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CONTENTS

Effect of nano zinc on haematological parameters of Wistar Rats

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ABSTRACT: Zinc is widely used in daily life, though it is considered comparatively safe; its pathological effects have not been documented. Limited investigations have been carried on effect of zinc nanoparticleson health of animals and man. Therefore, present study was designed to study the effect of zinc nano particles on haematological parametersof wistar rats.For this study, a total of 35 rats of six week age of both sexes approximately 50 gram weight weredivided randomly into two groups of 20 rats in Group I (control) and 15 rats in group II(treated). In treated group rats were given nano zinc particles into stomach by oral gavaging and dose of nano zinc was selected based on NOAEL dose of nano zinc oxide 31.25 mg/kg body weight of rat for a period of 90 days.Blood was collected from 5 rats from each group at 0th (only from G1 group), 30th, 60th and 90th DPT for hematological studies. Hematological parameters like haemoglobin (Hb), packed cell volume (PCV), mean corpuscular haemoglobin(MCH) and mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), total leucocyte count (TLC), absolute lymphocyte count and lymphocyte count showed decrease in values compared to control. Significant decrease was observed in mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV) and lymphocyte count. There was increase in values of total erythrocyte count (TEC), monocyte count, eosinophil count, neutrophil countand absolute neutrophil count (ANC) as compared to controlgroup. However, significant increase in total erythrocyte count (TEC), monocyte count and neutrophil count were observed.

Key words:Erthryocytic Indices,haematological parameters,Mean Corpuscular Haemoglobin, Wistar Rats,Zinc Nano Particles

Nanotoxicology refers to the study of the interactions of nanostructures with biological systems with an emphasis on elucidating the relationship between the physical and chemical properties e.g. size, shape, surface chemistry, composition and aggregation of nanostructures with induction of toxic biological responses (Maynard *et al.*, 2006). Nanostructures can enter the body through various routes such as intra-venous, dermal, subcutaneous, inhalation, intraperitoneal and oral. They enter the cells of the organ and reside in the cells for an unknown amount of time before moving to other organs or before getting excreted (Fischer and Chan, 2007). Interaction with biological systems can give rise to toxic effects like allergy (Maynard *et al.*, 2006), fibrosis (Nel *et al.*, 2006), deposition in different organs that can lead to organ failure, inflammation, cytotoxicity (Nel *et al.*, 2006), tissue damage (Singh *et al.*, 2009), ROS generation (Meng *et al.*, 2007), DNA damage (Singh *et al.*, 2009). Interaction of nanoparticles with lymphocytes and other cell types can grant to a varied spectrum of possible impacts, including inflammation and immunomodulation such as immunodeficiency, autoimmunity and hypersensitivity. (Ambwani *et al.*, 2015). Because Zinc Oxide Nano Particles (ZnO NPs) are the most commonly utilized nanomaterials in various consumer products. Many studies have shown the toxic effects of ZnO NPs in several experimental models, including cell lines, bacteria, nematodes, algae, plants, and fish (Heng *et al.*, 2010 and Lee *et al.*, 2012) and preliminary results indicated that affected organ systems may show inflammation, altered heart rate functions, and oxidative stress.

Ingested nanoparticles may be absorbed through the intestinal lining and translocate into the blood stream where they undergo first pass metabolism in the liver. Studies have revealed liver, kidney and spleen as the target organs for engineered nanoparticles after uptake by the gastrointestinal tract. Particularly, in vivo study is considered necessary to investigate the toxic effect of NPs in biological systems, which would stress the importance of local toxicity from the administration of NPs. For example, after oral administration of 30 nm ZnO NPs for 14 days to mice, ZnO NPs significantly accumulated in the liver and caused oxidative stress mediated by DNA

damage and apoptosis (Sharma *et al.*, 2012) Similarly, ZnO NPs caused impairment of mitochondria and cell membranes in rat kidneys after oral administration of ZnO NPs for 14 days (Yan *et al.*, 2012). Repeated application through dermal routes for 28 days decreases the collagen level at the site of application, which may be induced by oxidative stress (Surekha *et al.*,2012). Keeping in view the above facts, the present study has been planned to study the effects of nano zinc intoxication on haematological Parameters of Wistar rats of both sexes.

