have been reported to possess strong antioxidant activities in numerous in vitro studies (Saifi et al., 2021). In several cell models, including gastrointestinal epithelium, human breast line, neuronal cells, endothelium, and stem cells, CeO_2-NPs were capable of ROS scavenging and providing protection (Nelson et al., 2016). The heparin-functionalised nanoceria were found to be more effective compared to the bare nanoceria in terms of cellular uptake, residence time of the particles within cells, and ROS scavenging activity in activated human monocyte cells (Ting et al., 2013). In the study of Pagliari et al. (2012), long-term antioxidant activity of CeO_2-NPs at all concentrations (10 $\mu g \cdot mL^{-1}$, 25 $\mu g \cdot mL^{-1}$, and 50 $\mu g \cdot mL^{-1}$) was observed in vitro. The authors demonstrated that internalization of CeO_2-NPs into cardiac progenitor cells (CPCs) did not induce structural and functional cell modifications and did not cause cell structural damage while being able to protect CPCs from H_2O_2-induced cytotoxicity for at least 7 days. Rubio et al. (2016) indicated that pre-treatment of epithelial lung cell lines with CeO_2-NPs for 24 hours reduced mortality, exhibited anti-genotoxic effects, and increased the expression of antioxidant genes such as *Ho1*, *Sod2*, and *Gstp1* involved in the Nrf2 pathway.

The ROS-scavenging capacities of nanoceria were observed in the study of Rzigalinski et al. (2003) for the first time. In this paper, CeO2 nanoparticles act as a nanoenzyme or antioxidant modulating ROS and could prolong the lifespan of neuronal cells. Since then, nanoceria has gained extensive interest in the scientific community. As a result of in vivo research, CeO_2-NPs demonstrate promising efficacy as a therapeutic option for a variety of diseases, including reproductive, gastrointestinal, ophthalmologic, and neurological health (Thakur et al., 2019). Increasing evidence from in vivo studies suggests that CeO_2-NPs are primarily taken up by the liver and spleen, followed by the kidneys and lungs (Nelson et al., 2016). However, low to undetectable levels of naonoceria were found in the normal brain, and to deliver the therapeutic effect of nanoceria in neurons, lipid-based delivery systems were designed in some studies to cross the blood-brain barrier (Battaglini et al., 2018; Yang et al., 2022). It is worth mentioning that agglomeration of nanoceria is the basic process in the bloodstream and occurs due to the greater surface area and small size of particles that result in rapid clearance by macrophages before transporting them to target cells (Yang et al., 2022). Surface modification is recognized as the most commonly used protocol for reducing aggregation and increasing the circulation time of nanoceria in the bloodstream (Zhang et al., 2002). According to a previous study by Estevez et al. (2019), CeO_2-NPs stabilized with equal proportions of citrate acid (CA) and ethylenediamine

tetraacetic acid (EDTA) effectively reduce the formation of ROS and the severity of neuronal loss in three different models of cerebral ischemia.

There is evidence that a single-dose intake of nanoceria demonstrated a long-lasting antioxidant effect in biological systems. Notably, the therapeutic efficiency of nanoceria (5.8 μM) was similar to that of N-acetylcysteine (10 mM) at combating oxidative and nitrosamine damage in a mouse hippocampal brain slice model of ischemia (Yang et al., 2022; Estevez et al., 2011). Nevertheless, N-acetylcysteine is recognized as a potential H_2S-releasing donor, while nanoceria is actually a hydrogen donor. Traditional organic antioxidants can also promote ROS generation due to the single electron exchanging with reactive oxygen and nitrogen species (RONS) and transforming themselves into radicals (Yang et al., 2022). Mohammad et al. (2023) documented the effectiveness of intranasal administration of CeO_2-NPs in ameliorating oxidative stress, improving locomotor activity, and neuroinflammation against haloperidol-induced parkinsonism in rats. Tisi et al. (2022) indicated that CeO_2-NPs were able to reduce retinal neovascularization and reverse the detrimental effect of H_2O_2 on angiogenesis in vitro and in vivo in age-related macular degeneration. CeO_2 treatment led to a reduction in the expression of caspase-3 and p53 in the seminiferous tubules and increased antioxidant defense markers in testicular injury in rats (Yesil et al., 2023). The antioxidant properties of albumin-coated nanoceria were demonstrated

