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PII: S2215-017X(17)30223-0
DOI: https://doi.org/10.1016/j.btre.2017.11.008
Reference: BTRE 228

To appear in:

Received date: 2-8-2017
Revised date: 30-10-2017
Accepted date: 28-11-2017

Please cite this article as: S.Rajeshkumar, Poonam Naik, Synthesis and biomedical applications of Cerium oxide nanoparticles – A Review, Biotechnology Reports https://doi.org/10.1016/j.btre.2017.11.008

REVIEW

Synthesis and biomedical applications of Cerium oxide nanoparticles – A Review

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Highlights

- To synthesis the cerium oxide nanoparticles using different sources
- Biomedical applications of cerium oxide nanoparticles
- Applications of cerium oxide nanoparticles in toxicity studies

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.

Keywords: cerium oxide nanoparticles; Synthesis; Antibacterial activity, Antioxidant activity; Toxicity

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 enhances 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 cage-shaped protein called apoferritin. It was a two and three-D array formation using this protein. For the synthesis of nanoceria, apoferritin was considered as bio-template. The chemical reaction occurred at the cavity. There was oxidation of trivalent cerium ions and led to the formation of nanoceria as seen in case of iron oxide formation. It was confirmed as nanoceria with size 5.0 ± 0.7nm.There was salt bridge formation between the ferritins (each apoferritin containing a nanoparticle) by multivalent cerium ions. The best salt bridge formation led to a 2-D array of nanoceria containing ferritin and 3-D arrays acquiring ferritin with two different morphology i.e. Octahedral or prism structured [37].

2.2 Green method (from plant extracts)

The synthesis of nanoceria using different sources shown in table 1.Nanoceria can also be synthesized by using green methods. There are many studies which have reported the green synthesis of nanoceria using *Gloriosa superba L*. leaf extract. The nanoparticles formed were confirmed by XRD and was found to be spherical in shape [38].

Another study also addressed the synthesis of nanoceria using culture filtrate of *Curvularia lunata*. This even confirmed the spherical shape of the nanoparticles which ranged from 5-20 nm [39]. These synthesis nanoparticles also exhibited antibacterial activities against different bacterial species. However, it was concluded that nanoparticles couldn't penetrate the bacterial cells [40].

Recently, another study has also confirmed the use of *Acalypha indica* and Aloe vera plant leaf extract for the synthesis of nanoceria [41,42]. These extracts were used as a capping agent for the synthesis. The most recent study of nanoceria synthesis is acquired by using the flower extract of *Hibiscus sabdariffa* as a chelating agent. The characterization of nanoceria confirmed the size to be approx. 3.9 nm in diameter [43].

2.3 Synthesis from nutrients

The green approach synthesis is widely accepted by the researchers due to its reliable and eco-friendly purpose. Several studies have reported the synthesis of nanoceria using different nutrients like egg white protein [44]. It has been proposed that ovalbumin and lysozyme are the two proteins of egg white which acted as stabilizing agent for the synthesis of nanoceria. The mechanism of nanoparticles formation can be explained by the electrostatic interaction held between the cerium ions (Ce3+) and protein with opposite charge enhances the growth along with small stable isotropic nanoparticle formation [45].

However, another study also reveals the synthesis of nanoceria using honey. The presence of vitamins, carbohydrates, and enzymes in the honey matrix structure possess hydroxyl and amine groups. Therefore, it was extensively used as stabilizing and coating agent to the cerium species along with nanoceria which inhibited their crystal growth [46].

3. Applications of nanoceria

Nanoceria is having a lot of applications in the biomedical field shown in figure 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 1000µg L^{-1} per well in case of agar well diffusion method. Moreover, with concentration 200 and 400µg 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 supcapitata*. 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 20nm nanoceria was toxic towards cultured human lung cancer cells. Sulforhodamine B was used to check the cell viability when exposed to 3.5, 10.5 and 23.3µg/ml of nanoceria for 24, 48 and 72 hr. 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 hours the DNA single-strand broke when exposed to different concentrations of nanoceria (10,50,100,150µg/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 (1000mg/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 (unexposed) [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 10nm 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_2O_2, UV and $A\beta_{1-42}$ to find out the neuroprotective capacity. When compared to 7nm nanoceria, the level of cell death decreased in 10nm nanoceria which was induced by UV and H_2O_2. It was concluded that nanoceria depicted antioxidant activity and is size dependent. These nanoparticles protected the neurons from $A\beta_{1-42}$ 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 retained.

