Mutation Research 775–776 (2014) 7–19

Contents lists available at ScienceDirect

Mutation Research/Genetic Toxicology and Environmental Mutagenesis

journal homepage: www.elsevier.com/locate/gentox
Community address: www.elsevier.com/locate/mutres

Genotoxicity assessment of cerium oxide nanoparticles in female Wistar rats after acute oral exposure

Monika Kumari[a,b], Srinivas Indu Kumari[a], Sarika Srinivas Kalyan Kamal[c], Paramjit Grover[a,*]

[a] Toxicology Unit, Biology Division, Indian Institute of Chemical Technology, Hyderabad 500 007, Andhra Pradesh, India
[b] Department of Genetics, Osmania University, Osmania University Main Road, Hyderabad 500007, Andhra Pradesh, India
[c] Defence Metallurgical Research Laboratory, Kanchanbagh, Hyderabad 500058, Andhra Pradesh, India

ARTICLE INFO

Article history:
Received 26 May 2014
Received in revised form
19 September 2014
Accepted 22 September 2014
Available online 2 October 2014

Keywords:
CeO_2 nanoparticles
CeO_2 microparticles
Genotoxicity
Biochemical
Biodistribution
Wistar rats

ABSTRACT

Cerium oxide nanoparticles (CeO_2 NPs; nanoceria) have demonstrated excellent potential for commercial use in various arenas, such as in biomedical industry in cosmetics and as a fuel additive. However, limited knowledge exists regarding their potential toxicity. In this study, acute oral toxicity of CeO_2 NPs and their microparticles (MPs; bulk) was carried out in female albino Wistar rats. The CeO_2 NPs and CeO_2 MPs were characterized utilizing transmission electron microscopy (TEM), dynamic light scattering (DLS) and laser Doppler velocimetry (LDV) for the size, distribution and surface charge respectively. The genotoxicity studies were conducted using micronucleus test (MNT), comet and chromosomal aberration (CA) assays. Results revealed that at high dose (1000 mg/kg bw) CeO_2 NPs induced significant DNA damage in peripheral blood leukocytes (PBL) and liver cells, micronucleus formation in bone marrow and blood cells and total cytogenetic changes in bone marrow. However, significant genotoxicity was not observed at 500 and 100 mg/kg bw of CeO_2 NPs. The findings from biochemical assays depicted significant alterations in ALP and LDH activity in serum and GSH content in liver, kidneys and brain only at the high dose of CeO_2 NPs. Tissue biodistribution of both particles was analyzed by inductively coupled plasma optical emission spectrometer (ICP-OES). Bioaccumulation of nanoceria in all tissues was significant and dose-, time- and organ-dependent. Moreover, CeO_2 NPs exhibited higher tissue distribution along with greater clearance in large fractions through urine and feces than CeO_2 bulk, whereas, maximum amount of micro-sized CeO_2 got excreted in feces. The histopathological examination documented alterations in the liver due to exposure with CeO_2 NPs only. Hence, the results suggest that bioaccumulation of CeO_2 NPs may induce genotoxic effects. However, further research on long term fate and adverse effects of CeO_2 NPs is warranted.

1. Introduction

Nanomaterials (NMs) have been defined as natural, incidental or manufactured materials, in different states and in the size range of 1–100 nm [1]. These nanoparticles (NPs) commonly have different physico-chemical and electronic properties including much higher specific surface area, high surface reactivity and increased quantum effects, as a function of their size from those of the corresponding microparticles (MPs; bulk) [2]. The properties of NPs also include enhanced reactivity and greater ability to penetrate tissues and cell membranes [3]. Nanotechnological progress can enhance the quality of life, but it can also raise societal concerns as unavoidable perceived risks cannot be ignored. Thus, MPs that are considered safe need to be tested when in 'nano' form for their toxic effects. Several important health risks of NPs in human tissues such as lung,

Abbreviations: CeO_2, cerium oxide; NPs, nanoparticles; MPs, microparticles; TEM, transmission electron microscopy; DLS, dynamic light scattering; LDV, laser Doppler velocimetry; OECD, organization for economic co-operation and development; MNT, micronucleus test; CA assay, chromosomal aberration assay; ANOVA, analysis of variance; MN-PCEs, micronucleated polychromatic erythrocytes; PCEs, polychromatic erythrocytes; MI, mitotic index; TA, total aberrations; ROS, reactive oxygen species; ALP, alkaline phosphatase; LDH, lactate dehydrogenase; GSH, reduced glutathione; ICP-OES, inductively coupled plasma optical emission spectrometer.

* Corresponding author. Tel.: +91 40 27193135; fax: +91 40 27193227.
E-mail addresses: paramgrover@gmail.com, grover@iict.res.in, paramjit.grover@gmail.com (P. Grover).

http://dx.doi.org/10.1016/j.mrgentox.2014.09.009
1383-5718/

liver, kidneys and nervous system occur through the inhalation of nanoscale aerosols, contact with nano-structured surfaces, or by the consumption of foods with nanosized colloids [4]. Cerium oxide (CeO_2) is a rare earth oxide material from the lanthanide series of the periodic table which has been recently introduced for specialty applications. In CeO_2, a fraction of cerium (Ce) is in the Ce^{3+} form. The reduction in positive charge is compensated by a corresponding number of oxygen vacancies. The Ce^{3+}/Ce^{4+} redox reactions are responsible for the outstanding biological properties of CeO_2 NPs [5]. Owing to their large surface area to volume ratio, CeO_2 NPs (nanoceria) have a unique electronic structure and the reduction in the particle size results in the formation of surface oxygen vacancies, which endows it with the ability to exist in either Ce^{3+} or Ce^{4+} state on the particle surface [6]. The CeO_2 NPs are used in pharmaceutical industry [7], in nanotherapeutics [8], as polishing agents [9], UV-absorbing compounds in sunscreen [10] and UV-scattering agents in non-irritating lipsticks [11]. Nanoceria play several catalytic roles such as catalysts in the petroleum refining industry, as additives to promote combustion of diesel fuels [12], as subcatalysts for automotive exhaust cleaning [13] and as electrolytes in solid oxide fuel cells [14]. The broad range of applications of CeO_2 NPs increases the risk of human exposure and interactions with a variety of environmental factors with unknown health and safety implications. Organization for Economic Co-operation and Development (OECD) in the Environment Directorate added CeO_2 NPs in the list of 14 NMs for testing and identified it as commercially relevant to the global economic impact of nanotechnology and suggested that the most common route of CeO_2 exposure is likely to be through inhalation and/or ingestion [15]. There are several *in vitro* studies with CeO_2 NPs and their MPs in various cell lines [16–19]. Moreover, there are studies which have investigated CeO_2 NPs induced toxicity through different routes of exposure in rats such as inhalation [20], intratracheal instillation [21] and intravenous (iv) administration [22]. Gastrointestinal region is one of the most important portals of entry for NPs in humans and animals [3]. Hirst et al. [8] reported the biodistribution of nanoceria and their antioxidant capabilities after per-oral administration to mice. Nevertheless, there are no reports on the genotoxicity of CeO_2 NPs and their bulk on the animal system through oral route. Hence, this gave us the impetus to conduct an acute oral toxicity study to provide more inclusive information with regard to the fate of CeO_2 NPs and CeO_2 bulk after exposure to the gastrointestinal system and associated health effects. It is of utmost importance to investigate the physico-chemical characteristics of NMs followed by toxicological implications in the biological system. Therefore, in the present investigation we performed transmission electron microscope (TEM) analysis, dynamic light scattering (DLS) and laser Doppler velocimetry (LDV) studies to know the size, mean hydrodynamic diameter and zeta potential of CeO_2 NPs respectively. Acute oral toxicity study of CeO_2 NPs and CeO_2 MPs was performed following the "fixed dose method" in female albino Wistar rats. Further, genotoxicity, biochemical and biodistribution studies were conducted after acute oral treatment with CeO_2 NPs and its bulk. The genotoxicity studies included the most used comet assay, micronucleus test (MNT) and chromosomal aberration (CA) assay to depict the mechanisms of toxicity [23]. The comet assay is a versatile, simple and sensitive method to study the toxicity study of a broad range of compounds and is capable of measuring DNA damage in almost all organisms and cells [24]. In order to assess the clastogenic, aneugenic and epigenetic effects of compounds, the MNT is used to analyze the dysfunction of mitotic apparatus and chromosome damage. The CA test diagnoses agents that can cause numerical aberrations, structural chromosome or chromatid breaks, dicentrics and other abnormal chromosomes such as translocations which are implicated in the various human genetic diseases and cancers [23]. Therefore, the comet assay in the peripheral blood leukocytes (PBL) and liver, MNT in the blood and bone marrow and CA test in the bone marrow cells was carried out. Biochemical studies were performed to assess the activity of alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) in serum for liver toxicity, loss in cell membrane integrity and cell death respectively. Reduced glutathione (GSH) is the predominant antioxidant in the aqueous cytoplasm of cells and its production is primarily in the liver [25]. Hence, GSH content in liver, kidneys and brain of the treated rats was evaluated to get an insight of the oxidative stress status of the system. The biodistribution study of NMs is essential to assess the uptake and retention of NPs that enter target tissues or sites and for determining the anatomic fate, clearance and biological effects of these substances. Therefore, the effects of the test compounds on biodistribution were analyzed in the whole blood, liver, kidneys, heart, brain, spleen, urine and feces of rats using inductively coupled plasma optical emission spectrometer (ICP-OES). In the present study, the lowest treatment dose was intended to reflect the level of potential human exposure. However, the highest dose was utilized to obtain toxicity through accidental exposure to large amounts of CeO_2 NPs and to obtain detectable amounts of Ce after distribution in the animal as well. There are studies on aluminum oxide, zinc oxide (ZnO) and titanium oxide (TiO_2) NPs in which similar high doses were used to assess the toxicological effects through different routes [26–28].

2. Materials and methods

2.1. Nanoparticles and chemicals

CeO_2 NPs (CeO_2 < 25 nm, 99.95%, CAS No. 1306-38-3), CeO_2 MPs (CeO_2 < 5 μm, 99.90%, CAS No. 1306-38-3) were purchased from Sigma Chemical Co. Ltd. (St. Louis, MO, USA). Phosphate buffered saline (Ca^{2+}, Mg^{2+} free; PBS), cyclophosphamide (CP), normal melting agarose (NMA), low melting agarose (LMA) *etc.*, were also purchased from Sigma Chemical Co. Ltd. Other chemicals were purchased from Himedia, Mumbai, India.

2.2. Characterization of CeO_2 NPs and CeO_2 MPs

The particles were characterized using TEM, DLS and LDV to evaluate the size of the materials, size distribution, state of dispersion and zeta potential of the NMs in the Milli-Q water. Characterization of CeO_2 NPs and CeO_2 MPs was performed to assess the size and morphology using TEM (JEM-2100, JEOL, Japan). The images were obtained from TEM with an accelerating voltage of 120 kV. The TEM was equipped with a plunge freezer and cryo transfer holder to fix specimens in the frozen state and fitted with a Gatan 2Kx2K CCD camera for acquiring high-resolution images. Particles were suspended in Milli-Q water at a concentration of 0.01 mg/ml, and one drop of suspension was placed on a carbon-coated copper TEM grid and evaporated at room temperature. The software for advanced microscopy techniques was used for the digital TEM camera. This software was calibrated for nanoscale size measurements for the accurate examination of NPs. For the size measurement, 100 particles were calculated from random fields of view and images showing the general morphology of the particles. The size and surface charge of the CeO_2 NPs in Milli-Q water suspension was measured through DLS and LDV using a Malvern Zetasizer Nano-ZS (Malvern Instruments, United Kingdom). This device uses a 4 mW He–Ne 633 nm laser to analyze the samples and an electric field generator for the LDV measurements. The suspensions of freshly prepared CeO_2 NPs and MPs in Milli-Q water at the concentration of 40 μg/ml were ultra-sonicated using a probe sonicator (UPH 100, Germany) for 10 min at 90% amplitude. Further, the suspensions were diluted and adjusted to a lower concentration to acquire enough counts per second. The prepared samples were transferred to a 1.5 ml square cuvette for DLS measurements, and 1 ml of the suspension was transferred to a Malvern Clear Zeta Potential cell for LDV measurement. The mean NPs diameter was calculated using the same software program as utilized in the NPs distribution and the polydispersity index (PdI) was used to measure the size present in the solution. The PdI scale ranges from 0 to 1, where 0 corresponds to monodispersed and 1 corresponds to polydispersed state of particles.