MATERIALS AND METHODS

Five-week-old Wistar Rats of both sexes approximately of 50-gram weight were procured from Laboratory Animal Resources, Indian Veterinary Research Institute, Izatnagar, Bareilly, India. The rats were kept in Experimental Animal House of Department of Veterinary Pathology,Pantnagar and were fed standard recommended feed and RO water ad-libitum from start of experimentation upto 90 days of course of experiment. Rats were housed in stainless wire cages, two rats per cage, in the rats room, which was maintained at a temperature of 21.0°C–23.1°C with light hours from 8 am to 8 pm and good hygienic conditions. All animals were acclimated and quarantined for 7 days in an animal room of the department of Veterinary Pathology C.V.A.Sc. The rats were immunized with Ranikhet Disease Vaccine R2B strain at every 15 days interval from 0 day(six week of age) upto 90 days of course of experiment period.

The test compound, Nano zinc, of commercial grade (Sisco Research Laboratories Pvt. Ltd) used in the study was procured from local market. The Nano zinc used characterization; nano dispersion (50nm) and Molecular weight: 65.38.

Borosil glassware's viz : test tubes, flasks of various capacities, beakers, slides, pipettes etc were soaked in soap water overnight then washed and rinsed thrice with triple glass distilled water and then dried and sterilized in hot air oven prior to its use. Plastic wares viz: sterilized micropipette tips, centrifuge tubes; micro centrifuge tubes etc. were used during the study.

The experiment was conducted on 6 week old wistar rats divided randomly into two groups with 20 rats in G1 and 15 rats in G2 group. The rats of both the groups were immunized with 0.1 ml dose of Ranikhet Disease Vaccine R2B strain at every 15 days interval intraperitoneally started from 0 dayupto 90 days of experiment period. First group (G1) was kept as control and Second group (G2) was fed with nano zinc particles at the Non Observable Adverse Effect Level dose of zinc oxide nano particles that is 31.25 mg/kg body weight per day (Kim *et al.*,2014) from 0 day of experiment till 90 days post treatment (DPT), both treated and control group were given RO water ad-libitum.

Blood was collected from 5 rats from each group in clean, EDTA coated tubes at 0(only from G1 group), 30th, 60th and 90th DPT for hematological studies. Packed cell volume (PCV) wasestimated by microhematorit method. Haemoglobinwas estimated using Acid Haematin method (Hermann Sahli's). Total erythrocyte count (TEC) and total leucocyte count (TLC) were determined by hemocytometer with Hayem's RBC and Thomas WBC diluting fluid respectively. Differential leucocyte count (DLC) was done by preparing thin blood smear from a drop of fresh blood without anticoagulant. The smear was air dried and stained with Giemsa stain. The procedure was carried out by covering the stain with absolute methanol for 1 minute / fixation. Then methanol was drained and diluted Giemsa stain was poured on the smear and allowed to react for 30-40 minute. The stain from the slide was rinsed using tap water or distilled water and then the slides were air-dried and seen under 100 X with light microscope. The leucocytes were counted by zigzag method as described by Lucas and Jamroz (1961). Erythocytic indices were calculated as per the formula given below:

- Mean corpuscular volume MCV = $\frac{\text{PCV} + 10}{\text{TEC}}$ (expressed in femto liter)

- Mean corpuscular haemoglobin MCH = $\frac{Hb + 10}{TEC}$ (expressed in picogram)
- Mean corpuscular haemoglobin concentration MCHC = $\frac{Hb + 10}{PCV}$ (expressed in gm/dl)
- Absolute lymphocyte count = $\frac{\% \text{ lymphocyte}}{100}$ * TLC
- Absolute neutrophils count = $\frac{\% \text{ neutrophils}}{100}$ * TLC

Statistical Analysis

Done by as per Snedecor and Cochran (1994)

RESULTS AND DISCUSSION

Haemoglobin(Hb)

Mean haemoglobinof experimental rats in different groups at different time intervals are expressed in gm/dl and presented in Table 1. There was no significant difference in mean haemoglobin observed between group I and group II throughout the experiment at different time intervals. There was a slight 8.43%, non-significant increase in the mean haemoglobinof treated group as compared to controls at 30thDPT.

Table 1: Mean haemoglobin concentration (g/dl) in different groups of experimental rats at different time intervals (Mean ± SE)

DPT	Group		
	Control	Treated	Per cent increase or decrease value
0DPT	10.40±0.31	10.40±0.31	(0%)
30DPT	11.38±0.56	12.34±0.66	(8.43%)
60DPT	11.28±0.39	11.20±0.58	(-0.70%)
90DPT	13.80±0.43	13.18±1	(-4.49%)

Packed cell volume (PCV)

Mean PCV a value of experimental rats in different groups at different time intervals is expressed in per cent and presented in Table 2. There was no significant difference in mean packed cell volume observed between group I and group II throughout the experiment at different time intervals. There was 16.05% and 10.46%, non-significant increase in the mean packed cell volume of treated group as compared to controls at 30thDPT and 60th DPT, respectively. There was a slight 4.41%, non-significant decrease in the mean packed cell volume of treated group as compared to controls at 90thDPT.

