Review Article

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

Behnaz Karimi[1*], Saman Yousefi[2]

1. Department of Basic Science, Faculty of Veterinary Medicine, Shahrekord University, Shahrekord, Iran
2. Department of Pathobiology, Faculty of Veterinary Medicine, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran.

Use your device to scan and read the article online

How to Cite This Article Karimi, B., & Yousefi, S. (2025). A Brief Review of Synthesis Methods, Biological Activities, and Cytotoxicity of Cerium Oxide Nanoparticles. *Iranian Journal of Veterinary Medicine, 19*(3), 405-414. http://dx.doi.org/10.32598/ijvm.19.3.1005519

http://dx.doi.org/10.32598/ijvm.19.3.1005519

ABSTRACT

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

Keywords: Cerium oxide nanoparticles (CeO_2-NPs), Nanoceria, Antioxidant, Synthesis, Cytotoxic activity

Article info:
Received: 15 Feb 2024
Accepted: 23 Apr 2024
Available Online: 01 Jul 2025

*** Corresponding Author:**
Behnaz Karimi, Assistant Professor.
Address: *Department of Basic Science, Faculty of Veterinary Medicine, Shahrekord University, Shahrekord, Iran.*
Phone: *+98 (913) 1856234*
E-mail: *Behnazkarimi667@gmail.com, Karimibabaahmadi@sku.ac.ir*

Introduction

Nanotechnology is manipulating matter at atomic and molecular scales, ranging from 1 to 100 nm. It is 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 in medicine (Hossieni et al., 2024; Rajeshkumar & Naik, 2018; Dhall & Self, 2018). Due to their small size, nanoparticles have distinctive characteristics that distinguish them 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 that exists in both +3 and +4 oxidation states and can be found in cerium oxide (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 believed to have oxidizing properties. Therefore, cerium reacts with oxygen molecules to form CeO_2, which is also known as nanoceria (Abuid et al., 2020). CeO_2 is utilized as a UV absorber, polishing agent, gas sensor, fertilizer, 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 its ability to act as an antioxidant. 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 CeO_2's antioxidant activity. Cerium oxide nanoparticles (CeO_2-NPs) have received considerable attention in nanotechnology due to their valuable applications as reducing agents rather than catalysts (Saifi et al., 2021). Soluble Ce^{3+} salts (nitrate, acetate, and chloride) have been described to possess antiemetic, bacteriostatic, bactericidal, immunomodulatory, and antitumor activities, thus justifying their use in traditional medicinal therapies (Nelson et al., 2016). CeO_2-NPs have been shown to display an antioxidant activity expressed as oxidase, catalase (CAT) and superoxide dismutase (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 properties 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). Due to the high surface-to-volume ratio of nanoceria, which results in enhanced surface oxygen vacancies, their reactivity is particularly efficient at the nanoscale level. 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 exists only in the pure form of CeO_2 (Ce^{4+}) or Ce_2O_3 (Ce^{3+}) (Nelson et al., 2016).

This review begins with an overview of the methods commonly used to prepare CeO_2-NPs. Then, we present 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. Finally, we discuss the cytotoxicity of nanoceria.

Synthesis of Nanoceria

Researchers have described various methods, including hydrothermal, solvothermal, precipitation, spray pyrolysis, and microwaves, for synthesizing nanoceria (Table 1) (Rajeshkumar & Naik, 2018). The parameters of the technique, such as the amount of aging, temperature, and pH, were used to alter the properties of the resulting nanoparticles. By adjusting these factors, nanoparticles with a specific size, shape, stability, and oxidative ratio $3^+/4^+$ can be produced (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 the oxidizer (Abuid et al., 2020). The precipitation method is the most convenient technique that can be performed at both room and higher temperatures. The precipitation method was derived using two approaches. Hydrothermal synthesis refers to synthesis through chemical reactions, in which water plays a significant role as a solvent (Thakur et al., 2019). The hydrothermal crystallization method was first used by Masui et al. (2002) Based on their experiments, cerium chloride hexahydrate, citric acid, and ammonia water were used as precursors. The obtained nanoceria particles were spherical with an average diameter of 5 nm. This study used citric acid due to its inhibitory impact on particle growth. Solvothermal synthesis uses organic solvents in a high-pressure and

Table 1. Commonly used methods for synthesis of CeO_2-NPs

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)

high-temperature chamber to produce nanomaterials of various sizes (Nyoka et al., 2020). As expected, many traditional synthesis methods exhibit low biocompatibility. Green synthesis is an eco-friendly, sustainable, and cost-effective process. In the case of nanoceria, the green synthesis method involves a variety of approaches, such as plant-mediated synthesis, microorganism-mediated synthesis, and green chemistry-based techniques. It is also indicated that all green techniques have the same spherical nanoparticle form, but the size of the particles varies depending on the technique, ranging from 2 to 36 nm (Anvar et al., 2023; Sharmila et al., 2019). Additionally, nanoparticles are coated by several major active proteins in egg whites, such as ovalbumin and lysozyme. These proteins can simultaneously bind to both cationic and anionic metal complexes. The electrostatic interactions between cerium ions (Ce^{3+}) and proteins with opposite charges promote the developing and forming small, stable, and isotropic nanoparticles (Rajeshkumar & Naik, 2018).

Biological Properties of Nanoceria

CeO_2-NPs are well known for their powerful, broad-spectrum antioxidant, anti-inflammatory, and anticancer activities (Figure 1). Below is a list of some of the functions of nanoceria in biology and medicine.

