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EFFECTS OF ZINC OXIDE (ZNO) NANOPARTICLES ON HISTOPATHOLOGY OF KIDNEY AND HEMATOLOGICAL PARAMETER OF ALBINO RAT

Amara Akhtar[1]*, Hina Fatima[2], Ahmed Saqib[3], Lala Rukh Bano[4], Ammara[5], Hafiza Asma Talib[6], Rabia Khalid[7]

[1*2,3,4,5,6.7] Department of Zoology, Wildlife and Fisheries, University of Agriculture Faisalabad, Pakistan

***Corresponding Author:** Ahmed Saqib
*Department of Zoology, Wildlife and Fisheries, University of Agriculture Faisalabad, Pakistan
Email: ahmadsaqib0913@gmail.com

Abstract:
Zinc oxide nanoparticles (ZnO NPs) are widely prevalent in many facets of human life and have been used in a variety of sectors. As a result, questions have been raised regarding their potential negative effects. Although ZnO NP toxicity is well known, the effects of nanoparticle exposure are yet completely unknown. The present study is to evaluate the effects of zinc oxide nanoparticles on the hematological parameter and histopathology of kidney of rat. For this purpose twelve male albino rats were taken and acclimatized under laboratory condition for a week. After the acclimatized period the rats were divided into three groups. The control group T_1 was treated with no nanoparticles. The T_2 was treated with 50mg/kg and T_3 was treated with 100mg/kg zinc oxide nanoparticles. After three weeks of experimental period, the blood was drowned from the heart of rats. Results illustrated that in hematological analysis the count of RBCs HB and platelets were decreased in treatment (T_2) and (T_3) as compared to (T_1). Maximum decreased was found in T_3 with mean value of 3.68+0.23, 8.75+0.19 and 173.25+15.52 and minimum decreased was observed in T_2 with mean value of 4.13+0.11, 10+0.24 and 181+10.48 for RBCs, HB and platelets. While WBCs levels increased in T_2 and T_3 with mean values of 21.05+0.50 and 2.52+0.25 respectively. From these results, we can conclude that histopathological showed necrosis and detachment from basal membrane of kidney and congestion was also observed at some places.

Key words: Zinc Oxide Nanoparticles, Albino Rat, Hematological Parameter of Rat

1. INTRODUCTION

Nanotechnology is a developing field of science which has great development in nano sciences such as nanomedicine, nanomaterials, electrical engineering, biotechnology, chemistry, material sciences cosmetics and technology fields [1, 2]. The socioeconomic values of this field arc constantly rising all over the world and nanostructure particles have a major impact on almost every sector, mankind such as chemical industries, mechanics, drug gene delivery, optics, environmental health space industries, food and health care [3,4]. Nanotechnology gives full advantage to more than 1000 items or market segments such as cosmetics, sports and pigments [5].

Nanoparticles that are made up of noble metals like zinc, silver, platinum and gold are widely used in products that come in contact directly with the human body such as beauty products, skin care, toothpaste,

detergents, pharmaceuticals and therapeutic applications [6, 7]. Nanomedicines have a major impact in health care industry for treating different types of diseases so ecofriendly nanoparticle synthesis has been considered a key component for future generations to prevent different diseases [8].

Zinc oxide is an inorganic compound and the molecular formula of zinc oxide is ZnO. It is an odorless white powder that is insoluble in water and in the form of zincite mineral it is present in the earth crust [9]. After iron it is the second most abundant transition metal and the most abundant trace metal. It plays an effective role in the development of the human body, bones and metabolism of nutrients. !n the human body 2 to 3 g of zinc is present and the human body requires 10-15 mg per day [10]. Physiological and biochemical functions play an important role. There is very little amount absorbed in the gut and excreted in the environment in the form of feces that causes pollution. Nanoparticles of zinc oxide metal are the third most widely used particles, with approximately 550 and 33400 average production all over the world. At environmental level 31 p per kg of zinc oxide nanoparticles arc present in the soil and 76 to 760 p per litter in water [11].