2.3. Animals

Female albino Wistar rats, aged 6–8 weeks and weighing 80–120 g, were obtained from the National Institute of Nutrition, Hyderabad, India. The animals were acclimatized for 1 week in groups of five in polypropylene cages. The animals were fed with a standard laboratory pellet diet and reverse osmosis water was provided *ad libitum* and maintained under standard conditions of humidity (55–65%), temperature (22 ± 3 °C) and light (12 h light/12 h dark cycles). The study

was approved by the Institutional Animal Ethics Committee. Ethical clearance number for this study is IICT/BIO/TOX/PG/28/05/2012.

2.4. Acute oral toxicity study

The acute oral toxicity of CeO_2 NPs and CeO_2 MPs was assessed in accordance with the OECD guideline 420, known as the "acute oral toxicity-fixed dose method" [29]. According to the sighting study, a single rat was treated with an initial 5 mg/kg body weight (bw) dose. In case of no adverse symptoms and mortality, a second rat received a 50 mg/kg bw dose, followed by 300 mg/kg bw dose and a final dose of 2000 mg/kg bw in sequence. Rats were fasted overnight prior to dosing. There were no apparent toxic symptoms and mortality at any dose level in the sighting study, therefore, a limit test was carried out with four female rats using 2000 mg/kg bw dose for CeO_2 NPs and CeO_2 MPs. The test animals were kept under observation for a period of 14 days after dosing. The feed intake and bw was monitored daily during the observation period. Mortality, signs and symptoms if any, were recorded twice on the day of dosing and once each day thereafter. At the end of the test period the surviving animals were weighed and sacrificed for necropsy studies. Raw data (feed intake and bw) was noted daily for 14 days. Heart, liver, kidneys, spleen and brain tissues were collected for relative organ weight profile as well as histopathology studies.

2.5. Histopathological evaluation

Histopathological studies were conducted in the different organs *i.e.* heart, liver, spleen, brain and kidneys of female albino Wistar rats after acute exposure to 2000 mg/kg bw CeO_2 NPs and CeO_2 MPs to assess the changes in the tissue anatomy. After sacrifice, tissues were washed with 1% ice cold saline and fixed in 10% neutral buffered formalin. The formalin-fixed tissues were processed in a Leica TP 1020 tissue processor and then embedded in paraffin blocks using Leica EG 1160 paraffin embedder. The paraffin blocks were sliced into ribbons of 3 μm thick sections using a Microm HM 360 microtome and mounted on a glass microscope slide. After that, slides were stained in hemotoxylin and eosin (H & E) using a Microm HMS-70 stainer and examined with Nikon Eclipse E 800 microscope at ×400 magnification. A minimum of three random sections per slide and at least three different fields were assessed for histopathological damage.

2.6. Experimental design: genotoxicity, biochemical and biodistribution studies

The rats were randomly divided into three groups namely, positive control (for the genotoxicity studies), negative control and experimental groups. The experimental groups were further divided into three subgroups based on the acute toxicity data. Three different dose levels of CeO_2 NPs and CeO_2 MPs, (100, 500, and 1000 mg/kg bw), were used to assess genotoxicity, biochemical parameters and biodistribution. All groups contained five animals per test per sampling time. The doses for the experimental groups were obtained after diluting various doses of CeO_2 NPs and CeO_2 MPs in Milli-Q water after sonication using a probe sonicator for 10 min at 90% amplitude. The control groups were treated with 2 ml/kg bw of Milli-Q water, and the experimental groups were treated with CeO_2 NPs and CeO_2 MPs (100, 500, and 1000 mg/kg bw) through oral gavage. A known mutagen, CP was used as positive control at a dose of 40 mg/kg bw. The required volume was diluted up to 1 ml in Milli-Q water and injected intraperitoneally (ip) to rats under positive control group. All rats received a single dose and were sacrificed through cervical dislocation at the indicated sampling times. For the genotoxicity and biodistribution studies, sampling times were 4, 18, 24, 48 and 72 h based on assay guidelines. For biochemical parameters tissues samples were harvested at 24 and 72 h post treatment.

2.6.1. Comet assay

The alkaline comet assay was used for the assessment of DNA damage in the rats after acute exposure to the CeO_2 NPs and CeO_2 MPs. It was performed according the method described by Tice et al. [30] with slight modifications. Whole blood and liver samples were collected after 4, 24, 48 and 72 h after dosing. Blood withdrawal was done in EDTA coated tubes from retro-orbital plexus of rats. The comet assay in liver tissue was performed following the technique described by Miyamae et al. [31]. The tissue was removed from the rats after sacrificing at various time intervals, minced and suspended at ~100 mg/ml in chilled homogenizing buffer (pH 7.5) and homogenized gently at a speed of 500–800 rpm. Cell viability was determined by the trypan blue exclusion assay [32]. For each slide preparation, 10 μl of both whole blood (10,000–30,000 lymphocytes) and liver homogenate was used. Three slides were prepared for each experimental condition. In brief, microscope slides were pre-coated with 120 μl of 0.75% NMA in PBS and allowed to solidify overnight at 37 °C after putting a cover slip for a uniform layer. The second layer was prepared after pipetting 120 μl of 0.37% LMA containing blood or liver cells on the pre-coated slides and dried at 4 °C for 10 min. A third layer of plain 0.37% LMA (120 μl) was applied and a cover slip was quickly put to get an even layer and dried at 4 °C. After removing the cover slip, the slides were immersed in chilled lysis buffer (2.5 M NaCl, 0.1 M Na_2 EDTA, 0.2 M NaOH, 1% Triton X-100, 10% DMSO, pH 10.0) for 10 h at 4 °C. The slides were pre-soaked for 20 min in alkaline buffer (10 M NaOH, 200 mM Na_2 EDTA, pH > 13.0) and then electrophoresis was performed at 25 V adjusted at 300 mA for 20 min. The slides were neutralized twice in 0.4 M Tris buffer, pH 7.5, for 5 min and once in absolute methanol for 5 min. Coded slides were scored after staining with ethidium bromide (20 μg/ml) using a fluorescence microscope (Olympus, Shinjuku-ku, Tokyo, Japan) with a blue (488 nm) excitation filter and yellow (515 nm) emission (barrier) filter at ×400 magnification. A total of 150 randomly selected cells per rat (50 cells per slide) were used to measure the amount of DNA damage and expressed as percentage of DNA in the comet tail. Quantification of DNA breakage was realized by using a Comet Image Analysis System, version Komet 5.5 (Single cell Gel Electrophoresis analysis company, Andor Technology 2005, Kinetic Imaging Ltd., Nottingham, UK).

2.6.2. Micronucleus test

The MNT in the rat bone marrow cells was carried out following the method described by Schmid [33]. The bone marrow cells were extracted from thigh bone of rats following acute oral exposure for 24 and 48 h to CeO_2 NPs and CeO_2 MPs. The bone marrow was removed from both femurs by aspiration into hypotonic solution of 1% sodium citrate and centrifuged at approximately 1000 rpm for 5 min. The cell pellet was resuspended in a drop of 1% sodium citrate and a smear was prepared on a microscope slide and allowed to dry in humidified air overnight. The MNT in peripheral blood (PB) cells was performed according to the protocol described by Celik et al. [34] with some modifications and according to the OECD Guideline 474 [35]. Whole blood was collected from retro-orbital plexus of each rat at 48 and 72 h after the treatment from each group. Smears were prepared on clean microscope slides and air dried. The slides were fixed in methanol for 2 min and stained with 0.5% Giemsa stain (prepared in PBS) for 3 min. The stained slides were used for the assessment of the micronucleus (MN) occurrence. Three slides were made for each animal. The slides were microscopically analyzed at ×1000 magnification. Randomly, 2000 polychromatic erythrocytes (PCEs) per animal were selected from three slides and the frequencies of micronucleated PCEs (MN-PCEs) were recorded. In order to determine the ratio of PCEs to normochromatic erythrocytes (NCEs) in the bone marrow and PB cells, approximately 1000 cells from each animal were examined and the ratio was expressed as percentage: (PCEs × 100/PCEs + NCEs).

2.6.3. Chromosomal aberration assay

The method described by OECD Guideline 475 [36] and Adler [37] was used for CA analysis. The analysis was performed after 18 h and 24 h of oral treatment with the various doses (100, 500, 1000 mg/kg bw) of CeO_2 NPs and CeO_2 MPs in female Wistar rats. For analysis of cells in metaphase, cell division was arrested with a 0.01 ml/g bw dose of a mitotic inhibitor colchicine (0.020%) administered ip, 2 h prior to sacrifice. The bone marrow was collected from both femur and tibia bones in a hypotonic solution of 0.9% sodium citrate and centrifuged at 2000 rpm for 20 min. Cells were then fixed through three changes of ice-cold Carnoy's solution (methanol:acetic acid, 3:1 v/v) until the pellets were clean. After refrigeration for at least 24 h, cells were centrifuged and resuspended in fresh fixative *i.e.* Carnoy's solution. Two drops of the final cell suspension were dropped from a height of 30–40 cm onto a chilled slide kept in refrigerator for at least 1 h. The slides were gently blown and dried on a warm plate at 40 °C. Three slides for each animal were prepared and stained with 1% Giemsa for 2 h. Five hundred well spread metaphases per dose (100 per animal) were selected to detect the presence of CAs. The mitotic index (MI) was determined with 1000 or more cells.

2.6.4. Alkaline phosphatase activity assay

Serum (30 μl) collected by centrifuging blood without any anticoagulant at 1500 rpm for 10 min was used for the determination of ALP activity. This assay is based on kinetic reaction. The activity of ALP was determined following *p*-nitrophenyl phosphate (PNPP) method described in the diagnostic kit from Siemens Ltd., Vadodara, Gujarat. This method utilizes *p*-nitrophenyl phosphate that is hydrolyzed by ALP into a yellow colored product *p*-nitrophenol having maximum absorbance at 405 nm. The rate of reaction is directly proportional to the enzyme activity. The absorbance of the test was read against blank at 405 nm at intervals of 30 s for 2 min using a spectrophotometer (Spectra Max Plus, Molecular Devices, Sunnyvale, CA, USA). The enzyme activity was expressed as U/L using molar extinction coefficient of 18.75/mM/cm.

2.6.5. Lactate dehydrogenase activity

The activity of LDH was estimated in serum according to the procedures described by McQueen [38]. In a quartz cuvette, 1000 μl of Sorensen phosphate buffer, 100 μl NADH and 20 μl of serum was added and mixed well. Next, 150 μl sodium pyruvate was added and again mixed well. The absorbance was measured at 340 nm at 10 s interval for 2 min using a spectrophotometer. The LDH activity was expressed as μmoles/h/ml using molar extinction coefficient of 6.3/mM/cm.

2.6.6. Reduced glutathione content

The GSH content was measured using the method of Jollow et al. [39]. After 24 and 72 h of single acute oral exposure with CeO_2 NPs and CeO_2 MPs, rats were sacrificed and liver, kidneys and brain were removed. One gram of tissue from liver, kidneys and brain of treated and control rats was rinsed in ice cold physiological saline, perfused with cold KCl buffer (1.15% KCl and 0.5 mM EDTA) and homogenized in potassium phosphate buffer (KPB, 0.1 M, pH 7.4) using a Miccra D-1-high speed tissue homogenizer. An aliquot of 0.5 ml of each tissue homogenate was incubated with 0.5 ml of sulfosalicylic acid (4% w/v) for 1 h in ice and centrifuged at

Table 1
Characterization of cerium oxide nanoparticles (CeO_2 NPs) and CeO_2 microparticles (MPs).