Antioxidant and pro-oxidant activities

ROS are generated as byproducts during aerobic metabolism, and their elevated levels are frequently associated with oxidative stress (Miakhil et al., 2024). In this context, antioxidants are exogenous or endogenous substances that neutralize ROS or prevent their formation (Karimi et al., 2019; Schieber & Chandel, 2014). It has been demonstrated that metal and metal-based nanoparticles could be applied as pro-oxidants or as an antioxidant strategy (Hussain AlDulaimi, 2024; Dhall & Self, 2018). Ce ions can transform between trivalent and tetravalent oxidation states on the surface of CeO_2-NPs in an aqueous solution, which is considered to be the main reason for their unique regenerative antioxidant activity. The synthesis procedures, physicochemical properties, and chemical environment are all relevant factors that impact the catalytic antioxidant activities of CeO_2-NPs (Nelson et al., 2016). Nanoceria has been shown to act as a mimetic of SOD and CAT, effectively degrading superoxide (O_2^-) and hydrogen peroxide (H_2O_2) (Figure 1).

The application of CeONPs in cancer treatment can be explained by the loss of antioxidant ability of CeONPs under 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 CeO_2-NPs are linked to the shift of macrophage polarization from M1, which can exacerbate inflammatory responses, to M2, which possesses anti-inflammatory properties. The excellent ROS scavenging efficiency of CeO_2-NPs is attributed to their SOD- and CAT-mimetic activities.

Furthermore, CeO_2-NPs have oxidizing properties that depend on the pH of the solution. CNP has more SOD-mimetic than CAT-mimetic characteristics at acidic pH levels and possesses oxidase-like activity (peaking at

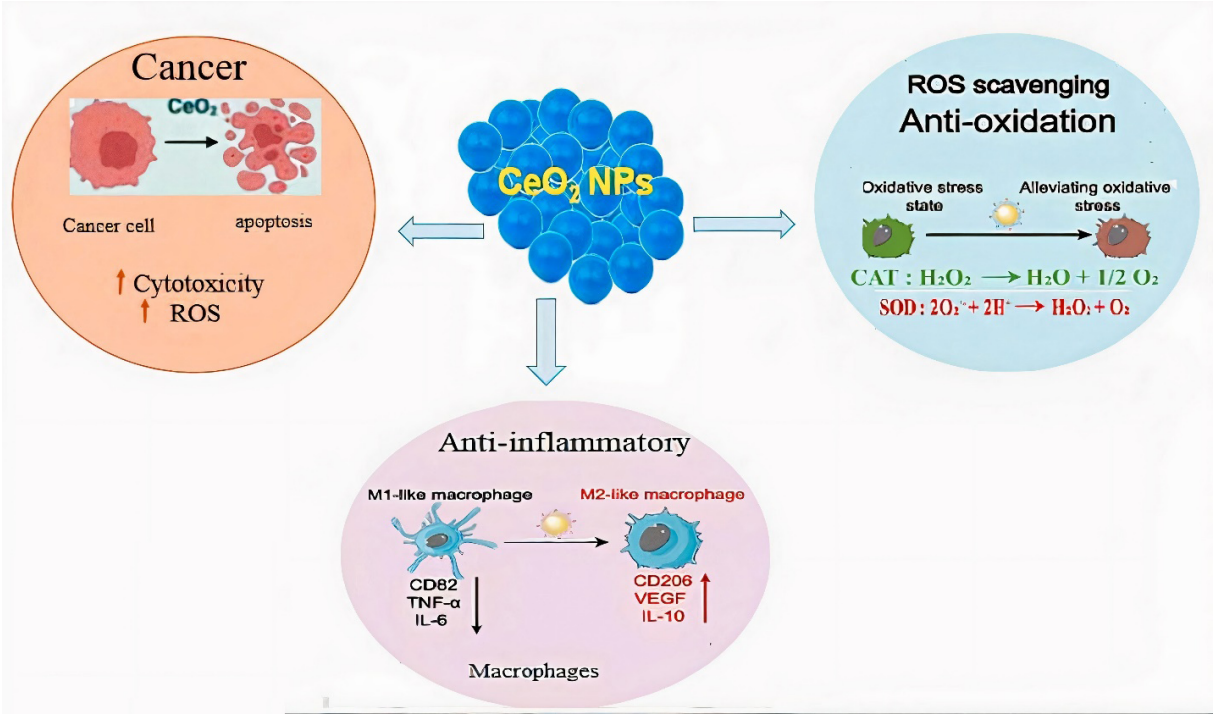

Figure 1. Graphical depiction of some of the functions and benefits of CeO_2-NPs in the field of biomedicine

pH 4). Depending on the particle's features and destination, CNP can be designed to behave as an oxidant or scavenger of various radicals (Abuid et al., 2020). Lowering the CNP environment's pH can convert CNP's antioxidant properties 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 of the solutions. The authors demonstrated that under neutral and alkaline conditions, dextran-coated CeO_2-NPs exhibited CAT-mimetic activity and were inactivated in acidic solutions (pH 4). Thakur et al. documented that the pro-oxidant effect of CeO_2-NPs within cancer cells can be explained by their acidic intracellular pH, which increases the SOD mimetic activity of CeO_2-NPs and inhibits their CAT mimetic activity, resulting in the accumulation of a large amount of H_2O_2 and apoptosis of cancer cells. However, in normal cells with a physiological pH, CeO_2-NPs exert a protective effect by scavenging both $O2^{\bullet-}$ and H_2O_2 due to SOD and CAT mimetic activities (Thakur et al., 2019). Figure 2 shows the mechanism of the pro-oxidant and antioxidant effects of nanoceria.

