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Effects of Zinc Oxide Nanoparticles on Biochemical Hematological Parameters and Liver Histopathology of Rabbit

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ABSTRACT

Zinc oxide nanoparticles have broad spectrum utilization in the nano-industry because of their distinct characteristics. Increased human exposure to nanoparticles has been observed through various products like dyes, additives, ceramics, beauty products rubber etc. **Objectives:** This study was carried out to evaluate the ZnO nanoparticle's toxic effects on hematological and biochemical parameters in lower and higher doses in a rabbit model. **Methods:** Thirty male rabbits were used and ten rabbits were assorted in each group. Groups included control and experimental group 1 (low dose group 50mg/kg) & and group 2 (high dose group 75 mg/kg). The oral administration of ZnO nanoparticles was given for 20 days. The parameters included body weight, blood glucose level, serum level of aspartate transaminase (AST), serum level of alanine transaminase (ALT), serum albumin, total cholesterol, triglycerides, hemoglobin, red blood cells, white blood cells and platelets. The parameters were measured on the 1st, 10th and 20th day of the experiment. **Results:** Nanoparticle administration resulted in a non-significant decrease in body weight and blood glucose level. Serum level aspartate transaminase in experimental group 2 was significantly increased. Triglycerides had shown a non-significant increase in experimental group 2. Non-significant decrease was observed in red blood cells and platelet count of both the experimental groups. Histopathological studies revealed damaged liver parenchyma and hepatocyte degeneration in the high-dose group. **Conclusions:** ZnO nanoparticle administration resulted in damage to liver histopathology. Its toxicity resulted in increased levels of triglycerides, AST and ALT due to liver damage.

INTRODUCTION

Nanotechnology is a novel field which has combined physics, chemistry and biology [1]. Zinc oxide is a white powder which is insoluble in water [2]. ZnO nanoparticles are inorganic compounds having unique physical and chemical properties and are used as multifunctional materials [3]. ZnO nanoparticles are frequently used in cosmetics, Opto-electronics, ceramics pigments etc. Toxicological studies have shown that nanoparticles have hazardous effects on environmental species as well as humans [4]. Oral administration of Zinc oxide nanoparticles results in the release of free Zn^{+2} ions in gastric acids and that is likely a toxic material. Various studies revealed that the liver is its main target organ [5]. Research on cell lines has shown that ZnO nanoparticles cause cytotoxicity. It is hypothesized that oxidative stress is one of the factors of its cytotoxicity [6]. A study on RGC-5 cells revealed that ZnO nanoparticles can minimize mitochondrial membrane potential. This is due to an increase in ROS (reactive oxygen species) which causes an over-expression of Caspase-12. The result is endoplasmic reticulum stress leading to cell death [7]. A study on commercial carp has shown that ZnO nanoparticles are also responsible for changes in serum proteins [8]. The use of nanomaterials is expanding day by day; therefore, it is necessary to find out the toxic effects of different nanomaterials [9]. The increasing usage of nanoparticles

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in various types of consumer products has pointed out concerns about their possible risks for workers, consumers and the environment as well. It has been estimated that about six million people are working in nanotechnology worldwide. It is important to go for animal investigations to explore the potential biomarker exposure, and find the link of biomarkers in experimental animals and humans [10]. The aims and objectives of the present study were to explore the impact of low-dose (50 mg/Kg) and high-dose (75 mg/ Kg) ZnO nanoparticles on body weight, blood glucose level, biochemical parameters and hematological parameters along with histopathological effects in a rabbit model. The rabbit model was selected because very little research has been carried out on rabbits to explore the effects of nanoparticles.

