EFFECT OF DOPED CERIUM OXIDE NANOPARTICLES ON THE HAEMATOLOGY OF RATS

R. Sovan[1], N. Vijayan[2] , C. B. Manomohan[3], V. Sajith[4] and K. B. Dhanush[5]
Department of Veterinary Pathology
College of Veterinary and Animal Sciences
Mannuthy- 680 651, Thrissur, Kerala.

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Abstract

Cerium is a rare earth lanthanide and a strong oxidizing agent. It has got a wide range of application from engendering to medicinal field. Potential amount of harm also reported about this particle. Recent days it is used as diesel fuel additive and doped with yttrium and zirconium nanoparticles to increase its oxygen caring capacity. Hence a study designed to study about the impact on the environment taking rat as model. A total no of 36 rats were taken and divided into six groups contains six animals each. Control animals served with normal saline and treatment animals administered intratracheally with 4 and 8 mg/kg body weight of this particles. The higher dose group showed increased levels of hemoglobin but other parameters had no significant difference among any group. The increased level of hemoglobin was suggestive of blood vascular changes.

Key Words: *Doped cerium, Diesel fuel, Intratracheal*

Nanotechnology is widely applied in almost all spheres of science and it is concerned with the manufacture of tailored nanostructures by manipulation of elements in their atomic and sub atomic level. Cerium, a rare earth lanthanide metal,is a pale yellow-white powder has got strong oxidizing properties. CeO_2 NPs is being use in treatment of cardiovascular, neurodegenerative and radiation induced tissue damage. Among metal based nanoparticles cerium oxide nanoparticles may be the most important materials with wide ranges of applications for solar cells, fuel cells, gas sensors, oxygen pumps, and glass/ceramic applications (Gao *et al.,* 2006).The use of CeO_2 NPs as a diesel fuel additive to reduce the ignition temperature of carbonaceous diesel exhaust particle (DEP) and subsequent reduction of the emission of particulate matter from diesel engine has been explored. Given the current industrial application, it is thought that the most common route of CeO_2 exposure is likely through inhalation and ingestion. Hence a systematic investigation about the effect of this particle in animal model was designed.

Material and Methods

Particles

Cerium Zirconium Yttrium oxide nanoparticles ($Ce_{ZrY}O_2$ NPs) of size 40-50 nm were obtained from School of Nanosciences and Technology, National Institute of

1. *M.V.Sc scholar*
2. *Professor and Head (Retd.)*
3. *Professor (Retd.)*
4. *Assistant Professor, School of Nanosciences and Technology, National Institute of Technology, Calicut*
5. *Assistant Professor*

Technology, Kozhikode. The powder was calcinated at 500˚C for 1 hour before use in order to prevent agglomeration. $CeZrYO_2$ NPs were suspended in normal saline (0.3ml) and used for the present study.

Animals

Adult Sprague-Dawley female rats weighing approximately 180-220g procured from Small Animal Breeding Station, College of Veterinary and Animal Sciences, Mannuthy were used for this study. The animals were acclimatized for twoweeks in the experimental animal house of Department of Veterinary Pathology prior to the experiment. The animals were given standard rat feed and clean water *ad libitum* during the study. The experiment was conducted for a period of 15 days after the approval of the Institutional Animal Ethics Committee of College of Veterinary and Animal Sciences, Mannuthy, according to the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and Prevention of Cruelty to Animals Act (Amendment 1988).

Experimental design

The rats were divided into six groups having six animals each and instillation was done as follows:

Group	Treatment
T_1	4mg/kg $Ce_{ZrY}O_2$ NPs (0.3ml with normal saline) and sacrifice on 3 days post inoculation (PI)
T_2	4mg/kg $Ce_{ZrY}O_2$ NPs (0.3ml with normal saline) and sacrifice on 15 days PI
T_3	8mg/kg $Ce_{ZrY}O_2$ NPs (0.3ml with normal saline) and sacrifice on 3 days PI
T_4	8mg/kg $Ce_{ZrY}O_2$ NPs (0.3ml with normal saline) and sacrifice on 15 days PI
T_5	Control(0.3ml normal saline) and sacrifice on 3 days PI
T_6	Control(0.3ml normal saline) and sacrifice on 15 days PI

Instillation

The instillation of the $CeZrYO_2$ NPs was done on the animals under general anesthesia. Anesthesia was induced by intraperitoneal injection of ketamine hydrochloride(70mg/kg) and xylazine hydrochloride (7mg/kg) combination. The rats were placed over an inclined board and intratracheal instillation was carried out.

