



## ACUTE AND SUB-ACUTE ORAL TOXICITY ASSESSMENT OF THE CERIUM OXIDE NANOPARTICLES IN WISTAR RATS

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### ABSTRACT

Cerium oxide is an oxide of the rare earth metal which is widely used for several disorders. However, there are no complete toxicity studies done on it. The purpose of present study is to evaluate acute and subacute toxicity of cerium oxide nano particles on Wistar albino rats. Both acute and subacute studies were done according to the Organization for Economic Cooperation and Development (OECD). In the acute toxicity study, Cerium oxides were administered to six rats at 2000 mg/kg, once orally and were observed for 48 hours. No toxic signs/mortality was observed. In the sub-acute study, Cerium oxide was administered once daily for 28 days to rats at 500, 1000 and 2000 mg/kg, orally. No toxic signs/mortality was observed. There were no significant changes ( $P < 0.01$ ) in the body weights, organ weights and haemato-biochemical parameters in any of the dose levels. No treatment related gross/histopathological lesions were observed. The present investigation demonstrated that the no observed adverse effect level was 2000 mg/kg body weight per day of Cerium oxide in rats and hence may be considered as non-toxic.

**Key words:** Cerium oxide, Acute toxicity, Sub-acute toxicity, Wistar rats.

### INTRODUCTION

Cerium oxide also known as ceric oxide, ceria or cerium dioxide is an oxide of the rare earth metal cerium. It is a pale yellow-white powder with the chemical formula  $CeO_2$ . Cerium oxide nanoparticles, considered one of the most interesting nanomaterials for their catalytic properties, show a promise for application in therapy. Due to the presence of oxygen vacancies on its surface and auto regenerative cycle of its two oxidation states,  $Ce^{3+}$  and  $Ce^{4+}$ , nanocerium can be used as an antioxidant agent. Because many disorders are associated with oxidative stress and inflammation, cerium oxide nanoparticles may be a tool for the treatment of these pathologies. Their application ranges from fighting

inflammation and cancer, to radiation protection of cells (Celadrol I *et al.*, 2011; Shah V *et al.*, 2012).

Organization for Economic Cooperation and Development (OECD) defined acute toxicity as the toxic effects which appeared shortly after giving one or more doses of the chemical substances in 24 h. Acute toxicity study is conducted to define the intrinsic toxicity of a chemical substance, sensitivity of a species towards the toxicity, target organs and information for risk assessment after acute exposure to the chemical substances (Paine AJ, Marrs TC, 2000) Sub-acute toxicity is defined by OECD as the adverse effects occurring as a result of the repeated daily dosing of chemical to experimental animals for part (not exceeding 10%) of the life span. These studies give information on the cumulative toxicity of a substance, target organs and the physiological and metabolic tolerance of a compound at low dose prolonged exposure (OECD, 1995).

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## MATERIALS AND METHODS

### Cerium oxide preparation

Nanocrystalline powders of cerium oxide were prepared from cerium (III) nitrate solution by a two-stage precipitation process which yielded weakly-agglomerated powders with a crystallite size smaller than 5 nm. Hydrogen peroxide was added to cerium nitrate at 5°C to slowly oxidize  $Ce^{3+}$  to  $Ce^{4+}$  and thereby initiate homogeneous precipitation with the formation of dense spherical agglomerates. The precipitation process was completed by the addition of ammonium hydroxide which disrupted the spherical agglomerates leaving a weakly-agglomerated powder of hydrated ceria. The process was completed by hydrothermal treatment at 180°C without increase in crystallite size (Boro D, Stephen P, 1999). It was dissolved in normal saline.

### Animals used

Healthy male and female Wistar albino rats at 8 weeks of age were purchased from Ghosh Enterprises, Kolkata. Females should be nulliparous and non-pregnant. At the commencement of its dosing, each animal should be between 8 and 12 weeks old. The temperature in the experimental animal room should be 22°C and Lighting should be artificial, the sequence being 12 hrs light and 12 hrs dark. The animals are housed individually. The study was performed following approval from Institutional Animal Ethical Committee (IAEC) of Shri Vishnu College of Pharmacy, Bhimavaram.

### Acute toxicity study

Acute toxicity studies were carried out using acute toxic class limit test dose guidelines 423 of Organization for Economic Co-operation and Development (OECD, 2001). Acute toxicity of the test drug was carried out, using groups of three Swiss albino mice; by administering a dose of 2000 mg/kg body weight, p.o., and the control group received normal saline, and observed all the animals closely for 24 hrs for any mortality. No mortality was observed.