METHODS

The study was conducted at the Physiology Lab of the Zoology Department, Lahore College for Women University, Lahore to find out the effects of ZnO nanoparticles on biochemical parameters including(serum levels of aspartate transaminase and alanine transaminase), hematological parameters (red blood cells, white blood cells, platelets and hemoglobin, blood glucose), body weight and histopathological effect of the liver. Experimental Model and Housing: Thirty adult and male rabbits were purchased from Tolinton Market, Lahore. The average weight was 1 kg to 1.5 kg approximately. The animals were kept in cages which were washed two times per week with 70% alcohol throughout the study in the animal house of the Zoology Department, Lahore College for Women University Lahore, in a well-aerated room with an optimum temperature and exposed to about 12-14 house/daylight program (the optimum environmental conditions required by the rabbit). Acclimatization of Experimental Animals: For acclimatization, rabbits were kept under observation in laboratory conditions for one week preceding the experimentation. They became acclimatized to new surroundings having free access to food and water. Their body weights were recorded throughout the week. A regular gain in body weight of rabbits was a healthy sign indicating that they were well adapted to the given environmental conditions. Animal Grouping: The animals were housed in standard steel cages, specific number of rabbits per section of the cage. Animals were randomly assorted into three groups, each group comprised of ten rabbits and were control group and experimental groups 1 and 2. Synthesis of Zinc Oxide nanoparticles: Characterized ZnO nanoparticles were obtained from the Physics Department, Lahore College for Women University, Lahore. Zinc oxide nanoparticles were synthesized by the Sol-gel method. Zinc oxide (ZnO) Nano-powders were prepared by mixing a methanol solution and Zinc acetate dehydrate and adding ammonia NH_4OH to adjust the pH value of the solution between 9 and 11. The resulting Nano-powders ZnO form a substrate material for the fabrication of ZnO varistors. The scanning electron micrograph showed that nanoparticles were polygonal and spherical in appearance as shown in Figure 1 showed Nanoparticles faceting with sizes of 20 nm and 70nm.

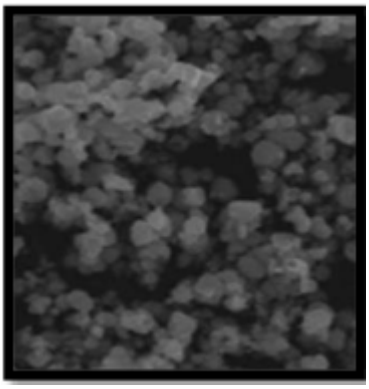

Figure 1: Scanning electron micrograph (SEM) ZnO nanoparticles [11]

Dose Treatment: The control group was given a normal diet i.e., rabbit feed and clean water and was not treated with nanoparticles. Experimental groups were given oral administration of Zinc oxide nanoparticles for twenty days. Group 1 of rabbits was given 50 milligrams per kilogram while group 2 was given 75 milligrams per kilogram. Blood Collection and Serum Separation: Blood samples were taken from the rabbit's marginal vein and transferred into EDTA tubes. For serum collection, blood samples were collected using a serum separator (SST) tube. The samples were subjected to centrifugation at 3000 rpm for 15 minutes. The serum was stored in labeled cryovials at -20 ˚C. Body Weight: Body weight in kilograms was determined by Redmond digital scale model no ZT740018. Blood Glucose Level: For measurement of glucose in the blood, Accusure glucometer model no TD-4183 was used. Test strips were placed in the glucometer and blood specimen was dropped at the strip edge. The reading was displayed on the monitor. Biochemical Analysis: The aspartate transaminase (AST) and alanine transaminase (ALT) and serum albumin were determined with the help of commercially available enzymatic test kits following the manufacturer's instructions with the help of URIT-800 chemistry analyzer. The lipid profile study included cholesterol and triglyceride. It was analyzed with the help of commercially available kits (Spinreact / CHOD- POD, Spain). Hematological Analysis: Hematological analysis included hemoglobin, red blood cells, white blood cells and

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platelets. Hematological assessment of blood samples was performed with an automated analyzer Sysmex XN- 430. Histological Analysis: For histopathological studies, the respective organ specimens (liver) were removed immediately after the Rabbit was sacrificed and fixed in phosphate buffer containing 4% formalin. The formalin-fixed biopsy specimens were embedded in paraffin, sections were prepared and stained with hematoxylin and eosin. Histological sections were observed with the help of an OPTIKA microscope (magnification 40X). Statistical Analysis: Data were analyzed using one-way analysis of variance (ANOVA), Tukey's HSD tests were used to test the significance differences between the mean values. The data were presented as the mean ± standard deviation for statistical analysis. P<0.05 was considered significant. The data were evaluated by SPSS (20 version) software. Graphs were plotted in Microsoft Excel XP worksheets 2018.