Particles	Size using TEM	DLS		LDV		
		Average diameter (nm)	PdI	Zeta potential, ζ (mV)	Electrophoretic mobility (μm·cm/s V)	pH
CeO_2 NPs	23.2 ± 1.92 nm	189.9 ± 17.1	0.299	−17.0	−1.15	7.0
CeO_2 MPs	3.26 ± 1.14 μm	ND	ND	ND	ND	7.0

CeO_2 NPs and CeO_2 MPs at the concentration of 40 μg/ml were dispersed in Milli-Q water and mixing was done *via* probe sonication for 10 min just before estimations. PdI, Polydispersity Index; DLS, Dynamic Light Scattering; LDV, Laser Doppler Velocimetry; ND, not detectable.

10,000 rpm for 10 min. The 0.4 ml aliquot of supernatant was mixed with 0.4 ml of DTNB (4 mg/ml in 5% sodium citrate) and 2.2 ml KPB (0.1 M, pH 7.4). The yellow color developed was read at 412 nm. The amount of GSH present was expressed as μg GSH/g wet weight of tissue.

2.6.7. Cerium content analysis in different tissues, blood, urine and feces

A biodistribution study of the CeO_2 NPs and CeO_2 MPs in the female Wistar rats was carried out in whole blood, liver, kidneys, heart, brain, spleen, urine and feces. The animals were placed in metabolic cages after treatment to collect the urine and feces samples and these samples were collected after 4, 24, 48 and 72 h of dosing. The samples were processed using the method of Gomez et al. [40]. The samples were pre-digested in nitric acid overnight, then heated at 80 °C for 10 h, followed by additional heating at 130–150 °C for 30 min. Subsequently, a volume of 0.5 ml of 70% perchloric acid was added, the samples were then again heated again for 4 h and evaporated nearly to dryness. Following digestion, the samples were filtered and 2% HNO_3 was added to a final volume of 5 ml for analysis. The standard solution of Ce was serially diluted to 100, 50, 10, 1 ppm. The wavelength of 418.66 nm was found to get intensity of the samples. The Ce content in the samples was determined using ICP-OES (JY Ultima, Jobin Vyon, France).

2.7. Statistical analysis

The statistical significant changes between treated and control groups were analyzed by one-way ANOVA. All results were expressed as mean and standard deviation (mean ± SD) of the mean. Multiple pair-wise comparisons were done using the Dunnett's multiple comparison post test and Student's '*t*' test to verify the significance of positive response. Statistical analyses were performed using GraphPad Instat Prism 3 Software package for windows (GraphPad Software, Inc., La Jolla, CA, USA). The statistical significance for all tests was set at $p < 0.05$.

3. Results

3.1. Characterization of CeO_2 NPs and CeO_2 MPs

The physico-chemical characteristics of CeO_2 particles were determined by TEM, DLS and LDV analysis. The data obtained is shown in Table 1. The mean size of CeO_2 NPs and CeO_2 MPs was calculated using TEM by measuring over 100 particles in random field. The size obtained of CeO_2 NPs and CeO_2 MPs was 23.2 ± 1.92 nm (Fig. 1A) and 3.26 ± 1.14 μm (Fig. 1B) respectively. The particles were found to be formed of polyhedron crystals. The hydrodynamic diameter and PdI of CeO_2 NPs in Milli-Q water suspension obtained by DLS was 189.9 ± 17.1 nm and 0.299 respectively. The result of DLS showed larger values than the NPs size measured by TEM, indicating that CeO_2 NPs formed larger agglomerates in water suspension. Zeta potential (ζ) and electrophoretic mobility of CeO_2 NPs in Milli-Q was determined by LDV and was found to be −17.0 mV and −1.15 μm cm/s V respectively at pH 7.0. In case of CeO_2 MPs DLS and LDV data was found to be out of the detection limit (Table 1).

3.2. Animal observation, food consumption, body weight and organ weight

Adverse signs, symptoms and mortality were not observed after 14 days of oral treatment with 5, 50, 300, 2000 mg/kg bw of CeO_2 NPs and CeO_2 MPs in female Wistar rats. However, rats treated with 2000 mg/kg bw of CeO_2 NPs showed dullness, irritation and moribund symptoms and an insignificant reduction in feed intake, bw gain and relative organ weight (data not shown). Mortality was not observed in rats receiving single doses of 2000 mg/kg bw of CeO_2 NPs and MPs. Hence, the acute toxicity of these compounds was greater than 2000 mg/kg bw, ranking these substances into category 5, as per OECD guideline 420 and the Globally Harmonized System.

3.3. Histopathological examinations

The slides of organs namely brain, heart, liver, spleen and kidneys stained with H & E were studied. All the organs were evaluated for any toxic changes as well as presence of any extraneous material deposits. Liver tissues obtained from rats treated with a single dose of 2000 mg/kg bw of CeO_2 NPs, showed tissue damage and focal areas of necrosis (Fig. 2B). Significant histopathological changes were not observed in kidneys, spleen and brain tissues with 2000 mg/kg bw (Fig. 2D, F and H). The sections of liver,

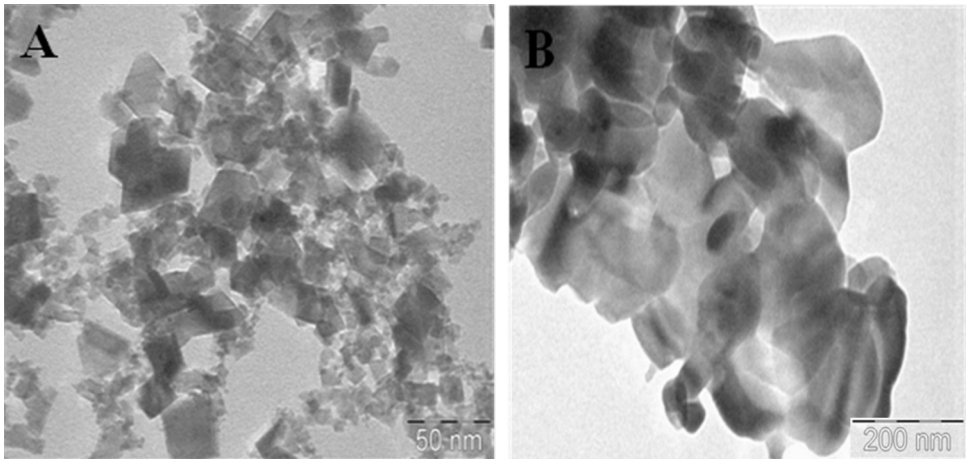

Fig. 1. Transmission electron microscopy (TEM) image of (A) cerium oxide nanoparticles (CeO_2 NPs) and (B) CeO_2 microparticles (MPs) in Milli-Q water for characterization of particles. Particles were scanned by TEM from JEOL, JEM-2100, Japan at voltage of 120 kV.

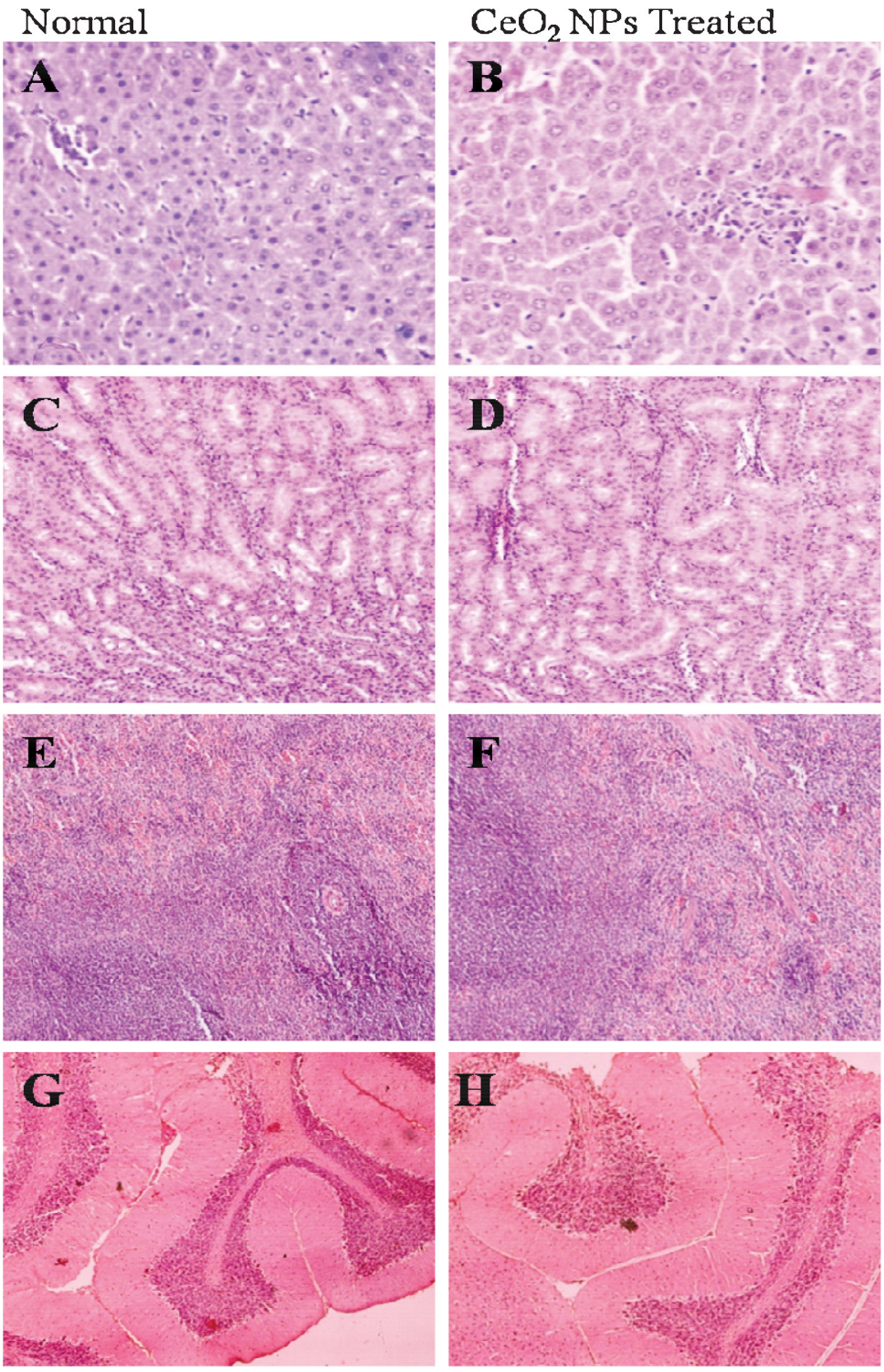

Fig. 2. Histopathology of liver, kidneys, spleen and brain tissues of rats after acute oral treatment with 2000 mg/kg bw CeO_2 NPs. A, C, E and G respectively showing normal architecture of liver, kidney, spleen and brain of control rats. (B) Focal area necrosis in liver, (D), (F) and (H) revealing no pathological alterations in kidney, spleen and brain of CeO_2 NPs treated group at ×400 magnification.

kidneys, spleen and brain of control rats are shown in Fig. 2A, C, E and G, respectively. However, rats exposed to 5, 50 and 300 mg/kg bw of CeO_2 NPs and 5, 50, 300 and 2000 mg/kg bw of CeO_2 MPs exhibited the normal architecture of liver, kidneys, spleen, brain and heart tissues (data not shown). Moreover, in the examined tissues there was no evidence of any extraneous material deposition.

