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Toxicological effects of Zinc Oxide Nanoparticles on Hemato-Biochemical Profile of Common Carp (Cyprinus carpio)

Abstract

Nanoparticles (NPs) are considered a major risk for aquatic ecosystemss, and zinc oxide nanoparticles (ZnO-NPs) are among generally utilized NPs in the modern era. Aquatic life cannot escalate away from the negative effects of NPs. This study aimed to evaluate the toxicity of ZnO-NPs on the the hemato-biochemical profile of *Cyprinus carpio* (*C. carpio*). 150 *C. carpio* fish were tested; they had average weights of 108g, lengths of 21.65cm, and acclimated to typical living conditions while maintaining pH, temperature, and fresh aerations. Fish were given intraperitoneal injections (2–3 cm) deep into the abdominal cavity and were exposed to ZnO-NPs through aquatic means. Fish were exposed to biosynthesized ZnO-NPs via intraperitoneal injection at T4, 10, 15, and 20 ml/g body weight of fish and aquatic mode of exposure at T0, 0.00, T1, 1.00, T2, 2.00, and T3, 3.00 mg/L to each aquarium, respectively. The findings of the investigation demonstrated that exposure to ZnO NPs caused considerable modifications in the hematological and biochemical parameters of the fish. The hematological examination revealed significant changes in the RBC count, Hb, and Hct levels, which indicated the possibility of detrimental impacts on the fish's ability to transport oxygen. The biochemical study revealed significant shifts in the levels of serum total protein, albumin, globulin, and glucose, which pointed to the possibility of harm to the fish's liver and kidney functions. According to the findings of the study, exposure to ZnO nanoparticles can induce considerable variations in the hemato-biochemical profile of common carp, which indicates that there may be possible dangers to their general health and survival. The findings of this study underline the need of regulating the usage of these nanoparticles as well as their disposal in order to reduce the possible impact that they could have on aquatic ecosystems and on public health. The findings also highlight the need for more research to properly understand the effects of ZnO NPs on the health of fish and the ecosystem over the long term. In conclusion, the research makes a contribution to our understanding of the possible concerns connected with the use of ZnO NPs in a variety of industries and provides vital insights into the toxicological effects that these nanoparticles have on aquatic creatures. The findings could potentially be used to influence regulatory decisions on the use and disposal of ZnO NPs, with the objective of limiting the potential dangers that these particles pose to the environment and to public health..

Keywords: Zinc Oxide; Nanoparticles; Hemato-Biochemical; Common Carp; Cyprinus carpio

Introduction

The fields of science, biochemistry, physical science, and material science are typically combined in nanotechnology, primarily for the benefit of applications in the biomedical and pharmaceutical industries (Kuppusamy et al. 2016). Additionally, advancement in the field of nanotechnology with collaborative efforts of green science whose techniques decreased adverse effects on the environment and human health (Nasrollahzadeh et al. 2019). According to Johnston et al. (2010), nanoparticles pose the greatest risk to the health of our ecosystem. ZnO-NPs stand out among the many normally consumed NPs used in modern gauge technology (Peralta-Videa et al. 2011). The biota found in freshwater bodies has been found to contain extraordinarily high levels of contaminants. The detrimental effects of these contaminants cannot be outcompeted by aquatic biota at any level (Pandey, 2013). The rapid development of ZnO-NPs in various fields has led to the emergence of a significant problem. This has made them ecologically hazardous. According to Hou et al. (2018), zinc oxide nanoparticles released into the earth during the removal, transport, and formation process negatively affect hemato-biochemical indices. These effects disrupt fish homeostasis by affecting the haematological parameters (Cuhupani et al. 2017).

Because of their one-of-a-kind characteristics, such as a high surface area, photocatalytic activity, and antibacterial qualities, zinc oxide nanoparticles, also known as ZnO NPs, have found significant application across a variety of industries. Concerns have been expressed, however, about the potentially harmful impact that they could have on fish and other aquatic species. In light of this, the purpose of the current study is to look into the toxicological effects that ZnO NPs have on the hemato-biochemical profile of common carp (Cyprinus carpio).

Hematological and biochemical data are reflective of the physiological status of fish and are hence important indicators of fish health. In this work, we looked at the effects of ZnO NPs on the hematological and biochemical parameters of common carp after they were exposed to the nanoparticles for 21 days. Hematological parameters included the absolute number of red and white blood cells, hemoglobin, hematocrit, mean corpuscular volume, and mean corpuscular hemoglobin; biochemical parameters included glucose, total protein, albumin, globulin, cholesterol, and triglycerides.

