Synthesis and toxicity assessment of environment friendly high yield ceria nanoparticles for biosafety

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Abstract

Different compounds at nanoscale level show more efficient behaviour because of increased surface area and optical properties. CeO_2 nanoparticles are of great importance for their unique properties. However, the extensive release of CeO_2 nanoparticles in the environment is also a serious problem that must be addressed as available data related to ceria toxicity is currently not comprehensive. The present study was aimed to evaluate the potential of CeO_2 nanoparticles in biomedical applications and assessment of their toxicity by using mutagenic and acute *in-vivo* approaches. High yield CeO_2 nanoparticles with spherical morphology and with an average size of 40nm were synthesized by adopting the alkaline fusion method under mild conditions. The synthesized CeO_2 nanoparticles showed antibacterial activity at different concentrations (50-500 µg/mL) against *E. coli*. The antioxidant properties of CeO_2 nanoparticles were determined, and CeO_2 nanoparticles show antioxidant behaviour that may be helpful for anti-cancer and anti-inflammatory drug preparation. Ames test confirms no mutagenicity at different concentrations of CeO_2 nanoparticles. Moreover, in the current study CeO_2 nanoparticles showed no toxicity for aquatic life even at a concentration of 100 mg/L in the same way the *in-vivo* toxicology was not evident even at the highest concentration ($\geqslant$5000 mg/kg BW for rats), no significant change was observed in haematological and biochemical parameters of control and CeO_2nanoparticles exposed rats. *In-vivo* dermal toxicity was not observed in rabbits at the application of 0.5 g CeO_2 nanoparticles. These results indicate the nontoxic nature of these nanomaterials. However, further experimentation is recommended to completely define the toxic potential of the nanomaterials.

Keywords: Green synthesis; Sustainable materials; Ceria nanoparticles; Nanotoxicity; Biosafety; Mutagenicity and LD_{50}.

1 Introduction

In nature, cerium (Ce) is present with other lanthanide elements in different minerals like alanite, bastanite, cerite, monazite and samarskite but bastanite and monazite are considered important commercially. Stability in Ce^{4+} state is a unique feature of Ce as other lanthanide elements are stable in the trivalent state only. Ce **(**placed in lanthanide series of elements in the periodic table**)** constitutes 0.0046% of earth crust and is therefore regarded as the most abundant rare earth metals [1]. The most important compound of Ce used commercially is CeO_2 [2]. Nanoparticles of CeO_2 are important as they are not only more efficient than non-nano sized Ce [3] but also have an important use in other applications as well. For example, in catalytic reactions[4], infrared filters coatings [5], buffer layer for many superconductors [6], for plastics manufacturing, used in Infrared absorbents and as sintering additives, oxidation resistant coatings. They have useful applications in oxygen pumps and also in oxygen sensors [1]. Alloys coating, electrolyte/ electrode materials in fuel cells [7]. In bearing balls, glasses and electronic devices [5] as UV absorbent because of their absorption at ~ 400 nm which is the strongest absorption ability for any known oxide [8].

The emergence of new diseases and microbial resistance against already available antibiotics demonstrates the need for novel drugs used against these pathogens and for the treatment of metabolic and other disorders [9]. Nanobiotics is relatively a new idea and different nanomaterials are available with strong potential to inhibit bacterial growth [10]. The prepared silver nanoparticles were screened for their antibacterial activity against *K. pneumonia, P. Aeruginosa, S. aureus and E. coli [11]*. CeO_2 nanoparticles also possess antibacterial activity [12-14]. Along with many other applications, CeO_2 nanoparticles were also studied in the medical field as anti-

inflammatory and anti-oxidant agents [15, 16] suggested the role of CeO_2 nanoparticles in the treatment of neurodegenerative disorders like trauma [17], ageing [18], Parkinson's and Alzheimer's diseases [19] and many others. The anti-inflammatory [20] and anti-angiogenic effects of CeO_2 nanoparticles were also proposed [21]. Moreover, one of the important bio applications of CeO2 nanoparticles is their biomedical applications in treatments of ischemia reperfusion injuries [22, 23].

The phenomenon that attracted the environmental biotechnologists' attention towards the rapidly growing field of nanotechnology was their hazardous side effects. Many of these nanomaterials were hazardous in nature and their toxic effects are spread over a wide range of species from aquatic organisms to mammalians on land and serious questions were raised about their fate in the environment and environmentalists showed their concern about the hazardous potential of these materials [24]. The toxic effects of a nano-product could be totally different after surface modification and producers and customers should keep this fact in mind before launching and using a new product [25]. Some reported toxicity effects are of fullerenes, q-dots, silver nanoparticles, a small contribution of carbon nanotubes, and different metals and metallic oxides such as Cu [26], Zn [27] and ZnO [28], SiO_2 [29] and TiO_2 [27]. As the concerns of scientific society are increasing about the toxicity of nanoparticles. Thus, different sophisticated protocols and technical approaches have been developed to study the adverse effects on different organisms.

Therefore, living systems, under investigation, are of different trophic levels from microbes to higher organisms, plants, and animals [30]. Comprehensive data related to CeO_2 nanoparticles is not present to date. On the basis of available data from different studies and EPA reports, the status

of these nanoparticles is still unclear [31]. Mostly reports related to CeO_2 nanoparticles are based on *in-vitro* studies [32-35], on plants [36] on *Cyriocosmus elegans*(spider) [37] on green algae (*Pseudokirchneriella subcapitata*) [38] and cyanobacterial (*Anabaena* CPB4337) strains [39].Therefore, in view of all the above facts, it is evident that CeO_2 nanoparticles have multiple applications in different industries and have great potential to be used in more fields in the future. However, excessive use and release of these nanoparticles in the environment are also subject to environmental safety and their toxic status is still controversial. The present study was an effort to find comprehensive results of different methods used in toxicological studies used for the assessment of the toxic potential of CeO_2 nanoparticles.

The toxicological evaluation of nanoparticles is normally carried out using the same assays as used in toxicity testing of chemicals. A major limitation, in the use, of these methods, is the novel physicochemical properties of nanomaterials that can make the results of these assays less reliable[40]. However, some nano products are ecofriendly and reportedly used to mitigate pollutants[41, 42]. Hence, the development of newly standardized protocols for the assessment of nanoparticle toxicity is necessary. Acute fish toxicity test, *In-vivo* toxicity testing, Oral LD_{50}, Intraperitoneal LD_{50}, Acute Dermal Irritation, Ocular LD_{50}, *In-vitro* toxicity testing and genotoxicity testing are the most common methods used in toxicology. The present study was focused to synthesize the nanoceria, their characterization, exploration of their application potential for antimicrobial and anti-inflammatory drug preparation and toxicological evaluation of these nanoparticles by using mutagenic, aquatic, and acute *In-vivo* toxicity tests.

2 Materials and methods

The detailed experimental plan of the current study is given graphically in Fig. 1. CeO_2 nanoparticles were synthesized by slightly modification of the method mentioned by [43]. A mixture of NaOH and KOH (2.51 g: 2.44 g respectively) was placed in a Teflon container and 1.1 g of Ce $(NO_3)_3$. $6H_2O$ was added. The container was placed in a vacuum oven pre-heated at 170 ˚C for 30 minutes. After 30 min the container was kept on a bench-top and cooled. Finally, the product washing step was done with milli-Q water thrice and then dried at 60 ˚C for 8 h.

2.1 Characterization of CeO_2 nanoparticles

CeO_2 nanoparticles were dispersed in deionized H_2O and characterized by using zetasizer nano [44], Field Emission Scanning Electron Microscopy (FESEM), UV-visible spectrophotometry, Atomic Force Microscopy [45] and X-ray Diffraction Analysis (XRD) [46].

2.2 Applications of CeO_2 nanoparticles

2.2.1 Antimicrobial activity assessment

The antimicrobial activity of CeO_2 nanoparticles was investigated by the disc diffusion method [47]. Fresh bacterial cultures of *Bacillus subtilis* and *E. coli* were used to detect the antimicrobial activity of CeO_2 nanoparticles. Penicillin G was used as a control. Different concentrations (50, 150, 250 and 500 µg/mL) were used to check the antimicrobial activity of different concentrations of these nanoparticles. The results were recorded the next day.

2.2.2 Determination of antioxidant activity

The antioxidant potential of synthesized CeO_2 nanoparticles was determined by a proposed method of Tian et al. [48]. Different concentrations (2.5-10 mg/mL) of ceria nanoparticles were prepared and mixed with 5ml of 0.2 M sodium phosphate buffer (pH 6.6) and 5ml of 1% potassium ferricyanide. Mixtures were placed in an incubator preheated at 50 ˚C for 20 minutes. Then 5 mL

of 10% TCA (trichloroacetic acid) was introduced and the mixture was centrifuged at 10,000 rpm for 10 minutes in refrigerated centrifuged (5 ˚C). The supernatant was taken and to dilute the mixture, 5 mL distilled water and 1 mL of 0.1% ferric chloride was added and absorbance was measured at 700 nm.