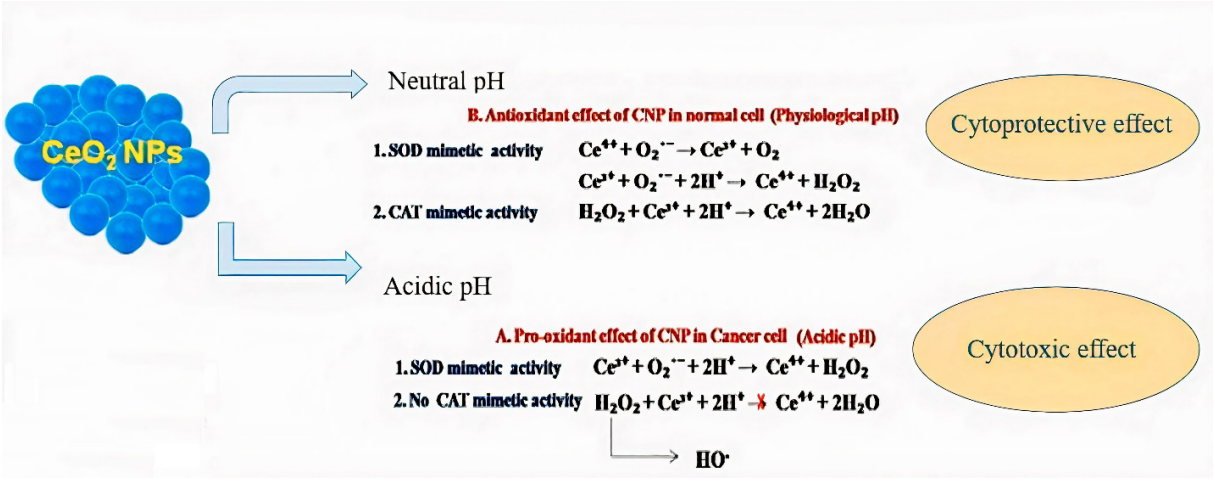

Figure 2. Mechanism of pro-oxidant and antioxidant effect of CeO_2-NPs

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

In the literature, CeO_2-NPs are recognized as crucial typical nanozymes (NZs) with different mimicking activities, such as catalase, SOD, oxidase, peroxidase, and phosphatase (Saifi et al., 2021). It has been reported that some NZs are preferred over natural enzymes due to their higher stability, longer lifetimes, and simple preparation (Mukherjee et al., 2023). Due to the NZ properties of CeO_2-NPs, they can convert several 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 could scavenge the hypochlorite anion, which is involved in the inflammatory process due to different oxidation states on the surface of the nanoparticles. The CAT-mimetic activity of CeO_2-NPs increased as the concentration of Ce^{4+} increased. In contrast, an elevated concentration of cerium in the 3^+ state is primarily responsible for superoxide scavenging (Nelson et al., 2016). Nevertheless, Dowding et al. (2012). indicated that the nitric oxide radical scavenging property was present in CeO_2-NPs with a lower level of cerium in the 3^+ state.

CNPs exhibit pro-oxidant effects in 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 hydrogen peroxide (H_2O_2) accumulates within cancer cells, triggering cancer cell death. Nanoceria mimics ROS-related enzymes that protect normal cells from oxidative stress at physiological pH.

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 the gastrointestinal epithelium, human breast line, neuronal cells, endothelium, and stem cells, CeO_2-NPs scavenge ROS and provide protection (Nelson et al., 2016). Heparin-functionalized nanoceria were more effective than 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 µg/mL, 25 µg/mL, and 50 µg/mL) 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 or cause cell structural damage while protecting cardiac progenitor cells (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 nuclear factor erythroid 2-related factor 2 (Nrf2) pathway.

The ROS-scavenging capacity of nanoceria was observed for the first time in a study by Rzigalinski et al. (2003) In this study, CeO_2 nanoparticles acted as nanoenzymes or antioxidants modulating ROS and can 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 have demonstrated promising efficacy as a therapeutic option for various 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 nanoceria were found in the normal brain, and to deliver the therapeutic effect of nanoceria in neurons, lipid-based delivery systems were designed 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 the particles that result in rapid clearance by macrophages before transporting them to target cells (Yang et al., 2022). Surface modification is 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 and ethylenediamine tetraacetic acid effectively reduced the formation of ROS and severity of neuronal loss in three different models of cerebral ischemia.

There is evidence that a single-dose intake of nanoceria has long-lasting antioxidant effects in biological sys-

tems. The therapeutic efficiency of nanoceria (5.8 µM) was similar to that of N-acetylcysteine (10 mM) in 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 H2S-releasing donor, while nanoceria is a hydrogen donor. Traditional organic antioxidants can also promote ROS generation due to the single electron exchange with reactive oxygen and nitrogen species, 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 in haloperidol-induced parkinsonism in rats. Tisi et al. (2022) indicated that CeO_2-NPs could 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 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 embryos. 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) protected the brain against the adverse effects of PQ by ameliorating lipid peroxidation, DNA damage, caspase-3 activity levels, and increasing the total antioxidant capacity, total thiol content, and messenger ribonucleic acid (mRNA) levels of neurogenesis markers, such as Nestin and Neurod1.