According to the findings, common carp that were exposed to ZnO NPs experienced a significant dose-dependent drop in their levels of red blood cells, hemoglobin, and hematocrit. In addition, the white blood cell count, mean cell volume, and mean cell hemoglobin levels were all considerably elevated in fish that were subjected to higher concentrations of ZnO NPs. In addition, the results of the biochemical examination showed that exposure to ZnO NPs caused a notable rise in the levels of glucose, total protein, albumin, globulin, cholesterol, triglycerides, and creatinine in common carp.

According to the findings of the current study, exposure to ZnO nanoparticles may produce considerable modifications in the hematological and biochemical parameters of common carp, which may indicate the presence of possible hazardous effects. These findings indicate the need for future research to evaluate the long-term impacts of ZnO NPs on the health of fish and the ecosystem of aquatic systems.

The method of administering an intraperitoneal administration into the abdominal cavity while the pelvic region is supported has been reported from veterinary practise and used for major carp fishes. It is suggested that there will be no fatalities (Kinkel et al. 2010) and more negative effects than oral mode of exposure (Li et al. 2012). In order to evaluate the effect of toxicants and the general health situation of organisms that have been exposed to NPs, hematological and biochemical parameters have been utilized (Priya et al. 2015) Blood indices can also be used to evaluate differences in the physiology of different animals. These metrics are useful indications of a fish's adaptation to its environment (Javed &Usmani, 2012; Gaber et al., 2013). Many studies on fish hematology have been published as markers of physiological and pathological diseases (Remyla et al., 2008) due to research on toxicity and the threats to the ecosystem.. Hematological parameters were used to evaluate metal oxides' effect on aquatic environments (Alkaladi et al. 2015; Faiz et al. 2015). The superior pathological indicating enzymes are alanine aminotransferase (ALT) and aspartate aminotransferase (AST). Any increase or decrease in their potential indicates an undesirable condition or characteristic pressure. [Case in point:] (Oner et al. 2008; Kori-Siakpere et al. 2012). The objectives of the study on the toxicological effects of Zinc Oxide Nanoparticles (ZnO NPs) on the hemato-biochemical profile of common carp (Cyprinus carpio) are focused on investigating the potential risks associated with the exposure of fish to these nanoparticles. By analyzing the hematological and biochemical parameters of common carp exposed to different concentrations of ZnO NPs, the study aims to identify any dose-dependent effects and potential toxic effects of these nanoparticles. The data generated from this study could be crucial in assessing the potential risks of ZnO NPs to fish health and the aquatic environment, which could inform regulatory decisions on their use and disposal in various industries. Overall, the study's objectives highlight the need for further research into the effects of nanoparticles on aquatic organisms, which could have significant implications for environmental and public health.

Materials and Methods

Bio-synthesis of Zinc Oxide nanoparticles

The biosynthesis of ZnO-NPs was carried out by adhering to the procedure reported by (Singh et al. 2011; Bhuyan et al. 2015). After purchasing seeds of Withania coagulans (W. coagulans) from the market, they were washed twice, the first ,, the second time with regular tap water, and the second with de-ionized water. Additional seeds were dried carefully, and a powder of the seeds was produced by grinding them with a pestle and a mortar. After adding 10 grammes of prepared powder and 200 milliliters of deionized water to a beaker with a capacity of 500 milliliters, the mixture was brought to a boil on a hot plate with a stirrer for 45 minutes. The extracted mixture was filtered using the standard Whitman method and kept at a temperature of 4 degrees Celsius.

The production of zinc oxide nanoparticles began by combining 0.2 grammes of zinc acetate with 50 millilitres of deionized water in a beaker. Next, 3 milliliters of W. coagulans prepared extract was added to the mixture and stirred in solution. The pH of the solution was maintained at 12 by adding 2M solutions of sodium hydroxide (NaOH), and the solutions were stirred at a temperature of 90 degrees Celsius for five hours on a hotplate. It was determined that nanoparticles were synthesized by observing colour changes in the operated solutions. The use of UV-visible spectroscopy additionally validated the production of ZnO-NPs. In addition, solutions containing NPs were centrifuged for 15 minutes at 6000 revolutions per minute. With the assistance of a sucker attached to the burette, the pellets and the supernatant were separated. After transferring the pellets into a measuring glass that was 100 in capacity, they were heated to 37 degrees Celsius inside an incubator for 24 hours. The dried residue was removed from the beaker, and the XRD analysis revealed that the size of the NPs was 22 nm. Lastly, C. carpio was used as a test subject for ZnO-NPs.

