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ORIGINAL ARTICLE

Effect of Low Dose (100mg/kg) of Zinc Telluride (ZnTe) on Hematology of Male Albino Mice *(Mus musculus)*

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ABSTRACT

Aim: ZnONPs have been identified from a variety of perspectives, including organic chemistry and toxicology, and under these circumstances, they were deemed to be among the most lethal nanoparticles. The aim of present study to access the effect of low dose (Zinc Telluride) on hematology of Male Albino Mice.

Method: Experiment was conducted for fifteen days and oral suspension of ZnTe (Zinc Telluride) was applied orally in the suspension form to male albino mice (*Mus musculus*). Two groups were devised as Treated and Control. Each group consisting of three treatment organisms. Two sample t-test was applied to evaluate the effects of ZnTe low dose (100mg/Kg) on albino mice (*Mus musculus*) hematocrit.

Results: Results revealed that the number of WBC, GRA and LYM increased non-significantly (p=0.50, 0.46 and 0.44 respectively) while MON decreased non-significantly (p = 0.42). The number of LYM% MON% and GRA% decreased non-significantly (p = 0.52, 0.54 and 0.41 respectively) as compared to the control group (Table1 and 2). While our results depicted that the number of RBC% decreased highly significantly (p=0.0071) in treated animals but HCB and HCT decreased highly significantly (p = 0.0022 and 0.0028 respectively) in treated group as compare to the control group. Results observed for MCV MCH and PCT decreased non-significantly (p=0.060, 0.13 and 0.054 respectively) as compare to the control group. While number of MCHC, RDW-SD MPV and PDW increased non-significantly (p=0.50, 0.79, 0.18 and 0.16) in treated animals.

Conclusion: It was also observed that there was no differences in weight gain in both control and treated group animals found. Further research is required to evaluate the long term effects of ZnTe on mammals and to investigate their remedies.

Keywords: ZnONPs, ZnTe, Hematology, Male Albino Mice

INTRODUCTION

Zinc oxide nanoparticles (ZnONPs) are a famous example of the numerous commonly used nanomaterials that are now available in standardized, measurable forms[1]. Nanoparticles (NPs) will be introduced to people and biological systems through both the planned and unplanned release of NPs into the environment, via design applications and sewage outflow[2]. ZnONPs have been discovered in different viewpoints, including organic chemistry and toxicology[3], and were declared a standout amongst the most unsafe nanoparticles under such circumstances[4].

Living beings when introduced to NPs stimulates associations among the synthetic and the organic framework it also offers gradient to disturbing impacts of biochemical versatile reactions[5]. Biomarkers, the natural reactions can be utilized for assessment of safety of living beings to attain those quick warnings of ecological disturbing influences[6-7].

In the most recent decade, a few epidemiological investigations have discovered high relationship of encompassing ultrafine particles (d<100 nm) with cardiovascular and respiratory infections of people[8]. It has shown that nanoparticles, for example, carbon, titanium oxide, and carbon nanotubes, may influence with more aggravation than bigger particles of similar materials at a same mass measurements conveyance[9].

Besides, nano Zinc oxide is supplemented to composts and creature nourishment as a hotspot for the micronutrient (Zinc) basic for numerous proteins[10]. It has likewise been connected in creature sustain as a contrasting option to anti-microbials and different medications[11]. Nano-ZnO has been numerous to fourth epoch as the component added substance. Post-expansion into creature encourage demonstrated enhanced cancer prevention agent limit, upgraded insusceptibility, lessened looseness of the bowels rate, enhanced creature generation execution, advanced creature development, enhanced item quality, improved capacity of protection, and expanded creature regenerative execution[12].

With the aforementioned facts in mind, we chose male albino mice (*Mus musculus*) as the experimental organism and placed them in the animal house of department of Life Sciences, IUB, in order to assess the potential effects of ZnTe nanoparticles on them. Experiment was conducted for fifteen days and oral suspension was applied as mentioned in methodology to these experimental organisms. ZnTe NP was availed by kind donation by Chemistry Department of Bahuddin Zakariya University of Multan. At the end of Experiment blood samples were collected to evaluate the possible effects of ZnTe NP on the Haematology of albino mice so that it could be possible to calculate the changes posed by the low dose (100ml/Kg) of these nanoparticles.

MATERIALS AND METHODS

ZnTe nanoparticles

A wet chemical synthesis technique was used to synthesize the ZnTe nanoparticles, which were then examined using TEM, XRD, and SAED to determine their composition. Debye Scherrer's equation was used to calculate the crystallite sizes of the synthesized nanoparticles, and it revealed that they were 6 nm in size[13].

