Synthesis and biomedical applications of Cerium oxide nanoparticles – A Review

S. Rajeshkumar*, Poonam Naik

Nano-Therapy Lab, School of Bio-Sciences and Technology, VIT University, Vellore, 632014, TN, India

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ABSTRACT

A cerium oxide nanoparticles (nanoceria) has a wide range of applications in different fields, especially biomedical division. As a matter of concern, it has a major impact on the human health and environment. The aim of this review is to address the different ways of synthesis of nanoceria using chemical and green synthesis methods and characterization and the applications of nanoceria for antioxidant, anticancer, antibacterial activities and toxicological studies including the most recent studies carried out in vivo and in vitro to study the problems. We have exclusively discussed on the toxicology of nanoceria exposed to the general public along with recent advances in the studies of antimicrobial, toxicity and anti-oxidant activity.

1. Introduction

Cerium belongs to lanthanide series and is rare earth metal (atomic number = 58). It is the most abundant rare earth metal which is present in two oxidation states i.e. +3 and +4 [1]. Cerium oxide is considered to be a lanthanide metal oxide and is used as an ultraviolet absorber [2,3], catalyst [4,5], polishing agent, gas sensors etc [6–10]. For commercial purpose, nanoceria plays a vital role in cosmetic products, consumer products, instruments and high technology. Moreover, they behave as very good oxide ion conductors in case of solid oxide fuel cells and used as a material in the electrode for gas sensors [11].

Recently, the importance of biomedical applications is growing as they exhibit protection against radiation, cellular damage mediated by toxicants and during pathological conditions such as brain or cardiac ischemia, neurological disorders or neurodegeneration of retina [12]. Naked nanoceria has poor solubility in the water leading to complications in biological applications. Many studies have come out with the polymer coating of nanomaterials which enhance the stability, biocompatibility and water solubility e.g. nanoceria coated with dextran exhibits antioxidant property [13].

Due to the extensive use, nanoceria is getting released to the environment and exposure to humans (mostly via inhalation) is a major concern. Contradictory results are found in the literature reporting the toxicity of nanoceria. Few papers addressed nanoceria to have low toxicity [14], and don’t mediate cytotoxicity or inflammation [15,16]. On the contrary, evidence from literature also depicts nanoceria trigger cell death. They trigger pro-oxidative effect due to reactive oxygen species (ROS) which cause damage to the cell and ultimately lead to cell death. Some studies addressed induction of oxidative stress caused by nanoceria either in vitro or in vivo [17] whereas they act as direct antioxidants and behave as free radical scavengers. It occurs by the interaction of superoxide radical, hydroxyl radical and hydrogen peroxide which restricts cell death due to oxidative stress. In addition to this, controversial results are also seen regarding oxidative stress. Studies have shown nanoceria either to exhibit pro-oxidative properties or antioxidant properties [18–20].

In this review, we focus and discuss the chemical and green synthesis of nanoceria and the underlying mechanisms in several studies like antimicrobial, toxicity (human health) such as cytotoxicity, genotoxicity, neurotoxicity, antioxidant activities (in vivo & in vitro) and biomedical applications.

2. Synthesis of nanoceria

Synthesis of nanoceria can be prepared by two means i.e. chemical method and green synthesis.

2.1. Chemical method

Many chemical methods are reported by researchers for the synthesis of nanoceria. Different have proved the synthesis of nanoceria by precipitation method [21–23] like co-precipitation [24] and chemical precipitation [25,26], microwave [27,28], sonochemical [29,30], hydrothermal [31–34], reverse-co-precipitation [35], microwave-hydrothermal method [36].

