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ORIGINAL RESEARCH PAPER

Impact of different quantity of Zinc oxide nanoparticles on growth and hematology of Mrigal *Cirrhinus mrigala*

Muthuswami Ruby Rajan*, Raja Rohini

Department of Biology The Gandhigram Rural Institute- Deemed to be University Gandhigram- 624 302, Tamil Nadu, India

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ABSTRACT

Zinc is essential for aquatic biota including fishes at a lower concentration, but when it reaches higher concentration it becomes toxic. The objectives of the present work were related to the impact of different quantities of zinc oxide nanoparticles on the growth and hematology of Mrigal *Cirrhinus mrigala*. Zinc oxide nanoparticles were synthesized via chemical precipitation method and characterized using UV-VIS, SEM, EDAX, FTIR, and XRD. Different quantities of zinc oxide nanoparticles such as 0, 5,10,15,20, and 25mg/100g were prepared by using a fish meal, groundnut oil cake, wheat flour, and tapioca flour. Feed utilization and hematological parameters of Mrigal were estimated after 21 days of feeding. UV-visible absorption spectra indicated that the peak absorbance of ZnO nanoparticles was observed at 500 nm. SEM shows that formed nanoparticles are clustered due to the adhesive nature of flower-shaped appearance. EDAX shows that the zinc oxide nanoparticles and the peaks are located between 1.0Kev and 8.5Kev. The FTIR spectrum of zinc oxide nanoparticles was analyzed in the range of 400-4000cm^{-1} and spectral bands were observed. The XRD results were viewed as the crystalline nature and average size of zinc oxide nanoparticles. Survival rate indicated that all Mrigal were healthy during 21 days except in feed II, IV, and V. The feed utilization and growth parameters are higher in feed IV. Hematological parameters such as hemoglobin, RBC, Hematocrit, MCV, MCH, and MCHC of Mrigal progressively increased and the WBC and platelets decreased with the increase in the quantity of Zinc Oxide nanoparticles. This study provides that 15mg of Zinc oxide nanoparticles incorporated feed was suitable for the growth and hematological parameters of Mrigal and it would be used in the feed of fishes as micronutrients.

Keywords: *Impact, Zinc oxide nanoparticles, growth, hematology, Mrigal*

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INTRODUCTION

Nanoparticles have enormous potential in controlling pathogens, improving the immunity and growth functions in aquaculture (Brintha and Ajitha, 2015) [1]. Among the numerous metal oxide nanoparticles, zinc oxide nanoparticles play a vital role in the nanotechnology field due to their specificity when compared to other metal oxide micro and nanoparticles. ZnO is a low-cost material that could be processed in many forms such as nanostructured thin films. Due to its easy processing in various forms, it is used in various applications from optoelectronics to energy conversion, photocatalysis, and sensors (Lucian Pislaru-Dănescu *et al* 2018) [2]. ZnO NPs having exciting properties such as high stability, anti-corrosion, photocatalytic, antimicrobial, and UV absorption properties are used in various products including sunscreens, food packaging, drug delivery, cosmetics, paint, plastic, ceramics, and building materials [Osmond and McCall 2010) [3], textiles with self-cleaning fibers, rubber and papers (Pandurangan and Kim 2015,

* Corresponding Author Email: *mrrrajanbio@gmail.com*

Saad *et al* 2016) [4,5]. It is also added to the diet and water as a micronutrient for the production of plankton and fish growth as a zinc source. Zinc is an essential trace element for finfish and plays a critical role in biological processes and physiological functions such as biosynthesis of hormones, enzymatic activity, and metabolism of proteins carbohydrates, and lipids (Muralisankar et al 2014) [6]. Large-scale production and huge use of zinc oxide lead to the direct and indirect release into an aquatic ecosystem which ultimately affects the aquatic biota. ZnO is one of the most harmful products present in the aquatic environment, due to its toxic and biochemical altering properties in aquatic organisms (Kahru and Dubourguier 2010)[7]. Fish, occupying high trophic levels in the aquatic ecosystem and being an important food source, are regarded as indicators of ZnO contamination in the aquatic environment (Agah et al 2009)[8]. Therefore, it is generally considered the most relevant organism for pollution monitoring in aquatic ecosystems. Metal oxides and metal oxide nanoparticles can accumulate in the different organs of fish through potential routes such as food or non-food particles, oral consumption, absorption via gill epithelia and skin (Ay *et al.,* 2009) [9] which leads to the damage of tissues and interfering the normal growth of aquatic biota. Among freshwater fishes, the Indian major carp Mrigal *Cirrhinus mrigala* is of great commercial importance because it is the most common fish consumed by the largest population in India. It is often cultured in polluted water and thus susceptible to exposure to different metal oxides (Debjit Das et al 2017) [10]. Hence, *Cirrhinus mrigala* acts as a good biological model for toxicological studies due to diverse characteristics, namely, high tolerance to stress and diseases and a wide variety of environmental conditions. The toxicants in an aquatic ecosystem are taken by fish and transported to the tissues and organs through the blood. Growth and hematological parameters are widely used as a health indicator in ecotoxicological studies because these parameters react before the toxicants enter the body of fish. Fish blood is a suitable way to determine and diagnose the toxicity of metal and metal oxide nanoparticles (Sevcikova et al 2016, Ates et al 2016) [11,12] and hematological analysis is excellent to assess the stress condition of aquatic organisms (Bahmani et al 2012) [13]. Monitoring changes in the activities of growth and hematological parameters may help to understand the stress-related homeostatic adjustments in fish exposed to xenobiotics. The present study has reported for the first time on the impact of zinc oxide nanoparticles on Mrigal *Cirrhinus mrigala* and its effect on the overall physiology of fish.