3.4. Genotoxicity analysis

3.4.1. Comet assay

The findings of the comet assay after acute oral treatment with 100, 500, 1000 mg/kg bw dose of CeO_2 NPs and CeO_2 MPs are depicted in Fig. 3. In all samples, the cell viability by the trypan blue exclusion technique was >90% (data not shown). There was

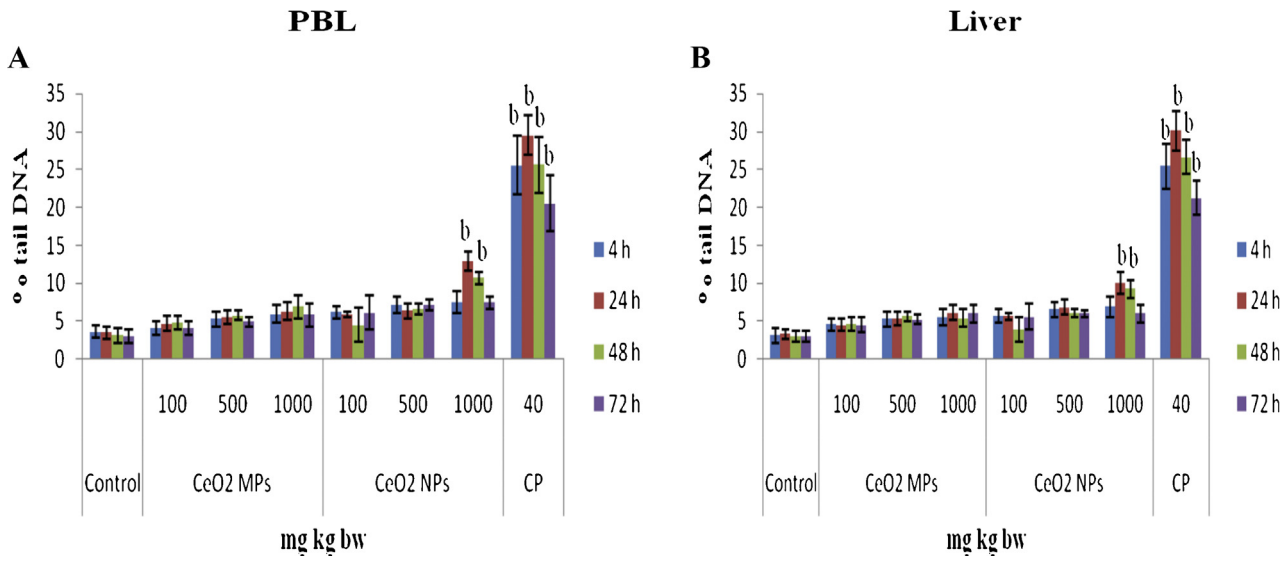

Fig. 3. The *in vivo* comet assay with CeO_2 NPs and CeO_2 MPs. (A) Mean % tail DNA in peripheral blood leukocytes (PBL) and (B) Mean % tail DNA in liver of female Wistar rats exposed orally to different doses of CeO_2 NPs and CeO_2 MPs at 4, 24, 48 and 72 h. Deionised water (control), cyclophosphamide (CP) as positive control, Data represented as mean ± S.D., significantly different from control at $^b p < 0.01$, $n = 5$ animals per group.

significant ($p < 0.01$) DNA damage at the highest dose of 1000 mg/kg bw in the PBL and liver cells of rats at the 24 and 48 h sampling times (Fig. 3A and B). However, no significant DNA damage was observed at the 4 and 72 h sampling times at any dose. A dose-dependent but insignificant increment in % tail DNA was noted in comparison to control group at all sampling times. Moreover, CeO_2 MPs did not induce significant DNA damage in PBL and liver of rats at all dose and time intervals. On the other hand, the mean % tail DNA in CP treated positive control rats was significantly ($p < 0.01$) higher compared with the control.

3.4.2. Micronucleus test

The bone marrow MNT was conducted after 24 h and 48 h of oral treatment with 100, 500 and 1000 mg/kg bw dose of CeO_2 NPs and CeO_2 MPs in female Wistar rats. After 24 h the MNT data indicated statistically significant ($p < 0.01$) increment in MN-PCEs frequency in the CeO_2 NPs treated groups with only higher dose of 1000 mg/kg bw in rats. At the same dose, after 48 h of oral exposure rats showed less significant ($p < 0.05$) enhancement (Table 2). However, CeO_2 MPs treated groups did not show any significant increase in the frequency of MN-PCEs. On the other hand, the CP (40 mg/kg bw) treated group induced a substantially significant ($p < 0.01$) effect on MN-PCEs frequency. The various doses of CeO_2 NPs and CeO_2 MPs exhibited an insignificant decrease in % PCEs in comparison to the negative control female rats with the MNT (Table 2). Similarly, the MNT was carried out in PB cells after 48 and 72 h of acute oral treatment with all the three doses of CeO_2 NPs and MPs. Significant ($p < 0.05$) changes in MN-PCEs in CeO_2 NPs treated group was observed only after 48 h at 1000 mg/kg bw. The positive control rats treated with CP induced very significant changes in MN-PCEs and % PCEs as well. All doses of CeO_2 NPs and CeO_2 MPs exhibited an insignificant decrease in % PCEs in comparison to the negative control rats (Table 3).

3.4.3. Chromosomal aberration assay

The results of the CA assay examined at 18 and 24 h after the oral administration of CeO_2 NPs and CeO_2 MPs with various doses (100, 500 and 1000 mg/kg bw) to female Wistar rats in bone marrow cells are shown in Tables 4 and 5 respectively. The CeO_2 NPs induced significant CAs at 1000 mg/kg bw dose at both time periods of exposure. Moreover, there was dose-dependent increase in numerical and structural changes. Total cytogenetic changes and aneuploidy were observed in bone marrow cells of CeO_2 NPs treated rats at 1000 and 500 mg/kg bw dose after single exposure at 18 and 24 h. The increase in aneuploidy was less significant ($p < 0.05$) at both 1000 and 500 mg/kg bw doses. However, there were no changes in polyploidy level and reciprocal translocations. The MI was used to conclude the rate of cell division. There was no significant decrease in MI after 18 and 24 h of oral exposure with CeO_2 NPs and CeO_2 MPs at any dose level. Further, there was no revelation of alterations in chromosome by CeO_2 MPs after acute oral study as compared with the control groups (Tables 4 and 5).

3.5. Biochemical parameters

3.5.1. Alkaline phosphatase activity assay

The activity of ALP in serum increased significantly ($p < 0.01$) at 1000 mg/kg bw dose of CeO_2 NPs after both exposure periods *i.e.* 24 and 72 h. The percent increase in activity was 31.77% and 26.18% respectively for 24 and 72 h of the exposure with 1000 mg/kg bw CeO_2 NPs. At 500 mg/kg bw dose of CeO_2 NPs, the significant ($p < 0.01$) increase (14.10%) was observed only at the 24 h sampling time. However, acute oral exposure of CeO_2 MPs at all dose levels and nanoceria at 100 mg/kg bw dose did not induce significant increase in ALP activity and percent increase was in the range of 0.1–5.6% when compared with control for both time periods (Fig. 4A).

3.5.2. Lactate dehydrogenase activity

The activity of serum LDH significantly increased ($p < 0.01$) at 1000 mg/kg bw dose of CeO_2 NPs at 24 and 72 h sampling periods. Percent increase in LDH activity with 1000 mg/kg bw dose of CeO_2 NPs with respect to control was noted as 86.81% and 74.84% after 24 and 72 h of acute exposure respectively (Fig. 4B).

3.5.3. Reduced glutathione content

The acute oral treatment with CeO_2 NPs for 24 and 72 h revealed that the GSH content in liver, kidneys and brain was inhibited in a dose-dependent manner compared with controls in female rats. Significant ($p < 0.01$) inhibition in the GSH content in liver

Table 2
The frequency of MN-PCEs and percent PCEs in female rat bone marrow cells treated with CeO_2 NPs and CeO_2 MPs at 24 and 48 h.

Treatments	Dose (mg/kg bw)	24 h		48 h	
		MN-PCEs frequency	% PCEs	MN-PCEs frequency	% PCEs
Control[A]		4.3 ± 1.53	40.0 ± 1.00	5.3 ± 1.53	34.3 ± 2.01
CeO_2 NPs	100	4.0 ± 1.00	40.2 ± 1.58	5.7 ± 1.53	32.0 ± 2.00
	500	6.0 ± 1.00	40.0 ± 3.14	8.0 ± 1.00	31.3 ± 2.08
	1000	11.0 ± 1.00[b]	37.1 ± 1.42	11.0 ± 1.00[a]	30.7 ± 1.51
CeO_2 MPs	100	2.4 ± 0.32	39.6 ± 1.31	2.1 ± 0.57	31.3 ± 3.78
	500	3.2 ± 0.53	40.3 ± 1.93	2.8 ± 0.15	33.9 ± 2.50
	1000	3.9 ± 0.36	38.5 ± 1.85	3.4 ± 0.40	29.1 ± 0.58
CP[B]	40	33.0 ± 3.61[b]	24.7 ± 1.53[b]	31.0 ± 3.61[b]	23.4 ± 0.93[b]

Data represented as mean ± S.D., significantly different from control [a]$p < 0.05$ and [b]$p < 0.01$, $n = 5$ animals per group.
[A] Deionised water (negative control).
[B] Cyclophosphamide (positive control).

Table 3
The frequency of MN-PCEs and percent PCEs in female rat peripheral blood treated with CeO_2 NPs and CeO_2 MPs at 48 and 72 h.

Treatments	Dose (mg/kg bw)	48 h		72 h	
		MN-PCEs frequency	% PCEs	MN-PCEs frequency	% PCEs
Control[A]		2.4 ± 0.59	3.9 ± 0.48	2.3 ± 0.42	4.0 ± 0.36
CeO_2 NPs	100	2.5 ± 0.40	3.7 ± 0.43	2.4 ± 0.40	3.7 ± 0.44
	500	3.1 ± 0.21	3.6 ± 0.25	3.0 ± 1.00	3.6 ± 0.30
	1000	3.9 ± 0.35[a]	3.1 ± 0.19	3.2 ± 0.35	3.2 ± 0.25
CeO_2 MPs	100	2.3 ± 0.44	3.9 ± 0.40	2.3 ± 0.26	3.8 ± 0.28
	500	2.8 ± 0.20	3.7 ± 0.26	2.9 ± 0.15	3.7 ± 0.36
	1000	3.0 ± 0.15	3.6 ± 0.26	2.9 ± 0.20	3.6 ± 0.41
CP[B]	40	11.3 ± 1.04[b]	1.9 ± 0.20[b]	11.2 ± 0.76[b]	2.0 ± 0.54[b]

Data represented as mean ± S.D., significantly different from control at [a]$p < 0.05$ and [b]$p < 0.01$, $n = 5$ animals per group.
[A] Deionised water (negative control).
[B] Cyclophosphamide (positive control).

at 1000 mg/kg bw dose of the CeO_2 NPs treated groups after 24 h was observed, whereas, less significant ($p < 0.05$) inhibition after 72 h as compared with the control was noted (Fig. 5A). In the kidneys and brain, CeO_2 NPs induced significant ($p < 0.05$) inhibition at 1000 mg/kg bw as compared with the control after 24 and 72 h (Fig. 5B and C). The percent change in liver (33.77% and 29.20%), kidneys (26.55% and 24.19%) and brain (14% and 13%) was noted after 24 and 72 h respectively at 1000 mg/kg bw dose. However, CeO_2 MPs at all doses and CeO_2 NPs at 500 and 100 mg/kg bw did not induce any significant alterations in GSH content in liver, kidneys and brain.