RESULTS

The body weight of rabbits in experimental group 1 did not differ significantly. High doses of nanoparticles caused rabbits of group 2 to lose weight (Table 1) (Figure 2A). The blood glucose levels were measured on the 1st, 10th and 20th day for each group. In the control group, it was 83.1 ± 2.50 mg/dL ± SEM, 81.2 ± 1.18 mg/dL ± SEM and 79.5 ± 1.73 mg/dL ± SEM respectively. In experimental group 1, it was found to be 82.8 ± 2.98 mg/dL ± SEM, 76.2 ± 1.54 mg/dL ± SEM and 69.9 ± 0.58 mg/dL ± SEM respectively. In experimental group 2, the values were found to be 83.9 ± 2.63 mg/dL ± SEM, 68.2 ± 1.37 mg/dL ± SEM and 62.9 ± 0.38 mg/dL ± SEM respectively. A non-significant decrease was observed in experimental groups (Table 1)(Figure 2B). The table showed AST value 52.00 ± 1.36***** U/L ± SEM on the 10th day and 64.37 ± 1.32*****U/L ± SEM was recorded on the 20th day. The value of AST in experimental group 2 was significantly increased (p<0.05) as compared to the control group. The value of AST in experimental group 1 was significantly increased as compared to the control group (Table 1) (Figure 2C). The blood was extracted on day 1st of the experiment and the serum concentration of ALT was found to be 63.35 ± 1.02 U/L ± SEM. Then on day 10, the concentration of ALT was 78.05 ± 0.88*U/L ± SEM while on day 20, the concentration of the ALT was observed to be elevating from the control reading, the value 86.35 ± 1.32*****U/L ± SEM. Serum albumin was raised non-significantly in both the experimental groups as compared to the control group (Table 1) (Figure 2D). The values of total cholesterol in the control group for experimental days were 75.0 ± 0.97 mg/dL ± SEM, 75.0 ± 0.10 mg/dL ± SEM and 75.1 ± 0.21 mg/dL ± SEM respectively. In experimental group 2, the values were found to be 77.43 ± 0.48 mg/dL ± SEM, 72.2 ± 1.5 mg/dL ± SEM and 81.5 ± 0.6 mg/dL ± SEM (Table 1) (Figure 2E). Total triglycerides were found to be 102.5 ± 0.5 mg/dL ± SEM, 102.9 ± 0.1 mg/dL ± SEM, and 103.1 ± 0.1 mg/dL ± SEM in the control group on the 1st, 10th and 20th day of the experiment. In experimental group 1, it was 106.2 ± 0.5 mg/dL ± SEM, 106.93 ± 0.43 mg/dL ± SEM and 106.8 ± 0.6 mg/dL ± SEM respectively. In experimental group 2, there was a non-significant increase with values 104.9 ± 0.4 mg/dL ± SEM, 109.10 ± 1.0 mg/dL ± SEM and 110 ± 0.6 mg/dL ± SEM. Hemoglobin of the control group ranged between 12.80 to 13.21 during the experiment. There was a non-significant decrease in both the experimental groups (Table 1) (Figure 2F). Red blood cell count ranged between 6.14-6.34 (x 10^{6}/µl) for the control group. Non-significant decrease was observed in both the experimental groups. White blood cell count on the 1st, 10th and 20th day of the experiment in the control group was found to be 6.19 ± 0.57 x 10^{3}/µl ± SEM, 6.53 ± 0.78 x 10^{3}/µl ± SEM and 6.63 ± 0.81 x 10^{3}/µl ± SEM. A non-significant increase was observed in both the experimental groups with 6.21 ± 0.33 x 10^{3}/µl ± SEM, 7.91 ± 0.39 x 10^{3}/µl ± SEM and 8.98 ± 0.44 x 10^{3}/µl ± SEM values in group 1 respectively while 6.18 ± 0.31 U/L ± SD, 9.57 ± 0.48 U/L ± SD and 10.14 ± 0.42 U/L ± SD values were observed in experimental group 2. The platelets count of the control group ranged between 417.4 ± 60.18 x 10^{3}/µl ± SEM and 424 ± 62.47 x 10^{3}/µl ± SEM. Non-significant decrease was observed after experimental days in both the experimental groups (Table 1). The liver of the control rabbit showed no marked changes in its histological structure. The liver appeared to be composed of hexagonal or pentagonal lobules with a central vein and peripheral hepatic triads or tetrads embedded in connective tissue. Histological studies revealed that on the 10th day, the liver cells of group 1 animals showed fewer histological changes. The liver parenchyma was damaged. On the 20th day of group 1, treated with ZnO nanoparticles 50mg/kg according to the body weight of rabbits showed that the hepatocytes exhibited densely stained cytoplasm with pyknotic nuclei. Hepatocytes were swollen, and Sinusoids were noted dilated.