- This study provides information about the toxic potential of zinc oxide nanoparticles.
- 15mg of Zinc oxide nanoparticles incorporated feed was suitable for the growth of Mrigal.
- Hematological parameters such as Red Blood Cells, Hemoglobin, Hematocrit, Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), and Mean Corpuscular Hemoglobin Concentration (MCHC) of Mrigal were increased with an increased quantity of ZnO nanoparticles in the feed.
- The impact of these pollutants must be assessed before it discharges into the aquatic environment to protect aquatic biota including fishes as well as human lives.

MATERIALS AND METHODS

Materials

For the present work, Mrigal fingerlings (0.72±0.2g) were collected from Aqua garden fish farm, Madurai, Tamil Nadu, India, and transported to the laboratory in polythene bags filled with oxygen from a cylinder. Fishes were acclimated in a round cement tank for 15 days at 28^{0}C. During acclimation, fishes were fed with trainee feed containing fish meal, groundnut oil cake, wheat flour, and rice bran in the form of dry pellets.

Synthesis of Zinc oxide nanoparticles

Synthesis of zinc oxide nanoparticles was carried out by simple precipitation method. For this study, 0.5M of zinc acetate ($Zn(CH_3COO)_2.2H_2O$) was dissolved in 100mL of distilled water and 1M of sodium hydroxide was also dissolved in 100mL of distilled water. Precipitation was done by mixing 1M of NaOH which is to be added dropwise to the 0.5M of zinc acetate solution under vigorous stirring. The process continued until the appearance of milky white precipitate. During this precipitating process, pH was increased from 7 to 14. Following the precipitation, the solution was centrifuged at 3000 rpm for 10 min and washed several times with distilled water and ethanol to remove the by-products. The supernatant was then removed and the pellet was dried. After drying, the precipitate was calcinated in a muffle furnace

Table 1. Composition of Different Components in Experimental feed (g/100gm) of Mrigal *Cirrhinus mrigala*

Ingredients	Experimental Feeds					
	Feed I (control)	Feed II	Feed III	Feed IV	Feed V	Feed VI
Fishmeal	36.2	36.2	36.2	36.2	36.2	36.2
GNOC*	36.2	36.2	36.2	36.2	36.2	36.2
Wheat flour	8.8	8.8	8.8	8.8	8.8	8.8
Tapioca	8.8	8.8	8.8	8.8	8.8	8.8
Fish oil	2	2	2	2	2	2
Sunflower oil	2	2	2	2	2	2
Supplevite mix	4	4	4	4	4	4
Sodium chloride	1	1	1	1	1	1
Sodium benzoate	1	1	1	1	1	1
ZnO nanoparticles	0	5mg	10mg	15mg	20mg	25mg

*GNOC- Groundnut Oil Cake

at 300ºC for 3 h. Finally, nano ZnO was grinded with mortar to be shaped into powder. Magnetic stirrer, centrifuge, and muffle furnace were used in synthesis.

Characterization of Synthesized ZnO Nanoparticles

The synthesized nanoparticles were characterized by UV-VIS Spectrophotometer (JASCO-V-530), Scanning Electron Microscopy (SEM) (LEO 1455 VP), Energy Dispersive X-ray detection instrument (EDAX) (HORIBA 8121-H), Fourier Transform Infrared Spectroscopy JASCO (FTIR- 6200), and X-Ray Diffraction (SHIMADZU Model XRD¬6000).

Experimental Feed preparation

The raw materials are selected based on the ability to supply nutrients. Fish meal and groundnut oil cake were used as a protein source; wheat flour and tapioca flour were used as carbohydrates sources, vegetable oil was used as binding agents, and a suppletive mix was also added. The components used for feed preparation were dried, powdered, and sieved through a 425-micron sieve. After knowing the protein content of major ingredients by the Micro-Kjeldahl method, the ingredients were weighed and mixed thoroughly with 130-150 ml of distilled water. The mixed feedstuff was put in the autoclave for 15 minutes at 100^{0}C and later on cooled. After cooling, fish oil, sunflower oil, suppletive mix, sodium chloride, sodium benzoate, and different quantity of Zinc Oxide nanoparticles (5, 10, 15, 20, 25 mg/100g) were mixed with the ingredients it was extruded with the help of a pelletizer. The pellets were dried at room temperature. The formulated feed was kept in an airtight container at -20^{0}C until used to prevent contamination (Table 1).