2.3 Mutagenicity of CeO_2 nanoparticles

For the detection of CeO_2 nanoparticles spontaneous mutation causing ability, the Ames test was performed [49]. The Mutagenicity index (M.I) was calculated using Eq. (1). A sample is considered mutagenic when M.I in the case of TA98 is 2.00 and 1.80 with TA 100 [50].

$$Mutagenicity\ index\ (M.I) = \frac{Number\ of\ revertant\ colonies\ in\ test\ plate}{Number\ of\ revertant\ colonies\ in\ the\ negative\ control\ plate} \quad (1)$$

2.4 Acute fish toxicity testing

Cyprinus carpio L., 1758 was obtained from a Fish farm, Satyana Road, Faisalabad, Pakistan. The specimens were approximately 3 months old, the average body length measured was 4.15 ± 0.75 cm and body weights 1.0 ± 0.25 g. After 15 days, fish were ready to be used in acute fish toxicity testing and OECD 203 guidelines were followed for this purpose. Three different concentrations (1, 50 and 100 mg/L) of commercially available non-nano-CeO_2 and synthesized CeO_2 nanoparticles were used in this study. CeO_2 is insoluble in water so it was dissolved in dilute nitric acid and pH of soln. was adjusted at 4 before its addition in water. The effect of nitric acid (used as a vehicle) was also determined. Change in activity and behaviour (including loss of balance, lying laterally, movement in a spiral fashion with jerks, open mouth and rapid opercular movements) and death rate of fish were observed after 0, 0.5. 1.0, 2.0, 4.0 6.0, 10.0, 24, 48, 72 and 96 h of exposure.

2.5 *In-vivo* toxicity testing

Acute oral toxicity assessment (oral LD50) was carried out by the administration of a single dose (of different concentrations up to 5000 mg/kg body weight of rat) were orally for the determination of median lethal dose LD_{50}. LD_{50} is considered a single dose to produce mortality in 50% of individuals) in rats. Guidelines of the Organization of Economic Cooperation and Development (OECD) 423 were followed to assess the toxicity of CeO_2 nanoparticles. Two sets of rats in triplicates were used in this study for each concentration. The rats' group administered with normal saline was used as a control. For intraperitoneal LD50 the maximum dose used in this study was 500 mg/L (as above this concentration, soln. was unable to pass through syringe of 28G1/2 gauge) was administered intra-peritoneal by following the OECD guidelines and rats were observed for a period of 14 days. Finally, rats were sacrificed for biochemical and haematological studies. The dermal irritation/toxicity testing was carried out in adult albino rabbits. A standard dose of 0.5 g of ceria nano-powder was applied on the skin of rabbits; normal saline was used as a control. Effects were observed after 1 h, 24 h and 14 days intervals.

3 Results and discussion

The field of nanotechnology is developing very rapidly in the last few decades due to its interesting applications in multiple disciplines. On the other hand, the threat to the environment and its living biota from these nanomaterials is also of great importance and must be addressed. Different efforts have been made in this regard, but satisfactory measures still needed to be taken. The present study aimed to synthesize CeO_2 nanoparticles by different methods, to characterize these as-synthesized nanoparticles, to explore their applications and their toxicological evaluation.

3.1 Structural analysis of CeO_2 nanoparticles

For the synthesis of CeO_2 nanoparticles, a modified alkaline fusion method proposed by Jin et al. [43] was adopted and pale-yellow nano-ceria powder was obtained. Dynamic light scattering **(**DLS) is mostly considered a suitable technique for the measurement of nanoparticles size and agglomeration in solution [51]. The average size of these nanoparticles, synthesized at 170 ˚C for 30 min was 40 nm that was similar to the size reported by Jin et al. [43]. In the modern era, several analytical techniques and instruments are used for the characterization of nanoparticles. For the determination of average size, surface charge, surface area, hydrodynamic diameter, elemental composition, agglomeration and porosity, various types of spectroscopic and microscopic techniques are available [52-54]. Fig. 2 illustrates the size distribution of synthesized CeO_2 nanoparticles characterized by photon correlation spectroscopy. Under UV-Visible spectrophotometer, newly synthesized CeO_2 nanoparticles absorb wavelength in a range of 310-340 nm (Fig. 2). Similar ranges for CeO_2 nanoparticles were previously reported [55-57].

The technique is focused on the whole area of the micrograph and therefore is used to create a surface image of the sample and also to generate estimate images with great depth of field. This UV-absorption phenomenon makes CeO_2 nanoparticles a suitable substance for their use in UV-filters and UV blocking cosmetics, showing maximum absorbance at a range of 310 to 330 nm, these findings are in line with the findings of Sohn et al.[58]. FESEM is very useful to study materials from both the qualitative and quantitative analytical aspects[59]. In the current study, FESEM reveals the information that CeO_2 nanoparticles were spherical in shape and weekly agglomerated (Fig. 3(a)) and AFM images showed that A 3-D image of ceria nanoparticles showed the maximum height of about 85 nm (Fig. 3(b)). X-ray diffraction (XRD) is a versatile, non-destructive technique that is used to analyze crystalline materials qualitatively and quantitatively.

The technique has been used to determine the overall structure of bulk materials, including lattice constants, the orientation of single and polycrystals, identification of unknown materials, stress, film thickness and texture etc.[60]. Structural identification of CeO_2 nanoparticles by means of X-ray diffraction in the range of angle 2θ between 20◦ and 80◦ as shown in Fig. 4, the peaks were obtained at (200), (202), (222), (313) and (402). It was indicated that the crystalline and cubic structure of CeO_2 nanoparticles of about 85 nm in size. Furthermore, by using XRD analysis, the structural arrangements of Ce and O atoms can be determined at various positions. For example, Fig. 5(a) shows the hexagonal structural model of Ce and O in a unit cell. The ball and stick type alternative arrangements of atoms are presented in Fig. 5(b). Fig. 5(c) shows the polyhedral present in the hexagonal structure of CeO_2. Doted surface type structural model around the ball and stick style of Ce and O atomic arrangement is shown in Fig. 6(d). Further, Fig. 6(e-h) shows the 3D representation of (200), (202), (222), (313) and (402) planes respectively [61-63].

3.2 Nanoparticles as nanobiotics

Treatment of different diseases with the help of different naturally available sources (plant extracts etc.) and laboratory synthesized chemical drugs is a common practice. The emergence of new diseases and microbial resistance against already available antibiotics demonstrate the need for novel drugs used against these pathogens and also for the treatment of metabolic and other disorders. Nanobiotics is relatively a new idea and different nanomaterials are available with strong potential [64, 65]to inhibit bacterial growth e.g; Silver monovalent ions [11], Silver oxide [66], Gold [67], Copper oxide [68] and Zinc oxide [52]nanoparticles are reported strong antibacterial agents against a wide range of bacterial strains e.g; *Bacillus subtilis*, B. Calmette-Guérin (BCG, used as surrogate for TB in anti TB drugs), *E. coli*, *Salmonella typhi*,

Staphulococcus aureus, *Psedomonas aueroginosa*, *Micrococcus luteus*, *Klebsiella pneumonia* and *Compylobacter jejuni* (Food borne pathogen). Recently, it was reported that oxygen-containing nano-shuttles can potentially use as anti-infecting nano-robots in upcoming graphene-based nano-biomedical applications [69].

The use of these nanoparticles in medicines [19] is a promising idea for the prevention of diseases e.g. colon cancer [59], fibrosarcoma [70], neurodegenerative disease [71]and anti-obesity [72]. Cytotoxic effects of CeO_2 nanoparticles were observed on *E. coli* and *Bacillus subtilis* and results showed the antibacterial activity of these nanoparticles at different concentrations (50, 150, 250 and 500 µg/mL) against *E. coli* (Fig. 6, 7). It was revealed that a gradual increase in antibacterial activity with an increase in the concentration of CeO_2 nanoparticles but these effects were more visible in *E. coli* (Fig. 6(a)). Thill et al. [73] worked on the toxicity of ceria nanoparticles on *E. coli* and on the basis of findings concluded that toxic behaviour of ceria nanoparticles is dependent on direct exposure of cells to these particles and in media bacterial cells may produce some chemicals that inhibit nanoparticles aggregation around and side the cells and as a result, prevention of growth inhibition occurs. These results suggest a possible role of CeO_2 nanoparticles in the development of nanobiotics. Similar findings, related to the antibacterial activity of CeO_2 nanoparticles against *E. coli* are also previously reported.

Furthermore, Villa et al. [14] worked on the toxicity of CeO_2 nanoparticles on *E. coli* and on the basis of findings concluded that the toxic behaviour of CeO_2 nanoparticles is dependent on direct exposure of cells to these particles and in media bacterial cells may produce some chemicals that inhibit nanoparticles aggregation around and inside the cells and in the result of it, prevention of

growth inhibition occurs [14]. Pelletier *et al*. focused on bacterial toxicity and visualized the toxic effects of CeO_2 nanoparticles based on different particle sizes and growth, different media and at variable pH [13]. *B. subtilis* and *E. coli* were used as model Gram-positive and negative bacterial strain respectively while a metal-reducing Gram-negative strain, *Shewanella oneidensis*, was also used.