A novel method for the synthesis of nanoceria is done by using the...
Table 1
Synthesis and applications of nanoceria.

| S. no | Synthesis route | Size | Applications | Reference |
|-------|----------------|------|--------------|-----------|
| 1     | co-precipitation method | 20 nm (TEM and XRD) | – | [24] |
| 2     | Commercial nanoceria | 8–20 nm (TEM) | molecular mechanism of cytotoxicity on lung adenocarcinoma (AS49) cells | [55] |
| 3     | hydrothermal process | 3.1 nm (TEM) | High oxidation activity | [34] |
| 4     | Fungal culture filtrate of Curvularia lunata | 5 to 20 nm (TEM) | Antibacterial activity against Gram positive (Staphylococcus aureus, Streptococcus pneumoniae and Bacillus subtilis) and three Gram negative bacteria (Pseudomonas aeruginosa, Proteus vulgaris and Klebsiella pneumoniae) | [39] |
| 5     | Leaves of Aloe barbadensis Miller plant | 63.6 nm (dynamic light scattering analysis) | – | [42] |
| 6     | Precipitation method using ammonium water and oxalic acid as precipiant | 100–300 nm (SEM) | – | [21] |
| 7     | Gloriosa superba L. leaf extract | 5 nm (TEM) | Antibacterial activity against both gram positive and gram-negative bacteria | [38] |
| 8     | Acalypha indica leaf extract | 25–30 nm (TEM and XRD) | Antibacterial activity | [41] |
| 9     | Olea europaea leaf extract | 24 nm (SEM and TEM) | Antibacterial and antifungal activity against Gram-positive (G + ve) (Staphylococcus aureus ATCC 6538) and Gram-negative (G – ve) (Escherichia coli ATCC 15224, Pseudomonas aeruginosa ATCC 15442, Klebsiella pneumoniae ATCC BAA 1706) strains and Muco species (FCBP-0300), Aspergillus flavus (FCBP-0064), Fusarium solani (FCBP-434), and Aspergillus niger (FCBP-0198) | [40] |
| 10    | Hibiscus Sabdariffa’s flower aqueous extract | 3.9 nm (HR TEM and XRD) | Stability, surface morphology, chemical bonding and chemical valance states are studies | [43] |
| 11    | Fresh egg white | 25 nm (FE TEM) | non-toxic effect of concentration up to 800 μg/ml on human periodontal fibroblasts cells | [44] |

Nanoceria is having a lot of applications in the biomedical field shown in Fig. 1.
3.1. Antibacterial activity

Many studies have confirmed that nanoceria also showed antibacterial activity against Pseudomonas aeruginosa through agar well diffusion and broth dilution method. The experimental data confirmed that there was a complete zone of inhibition in case of P. aeruginosa (NCIM-2242) with the increase in the concentration of nanoceria i.e. 500,750 and 1000 μg L⁻¹ per well in case of agar well diffusion method. Moreover, with concentration 200 and 400 μg L⁻¹ against P. aeruginosa (NCIM-2242) the antibacterial activity was confirmed by broth dilution method [47].

In addition to this, another study also addressed that at lower temperature antibacterial activity was seen including E.Coli, B.subtilis, Shewanellaoneidensis and Pseudokirchneriella supercapitata. The probable mechanism behind this activity was due to the action of reactive oxygen species (ROS) [48].

3.2. Toxicity studies (Impact on human health)

The impact of nanoceria in the human health has brought keen interest among the researchers. There are two main routes through which nanoceria are exposed to the public i.e. inhalation and ingestion. Moreover, the inhaled cerium exits the respiratory tract mediated by different pathways and at different rates which depend on the body fluids solubility [49,50]. After the process of ingestion, cerium is excreted in the feces.

As nanoceria get poorly absorbed in the intestine, the exposure through inhalation is a major concern than ingestion. After the inhalation, the lungs and lymph nodes associated with it are the major targets. It may so happen that other organs might get affected. When the nanoceria get absorbed through circulation, it may also get distributed in other organs like liver, spleen, and kidney. Therefore, with variation in the size of nanoparticles can reach to different target areas of respiratory tract where it gets absorbed.

Researchers have also confirmed that nanoceria is poorly absorbed in the digestive system. Through oral route of exposure, the solubility of cerium oxide nanoparticle is very less when compared to other forms. So, it is probably thought that acute toxicity is less even though when transformed into soluble forms when absorbed by the body [51].

3.3. Cytotoxicity

Nanoceria was also the reason to cause cytotoxicity and oxidative stress. The 20 nm nanoceria was toxic towards cultured human lung cancer cells. Sulfurhodamine B was used to check the cell viability when exposed to 3.5, 10.5 and 23.3 μg/ml of nanoceria for 24, 48 and 72 h. There was a decrease in cell viability with respect to the dosage of nanoparticles and exposure time.