METHODS

Experimental Design for Growth studies

For the present study uniform and size of *Cirrhinus mrigala* (0.72±0.2g) were selected and then the fishes were introduced in the trough having a capacity of 15 liters. Ten fishes were introduced in each trough. For each treatment triplicates were maintained. A total of 180 fishes were used for the study. During rearing the fishes were fed on an ad-libitum diet of the prepared feed twice a day for 1 hour each from 9-10 cm. The unfed were collected after one hour of feeding without disturbing the fishes. The unfed was dried to constant weight. The fecal matter was collected daily before changing the water with the least disturbance to the fishes and dried at 95^{0}C. Approximately 70% of the water in the tank is replaced with tap water without chlorination. The experiment was continued for 21 days. On the 21st-day growth, parameters were calculated. For hematological analysis blood samples were collected from a cordial vein on the right side of the fish. Complete blood parameters such as White Blood Cells (WBC), Haemoglobin, Red Blood Cells (RBC), Hematocrit (PVC), Mean Corpuscular Volume (MCV), Mean corpuscular

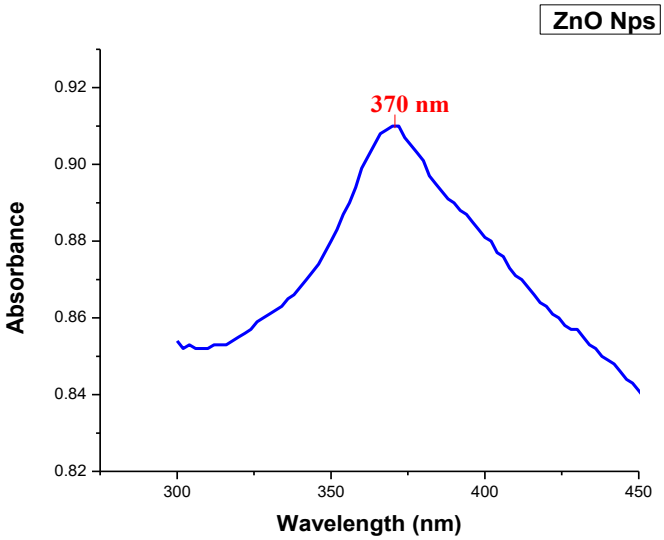

Fig. 1. UV-Visible image of Zinc oxide Nanoparticles

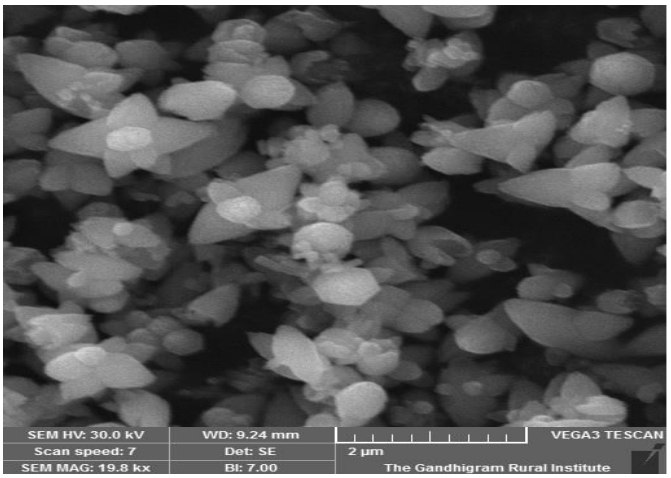

Fig. 2. SEM Image of Zinc Oxide Nanoparticles

hemoglobin (MCH), Mean corpuscular hemoglobin concentration (MCHC), and Platelets count were estimated.

RESULTS AND DISCUSSION

Characterization of Zinc oxide nanoparticles

The primary characterization of synthesized zinc oxide nanoparticles was analyzed by using UV-VIS Spectrophotometer and the peak absorbance was observed at 370 nm (Fig.1). Jamdagni et al (2018) [14] reported a UV-Visible spectrum of chemically synthesized ZnO nanopowder was obtained upon resuspension in sterile deionized water and a sharp absorption peak was observed at 370 nm. Ahmadi Shadmehri et al (2019) [15] also reported that the ZnO-NPs have a strong absorption maximum at a wavelength of 362 nm. The UV-Vis spectra of ZnO NPs synthesized using zinc nitrate and zinc acetate were observed at 380nm (Shubhangi Moharekar et al 2014) [16]. Scanning electron microscopy shows that the nanoparticles formed are clustered due to the adhesive nature of the flower-shaped appearance shown in Fig. 2. Jeyabharathi et al., (2017) [17] reported that the SEM image of zinc oxide nanoparticles synthesized using zinc acetate is spherical in shape with more aggregation. The elemental composition of zinc oxide nanoparticles was determined by using EDX analysis and