Animals

We bought 4-week-old adult male albino mice (*Mus musculus*) from the institute of pharmacy and pharmacology, Bahuddin Zakariya University, Multan, Pakistan. The rats were randomly allocated to woody cages (one animal per cage) and acclimatized for 15 days at a temperature of 22°C±1°C and 50%±1% humidity with a 10hrs artificial light and access ad libitum to fresh water and a mouse diet for 15 days before the experiment. The experiments had been accomplished following the research protocols recognized by the ethical committee of the Islamia University of Bahawalpur, Pakistan.

Fig. 1: Mice rearing cages for experimental work.

Control group, Treated group I and Treated group II

Mus musculus were randomly divided into three groups;

1. Control group: *Mus musculus* of this group were administered 0.9 % NaCl saline solution orally for two weeks. Control group didn't receive any other treatment throughout the whole period of the experiment.
2. Treated Group-I (low-dose ZnTe): *Mus musculus* of this group received 100mg/kg body weight of Zinc Telluride nanoparticles orally by a gastric tube once per day for two weeks

Zinc Telluride Nanoparticles Solution: To prepare a stock solution of zinc telluride nanoparticles, 12mg was dissolved in 100l and 200l of distilled water. The dosage was determined by the relative sizes of the mice. For a 30g mouse, a dose of 0.036µl will be applied from the stock solution[14]. A similar process was repeated for the control group, which got the standard saline solution.

After 15 days (24 hours from the last dose) mice from control and treated group were subjected to;

Blood samples collection: Following the inter peritoneal injections for 15 days, mice were anaesthetized with Chloroform and blood was taken either from direct cardiac puncture or through retro-orbital sinus. About 2ml blood samples were taken in clean test tubes without anticoagulant and permitted to clot for 25 min at 26°C after which serum was detached by centrifugation of blood 2800 rpm for 16 min. Additional 1ml blood samples were taken into test tubes with 25 micro EDTA to determine hematological parameter and serum biochemical profiling[15].

Serological and Hematological Parameters: Blood samples from treated group and control group of male albino mice were taken in Eppendorf tubes and centrifuged at 14000RPM for 8 minutes. Both nanomaterials treated and untreated mice blood samples were used to determine several hematology parameters comprising hemoglobin (Hgb), total red blood cells (RBC), hematocrit (HCT), mean cell hemoglobin (MCH), red blood cell distribution width (RDW), and mean cell volume (MCV), and mean cell hemoglobin concentration (MCHC). Other parameters including monocytes, lymphocytes, granulocytes, total white blood cells, and platelets counting, mean platelet volume (MPV), Lymphocytes(LYM),thrombocrit (PCT), platelet distribution width (PDW), Granulocytes (GRA), Monocytes(MON), Lymphocytes percentage (LYM%), Granulocytes percentage (GRA%), Monocytes percentage (MON %), Platelet count (PLT), and Red cell distribution-Standard deviation (RDW-SD). All of these examinations were carried out at the Bhutta Pathological Lab, close to the Children Complex (Hospital) Multan.

Protective measures: Before treating mice with stock solution, every time we used hand gloves, hand towels and laboratory mask as a protective measures. We used micropipette with labelled amount of stock solution to treat mice so to attain accuracy in doses. After every treatment we washed hands with antiseptic hand wash.

A B

Fig. 2 (A & B): Showing treatment of ZnTe to mal albino mice in the laboratory.

RESULTS

Two sample t-test was applied to evaluate the effects of ZnTe low dose (100mg/Kg) on albino mice (*Mus musculus*) hematocrit with comparison to control group, treated orally with ZnTe solution for fifteen days. Our results revealed that the number of WBC increased non-significantly (p=0.46) as compared to control group. Our results showed that the number of LYM increased in treated group non-significantly (p = 0.44) as compared to control group. Similarly, the results indicated that the number of MON decreased non-significantly (p=0.42) as compared to control group. Results exposed that the number of GRA increased non-significantly (p=0.50) in treated group as compared to control group. Our results directed that the number of LYM% decreased non-significantly (p=0.52) as compared to the control group. Our results directed that the number of MON% decreased non-significantly (p=0.54) in treated group in relation to the control group. Results displayed that the number of GRA% decreased non-significantly (p=0.41) in treated animals as compare to the control group (Table1 and 2 and Fig.3).

Table 1: T-Test results for different hematological parameters of Male albino mice (*Mus musculus*).