both in vitro and in vivo in human lung epithelial cells (L-132) and zebrafish embryo. Ranjbar et al. (2018) suggest a neuroprotective and antioxidant role for CeO_2-NPs in paraquat-induced brain injury in rats. They showed that CeO_2-NPs (15 and 30 mg/kg doses) protect the brain against the adverse effects of PQ by ameliorating lipid peroxidation, DNA damage, caspase-3 activity levels, and increasing total antioxidant capacity, total thiol molecule content, as well as mRNA levels of neurogenesis markers such as *Nestin* and *Neurod1*.

Anti-inflammatory effects

According to several studies, CeO_2-NPs have strong anti-inflammatory effects. Hirst et al. (2009) showed that treatment of J774A.1 murine macrophage cells with cerium oxide nanoparticles inhibited inflammation through a reduction in iNOS protein and mRNA levels. Further, the authors reported that it is nontoxic in the tissues of C57BLK6 mice following intravenous injections. It was the first study to suggest CeO_2-NPs as a promising anti-inflammatory therapeutic agent. Subsequently, numerous in vitro and in vivo reports have emerged suggesting that CeO_2-NPs have the potential to improve many inflammatory disorders, including neurodegenerative, autoimmune diseases, liver inflammation, gastrointestinal disorders, bacterial

infections, and traumatic injuries (Corsi et al., 2023). Recently, Wei et al. (2021) indicated that CeO_2-NPs exerted a protective effect in macrophages by reducing pro-inflammatory gene expression under chronic inflammatory conditions. Kwon et al. (2016) investigated the effect of mitochondria-selective targeting of triphenylphosphonium (TPP)-conjugated CeO_2-NPs on suppressing the pathogenesis of Alzheimer's disease using an in vivo 5XFAD mouse model. The results showed that TPP-ceria NPs can alleviate brain inflammation by reducing mitochondrial ROS levels and ameliorating neuronal loss in these mice. A new formulation of europium-doped CeO2NPs (EuCeO2NPs) acted as an immunomodulator for Alzheimer 's disease by reducing proinflammatory cytokines and facilitating phagocytosis and intracellular clearance of amyloid beta plaques in LPS-treated BV2 microglia cells (Machhi et al., 2022). Recently, Zavvari et al. (2020) observed that intrahippocampal injection of CeO2 NPs exerts a neuroprotective effect in the stress-induced model of depression by improving hippocampal cell viability, cell proliferation, and neurite formation. This therapeutic effect was accompanied by suppression of the hippocampal IL-6, and malondialdehyde (MDA) productionKalashnikova et al. (2020) administered albumin-cerium oxide nanoparticles in a collagen-induced arthritis (CIA) mouse model and reported that nanoparticles accumulated in synovial tissues of joints and effectively inhibited inflammation *via* reducing hypoxia, scavenging excessive ROS, and shifting the

activity of macrophages towards M2-like polarization, which has high phagocytosis capacity. M1 macrophages are involved in initiating and sustaining inflammatory responses, whereas M2 macrophages have modulatory activity and exert anti-inflammatory effects. They also conceived that the therapeutic effect of nanoparticles was comparable to that of methotrexate, the gold standard treatment for RA. CeO_2 nanoparticles were demonstrated to have an in vitro *cartilage*-protecting *effect* by preventing H_2O_2-induced chondrocyte injury and promoting the expression of genes involved in collagen and aggrecan deposition (Lin et al., 2020). The main reason for the protective effect of CeO_2-NPs in temporomandibulandar joint osteoarthritis is activation of Nrf2/HO1 pathway, as indicated by Xiong et al. (2023). Surprisingly, in terms of multiple sclerosis (MS), combined treatment of nanoceria with lenalidomide could reduce demyelination and CNS inflammation in mice (Eitan et al., 2015). The *therapeutic potential* of CeO_2-NPs in experimental chronic liver diseases was indicated in the study of Oró et al. (2016). According to their findings, *cerium oxide nanoparticles reduced steatosis* and *portal hypertension* and *displayed anti-inflammatory properties* in *rats* with *liver fibrosis.* The effectiveness of CeO_2-NPs against stress-induced gastric mucosal lesions in rats was also investigated. A possible mechanism by which CeO_2-NPs combats inflammation is associated with the inhibition of the Nrf2/NF-κB signaling pathway. Furthermore, phosphorylation of