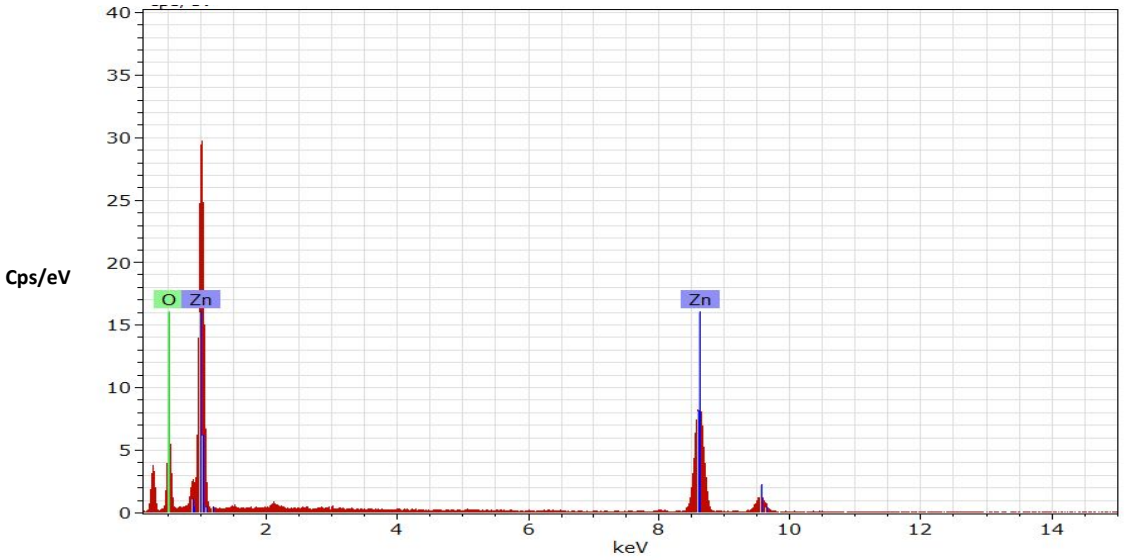

Fig. 3. EDAX Image of Zinc oxide Nanoparticles

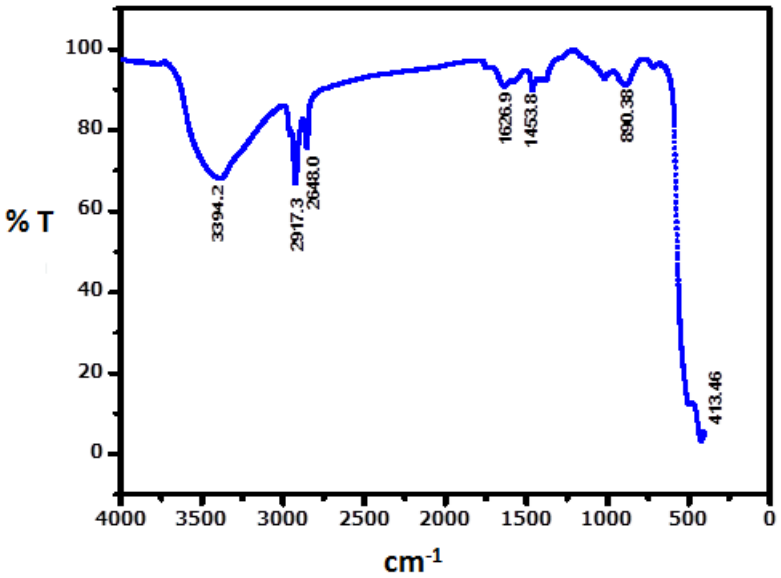

Fig. 4. FTIR Image of Zinc oxide Nanoparticles

zinc is higher when compared to oxygen (Fig. 3). EDAX result was viewed as the zinc oxide nanoparticles and the peaks are located between 1.0Kev and 8.5Kev. Sadhan Kumar Chaudhuri and Lalit Malodia (2017) [18] reported that EDAX analysis was carried out to determine the elemental composition and stereochemistry of the synthesized zinc oxide nanoparticles. The FT-IR measurement was carried out for identifying the possible bio-chemicals responsible for the formation of ZnO_2 zinc oxide nanoparticles. This characterization was used to identify the functional groups of the bioactive components based on the peak value in the region of infrared radiation. Zinc oxide formation was confirmed 3394.2, 2917.3, 2648.0, 1626.9, and 413.46 cm^{-1} bands have amide, C-O bond, C=C alkynes, diketones, and halogen compounds (Fig.4). Synthesized Zinc Oxide nanoparticles have functional groups such as the Amide group, Alkyn Halides group, Diketones, and halogen group. The peak of 413 cm^{-1} indicates the presence of zinc oxide was confirmed by the synthesis