In this study, methods like disk diffusion test, minimal inhibitory concentration (MIC) determination, CFU measurement, live/dead viability assay, Microarray hybridization and analysis were used, and the obtained results indicate the antibacterial ability of these nanoparticles against *B. subtilis* and *E. coli* at concentrations in the range of 50 to 150 mg/L in minimal media M9. Growth inhibition was not observed in LB broth media that may be because of the release of bacterial compounds in media and inhibiting nanoparticle aggregation around and into the bacterial cells. No effects were observed on *S. oneidensis* growth [13]. The antibacterial activity of nanoparticles depends on different parameters like size, dispersion rate, concentration, media and cell wall composition of bacteria [13, 14].

3.3 Reactive oxygen species scavenging

Along with many other applications, nanoparticles were also used in the medical field as anti-inflammatory and anti-oxidant agents. These nanoparticles actually act as ROS (reactive oxygen species) scavengers and hence are of great importance in the treatment of cancer therapy and their non-toxic behaviour, even at high concentrations, make them promising substances in nanobiotics. Singh et al.[3] suggested the role of ceria nanoparticles in the treatment of neurodegenerative disorders like trauma, ageing, Parkinson’s and Alzheimer’s diseases and many others. The antioxidant properties of these nanoparticles make them novel nano-pharmaceutics in near future.

Hence, antioxidant properties of these CeO_2 nanoparticles were also explored and results confirmed that the reduced ability of these nanoparticles is an indication of their antioxidant potential [48]. Fig. 8 illustrates the graphical representation of the gradual increase in reduction activity with a gradual increase in concentration. Antioxidant activity of CeO_2 nanoparticles was also reported in other studies and they act as intracellular ROS scavengers [74] therefore it can be predicted that these particles can be used for anti-cancer and anti-inflammatory drugs preparation however, data related to the antioxidant potential of these nanoparticles and about toxic effects is still not sufficient and extensive research is required in this area [75, 76].

3.4 Mutagenicity analysis

Genotoxic tests both *in-vitro* and *in-vivo* is used for the identification of compounds that cause genetic damage for example DNA and chromosomal damage or gene mutations etc. Compounds for which genotoxic test results are positive could be human carcinogenic and mutagenic as they have the potential to cause cancer or heritable defects Food and Drug Administration Authority (FDA), USA and many other regulatory agencies have some recommended series of toxicological tests (including mutagenicity testing) required for the regulation of newly commercialized products (e.g; pharmaceutics, food items and other chemicals) prior to their release into the market. Mutagenesis is a spontaneous change in genetic material due to chemical or physical agents in the next generation that is different in a heritable way from their predecessor.

Genotoxicity testing is useful for the detection of carcinogenic potential of different chemical compounds. The Ames test developed from the screening and selection of numerous histidine/ tryptophan mutants that were sensitive to reverse mutation by a variety of chemical mutagens[77,

78]. The test was particularly designed to detect those chemical substances that can cause mutations. The test has worldwide importance as an initial screening test to find out the mutagenic potential of new chemicals and pharmaceutical drugs because there is a high extrapolative value for rodent carcinogenicity when we obtain a mutagenic response.

Ames test is one of the most commonly practiced methods used to determine the mutagenicity of different chemicals. In the present study, the mutagenicity of CeO_2 nanoparticles at three different concentrations (50, 150 and 500 µg/ plate) was determined and the result showed the non-mutagenic behaviour of CeO_2 nanoparticles. The results of CeO_2 nanoparticles are presented in Table 1. A comparison of CeO_2 nanoparticles with a positive and negative control in Fig.10 clearly indicates the non-mutagenic nature of these nanoparticles. To date, no studies related to the evaluation of CeO_2 nanoparticles mutagenicity by Ames test is present and more work is needed to evaluate the mutagenic level of these nanoparticles.

3.5 *In-vivo* toxicological findings

Model organisms (mice, rats and rabbits etc.) are routinely used to assess the safety levels of food and drug additives, pesticides, fertilizers and industrial chemicals. Short term and long term exposures through different modes are used to determine the toxicity of the chemical compound. The *in-vivo* studies are performed under the strict guidelines of FDA (Food and Drug Administration) and other regulatory agencies (OECD or OCED). Acute Fish toxicity test was performed and effects of different concentrations (1, 50 and 100 mg/L) were observed and results indicate that CeO_2 nanoparticles are non-toxic at concentration $\leq$100 mg/L as no mortality or change in behaviour was observed during the exposure time (96 h) (Table 2). Data related to fish toxicity (*in-vivo*) is not available.

However, Hoecke et al. [78] also reported the nontoxic behaviour of these nanoparticles in aquatic toxicity assessment of CeO_2 nanoparticles for *Thamnocephalus platyurus, Daphnia magna* and embryos of *Danio rerio* (zebra fish). Comet assay and micronucleus test may be useful in determining the damage at DNA or Chromosomal level. CeO_2 nanoparticles were non-toxic even at a concentration of 5000 mg/Kg when administered orally in rats. Food and water intake, weekly body weight measurements and daily behaviour observation showed no difference between rats of test groups to the control group (untreated). All rats were healthy and alive. No clinical or behavioural changes were observed. Meanwhile, some nanomaterials are very toxic at a very low dose 2000 μg/mL of nano-graphene oxide in mice [79], in the same way remarkable toxicities were observed at higher concentrations of 1–100 μg/mL of cadmium based quantum dots in mice [80]. Therefore, the results of current study revealed that CeO_2 nanoparticles were non-toxic. Although systematic experiments are required for complete risk assessment of CeO_2 nanoparticles at different dose levels.

Another approach, Intraperitoneal LD_{50}, was also used to determine the toxic status of CeO_2 nanoparticles. Rats were injected intraperitoneal up to a max concentration of 500mg/kg and observed for mortality and behavioural changes for a time period of 24 h. All rats survived and showed no difference in behavioural changes to the control group (Table 3). Comet assay, micronucleus test, and blood analysis can be more helpful techniques in determining the effects of these CeO_2 nanoparticles on the organism [81] reported the first *in-vivo* cytotoxic study of europium-doped Gd_2O_3 (a member of lanthanide series) nanotubes in mice that were injected intraperitoneally. According to Liu et al. [81], Gadolinium (Gd) is non-toxic at low and medium

doses but shows some changes in blood chemistry at high doses and also at higher concentrations its accumulation in the spleen and kidney causes damage to these tissues.

Rats were sacrificed and blood was used for blood biochemistry and haematological studies. Treated and control groups showed no differences in haematology response variables. All parameters fall within the accepted limits of normal variation (Table 4). No significant differences were observed in serum biochemical parameters in tested and control groups as all the parameters fall within the accepted limits of normal variations. The values of different parameters are given in Table 5. Comet assay, micronucleus test, and tissue histopathology can be more useful in determining the risk assessment of these nanoparticles. To date, no details about Oral LD_{50} of CeO_2 nanoparticles are available but this study gives an idea that CeO_2 nanoparticles cause no acute illness when administered orally even at high concentrations.

Chen et al. [82], reported the oral LD_{50} for Copper that is 413 mg/kg and they also investigated and discovered that LD_{50} was the same for nano and micro-sized CuO. In-vivo toxicological studies of different other metal oxides have been carried out and scientists have reported the toxic and nontoxic behaviour of these compounds. Different in-vivo toxicological studies for orally administered nanoparticles (metals and their oxides) are available for Al_2O_3, Fe_2O_3, ZnO and CuO. Different nano-sized metal and their oxides can cause chromosomal aberrations, oxidative damage to DNA, breaks and mutations in the DNA strand. However, due to the presence of many contradictory factors in literature, it's not easy to draw any conclusion about the toxic nature of these nano-materials

As CeO_2 nanoparticles absorb UV, therefore, they are used in UV filters and can be used in UV blocking creams [55, 56]. To determine the effect of CeO_2 nanoparticles on the skin an acute dermal irritation test was carried out. Rabbit skin was observed for dermal irritation after 1 h, 24 h and daily observations up to the end of the observation period (14 days). No significant or minor effects like Oedema or Erythma were observed on the skin and daily activities of rabbits also remained unaffected Fig. 11. These results suggest that the application of these CeO_2 nanoparticles on the skin is not dangerous. Although no *in-vivo* studies are present in the literature to support this finding however in-vitro studies on different cell lines indicate that short term exposure to these nanoparticles is non-toxic [83].

4 Conclusion

In conclusion, CeO_2 nanoparticles were synthesized successfully by the alkaline diffusion method. UV-visible Spectrophotometry, Field Emission Scanning Electron Microscopy, Atomic Force Microscopy and X-ray Diffraction Spectroscopy revealed the spherical shape of CeO_2 nanoparticles with an average size of 40 nm. This small size and higher surface area of synthesized CeO_2 nanoparticles showed enhanced antibacterial (*E. coli*) and antioxidant activity. Ames test confirms the non-mutagenic effects of these nanoparticles. CeO_2 nanoparticles proved nontoxic even at a higher concentration by toxicology evaluation in aquatic life (fish) and results revealed that the CeO_2 nanoparticles showed no toxicity for *Cyprinus carpio* even at a concentration of 100 mg/L. Acute *in-vivo* toxicity assessment indicates the non-hazardous behaviour of these nanoparticles against mice, rat and rabbit and *in-vivo* toxicology was not evident even at the highest concentration ($\geqslant$5000 mg/kg BW for rats), no significant change was observed in haematological and biochemical parameters of control and CeO_2 nanoparticles exposed rats. *In-vivo* dermal toxicity was not observed in rabbits at the application of 0.5 g CeO_2 nanoparticles. The current

study provides the baseline information regarding CeO_2 nanoparticles which will be helpful for the application of these nanomaterials. Furthermore, acute, sub-acute, chronic, *in-vitro* and genotoxicity testing can enhance the safety of CeO_2 nanoparticles for useful applications such as their role in nano-biotics, cancer therapeutics and solid oxide fuel cells. However, similar comprehensive studies are required for other nanomaterials for their risk assessment.