Table 1: Mean ± SEM values of parameters

		Animal Groups		
Parameters	**Experimental Days**	**Control Group n=10**	**Experimental Group 1 (EG 1) Dose= (50mg/kg) n=10**	**Experimental Group 2 (EG 2) Dose= (75mg/kg) n=10**
Body weight (BW)(Kg)	1st DE	1.09 ± 0.26	1.32 ± 1.23	1.54 ± 1.39
	10th DE	1.14 ± 0.43	1.28 ± 0.67	1.37 ± 3.16
	20th DE	1.36 ± 0.62	1.36 ± 0.51	1.12 ± 1.55
Blood Glucose (mg/dL)	1st DE	83.1 ± 2.50	82.8 ± 2.98	83.9 ± 2.63
	10th DE	81.2 ± 1.18	76.2 ± 1.54	68.2 ± 1.37
	20th DE	79.5 ± 1.73	69.9 ± 0.58	62.9 ± 0.38

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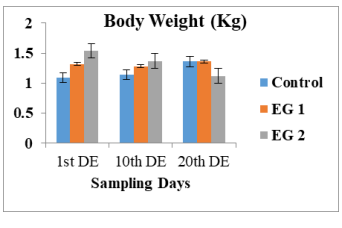
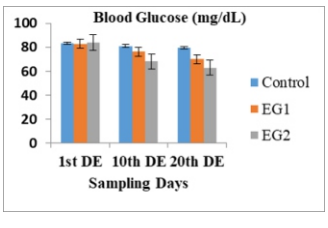
Parameters	Experimental Days	Animal Groups: Control Group n=10	Experimental Group 1 (EG 1) Dose= (50mg/kg) n=10	Experimental Group 2 (EG 2) Dose= (75mg/kg) n=10
Body weight (BW)(Kg)	1st DE	1.09 ± 0.26	1.32 ± 1.23	1.54 ± 1.39
	10th DE	1.14 ± 0.43	1.28 ± 0.67	1.37 ± 3.16
	20th DE	1.36 ± 0.62	1.36 ± 0.51	1.12 ± 1.55
Blood Glucose (mg/dL)	1st DE	83.1 ± 2.50	82.8 ± 2.98	83.9 ± 2.63
	10th DE	81.2 ± 1.18	76.2 ± 1.54	68.2 ± 1.37
	20th DE	79.5 ± 1.73	69.9 ± 0.58	62.9 ± 0.38
Serum level aspartate transaminase (AST)(U/L)	1st DE	45.42 ± 0.76	46.25 ± 0.56	46.12 ± 1.02
	10th DE	43.24 ± 1.02	49.19 ± 0.46	52.00 ± 1.36*
	20th DE	45.76 ± 0.86	52.14 ± 0.42*	64.37 ± 1.32*
Serum level alanine transaminase (ALT)(U/L)	1st DE	64.42 ± 0.92	65.27 ± 0.29	63.35 ± 1.02
	10th DE	65.25 ± 0.75	69.00 ± 0.46	78.05 ± 0.88*
	20th DE	63.78 ± 0.86	77.31 ± 0.93*	86.35 ± 0.56*
Serum Albumin (g/dL)	1st DE	2.44 ± 0.061	2.79 ± 0.02	3.20 ± 0.05*
	10th DE	2.41 ± 0.01	2.61 ± 0.21	3.10 ± 0.06
	20th DE	2.42 ± 0.02	2.8 ± 0.10	3.12 ± 0.12
Total cholesterol (mg/dL)	1st DE	75.0 ± 0.97	77.1 ± 0.49	77.4 ± 0.48
	10th DE	75.0 ± 0.10	72.7 ± 1.5	72.2 ± 1.5
	20th DE	75.1 ± 0.21	69.0 ± 1.1	81.5 ± 0.6
Triglycerides (mg/dL)	1st DE	102.5 ± 0.5	106.2 ± 0.5	104.9 ± 0.4
	10th DE	102.9 ± 0.1	106.93 ± 0.43	109.10 ± 1.0
	20th DE	103.1 ± 0.1	106.8 ± 0.6	110 ± 0.6
Hemoglobin (Hb)(g/dL)	1st DE	12.80 ± 1.27	16.80 ± 0.93	17.77 ± 0.36
	10th DE	12.93 ± 0.91	14.98 ± 0.68	15.65 ± 0.56
	20th DE	13.21 ± 0.84	13.09 ± 1.71	12.98 ± 1.3
Red blood Cells (RBCs) ($x 10^6$/µl)	1st DE	6.34 ± 0.61	5.90 ± 0.40	5.94 ± 0.43
	10th DE	6.28 ± 0.52	5.21 ± 0.31	3.44 ± 0.26*
	20th DE	6.18 ± 0.40	4.21 ± 0.27	3.01 ± 0.34*
White blood Cells (WBCs) ($x 10^3$/µl)	1st DE	6.19 ± 0.57	6.21 ± 0.33	6.18 ± 0.31
	10th DE	6.53 ± 0.78	7.91 ± 0.39	9.57 ± 0.48
	20th DE	6.63 ± 0.81	8.98 ± 0.44	10.14 ± 0.42*
Platelets (PLT) ($x 10^3$/µl)	1st DE	421.80 ±62.23	428.8 ± 63.21	427.4 ± 63.20
	10th DE	417.40 ±60.18	386 ± 57.06	320 ± 53.51
	20th DE	424 ± 62.47	348 ± 55.14	291.6 ± 46.74