Table 2: Mean packed cell volume (%) in different groups of experimental rats at different time intervals (Mean ± SE)

DPT	Group		
	Control	Treated	Per cent increase or decrease value
0DPT	34.4±1.57	34.4±1.57	(0%)
30DPT	32.4±1.21	37.6±2.5	(16.05%)
60DPT	34.4±0.68	38.0±2.41	(10.46%)
90DPT	40.8±1.02	39.0±1.48	(-4.41%)

Total erythrocyte count (TEC)

The Mean values of TEC of experimental rats in different groups at different time intervals are presented in Table 3 and expressed in 10^6/µl. The TEC at 30th DPT was significantly different. The TEC value of treated group is significantly higher than control group at 30thDPT.

Table 3: Mean total erythrocyte count (10^6/µl) in different groups of experimental rats at different time intervals (Mean ± SE)

DPT	Group		
	Control	Treated	Per cent increase or decrease value
0DPT	2.840±0.17	2.840±0.17	(0%)
30DPT*	3.974^b±0.13	5.040^a±0.22	(21.15%)
60DPT	6.246±0.3	4.784±1.15	(-30.56%)
90DPT	6.708±0.57	6.236±0.47	(-7.56%)

*Different alphabetic letters (a and b) indicate significant ($P<0.05$) difference when compared horizontally within the same row. (DPT= Days post treatment).

Mean corpuscular volume(MCV)

Average MCV of experimental rats in different groups at different time intervals are presented in Table 4 and expressed in fl. The MCV showed significant difference at 30th DPT and 60th DPT. The MCV value of treated group is significantly lower than control group at 30th DPT and 60th DPT. The MCV of treated group was decreased by 14.73% and 41.39% as compared to control group at 30th

DPT and 60th DPT,respectively.

Table 4: Mean corpuscular volume (MCV) (fl) in different groups of experimental rats at different time intervals (Mean ± SE)

DPT	Group		
	Control	Treated	Per cent increase or decrease value
0DPT	141.200±4.76	141.200±4.76	(0%)
30DPT*	75.483[a]±3.27	64.364[b]±2.07	(-14.73%)
60DPT*	96.182[a]±29	56.364[b]±3.05	(-41.39%)
90DPT	63.674±1.34	60.900±2.43	(-4.35%)

*Different alphabetic letters (a and b) indicate significant (P<0.05) difference when compared horizontally within the same row. (DPT= Days post treatment).

Mean corpuscular Haemoglobin(MCH)

Average values of MCH of experimental rats in different groups at different time intervals are presented in Table 5 and expressed in pg. The MCH was significantly different at 30th DPT and 60th DPT. The MCH of treated group was 6.80% and 44.79% lower at 30th DPT and 60th DPT, respectively as compared to control.

Table 5: Mean corpuscular haemoglobin (MCH) (pg) in different groups of experimental rats at different time intervals (Mean ± SE)

DPT	Group		
	Control	Treated	Per cent increase or decrease value
0DPT	40.570±1.29	40.570±1.29	(0%)
30DPT*	24.588[a]±1.36	22.916[b]±0.82	(-6.80%)
60DPT*	33.252[a]±9.63	18.356[b]±1.23	(-44.79%)
90DPT	21.795±0.65	21.222±0.89	(-2.62%)

*Different alphabetic letters (a and b) indicate significant (P<0.05) difference when compared horizontally within the same row. (DPT= Days post treatment).

Mean corpuscular haemoglobin concentration (MCHC)

Mean MCHC values of experimental rats in different groups at different time intervals are presented in Table 6 and expressed in g/dl. There was a significant difference in the mean value of MCHC at 60th DPT. A significant reduction of 13.27 % was observed as compared to control at 60thDPT.

Table 6: Mean corpuscular haemoglobin concentration (MCHC) (g/dl) in different groups of experimental rats at different time intervals (Mean ± SE)

DPT	Group		
	Control	Treated	Per cent increase or decrease value
0DPT	26.16±0.64	26.1688±0.64	(0%)
30DPT	34.930±0.66	33.134±2.01	(-5.42%)
60DPT*	31.560[a]±1.15	27.862[b]±0.32	(-13.27%)
90DPT	35.082±1.17	33.112±1.14	(-5.94%)

*Different alphabetic letters (a and b) indicate significant (P<0.05) difference when compared horizontally within the same row. (DPT= Days post treatment).