Table 1: Mean ± SE values of hematological parameters of rats instilled with $Ce_{ZrY}O_2$ NPs and normal saline

Parameters	T_1	T_2	T_3	T_4	T_5	T_6	p- value
TLC(10^3/mm^3)	12.23±1.37	11.87±1.50	10.43±1.50	8.87±0.78	10.47±1.86	14.8±1.86	>0.05
Hb (%)	11.6^{d}±1.48	16.67^{ab}±1.31	12.17^{cd}±0.50	17.93^{a}±0.56	13.21^{b}±0.71	14.83^{b}±0.34	<0.01
ESR(mm/hr)	1.66 ±0.33	1.18±0.27	1.02±0.11	1.27±0.24	1.17±0.05	0.83±0.08	>0.05

(N.B: p-value > 0.05 = nonsignificant; p-value < 0.05= significant at 5% level; p-value < 0.01= significant at 1% level Means bearing same superscript within a row does not differ significantly)

Table 2: Mean ± SE differential leukocyte percentage (%) of rats instilled with $Ce_{ZrY}O_2$ NPs and normal saline

Parameters	T_1	T_2	T_3	T_4	T_5	T_6	p- value
Lymphocyte	74.80±2.09	71.40±4.31	77.13±2.18	70.57±4.15	68.92±3.62	72.85±3.67	>0.05
Monocyte	10.40±0.92	8.92±1.08	10.72±1.47	11.05±1.46	8.22±1.29	9.33±0.94	>0.05
Granulocyte	14.80±1.51	19.68±3.38	12.15±1.25	18.38±3.79	22.87±3.32	17.82±3.69	>0.05

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ESR(mm/hr)	1.66 ±0.33	1.18±0.27	1.02±0.11	1.27±0.24	1.17±0.05	0.83±0.08	>0.05

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Table 2: Mean ± SE differential leukocyte percentage (%) of rats instilled with $Ce_{ZrY}O_2$ NPs and normal saline

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Hematological Parameters

1ml of blood was collected from the retro-orbital plexus under diethyl ether anesthesia using heparinized capillary tubes in eppendorf tube containing ethylene diamine tetra acetic acid (EDTA) as anticoagulant at the rate of 2 mg/ml. Total Leukocyte count (TLC), Differential Leukocyte count (DLC), haemoglobin (Hb), was estimated using automatic analyzer, (Orphee, Switzerland model: Mythic 18 Vet.). Erythrocyte sedimentation rate(ESR) was estimated by Wintrobe hematocrit tube method (Benjamin 1985).

Results and Discussion

Total leukocyte count (TLC)

There was no significant difference between the total leukocyte count of any of the groups. The mean total leukocyte count values of the experimental groups is presented in the table no.1 (p>0.05)

Differential leukocyte count (DLC)

The mean differential leukocyte count values presented in table no.2. the values showed no significant difference between the six groups (p>0.05)

Haemoglobin (Hb)

The mean values of haemoglobin of the groups T_1, T_2, T_3, T_4, T_5 and T_6 are presented in the table no.1. Significant increase was observed in the Hb level of T_4 group (17.93±0.56 g/dl) *i.e.* the rats instilled with $CeZrYO_2$ NPs at a dose rate of 8mg/kg body weight and sacrificed after 15day PI. There was significant decrease in the group T_1(11.6±1.48g/dl) *i.e.* the rats instilled with $CeZrYO_2$ NPs at a dose rate of 4mg/kg body weight and sacrificed after 3 day PI at 5% level (p<0.05).

Erythrocyte sedimentation rate (ESR)

The mean ESR values showed no significant difference among any of the six groups. (Table 1). There was no significant difference in total leukocyte count and mean differential leukocyte count of any of the six groups. This is in accordance with the observation of Hamrahi *et al.* (2012) and Park *et al.* (2009) that there was no significant alteration in WBC count after intra peritoneal and oral injection of CeO_2 NPs at different dose rate and also observed same after oral administration of this particles. This along with our observation confirms that small dosage of CeO_2 NPs does not affect the WBC count irrespective of route of administration. There

was significant increase in haemoglobin level in T_4 group *i.e.* the rats instilled with $CeZrYO_2$ NPs at a dose rate of 8mg/kg body weight which was sacrificed after 15day PI and there was significant decrease in the group T_1 *i.e.* the rats instilled with $CeZrYO_2$ NPs at a dose rate of 4mg/kg body weight which was sacrificed after 3 days. Decreased hematocrit values, mean RBC count were reported by Hamrahi *et al.* (2012) on intraperitoneal administration of CeO_2 NPs whereas Park *et al*. (2009) did not observe any effect on oral administration. The results of the present study showed increased Hb level in prolonged cases of high dose group and decrease in early cases of low dose groups. The mean ESR values showed no significant difference among any of the six groups.

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