It was observed that the cerium oxide was not lethal to the rats even at 2000mg/kg dose. Hence 1/10<sup>th</sup> (200mg/kg) selected as maximum safety dose. The toxicological effects were assessed on the basis of mortality and behavioral changes, the observation occurred during 48 hours. Animals were observed individually once during the first 30 minutes after dosing, periodically during the first 24 hours (with special attention given during the first 4 hours) Observations included changes in skin and fur, eyes and mucous membranes, and behavior pattern, and also observed for tremors, convulsions, salivation, diarrhea, lethargy, sleep and coma.

The rats are weighed on day 1, 7 & 14. The rats were sacrificed after 14 days and the liver kidneys were

taken out and fixed in neutral buffered formalin for 72 Hrs. They were then processed and stained with hematoxylin and eosin stain for histopathology examination. The slides were examined single-blindly by a qualified pathologist.

### Sub-acute toxicity study

Sub-acute toxicity study was conducted according to OECD Test guideline 407 (OECD, 1995). The Cerium oxide Nano particles at the dose of 200 mg/kg body weight were administered orally to one group of six rats respectively to every 24 h for 28 days and control received vehicle at the same volume. The toxic manifestation such as body weight, mortality, food and water intake was monitored. After 28 days all surviving animals were fasted overnight and anaesthetized with ether. The heparinized blood samples were collected for determining hematological parameters and the serum from non-heparinized blood was carefully collected for determining clinical blood chemistry. Animals were sacrificed after blood collection and the internal organs were removed and weighed to determine the relative organ weights and observed for gross lesions. The internal organs were preserved in 10% buffered formaldehyde solution for histological examination. The slides were examined single-blindly by a qualified pathologist.

### Statistical analysis

Graph pad prism5 software was used for statistical interpretation. Data are expressed as means  $\pm$  S.E.M. and were analysed using by one way ANOVA, Bonferroni's multiple comparison test. A  $P$ -value  $\leq 0.05$  and  $P$ -value  $\leq 0.001$  was considered statistically significant.

## RESULTS AND DISCUSSION

### Acute toxicity study

Acute toxicity studies were conducted in albino mice by using OECD-423 guidelines, in dose increasing method for Cerium oxide Nano particles. Animals are treated with higher dose of cerium oxide 2000 mg/kg. It was found that even at 2000mg/kg dose did not manifest any significant abnormal signs, mortality, behavioral changes, body weight changes (Table no:1). Toxicity signs such as Tremors, Salivation, Diarrhea and Lethargy were not observed. Confirming practically these are not has toxic activity. Histopathological examination of the liver kidney and heart tissues did not show any pathological changes.

### Sub-acute toxicity

In this study, sub-acute toxicity (28 days continuous drug administration) studies shown that there is no significance toxicity in CeO<sub>2</sub> Nano particles treated group compared to normal group.

In these toxicity studies shown, there is no sign of behavioral changes and external characters like mucous membrane, fur, skin etc. There were also no significant differences in the hematological parameters between the

control and treated groups. Peripheral blood smear showed normal morphology of red blood cells in all the rats (Table 2). Histopathological examination did not show any pathological change in the liver, kidney and heart.

**Table 1. Behavioral observations for test group (2000 mg/kg body weight) of Cerium Nano particles**

Observations	30 min	1 hr	4 hrs	8 hrs	24 hrs	48hrs
Skin & Fur	Normal	Normal	Normal	Normal	Normal	Normal
Eyes	Normal	Normal	Normal	Normal	Normal	Normal
Mucous membrane	Normal	Normal	Normal	Normal	Normal	Normal
Behavioral pattern	Normal	Normal	Normal	Normal	Normal	Normal
Tremors	Nil	Nil	Nil	Nil	Nil	Nil
Salivation	Normal	Normal	Normal	Normal	Normal	Normal
Diarrhea	Nil	Nil	Nil	Nil	Nil	Nil
Lethargy	Nil	Nil	Nil	Nil	Nil	Nil
Sleep	Normal	Normal	Normal	Normal	Normal	Normal
Coma	Nil	Nil	Nil	Nil	Nil	Nil