Anti-inflammatory effects

According to several studies, CeO_2-NPs exhibit strong anti-inflammatory effects. Hirst et al. (2009) showed the treatment of J774A.1 murine macrophage cells with CNPs inhibited inflammation by reducing inducible nitric oxide synthase (iNOS) protein and messenger ribonucleic acid (mRNA) levels. Furthermore, the authors reported that it is nontoxic in the tissues of C57BLK6 mice following intravenous injections. This is the first study to suggest CeO_2-NPs are a promising anti-inflammatory therapeutic agent. Subsequently, numerous in vitro and in vivo reports have suggested that CeO_2-NPs can potentially improve many inflammatory disorders, including neurodegenerative and 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 protected 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 alleviated brain inflammation by reducing mitochondrial ROS levels and ameliorating neuronal loss in these mice. A new formulation of europium-doped CeO_2NPs ($EuCeO_2$NPs) 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 microglial cells (Machhi et al., 2022). Recently, Zavvari et al. (2020) observed that intrahippocampal injection of CeO_2 NPs exerted a neuroprotective effect in a stress-induced model of depression by improving hippocampal cell viability, cell proliferation, and neurite formation. This therapeutic effect was accompanied by suppression of hippocampal IL-6 and malondialdehyde production. Kalashnikova et al. (2020) administered albumin-CNPs in a collagen-induced arthritis 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 initiate and sustain inflammatory responses, whereas M2 macrophages have modulatory activities and exert anti-inflammatory effects. They also reported that nanoparticles' therapeutic effect was comparable to methotrexate, the gold standard treatment for rheumatoid arthritis (RA). CeO_2 nanoparticles were demonstrated to have an in vitro cartilageprotecting effect by preventing H_2O_2-induced chondrocyte injury and promoting gene expression 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 the activation of the Nrf2/HO1 pathway, as indicated by Xiong et al. (2023) Combined treatment with nanoceria and lenalidomide in multiple sclerosis reduced mice's demyelination and central nervous system (CNS) inflammation (Eitan et al., 2015). The therapeutic potential of CeO_2-NPs in experimental chronic liver disease was demonstrated by Oró et al. (2016) According to their results, CNPs 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 investigated. A possible mechanism by which CeO_2-NPs combat inflammation is associated with inhibiting the Nrf2/NF-κB signaling pathway. Furthermore, the phosphorylation of ERK1/2,

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

Anticancer effects

Considering the biological importance of nanoceria, it has offered a promising new approach to cancer detection and treatment. However, like any other antioxidant, it can exhibit anti- and pro-oxidative effects depending on environmental conditions, such as the 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). Due to the oxidase-like activity of nanoceria at acidic pH and lower pH values in cancer cells as opposed to normal cells, nanoceria can exert direct effects on cancer cells and increase intracellular ROS levels, leading to insufficient mitochondrial quality control, DNA damage, and membrane damage (Saifi et al., 2021). Recently, CNPs have been shown to cause changes in mitochondrial bioenergetics, dynamics, and the number of cristae, leading to lower viability of tumor cells. Increased superoxide production was reported in the mitochondria of A375 cells as a substrate for nanoceria SOD-mimetic activity, producing H_2O_2 (Aplak et al., 2020). This indicates that nanoceria induce apoptotic cell death in various cancer cells by activating the p53-dependent mitochondrial signaling pathway (Tang et al., 2023). Nanoceria are thought to activate intrinsic apoptotic pathways by releasing Cyt c levels, and activating caspases-3 and -9, and reducing cell survival by preventing cells from entering mitosis (Tang et al., 2023). Furthermore, administration of CeO_2-NPs decreased the Ras/Raf/MAPK kinase/ERK signaling pathways involved in cell survival and accelerated apoptosis in experimental hepatocellular carcinoma in rats. However, in addition to the direct mechanism, some indirect effects of nanoceria have also been reported to affect tumor cell invasiveness, angiogenesis, and metastasis (Saifi et al., 2021). Giri et al. (2013) reported that in vivo treatment with nanoceria inhibited ovarian tumor growth and metastasis even at low doses (0.1 mg/kg). Thus, using nanoceria as vehicles 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 and CeO_2-NPs, could provide a potential platform and promising strategy for cancer therapy. The higher efficacy of CeO_2-NPs combined with doxorubicin might be due to cytotoxicity, oxidative damage, and induction of 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 CeO_2-NPs

Despite the therapeutic effects of nanoceria, their biological safety, such as potential toxicity, needs to be evaluated. The main challenges associated with using CeO_2 nanoparticles are potential toxicological effects on living organisms. Additionally, CNPs can form aggregates, as reported by Naiel et al. (2022) This allows particles to settle in aquatic environments, posing significant risks to exposed aquatic organisms and, ultimately, to human health. Most in vitro studies have demonstrated that the toxicity of CeO_2-NPs increases with nanoparticle size, and ROS production is dose- and size-dependent. Generally, owing to their small size, CeO_2-NPs are 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. If used at concentrations above the upper limit of the therapeutic range, they will be harmful to cells, tissues, and organs (Hosseini et al., 2020). According to a study by Rajeshkumar and Naike (2018), 20 nm nanoceria demonstrated cytotoxicity exclusively against lung cancer cells, and cell viability decreased about the nanoparticle dosage and exposure duration. Several studies have also reported different types of toxicity, including cytotoxicity, genotoxicity, and neurotoxicity, which are more common in in vivo models than in vitro models (De Marzi et al., 2013; Rajeshkumar et al., 2018). Regarding tissue toxicity, a major accumulation of nanoceria was found in organs, including the liver, spleen, lungs, kidney,s and brain (Battaglini et al., 2018). Evidence shows that nanoceria can display anti- and pro-oxidant activities, thereby avoiding the need for well-characterized particles to reduce their toxicity before delivery into the body (Yang et al., 2022).