3.6. Tissue biodistribution of cerium particles

The biodistribution of Ce after acute exposure of CeO_2 NPs and CeO_2 MPs for 4, 24, 48 and 72 h at various doses 100, 500 and 1000 mg/kg bw in female Wistar are shown in Fig. 6. There was significant accumulation of Ce in all organs and

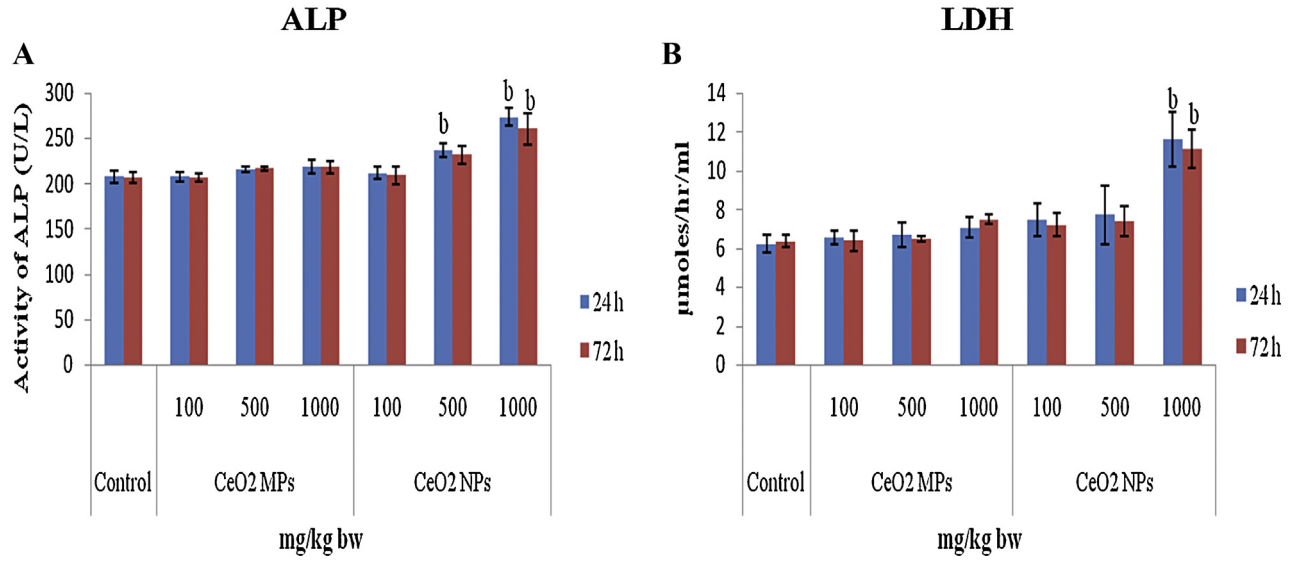

Fig. 4. Acute oral exposure effects of CeO_2 NPs and CeO_2 MPs on (A) alkaline phosphatase (ALP) and (B) lactate dehydrogenase (LDH) in serum of female albino Wistar rats after 24 and 72 h of treatment. Data represented as mean ± S.D., significantly different from control at [b]$p < 0.01$, $n = 5$ animals per group.

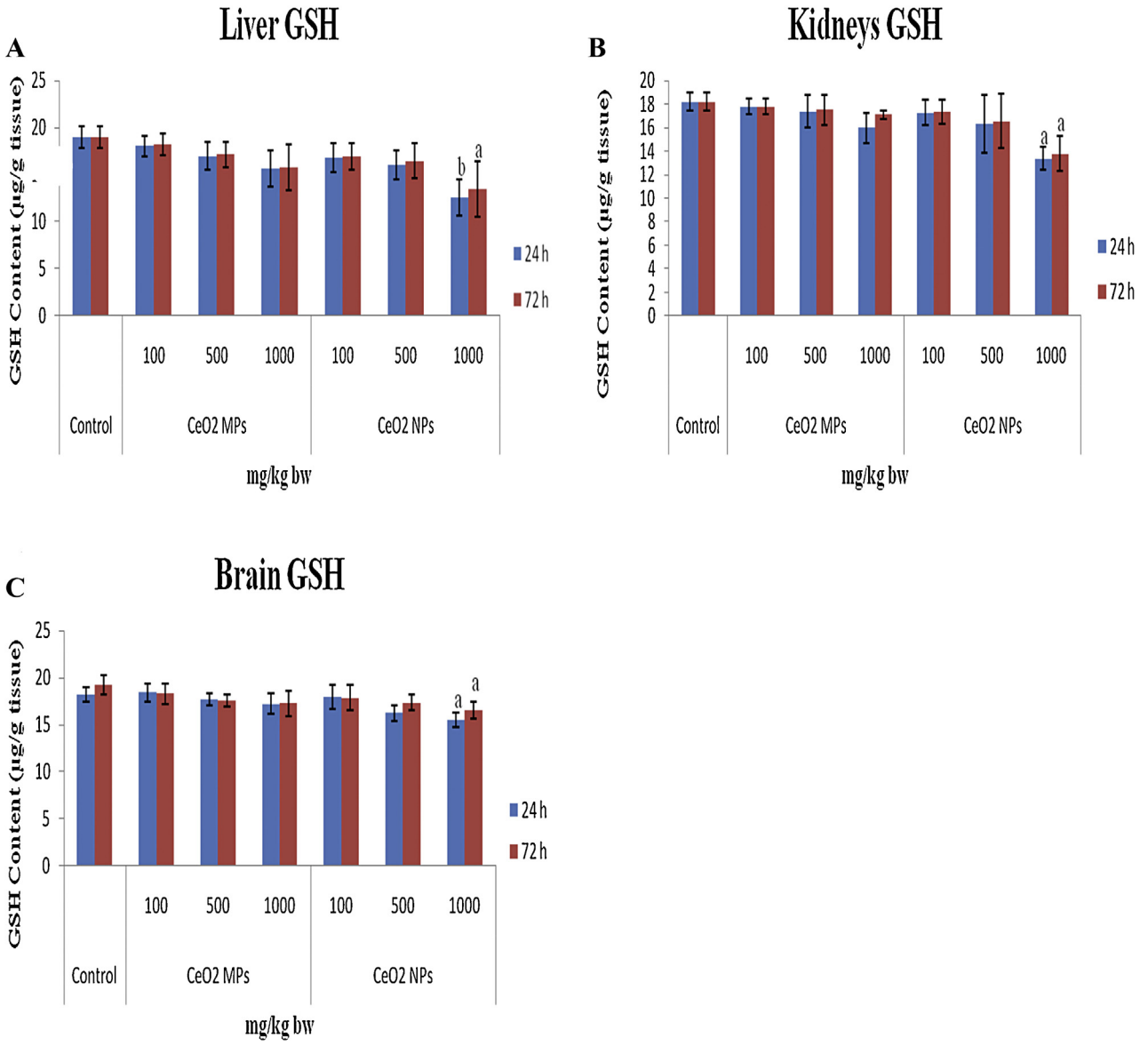

Fig. 5. Alterations in reduced glutathione (GSH) content in (A) liver, (B) kidneys and (C) brain after 24 and 72 h of acute oral treatment with CeO_2 NPs and CeO_2 MPs. Data represented as mean ± S.D., significantly different from control at $^{a}p < 0.05$, $^{b}p < 0.01$, $n = 5$ animals per group.

blood in the groups of animals treated with CeO_2 NPs. The maximum accumulation of Ce was observed at the 24 h sampling time in tissues such as liver, kidneys, brain, spleen, heart and blood (Fig. 6A–F). The order of accumulation of Ce was liver > kidneys > spleen = heart > blood > brain. After 24 h, Ce concentration started to decline in time-dependent manner at 48 and 72 h, but was still significant in comparison to control. The Ce concentration was significant at 4 h in liver, kidneys, spleen and blood at 1000 and 500 mg/kg bw, whereas, at low dose (100 mg/kg bw) only in kidneys (Fig. 6B). This indicated that as the days passed the concentration of particles got diluted. In contrast, CeO_2 MPs treated group did not exhibit significant Ce accumulation in any tissue. The excretion of Ce in urine was significant at all doses of CeO_2 NPs at all sampling time in dose- and time-dependent order unlike CeO_2 MPs treated group where there was no significant removal of Ce (Fig. 5G). In feces, maximum amount of both particles *i.e.* CeO_2 NPs and MPs got cleared significantly and clearance was rapidly reduced from 24 to 72 h (Fig. 5H). Probably due to their larger size, CeO_2 MPs treated rats showed more excretion in feces only.

4. Discussion

The CeO_2 NPs are considered as one of the most interesting NPs because of their several applications. Thus, the aim of this study was to study the genotoxic ability of CeO_2 NPs and compare it with their bulk particles by oral route in female Wistar rats. The physico-chemical characterization is considered as the primary requirement for the study of NPs induced toxicity. Hence, the mean size of CeO_2 NPs and CeO_2 MPs was calculated and found to be 23.2 nm and 3.26 μm respectively using TEM. A study with CeO_2 NPs has demonstrated that the NMs elicited more pronounced toxicity than their bulk counterparts [41]. The oral administration of different doses of CeO_2 NPs and CeO_2 MPs in rats did not cause any obvious adverse effects in a 14 day acute toxicity study. However, histopathological analysis of nanoceria treated rats with a 2000 mg/kg bw dose showed focal area necrosis in liver. This may be due to the fact that liver is the first organ to be exposed to NPs after entry into the circulation system because of its major role in biotransformation of toxins and foreign materials. Consistent

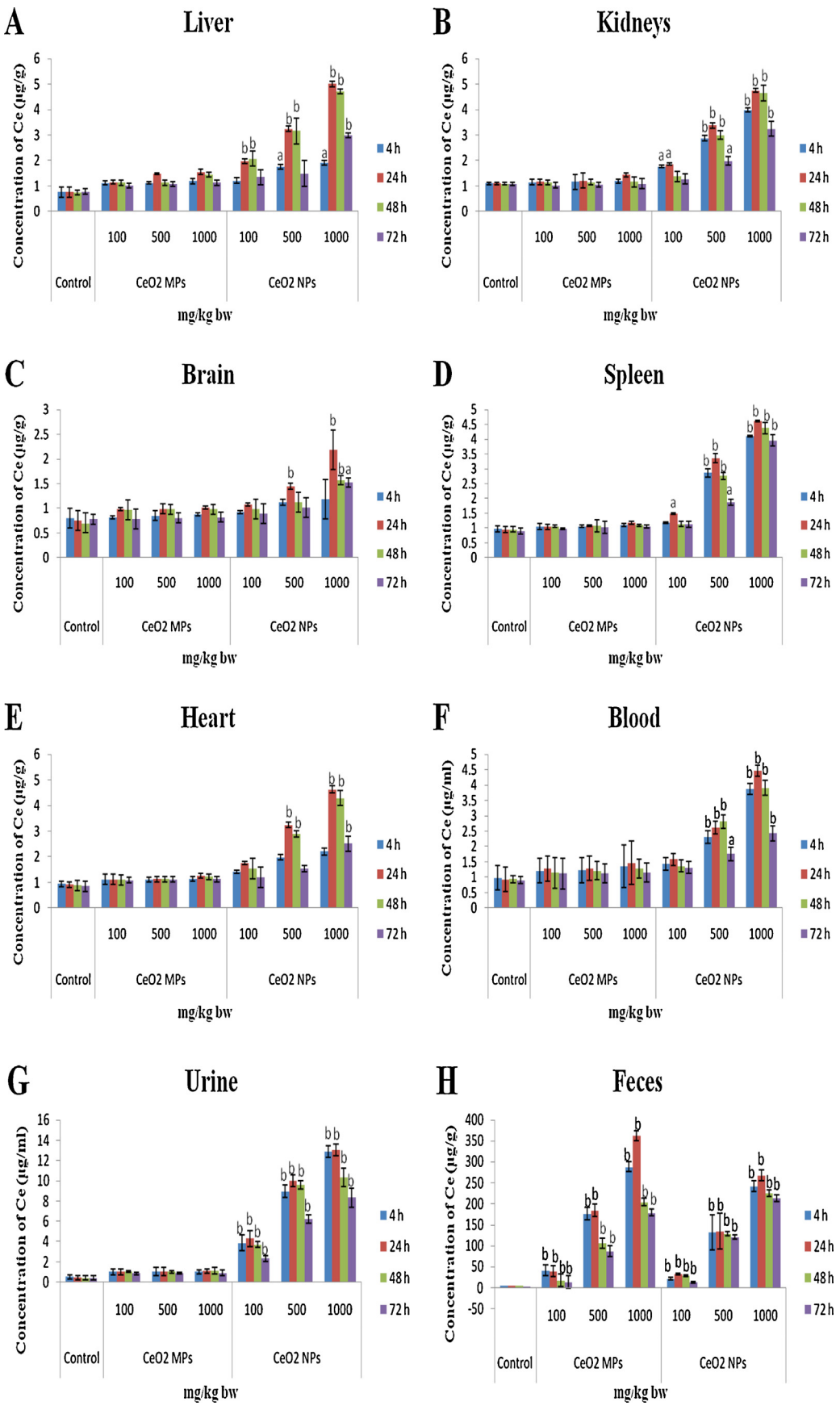

Fig. 6. Tissue distribution of Cerium measured by ICP-OES in (A) liver, (B) kidneys, (C) brain, (D) spleen, (E) heart, (F) blood, (G) urine and (H) feces of rats after 4, 24, 48 and 72 h of single oral administration with 1000 mg/kg, 500 mg/kg and 100 mg/kg bw of CeO_2 NPs and CeO_2 MPs. Significantly different from control at $^{a}p < 0.05$, $^{b}p < 0.01$, $n = 5$ animals per group.