### SUB- ACUTE TOXICITY STUDIES

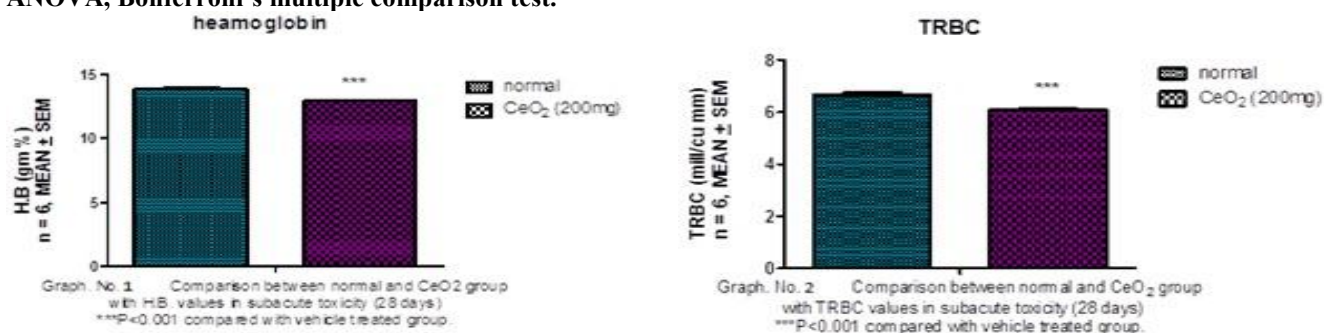
**Table 2. Sub-acute toxicity Hematological parameters in control and test animals**