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References

1. Yousefi, T., A.N. Golikand, and M.H. Mashhadizadeh, Synthesis and characterization of cerium oxide nano-particles in chloride bath: Effect of the H2O2 concentration and bath temperature on morphology. Materials science in semiconductor processing, 2013. **16**(6): p. 1943-1948.
2. Liu, X., et al., A stearic Acid/CeO2 bilayer coating on AZ31B magnesium alloy with superhydrophobic and self-cleaning properties for corrosion inhibition. Journal of Alloys and Compounds, 2020. **834**: p. 155210.
3. Singh, S., et al., A phosphate-dependent shift in redox state of cerium oxide nanoparticles and its effects on catalytic properties. Biomaterials, 2011. **32**(28): p. 6745-6753.
4. Tan, J., et al., Facile preparation of Mn-doped CeO2 Submicrorods by composite-hydroxide-salt-mediated approach and their magnetic property. Materials Research, 2013. **16**(4): p. 689-694.
5. Wu, M., et al., Vacuum ultraviolet (VUV)-based photocatalytic oxidation for toluene degradation over pure CeO2. Chemical Engineering Science, 2019. **200**: p. 203-213.
6. Calvache-Muñoz, J. and J.E. Rodríguez-Páez, Removal of Rhodamine 6G in the absence of UV radiation using ceria nanoparticles (CeO2-NPs). Journal of Environmental Chemical Engineering, 2019: p. 103518.
7. Miseki, Y. and K. Sayama, Photocatalytic water splitting for Solar hydrogen production using the carbonate effect and the Z-scheme reaction. Advanced Energy Materials, 2019. **9**(23): p. 1801294.
8. Sadeghifar, H. and A. Ragauskas, Lignin as a UV light blocker—a review. Polymers, 2020. **12**(5): p. 1134.
9. Kalaycıoğlu, Z., et al., Antibacterial nano cerium oxide/chitosan/cellulose acetate composite films as potential wound dressing. European Polymer Journal, 2020. **133**: p. 109777.
10. Tyagi, P.K., et al., Estimation of toxic effects of chemically and biologically synthesized silver nanoparticles on human gut microflora containing Bacillus subtilis. Journal of Toxicology Environmental Health Sciences, 2013. **5**(9): p. 172-177.
11. Alheety, M.A., et al., Ag(I)-benzisothiazolinone complex: synthesis, characterization, H2 storage ability, nano transformation to different Ag nanostructures and Ag nanoflakes antimicrobial activity. Materials Research Express, 2019. **6**(12): p. 125071.
12. Wason, M.S. and J. Zhao, Cerium oxide nanoparticles: potential applications for cancer and other diseases. American journal of translational research, 2013. **5**(2): p. 126.
13. Pelletier, D.A., et al., Effects of engineered cerium oxide nanoparticles on bacterial growth and viability. Applied environmental microbiology, 2010. **76**(24): p. 7981-7989.
14. Villa, S., et al., Natural molecule coatings modify the fate of cerium dioxide nanoparticles in water and their ecotoxicity to Daphnia magna. Environmental Pollution, 2020. **257**: p. 113597.
15. Solanki, P.R., et al., Nanostructured cerium oxide film for triglyceride sensor. Sensors Actuators B: Chemical, 2009. **141**(2): p. 551-556.
16. Akbari, A., et al., Zinc-doped cerium oxide nanoparticles: Sol-gel synthesis, characterization, and investigation of their in vitro cytotoxicity effects. Journal of Molecular Structure, 2017. **1149**: p. 771-776.

17. Vissers, C., G.-l. Ming, and H. Song, Nanoparticle technology and stem cell therapy team up against neurodegenerative disorders. Advanced drug delivery reviews, 2019. **148**: p. 239-251.
18. Spivak, N.Y., et al., Ceria nanoparticles boost activity of aged murine oocytes. Nano Biomedicine & Engineering, 2012. **4**(4).
19. Nyoka, M., et al., Synthesis of cerium oxide nanoparticles using various methods: implications for biomedical applications. Nanomaterials, 2020. **10**(2): p. 242.
20. Lord, M.S., et al., Cellular uptake and reactive oxygen species modulation of cerium oxide nanoparticles in human monocyte cell line U937. Biomaterials, 2012. **33**(31): p. 7915-7924.
21. Abdalla, A.M., et al., Current challenges of cancer anti-angiogenic therapy and the promise of nanotherapeutics. Theranostics, 2018. **8**(2): p. 533.
22. Amani, H., et al., Antioxidant nanomaterials in advanced diagnoses and treatments of ischemia reperfusion injuries. Journal of Materials Chemistry B, 2017. **5**(48): p. 9452-9476.
23. Ni, D., et al., Ceria nanoparticles meet hepatic ischemia-reperfusion injury: the perfect imperfection. Advanced Materials, 2019. **31**(40): p. 1902956.
24. Gothandam, K., et al., Environmental Biotechnology Vol. 3. 2020: Springer.
25. Salas, P., et al., The role of size and protein shells in the toxicity to algal photosynthesis induced by ionic silver delivered from silver nanoparticles. Science of The Total Environment, 2019. **692**: p. 233-239.
26. Jin, Z., et al., Dopamine-modified poly (styrene) nanospheres as new high-speed adsorbents for copper-ions having enhanced smoke-toxicity-suppression and flame-retardancy. Journal of Colloid and Interface Science, 2021. **582**: p. 619-630.
27. Lu, J., et al., TiO2 nanoparticles enhanced bioaccumulation and toxic performance of PAHs via trophic transfer. Journal of Hazardous Materials, 2021. **407**: p. 124834.
28. Zhu, P., et al., Enhanced photocatalytic NO removal and toxic NO2 production inhibition over ZIF-8-derived ZnO nanoparticles with controllable amount of oxygen vacancies. Chinese Journal of Catalysis, 2021. **42**(1): p. 175-183.
29. Puerari, R.C., et al., Acute and chronic toxicity of amine-functionalized SiO2 nanostructures toward Daphnia magna. Ecotoxicology and Environmental Safety, 2021. **212**: p. 111979.
30. Qu, X., et al., Defect enrich ultrathin TiO2 nanosheets for rapid adsorption and visible light mediated PPCPs degradation. Chemosphere, 2021. **268**: p. 128782.
31. Souza, M.R., R. Mazaro-Costa, and T.L. Rocha, Can nanomaterials induce reproductive toxicity in male mammals? A historical and critical review. Science of The Total Environment, 2021: p. 144354.
32. Gaiser, B.K., et al., Interspecies comparisons on the uptake and toxicity of silver and cerium dioxide nanoparticles. Environmental Toxicology Chemistry, 2012. **31**(1): p. 144-154.
33. Gaiser, B.K., et al., Assessing exposure, uptake and toxicity of silver and cerium dioxide nanoparticles from contaminated environments. Environmental Health, 2009. **8**(1): p. S2.
34. Chen, B., et al., Integration of sub-organ quantitative imaging LA-ICP-MS and fractionation reveals differences in translocation and transformation of CeO2 and Ce3+ in mice. Analytica chimica acta, 2019. **1082**: p. 18-29.