Exposure of nanoparticles during their production causes zinc fever which is defined by different symptoms such as cough, irritation in the throat, flue and respiratory diseases. Recently ZnO nanoparticles of 2.5 and 1.0mghn^3 were administered in volunteers by inhalation that causes systemic inflammation. Systemic diseases, inflammation, alveolar epithelial damage, pneumonia, changes in tissues of the lungs and eytotoxicity in the lungs can be caused by inhalation of these nanoparticles [12, 13].
ZnO nanoparticles are more harmful then other metallic oxide nanoparticles because of their shedding iron effect These nanoparticles cause neurological effects in the form of changes in the spatial learning and loss of memory [14]. Exposure of ZnO nanoparticles in humans causes increase or decrease blood pressure and heart rate [15]. Apoptosis in clone carcinoma cells through oxidative damage which leads to cytotoxicity through immune diseases and mitochondrial membrane modifications. Blood parameters such as leukocytes and C-reactive protein (Hsrp) amount increase due to the induction of ZnO nanoparticles [16]

Toxic effect of ZnO nanoparticle is because of their possible function in causing oxidative stress. Reactive oxygen species (ROS) are produced because of oxidative stress. The incomplete breakdown of molecular oxygen produces reactive oxygen species (ROS) such as (0_2) Superoxide anion, (H_20_2) hydrogen peroxide, hydroxyl radical (OH) hydroxyl radical and singlet oxygen ($1O_2$). Renal cells as well as inflammatory bone marrow-derived cells infiltrating the renal tissue create them. Regardless of their source ROS may damage the glomerular basement membrane and affect the activities of glomerular and tubular cells, independent of their source [17, 18]. Most of the environmental particles eliminated from the blood with the help of kidney and liver [19]. Increased level of creatinine and urea in the blood is the indicator of the renal damage [20, 21].

2. MATERIALS AND METHOD

Nanoparticles are particle with a size less than 100. There is a strong interaction between nanoparticles and human health. The nanoparticle of zinc oxide has toxic properties and induced changes in kidney biochemical parameters and hematology. Three groups were made in which 12 albino rats were distributed and these groups were designated as T_1, T_2 and T_3. T_1 was control group while the other two were experimental groups. T_2 contained 50 mg/kg nanoparticles while T_3 contained 100mg/kg nanoparticles.

2.1 Collection and Preparation of Leaf Extract

Peepal tree leaves were collected from the University of Agriculture, Faisalabad area. Then to eliminate dust particles leaves were washed with tap water and distilled water and dried at the oven

for 1 hour. And these leaves were grinded with mortar and pestle and 20 g powder was boiled in 100 ml water and cool for 1 hour. Then it was filtered to obtain the leaf extract.

2.2 Preparation of Zinc Oxide Nanoparticles

Zinc oxide nanoparticles were prepared by Heer *et al.* [22]. Boiling of 50ml extract of leaves of peepal tree was take place. Then it was added to a 250 ml conical flask containing a 5 g solution of zinc chloride salt. A magnetic stirrer was used until the deep yellow paste was changed to white powder and covered this flask with aluminum foil and stirred it overnight at 65^0C. Then solution color was changed from dark yellow to white and it was centrifuged at 3000rpm and the obtained mixture was dried at 65^0C. The precipitate was ground to fine powdered and the particles was further characterized.

2.3 Experimental Animal

In the experimental study of 3 weeks, 2-month-old male albino rats were used. From the animal house of the national institute of Food Science and Technology, University of Agriculture, Faisalabad 12 albino rats were taken. These rats were acclimatized for a period of one week in a stainless-steel cage in the animal house at a room temperature of 25^0C while these rats were fed on standard diet.

2.4 Experimental Feed

The standard feed was given in the form of a pellet. Distilled water was given to them in bottles with rubber cork on its mouth and an iron pipe was inserted into it. Water was available for 24 hours and was changed on daily basis.

