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EFFECTS OF SUBLETHAL ZINC OXIDE NANOPARTICLE EXPOSURE ON HEMATOLOGICAL AND BIOCHEMICAL PARAMETERS OF COMMON CARP (*CYPRINUS CARPIO L.*)

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ABSTRACT

Concerns have been raised about the potential environmental problems related to ZnO nanoparticles (ZnO NPs) entering aquatic systems as a result of their expanding use in various areas. With a high potential for release into aquatic habitats, zinc oxide nanoparticles (ZnO NP) are widely employed in a variety of consumer items such as sunscreens and cosmetics. The current study looked at how ZnO nanoparticles affected blood parameters like white blood cells (WBC), red blood cells (RBC), hemoglobin (Hb), packed cell volume (PCV), mean cell volume (MCV), mean cell hemoglobin concentration (MCHC), and mean cell hemoglobin (MCH), as well as biochemical parameters like total protein (TP), alanine transaminase (ALT), alkaline phosphatase (ALP), Lactate Dehydrogenase (LDH) and aspartate transaminase (AST).The results revealed that fish (*Cyprinus carpio*) exposed to different doses of ZnO nanoparticles (250 and 500 mg\L) for six weeks showed a significant decrease in (PCV, MCV, MCHC, RBC, Hb, total protein), whereas the parameters WBC, LDH, ALP, AST, and ALT presented a significant increase at the higher concentration.

Keywords: ZnO NP, blood parameters, biochemical parameters, *Cyprinus carpio*.

1. Introduction

Nanoparticles are being produced and employed more frequently in everyday consumer goods, including textiles, medications, and cosmetics, in addition to processes for cleaning up pollution(Masciangioli et al.,2003 ; Nohynek et al.,2007). Due to their exceptional qualities in products including cosmetics, skincare products, paints, ceramic materials, photocatalysis, UV filters, and biosensors, metal oxide (ZnO) nanoparticles have drawn attention from researchers throughout the world(Handy et al.,2008 ; Yu et al.,2011 ; Al-khafaji et al.,2011). Numerous studies have examined the potential negative consequences of a few nanoparticles in creatures, including people and mammals (Oberdörster et al.,2005 ; Moore ,2006).

Biomarkers hold potential in the environmental context as crucial indications showing that the toxic metals possess gotten into organisms, have indeed been dispersed between tissues, and are having a harmful effect on important targets (Huseen and Mohammed ,2019) Fish are important in determining the possible risk of toxicants in the aquatic system (Lakra et al.,2009). Thus, there may be a significant ecological significance to knowing fish behavior, uptake, and responses to toxicants. The majority of the generic biomarker requirements seem to be presenting directly applicable on the way to some fish (*Cyprinus carpio*) as biomarkers (Stegeman et al.,2018). The usage of proper biomarkers using various degrees of particularity be present a crucial factor of monitoring systems that rely based on such biomarkers (Sarkar et al.,2006). Therefore,

the purpose of this research is to determine how ZnO NP affects the hematologic and biochemical parameters of common carp. Additionally, the purpose of the study to assess the effectiveness of the variables employed as biomarkers to assess the study animals' sensitivity or harmful effect status.

An efficient biomarker for assessing the toxicity of aquatic creatures is C. *carpio L*. , A study by Showy et al (2019),demonstrates that the hematological parameters are changed by subchronic toxicant exposures of C. carpio , was subjected to toxicants in sublethal quantities for six weeks, and their hematological, biochemical, and histological data were analyzed. Chlorpyrifos toxicity in aquatic creatures can be effectively tracked using chemical and hematological characteristics as biomarkers. Fish showed a significant reduction in PCV, MCH, RBC, Hematocrit, T3, T4, and total protein at three doses. At the two higher concentrations, there was a significant rise in WBC, creatinine, GOT, and GPTThe aim of this research is to determine the effect of zinc oxide nanoparticles on *Cyprinus carpio L*, given their importance in the aquatic environment, and to ascertain the extent to which this type of fish is affected through hematological and biochemical tests.

2. Materials and methods

2.1. Zinc oxide (ZnO) nanoparticles

The ZnO nanoparticles were created by SIGMA-Aldrich and are extremely pure (99.8%). (10-30nm). Before exposing fish to nanoparticle suspensions, aerated, single-distilled water is sonicated in a bath at a frequency of 33 kHz for one hour at a time(Taurozzi et al.,2011).The fish were treated with three concentrations of ZnO nanoparticles that were computed using 1/15th, and 1/7th of the 96 LC50 value which determined in this study, which were = 250 mg/L and 500 mg/L correspondingly for six weeks.