35. Cotena, M., et al., In Vitro Co-Exposure to CeO2 Nanomaterials from Diesel Engine Exhaust and Benzo (a) Pyrene Induces Additive DNA Damage in Sperm and Cumulus Cells but Not in Oocytes. Nanomaterials, 2021. **11**(2): p. 478.
36. Hamidian, K., et al., Photocatalytic performance on degradation of Acid Orange 7 dye using biosynthesized un-doped and Co doped CeO2 nanoparticles. Materials Research Bulletin, 2021. **138**: p. 111206.
37. Arnold, M., et al., Cerium oxide nanoparticles are more toxic than equimolar bulk cerium oxide in Caenorhabditis elegans. Archives of environmental contamination toxicology, 2013. **65**(2): p. 224-233.
38. Roy, B., et al., Antibiotic tetracycline enhanced the toxic potential of photo catalytically active P25 titanium dioxide nanoparticles towards freshwater algae Scenedesmus obliquus. Chemosphere, 2021. **267**: p. 128923.
39. El-Belely, E., et al., Green Synthesis of Zinc Oxide Nanoparticles (ZnO-NPs) Using Arthrospira platensis (Class: Cyanophyceae) and Evaluation of their Biomedical Activities. Nanomaterials 2021, 11, 95. 2021, s Note: MDPI stays neu-tral with regard to jurisdictional clai-ms in
40. Alheety, M.A., et al., Eco-friendly C60-SESMP-Fe3O4 inorganic magnetizable nanocomposite as high-performance adsorbent for magnetic removal of arsenic from crude oil and water samples. Materials Chemistry and Physics, 2019. **231**: p. 292-300.
41. Alheety, M.A., et al., A novel synthesis of MnO2, nanoflowers as an efficient heterogeneous catalyst for oxidative desulfurization of thiophenes. Nano-Structures & Nano-Objects, 2019. **20**: p. 100392.
42. Abd, A.N., A.H. Al-Agha, and M.A. Alheety, Addition of some primary and secondary amines to graphene oxide, and studying their effect on increasing its electrical properties. Baghdad Science Journal, 2016. **13**(1).
43. Jin, H., et al., Synthesis and conductivity of cerium oxide nanoparticles. Materials Letters, 2010. **64**(11): p. 1254-1256.
44. Tiwari, A.K., et al., 4S consideration (synthesis, sonication, surfactant, stability) for the thermal conductivity of CeO2 with MWCNT and water based hybrid nanofluid: An experimental assessment. Colloids and Surfaces A: Physicochemical and Engineering Aspects, 2021. **610**: p. 125918.
45. Xie, L., et al., Mechanical wear behavior between CeO2 (100), CeO2 (110), CeO2 (111), and silicon studied through atomic force microscopy. Tribology International, 2021. **153**: p. 106616.
46. Poornaprakash, B., et al., Enhanced photoluminescence characteristics and intrinsic ferromagnetism in Co-substituted CeO2 nanoparticles. Materials Science in Semiconductor Processing, 2021. **123**: p. 105566.
47. Sebastiammal, S., et al., Annona muricatainspired synthesis of CeO2 nanoparticles and their antimicrobial activity. Materials Today: Proceedings, 2019. **9**: p. 627-632.
48. Tian, X., et al., Exploration of the hepatoprotective chemical base of an orally administered herbal formulation (YCHT) in normal and CCl4-intoxicated liver injury rats. Part 2: hepatic disposition in vivo and hepatoprotective activity in vitro. Journal of ethnopharmacology, 2019. **236**: p. 161-172.
49. Anjum, R., et al., Assessment of mutagenic potential of pyrolysis biochars by Ames Salmonella/mammalian-microsomal mutagenicity test. Ecotoxicology and environmental safety, 2014. **107**: p. 306-312.

50. Zhang, X., Y. Peng, and C. Wu, Chicken embryonic toxicity and potential in vitro estrogenic and mutagenic activity of carvacrol and thymol in low dose/concentration. Food and Chemical Toxicology, 2021. **150**: p. 112038.
51. Li, X., et al., Impact of CeO2 nanoparticles on the aggregation kinetics and stability of polystyrene nanoplastics: Importance of surface functionalization and solution chemistry. Water Research, 2020. **186**: p. 116324.
52. Zhu, K., et al., Antagonistic effect of zinc oxide nanoparticle and surfactant on anaerobic digestion: Focusing on the microbial community changes and interactive mechanism. Bioresource technology, 2020. **297**: p. 122382.
53. Al-Janabi, A.S., A. Osama'a, and M.A. Alheety, Novel Mercury (II) 1-Phenyl-1H-tetrazol-5-thiol and carbon nanotube complexes: synthesis, characterization and H2 storage capacities. Chemical Data Collections, 2020. **28**: p. 100399.
54. Majeed, A.H., et al., Poly (o-Phenylenediamine-GO-TiO2) nanocomposite: modulation, characterization and thermodynamic calculations on its H2 storage capacity. Chemical Data Collections, 2020. **28**: p. 100450.
55. Truffault, L., et al., Application of nanostructured Ca doped CeO2 for ultraviolet filtration. Materials Research Bulletin, 2010. **45**(5): p. 527-535.
56. Masui, T., et al., Characterization of cerium (IV) oxide ultrafine particles prepared using reversed micelles. Chemistry of Materials, 1997. **9**(10): p. 2197-2204.
57. Abid, S.A., et al., Antibacterial and cytotoxic activities of cerium oxide nanoparticles prepared by laser ablation in liquid. Environmental Science and Pollution Research, 2020. **27**: p. 30479-30489.
58. Sohn, M., et al., Effect of emollients on UV filter absorbance and sunscreen efficiency. Journal of Photochemistry and Photobiology B: Biology, 2020. **205**: p. 111818.
59. Miri, A., M. Darroudi, and M. Sarani, Biosynthesis of cerium oxide nanoparticles and its cytotoxicity survey against colon cancer cell line. Applied Organometallic Chemistry, 2020. **34**(1): p. e5308.
60. Nithya, P. and M. Sundrarajan, Ionic liquid functionalized biogenic synthesis of AgAu bimetal doped CeO2 nanoparticles from Justicia adhatoda for pharmaceutical applications: Antibacterial and anti-cancer activities. Journal of Photochemistry and Photobiology B: Biology, 2020. **202**: p. 111706.
61. Sehar, S., et al., Thermodynamic and kinetic study of synthesised graphene oxide-CuO nanocomposites: A way forward to fuel additive and photocatalytic potentials. Journal of Molecular Liquids, 2020. **313**: p. 113494.
62. Rasheed, S., et al., Hydrothermally engineered Ni–CuC hybrid nanocomposites: Structural and morphological investigations with potential fuel catalytic applications. Materials Chemistry and Physics, 2021: p. 124837.
63. Sadiq, H., et al., Green synthesis of ZnO nanoparticles from Syzygium Cumini leaves extract with robust photocatalysis applications. Journal of Molecular Liquids, 2021. **335**: p. 116567.
64. Batalha, I.L., et al., Polymeric nanobiotics as a novel treatment for mycobacterial infections. Journal of Controlled Release, 2019. **314**: p. 116-124.
65. Wagi, S. and A. Ahmed, Bacterial nanobiotic potential. Green Processing and Synthesis, 2020. **9**(1): p. 203-211.
66. Awwad, A.M., et al., Green synthesis, characterization of silver sulfide nanoparticles and antibacterial activity evaluation. Chem. Int, 2020. **6**(1): p. 42-48.

67. Salam, F.D., et al., Anti-bacterial and anti-biofilm efficacies of bioinspired gold nanoparticles. Materials Letters, 2020. **261**: p. 126998.
68. Menazea, A. and M. Ahmed, Silver and copper oxide nanoparticles-decorated graphene oxide via pulsed laser ablation technique: Preparation, characterization, and photoactivated antibacterial activity. Nano-Structures & Nano-Objects, 2020. **22**: p. 100464.
69. Jannesari, M., et al., Graphene/CuO2 Nanoshuttles with Controllable Release of Oxygen Nanobubbles Promoting Interruption of Bacterial Respiration. ACS Applied Materials & Interfaces, 2020. **12**(32): p. 35813-35825.
70. Nourmohammadi, E., et al., Evaluation of anticancer effects of cerium oxide nanoparticles on mouse fibrosarcoma cell line. Journal of cellular physiology, 2019. **234**(4): p. 4987-4996.
71. Naz, S., et al., Cerium oxide nanoparticles: a 'radical'approach to neurodegenerative disease treatment. Nanomedicine, 2017. **12**(5): p. 545-553.
72. Rocca, A., et al., Pilot in vivo investigation of cerium oxide nanoparticles as a novel anti-obesity pharmaceutical formulation. Nanomedicine: nanotechnology, biology and medicine, 2015. **11**(7): p. 1725-1734.
73. Thill, A., et al., Cytotoxicity of CeO2 nanoparticles for Escherichia coli. Physico-chemical insight of the cytotoxicity mechanism. Environmental science & technology, 2006. **40**(19): p. 6151-6156.
74. Lord, M.S., et al., Anti-angiogenic activity of heparin functionalised cerium oxide nanoparticles. Biomaterials, 2013. **34**(34): p. 8808-8818.
75. Kashyap, D., et al. Natural product-based nanoformulations for cancer therapy: Opportunities and challenges. in Seminars in cancer biology. 2019. Elsevier.
76. Hirst, S.M., et al., Anti-inflammatory properties of cerium oxide nanoparticles. Small, 2009. **5**(24): p. 2848-2856.
77. Maron, D.M. and B.N. Ames, Revised methods for the Salmonella mutagenicity test. Mutat Res, 1983. **113**(3-4): p. 173-215.
78. Hoecke, K.V., et al., Fate and effects of CeO2 nanoparticles in aquatic ecotoxicity tests. Environmental science technology, 2009. **43**(12): p. 4537-4546.
79. Akhavan, O., et al., Dose-dependent effects of nanoscale graphene oxide on reproduction capability of mammals. Carbon, 2015. **95**: p. 309-317.
80. Akhavan, O., et al., Influence of heavy nanocrystals on spermatozoa and fertility of mammals. Materials Science and Engineering: C, 2016. **69**: p. 52-59.
81. Liu, H., et al., Biodistribution and toxicity assessment of europium-doped Gd 2 O 3 nanotubes in mice after intraperitoneal injection. Journal of nanoparticle research, 2014. **16**(3): p. 2303.
82. Chen, Z., et al., Acute toxicological effects of copper nanoparticles in vivo. Toxicology letters, 2006. **163**(2): p. 109-120.
83. De Marzi, L., et al., Cytotoxicity and genotoxicity of ceria nanoparticles on different cell lines in vitro. International journal of molecular sciences, 2013. **14**(2): p. 3065-3077.