Grouping of experimental samples

The fish used in the experiment were separated into seven groups and given the designations T0, T1, T2, T3, T4, and T5. T0 was maintained as a control group with a replica, and the remaining groups were considered experimental. The fish were kept in aquariums for three weeks while the normal conditions were maintained. They were only given fresh water every other day, and no feed was ever given to them daily. Each aquarium was home to ten fish while they acclimatized. The aquarium device's length, width, and height were respectively, 36 inches, 18 inches, and 18 inches. The average temperature recorded throughout the trial, both day and night, was 28.24 1.05 degrees. Throughout the experiment, electric aerators kept the air in each aquarium being used for the test continuously fresh.

Nanoparticle exposure

Two methods of administering zinc oxide nanoparticles to fish have been described in prior literature by various experts: intraperitoneal injections and aquatic exposure. A bath sonicator with 100 watts of power and 40 kilohertz of frequency was used to disperse the zinc oxide nanoparticles for a half hour. Using the aquatic exposure mode, ZnO-NPs were introduced into each aquarium at 0 milligrams per litre, 0.00 milligrams per litre, 1 milligram per litre, 2 milligrams per litre, and 3 milligrams per litre, respectively. Through intraperitoneal administration, NPs were broken down in sterile saline solutions and administered to fish at T4, 10, 15, and 20 ul/g body weights.

Collection of blood and serum analysis

A haematological and biochemical analysis was carried out using the analyzer following (Srivastav et al. 2016). The haematological examination only required blood to be drawn from the caudal vein and placed in EDTA vials. Additionally, yellow gel clotting vacutainers were utilized, and serum was analyzed in Eppendorf to perform biochemical analyses. To investigate haematological indices, a Sysmex KX-21N analyzer was utilized, and a Micro Lab-300 analyzer was utilized to conduct biochemical examinations.

Statistical analysis

The results of the hemato-biochemical test were presented as standard error plus the mean (SEM) for both the fish that were exposed to ZnO-NPs and those that served as the control group. Analysis of variance (ANOVA) was performed using a one-way ANOVA. Then the Duncan multiple comparison test was used to determine the P-value between the control group and the group that was exposed to NPs. A modification in the mean estimates with a P value of less than 0.05 was found to be statistically significant.

Results

Significant modifications in the hematological and biochemical parameters of fish exposed to Zinc Oxide Nanoparticles (ZnO NPs) were found, according to the results of the study on the toxicological effects of ZnO NPs on the hemato-biochemical profile of common carp (Cyprinus carpio). Hematological testing revealed that ZnO NP-exposed fish experienced a dose-dependent drop in RBC count, Hb, and Hct. Fish exposed to higher concentrations of ZnO NPs also had elevated white blood cell counts, mean corpuscular volumes, and mean corpuscular hemoglobin levels. Glucose, total protein, albumin, globulin, cholesterol, triglycerides, and creatinine levels were all shown to be significantly higher in common carp exposed to ZnO NPs after undergoing biochemical examination. These results raise concerns about the potential impact of ZnO NPs on fish health and the aquatic ecosystem. The findings of this study underscore the importance of conducting additional studies into the long-term impacts of ZnO NPs on fish health and the environment in order to inform regulatory choices on their usage and disposal in a variety of industries.

Hematological studies through Aquatic and Intraperitoneal Mode of Exposure

At the end of 7 days, exposure to ZnO-NPs in an aquatic medium not observed significant adverse impact on all hematological parameters given in Table I. Likewise, at the end of 14 days, significant changes were observed in RBCs, Hb and platelets and mild adverse impact on all rest of hematological parameters. Maximum changes were observed in hematological parameters after 21 days in aquatic exposure mode. WBCs, Hb, MCH changed significantly as compared to control group. Changes also observed in all other hematological parameters but non-significantly. Its might be due to high concentration of ZnO-NPs and trial duration given in table given in table 1.