2.2. Experimental design

Cyprinus carpio fish (weight 120±5 g) These fish, with an average length of 15-18 cm, were obtained from local fish hatchery incubators (the city of Madaan, south of Baghdad) and kept in a glass aquarium (60*40*50 cm) provided with de-chlorinated water with 7.5 pH and 23±2 °C as well as with feeding and constant oxygen delivery, for one week, which was stopped at 24 hours before the experiment began. Every 24 hours, tank water was replaced and a fresh solution of ZnO nanoparticles was added (USEPA), as well as the use of an air pump to keep the dissolved oxygen concentration at an optimal level and the concentration of ZnO nanoparticles constant.

2.3. Taking blood samples

To keep the animals alive while appropriately drawing blood, fish are anesthetized. This stops hemolysis. A cardiac puncture was used to extract blood, which was then put into EDTA tubes (Mgbenka et al.,2003). Serum was separated for the biochemical and hormonal assays in a plastic test tube and for hematological tests in a hypodermic syringe that had been previously washed with the anticoagulant heparin. Using an automated pipette, the serum was transferred into Eppendorf tubes after being allowed to coagulate for 10 minutes and then centrifuged for 15 min at 4000 rpm.

2.4. Statistical Analysis

In this study, the Statistical Analysis system (SAS,2012) ,used to assess the impact of various factors on study parameters and the significant comparisons of means was made using the least significant difference (LSD) test (ANOVA).

3. Results and discussion

3.1. Changes in blood parameters

When diagnosing fish physiology in a clinic, hematological approaches have been used to determine the sub lethal effects of contaminants(Bhagwant and Bhikajee,2000).Blood parameters can be utilized to diagnose the functional and structural health of fish exposed to toxins because they are thought to be reliable physiological indicators of overall body conditions (Seriani et al.,2011).

Table 1 show changes in the hematological and biochemical parameters of common carp subjected to different ZnO NPs doses. The result of WBC in this study ranged from (125.50 Cell $x10^3/mm^3$) in the third week in a concentration (250 mg/l) to (278.63 Cell $x10^3/mm^3$) in the sixth week in a concentration (500 mg/l), while the control (97.63 Cell $x10^3/mm^3$). The findings of the analysis of variance demonstrate that nano-ZnO has a substantial impact, especially after a 6-week period in 500 mg/l. The results showed an increase in the number of leukocytes, which was statistically significant ($p<0.05$). After treatment, the number of leukocyte cells in C. carpio increased, indicating an immunological response to the ZnO NP. This is in conformity with the results of (Alamanda *et al*.,2007). who consider that a rise in overall leukocyte count signals a body's reaction to pathogenic antigens.

After three weeks, blood samples exposed to 250 mg/l had the highest mean RBC value (1.53 Cell $x10^3/mm^3$), whereas blood samples exposed to 500 mg/l had the lowest mean value (1.17 Cell $x10^3$ $/mm^3$). The mean of the control blood sample was (1.83 Cell $x10^3/mm^3$). After a 6-week period of exposure, all RBC concentrations showed a significant decrease. The reduction in RBC count during the sub-chronic treatments in this study could have been caused by severe anemia or the hemolyzing effect of the toxicant ZnO-NP on the red blood cell membrane. Alternately, the concentration of toxicants in the gill region may harm the gill structure, which would result in cell lysis, and toxicant-induced disruption of osmoregulation would cause RBC levels to drop (Kavitha et al.,2010 ; Saravanan et al.,2011).

The maximum mean Hb value (4.20 ±0.63) was detected in fish samples that which treated with 250 mg/L ZnO nanoparticles after being exposed for three weeks. whereas, the minimum mean value (1.51 ±0.35) detected in fish samples medicated with 500 mg/L ZnO nanoparticles after six weeks of treatment compared with the control group (6.80 ±0.05). Statistical analysis revealed a significant decline in Hb during the final three weeks of exposure. Reduced hemoglobin concentrations show that perhaps the fish's capacity to give its tissues the right amount of oxygen has significantly decreased, which has led to a drop in physical activity (Nussey et al.,1995).