List of Tables

Table 1. The number of reverse mutants induced by CeO_2 nanoparticles to *S. typhimurium.*

Dose (µg/ plate)	Number of revertants/ Plate (Mean ± SD*)	
	TA98	**TA100**
NC	41.23 ± 2.09	152.4 ± 3.08
50	35.34 ± 3.1	160.37 ± 5.2
100	39.7 ± 4.5	158. 54 ± 3.8
500	46.56 ± 2.8	165.12 ± 4.1
PC	546.2 ± 5. 1	821.20 ± 3.1

*SD = standard deviation, NC = negative control (Distilled water), PC = Positive control (TA98:$K_2Cr_2O_7$, TA100: NaN_3**)**

632

633 **Table 2.** Impact of CeO_2 nanoparticles and nitric acid on the behavioural pattern of *Cyprinus*
634 *carpio* L., 1758 (common crap) exposed to different concentrations up to 96 h.

Parameters	**Vehicle (0.1%)**	**Nonnano-ceria (mg/L)**			**CeO_2 nanoparticles (mg/L)**		
	HNO_3	1	50	100	1	50	100
Anxiety	-	-	-	-	-	-	-
Rate of swimming	-	-	-	-	-	-	-
Loss of balance	-	-	-	-	-	-	-
Lying laterally	-	-	-	-	-	-	-
Opercular activity	-	-	-	-	-	-	-
Movements in circular form with jerks	-	-	-	-	-	-	-

635
636 HNO_3 = nitric acid, - (sign indicates normal behaviour) +, ++, +++ (signs indicate the levels of parameters).
637

638 **Table 3.** Acute Intraperitoneal LD_{50} of CeO_2 nanoparticles.

Group		Mean body weight (gm)	Cumulative Mortality	Acute intraperitoneal LD50 (mg/kg)
Test (CeO_2 NP)	Female	198± 7	0/6	> 500
Control (normal diet)	Female	185±2	0/6	> 500

639 Note: Data based on females in the group (n=6/group)

640

641 **Table 4.** Haematological parameters of control and CeO_2 nanoparticles (5000 mg/kg) exposed
642 mice.

Parameters	Treated group	Control
Haemoglobin (g/ dl)	15.8 ± 0.14	14.7 ± 1.4
Haematocrite (%)	36.85 ± 0.77	36.65 ± 4.16
Total RBC count (M/ µL)	6.58 ± .09	6.51 ± 0.7
M.C.V.	55.95 ± 0.56	56.42 ± 2.43
M.C.H (PG)	24 ± 0.5	22.58 ± 1.04
M.C.H.C (G/dl)	42.9 ± 0.56	40.01 ± 0.88
Total WBC count (K/ µL)	6.1 ± 1.13	9.27 ± 3.63
Neutrophiles (%)	10 ± 7.07	14.3 ± 4.2
Lymphocytes (%)	90 ± 7.07	82.3 ± 3.4
Monocytes (%)	0	1.5 ±1.2
Eosinophils (%)	0	1.83 ± 0.98
Basophils (%)	0	0
platelets count (K/ µL)	663.5 ± 23.3	655.67 ± 70.9
ESR (mm)	5.5 ± 0.7	6.67 ± 1.6

643

644

645 **Table 5.** Clinical biochemistry evaluations of CeO_2 nanoparticles exposed mice.

Blood biochemistry	**Treated**	**Control**
Blood urea (mg/dl)	21.3 ± 5.6	18 ± 4.07
Serum creatinine (mg/dl)	0.46 ± 0.05	0.4 ± 0.11
ALT (SGPT) (U/L)	60.33 ± 13. 79	57.4 ± 5.70
Blood glucose random (mg/dl)	98.33 ± 10.4	95.5 ± 13.5
Blood urea nitrogen (BUN) (mg/ dl)	10 ± 2.64	8 ± 1.80

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List of Figure

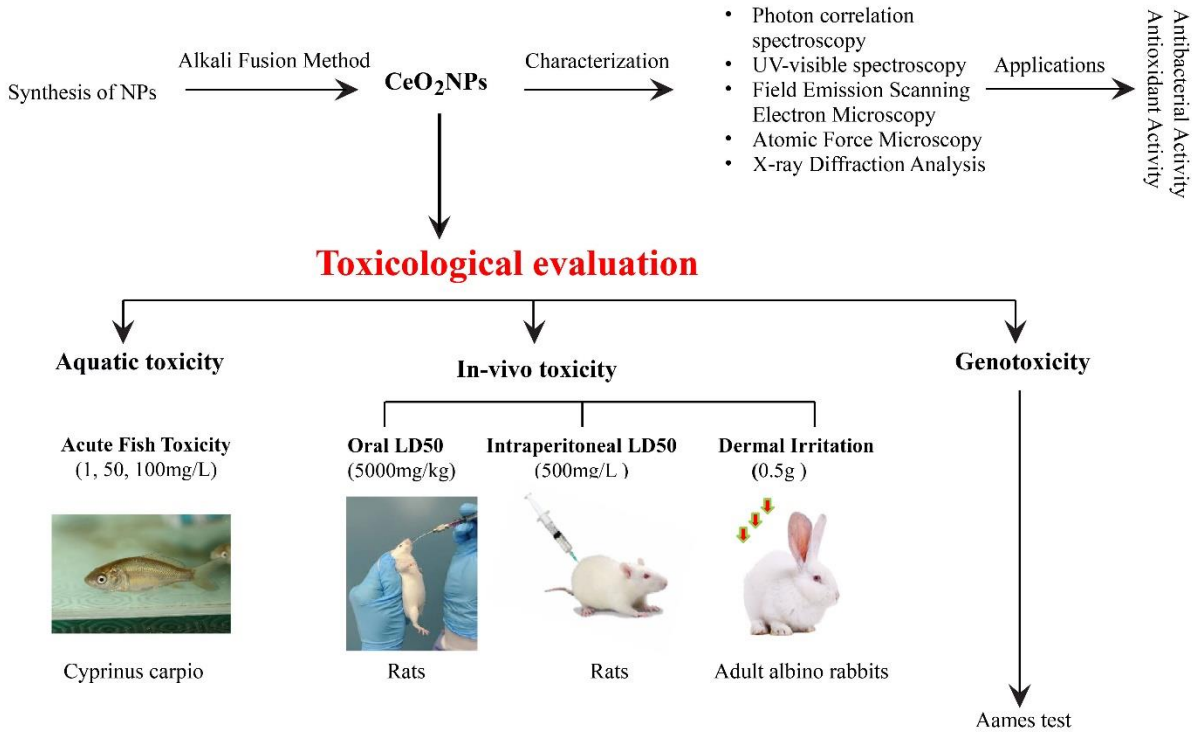


Fig. 1. Schematic diagram showing the present study layout.