There was a quantitative assessment of total ROS, malondialdehyde, α-tocopherol, glutathione and lactate dehydrogenase which were the indicators of oxidative stress and cytotoxicity. Ultimately, there was a reduced level of glutathione and α-tocopherol. Free radicals were generated due to nanoparticle exposure and increase in oxidative stress led to high level of lactate dehydrogenase and malondialdehyde which showed clear indication towards cell membrane damage and lipid peroxidation [52].

In addition to the above study, nanoceria also caused cytotoxicity towards prostate cancer cell lines (PC-3) which was confirmed by MTT assay. But these were non-toxic towards normal cell lines (L929). The fluorescent dye rhodamine-123 conjugated with nanoceria which confirmed the cellular uptake followed by the optical detection [53].

3.4. Genotoxicity

Human bronchial epithelial cells (BEAS-2B) were cultured in KGM (Keratinocyte growth medium) defined the medium. Comet assay confirmed that after 24 h the DNA single-strand broke when exposed to different concentrations of nanoceria (10,50,100,150 μg/ml) [54].

Recently, a study explained the molecular mechanism behind the toxicity of nanoceria on lung adenocarcinoma (A549) cells. These nanoparticles were solely responsible for morphological changes in A549 cells. Moreover, it led to cell apoptosis, due to increase in annexin-V positive cells and loss in mitochondrial membrane potential. These were confirmed by immunoblot analysis of BAX, Bcl-2, Cyt-C, AIF, caspase-3, and caspase-9. Hence, reactive oxygen species induced DNA damage and cell cycle arrest which caused apoptotic cell death in A549 cells due to nanoceria [55].

Another genotoxicity study was carried out in female albino Wistar rats when exposed to nanoceria using comet and chromosomal aberration (CA) assay and micronucleus test (MNT). It was concluded from the results that with high dose (1000 mg/kg BW) of nanoceria mediated DNA damage in liver cells and peripheral blood leukocytes (PBL). It further led to cytogenetic changes and micronucleus formation in bone cells and bone marrow [56].

In addition to this, another study consisted of the cytotoxic and genotoxic study of nanoceria in human neuroblastoma cell line (IMR 32). Nanoceria caused cytotoxicity which was confirmed by lactate dehydrogenase assays and 3-[4,5-dimethylthiazol-2-yl]-2, 5-diphenyl tetrazolium bromide whereas genotoxicity assessment was confirmed using the cytokines-block micronucleus and comet assays. It was concluded that ROS were involved in the toxicity of nanoceria [57].

3.5. Neurotoxicity

Delivery of a targeted drug is a major concern and the most difficult job in neuroscience due to the fact that the blood-brain barrier (BBB) blocks most of the molecules and acts as a selective filter. In vitro and in vivo study confirmed that nanoparticles were used as carriers to move across the BBB i.e. the drug called suramin was used to cure the infection caused by the African trypanosomes which are the extracellular parasites. Presently, there is the availability of very few toxic drugs for this disease.

Therefore, the study was carried out to understand the responsive action of the brain that instructs the administration of suramin into the intracerebral region. Results have shown that the nanoceria which was fluorescently tagged when IV injected into mice induced nanoparticles accumulation in the liver and spleen. Moreover, very less penetration was seen in the brain.

Another in vitro and in vivo study elucidated neurotoxic effect caused due to nanoceria when exposed to serotonin (5-HT) which plays a vital role as a neurotransmitter. In vitro study of 5-HT demonstrated that nanoceria interacted with 5-HT and formed a 5-HT nanoceria complex. And in vitro study carried out in live zebrafish embryos depicted the lower level of 5-HT in the intestine due to prolonged exposure for more than 3 days. Therefore the exposure of 20 and 50 ppm nanoparticles decreased the 5-HT level to 20.5( ± 1.3) and 5.3( ± 1.5) nM respectively when exposed to 30.8 ( ± 3.4) nM in control embryos (un-exposed) [58].