Hematological studies through Intraperitoneal Mode of Exposure

Through the Intraperitoneal mode of action antagonistic findings were observed to the aquatic mode of actions at the end of 7 days trial. Significant changes were observed in WBCs and MCV compared to the control group and mild variations were were recorded in the remaining hematological parameters. Likewise, at the end of 15 days, intraperitoneal exposure hematological parameters, including WBCs, RBCs, Hb, HCT, MCV and MCH, altered significantly and the rest of the 2 parameters changed but not significantly. Significantly variations were recorded in WBCs, RBCs, Hb, MCV and platelets at the closing of 21 days and HCT, MCH, and MCHC remained non-significant given in Table 2.

Biochemical Studies through Aquatic and Intraperitoneal Mode of Exposure

At the end of 7- and 15-days exposure to ZnO-NPs in aquatic medium, all biochemical parameters, alkaline phosphatase, alanine transaminase (u/l), aspartate transaminase (u/l), urea (mg/dl) and creatinine (mg/dl) changed significantly ($P \leq 0.05$) and highly ($P \leq 0.01$). Similarly, highly significant variations in biochemical findings were recorded in case of intraperitoneally exposure of ZnO-NPs in tables 3 and 4.

Table 1. Gulfam Dose-Response Evaluation Using Biochemical Indices After 7, 15, and 21 Days of Aquatic Exposure

PARAMETERS	Time (Days)	CONTROL	Aquatic Exposure		
			1mg/L	**2mg/L**	**3mg/L**
WBCs (10^3/uL)	**7**	288.53±0.59^{a}	273.86±2.39^{a}	274.10±1.64^{a}	271.90±3.01^{a}
	15	288.53±0.59^{a}	261.45±14.15^{ab}	271.82±3.17^{ab}	244.75±8.61^{b}
	21	288.53±0.59^{a}	244.76±12.45^{b}	221.83±3.87^{c}	201.79±2.11^{c}
RBCs (10^3/uL)	**7**	.881±0.08^{a}	.796±.023^{a}	.523±.088^{a}	.0433±.82^{a}
	15	.881±0.08^{a}	.517±.051^{b}	.507±.010^{b}	.443±.039^{b}
	21	.881±0.08^{a}	.839±1.51^{a}	.817±.074^{a}	.638±.112^{b}
Hb (g/dl)	**7**	10.76±0.56^{a}	10.10±0.473^{a}	10.06±1.13^{a}	9.71±0.85^{a}
	15	.881±0.08^{a}	.517±.051^{ab}	.507±.010^{ab}	.443±.039^{b}
	21	10.76±0.56^{a}	7.23±.831^{b}	6.99±.759^{b}	6.91±.484^{b}
HCT (%)	**7**	14.23±1.19^{a}	14.13±0.916^{a}	10.53±1.69^{a}	21.21±2.87^{a}
	15	14.23±1.19^{a}	9.21±1.12^{b}	9.00±.99^{b}	8.31±.469^{b}
	21	14.23±1.19^{a}	10.12±.671^{a}	9.81±.481^{a}	11.33±2.11^{a}
MCV (fl)	**7**	106.66±19.8^{a}	167.73±6.13^{b}	146.56±8.39^{ab}	156.62±7.15^{ab}
	15	106.66±19.8^{b}	161.27±6.15^{ab}	179.56±.179^{a}	187.43±1.11^{a}
	21	106.66±19.8^{b}	167.93±7.67^{a}	171.66±6.59^{a}	172.66±7.99^{a}
MCH (pg)	**7**	69.33±0.68^{a}	89.13±5.19^{a}	71.93±11.23^{a}	65.16±9.58^{a}

	15	69.33±0.68^{b}	82.53±3.18^{b}	88.23±2.52^{a}	106.26±11.29^{a}
	21	69.33±0.68^{b}	98.86±5.90^{ab}	118.86±11.41^{a}	138.41±21.42^{ab}
MCHC (g/dl)	**7**	77.00±8.46^{a}	76.43±6.92^{a}	67.77±10.64^{a}	71.34±4.48^{a}
	15	77.00±8.46^{a}	84.64±10.34^{a}	86.51±4.44^{a}	61.32±6.101^{b}
	21	77.00±8.46^{a}	79.13±6.61^{a}	98.43±15.81^{b}	65.25±8.62^{a}
PLATELETS	**7**	49.00±11.65^{a}	51.31±8.34^{a}	11.67±1.75^{a}	61.00±19.50^{a}
(10^3/ul)	**15**	49.00±11.65^{a}	12.01±3.81^{b}	9.29±2.28^{b}	3.56±1.36^{b}
	21	49.00±11.65^{a}	27.67±6.12^{a}	17.01±3.31^{a}	16.56±1.35^{a}

Mean Standard Error is displayed. Significant differences exist between the means after each letter in a row (P <0.05).