Conclusion

In summary, we present a brief overview of the synthesis method, biological activities, and cytotoxic effects of nanoceria. Due to the self-regeneration of their surface, they mimic multi-enzymatic catalytic activities and exhibit ubiquitous antioxidant properties in natural envi-

ronments. Hence, oxidative stress can potentially trigger inflammation, and 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 medical advancements. It is crucial to note that various factors, including particle shape, size, structure, and solvents, can significantly impact the properties of CNPs. However, many studies lack sufficient details regarding these factors. The toxicity of nanoceria, which their charge and size-switchable properties can modulate, is the primary concern of their use. Although 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 studies are required to understand these potential applications better and develop them.

Ethical Considerations

Compliance with ethical guidelines

There were no ethical considerations to be considered in this research.

Funding

This research did not receive any grant from funding agencies in the public, commercial, or non-profit sectors.

Authors' contributions

All authors equally contributed to preparing this article.

Conflict of interest

The authors declared no of interest.

Acknowledgments

The authors thank Shahrekord University, Shahrekord, Iran, for its valuable support.

References

Abuid, N. J., Gattás-Asfura, K. M., LaShoto, D. J., Poulos, A. M., & Stabler, C. L. (2020). Biomedical applications of cerium oxide nanoparticles: A potent redox modulator and drug delivery agent. *Nanoparticles for Biomedical Applications, 220,* 283-301. [DOI:10.1016/B978-0-12-816662-8.00017-5]

Alpaslan, E., Geilich, B. M., Yazici, H., & Webster, T. J. (2017). PH-Controlled Cerium Oxide Nanoparticle Inhibition of Both Gram-Positive and Gram-Negative Bacteria Growth. *Scientific Reports, 7,* 45859. [DOI:10.1038/srep45859] [PMID]

Anvar, S. A. A., Nowruzi, B. and Afshari, G. (2023). A review of the application of nanoparticles biosynthesized by microalgae and cyanobacteria in medical and veterinary sciences. *Iranian Journal of Veterinary Medicine, 17*(1), 1-18. [DOI:10.32598/IJVM.17.1.1005309]

Aplak, E., von Montfort, C., Haasler, L., Stucki, D., Steckel, B., & Reichert, A. S., et al. (2020). CNP mediated selective toxicity on melanoma cells is accompanied by mitochondrial dysfunction. *Plos One, 15*(1), e0227926. [DOI:10.1371/journal.pone.0227926] [PMID]

Battaglini, M., Tapeinos, C., Cavaliere, I., Marino, A., Ancona, A., & Garino, N., et al. (2019). Design, fabrication, and in vitro evaluation of nanoceria-loaded nanostructured lipid carriers for the treatment of neurological diseases. *ACS biomaterials science & engineering, 5*(2), 670–682.[DOI:10.1021/acsbiomaterials.8b01033] [PMID]

Corsi, F., Deidda Tarquini, G., Urbani, M., Bejarano, I., Traversa, E., & Ghibelli, L. (2023). The impressive anti-inflammatory activity of cerium oxide nanoparticles: More than Redox?. *Nanomaterials (Basel, Switzerland), 13*(20), 2803. [DOI:10.3390/nano13202803] [PMID]

De Marzi, L., Monaco, A., De Lapuente, J., Ramos, D., Borras, M., & Di Gioacchino, M., et al. (2013). Cytotoxicity and genotoxicity of ceria nanoparticles on different cell lines in vitro. *International Journal of Molecular Sciences, 14*(2), 3065-3077. [DOI:10.3390/ijms14023065] [PMID]

Dhall, A., Self, W. (2018). Cerium oxide nanoparticles: A brief review of their synthesis methods and biomedical applications. *Antioxidants (Basel, Switzerland), 7*(8), 97. [DOI:10.3390/antiox7080097] [PMID]

Dowding, J. M., Dosani, T., Kumar, A., Seal, S., & Self, W. T. (2012). Cerium oxide nanoparticles scavenge nitric oxide radical (˙ NO). *Chemical Communications, 48*(40), 4896-4898. [DOI:10.1039/c2cc30485f] [PMID]

Eitan, E., Hutchison, E. R., Greig, N. H., Tweedie, D., Celik, H., & Ghosh, S., et al. (2015). Combination therapy with lenalidomide and nanoceria ameliorates CNS autoimmunity. *Experimental Neurology, 273,* 151-160. [DOI:10.1016/j.expneurol.2015.08.008] [PMID]

Estevez, A. Y., Ganesana, M., Trentini, J. F., Olson, J. E., Li, G., & Boateng, Y. O., et al. (2019). Antioxidant enzyme-mimetic activity and neuroprotective effects of cerium oxide nanoparticles stabilized with various ratios of citric acid and EDTA. *Biomolecules, 9*(10), 562. [DOI:10.3390/biom9100562] [PMID]