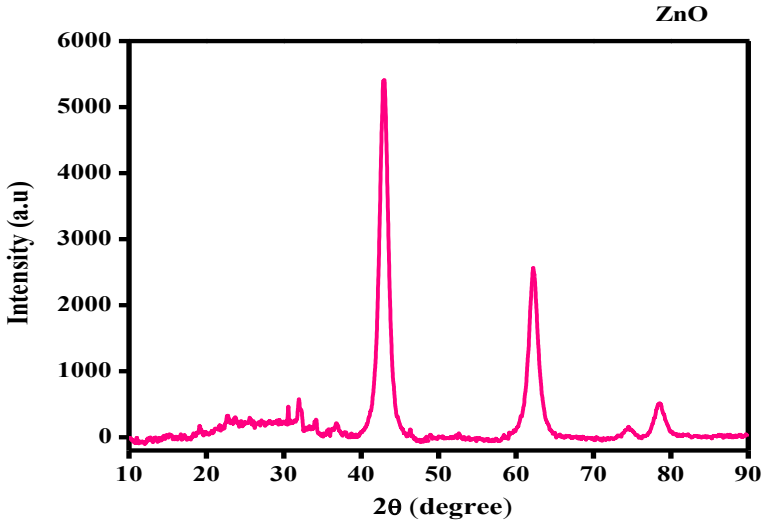

Fig. 5. XRD Image of Zinc oxide Nanoparticles

of zinc oxide nanoparticles. Sheikh et al (2017) [19] reported a peak in the range of 400 cm^{-1} proved the presence of ZnO nanoparticles. Maribel Guzman et al., (2018) [20]reported that the FTIR spectra of nanoparticles show absorption bands at 483 and 507.31cm-1 corresponding to the stretching vibrations of the Zn-O bond. The XRD results were viewed that the average size of zinc oxide nanoparticles is10nm(Fig.5). The 2θ values of 31.9,34.5,36.3,47.6,56.7,62.9, 66.5,68.4,69.1, 2.6,77.3,81.5,89.2 in the reference elemental zinc which are attributed 016, 310, 356, 313, 325, 301, 466, 356, 261, 362, 956, 609, 262, 670 crystallographic plane of face-centered cubic zinc crystals. The peak related to the ZnO structure indicates a good polycrystalline nature of the deposited film. These films have shown the formation of ZnO nanoparticles. Purwaningsih et al., (2015)[21] also reported γ- ZnO, and the particle size was calculated using the Scherrer's equation estimated at around 10 nm. To evaluate and identify the reaction product XRD viewed the crystal size analysis and XRD diffraction 2θ value (Haritha et al 2017)[22]. Brintha and Ajitha, (2015) [1]reported that the main XRD diffraction peak of ZnO NPs synthesized by aqueous solution method at 2θ value of 36.1769°, β value 0.7073 is from ZnO and the crystalline size was 13nm which is in good agreement with the standard JCPDS (Card No. 36- 1451) and also found to be in accordance with previous reports (Molahasani et al 2013, Jegan, et.al 2012)[23,24].

Growth Studies

The growth parameters are presented in Table 2. The Survival Rate of *Cirrhinus mrigala* was 100% in feed containing 0, 5, and 20 mg of zinc oxide nanoparticles, and 80, 90, and 90% were observed in 10, 15, and 25 mg. Feed consumption and feed conversion efficiency of Mrigal were higher in feed IV (3.56±0.27 and 0.16±0.01) containing 15 mg of zinc oxide nanoparticles. Onuegbu et al (2018) [25] reported an increase in the concentration of Zinc Oxide nanoparticles with feed consumption and feed conversion in African Catfish fingerlings. The feed conversion ratio was good in Ex. Feed III (2.15). Faiz et al (2015) [26] reported that the feed conversion ratio was higher in ZnO nanoparticles incorporated feed of juvenile grass carp. Growth and Specific growth rate were higher in feed IV (20mg/g^{-1}) when compared to control and were significantly increased. Thangapandiyan and Monika(2020)[27] reported that the growth improved *Labeo rohita* when green synthesized zinc oxide nanoparticles were incorporated in the feed. Muralisankar *et al*.,(2016)[28] reported that the growth of *Macrobrachium rosenbergii* was higher in copper supplemented feed. Aziz et al(2020)[29] reported a higher specific growth rate of *Labeo rohita* fingerlings fed a diet containing 20 mg ZnO-NP per kg. *The assimilation* and metabolism of Mrigal were increased with an increase in ZnO nanoparticles in the feed. The gross and net growth efficiency was higher in feed VI and significantly varied (Table 3).

Table. 2.Feed utilization and Growth parameters of Mrigal in relation to different quantity of Zinc oxide nanoparticles. Each value is the average (±SD) performance of 5 individuals in triplicates reared for 21 days

Parameters	Experimental Feed					
	Feed (control)	Feed II (5mg)	Feed III (10mg)	Feed IV (15mg)	Feed V (20 mg)	Feed VI (25mg)
Survival Rate (%)	70	80	50	100	90	60
Feed Consumption(FC) (g/g live wt/21 days)	1.8±0.24[a]	2.03±0.28[b]	2.43±0.46[c]	3.56±0.28[d]	3.23±0.32[e]	2.16±0.27[f]
Feed Conversion Efficiency(FCE)	0.09±0.06	0.06±0.05	0.12±0.03	0.16±0.01	0.08±0.02	0.2±0.08
Feed Conservation Ratio(FCO)	4.82±0.61	5.26±0.23	6.52±0.52	3.15±0.35	4.93±0.56	6.33±0.72
Growth(G) gm/gm live wt/21 days	0.25±0.05[a]	0.26±0.04[b]	0.33±0.05[c]	0.71±0.06[d]	0.67±0.07[e]	0.45±0.05[f]
Specific Growth Rate(SGR) (%)	1.81±0.21	1.5±0.15	2.3±0.32	4.6±0.41	3.5±0.22	2.6±0.16
Assimilation(A)	1.9±0.56	0.98±0.65	1.43±0.81	1.46±0.90	1.63±2.6	2.33±0.95
Metabolism(M)	1.73+0.47	1.5+0.57	1.3+0.79	2.0+0.46	1.9+0.63	1.6+0.65
Gross Growth Efficiency(GGE) (%)	28.7+2.9[a]	36.5+2.1[b]	28.8+3.27[c]	40.63+1.3[d]	34.03+5.2[e]	43.6+1.27[f]
Net Growth Efficiency (NGE) (%)	29.9+2.89[a]	39.2+2.28[b]	45.8+1.72[c]	45.7+4.5[d]	52.3+2.1[e]	53.5+1.24[f]