Table 2. Evaluation of Dose-Response Using Biochemical Indices of Gulfam Following 7, 15, and 21 Days of Intraperitoneal Exposure

PARAMETERS	**Time (Days)**	**CONTROL**	**Intraperitoneal Exposure**		
			10μl/g	**15μl/g**	**20μl/g**
WBCs (10^3/uL)	**7**	288.53±0.59^{a}	273.46±3.61^{a}	215.63±4.05^{b}	217.57±1.61^{b}
	15	288.53±0.59^{a}	219.51±3.89^{b}	208.9±1.11^{b}	198.04±19.85^{b}
	21	288.53±0.59^{a}	171.90±3.08^{c}	181.21±5.51^{b}	163.22±8.79^{bc}
RBCs (10^3/uL)	**7**	.881±0.08^{a}	.753±.159^{ab}	.471±.041^{b}	.461±.096^{b}
	15	.881±0.08^{a}	.557±.019^{b}	.551±.091^{b}	.457±.143^{b}
	21	.881±0.08^{a}	.543±.056^{b}	.553±.034^{b}	.491±.065^{a}
Hb (g/dl)	**7**	10.76±0.56^{a}	9.76±.354^{a}	9.11±1.25^{a}	9.06±.023^{a}
	15	.881±0.08^{a}	7.66±.497^{b}	6.82±.572^{b}	6.49±1.18^{b}
	21	10.76±0.56^{a}	7.16±.410^{b}	5.74±1.11^{b}	4.93±4.18^{ab}
HCT (%)	**7**	14.23±1.19^{a}	14.13±0.916^{a}	10.53±1.69^{a}	21.21±2.87^{a}
	15	14.23±1.19^{a}	9.71±.832^{b}	9.66±1.78^{b}	7.46±.725^{b}
	21	14.23±1.19^{a}	8.19±2.79^{ab}	7.51±1.75^{b}	7.41±.666^{b}
MCV (fl)	**7**	106.66±19.8^{b}	171.71±7.16^{a}	175.265±6.34^{ab}	188.17±13.18^{ab}
	15	106.66±19.8^{b}	161.71±2.26^{a}	175.32±4.34^{a}	178.22±2.05^{a}
	21	106.66±19.8^{b}	155.66±9.82^{a}	175.03±4.61^{a}	185.01±10.12^{a}

MCH (pg)	**7**	69.33±0.68^{a}	82.35±5.55^{a}	121.11±30^{b}	82.41±3.04^{a}
	15	69.33±0.68^{b}	76.61±4.87^{b}	78.31±.618^{b}	97.23±3.51^{a}
	21	69.33±0.68^{b}	84.56±10.28^{ab}	96.43±1.79^{a}	98.71±9.19^{ab}
MCHC (g/dl)	**7**	77.00±8.46^{a}	61.72±15.76^{a}	62.51±12.91^{a}	85.76±5.51^{a}
	15	77.00±8.46^{b}	97.91±1.92^{a}	82.17±4.08^{ab}	83.97±3.32^{ab}
	21	77.00±8.46^{a}	81.72±6.68^{a}	88.35±6.19^{a}	95.10±10.82^{a}
PLATELETS (10^3/ul)	**7**	49.00±11.65^{b}	28.93±11.59^{a}	23.61±6.51^{a}	29.31±7.24^{a}
	15	49.00±11.65^{a}	26.10±1.15^{ab}	25.56±2.71^{ab}	14.43±1.77^{b}
	21	49.00±11.65^{a}	24.31±4.57^{b}	21.13±.313^{b}	13.10±3.54^{b}

Mean Standard Error is displayed. Significant differences exist between the means after each letter in a row (P <0.05).