Estevez, A. Y., Pritchard, S., Harper, K., Aston, J. W., Lynch, A., & Lucky, J. J., et al. (2011). Neuroprotective mechanisms of cerium oxide nanoparticles in a mouse hippocampal brain slice model of ischemia. *Free Radical Biology and Medicine, 51*(6), 1155-1163. [DOI:10.1016/j.freeradbiomed.2011.06.006] [PMID]

Giri, S., Karakoti, A., Graham, R. P., Maguire, J. L., Reilly, C. M., & Seal, S., et al. (2013). Nanoceria: A rare-earth nanoparticle as a novel anti-angiogenic therapeutic agent in ovarian cancer. *Plos One, 8*(1), e54578. [DOI:10.1371/journal.pone.0054578] [PMID]

Hirst, S. M., Karakoti, A. S., Tyler, R. D., Sriranganathan, N., Seal, S., & Reilly, C. M. (2009). Anti-inflammatory properties of cerium oxide nanoparticles. *Small (Weinheim an der Bergstrasse, Germany), 5*(24), 2848–2856. [DOI:10.1002/smll.200901048] [PMID]

Hosseini, M., & Mozafari, M. (2020). Cerium oxide nanoparticles: Recent advances in tissue engineering. *Materials (Basel, Switzerland), 13*(14), 3072. [DOI:10.3390/ma13143072] [PMID]

Hossieni, M., Kiani, S. J., Tavakoli, A., Kachooei, A., Habib, Z., & Monavari, S. H. (2024). In vitro inhibition of rotavirus multiplication by copper oxide nanoparticles. *Archives of Razi Institute, 79*(1), 83-91 [DOI:10.32592/ARI.2024.79.1.83] [PMID]

Hussain AlDulaimi, L. (2024). Effect of Oxidative Stress on Histological and Immunohistochemical Changes in Testes of Albino Mice. *Iranian Journal of Veterinary Medicine, 18*(2), 187-194. [DOI:10.32598/IJVM.18.2.1005459]

Karimi, B., Ashrafi, M., Shomali, T., & Yektaseresht, A. (2019). Therapeutic effect of simvastatin on DMBA-induced breast cancer in mice. *Fundamental & Clinical Pharmacology, 33*(1), 84-93. [DOI:10.1111/fcp.12397] [PMID]

Kalashnikova, I., Chung, S. J., Nafiujjaman, M., Hill, M. L., Siziba, M. E., & Contag, C. H., et al. (2020). Ceria-based nanotheranostic agent for rheumatoid arthritis. *Theranostics, 10*(26), 11863–11880. [DOI:10.7150/thno.49069] [PMID]

Kannan, S. K., & Sundrarajan, A. M. (2014). A Green Approach for the Synthesis of a Cerium Oxide Nanoparticle: Characterization and Antibacterial Activity. *International Journal of Nanoscience, 13*(3), 1450018 [DOI:10.1142/S0219581X14500185]

Karakoti, A. S., Munusamy, P., Hostetler, K., Kodali, V., Kuchibhatla, S., & Orr, G., et al. (2012). Preparation and characterization challenges to understanding environmental and biological impacts of ceria nanoparticles. *Surface and Interface Analysis, 44*(5),882–889. [DOI:10.1002/sia.5006] [PMID]

Karimi, B., Mardani, M., Kaboutari, J., Javdani, M., Albadi, J., & Shirian, S. (2024). Green synthesis of copper oxide nanoparticles using Artemisia annua aqueous extract and its characterization, antioxidant, and burn wound healing activities. *Chemical Papers, 78*(1), 231-243. [DOI:10.1007/s11696-023-03069-8]

Kumari, M., Singh, S. P., Chinde, S., Rahman, M. F., Mahboob, M., & Grover, P. (2014). Toxicity study of cerium oxide nanoparticles in human neuroblastoma cells. *International Journal of Toxicology, 33*(2), 86-97. [DOI:10.1177/1091581814522305] [PMID]

Kwon, H. J., Cha, M. Y., Kim, D., Kim, D. K., Soh, M., & Shin, K., et al. (2016). Mitochondria-targeting ceria nanoparticles as antioxidants for Alzheimer›s disease. *ACS Nano, 10*(2), 2860–2870. [DOI:10.1021/acsnano.5b08045] [PMID]

Lin, Y. W., Fang, C. H., Meng, F. Q., Ke, C. J., & Lin, F. H. (2020). Hyaluronic acid loaded with cerium oxide nanoparticles as antioxidant in hydrogen peroxide induced chondrocytes injury: An in vitro osteoarthritis model. *Molecules, 25*(19), 4407. [DOI:10.3390/molecules25194407] [PMID]

López, J. M., Gilbank A. L., García T., Solsona B., Agouram S., & Torrente-Murciano L. (2015). The prevalence of surface oxygen vacancies over the mobility of bulk oxygen in nanostructured ceria for the total toluene oxidation. *Applied Catalysis B: Environmental, 174-175,* 403-412. [DOI:10.1016/j.apcatb.2015.03.017]

Lu, M., Zhang, Y., Wang, Y., Jiang, M., & Yao, X. (2016). Insight into several factors that affect the conversion between antioxidant and oxidant activities of nanoceria. *ACS Applied Materials & Interfaces, 8*(36), 23580–23590. [DOI:10.1021/acsami.6b08219] [PMID]

Ma, Y., Gao, W., Zhang, Z., Zhang, S., Tian, Z., & Liu, Y., et al. (2018). Regulating the surface of nanoceria and its applications in heterogeneous catalysis. *Surface Science Reports, 73*(1), 1e36. [DOI:10.1016/j.surfrep.2018.02.001]