Feed consumption	Growth	Gross growth efficiency	Net growth efficiency
a vs b (P>0.05) S	a vs b (P>0.05) S	a vs b (P>0.05) S	a vs b (P>0.05) S
a vs c (P>0.05) S	a vs c (P>0.05) S	a vs c (P>0.05) S	a vs c (P>0.05) S
avs d (P>0.05) S	a vs d (P>0.05) S	a vs d (P>0.05) S	a vs d (P>0.05) S
avs e (P>0.05) S	a vs e (P>0.05) S	a vs e (P>0.05) S	a vs e (P>0.05) S
avs f (P>0.05) S	a vs f (P>0.05) S	a vs f (P>0.05) S	a vs f (P>0.05) S

Table.3. ANOVA (Analysis of Variance) of Growth parameters (Feed consumption, growth, gross growth efficiency, net growth efficiency) of Mrigal *Cirrhinus mrigala*

S.NO	Parameters	Source	SS	Df	MS	F	PROB
1.	Feed Consumption	Columns	1.1295	02	0.28321	5.06	0.0112
			0.4005				
		Errors	1.25743	10	0.06005		
		Total	3.44055	11			S
2.	Growth	Columns	0.64065	04	0.10316	7.25	0.00084
			0.2553				
		Errors	0.68665	9	0.9237		
		Total	2.1365	14			S
3.	Gross Growth Efficiency	Columns	1029.08	03	348.78	7.65	0.0015
			548.87				
		Errors	14354.87	10	49.765		
		Total	3213.52	13			S
4.	Net Growth Efficiency	Columns	1952.44	04		7.78	0.0005
			394.82		315.98		
		Errors	1128.5	13	30.897		
		Total	3446.03	10			S

Hematological Analysis

The hematological analysis acts as a rapid and economical method for assessing metal oxide toxicity on fishes. Shah and Altindag, (2005)[30] reported that the hematological parameters such as hematocrit, Hb, RBC, and WBC are used to assess the functional status of the oxygen-carrying capacity of the bloodstream and have been used

Table. 4. Hematological parameters of Mrigal exposed to ZnO nanoparticles

Blood parameters	Feed-1	Feed-2	Feed-3	Feed-4	Feed-5	Feed-6
White Blood Cells (WBC)(cells/cumm)	10,000± 25	17,600±30	13200±27	11700±±25	8200± 32	5400± 24
Hemoglobin(gm/Di)	0.4±0.37	1.0±0.25	1.0±0.11	1.1±0.08	1.8±0.12	2.9±0.14
Red Blood Cells (RBC)(Millions/cumm)	0.0	0.2±0.06	0.3±0.02	0.3±0.04	0.4±0.02	0.54±0.06
Hematocrit(PCV) (%)	0.0	2.86±0.16	0.4±0.12	0.56±0.14	5.8±0.13	6.7±0.11
Mean Corpusular Volume (MCV)	0.1±0.02	1.0±0.03	0.19±0.01	1.87±0.05	2.56±0.04	4.98±0.06
Mean Corpuscular Hemoglobin (MCH)	0.0	1.76±0.03	3.02±0.02	6.09±0.01	7.00±0.07	9.0±0.05
Mean Corpuscular Hemoglobin Concentration(MCHC)	0.2±0.01	35±2.0	37±6.2	38±4.0	40±5.0	42±3.0
Platelets Count (Cells/cumm)	49000±28	73,000±31	72000±29	61000±34	38000±32	26000±29

as an indicator of metal pollution in the aquatic environment. In the present study, hemoglobin, RBC, Hematocrit, MCV, MCH, MCHC of Mrigal progressively increased and WBC and platelets decreased with an increase in the quantity of Zinc Oxide nanoparticles (Table 4). Fırat, (2007)[31] reported that the decrease in WBC count could be associated with the cortisol hormones which play an important role in the prevention and healing of inflammation on fish by the introduction of toxicants. Paria Akbary et al., (2018)[32] reported that the Hb, Hct, and RBC counts are decreased and WBC count significantly increased compared to the control in grey mullet fish exposed to sub-lethal concentration of copper oxide nanoparticles. Ali et al., (2015)[33] reported the increase of blood parameters when compared to control with a high concentration of selenium nanoparticles supplemented feed to African catfish, *Clarius gariepinus*. Aasma Noureen et al., (2018)[34] reported significant variation in blood parameters such as MCH, MCV, MCHC, Hct, and RBC levels of *C. carpio* exposed to both bulk and CuO NPs when compared to the control group after 14 days of fish exposure. Latifeh Chupani et al., (2018) [35] reported that when common carp (*Cyprinus carpio* L.) exposed to diet-born ZnO nanoparticles had no effects on hematology.