Table 3. Biochemical Indices of Gulfam Exposure at 7 and 15 Days as a Dose-Response Measure

PARAMETERS	**Time (Days)**	**CONTROL**	**Aquatic Exposure**		
			1mg/L	**2mg/L**	**3mg/L**
ALP (U/L)	**7**	18.54±2.62^{b}	55.01±16.47^{b}	122.10±9.18^{a}	145.11±13.94^{a}
	15	18.54±2.62^{b}	108.23±10.15^{ab}	169.33±19.81^{a}	219.56±16.18^{ab}
ALT (U/L)	**7**	11.71±1.86^{c}	34.67±8.86^{b}	71.34±.656^{a}	86.30±2.78^{a}
	15	11.71±1.86^{d}	35.31±2.91^{c}	137.67±2.34^{a}	166.01±12.33^{b}
AST (U/L)	**7**	22.91±8.19^{b}	40.23±9.59^{b}	79.65±18.44^{ab}	125.01±8.67^{a}
	15	22.91±8.19^{b}	51.10±3.69^{b}	92.56±13.58^{a}	125.65±11.60^{a}
UREA (mg/dl)	**7**	5.10±.567^{c}	9.43±1.35^{b}	7.21±.675^{bc}	17.02±.544^{a}
	15	5.10±.567^{a}	6.86±1.20^{b}	14.17±.431^{a}	15.04±.568^{a}
CREATININE (mg/dl)	**7**	.643±.034^{c}	.749±.090^{c}	2.26±.268^{b}	6.51±.231^{a}
	15	.643±.034^{b}	2.69±.087^{b}	6.92±1.71^{a}	8.41±.844^{a}

Mean Standard Error is displayed. Significant differences exist between the means after each letter in a row (P <0.05).

Table: 4 Gulfam dose-response analysis using biochemical indices following 7 and 15 days of intraperitoneal administration

PARAMETERS	Time (Days)	CONTROL	Intraperitoneal Exposure		
			10μl/g	**15μl/g**	**20μl/g**
ALP (U/L)	**7**	18.54±2.62[b]	57.23±11.31[ab]	75.32±18.67[ab]	111.30±17.42[a]
	15	18.54±2.62[b]	51.06±2.89[b]	238.10±21.56[a]	361.02±16.28[a]
ALT (U/L)	**7**	11.71±1.86[b]	17.62±2.61[b]	21.64±2.61[b]	63.56±4.41[a]
	15	11.71±1.86[b]	29.33±2.81[b]	81.67±20.58[a]	135.56±8.768[a]
AST (U/L)	**7**	22.91±8.19[d]	63.02±8.31[c]	143.31±6.78[b]	248.12±9.65[a]
	15	22.91±8.19[a]	107.61±11.89[a]	168.65±17.61[ab]	199.20±20.79[a]
UREA (mg/dl)	**7**	5.10±.567[b]	9.19±.617[a]	9.74±1.20[a]	9.77±.501[a]
	15	5.10±.567[c]	11.91±.655[b]	16.91±1.13[a]	19.51±.861[a]
CREATININE (mg/dl)	**7**	.643±.034[b]	.946±.118[b]	1.31±.229[b]	4.82±1.13[a]
	15	.643±.034[c]	2.51±.899[c]	5.19±.586[b]	8.56±.990[a]

Mean Standard Error is displayed. Significant differences exist between the means after each letter in a row ($P < 0.05$).

Discussion

Zinc oxide nanoparticles (ZnO NPs) have recently received a lot of interest because of their many potential uses in products for the general public and in manufacturing. However, there are worries that they could be hazardous to marine life. To determine the safety of ZnO NPs, researchers have examined the fish's blood chemistry by studying the common carp (Cyprinus carpio). ZnO NPs can cause changes in common carp hemato-biochemical parameters, according to research. There have been observations of changes in hematological parameters like red blood cell (RBC) count, hemoglobin (Hb) concentration, and hematocrit (Hct) levels. ZnO NP exposure has been linked to elevated red blood cell (RBC) count, Hb concentration, and Hct, which may indicate a stimulation of erythropoiesis. This may be a protective mechanism against the harmful effects of the nanoparticles' oxidative stress. A drop in RBC count, Hb concentration, and Hct levels may indicate hematopoietic system damage in fish exposed to ZnO NPs for longer periods of time or at higher concentrations. In addition, ZnO NPs were discovered to alter the metabolic composition of common carp. Altered activity of enzymes involved in a variety of metabolic processes have been observed after exposure to ZnO NPs. These include alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP). Hepatotoxicity is indicated by elevated levels of liver enzymes ALT, AST, and ALP, hence ZnO NPs may be harmful to common carp in this regard. Total protein, albumin, and glucose levels have all been shown to change, pointing to disruptions in protein metabolism and glucose homeostasis. ZnO NPs are able to cause toxicological effects on the hemato-biochemical profile of common carp because of their tiny size and large surface area, which allow them to penetrate many different tissues and organs. ZnO NPs, once ingested by fish, can cause hematological and metabolic changes by inducing oxidative stress, disrupting cellular functioning, and triggering inflammatory responses. Zinc ions released from ZnO NPs may also contribute to the harmful consequences seen, as zinc buildup in excess can interfere with the proper operation of biological systems.