Machhi, J., Yeapuri, P., Markovic, M., Patel, M., Yan, W., & Lu, Y., et al. (2022). Europium-doped cerium oxide nanoparticles for microglial amyloid beta clearance and homeostasis. *ACS Chemical Neuroscience, 13*(8), 1232-1244. [DOI:10.1021/acschemneuro.1c00847] [PMID]

Masui, T., Hirai, H., Imanaka, N., Adachi, G., Sakata, T., & Mori, H. (2002). Synthesis of cerium oxide nanoparticles by hydrothermal crystallization with citric acid. *Journal of Materials Science Letters, 21,* 489-491. [DOI:10.1023/A:1015342925372]

Miakhil, A., Kamkar, A., & Banuree, S. A. H. (2024). Physicochemical Properties and Antioxidant Activity of Honey Brands Distributed in Tehran City, Iran. *Iranian Journal of Veterinary Medicine, 18*(2), 243-252. [DOI:10.32598/IJVM.18.2.1005348]

Mohammad, Khan, U. A., Warsi, M. H., Alkreathy, H. M., Karim, S., & Jain, G. K., et al. (2023). Intranasal cerium oxide nanoparticles improve locomotor activity and reduces oxidative stress and neuroinflammation in haloperidol-induced parkinsonism in rats. *Frontiers in Pharmacology, 14,* 1188470. [DOI:10.3389/fphar.2023.1188470] [PMID]

Mukherjee, A., Ashrafi, A. M., Bytesnikova, Z., Svec, P., Richtera, L., & Adam, V. (2023). An investigation on the multiple Roles of Ceo_2 nanoparticle in electrochemical sensing: Biomimetic activity and electron acceptor. *Journal of Electroanalytical Chemistry, 935,* 117301. [DOI:10.1016/j.jelechem.2023.117301]

Naiel, M. A., Abdel-Latif, H. M., Abd El-Hack, M. E., Khafaga, A. F., Elnesr, S. S., & Dawood, M. A., et al. (2022). The applications of cerium oxide nanoform and its ecotoxicity in the aquatic environment: an updated insight. *Aquatic Living Resources, 35,* 9. [DOI:10.1051/alr/2022008]

Nelson, B. C., Johnson, M. E., Walker, M. L., Riley, K. R., & Sims, C. M. (2016). Antioxidant cerium oxide nanoparticles in biology and medicine. *Antioxidants, 5*(2), 15. [DOI:10.3390/antiox5020015] [PMID]

Nyoka, M., Choonara, Y. E., Kumar, P., Kondiah, P. P., & Pillay, V. (2020). Synthesis of cerium oxide nanoparticles using various methods: Implications for biomedical applications. *Nanomaterials (Basel, Switzerland), 10*(2), 242. [DOI:10.3390/nano10020242] [PMID]

Oró, D., Yudina, T., Fernández-Varo, G., Casals, E., Reichenbach, V., & Casals, G., et al. (2016). Cerium oxide nanoparticles reduce steatosis, portal hypertension and display anti-inflammatory properties in rats with liver fibrosis. *Journal of Hepatology, 64*(3), 691-698. [DOI:10.1016/j.jhep.2015.10.020] [PMID]

Pagliari, F., Mandoli, C., Forte, G., Magnani, E., Pagliari, S., & Nardone, G., et al. (2012). Cerium oxide nanoparticles protect cardiac progenitor cells from oxidative stress. *ACS nano, 6*(5), 3767–3775.[DOI:10.1021/nn2048069] [PMID]

Peidaei, F., Ahari, H., Anvar, A., & Ataei, M. (2021). Nanotechnology in Food Packaging and Storage: A Review. *Iranian Journal of Veterinary Medicine, 15*(2), 122-154. [Link]

Perez, J. M., Asati, A., Nath, S., & Kaittanis, C. (2008). Synthesis of biocompatible dextran-coated nanoceria with pH-dependent antioxidant properties. *Small (Weinheim an der Bergstrasse, Germany), 4*(5), 552–556. [DOI:10.1002/smll.200700824] [PMID]

Pulido-Reyes, G., Das, S., Leganés, F., Silva, S. O., Wu, S., & Self, W., et al. (2016). Hypochlorite scavenging activity of cerium oxide nanoparticles. *RSC Advances, 6*(67), 62911-62915. [Link]

Rajeshkumar, S., & Naik, P. (2017). Synthesis and biomedical applications of cerium oxide nanoparticles-a review. *Biotechnology Reports, 17,* 1-5. [DOI:10.1016/j.btre.2017.11.008] [PMID]

Ramachandran, M., Subadevi, R., & Sivakumar, M. (2018). Role of pH on synthesis and characterization of cerium oxide (CeO2) nano particles by modified co-precipitation method. *Vacuum, 161,* 220-224. [DOI:10.1016/j.vacuum.2018.12.002]

Ranjbar, A., Soleimani Asl, S., Firozian, F., Heidary Dartoti, H., Seyedabadi, S., & Taheri Azandariani, M., et al. (2018). Role of cerium oxide nanoparticles in a paraquat-induced model of oxidative stress: emergence of neuroprotective results in the brain. *Journal of Molecular Neuroscience, 66*(3), 420–427. [DOI:10.1007/s12031-018-1191-2] [PMID]

Rubio, L., Annangi, B., Vila, L., Hernández, A., & Marcos, R. (2016). Antioxidant and anti-genotoxic properties of cerium oxide nanoparticles in a pulmonary-like cell system. *Archives of Toxicology, 90,* 269-278. [DOI:10.1007/s00204-015-1468-y] [PMID]