ERK1/2, p-38, and JNK MAP kinases, in addition to NF-κB, has been shown to be reduced by CeO_2-NPs. Considering that inflammation is largely caused by oxidative stress and due to SOD and catalase mimetic effects of CeO_2-NPs and other various functions of CeO_2-NPs such as phosphatase, haloperoxidase, photolyase, and oxidase-like activities, it could be generally understood that the antioxidant activity of CeO_2-NPs is primarily responsible for their anti-inflammatory effects (Corsi et al., 2023).

Anticancer effects

In considering the biological importance of nanoceria, it has offered a promising new approach to cancer detection and treatment in the literature. However, like any other antioxidant, it can exhibit both anti- and pro-oxidative effects depending on environmental conditions such as pH and oxygen tension gradient. Nanoceria can simultaneously induce ROS production in tumor cells while acting as an antioxidant and protecting neighboring tissues from oxidative stress (Tang et al., 2023). Generally, due to the oxidase-like activity of nanoceria at acidic pH and lower pH values of cancer cells as opposed to normal cells, nanoceria can exert directly on the cancer cells and increase intracellular ROS levels, leading to insufficient mitochondrial quality

control, DNA damage, and membrane damage (Saifi et al., 2021). Recently, cerium oxide nanoparticles have been shown to cause changes in mitochondrial bioenergetics, dynamics, and the number of cristae, leading to a lower viability of tumor cells. The increased superoxide production was reported in the mitochondria of A375 cells as the substrate for nanoceria SOD-mimetic activity producing H2O2 (Aplak et al., 2020). It is indicated that nanoceria provoke apoptotic cell death in a variety of cancer cells through the activation of the p53-dependent mitochondrial signaling pathway (Tang et al., 2023). Nanoceria is thought to activate intrinsic apoptotic pathways by releasing Cyt c levels and activating caspases-3 and -9 and reduce cell survival by preventing cells from entering mitosis (Tang et al., 2023). Further, administration of CeO2-NPs could decrease Ras/Raf/MAPK kinase/ERK signaling pathways involved in cell survival and accelerate apoptosis in experimental hepatocellular carcinoma in rats. However, apart from the direct mechanism, some indirect effects of nanoceria have also been reported that affect tumor cell invasiveness, angiogenesis, and metastasis (Saifi et al., 2021). Giri et al. (2013), reported that in vivo treatment of nanoceria resulted in inhibiting ovarian tumor growth and metastasis even in low doses (0.1 mg/kg). Thus, the use of nanoceria as a vehicle in *drug delivery* systems has several advantages for cancer theranostic applications. Sack et al. (2014) demonstrated that a combination of classical chemotherapeutics, such as doxorubicin with CeO_2-

NPs, could provide a potential platform and promising strategy in cancer therapy. The higher efficacy of CeO_2-NPs combined with doxorubicin might be due to cytotoxicity and oxidative damage as well as inducing apoptosis in A375 melanoma cells. However, in comparison to cells that received doxorubicin alone, co-incubation with CeO_2-NPs did not induce DNA damage.

Toxicity of CeO2 nanoparticles

Despite the therapeutic effects of nanoceria, their biological safety problems, such as potential toxicity, need to be evaluated. The potential toxicological effects on living organisms are the main challenges associated with CeO2 nanoparticles. Additionally, cerium oxide nanoparticles have the capacity to form aggregates, as shown by Naiel et al. (2022). This allows the particles to settle in aquatic environments, posing significant risks to the exposed aquatic organisms and, ultimately, to human health. Most in vitro studies *have* demonstrated that the toxicity of CeO_2-NPs increased with nanoparticle size, and even ROS production was dose and size-dependent. Generally, owing to their smaller size, CeO_2-NPs were found to be more toxic than cerium oxide microparticles (MPs) (Kumari et al., 2014). Moreover, the variable cytotoxicity of CeO_2-NPs is also due to other parameters such as aggregation, aspect ratio, surface charge, entry rate, cell