In this investigation, the mean PCV value varied between (36.50 ±0.06%) was observed in blood samples exposed to 250 mg/l of ZnO NPs after six weeks, and (22.43 ±0.06%) identified in the blood samples treated with 500 mg/l ZnO NPs after six weeks. However, when compared to control (36.50 ±0.06 %), the results are much lower in the last weeks of exposure. The lysis of erythrocytes could possibly be responsible for the noticed changes in Hb and PCV rates within fish(Ovie and Ewoma ,2008).As a result, a large decrease in these parameters indicates severe anemia.

When it comes to MCV blood, which indicates the health or size of RBCs (Alwan et al.,2009).After six weeks, the maximum value (103.93 ±6.11 fl/cell) was recorded in a sample of common carp treated with 500 mg/L ZnO, whereas the mean value (172.00 ±1.60 fl/cell) was obtained in a sample of those treated with 250 mg/L ZnO. Only after the last week of exposure did the MCV data show a significant decrease in high

concentration comparing with control group which was (246.76 ±0.03).

Mean corpuscular hemoglobin (MCH) measures the typical amount of hemoglobin in a red blood cell(Svoboda et al.,2001). The lowest mean value (72.60 ±4.75 Pg/cell) was found in a carp group treated with 250 mg/L ZnO after three weeks of treatment, in contrast to the largest mean value (78.03 ±0.42 Pg/cell) was discovered in a carp sample treated with 250 mg/L after treated for six weeks, and the mean of control group samples were (62.33 ±0.03 Pg/cell). Only in the 250 mg/l, 500 mg/l, and control groups did MCH data demonstrate a significant difference after six weeks of exposure. The significant alteration to MCH values obtained might be related to a decrease in intracellular blood iron, causing a decrease in the blood's ability to carry oxygen and eventually causing erythropoiesis.

The lowest mean MCHC value (251.33 ±28.08 %) was discovered in a blood sample treated with 500 m/L for six weeks of exposure, while the highest value (325.33 ±4.80 %) was identified in a sample subjected to 250 m/L ZnO after three weeks in this study. The control sample, on the other hand, had a mean of (162.80 ±0.05 %). According to statistical analysis, when fish were exposed to 500 mg/l of ZnO for 6 weeks, the MCHC result revealed a substantial increased. The higher MCH and MCHC values in the blood of *Cyprinus carpio* are consistent with the changes in erythrocyte quantity brought on by the degradation of RBC and the lower Hb level for each cell (Sakthivel et al.,1990). Table (1).

Table 1- Shows the mean and standard deviation of multiple blood samples taken from (*C. carpio*) that were exposed to various ZnO NPs concentrations for three and six weeks.

	3 weeks			6 weeks		
	250 mg/l	500 mg/l	control	250 mg/l	500 mg/l	control
WBC Cell * 10^3	125.50 ±0.23 B	131.33 ±0.06 A	97.63 ±0.07 C	169.60 ±20.80 AB	278.63 ±60.83 A	97.63 ±0.07 B
RBC Cell * 10^3	1.53 ±0.14 AB	1.39 ±0.04 B	1.83 ±0.03 A	1.31 ±0.05 B	1.17 ±0.02 C	1.83 ±0.03 A
Hb (g/dl)	4.20 ±0.63 B	4.50 ±0.43 B	6.80 ±0.05 A	2.33 ±0.39 B	1.51 ±0.35 B	6.80 ±0.05 A
PCV (%)	31.00 ±0.70 B	23.40 ±0.44 C	36.50 ±0.06 A	22.80 ±0.37 B	22.43 ±0.06 B	36.50 ±0.06 A
MCV (FI)	172.00 ±1.60 B	165.53 ±4.88 B	246.76 ±0.03 A	150.60 ±20.73 B	103.93 ±6.11 C	246.76 ±0.03 A
MCH (Pg)	72.60 ±4.75 A	76.40 ±5.91 A	62.33 ±0.03 A	78.03 ±0.42 A	74.26 ±2.91 A	62.33 ±0.03 B
MCHC (%)	325.33 ±4.80 A	292.67 ±12.28 B	162.80 ±0.05 C	249.33 ±31.38 A	251.33 ±28.08 A	162.80 ±0.05 B

3.2. Biochemical tests

Total protein test results ranged from (2.53 ±0.14 g/dl) in a common carp sample treated with 250 mg/L ZnO NPs during the third week to (0.766 ±0.03 g/dl) in a sample exposed to 500 mg/l ZnO NPs during the final week of exposure, compared to (5.23 ±0.03) g/dl in a control sample.