1st DE= 1st day of experiment, 10th DE= 10th day of experiment and 20th DE= 20th day of experiment

Values represent the Mean ± SEM of animals* p<0.05 indicates a significant difference as compared to control ** p<0.01 indicates a highly significant difference as compared to control





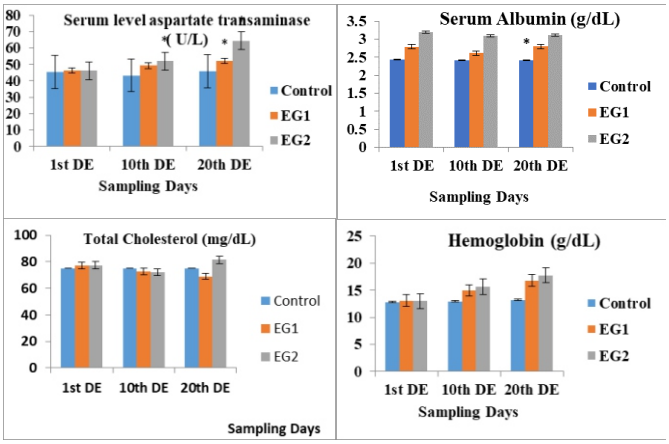


Figure 2: Effect of ZnO nanoparticles (50 & 75 mg/kg) on body weight (A), blood glucose level (B), AST (C), serum albumin (D), total cholesterol (E) and hemoglobin (F) of control, experimental group 1 and 2 of rabbits. Each value represents mean ± SEM. * p<0.05 indicates a significant difference as compared to the control ** p<0.01 indicates a highly significant difference as compared to control.

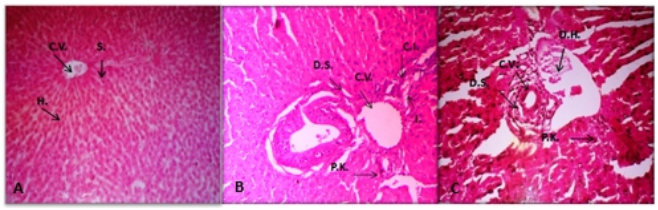


Figure 3: Photomicrographs of T.S. of the liver (A to C) at 40 X, A=Control, B= group 1 (50mg/kg of ZnO nanoparticles) on day 10, C= group 2 (75mg/kg of ZnO nanoparticles) 20th day. (C.V.) Central Vein, (S.) Sinusoids, (H.) Hepatocytes, (P.K.) pyknosis, (I.) infiltration, (D.S.) Dilated Sinusoid, (C. I.) Cellular inflammation and (D.H.) degenerating hepatocytes