According to the current study's findings, ZnO-NPS harmed the fish that live in freshwater when the exposure time was too long or the concentration of NPs was too high. The hemato-biochemical parameters of the fish subjected to the experiment showed significant changes. Both Monteiro et al. 2010 and Plessl et al. 2017 reported similar findings, namely that fish are susceptible to metabolising metals and oxides of metals, as well as aggregating them. After that, it was stated by Naigaga et al. 2011 and Klobucar et al. 2010 that fish can be used as a biomarker of contaminated freshwater and saltwater environments. The presence of zinc oxide nanoparticles significantly impacts the haematological indices of aquatic fish (Faiz et al. 2015).

Similar results were found for haematological biomarkers in major carp when fish were treated with various doses of ZnO-NPs, as reported by Kori-Siakpere&Ubogu (2008; Hedayati, 2015; Faiz et al. 2015). When compared to the control group, Cyprinus carpio that had been given ZnO-NPs experienced a significant drop in their RBCs, HCT, and Hb levels after receiving either intraperitoneal injections or intravenous administration of the nanoparticles. Additionally, Abdel-Khaleket et al. 2016 found that the levels of RBCs, Hb, and HCT had significantly changed in O. niloticus. Red blood cell production failure, poor osmoregulation, and poor internal blood flow could all decrease the total number of RBCs in a high-pressure environment (Abhijith et al. 2012). According to Pamila et al. (1991), toxicants may have an inhibitory effect on the profile of the catalyst, which is responsible for the mixture of Hb, which could explain why fish treated with toxicants had lower levels of Hb. A lower HCT fixation also shows cell shrinkage due to the stress that toxicants put on erythropoietin tissue (Saravananet al. 2011). Red blood cells' structural bend under the force of the metal causes a decrease in RBC count, which in turn causes a decrease in HCT and Hb levels in the blood (Venkatachalam&Natarajan, 2014).

In this study both the aquatic and intra - peritoneal routes of NPs introduction resulted in a significant rise in white blood cells in ZnO-treated fish, which needed to be monitored and managed. The comparative results were found by Soltani et al. (2016) after they discovered that ZnO-NPs were harmful to Capoeta gracilis. White blood cells (WBCs) commonly aid the body's defence mechanisms in its struggle against toxic substances (Abhijith et al. 2012). Investigating WBC levels is one of the best ways to assess the structure of the immune system, according to Tavares-Dias (2007). In response to contact with nanomaterials, extreme caution is required because there is evidence of an increase in white blood cells (Abhijith et al. 2012). Because of the high level of contamination in the body tissues and the intense physical weight, the risk of annihilation increases in direct proportion to the white blood cell count (WBC) (Singh et al. 2008). During this study, significant (P 0.05) shifts were observed in the MCV, MCH, and MCHC of fishes that had been subjected to ZnO-NPs as compared to the group that was used as the control. Soltani et al. (2016) also looked into the impact of ZnO-NPs, and their findings are similar to those of the current study. In response to the stress applied to the nanomaterials, they claimed that a damaging film of RBCs formed due to hemolysis. After a potentially lethal exposure, Saravanan et al. found an increase in macrocytic disease due to an expansion in macrocytic cell volume and macrocytic cell height (2011).

The changes in cardiovascular catalysts (AST), liver enzymes (ALP, ALT), and levels of urea and creatinine also provided evidence of the detrimental effects of nanoparticles on fish health (Karthikeyeni et al. 2013; Rajkumar et al. 2016). Toxins are present in aquatic ecosystems, which has detrimental effects at the cellular and subatomic levels. As a result, aquatic life's biochemical indices vary significantly (Chowdhury et al. 2004).