4. Antioxidant activity

The most recent study described that when nanoceria was conjugated with levan, it depicted antioxidant activity. Levan coated nanoceria were synthesized using the system called one pot-and green synthesis. Levan acted as reducing and stabilizing agent. Moreover, there was a reduction in the level of ROS when levan coated nanoparticles were treated with hydrogen peroxide which stimulated NIH3T3 cells. Therefore, levan coated nanoparticles were beneficial towards the disease induced by ROS [59].

Another study has demonstrated that an average 10 nm size nanoceria extended the lifespan and preserved the neuronal function expressed in brain cell cultures. It was examined that the impact of Fe-
doped nanoceria (6%Fe) was proved out to be less effective as compared to nanoceria.

The examination of 3 groups of nanoceria was done using H$_2$O$_2$, UV and A$_β_{1,2}$ to find out the neuroprotective capacity. When compared to 7 nm nanoceria, the level of cell death decreased in 10 nm nanoceria which was induced by UV and H$_2$O$_2$. It was concluded that nanoceria depicted antioxidant activity and is size dependent. These nanoparticles protected the neurons from A$_β_{1,2}$ toxicity and damage from free radicals [60].

4.1. In vitro study

In vitro studies are the evidence which proves nanoceria to be best antioxidants. They show ROS scavenging which protects different cells like stem [61], neuronal [62,63], human breast [64], gastrointestinal epithelium [65] and endothelial [66]. Another study expressed that the drug doxorubicin had antitumor activity in human melanoma cells [67]. In A375 human melanoma cell line, cytotoxicity was seen and the cell viability decreased due to co-incubation of nanoceria and the drug doxorubicin. Anti-tumor activity and induced apoptosis were seen in A375 cell line but didn’t cause DNA damage.

In addition to this, researchers have reported that when PC12 neuron-like cells were incubated with an increase in the concentration of nanoceria, it was seen that PC12 cells depicted no deficiency in their metabolic activity and cell differentiation capabilities were preserved. Moreover, there was an increase in neuronal length when cells were exposed to nanoceria. Further, there was a reduction in the production of ROS when stimulated with hydrogen peroxide. An increase in the production of dopamine was also seen [68].

Cytotoxicity assay of nanoceria was carried with human breast cancer (MCF-7) and fibrosarcoma (HT-1080) cells. No cell death was seen when the cells were treated with 20 μg mL$^{-1}$, 50 μg mL$^{-1}$, 100 μg mL$^{-1}$ and 200 μg mL$^{-1}$ concentration of nanoparticles. These nanoparticles treated cells lead to increase in the production of glutathione (GSH) and decrease the depletion of GSH caused due to hydrogen peroxide [69].

4.2. In vivo study

Many in vivo animal studies were carried out using rats and mice to understand the involvement of nanoceria in organs like liver, spleen, kidneys, lungs, and brain [70–74]. Another study reflected the absorption of 30 nm in the liver and spleen via time and dose dependent manner [75]. And study was examined to understand the effects of 5 nm vs. 30 nm nanoceria in terms of size, shape and dose. But no difference was seen on the basis of retention and bio-distribution [76].

Very few studies were carried out with non-rodent models e.g. Drosophila melanogaster was chosen for in vivo study with nanoceria. It was confirmed that the uptake of nanoceria was seen in microvilli, interior parts of the intestine, intestinal lumen, hemolymph tissues and cytoplasm of intestinal cells. This was caused due to the ingestion of nanoceria as food, which passed through the intestine followed by the absorption in the mid-gut cells [77]. In addition to this, Caenorhabditis elegans was chosen as a model organism which was exposed to different charged surface coated nanoceria. It was observed that different surface coated nanoparticles had different uptake. Positively charged showed the best candidacy with highest bio-accumulation when compared to negative and neutral particles [78].

Several in vivo studies are carried out with plant crops like rice [79], wheat, sunflower, pumpkin [80], alfalfa, corn, tomato [81], kidney bean [82], radish [83,84], cucumber [85], Rubia cordifolia [86] to study the uptake of nanoceria. Results have shown the highest uptake in roots as compared to other parts of plants like leaves shoots etc. [80–83]. This is caused due to several factors like nanoceria size [80,83,85], agglomeration [85,80] and concentration [81,82] that lead to the uptake and distribution of nanoparticles.