Total leucocytes count(TLC)

Mean TLC values of experimental rats in different groups at different time intervals are expressed in 10^3/µl ofblood and presented in Table 7. There was no significant difference in mean TLC values observed between group I and group II throughout the experiment at different time intervals. At 60th DPT the TLC value of treated group was increased by 17.88 % as compared to control group, it was found statistically non-significant. There was 18.22% and 9.05%, non-significant decrease in the mean TLC of treated group as compared to controls at 30th DPT and 90th DPT, respectively.

Table 7: Mean total leucocyte count (TLC) (10^3/µl) in different groups of experimental rats at different time intervals (Mean ± SE)

DPT	Group		
	Control	Treated	Per cent increase or decrease value
0DPT	12.348±0.6	12.348±0.6	(0%)
30DPT	12.432±0.51	10.166±1.26	(-18.22%)
60DPT	4.25±0.92	5.01±2.33	(17.88%)
90DPT	6.861±0.41	6.24±0.96	(-9.05%)

Differential Leucocytecount

The variation in numbers of different white blood corpuscles during the study are described below.

Neutrophil

Neutrophil count of experimental rats in different groups at different time intervals are presented in Table 8 and expressed in per cent. Mean neutrophil count value was significantly different at 60th DPT

and 90th DPT. The neutrophil count of treated group at 60th DPT and 90th DPT were respectively 14.63% and 36.84% higher as compared to control group.

Table 8: Mean neutrophils count (%) in different groups of experimental rats at different time intervals (Mean ± SE)

DPT	Group		
	Control	Treated	Per cent increase or decrease value
0DPT	15.8±1.88	15.8±1.88	(0%)
30DPT	26.6±1.12	28±1.85	(5.26%)
60DPT*	32.8^{b}±1.66	37.6^{a}±1.89	(14.63%)
90DPT*	22.8^{b}±1.16	31.2^{a}±1.85	(36.84%)

*Different alphabetic letters (a and b) indicate significant (P<0.05) difference when compared horizontally within the same row. (DPT= Days post treatment).

Lymphocyte

Table 9 shows mean lymphocyte count of experimental rats in different groups at different time intervals and is expressed in per cent. There wassignificant difference in lymphocyte count at 60th DPT and 90th DPT. The lymphocyte count of treated group at 60th DPT was 14.63 % lower than the control group and 90th DPT was 10.14% higher than the control group.

Table 9: Mean lymphocyte count (%) in different groups of experimental rats at different time intervals (Mean ± SE)

DPT	Group		
	Control	Treated	Per cent increase or decrease value
0DPT	80.2±1.56	80.2±1.56	(0%)
30DPT	69.0±1.14	66.4±10.66	(-3.91%)
60DPT*	37.6^{a}±1.89	32.8^{b}±1.66	(-14.63%)
90DPT*	63.8^{b}±1.36	71.0^{a}±0.89	(10.14%)

*Different alphabetic letters (a and b) indicate significant (P<0.05) difference when compared horizontally within the same row. (DPT= Days post treatment).

Monocyte

The monocyte values of experimental chicken are expressed in per cent and presented in Table 10. A significant difference in monocyte count was observed at 30th DPT, 60th DPT and 90th DPT. The monocyte count of treated group at 30thDPT, 60th DPT and 90th DPT were respectively 22.72%, 42.42% and 41.67% higher than the controlgroup.

Table 10: Mean monocyte count (%) in different groups of experimental rats at different time intervals (Mean ± SE)

DPT	Group		
	Control	Treated	Per cent increase or decrease value
0DPT	2.8±0.37	2.8±0.37	(0%)
30DPT*	3.4^{b}±0.51	4.4^{a}±0.24	(22.72%)
60DPT*	3.8^{b}±0.37	6.6^{a}±0.51	(42.42%)
90DPT*	2.8^{b}±0.37	4.8^{a}±0.66	(41.67%)

*Different alphabetic letters (a and b) indicate significant (P<0.05) difference when compared horizontally within the same row. (DPT= Days post treatment).

Eosinophil

The values of eosinophil in experimental rats are presented in Table 11 and expressed in per cent. There was no significant difference observed at different time interval throughout the experiment. In treated group, an increase of 25.00 % was observed in eosinophil count at 90th DPT as compare to control group.