Table 1. Different Groups and their Compositions

SR. No.	Groups	Composition
1	T_1	Standard feed+ distilled water without zinc oxide nanoparticles
2	T_2	50mg/kg zinc oxide nanoparticles
3	T_3	100mg/kg zinc oxide nanoparticles

2.5 Experimental Protocol

From the animal house of the national institute of Food Science and Technology, University of Agriculture, Faisalabad 12 albino rats were taken. After acclimatization of one week, these rats were divided into two experimental group and one control group. Nanoparticles were weighed in grams by electrical balance and mixed in water with the help of magnetic stirrer. With the help of gavage, nanoparticles were provided to them orally.

2.6 Dissection of Animal

At the end of the experiment, rats were anesthetized by chloroform and dissected. With the help of pins, hands and limbs were fixed and skin was removed with the help of scissor.

2.7 Hematological Analysis

After dissection, 3ml blood sample was taken with the help of syringe. Blood was taken into tube that contained EDTA to analyze the hematological parameters such as red blood cells, white blood cells and platelets.

2.8 Determination of Hemoglobin Content

Drabkin method was used to determine the hemoglobin content. For this procedure, drabkin solution was used whose pH ranged from 7.0-7.4 and was yellow in color. With the help of pipette this solution was taken and transferred to test tube. Then blood sample was taken by micropipette and added into

drabkin solution. Then this solution was further diluted. Leave it for 10 minutes for the completion of reaction.

2.9 Determination of RBCS Count

The method was used to determine the RBCs count. RBCs diluting fluid consists of 4-5 g sodium sulphate, 0.4-0.5 g mercury chloride, 0.3-0.5 g sodium chloride and 150-200 g distilled water. With diluting fluid of RBCs, blood sample was taken. This fluid was drawn up to mark 101 then carefully inserted one drop of blood to both end of the cover glass. Then 40X microscopes were used to measure the RBCs count.

2.10 Determination of White Blood Cells

It was determined by counter chamber method described by Jeffe. The apparatus used for this procedure was WBCs pipette and WBCs diluting solution that was Turk solution and it was composed of 0.4-1% glacial acetic acid, methyl violet and 50-100g distilled water. With diluting solution of WBCs, blood sample was diluted. This fluid was drawn up to mark 101 then carefully inserted one drop of blood to both end of the cover glass. Then 10 X microscopes were used to measure the WBCs count.

2.11 Biochemical Analysis of Kidney

At the end of the experiment, first blood was collected from heart then it was collected from tubes. By utilizing gel containing tubes, serum was separated. The urea and creatinine quantity were assessed by utilizing commercial kit of RFT (Renal function test). This test was necessary to determine the kidney functioning.

2.12 Creatinine Serum Test

Jeffes method described Breyer and Qi [23] was used to determine the creatinine serum test. The reagent which is used for this procedure was picric acid, sodium hydroxide and creatinine standard. The blood sample was taken into gel containing tube and serum was separated. Then equal quantity of each reagent was taken into tube and also added blood serum in it. By utilizing the autoanalyzer, reading of creatinine was noted.

2.13 Urea Serum Test

Berthelot method described by Fawcett *et al*. [24] was used to determine the urea serum test. The reagent used for this procedure was urea chromogen reagent, urea enzyme reagent and urea standard. Three test tubes were taken and labeled as blank, standard and test. Then reagent was added to the test tube and the autoanalyzer was fixed. Blank test tube was set with distilled water and standard test tube was set with standard solution. Then blood sample was added and reading was noted.