5. Conclusion

The effect of nanoceria is a major concern among the researchers on the human health. We have discussed the overall processes and a recent synthesis of nanoceria via chemical and green methods. Synthesis using parts of plants extract is carried out for several years but we have included the most recent synthesis using flower extract that acts as chelating agent. Another recent chemical synthesis includes the preparation of nanoceria from a protein supra-molecule called apoferritin.

We have focused on the positive and negative impacts of nanoceria on different living organism model e.g. rat, mice, human cancer cell lines and non-rodent models. Controversy is seen in the study of nanoceria in the application of antioxidant property and toxicity analysis. However, mostly the studies focus on the toxicity of nanoceria on human health and different types of toxicity including cytotoxicity, genotoxicity, and neurotoxicity. We have exclusively focused on the antioxidant activities which are carried out both in vitro and in vivo. Many in vitro studies have concluded that nanoceria can be considered as safer nanoparticles as compared to the in vivo models.

We concluded that nanoceria was toxic towards human cancer cell lines. They can lead to the release of free radicals and oxidative stress ultimately leading to cell membrane damage and lipid peroxidation. However, ROS mediated DNA damage and cell cycle arrest. Finally, nanoceria can be used for several biomedical applications mostly for ROS related diseases like cardiac diseases, Alzheimer’s disease, and cancer. So, the ROS scavenging nanoceria can be considered as an alternative therapy for oxidative stress and several diseases and disorders.

References

[1] Casandra Kornvik, et al., Superoxide dismutase mimetic properties exhibited by vacancy engineered ceria nanoparticles, Chem. Commun. 10 (2007) 1056–1058.
[2] Ngoc Thiem Dao, et al., UV absorption by nanoceria/epoxy composite thin films, Adv. Nat. Sci.: Nanosci. Nanotechnol. 2 (4) (2011) 045013.
[3] N.M. Zhlobok, et al., UV shielding property, photocatalytic activity and photo-cytotoxicity of ceria colloidal solutions, J. Photochem. Photobiol. B 102 (1) (2011) 32–38.
[4] Trovarelli Alessandro, Alessandro Catalytic properties of ceria and CeO$_2$ containing materials, Catal. Rev. 38 (4) (1996) 439–520.
[5] Ching-Huei Wang, Shih-Shyung Lin, Preparing an active cerium oxide catalyst for the catalytic incineration of aromatic hydrocarbons, Appl. Catal. A: Gen. 268 (1) (2004) 227–233.
[6] Blandine Courbiere, et al., Ultrastructural interactions and genotoxicity assay of cerium dioxide nanoparticles on mouse oocytes, Int. J. Mol. Sci. 14 (11) (2013) 21613–21628.
[7] Laura De Marzi, et al., Cytotoxicity and genotoxicity of ceria nanoparticles on different cell lines in vitro, Int. J. Mol. Sci. 14 (2) (2013) 3065–3077.
[8] Philip Demokritou, et al., An in vivo and in vitro toxicological characterisation of realistic nanscale CeO$_2$ inhalation exposures, Nanotoxicology 7 (8) (2013) 1338–1350.
[9] Lu Peng, et al., Comparative pulmonary toxicity of two ceria nanoparticles with the same primary size, Int. J. Mol. Sci. 15 (4) (2014) 6072–6085.
[10] Gerardo Pulido-Reyes, et al., Untangling the biological effects of nanoceria: the role of surface valence states, Sci. Rep. 5 (2015) 15613.
[11] Jessica T. Dahle, Yuji Araki, Environmental geochemistry of cerium: applications and toxicology of nanoceria, Int. J. Environ. Res. Public Health 12 (2) (2015) 1253–1279.
[12] Marcel Culcaet, et al., EPR spin trapping evaluation of ROS production in human fibroblasts exposed to nanoceria: evidence for NADPH oxidase and mitochondrial stimulation, Chem. Biol. Interact. 199 (3) (2012) 161–176.
[13] J. Manuel Perez, et al., Synthesis of biocompatible dextran-coated nanoceria with pH-dependent antioxidant properties, Small 4 (5) (2008) 552–556.
[14] Martin Urner, et al., Inflammatory response of lung macrophages and epithelial cells after exposure to redox active nanoparticles: effect of solubility and antioxidant treatment, Environ. Sci. Technol. 48 (23) (2014) 13960–13968.
[15] Matthias Fischella, et al., Toxicity evaluation of manufactured CeO$_2$ nanoparticles before and after alteration: combined physicochemical and whole-genome expression analysis in Caco-2 cells, BMC Genomics 15 (1) (2014) 700.
[16] P. Leonardo Franchi, et al., Cyto- and genotoxic effects of mesoscopic nanoparticles in untransformed human fibroblast, Toxicol. In Vitro 29 (7) (2015) 1319–1331.
[17] Milica Pečić, et al., Anti-cancer effects of nanoceria and its intracellular redox activity, Chem. Biol. Interact. 232 (2015) 85–91.
[18] Megan S. Lord, et al., Cellular uptake and reactive oxygen species modulation of nanoceria in human monocyte cell line U937, Biomaterials 33 (31) (2012) 7915–7924.
[19] P. Rosenkranz, et al., Effects of nanoceria to fish and mammalian cell lines: an assessment of cytotoxicity and methodology, Toxicol. In Vitro 26 (6) (2012) 888–896.
[20] Tian Xia, et al., Comparison of the mechanism of toxicity of zinc oxide and
nanoceria based on dissolution and oxidative stress properties, ACS Nano 2 (10) (2008) 2121.