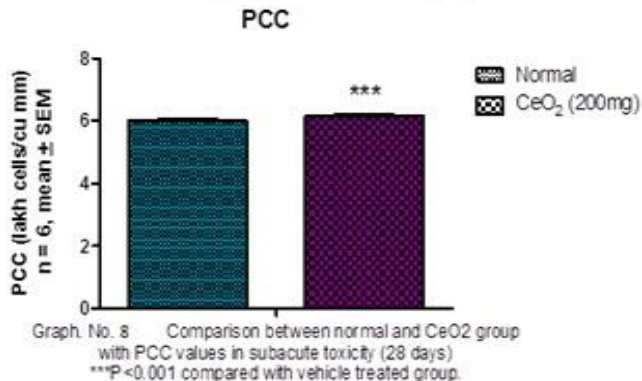
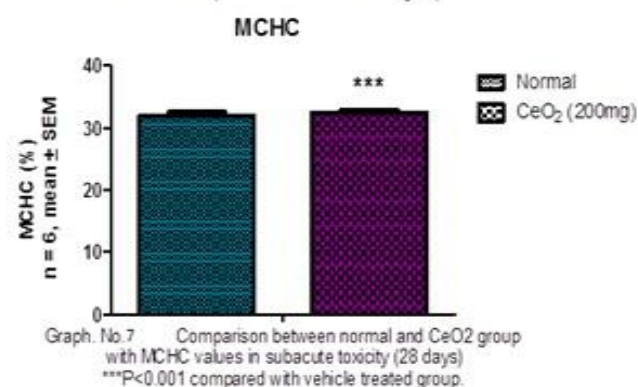
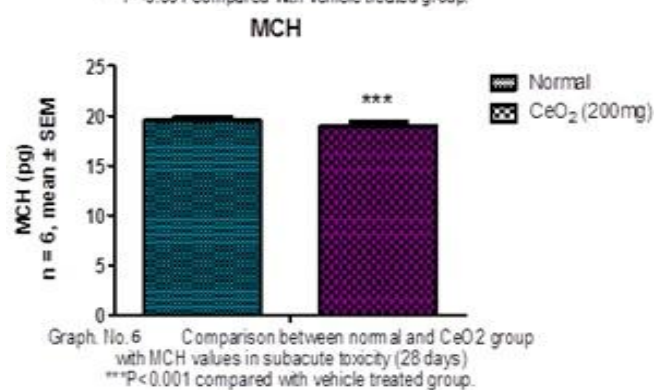
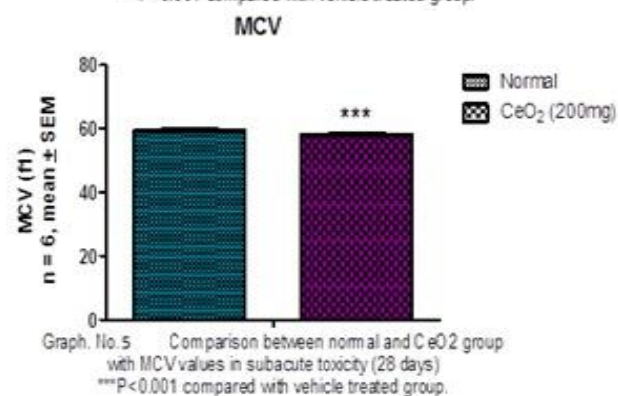
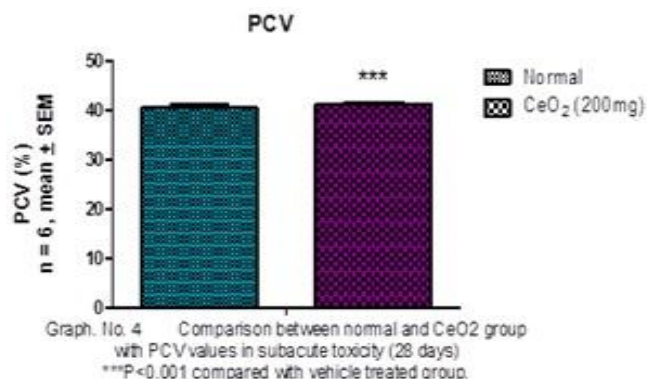
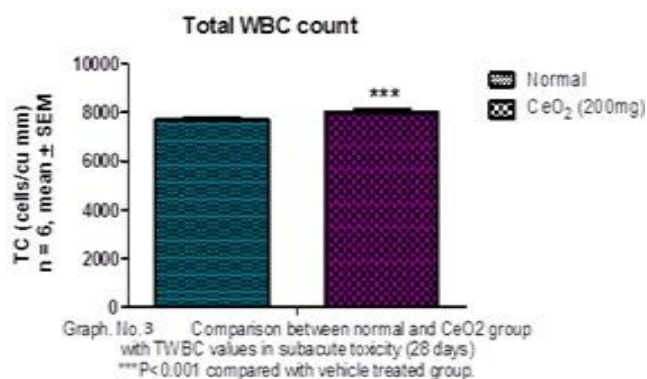
Parameters	Normal	CeO <sub>2</sub> (200mg/kg)
Haemoglobin (Gm %)	13.867 ± 0.076	12.933 ± 0.042***
TRBC (mill/cu mm)	6.65 ± 0.076	6.067 ± 0.033***
TWBC (cells/cu mm)	7691.66 ± 71.20	8016.66 ± 107.755***
ESR (mm )	1±0	1±0
PCV (%)	40.5 ± 0.764	41.167 ± 0.477***
MCV (fl)	59.50 ± 0.428	58.167 ± 0.477***
MCH (pg)	19.5 ± 0.428	19.0 ± 0.365***
MCHC (%)	32.0 ± 0.5777	32.5 ± 0.428***
PCC (lakh cells/cu mm)	6.008 ± 0.049	6.158 ± 0.042***

**Table 3. Biochemical parameters**

Parameters	Control	Test(200mg/kg)
ALT (IUL <sup>-1</sup> )	64.12 ± 2.38	59.63 ± 2.06
ASP (IUL <sup>-1</sup> )	239.75 ± 24.27	219.25 ± 18.86
ALP (IUL <sup>-1</sup> )	221.75 ± 38.96	157.25 ± 18.39
Total bilirubin (µmol L <sup>-1</sup> )	1.75 ± 38.96	1.75 ± 0.16
Total Protein(g L <sup>-1</sup> )	73.75 ± 1.61	75.25 ± 1.05
Creatinine (µmol L <sup>-1</sup> )	52.22 ± 0.68	52.60 ± 0.71

**Fig 1. Values represent the mean ± S.E.M., N= 6, \*\*\*P<0.001 compared with vehicle treated group by one way ANOVA, Bonferroni's multiple comparison test.**





## CONCLUSION

Based on our toxicity study, the oral LD<sub>50</sub> for cerium oxide was more than 2000mg/kg<sup>-1</sup>. We may be the first to report on the effects of cerium oxide on liver, kidney and heart of rat model. In sub-acute toxicity studies concluded that there is no significant changes in biological and histological parameters of rats treated with

CeO<sub>2</sub>Nano particles compared to normal rats.

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