3. RESULTS AND DISSCUSSION

TABLE 2. Comparison of Mean ±SD of Hematological Parameters of Treatments

Parameters	**Treatment no 1 (0mg/Kg)**	**Treatment no 2 (50mg/kg)**	**Treatment no 3 (100mg/Kg)**
RBCs(10^6/µL)	7.62±0.19(10^6/µL)	4.13±0.11(10^6/µL)	3.68±0.23(10^6/µL)
WBCs(10^3/µL)	9.64±0.19(10^3/µL)	11.56±0.35(10^3/µL)	12.99±0.131(10^3/µL)
PLATELETs(10^3/µL)	260.5±5.80(10^3/µL)	181±10.48(10^3/µL)	173.25±15.52(10^3/µL
Hemoglobin (g/dL)	12±0.35(g/dL)	10±0.24(g/dL)	8.75±0.19(g/dL)

The result obtained is shown in the table in which the mean result of hematological parameters such as RBCs, WBCs, Platelets, and Hemoglobin of treatment 19control) without ZnO nanoparticles were compared with the mean value of the treatment T_2 and treatment T_3 (exposed to ZnO nanoparticles). The mean results for treatment 1 of RBCs, WBCs, PLT, and Hb were 7.62±0.19 (10^6/µL), 9.64±0.19

(10^3/µL), 260.5±5.80 (10^3/µL), 12±0.35 (g/dL) respectively. The mean result for treatment 2 and treatment 3 of RBCs, WBCs, Platelets, and Hemoglobin were 4.13±0.11 (10^6/µL) 3.68±0.23 (10^6/µL), 11.56±0.35 (10^3/µL), 12.99±0.131 (10^3/µL), 181±10.48 (10^3/µL), 173.25±15.52 (10^3/µL, 10±0.24 (g/dL), 8.75±0.19 (g/dL).

Table 3. Comparison of Mean ±SD of Biochemical Parameters Creatinine and Urea in Rats of Treatments

Parameters	**Treatment 1(control omg/kg)**	**Treatment 2(50mg/kg)**	**Treatment 3(100mg/kg)**
Creatinine (mg/dL)	17.8425±0.13301	19.66±0.498865	21.05±0.503488
urea(mg/dL)	0.925±0.457347	1.675±0.170783	2.525±0.25

The results obtained showed in table in which the mean results of biochemical parameters such as creatinine, urea of treatment 1 without ZnO nanoparticles were compared with means of the treatment 2 and treatment 3(exposed to ZnO nanoparticles). The mean results for the treatment 1 of creatinine and urea were 17.8425±0.13301 (mg/dL), 0.925±0.457347 (mg/dL) respectively. The mean results for the treatment 2 and treatment 3 of creatinine, urea was 19.66±0.498865, 1. 675±0.170783, 21.05±0.503488, .2.525±0.25 The aim of this study was the assessment of hematological parameters and kidney damage markers in albino rats exposed to nanoparticles of ZnO. Present study presents that effect of ZnO nanoparticles on RBCs of rats showed a significant ($P<0.05$) decrease with mean value of 4.13 ±0.11 and 3.68±0.23 respectively in T_2 and T_3 comparison to control group with mean value of 7.62±0.43. ZnO nanoparticles at elevated doses lead to reduction in blood cell count due to the increased oxidative stress, inhibition of the activity in the cell, reduced cellular antioxidant and stimulation of antimitotic characteristics. The production of free radicles affected by nanoparticles is the primary cause of RBC destruction. This may be due to the ability of nanoparticles to pass through cell membrane even in cells that are not involved in phagocytosis such as RBCs and are thus responsible for their demise. There are four routes for the integration of RBCs channels, fluidity of membranes, endocytosis and adhesive interaction.

The current study showed a significant ($p<0.05$) increase in white blood cells (WBCs)count in Treatment (T_2) and Treatment (T_3) with a mean value of 11.56±0.35, 12.99±0.131 respectively when compared to the control treatment (T_1) with 9.64±0.19 mean value. White blood cells are the important body defender cells that enhance the immune response to nanoparticles. Their numbers are increased because these function as phagocytic cells. This may be due to the access of ZnO nanoparticles in the lymphatic system which results in the inflammation of lymph nodes. ZnO nanoparticles enter the lymphatic system and result in the inflammation of lymph nodes. This lymph node inflammation is very useful in increasing the number of WBCs that fight ZnO nanoparticles.