culture environment, type, and storage conditions. In particular, CeO_2-NPs exhibit toxic effects in a concentration and time-dependent manner, and if used at a concentration above the upper limit of the therapeutic range, they will be harmful to cells, tissues, and organs (Hosseini et al., 2020). According to the study of Rajeshkumar et al. (2018), the 20 nm nanoceria demonstrated cytotoxicity exclusively against lung cancer cells, and cell viability decreased in relation to the nanoparticle dosage and exposure duration. Several studies also have reported different types of toxicity including cytotoxicity, genotoxicity, and neurotoxicity that were more common in vivo models compared to in vitro models (De Marzi et al., 2013; Rajeshkumar et al., 2018). In terms of tissue toxicity, a major accumulation of nanoceria was found in organs including the liver, spleen, lungs, kidney and brain (Battaglini et al., 2018). Generally, there is evidence that nanoceria *can display both anti- and* pro-oxidant activities, thereby to avoid that particles need to be well characterized in order to reduce their toxicity before being delivered into the body (Yang et al., 2022).

Conclusion and future perspectives

In summary, we presented a brief overview of the synthesis method, biological activities, and cytotoxicity effects of nanoceria. Due to the self-regeneration of their surface, they mimic multi-

enzymatic catalytic activities and exhibit ubiquitous antioxidant properties in natural environment. Hence, oxidative stress can potentially trigger inflammation, so the antioxidant activities of nanoceria contribute to their anti-inflammatory activities. These activities make nanoceria a novel therapeutic agent for a wide range of applications and hold promise for advancements in *medicine*. It is important to note that various factors, including particle shape, size, structure, and solvents, can significantly impact the properties of cerium oxide nanoparticles. However, many studies lack sufficient details regarding these factors. In general, the toxicity of nanoceria is the primary concern of their use, which can be modulated by their charge and size-switchable properties. While considerable research has been conducted on the antioxidant activity of nanoceria, there are still some knowledge gaps, specifically in finding a useful scaffold for tissue regeneration, antidiabetic agents, and gene delivery agents. Further exploration is needed to better understand and develop these potential applications.

Conflict of interest

The authors declared no conflict of interest.

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مروری کوتاه بر روش های سنتز، فعالیت های بیولوژیک و سمیت سلولی نانوذرات اکسید سریم

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چکیده:

نانوذرات اکسید سریم (**CeO_2-NPs** یا نانوسریا) یکی از منحصربه‌فردترین و امیدوارکننده‌ترین نانومواد لانتانیدی هستند که دارای ویژگی‌های منحصربه‌فردی نظیر فعالیت ردوکس، ظرفیت ذخیره‌سازی اکسیژن و توانایی مهار رادیکال‌های آزاد هستند. توانایی آنها در بازسازی سطح خود، آنها را به یک کاندید بالقوه در زمینه های مختلف، به ویژه بخش زیست پزشکی تبدیل می کند. مکانیسم‌های محافظتی نانوسریا در برابر استرس اکسیداتیو شامل حذف مستقیم رادیکال‌ها با تقلید از فعالیت کاتالیستی آنزیم‌های ردوکس است. نانوسریا می‌تواند به عنوان یک شمشیر دو

لبه عمل کند و بسته به ویژگی‌های سطح و محیط، هم خواص اکسیدانی و هم آنتی‌اکسیدانی را نشان دهد. با وجود پتانسیل نانوذرات اکسید سریم برای کاربردهای بالینی، مطالعات متناقضی نیز وجود دارد که سمیت بالقوه آنها را گزارش می کند. این مقاله مروری به شرح: (1) روش های سنتز نانوذرات اکسید سریم (2) کاربردهای نانوسریا در فعالیت های آنتی اکسیدانی، ضد التهابی و ضد سرطانی، شامل جدیدترین مطالعات انجام شده در شرایط درون تنی و برون تنی و (3) فعالیت سیتوتوکسیک آنها می پردازد.

کلیدواژها: نانوذرات اکسید سریم، نانوسریا، آنتی اکسیدان، سنتز، فعالیت سیتوتوکسیک