[21] Y.H. Liu, et al., Synthesis and character of cerium oxide (CeO₂) nanoparticles by the precipitation method, Metalurgija 52 (4) (2014) 463–465.

[22] Qiu Li Zhang, Yang Mao Zhi, Bing Jun Ding, Synthesis of nanoceria by the pre-cipitation method, Rev. Funct. Mater. 12 (2012) 200–203.

[23] N. Thovhogi, A. Diallo, A. Gurib-Fakim, M. Maaza, Nanoparticles green synthesis by precipitation method, Ultrason. Sonochem. 18 (5) (2011) 1118–1123.

[24] Gianni Ciofani, et al., Efects of nanoceria on PC12 neuronal-like cells: proliferation, differentiation, and dopamine secretion, Toxicol. In Vitro 28 (3) (2014) 635–642.

[25] Robert A. Yokel, et al., Biodistribution and oxidative stress effects of a systemically-introduced commercial ceria engineered nanomaterial, Nanotoxicology 3 (3) (2009) 234–248.

[26] Robert A. Yokel, et al., Nanoceria biodistribution and retention in the rat after its intratracheal administration greatly influenced by dosing schedule, dose, or particle shape, Environ. Sci. Nano 1 (6) (2014) 549–560.

[27] Mohamed Alarabey, et al., Antioxidant and antigenotoxic properties of CeO₂ NPs and cerium sulphate: studies with Drosophila melanogaster as a promising in vivo model of cerium, Toxic. In Vitro 29 (6) (2015) 156–166.

[28] Blanche Collin, et al., Influence of natural organic matter and surface charge on the toxicity and bioaccumulation of functionalized ceria nanoparticles in Caenorhabditis elegans, Environ. Sci. Technol. 48 (11) (2014) 6289–6299.

[29] Cyren M. Rico, et al., Effect of nanoceria on rice: a study involving the antioxidant defense system and in vivo fluorescence imaging, Environ. Sci. Technol. 47 (11) (2013) 5625–5642.

[30] Franziska Schwabe, et al., Dissolved cerium contributes to uptake of Ce in the presence of differently sized CeO₂ nanoparticles by three crop plants, Metalloccins 7 (3) (2015) 466–477.

[31] Maritha L. López-Moreno, et al., X-ray absorption spectroscopy (XAS) corroboration of the uptake and storage of CeO₂ nanoparticles and assessment of their differential toxicity in four edible plant species, J. Agric. Food Chem. 58 (9) (2010) 3689–3693.

[32] Suryakant Majumda, Environ. Sci. Technol. 48 (2) (2014) 1280–1289.

[33] Weisheng Lin, et al., Toxicity of nanoceria in human lung cancer cells, Int. J. Toxicol. 25 (6) (2006) 451–457.

[34] G. Remu, et al., Development of nanoceria and its cytotoxicity in prostate cancer cells, Adv. Sci. Lett. 6 (1) (2012) 17–25.