The highest AST mean value (706.60 ±39.52 U/l) was discovered in a sample of common carp treated with 500 mg/l after six weeks of treatment, whereas the lowest value (90.90 ±1.65 U/l) was obtained in a sample of that subjected to 250 mg/l for three weeks of exposure, and the control group sample recorded a mean value of (32.53 ±0.03 U/l).

The mean ALT levels in common carp samples treated with 250 mg/l ZnO NPs after three weeks of exposure ranged from (22.06 ±1.03 U/l) to (38.00 ±0.82 U/l) in those exposed to 500 mg/l ZnO NPs after six weeks of exposure. Increased ALT and AST activity in tissues of ZnO Nanoparticles exposed fish could indicate a faster rate of protein digestion in cells (Fazilati ,2013).discovered that rats treated with ZnO NPs had serum rates of AST as well as ALT activity that were raised, and Taheri *et al*.,2017).They've been discovered the activities of both (AST) and (LDH) in the liver, as well as (ALT), (ALP), and LDH in the kidney, were significantly raised by ZnO NPs (15 mg kg-1). In contrast, in chicken broilers treated with ZnO NPs as a dietary supplement, however, plasma ALT and AST activity were shown to be lower (Fathi ,2016).

The highest ALP mean value (108.76 ±11.68 U/l) was discovered in a sample of common carp treated with 500 mg/l for six weeks of treatment, whereas the lowermost value (67.33 ±0.40 U/l) was obtained in a group that treated with 250 mg/l for three weeks exposure, and the control group had a mean value of (37.87 ±0.03 U/l). Statistical analysis of the ALP results

revealed that all concentrations of exposure result in a significant increase in ALP. In comparison to the control group, the concentrations of ALP increased significantly ($p \leq 0.05$) in ZnO NPs treated groups after 3 and 6 weeks after treated.

Regarding to LDH, the present study demonstrates the mean ALT levels in common carp samples treated with 250 mg/l ZnO NPs after three weeks of exposure ranged from (470.60 ±4.82 U/l) to (1963.00 ±15.39 U/l) in those exposed to 500 mg/l ZnO NPs after six weeks of exposure. These results were all greater than the control sample (145.60 ±0.11 U/l). However, analysis of variance shows a substantial rise in 250 and 500 mg/l during the third and sixth weeks of exposure. The statically analysis shows significant differences ($p \leq 0.05$) among treatment groups. Metabolic stress may produce an elevated LDH activity in the liver and kidney and ZnO NPs raised AST, ALT, ALP, and LDH activities according to (Muthuraman &Kim,2015)Table2

Table 2- Shows the mean and standard deviation of biochemical tests taken from (*C. carpio*) that were exposed to various ZnO NPs concentrations for three and six weeks.

	3 weeks			**6 weeks**		
	250 mg/l	500 mg/l	control	250 mg/l	500 mg/l	control
TP (g/dl)	2.53 ±0.14 B	1.87 ±0.03 C	5.23 ±0.03 A	1.30 ±0.10 B	0.766 ±0.03 C	5.23 ±0.03 A
AST (U/L)	90.90 ±1.65 B	194.30 ±38.10 A	32.43 ±0.03 B	364.90 ±36.54 B	706.60 ±39.52 A	32.53 ±0.03 C
ALT (U/L)	22.06 ±1.03 B	25.50 ±0.30 A	16.67 ±0.11 C	33.73 ±0.80 B	38.00 ±0.82 A	16.67 ±0.11 C
ALP (U/L)	67.33 ±0.40 B	79.86 ±3.72 A	37.87 ±0.03 C	80.63 ±3.61 B	108.76 ±11.68 A	37.87 ±0.03 C
LDH (U/L)	470.60 ±4.82 B	650.13 ±0.03 A	145.60 ±0.11 C	1189.00 ±91.18 B	1963.00 ±15.39 A	145.60 0.06 C

4. Conclusions

The results of the current study show that *Cyprinus carpio* L.'s biological responses to treatment to sub-lethal doses of zinc oxide nanoparticles are significantly altered. These findings suggest that the accumulation of zinc oxide nanoparticles in water bodies ecosystems perhaps dangerous to the survival and viability of *Cyprinus carpio*. as well as likely other aquatic organisms.

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