CONCLUSIONS

From the present study, the impact of Zinc oxide nanoparticles on Mrigal Cirrhinus mrigala was concluded with the following findings:

1. ZnO NPs were synthesized by chemical precipitation method and characterized by UV-Vis, SEM, EDAX, FT-IR, and XRD.
2. Zinc oxide nanoparticles were analyzed by using UV-VIS Spectrophotometer and the peak absorbance was observed at 370 nm.
3. Scanning electron microscopy shows that nanoparticles are flower-shaped.
4. EDAX result was viewed as the zinc oxide nanoparticles and the peaks are located between 1.0Kev and 8.5Kev.
5. FTIR data confirm the presence of ZnO in the powder sample.
6. XRD shows that the average size of zinc oxide nanoparticles is10nm.
7. 15mg of Zinc oxide nanoparticles in the feed was suitable for the growth of Mrigal.
8. Most of the hematological parameters increased with an increase in the quantity of zinc oxide nanoparticles.

So zinc oxide nanoparticles will be used in the feed of fishes as micronutrients.

AUTHOR'S CONTRIBUTION

Raja Rohini - Laboratory experiments were conducted starting from ZnO nanoparticles preparation, characterization, collection of fish, growth studies, and blood collection for hematological analysis.**Muthuswami Ruby Rajan** - The research work was formulated and guidance was given to the first author for execution.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Brintha S, Ajitha M. Synthesis and characterization of ZnO nanoparticles via aqueous solution, sol-gel and hydrothermal methods. IOSR J Appl Chem. 2015;8(11):66-72.
2. Pîslaru-Dănescu L, Chitanu E, El-Leathey L-A, Marinescu V, Marin D, Sbârcea B-G. Synthesis and Characterization of Antireflective ZnO Nanoparticles Coatings Used for Energy Improving Efficiency of Silicone Solar Cells. Electron Mater Lett. 2018;14(3):376-86.
3. Osmond MJ, McCall MJ. Zinc oxide nanoparticles in modern sunscreens: An analysis of potential exposure and hazard. Nanotoxicology. 2010;4(1):15-41.
4. Pandurangan M, Kim DH. In vitro toxicity of zinc oxide nanoparticles: a review. J Nanopart Res. 2015;17(3):158.
5. Saad SR, Mahmed N, Abdullah MMAB, Sandu AV. Self-Cleaning Technology in Fabric: A Review. IOP Conference Series: Materials Science and Engineering. 2016;133:012028.
6. Muralisankar T, Bhavan PS, Radhakrishnan S, Seenivasan C, Manickam N, Srinivasan V. Dietary Supplementation of Zinc Nanoparticles and Its Influence on Biology, Physiology and Immune Responses of the Freshwater Prawn, Macrobrachium rosenbergii. Biol Trace Elem Res. 2014;160(1):56-66.
7. Kahru A, Dubourguier H-C. From ecotoxicology to nanoecotoxicology. TOXICOLOGY. 2010;269(2):105-19.
8. Agah H, Leermakers M, Elskens M, Fatemi SMR, Baeyens W. Accumulation of trace metals in the muscle and liver tissues of five fish species from the Persian Gulf. Environ Monit Assess. 2008;157(1):499.
9. Ay T, Fawole O, Adewoye S, Ogundiran M. Bioconcentration of metals in the body muscle and gut of Clarias gariepinus exposed to sublethal concentrations of soap and detergent effluent. Journal of Cell and Animal Biology. 2009;3(8):113-8.
10. Das D, Moniruzzaman M, Sarbajna A, Chakraborty SB. Effect of heavy metals on tissue-specific antioxidant response in Indian major carps. Environ Sci Pollut Res. 2017;24(22):18010-24.
11. Sevcikova M, Modra H, Blahova J, Dobsikova R, Plhalova L, Zitka O, et al. Biochemical, haematological and oxidative stress responses of common carp (Cyprinus carpio L.) after sub-chronic exposure to copper. VET MED-CZECH. 2016;61(1).
12. Ates M, Demir V, Arslan Z, Kaya H, Yılmaz S, Camas M. Chronic exposure of tilapia (Oreochromis niloticus) to iron oxide nanoparticles: Effects of particle morphology on accumulation, elimination, hematology and immune responses. Aquat Toxicol. 2016;177:22-32.
13. Bahmani M, Kazemi R, Donskaya P. A comparative study of some hematological features in young reared sturgeons (Acipenser persicus and Huso huso). FISH PHYSIOL BIOCHEM. 2001;24(2):135-40.
14. Jamdagni P, Rana JS, Khatri P, Nehra K. Comparative account of antifungal activity of green and chemically synthesized Zinc Oxide nanoparticles in combination with agricultural fungicides. International Journal of Nano Dimension. 2018;9(2):198-208.
15. Carrouel F, Viennot S, Ottolenghi L, Gaillard C, Bourgeois D. Nanoparticles as Anti-Microbial, Anti-Inflammatory, and Remineralizing Agents in Oral Care Cosmetics: A Review of the Current Situation. Nanomaterials. 2020;10(1).
16. Moharekar S, Raskar P, Wani A, Moharekar S. Synthesis and comparative study of zinc oxide nanoparticles with and without capping of pectin and its application. World Journal of Pharmacy and Pharmaceutical Sciences. 2014;3(7):1255-67.
17. Jeyabharathi S, Kalishwaralal K, Sundar K, Muthukumaran A. Synthesis of zinc oxide nanoparticles (ZnONPs) by aqueous extract of Amaranthus caudatus and evaluation of their toxicity and antimicrobial activity. Mater Lett. 2017;209:295-8.
18. Chaudhuri SK, Malodia L. Biosynthesis of zinc oxide nanoparticles using leaf extract of Calotropis gigantea: characterization and its evaluation on tree seedling growth in nursery stage. Applied Nanoscience. 2017;7(8):501-12.
19. Sheikh M, Asghari M, Afsari M. Effect of nano Zinc Oxide on gas permeation through mixed matrix poly (Amide-6-b-Ethylene Oxide)-based membranes. International Journal of Nano Dimension. 2017;8(1):31-9.
20. Becheri A, Dürr M, Lo Nostro P, Baglioni P. Synthesis and characterization of zinc oxide nanoparticles: application to textiles as UV-absorbers. J Nanopart Res. 2008;10(4):679-89.
21. Purwaningsih SY, Pratapa S, Triwikantoro, Darminto. Synthesis of nano-sized ZnO particles by co-precipitation method with variation of heating time. AIP CONF PROC. 2016;1710(1):030040.
22. Manyasree D, Kiranmayi P, Venkata RK. Characterization and antibacterial activity of ZnO nanoparticles synthesized by co-precipitation method. Int J App Pharm. 2018;10(6):224-8.
23. Molahasani N, SEYEDSADJADI M, Zare K. Correlation of morphology and luminescent properties of ZnO nano particles to different surfactants via hydrothermal method. 2013.
24. Jegan A, Ramasubbu A, Karunakaran K, Vasanthkumar S. Synthesis and characterization of zinc oxide–agar nanocomposite. 2012.
25. Onuegbu C, Aggarwal A, Singh N. ZnO nanoparticles as feed supplement on growth performance of cultured African catfish fingerlings. 2018.
26. Faiz H, Zuberi A, Nazir S, Rauf M, Younus N. Zinc oxide, zinc sulfate and zinc oxide nanoparticles as source of dietary zinc: comparative effects on growth and hematological indices of juvenile grass carp (Ctenopharyngodon idella). International Journal of Agriculture and Biology. 2015;17(3).
27. Thangapandiyan S, Monika S. Green Synthesized Zinc Oxide Nanoparticles as Feed Additives to Improve Growth, Biochemical, and Hematological Parameters in Freshwater Fish Labeo rohita. Biol Trace Elem Res. 2020;195(2):636-47.
28. Muralisankar T, Saravana Bhavan P, Radhakrishnan S, Seenivasan C, Srinivasan V. The effect of copper nanoparticles supplementation on freshwater prawn Macrobrachium rosenbergii post larvae. J Trace Elem Med Biol. 2016;34:39-49.