Table 4
Chromosome aberrations and percent mitotic index observed in bone marrow cells of female Wistar rats treated with different doses of CeO_2 NPs and CeO_2 MPs at 18 h.

Dose (mg/kg bw)	MI (%) M ± SD	Chromosomal aberrations							Total Cytogenetic changes	TA + gaps M ± SD	TA − gaps M ± SD
		Numerical aberrations			Structural aberrations						
		Aneuploidy	Polyploidy	Gaps	Breaks	Minutes	Acentric fragments	Reciprocal translocations			
Control[A]	5.30 ± 1.1	0.33 ± 0.3	0.0 ± 0.0	0.53 ± 0.5	0.50 ± 0.5	0.20 ± 0.3	0.23 ± 0.2	0.0 ± 0.0	1.79 ± 1.3	1.46 ± 0.7	0.93 ± 0.3
CeO_2 NPs											
100	5.14 ± 1.2	2.00 ± 1.0	0.0 ± 0.0	0.97 ± 0.6	0.67 ± 0.3	0.57 ± 0.2	0.47 ± 0.2	0.0 ± 0.0	4.68 ± 1.2	2.68 ± 1.0	1.71 ± 0.6
500	4.98 ± 1.7	3.00 ± 1.0[a]	0.0 ± 0.0	1.27 ± 0.8	0.90 ± 0.4	0.77 ± 0.3	0.70 ± 0.3	0.0 ± 0.0	6.64 ± 0.6[a]	3.64 ± 1.6	2.37 ± 0.8
1000	3.76 ± 0.6	5.33 ± 1.5[a]	0.0 ± 0.0	2.27 ± 0.7	1.70 ± 0.5	1.60 ± 0.3	1.30 ± 0.3	0.0 ± 0.0	12.2 ± 1.1[b]	6.87 ± 0.5[b]	4.60 ± 0.6[a]
CeO_2 MPs											
100	5.31 ± 1.4	0.67 ± 0.6	0.0 ± 0.0	0.77 ± 0.4	0.27 ± 0.3	0.23 ± 0.2	0.30 ± 0.1	0.0 ± 0.0	2.24 ± 1.1	1.57 ± 0.6	0.80 ± 0.6
500	5.24 ± 1.5	1.00 ± 1.0	0.0 ± 0.0	1.27 ± 0.6	0.57 ± 0.4	0.50 ± 0.3	0.43 ± 0.2	0.0 ± 0.0	3.77 ± 1.3	2.77 ± 0.6	1.50 ± 0.9
1000	4.91 ± 0.8	2.00 ± 1.0	0.0 ± 0.0	1.57 ± 0.5	0.97 ± 0.2	0.90 ± 0.1	0.73 ± 0.2	0.0 ± 0.0	6.17 ± 1.0	4.17 ± 0.2	2.60 ± 0.3
CP[B]	3.24 ± 0.9	35.33 ± 5.5[b]	2.2 ± 0.7[b]	10.7 ± 2.1[b]	12.0 ± 2.7[b]	13.16 ± 1.8[b]	12.33 ± 3.1[b]	1.3 ± 0.3[b]	87.02 ± 13.1[b]	49.49 ± 8.7[b]	38.79 ± 6.6[b]

Significantly different from control at [a]$p < 0.05$, [b]$p < 0.01$. One hundred metaphases were analyzed per animal; $n = 5$ animals per group. Total cytogenetic changes = numerical aberrations and structural aberrations.
MI, mitotic index; data represented as mean ± standard deviation; TA, total aberrations = structural aberrations.
[A] Negative control (deionised water).
[B] Cyclophosphamide (40 mg/kg bw).

Table 5
Chromosome aberrations and percent mitotic index observed in bone marrow cells of female Wistar rats treated with different doses of CeO_2 NPs and CeO_2 MPs at 24 h.

Dose (mg/kg bw)	MI (%) M ± SD	Chromosomal aberrations							Total Cytogenetic changes	TA + gaps M ± SD	TA − gaps M ± SD
		Numerical aberrations			Structural aberrations						
		Aneuploidy	Polyploidy	Gaps	Breaks	Minutes	Acentric Fragments	Reciprocal translocations			
Control[A]	5.89 ± 1.0	0.50 ± 0.5	0.0 ± 0.0	0.60 ± 0.4	0.53 ± 0.5	0.23 ± 0.2	0.20 ± 0.1	0.0 ± 0.0	2.06 ± 1.2	1.56 ± 0.6	0.96 ± 0.4
CeO_2 NPs											
100	5.29 ± 1.4	2.10 ± 1.0	0.0 ± 0.0	1.10 ± 0.4	0.77 ± 0.2	0.60 ± 0.2	0.50 ± 0.2	0.0 ± 0.0	5.07 ± 0.8	2.97 ± 0.6	1.87 ± 0.6
500	5.21 ± 1.5	3.27 ± 0.6[a]	0.0 ± 0.0	1.40 ± 0.6	1.00 ± 0.5	0.80 ± 0.3	0.73 ± 0.3	0.0 ± 0.0	7.20 ± 0.9[a]	3.93 ± 1.5	2.53 ± 0.9
1000	3.72 ± 0.5	5.70 ± 1.3[a]	0.0 ± 0.0	2.40 ± 0.7	1.80 ± 0.6	1.67 ± 0.2	1.33 ± 0.3	0.0 ± 0.0	12.90 ± 0.4[b]	7.20 ± 0.85[b]	4.80 ± 0.7[a]
CeO_2 MPs											
100	5.28 ± 1.2	0.83 ± 0.8	0.0 ± 0.0	0.87 ± 0.2	0.30 ± 0.2	0.27 ± 0.2	0.27 ± 0.2	0.0 ± 0.0	2.54 ± 1.2	1.71 ± 0.5	0.84 ± 0.5
500	5.06 ± 1.7	1.30 ± 0.6	0.0 ± 0.0	1.43 ± 0.6	0.63 ± 0.4	0.53 ± .25	0.40 ± 0.3	0.0 ± 0.0	4.29 ± 0.8	2.99 ± 0.8	1.56 ± 0.9
1000	4.86 ± 0.7	2.3 ± 0.8	0.0 ± 0.0	1.67 ± 0.3	1.03 ± 0.3	0.93 ± 0.2	0.70 ± 0.3	0.0 ± 0.0	6.63 ± 0.9	4.33 ± 0.2	2.66 ± 0.4
CP[B]	3.42 ± 0.8	37.33 ± 6.8[b]	2.63 ± 0.6[b]	10.93 ± 2[b]	12.67 ± 2.5[b]	13.5 ± 2.3[b]	12.67 ± 2.5[b]	1.4 ± 0.3[b]	91.13 ± 13.9[b]	51.17 ± 7.9[b]	40.24 ± 6.0[b]

Significantly different from control at [a]$p < 0.05$, [b]$p < 0.01$. One hundred metaphases were analyzed per animal; $n = 5$ animals per group. Total cytogenetic changes = numerical aberrations and structural aberrations.
MI, mitotic index; data represented as mean ± standard deviation; TA, total aberration = structural aberrations.
[A] Negative control (deionised water).
[B] Cyclophosphamide (40 mg/kg bw).

with our results, an investigation found that single intratracheal instillation of CeO_2 NPs in male Sprague Dawley rats induced dose-dependent liver damage such as hydropic degeneration, hepatocyte enlargement, sinusoidal dilatation and accumulation of granular material, but no histopathological alterations in the kidneys, spleen and heart [42]. Toxicity tests are not designed to show that a substance is safe, rather to characterize what toxic effects a chemical can produce. Hence, a wide dose range was taken. Further, the doses were selected on the basis of a range finding study. Three dose levels were used ranging from maximum to little or no toxicity. The highest dose was the dose producing signs or some indication of toxicity. Moreover, the minimum dose of 100 mg/kg bw was intended to reflect likely human exposure. The workers engaged in the manufacturing the NPs can be exposed unintentionally by hand to mouth transfer of NPs. Further, some of the NPs can be swallowed and reach the gastrointestinal tract. Further, the highest dose of 1000 mg/kg bw was chosen to see the effect when large quantities of NPs are released accidentally into the environment, and they enter the human body. Genotoxicity is a fundamental parameter to assess noxious effects of NPs as mankind's concern about genetic diseases and cancers is well known. In the present study, CeO_2 NPs and CeO_2 MPs were found to cause significant increase in % tail DNA damage in PBL and liver at 1000 mg/kg bw in comparison to control, by the comet assay at 24 and 48 h sampling period. The gradual decline in the % tail DNA with time may be due to the action of complex DNA repair following removal of the DNA damaging agent [43]. The formation of MN in MN-PCEs in bone marrow and PB cells was observed to be time-dependent and increased significantly only at 1000 mg/kg bw of CeO_2 NPs. The % PCEs calculated in the CeO_2 NPs and CeO_2 MPs treated groups did not show any significant reduction compared with the control group, suggesting that cell death had not occurred in any of the treated groups. Similarly, significant total cytogenetic changes with CA assay were also noted only at high dose of CeO_2 NPs. In support of our result, there are some *in vitro* studies with CeO_2 NPs demonstrating DNA damage through comet assay. For example, 7 nm CeO_2 induced DNA damage and chromosome damage in normal human fibroblasts after 2 h of exposure at broad concentration range of 0.6–1200 μg/ml [44]. Further, Kumari et al. [19] reported significant DNA damage and MN formation in human neuroblastoma cells (IMR32) after exposure with 25 nm CeO_2 NPs for 24 h at 200 μg/ml dose. Studies on the effects of nanoceria after acute oral exposure in the intact mammals using genotoxicity assays were not found in literature. However, similar studies using different NMs have demonstrated size-dependent genotoxicity [26,45–47]. Our results suggest that CeO_2 NPs appear to be more genotoxic than their MPs. The NPs have greater surface area in comparison to their total mass, which increases the likely hood of interaction with the biomolecules present and as a result, lead to adverse responses [48].