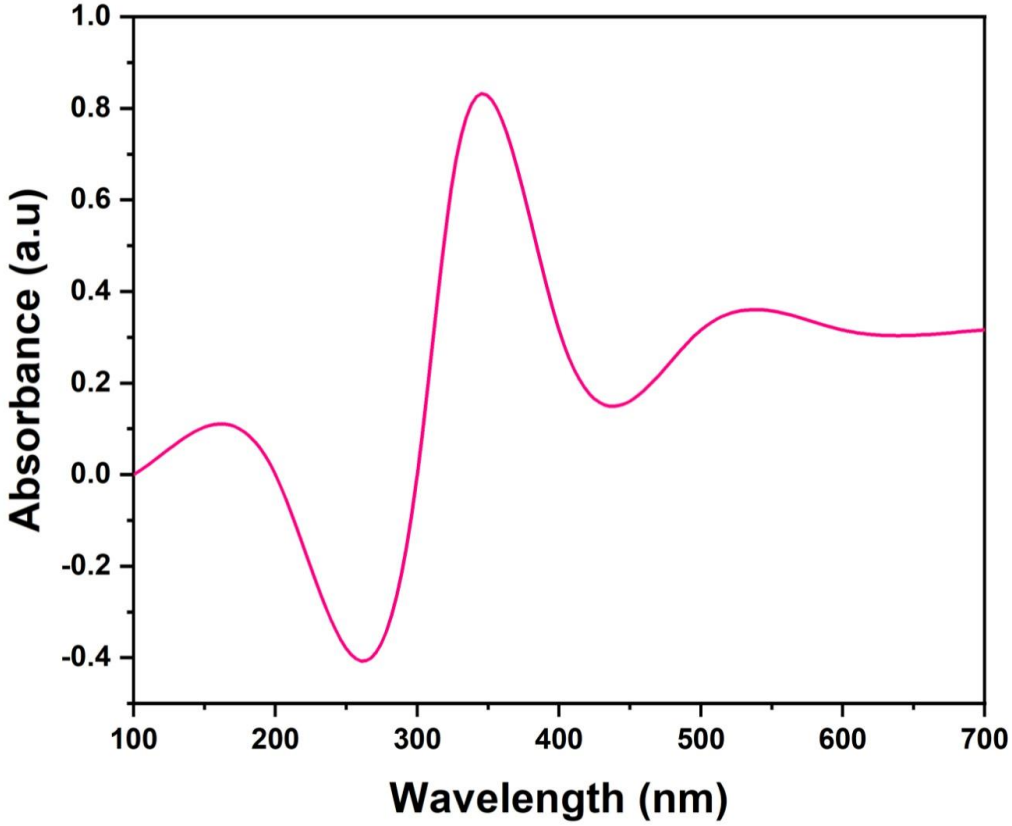
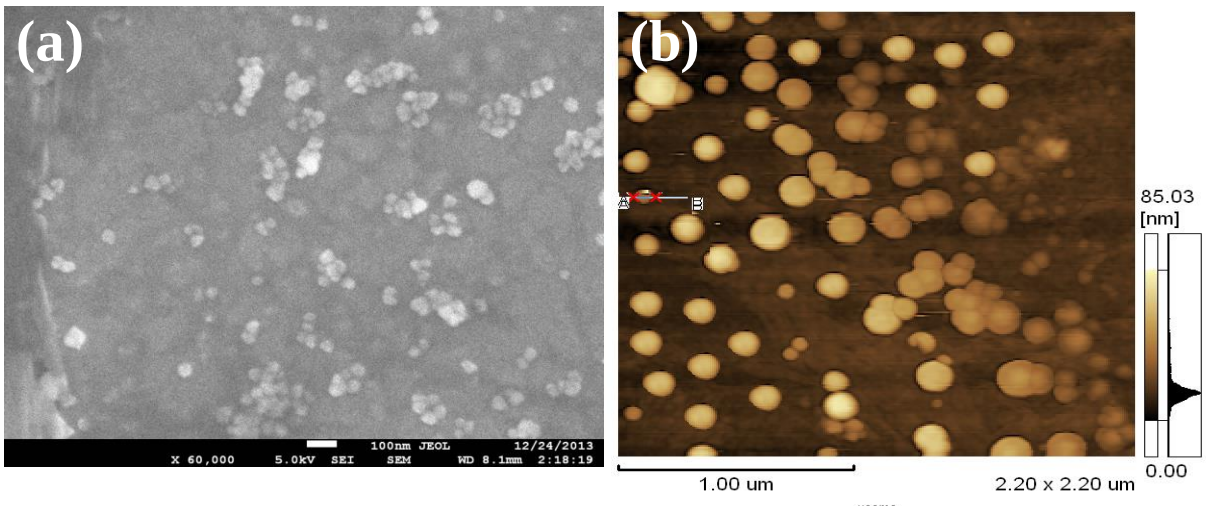


Fig. 2. UV-Visible spectrophotometry showing the UV absorbance by CeO_2 nanoparticles in a range of 310-400 nm with the highest peak at 324 nm.



659

660 **Fig. 3.** Morphological analysis of CeO_2 nanoparticles synthesized by alkaline fusion method; (a)
661 FESEM image and (b) AFM topographic image.
662

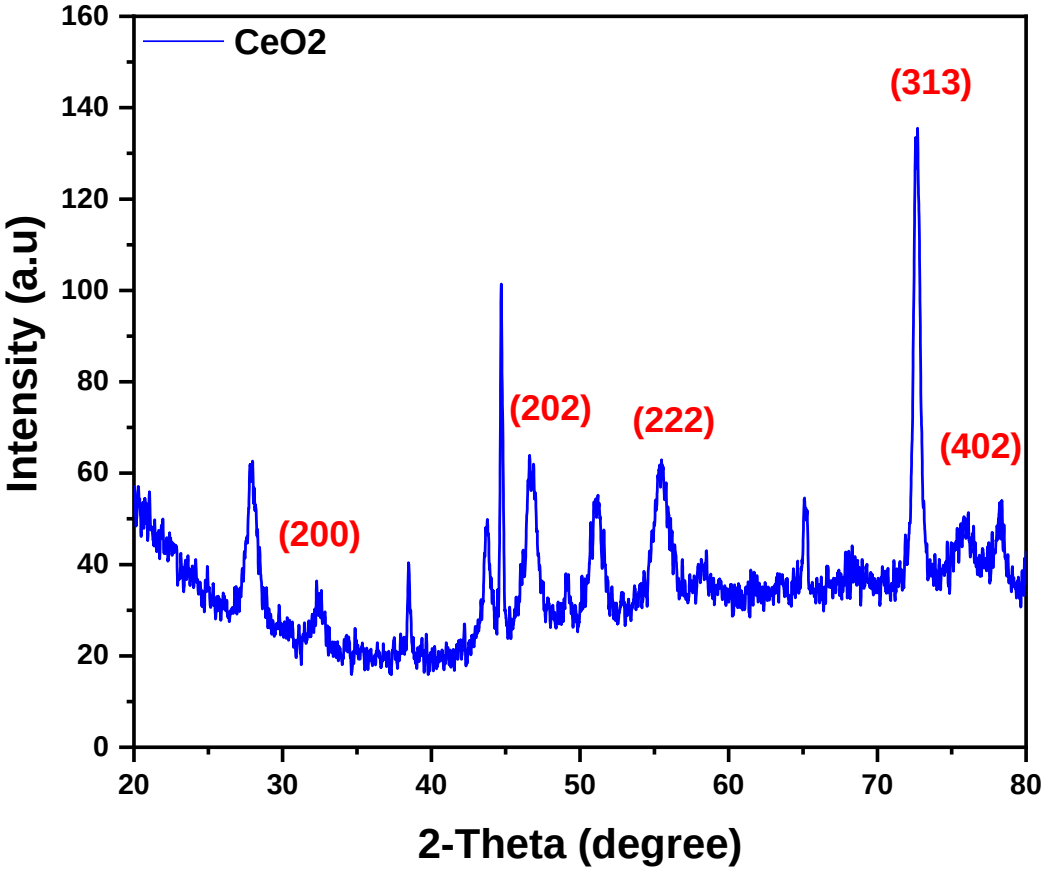


Fig. 4. X-ray diffraction pattern of ceria nanoparticles shows different peaks.

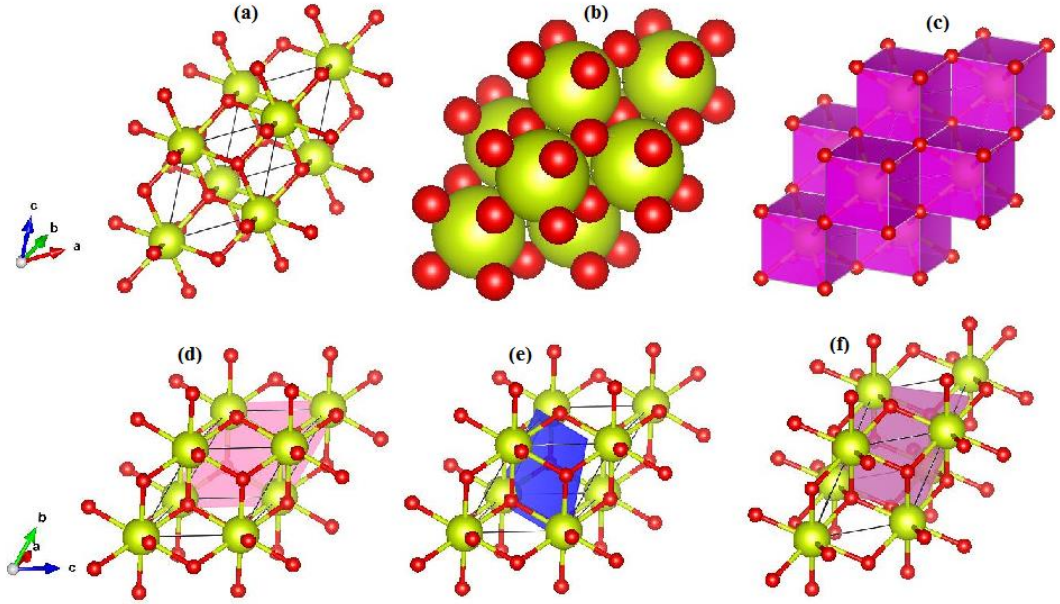
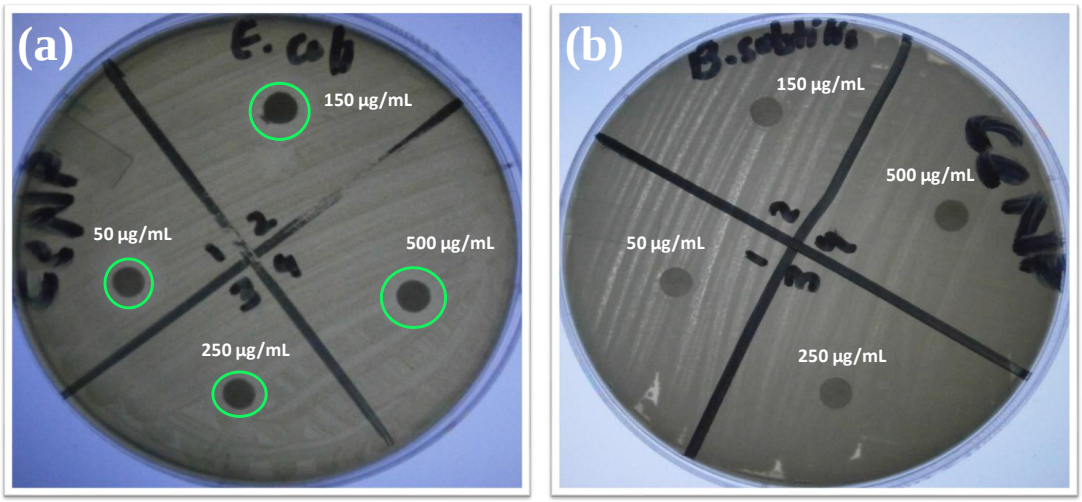


Fig. 5. Structural representation; (a) Positions of Ce atoms bonded with O atoms in a cubic unit cell, (b) Doted surface of unit cell atoms, (c) Polyhedrons in a unit cell, (d) Ce bonding with O at interstitial positions, (e) (222) Plane, (f) (313) Plane, (g) (402) Plane and (h) All planes in one cubic unit cell at 3.51 Å from origin.