29. Mondal AH, Behera T, Swain P, Das R, Sahoo SN, Mishra SS, et al. Nano zinc vis-à-vis inorganic Zinc as feed additives: Effects on growth, activity of hepatic enzymes and non-specific immunity in rohu, Labeo rohita (Hamilton) fingerlings. Aquacult Nutr. 2020;26(4):1211-22.
30. Shah SL, ALTINDAĞ A. Effects of Heavy Metal Accumulation on the 96-h LC_50 Values in Tench Tinca tinca L., 1758. Turkish Journal of Veterinary and Animal Sciences. 2005;29(1):139-44.
31. Fırat Ö. Effects of metal (Zn, Cd) and metal mixtures (Zn+ Cd) on physiological and biochemical parameters in blood tissues of Oreochromis niloticus: PhD Thesis, Çukurova University, Turkey; 2007.
32. Akbary P, Sartipi Yarahmadi S, Jahanbakhshi A. Hematological, hepatic enzymes' activity and oxidative stress responses of gray mullet (Mugil cephalus) after sub-acute exposure to copper oxide. Environ Sci Pollut Res. 2018;25(2):1800-8.
33. Alkaladi A, El-Deen NAMN, Afifi M, Zinadah OAA. Hematological and biochemical investigations on the effect of vitamin E and C on Oreochromis niloticus exposed to zinc oxide nanoparticles. Saudi Journal of Biological Sciences. 2015;22(5):556-63.
34. Noureen A, Jabeen F, Tabish TA, Yaqub S, Ali M, Chaudhry AS. Assessment of copper nanoparticles (Cu-NPs) and copper (II) oxide (CuO) induced hemato- and hepatotoxicity inCyprinus carpio. NANOTECHNOLOGY. 2018;29(14):144003.
35. Chupani L, Niksirat H, Velíšek J, Stará A, Hradilová Š, Kolařík J, et al. Chronic dietary toxicity of zinc oxide nanoparticles in common carp (Cyprinus carpio L.): Tissue accumulation and physiological responses. ECOTOX ENVIRON SAFE. 2018;147:110-6.