In the present study the rats that were treated with low and high dose of nanoparticles of ZnO indicated significant ($p<0.05$) decrease in the concentration of hemoglobin (Hb) of male albino rats of the treatment T_2 and treatment T_3 with mean values of 10±0.24 and 8.75±0.19 respectively as compared to Treatment T_1(control) with a mean value of 12±0.35.This may be due to nanoparticles which suppress the ability of bone marrow to produce new cells that cause a decrease in blood cell count and a decrease in hemoglobin level in blood. The hemolysis of RBCs may also be induced by ZnO nanoparticles which results in a decrease in hemoglobin level due to the loss of water from RBCs.

Results of the present study showed that Treatment T_2 and Treatment T_3 were treated with low dose (50mg/Kg) and high dose (100MG/Kg) of ZnO nanoparticles respectively showed a significant ($p<0.05$) reduction in platelets count with mean value of 181±10.48 and 173.25±15.52 respectively when compared to the rats of control treatment T_1 with mean values of 260.5±80. This may be due to the ability of the nanoparticles to absorb platelets and the formation of blood clots. The rats developed

thrombocytopenia after being exposed to ZnO nanoparticles as indicated by a significant decrease in platelet count. Megakaryocytes are platelet-producing cells found in the bone marrow. In the bone marrow megakaryocytes are broken down into smaller platelets. The level of platelets in the blood may be decreased because of megakaryocyte destruction.

In the case of kidney functioning the concentration of creatinine and urea were examined in the kidney of male albino rats that were treated with nanoparticles of ZnO and the rats that were not treated with ZnO nanoparticles. The present study showed that creatinine was significantly ($p>0.05$) increased in T_2 (19.66±0.49) and T_3(21.05±0.50) of male albino rats that were treated with nanoparticles of ZnO for twenty-one days as compared to control group T_1 with mean value (17.84±0.13). Blood urea nitrogen was also significantly increased in T_2 (1.67±0.17) and T_3 (2.52±0.25) as compared to the control group (0.925±0.45). ZnO nanoparticles caused toxicological symptoms such as increased serum blood urea nitrogen, and increased creatinine and gastrointestinal symptoms may be due to asserting potential renal damage Histopathological examination further confirms the damage caused by the exposure of nanoparticles of ZnO on the kidney. A mild degree of congestion is present throughout the renal parenchyma. The nuclei of the tubular epithelium cells are congested in some places while in other places these are normal. Glomeruli are artifact and a mild degree of cellular infiltration is also present. The rats of T_3 Treatment that were treated with a high dose (100mg/Kg) of ZnO nanoparticles showed that cells of renal tissues necrosis and detached from the basal membrane and swelling in the proximal tubule of epithelial cells and a mild degree of infiltration. Mild to moderate degrees of necrotic changes are present that were indicated by condensed nuclei. The current studies' histopathology results were indicated by condensed nuclei.

4. CONCLUSION

The researchers came to the conclusion that ZnO nanoparticles added to animal feed at the doses investigated had negative effects on hematologic parameters, cytokines, oxidative stress, cytochrome enzymes, liver enzymes, histologic parameters of rat liver, alterations in body and organ weight and liver function along with kidney function enzymes. The high dose of ZnO nanoparticles (100 mg/Kg) caused necrosis in the renal tissues, which were then separated from the basal membrane, and minor infiltration as well as swelling of the epithelial cells in the proximal tubule. In rats exposed to ZnO-NPs, we looked into dose-dependent increases in hemotoxicity, hepatotoxicity and renotoxicity. Further research is needed to understand the pathophysiology of these particles as well as the potential hazards they may provide to different organs.

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6. AUTHOR CONTRIBUTION

All the authors contributed equally.

7. FUNDING

Not applicable.

8. CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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