Parameter	Group	Mean	StDev.	t-value	P-value
WBC	Control	10.167	0.907	-0.91	0.46
	Treated	23.1	24.5		
LYM	Control	7.933	0.306	-0.95	0.44
	Treated	20.4	22.8		
MON	Control	16.3	27.5	1.00	0.42
	Treated	0.500	0.346		
GRA	Control	1.667	0.208	-0.82	0.50
	Treated	2.87	2.51		
LYM%	Control	86.20	6.17	0.73	0.52
	Treated	83.10	4.10		
MON%	Control	3.333	0.321	0.72	0.54
	Treated	2.87	1.07		
GRA%	Control	16.33	1.53	1.04	0.41
	Treated	14.03	3.49		
RBC	Control	11.723	0.585	6.59	0.0071**
	Treated	8.903	0.455		
HGB	Control	18.333	0.404	9.93	0.0022**
	Treated	13.700	0.700		
HCT	Control	56.40	1.90	9.07	0.0028**
	Treated	41.70	2.07		
MCV	Control	53.43	2.63	3.90	0.060
	Treated	46.87	1.27		
MCH	Control	17.167	0.907	2.49	0.13
	Treated	15.767	0.351		
MCHC	Control	32.333	0.764	-0.76	0.50
	Treated	32.833	0.839		
RDW	Control	22.100	0.794	-0.30	0.79
	Treated	22.60	2.76		
RDW-SD	Control	41.233	0.802	1.20	0.35
	Treated	38.27	4.22		
PLT	Control	1080.3	53.7	4.89	0.039*
	Treated	678	132		
MPV	Control	7.4333	0.0577	-2.03	0.18
	Treated	7.967	0.451		
PCT	Control	0.8213	0.0758	3.09	0.054
	Treated	0.544	0.136		
PDW	Control	22.000	0.693	-2.17	0.16
	Treated	24.83	2.15		

While our results depicted that the number of RBC% decreased highly significantly (p = 0.0071) in treated animals as compare to the control group. The results portrayed that HGB decreased highly significantly (p=0.002) in treated group as compare to the control group. Similarly, results represented that HCT decreased highly significantly (p=0.0028) in treated group as compare to the control group. The results signified that MCV decreased non-significantly (p=0.060) as compare to the control group. Our results indicated that MCH decreased non-significantly (p=0.13) when compared to the control group. Our results showed that the number of MCHC increased non-significantly (p=0.50) in treated animals as compared to control group. Our results exhibited that the number of RDW increment in treated animals non-significantly (p = 0.79) as linked to control group. Our results signposted that RDW-SD decreased non-significantly (p=0.35) in treated as compare to the control group. Our results indicated that PLT decreased significantly (p=0.039) in treated group as compare to the control group. Our results exposed that the number of MPV increased non-significantly (p=0.18) in control animals as compared to control group. Our results directed that the number of PCT decreased non-significantly (p=0.054) in treated as compared to the control group. Our results depicted that the number of PDW non-significantly increased (p=0.16) in treated animals in relation to the control group (Table1 and 2 and Fig.4).

Our results does not show any change in weight of the mice. It neither decreased nor increased during and after the trial period.

Table 2: Hematology of male albino mice (*Mus musculus*) treated with low dose (100mg/Kg) of ZnTe-NP.

Group	WBC	LYM	MON	GRA	LYM%	MON%	GRA%	RBC	HGB	HCT	MCV	MCH	MCHC	RDW	RDW-SD	PLT	MPV	PCT	PDW
C1	9.5	7.6	0.4	1.5	80.3	3.7	16	11.07	17.9	56.9	51.4	16.2	31.5	21.8	40.4	1020	7.4	0.734	22.4
C2	9.8	8	0.45	1.6	85.7	3.2	18	11.9	18.4	58	52.5	18	33	23	42	1123	7.5	0.86	21.2
C3	11.2	8.2	48	1.9	92.6	3.1	15	12.2	18.7	54.3	56.4	17.3	32.5	21.5	41.3	1098	7.4	0.87	22.4
T1	7.3	6.1	0.2	0.9	84	3	13	8.43	12.6	40.7	48.3	14.9	31	19.7	33.1	773	7.6	0.587	20.7
T2	32.6	29.2	0.6	2.8	89.6	1.7	8.7	7.76	12.5	40.3	51.9	16.1	31.2	18	32.9	850	6.9	0.598	16.4
T3	6.7	5.5	0.2	1	82.5	2.8	14.7	7.47	12.3	40.1	53.7	16.5	30.7	17.2	33.3	678	7.5	0.508	14.6

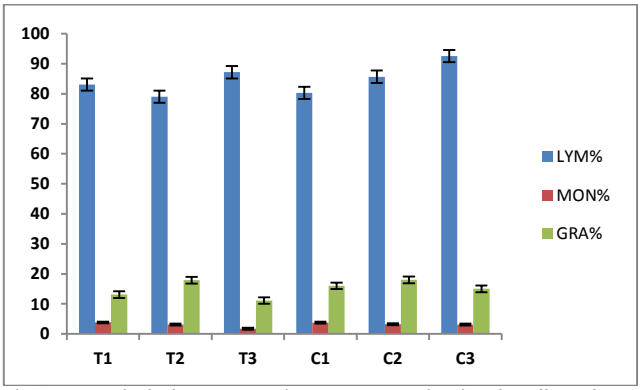

Fig.3: Hematological parameters in percentages showing the effect of Low Dose (100mg/kg) of ZnTe-NP on male Albino mice *(Mus musculus)*