Both AST and ALT levels significantly increased in Sebastes schlegeli after 40 days of infrequent exposure to NPs, as observed by Kim and Kang (2004). Longer introduction terms and longer periods of fixation were found to be related to this increase. Serum AST and ALT exercises in O. niloticus exposed to different concentrations of Zn compared with the control at 7 and 14 days (Firat&Kargin 2010; Alkaladi et al. 2015; Taheri et al. 2017) were found to be significantly higher after treatment with ZnO for both short and long periods (Younis et al. According to Elghobashy et al. (2001), fish caught in the lakes and rivers of the Nile River had higher serum urea levels. They explained this rise in metal toxicity, which results in neurotic changes in the kidney's glomerulus filtration repeat, as the cause of this increase. Through a renal working test, Alkaladi et al. (2015) found an increase in the amount of creatinine fixation in O. niloticus. The findings of this discovery were comparable to the findings of the present examination. Toxic effects of zinc oxide were found to increase the rate at which urea was fixed in tilapia, as discovered by Abdel-Tawwab et al. (2011). This increase was inversely related to the concentration of zinc oxide and the exposure time.

The findings of the study on the toxicological effects of Zinc Oxide Nanoparticles (ZnO NPs) on the hemato-biochemical profile of common carp (Cyprinus carpio) provide useful insights into the possible concerns that these nanoparticles pose to the health of fish and the aquatic environment. According to the findings, being exposed to ZnO NPs might cause considerable changes in the hematological and biochemical parameters of common carp. These changes may have an effect on the general health of fish as well as their chances of survival, and they may also destabilize aquatic ecosystems.

The dose-dependent drop in red blood cell count, hemoglobin level, and hemoglobin concentration that was observed in fish after exposure to ZnO NPs was one of the most important discoveries made by the research team. According to these findings, ZnO NPs could have detrimental impacts on the oxygen-carrying capacity of fish, which could have implications for the fish's general health and ability to survive. Additionally, the considerable increase in glucose, total protein, albumin, globulin, cholesterol, triglycerides, and creatinine levels in common carp that were exposed to ZnO NPs indicates that these nanoparticles could alter the metabolism of fish as well as the function of their organs.

The findings of the study indicate the necessity for additional research into the effects that ZnO NPs have over the long term on the health of fish and the ecosystem. In addition, the study highlights the significance of controlling the usage of these nanoparticles as well as their disposal in order to limit the possible threats that these particles provide to aquatic creatures and ecosystems. The findings of this study could be utilized to guide regulatory decisions on the use of ZnO NPs in a variety of industries, including the cosmetics industry, the food packaging industry, and the textile industry, all of which make frequent use of these nanoparticles.

In conclusion, the research on the toxicological effects of zinc oxide nanoparticles on the hemato-biochemical profile of common carp sheds light on the possible dangers that these nanoparticles pose to the health of fish and the ecology of aquatic ecosystems. The findings highlight how important it is to regulate the usage of these nanoparticles as well as their disposal in order to reduce the possible impact that they could have on aquatic ecosystems and on public health. More research is required if we are going to have a complete understanding of the long-term consequences that ZnO NPs have on the health of fish and the ecosystem.

Conclusion

The research that was conducted on the toxicological effects of zinc oxide nanoparticles (ZnO NPs) on the hemato-biochemical profile of common carp (Cyprinus carpio) uncovered crucial insights into the potential dangers that are connected with exposure to these nanoparticles. According to the findings of this study, nanoparticles of ZnO have the potential to induce considerable changes in the hematological and biochemical parameters of fish, which may have an effect on the fish's general health and ability to survive.

The findings of this study indicate the necessity for additional research into the effects that ZnO NPs have over the long term on the health of fish and the ecosystem. In addition, the findings highlight how important it is to regulate the usage of these nanoparticles as well as their disposal in order to reduce the possible impact that these particles could have on aquatic ecosystems and on public health.

In general, the research conducted on the toxicological effects of Zinc Oxide Nanoparticles on the hemato-biochemical profile of common carp contributes to our awareness of the possible dangers that are linked with the usage of these nanoparticles in a variety of different industries. The findings of the study could potentially be used to inform regulatory decisions on the use and disposal of ZnO NPs, with the objective of limiting the potential dangers that these particles pose to the environment and to public health. The long-term impacts of ZnO NPs on aquatic creatures and ecosystems need to be investigated further in order to gain a complete understanding of these effects, which may have substantial repercussions for environmental and public health..

Conflict of interest

Authors declare there is no conflict of interest.

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