Table 11: Mean eosinophil count (%) in different groups of experimental rats at different time intervals (Mean ± SE)

DPT	Group		
	Control	Treated	Per cent increase or decrease value
0DPT	1.2±0.2	1.2±0.2	(0%)
30DPT	1.2±0.2	1.4±0.24	(16.67%)
60DPT	1.2±0.2	1.4±0.24	(16.67%)
90DPT	0.8±0.37	1.0±0.32	(25%)

Absolute lymphocyte count(ALC)

Absolute lymphocyte count values of experimental rats are expressed in 10^3/µl and shown in Table 12. There was no significant difference observed at different time interval throughout the experiment. There was non-significant decreased of 8.10% in absolute lymphocyte count of treated group at 90th DPT as compared to control group.

Absolute neutrophil count(ANC)

Absolute neutrophil count values of experimental rats in different groups are shown in Table 13 and expressed in 10^3/µl. The value of ANC was

Table 12: Mean absolute lymphocyte count (ALC, 10^3/µl) in different groups of experimental rats at different time intervals (Mean ± SE)

DPT	Group		
	Control	Treated	Per cent increase or decrease value
0DPT	11.811±0.47	11.811±0.47	(0%)
30DPT	8.697±0.32	6.976±1.45	(-19.79%)
60DPT	4.019±0.81	3.842±1.38	(-4.403%)
90DPT	4.459±0.21	4.098±0.22	(-8.10%)

significantly different at 30th DPT. The value of ANC was significantly increased by 38.96 % in treated group at 30th DPT as a compared to control group.

Table 13: Mean absolute neutrophils count (ANC, 10^3/µl) in different groups of experimental rats at different time intervals (Mean ± SE)

DPT	Group		
	Control	Treated	Per cent increase or decrease value
0DPT	1.484±0.25	1.484±0.25	(0%)
30DPT*	2.425^{b}±0.79	3.370^{a}±0.12	(38.96%)
60DPT	1.939±0.76	2.166±0.78	(11.70%)
90DPT	1.256±0.11	1.688±0.22	(34.39%)

*Different alphabetic letters (a and b) indicate significant ($P<0.05$) difference when compared horizontally within the same row. (DPT= Days post treatment).

The present study of various haematological parameters revealed the decrease in the haemoglobin (Hb) in nano zinc treated group. However, this decrease was not statistically significant.Thepacked cell volume (PCV), total erythrocyte count (TEC) were increased in nano zinc treated group but this was also non significant statistically. The significant decrease in mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV) and mean corpuscular haemoglobinconcentration (MCHC) were revealedin nano zinc treated group. There was non-significant decrease in total leucocyte count (TLC) and absolute lymphocyte count (ALC). There was non-significant increase in absolute neutrophil count (ANC). The mean per cent neutrophil count and monocyte count was significantly higher in nano zinc treated group, while mean per cent lymphocyte count was significantly lower in nano zinc treated group.The non-significant increase in per cent eosinophil count was observed in nano zinc treated group.The rats of treated groups were not so much dehydrated during experiment.

These results are in confirmation with Yan *et al.* (2012)in zinc fed rats and reported that high zinc diet resulted in iron deficiency anemia and this zinc-induced anemia was related to a decrease in the levels of Hb, MCV and MCH. Kim *et al.* (2009)in rats and Suganthi *et al.* (2015)in fish reported decrease in mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC) in nano zinc oxide treated group. Results of present study are in agreement with the result of Tang *et al.* (2016)in rats they observed that neutrophil count and monocyte count was significantly higher and lymphocyte count was significantly lower in sub lethal dose treated group than the corresponding control group. They also propose that the neutrophil and granulocyte counts reflect an inflammatory reaction, and the decreased numbers of lymphocyte may be indicative of immune dysfunction. Sayes *et al.* (2007) in his experiment had been demonstrated that the exposure to ZnO nanoparticles in rat after 7 days is accompanied with signs of toxicity including the increased level of LDH enzyme, inflammation and increasing the number of neutrophils in blood. Based on the above findings, it was concluded that nano zinc causes ill effects on the health status of rats even at NOAEL dose of nano zinc oxide. It induces alteration in haematological parameters and also structural and functional alteration in various organs of the body and thus causes health hazards. It is suggested that further studies should be carried out in different animal models using varied doses and increased duration to exactly find out the haematological and pathological alterations.

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