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 $20\mu g.mL^{-1}$, $50\mu g.mL^{-1}$,$100\mu g.mL^{-1}$ and $200\mu g.mL^{-1}$ 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 ~ 30nm in the liver and spleen via time and dose dependent manner [75]. And study was examined to understand the effects of 5nm 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.

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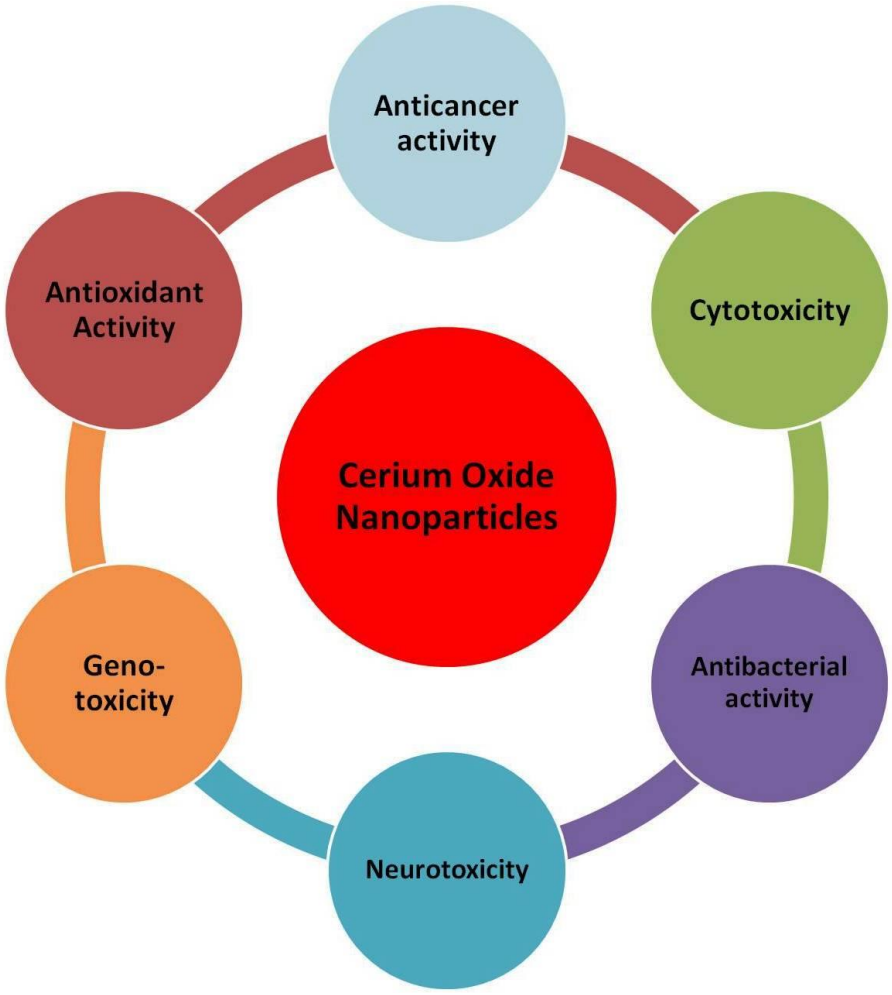

Figure 1: Applications of Nanoceria

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	8nmto 20nm (TEM)	molecular mechanism of cytotoxicity on lung adenocarcinoma (A549) 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 ammonia water and oxalic acid as precipitant	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	[40]

			15224, *Pseudomonas aeruginosa* ATCC 15442, *Klebsiella pneumoniae* ATCC-BAA 1706) strains and *Mucor* species (FCBP-0300), *Aspergillus flavus* (FCBP-0064), *Fusarium solani* (FCBP-434), and *Aspergillus niger* (FCBP-0198)	
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 SEM)	non-toxic effect of concentration up to 800 µg/ml on human periodontal fibroblasts cells	[44]