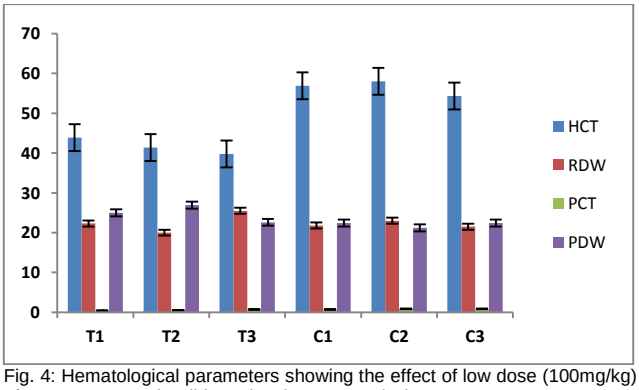

Fig. 4: Hematological parameters showing the effect of low dose (100mg/kg) of ZnTe-NP on male Albino mice *(Mus musculus)*

DISSCUSSION

The results showed non-significant increase in WBC count in male albino mice (*Mus musculus*) when orally treated with Low dose (100mg/kg) of Zinc Telluroid (ZnTe) nanoparticles. The results are similar to those of Xu et al.[16] who reported increase in WBC due to a reflect of an inflammatory reaction and increase in number of monocytes when exposed to ZnO nanoparticles. Results exhibited non-significant increase in LYM and GRA in male albino mice *(Mus musculus)* which were orally treated with low dose (100mg/kg) of ZnTe[17].These results showed divergence from results of Ko *et al.*[18] he reported decrease in LYM level when exposed to ZnOAE these fluctuations were significant and exhibited a clear cut dose response association.

According to our results MON decreased non-significantly when *Mus musculus* were treated orally with low dose (100mg/kg) of ZnTe. These findings showed discrepancy to those of Park *et al*. [19] who exposed Sprague Dawley rats to positively charged zinc oxide nanoparticles and observed increase in MON values due to anaemic. Total RBC count decreased significantly in Zinc Telluride (ZnTe) nanoparticles treated male Albino Mice as compare to that of the controlled group mice. Our findings are in contrast to Nitsche *et al*.[20] who had elevated levels of RBC cells in mice which were exposed to nano- and sub micro-scaled zinc oxide powder. They concluded that these changes are due to hypoxia, dehydration, or a disease known as polycythemia. A highly significant decrease in HBG in male albino mice when exposed to low dose 100mg/kg of ZnTe .The results enclosed similarity to Ko *et al.*[18] that a diet with more zinc contents resulting in iron deficiency anemia and that zinc-induced anemia may associated with decreases in HGB.

HCT, MCH and MCV level decreased highly significantly when Albino mice were given the treatment of Zinc Telluride low dose (100mg/kg).The results are in line with Je-Won Ko *et al*. [18] who reported decline in HCT when induced with ZnO. According to Ko *et al.*[18] who discussed the reduction in MCV when treated with ZnOAE100(+)[21].The MCHC value increased non-significantly in our results when albino mice were exposed toLow dose(100mg/kg) of Zinc Telluride. These results are in contrast with the findings of Wang*et al*. [22] who reported the decrease MCHC when rats were induced ZnO nanoparticles powder. The decrease is associated with anemia which is caused by the unnecessary dietary zinc in animals which encourage deficiencies of iron and copper and then form growth retardation and anemia.

Moreover, these results showcased increase in RDW, PDW and MPV value when mice were orally treated with Low dose of Zinc Telluride nanoparticles. Results are alike those of Kante *et al*. [23] who concluded that RDW is increased in rats when exposed to Zn powder in N-Zn nanoscale and microscale M-Zn. A non-significant decrease in Plateletcrit (PCT), PLT and RDW-SD in ZnTe treated mice as compare to control group mice. Our results are in line with Muhlestin *et al.*[24] as they had reported decrease in RDW-SD level in females which further resulted in various health issues.

CONCLUSION

It is concluded that when low dose (100mg/kg) of zinc nanoparticles (ZnTe) was injected to male albino mice (*Mus musculus*) there was gradual decrease in LYM, MON, LYM%, MON%, GRA%, RBC%, HCB, HCT, MCV, MCH and PCT. On the other hand there was a gradual increase in WBC,GRA,LYM,MCHC,RDW-SD,MPV and PDW.

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