Rzigalinski, BA., Bailey, D., Chow, L., Kuiry, S., Patil, S., Merchant, S., et al. (2003). Cerium oxide nanoparticles increase the lifespan of cultured brain cells and protect against free radical and mechanical trauma. *Faseb Journal, 17*(4), A606. [Link]

Sack, M., Alili, L., Karaman, E., Das, S., Gupta, A., & Seal, S., et al. (2014). Combination of conventional chemotherapeutics with redox-active cerium oxide nanoparticles-a novel aspect in cancer therapy. *Molecular Cancer Therapeutics, 13*(7), 1740–1749. [DOI:10.1158/1535-7163.MCT-13-0950] [PMID]

Saifi, M. A., Seal, S., & Godugu, C. (2021). Nanoceria, the versatile nanoparticles: Promising biomedical applications. *Journal of Controlled Release, 338,* 164-189. [DOI:10.1016/j.jconrel.2021.08.033] [PMID]

Schieber, M., & Chandel, N.S. (2014). ROS function in redox signaling and oxidative stress. *Current Biology, 24*(10), R453-R462. [DOI:10.1016/j.cub.2014.03.034] [PMID]

Sharmila, G., Muthukumaran, C., Saraswathi, H., Sangeetha, E., Soundarya, S., & Kumar, N. M. (2019). Green synthesis, characterization and biological activities of nanoceria. *Ceramics International, 45*(9), 12382-12386. [DOI:10.1016/j.ceramint.2019.03.164]

Singh, K. R., Nayak, V., Sarkar, T., & Singh, R. P. (2020). Cerium oxide nanoparticles: Properties, biosynthesis and biomedical application. *RSC Advances, 10*(45), 27194–27214. [DOI:10.1039/D0RA04736H] [PMID]

Tang, J. L., Moonshi, S. S., & Ta, H. T. (2023). Nanoceria: An innovative strategy for cancer treatment. *Cellular and Molecular Life Sciences: CMLS, 80*(2), 46. [DOI:10.1007/s00018-023-04694-y] [PMID]

Thakur, N., Manna, P., & Das, J. (2019). Synthesis and biomedical applications of nanoceria, a redox active nanoparticle. *Journal of Nanobiotechnology, 17*(1), 84. [DOI:10.1186/s12951-019-0516-9] [PMID]

Ting, S. R., Whitelock, J. M., Tomic, R., Gunawan, C., Teoh, W. Y., & Amal, R., et al. (2013). Cellular uptake and activity of heparin functionalised cerium oxide nanoparticles in monocytes. *Biomaterials, 34*(17), 4377-4386. [DOI:10.1016/j.biomaterials.2013.02.042] [PMID]

Tisi, A., Pulcini, F., Carozza, G., Mattei, V., Flati, V., & Passacantando, M., et al. (2022). Antioxidant properties of cerium oxide nanoparticles prevent retinal neovascular alterations in vitro and in vivo. *Antioxidants, 11*(6), 1133. [DOI:10.3390/antiox11061133] [PMID]

Tok, A. I. Y., Boey, F. Y.C., Dong, Z., & Sun, X. L. (2007). Hydrothermal synthesis of CeO_2 nano-particles. *Journal of Materials Processing Technology, 190*(1-3), 217-222. [DOI:10.1016/j.jmatprotec.2007.02.042]

Wei, F., Neal, C. J., Sakthivel, T. S., Kean, T., Seal, S., & Coathup, M. J. (2021). Multi-functional cerium oxide nanoparticles regulate inflammation and enhance osteogenesis. *Materials Science and Engineering: C, 124,* 112041. [DOI:10.1016/j.msec.2021.112041]

Xiong, L., Bao, H., Li, S., Gu, D., Li, Y., & Yin, Q., et al. (2023). Cerium oxide nanoparticles protect against chondrocytes and cartilage explants from oxidative stress via Nrf2/HO-1 pathway in temporomandibular joint osteoarthritis. *Frontiers in Bioengineering and Biotechnology, 11,* 1076240. [DOI:10.3389/fbioe.2023.1076240] [PMID]

Yang, W., Zhang, M., He, J., Gong, M., Sun, J., & Yang, X. (2022). Central nervous system injury meets nanoceria: opportunities and challenges. *Regenerative Biomaterials, 9,* rbac037. [DOI:10.1093/rb/rbac037] [PMID]

Yesil, S., Ozdemir, C., Arslan, M., Gundogdu, A. C., Kavutcu, M., & Atan, A. (2023). Protective effect of cerium oxide on testicular function and oxidative stress after torsion/detorsion in adult male rats. *Experimental and Therapeutic Medicine, 25*(1), 1. [DOI:10.3892/etm.2022.11700] [PMID]

Zavvari, F., Nahavandi, A., & Shahbazi, A. (2020). Neuroprotective effects of cerium oxide nanoparticles on experimental stress-induced depression in male rats. *Journal of Chemical Neuroanatomy, 106,* 101799. [DOI:10.1016/j.jchemneu.2020.101799] [PMID]

Zhang, Y., Kohler, N., & Zhang, M. (2002). Surface modification of superparamagnetic magnetite nanoparticles and their intracellular uptake. *Biomaterials, 23*(7), 1553–1561. [DOI:10.1016/S0142-9612(01)00267-8] [PMID]