DISCUSSION

Earlier, the effects of ZnO nanoparticles had not been studied in detail in rabbit models. The present study attempted to explore the effects of ZnO nanoparticles on rabbits. There was no specific change in the body weight of rabbits of experimental Group 1. The decrease in weight of rabbits of experimental Group 2 was due to loss of water from cells, reduction in bone mass and decrease in the ability to take excess food and water as a result of Zinc Oxide nanoparticles. While the body weight of the control group has remained the same. Blood glucose levels showed a non-significant decrease in experimental groups. The result of glucose depletion was due to ZnO nanoparticle administration and that is comparable with previous studies on rats [12]. A non-significant increase in total cholesterol and triglycerides had been observed in experimental groups which is related to liver dysfunction. A similar report had been presented earlier [13]. A significant decrease in red blood cells and a non-significant decrease in platelets were observed in the experimental groups as

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compared to the control group. A similar result had been reported in rabbits treated with nanoparticles for 25 days [14]. White blood cells showed a significant increase in experimental group 2 due to the high dose of nanoparticles. This result got support from similar research carried out on rats [15]. Histological studies revealed that the liver cell showed distortion in the hepatocytes of neutrophils was also prominent. Necrosis was more prominent. Sinusoids were dilated. Cytoplasm was also vacuolated. In group 2, degeneration of hepatocytes was observed. A similar picture of histopathological changes had been reported earlier [16]. In another study, researchers explored the immunomodulatory and antiparasitic effects of zinc oxide nanoparticles loaded with garlic extract compared with pure garlic extract in rabbit hepatic coccidiosis. They divided sixty male rabbits into four groups, including a control group and an infected group treated with garlic extract or garlic extract nanoparticles. Results demonstrated that both treatments improved hematological profiles, decreased liver enzyme levels, and reduced markers of oxidative stress compared to the infected, untreated group. Furthermore, both treatments effectively reduced fecal oocyst counts, indicating their antiparasitic efficacy [17]. Another study investigated the potential adverse effects of orally administered zinc oxide nanoparticles (ZnO-NPs) with a particle size of 30 ± 5 nm on rats over ten weeks. There were several remarkable results and there was decreased body weight from the sixth week onwards and an increased activity of serum markers such as AST, ALT, creatinine, and uric acid. Plasma glucose levels were largely unaffected by the addition of ZnO-NPs. These results suggested that long-term exposure to ZnO-NPs of this size can induce genotoxicity and cytotoxicity in the bone marrow, liver, and kidney of mice, which was associated with the widespread use of ZnO-NPs in food and fertilizers [18]. In another study, the effects of zinc oxide nanoparticles (ZnO-NPs) on performance, blood parameters, carcass characteristics and meat quality of New Zealand white rabbits reared under warm conditions. Results showed that ZnO-NP supplementation, ranging from 20 to 80 mg/kg, had several beneficial effects and led to increased body weight gain, body weight gain, and feed intake linearly, while also improving feed conversion ratio [19]. In a five-week experimental study, the effects of dietary supplementation with zinc oxide nanoparticles (ZnONPs) synthesized by the endophytic fungus on the performance and health parameters of broiler chicks were studied. There were 108 commercial broiler chicks administered into three dietary groups, with different levels of ZnONPs (0, 40, and 60 mg/kg diet). The results showed significant improvement in body weight, feed consumption, feed conversion ratio, and performance index in the ZnONPs supplementation groups compared to the control. These results suggested that supplementation of ZnONPs at 40 mg/kg dose was particularly promising, enhancing broiler performance and physical well-being [20].

CONCLUSIONS

ZnO nanoparticle administrations have shown changes in biochemical, hematological parameters and liver histopathology in experimental groups. These parameters are related to liver functioning. Vacuolar degeneration, necrosis (N), pyknosis (P), Infiltration, and sinusoidal dilation have been observed in the liver of experimental groups. The degree of degenerative changes was more pronounced as zinc oxide nanoparticle concentration increased. High levels of AST, ALT and triglycerides coordinated with the damaged condition of the liver. Non-significant decreases in hemoglobin and red blood cells are related to one another as hemoglobin is found in red blood cells.

Authors Contribution

Conceptualization: FA, SH
Methodology: FA
Formal analysis: SI
Writing and editing: AA, SE, HA, FA, SH

All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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