In the present study, it was observed that single doses of CeO_2 NPs significantly increased the activities of ALP enzyme in the serum of the exposed rats at 1000 mg/kg bw. Similarly, enhancement in the LDH levels in the serum after CeO_2 NPs exposure was dose-dependent, suggesting that there might be injuries to the tissues. The LDH levels increased significantly in rats treated with higher doses of CeO_2 NPs indicating cytotoxicity. Correspondingly, in the liver, kidneys and brain, the GSH content declined significantly at 1000 mg/kg bw dose. There are reports demonstrating the interaction of NPs with proteins and enzymes and thus interfering with the antioxidant defense mechanism, leading to ROS generation and subsequent apoptosis and necrosis [49]. In the study performed by Rosenkranz et al. [50], using the LDH assay, 8 nm ceria particles induced membrane damage and hence, cytotoxicity occurred only at highest concentration of 100 μg/ml after 72 h exposure to H4IIE rat hepatoma cells. Similarly, cell membrane breakage after 72 h exposure to 20 nm CeO_2 NPs was observed in A549 cells along with decrease of cellular GSH levels [51]. Glutathione plays an important role in antioxidant defense, nutrient metabolism and regulation of cellular events and its deficiency contributes to oxidative stress, which plays a key role in aging and the pathogenesis of many diseases [52]. GSH effectively scavenges free radicals and other ROS (*e.g.* hydroxyl radical, lipid peroxyl radical, peroxynitrite and H_2O_2) directly and indirectly through enzymatic reactions [53]. In the current study, the positive results of oxidative effect at high doses signify that CeO_2 NPs are of low efficiency in inducing oxidative changes. The decrease in intracellular GSH level was evident in the BEAS-2B cells derived from human bronchus epithelial cells after 24 h exposure to 40 μg/ml of 30 nm CeO_2 NPs [17]. Moreover, significant increment in LDH activity and reduction in GSH content was found in IMR32 with 100 and 200 μg/ml of CeO_2 NPs after 24 h exposure [19]. Small NPs have increased active surface that might allow harmful chemical reactions such as ROS generation. Endocytosis mechanisms, quantity of cellular uptake and effectiveness of NPs processing in the endocytotic pathway are also affected by the size of the NPs [54]. In the current tissue biodistribution study upon oral exposure, CeO_2 NPs were significantly distributed and destined to the liver, spleen, kidneys, heart and brain thus providing evidence that they could be transported to other sites subsequent to exposure due to their translocation into the blood. The most avid uptake and retention of NPs was detected in the liver, kidneys and spleen. The distribution was in a dose- and in a time-dependent order, as the amount absorbed increased with higher the dose administered. In corroboration with our results, ZnO NPs (2.5 g/kg bw) were shown to be distributed in liver, spleen and kidneys of mice after oral administration and greater accumulation in liver resulted in histopathological necrosis [55,56]. Moreover, absorptivity and tissue biodistribution of ZnO NPs was reported to be more prominent after oral treatment than ip administration [55]. Primary retention of 30 nm CeO_2 NPs in various organs of rats after single iv infusion was demonstrated by Yokel et al. [57] as well. The excretion data showed that a small quantity of NPs was removed in the urine, whereas, large amounts of NPs as well as MPs were excreted in the feces at all the sampling times. Significant amounts of nanoceria in urine were observed because the size of the NPs was smaller than the average diameter of glomerular filtration pore of the rats which is 75 nm. However, there were no apparent pathological alterations in the renal tissue compared to the untreated controls. The probable mechanism underlying the distribution could be that the CeO_2 NPs may have been phagocytized and transported to the liver or spleen by macrophages to reduce the filtered amount or bound to heme in RBCs [8]. The biochemical alterations such as reduction in liver, kidneys and brain GSH content and increased activity of serum ALP and LDH can be probably explained by the accumulation of nanoceria. However, these alterations could result as morphological alterations only in liver. Comparative absorption after oral administration of nanoceria was reported to be lesser than the systemic or pulmonary administration [58], even though the impact of NPs on the gastrointestinal system and their fate is imperative. In accordance to our results, 25 and 80 nm TiO_2 NPs and 58 nm zinc powder at 5 g/kg bw dose after 14 days of acute oral exposure to mice were found to be translocated into the blood and thereafter distributed to the organs including the liver, spleen, lungs and kidneys leading to several hepatic alterations [59,60]. Similarly, nano-copper (23.5 nm) when orally administered to mice for 72 h at 413 mg/kg bw dose induced grave toxicological effects and heavy injuries to the kidneys, liver and spleen, whereas, micro-copper particles did not [61]. Unlike systemic tissues like liver, kidneys and spleen rich in mononuclear phagocyte cells, it was noticed that in the brain there was comparatively less uptake, retention and no histological changes in tissue architecture. Yokel et al. [57] also reported similar result with iv infusion of 30 nm nanoceria. There are no reports

addressing the acute oral exposure with this particles, however, other routes of exposures have been studied. The iv infusion of 5 nm ceria in rats revealed hepatic oxidative stress resulting in alteration in hepatic structure after bioretention in different hepatic cells after 1 h and 24 h of exposure [22]. Moreover, adverse hepatic responses were evident after single iv infusion of an aqueous dispersion of 85 mg/kg, 30 nm nanoceria into Sprague Dawley rats [62]. As can be inferred from our results, the *in vivo* CeO_2 toxicity was directly connected to the biodistribution and bioaccumulation times, and both were size-dependent. Moreover, adverse effects of nanoceria may be closely linked with the size associated capacity to enter in the biological system without any difficulty [48].

There are several direct interaction and indirect mechanisms that can subsequently promote genotoxicity upon exposure with NPs. Direct interaction of NPs occur due to their potency to attack the phosphate group to catalyze break and indirect through inflammation and ROS generation [63,64]. Hence, the most probable and valid mechanism of genotoxicity upon nano-, micro- and ultrafine-particles exposure was suggested to be oxidative stress induced DNA damage [3]. Intracellular ROS generated in the metabolizing cells could attack DNA base guanine and form 8-OHdG lesions after unbalancing the redox potential in the cellular environment and these 8-OHdG lesions may have mutagenic potential [65].

5. Conclusions

In summary, results of the present study implicated the size, dose and time of bioaccumulation of the CeO_2 NPs for its toxicity. Relatively low toxicity of CeO_2 NPs was observed through the acute oral exposure, whereas, CeO_2 MPs did not incite any toxicity attributes in comparison to controls. The findings demonstrated that CeO_2 NPs induced genotoxicity, biochemical alterations and cytotoxicity at the high dose level. The concurrence of DNA damage and chromosomal changes with the reduction of GSH content suggested the role of oxidative stress cascade in eliciting toxicity in liver and other organs after the progression from circulatory system. Exposure to CeO_2 NPs clearly induced hepatic damage and mild alterations in kidneys. Although the complete understanding of NPs toxicity is still a way ahead, however, the possible mechanism might be inflammation mediated either by ROS generated by the NPs or the NPs themselves. Moreover, bioaccumulation of CeO_2 NPs was found to be more pronounced in comparison to CeO_2 MPs. Nevertheless, there is always a wide scope for more rigorous toxicity study in order to get firm and final conclusion.

Conflict of interest statement

There is no conflict of interest related to this research.

Acknowledgments

This research was supported by Asian Office of Aerospace Research and Development (AOARD), Japan under the Grant no. FA2386-11-1-4085. Moreover, we express our sincere thanks to the Director, IICT, Hyderabad for providing facility to execute this study. Further, Monika Kumari (SRF) is grateful to University Grant Commission, India for the award of fellowship.

References

[1] Anon, Commission recommendation on the definition of nanomaterial. Official Journal of the European Union, L 275/38 (2011).
[2] M.F. Hochella, S.K. Lower, P.A. Maurice, R.L. Penn, N. Sahai, D.L. Sparks, B.S. Twining, Nanominerals, mineral nanoparticles, and earth systems, Science 319 (2008) 1631–1635.
[3] G. Oberdorster, E. Oberdorster, J. Oberdorster, Nanotoxicology: an emerging discipline evolving from studies of ultrafine particles, Environ. Health Perspect. 113 (2005) 823–839.
[4] M.C. Roco, National science foundation U.S. national science and technology council subcommittee on nanoscale science, engineering, and technology. Responsible of Nanotechnology. How the U.S. government is dealing with the immediate and long-term issues of this new technology, Environ. Sci. Technol. (2005) 107A.
[5] I. Celardo, M. De Nicola, C. Mandoli, J.Z. Pedersen, E. Traversa, L. Ghibelli, Ce^{3+} ions determine redox-dependent anti-apoptotic effect of cerium oxide nanoparticles, ACS Nano 5 (2011) 4537–4549.
[6] T. Naganuma, E. Traversa, Stability of the Ce^{3+} valence state in cerium oxide nanoparticle layers, Nanoscale 4 (2012) 4950–4953.
[7] I. Celardo, J.Z. Pedersen, E. Traversa, L. Ghibelli, Pharmacological potential of cerium oxide nanoparticles, Nanoscale 3 (2011) 1411–1420.
[8] S.M. Hirst, A. Karakoti, S. Singh, W. Self, R. Tyler, S. Seal, C.M. Reilly, Biodistribution and *in vivo* antioxidant effects of cerium oxide nanoparticles in mice, Environ. Toxicol. 28 (2013) 107–118.
[9] T. Yu, Y.I. Park, M.C. Kang, J. Joo, J.K. Park, H.Y. Won, J.J. Kim, T. Hyeon, Large-scale synthesis of water dispersible ceria nano crystals by a simple sol–gel process and their use as a chemical mechanical planarization slurry, Eur. J. Inorg. Chem. 2008 (2008) 855–858.
[10] W. Wu, S.S. Li, S. Liao, F. Xiang, X.H. Wu, Preparation of new sunscreen materials $Ce_{1-x}Zn_xO_{2-x}$ via solid-state reaction at room temperature and study on their properties, Rare Metals 29 (2010) 149–153.
[11] Y. Kawamoto, Y. Tanabe, [assignee: Nippon Shikizai Inc.]. 2005 pat. Lipstick composition having minimized skin irritation (Japan). Japanese Patent No. 2005220099 A2. Japan Kokai Tokyo Koho, 8 pp. Abstract from Toxcenter, 232470.
[12] B. Park, K. Donaldson, R. Duffin, L. Tran, F. Kelly, I. Mudway, J.P. Morin, R. Guest, P. Jenkinson, Z. Samaras, M. Giannouli, H. Kouridis, P. Martin, Hazard and risk assessment of a nanoparticulate cerium oxide-based diesel fuel additive – a case study, Inhal. Toxicol. 20 (2008) 547–566.
[13] T. Masui, T. Ozaki, K. Machida, G. Adachi, Preparation of ceria-zirconia Sub-catalysts for automotive exhaust cleaning, J. Alloys Compd. 303 (2000) 49–55.
[14] J.H. Joo, G.M. Choi, Micro-solid oxide fuel cell using thick-film ceria, Solid State Ion 180 (2009) 839–842.
[15] OECD, List of Manufactured Nanomaterials and List of Endpoints for Phase One of the Sponsorship Programme for the Testing of Manufactured Nanomaterials: Revision. Environment Directorate, Pesticides and Biotechnology, Organisation for Economic Co-operation and Development. Paris, 2010, pp. 16, ENV/JM/MONO 46. Available at: http://search.oecd.org/officialdocuments/displaydocumentpdf/?cote=env/jm/mono(2009)20/rev&doclanguage=en
[16] S. Hussain, F. Al-Nsour, A.B. Rice, J. Marshburn, B. Yingling, Z. Ji, J.I. Zink, N.J. Walker, S. Garantziotis, Cerium dioxide nanoparticles induce apoptosis and autophagy in human peripheral blood monocytes, ACS Nano 6 (2012) 5820–5829.
[17] E.J. Park, J. Choi, Y.K. Park, K. Park, Oxidative stress induced by cerium oxide nanoparticles in cultured BEAS-2B cells, Toxicology 245 (2008) 90–100.
[18] H.J. Eom, J. Choi, Oxidative stress of CeO_2 nanoparticles via p38-Nrf-2 signaling pathway in human bronchial epithelial cell, Beas-2B, Toxicol. Lett. 187 (2009) 77–83.
[19] M. Kumari, S.P. Singh, S. Chinde, M.F. Rahman, M. Mahboob, P. Grover, Toxicity study of cerium oxide nanoparticles in human neuroblastoma cells, Int. J. Toxicol. 33 (2014) 86–97.
[20] A. Srinivas, P.J. Rao, G. Selvam, P.B. Murthy, P.N. Reddy, Acute inhalation toxicity of cerium oxide nanoparticles in rats, Toxicol. Lett. 28 (2011) 105–115.
[21] J.Y. Ma, R.R. Mercer, M. Barger, D. Schwegler-Berry, J. Scabilloni, J.K. Ma, V. Castranova, Induction of pulmonary fibrosis by cerium oxide nanoparticles, Toxicol. Appl. Pharmacol. 262 (2012) 255–264.
[22] M.T. Tseng, X. Lu, X. Duan, S.S. Hardas, R. Sultana, P. Wu, J.M. Unrine, U. Graham, D.A. Butterfield, E.A. Grulke, R.A. Yokel, Alteration of hepatic structure and oxidative stress induced by intravenous nanoceria, Toxicol. Appl. Pharmacol. 260 (2012) 173–182.
[23] Z. Magdolenova, A. Collins, A. Kumar, A. Dhawan, V. Stone, M. Dusinska, Mechanisms of genotoxicity. A review of *in vitro* and *in vivo* studies with engineered nanoparticles, Nanotoxicology 8 (2014) 233–278.
[24] P.L. Olive, J.P. Banath, The comet assay: a method to measure DNA damage in individual cells, Nat. Protoc. 1 (2006) 23–29.
[25] G. Wu, Y.Z. Fang, S. Yang, J.R. Lupton, N.D. Turner, Glutathione metabolism and its implications for health, J. Nutr. 134 (2004) 489–492.
[26] G.M. Morsy, K.S. Abou El-Ala, A.A. Ali, Studies on fate and toxicity of nanoalumina in male albino rats: lethality, bioaccumulation and genotoxicity, Toxicol. Ind. Health. (2013) [Epub ahead of print].
[27] M. Baek, H.E. Chung, J. Yu, J.A. Lee, S.M. Paek, J.K. Lee, J. Jeong, J.H. Choy, S.J. Choi, Pharmacokinetics, tissue distribution, and excretion of zinc oxide nanoparticles, Int. J. Nanomed. 7 (2012) 3081–3097.
[28] T.H. Umbreit, S. Francke-Carroll, J.L. Weaver, T.J. Miller, P.L. Goering, N. Sadrieh, M.E. Stratmeyer, Tissue distribution and histopathological effects of titanium dioxide nanoparticles after intravenous or subcutaneous injection in mice, J. Appl. Toxicol. 32 (2012) 350–357.
[29] OECD Guideline 420, Guideline for the Testing of Chemicals: Acute Oral Toxicity - Fixed Dose Procedure. Organization for Economic Cooperation and Development: Paris (2001).
[30] R.R. Tice, E. Agurell, D. Anderson, B. Burlinson, A. Hartmann, H. Kobayashi, Y. Miyamae, E. Rojas, J.C. Ryu, Y.F. Sasaki, The single cell gel/comet assay:

Guidelines for *in vitro* and *in vivo* genetic toxicology testing, Environ. Mol. Mutagen. 35 (2000) 206–221.
[31] Y. Miyamae, M. Yamamoto, Y.F. Sasaki, H. Kobayashi, M. Igarashi-Soga, K. Shimoi, M. Hayashi, Evaluation of a tissue homogenization technique that isolates nuclei for the *in vivo* single cell gel electrophoresis (comet) assay: a collaborative study by five laboratories, Mutat. Res. 418 (1998) 131–140.
[32] B.L. Pool-Zobel, N. Lotzmann, M. Knoll, F. Kuchenmeister, R. Lambertz, U. Leucht, H.G. Schroder, P. Schmezer, Detection of genotoxic effects in human gastric and nasal mucosa cells isolated from biopsy samples, Environ. Mol. Mutagen. 24 (1994) 23–45.
[33] W. Schmid, The micronucleus test, Mutat. Res. 31 (1975) 9–15.
[34] A. Celik, O. Ogenler, U. Comelekoglu, The evaluation of micronucleus frequency by acridine orange fluorescent staining in peripheral blood of rats treated with lead acetate, Mutagenesis 20 (2005) 411–415.
[35] OECD Guidelines 474, Guidelines for genetic toxicology: micronucleus test. Organization for Economic Cooperation and Development: Paris, 1997.
[36] OECD Guidelines 475, Guidelines for genetic toxicology: in vivo mammalian bone marrow cytogenetic test-chromosome analysis. Organization for Economic Cooperation and Development: Paris, 1997.
[37] I.D. Adler, Cytogenetic tests in mammals, in: S. Venitt, J. Parry (Eds.), Mutagenicity Testing: A Practical Approach, IRL Press, Oxford, 1984, pp. 273–306.
[38] M.J. McQueen, Optimal assay of LDH and α-HBD at 37 °C, Ann. Clin. Biochem. 9 (1972) 21–25.
[39] D.J. Jollow, J.R. Mitchell, N. Zampaglione, J.R. Gillete, Bromobenzene induced liver necrosis. Protective role of glutathione and evidence for 3,4-bromobenzeneoxide as the hepatotoxic metabolite, Pharmacology 11 (1974) 151–169.
[40] M. Gomez, D.J. Sanchez, J.M. Llobet, J. Corbella, J.L. Domingo, The effect of age on aluminum retention in rats, Toxicology 116 (1997) 1–8.
[41] M.C. Arnold, A.R. Badireddy, M.R. Wiesner, R.T.D. Giulio, J.N. Meyer, Cerium oxide nanoparticles are more toxic than equimolar bulk cerium oxide in *Caenorhabditis elegans*, Arch. Environ. Contam. Toxicol. 65 (2013) 224–233.
[42] S.K. Nalabotu, M.B. Kolli, W.E. Triest, J.Y. Ma, N.D.P.K. Manne, A. Katta, H.S. Addagarla, K.M. Rice, E.R. Blough, Intratracheal instillation of cerium oxide nanoparticles induces hepatic toxicity in male Sprague-Dawley rats, Int. J. Nanomed. 6 (2011) 2327–2335.
[43] H.L. Karlsson, The comet assay in nanotoxicology research, Anal. Bioanal. Chem. 398 (2010) 651–666.
[44] M. Auffan, J. Rose, T. Orsiere, M. De Meo, A. Thill, O. Zeyons, O. Proux, A. Masion, P. Chaurand, O. Spalla, A. Botta, M.R. Wiesner, J.Y. Bottero, CeO_2 nanoparticles induce DNA damage towards human dermal fibroblasts *in vitro*, Nanotoxicology 3 (2009) 161–171.
[45] S.P. Singh, M. Kumari, S.I. Kumari, M.F. Rahman, S.S.K. Kamal, M. Mahboob, P. Grover, Genotoxicity of nano- and micron-sized manganese oxide in rats after acute oral treatment, Mutat. Res. 754 (2013) 39–50.
[46] B. Trouiller, R. Reliene, A. Westbrook, P. Solaimani, R.H. Schiest, Titanium dioxide nanoparticles induce DNA damage and genetic instability *in vivo* in mice, Cancer Res. 69 (2009) 8784–8789.
[47] H.R. Hamad, Modulation of the genotoxic effect induced by titanium dioxide nanoparticles in mice (*Mus musculus*) (PhD Thesis), Cairo University, Faculty of Science, Zoology Department, Egypt, 2012.
[48] L. Shang, K. Nienhaus, G.U. Nienhaus, Engineered nanoparticles interacting with cells: size matters, J. Nanobiotechnol. 12 (2014) 5.
[49] A.M. Schrand, M.F. Rahman, S.M. Hussain, J.J. Schlager, D.A. Smith, S.F. Ali, Metal based nanoparticles and their toxicity assessment, Wiley Interdiscip. Rev. Nanomed. Nanobiotechnol. 2 (2010) 544–568.
[50] P. Rosenkranz, M.L. Fernandez-Cruz, E. Conde, M.B. Ramirez-Fernandez, J.C. Flores, M. Fernandez, J.M. Navas, Effects of cerium oxide nanoparticles to fish and mammalian cell lines: an assessment of cytotoxicity and methodology, Toxicol. In Vitro 26 (2012) 888–896.
[51] W. Lin, Y.W. Huang, X.D. Zhou, Y. Ma, Toxicity of cerium oxide nanoparticles in human lung cancer cells, Int. J. Toxicol. 25 (2006) 451–457.
[52] D.M. Townsend, K.D. Tew, H. Tapiero, The importance of glutathione in human disease, Biomed. Pharmacother. 57 (2003) 145–155.
[53] Y.Z. Fang, S. Yang, G. Wu, Free radicals, antioxidants, and nutrition, Nutrition 18 (2002) 872–879.
[54] A. Nel, T. Xia, L. Madler, N. Li, Toxic potential of materials at the nanolevel, Science 311 (2006) 622–627.
[55] C.H. Li, C.C. Shen, Y.W. Cheng, S.H. Huang, C.C. Wu, C.C. Kao, J.W. Liao, J.J. Kang, Organ biodistribution, clearance, and genotoxicity of orally administered zinc oxide nanoparticles in mice, Nanotoxicology 6 (2012) 746–756.
[56] S. Pasupuleti, S. Alapati, S. Ganapathy, G. Anumolu, N.R. Pully, B.M. Prakhya, Toxicity of zinc oxide nanoparticles through oral route, Toxicol. Ind. Health 28 (2012) 675–686.
[57] R.A. Yokel, T.C. Au, R. MacPhail, S.S. Hardas, D.A. Butterfield, R. Sultana, M. Goodman, M.T. Tseng, M. Dan, H. Haghnazar, J.M. Unrine, U.M. Graham, P. Wu, E.A. Grulke, Distribution, elimination, and biopersistance to 90 days of a systemically introduced 30 nm ceria engineered nanomaterial in rats, Toxicol. Sci. 127 (2012) 256–268.
[58] J.K. Miller, W.P. Byrne, Absorption, excretion, and tissue distribution of orally and intravenously administered radiocerium as affected by EDTA, J. Dairy Sci. 53 (1970) 171–175.
[59] J. Wang, G. Zhou, C. Chen, H. Yu, T. Wang, Y. Ma, G. Jia, Y. Gao, B. Li, J. Sun, Y. Li, F. Jiao, Y. Zhao, Z. Chai, Acute toxicity and biodistribution of different sized titanium dioxide particles in mice after oral administration, Toxicol. Lett. 168 (2007) 176–185.
[60] B. Wang, W.Y. Feng, T.C. Wang, G. Jia, M. Wang, J.W. Shi, F. Zhang, Y.L. Zhao, Z.F. Chai, Acute toxicity of nano- and micro-scale zinc powder in healthy adult mice, Toxicol. Lett. 161 (2006) 115–123.
[61] Z. Chen, H. Meng, G. Xing, C. Chena, Y. Zhaoa, G. Jia, T. Wang, H. Yuan, C. Ye, F. Zhaoa, Z. Chai, C. Zhuc, X. Fang, B. Mac, L. Wan, Acute toxicological effects of copper nanoparticles *in vivo*, Toxicol. Lett. 163 (2006) 109–120.
[62] M.T. Tseng, Q. Fu, K. Lor, G.R. Fernandez-Botran, Z.B. Deng, U. Graham, D.A. Butterfield, E.A. Grulke, R.A. Yokel, Persistent hepatic structural alterations following nanoceria vascular infusion in the rat, Toxicol. Pathol. 42 (2014) 984–996.
[63] S.Q. Li, H. Zhu, R.R. Zhu, X.Y. Sun, S.D. Yao, S.L. Wang, Impact and mechanism of TiO_2 nanoparticles on DNA synthesis *in vitro*, Sci. China Ser. B Chem. 51 (2008) 367–372.
[64] H.W. Chen, S.F. Su, C.T. Chien, W.H. Lin, S.L. Yu, C.C. Chou, J.J. Chen, P.C. Yang, Titanium dioxide nanoparticles induce emphysema-like lung injury in mice, FASEB J. 20 (2006) 2393–2395.
[65] N. Singh, B. Manshian, G.J. Jenkins, S.M. Griffiths, P.M. Williams, T.G. Maffeis, C.J. Wright, S.H. Doak, NanoGenotoxicology: the DNA damaging potential of engineered nanomaterials, Biomaterials 30 (2009) 3891–3914.