678

679 **Fig. 6.** Circles show the zone of inhibition against different concentrations of ceria nanoparticles
680 in plate seeded with (a) *E. coli* (b) *B. subtilis*.
681

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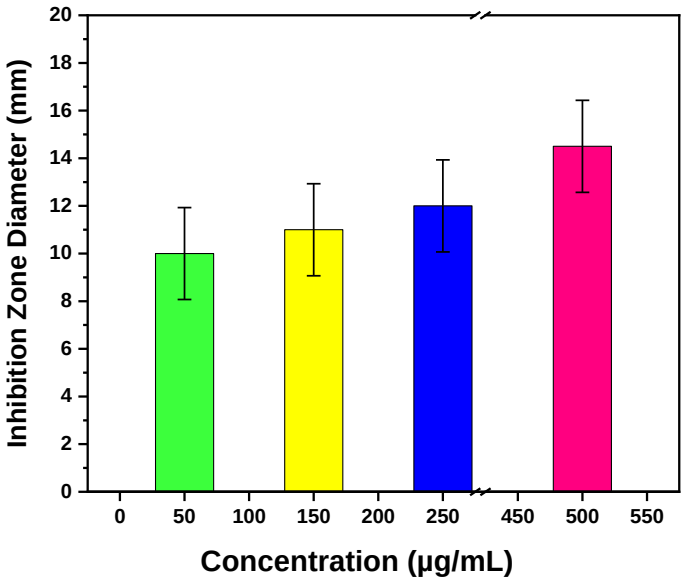

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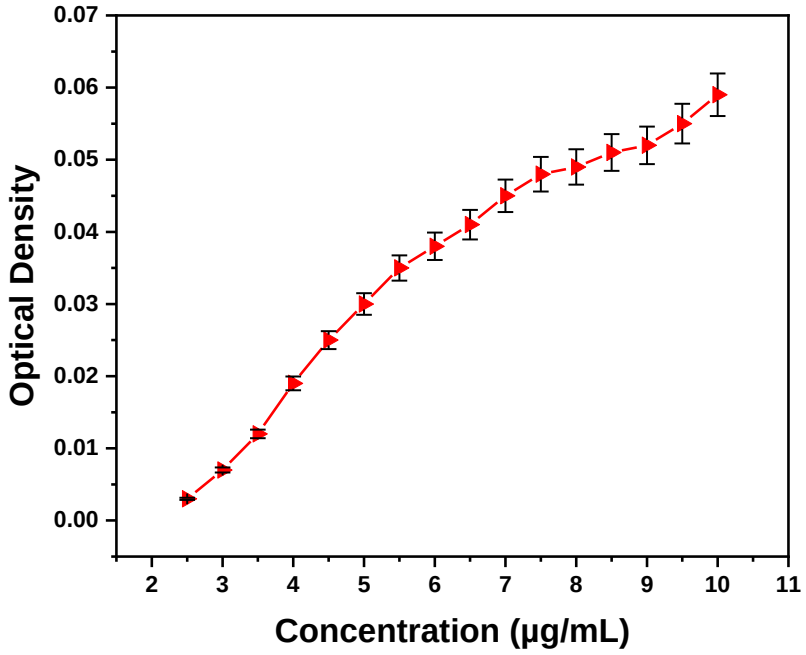
685

686 **Fig. 7.** Graphical representation of antibacterial activity showing a greater zone of inhibition with
687 increasing concentration (DIZ: diameter of inhibition zone).
688



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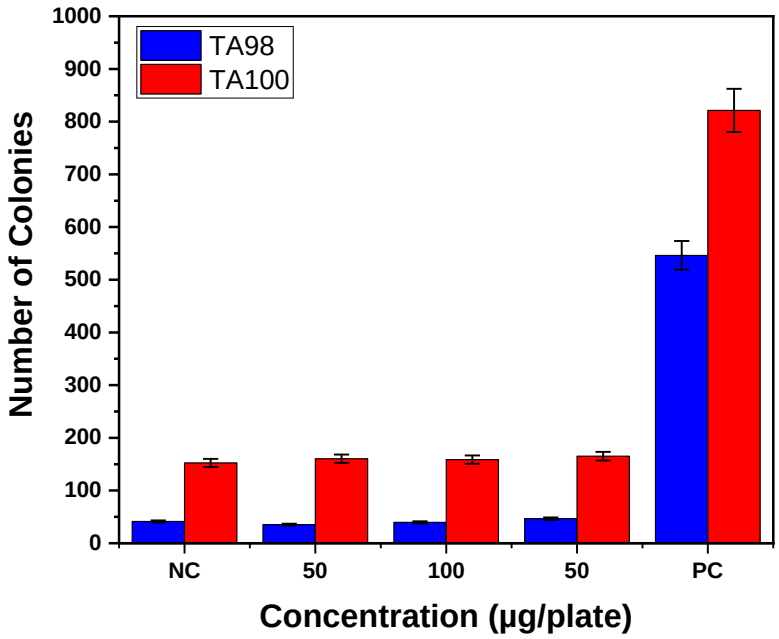
690

691 **Fig. 8.** A line graph showing the direct association of reduction ability with an increase in the
692 concentration of CeO_2 nanoparticles (OD: Optical density).
693



694

695 **Fig. 9.** The number of reverse mutants induced by CeO_2 nanoparticles to *S. typhimurium* TA98
696 and TA100.
697

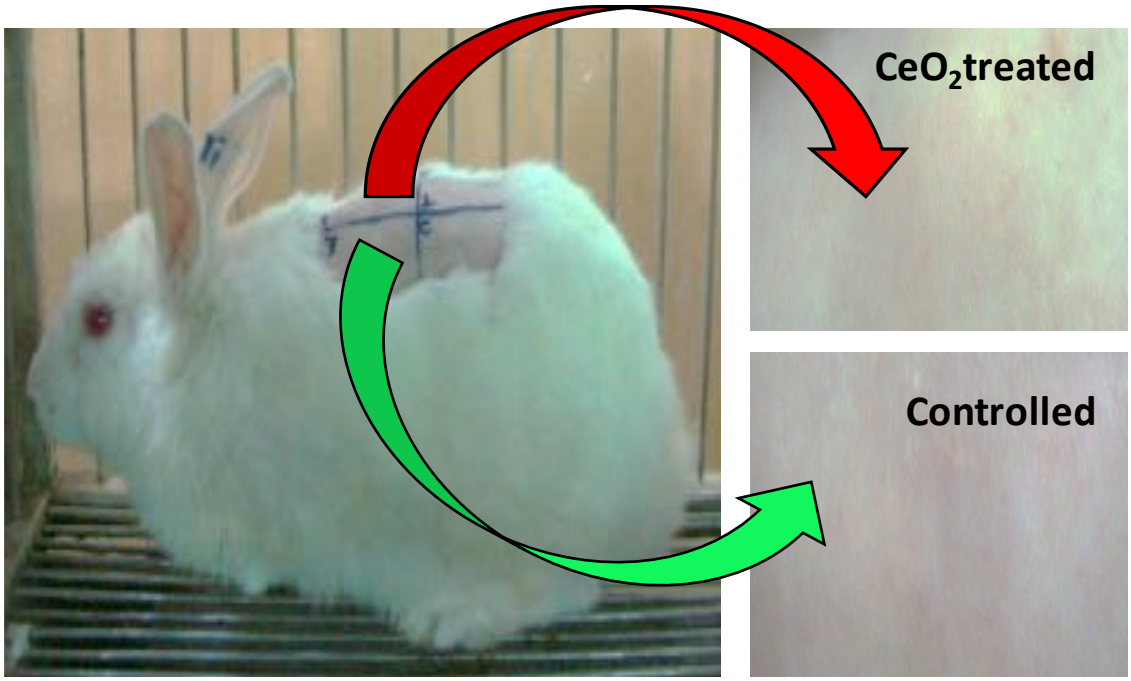


Fig. 10. No significant or minor dermal irritation like edema/erythema after 14 days. Treated with CeO_2 and control (normal saline).