EFFECTS OF SUB-ACUTE EXPOSURE OF ZINC OXIDE ON HEMATOLOGICAL AND BIOCHEMICAL CHANGES IN *LABEO ROHITA*

Keerthika V, Ananth A and M R Rajan *
Department of Biology, The Gandhigram Rural Institute (Deemed to be University), Gandhigram-624302, Tamil Nadu, India.
* Corresponding author

Abstract **- The aquatic environment was affected by the increasing use of metal oxide compounds in modern industrial world. The present study was performed to investigate the effect of sub-acute exposure of zinc oxide on hematological and biochemical changes in *Labeo rohita*. The acute toxicity (LC_{50}) of zinc oxide exposed to *Labeo rohita* for 96hrs was 3.95mg/L. Based on acute toxicity test, 0, 1/10, 1/50 and 1/100th of LC_{50} concentration were selected for treatments T_0, T_1, T_2 and T_3 for present sub-acute study. At the end of 7th and 14th day, haematological and biochemical parameters in gill, muscle and liver of fish were analyzed. The RBC, hemoglobin and hematocrit count was significantly decreased ($p<0.05$) compared to that of control and WBC and platelets level was significantly higher than control during the exposure period. The protein, carbohydrate and lipid content were reduced with significant level ($p<0.05$) compared to the respective control group on 14th day of exposure period. Hence, this study revealed that the zinc oxide at sub-acute concentration affects hematological and biochemical parameters of *Labeo rohita*.**

Keywords: Zinc oxide, LC_{50}, sub-acute, hematological, biochemical, *Labeo rohita*.

1. Introduction:

Urbanization and industrialization has resulted increasing pollution by the discharge of industrial waste, mainly sediments and water to the environment (Fu and Wang, 2011). However, these anthropogenic activities are mobilizing and discharging high levels of pollutants, especially heavy metal oxides into the aquatic environment. Metal contamination of the environment results both from natural sources and industrial activities. Metal oxides contamination of aquatic ecosystem has long been recognized as a serious problem. Further potential sources of human exposure include consumer products and industrial waste as well as the working environment. Low concentrations of some heavy metals are essential for aquatic animals. However, at high concentration it accumulate in different organs, damage tissues and interfere with the normal growth (Alkarkhi *et al.*, 2009) and it may have lethal effect on environment and diversity of aquatic organism (Charjan, 1997, Farombi *et al.*, 2007). Because of these impacts, monitoring of heavy metals has received worldwide significant attention in the field and under laboratory conditions (Barnhoorn and Van Vuren, 2004). Certain heavy metal oxides are common in our environment and trace amounts are required for human wellbeing such as iron oxide (Fe_2O_3), CuO and ZnO. Hayat *et al.*, (2007) reported that the zinc oxide and copper oxide ultimately reach the water bodies and adversely affect the growth, reproduction, physiology and survival of aquatic life.

Zinc oxide is one of the most abundantly used metal oxide in cosmetics, concrete, ceramic, glass compositions and sunscreens as it efficiently absorb UV light and also do not scatter visible light. This makes transparent and more aesthetically acceptable compared to other metal oxides. It is one of an environmental water pollutant, added to diet and water as a micronutrient for increase of plankton production and fish growth as zinc source (Hallajian *et al.*, 2013), due to wide application directly or

indirectly released in to the aquatic ecosystem. Hence it leads to toxic to aquatic organisms. Among aquatic organisms, fish cannot escape from the detrimental effects of these pollutants, and are therefore generally considered to be the most relevant organism for pollution monitoring in aquatic ecosystems (Van der Oost *et al.,* 2003). The fish constitutes a valuable commodity from the point of human consumption. So heavy metal contamination of freshwater bodies and aquatic biota becomes a serious concern from human health point of view. Generally, acute toxicity (LC_{50}) is usually from a sudden or unexpected exposure to a relatively high concentration of chemicals in a short period of exposure, consequently, acute symptoms can appears after exposure (Ahmed *et al.,* 2013). LC_{50}-based acute toxicity testing was conventional method because the exposure duration is set for 96 h as it estimates the lethal concentration at 50% as less variability than those at higher or lower centimes (Finney, 1947).

Several experiments on aquatic organisms are shown to demonstrate that the presence of toxicants in an exceedingly medium result in attenuated fertility, physiological changes, behavior abnormalities, and an increased mortality rate. Based on LC_{50} toxicity, sub lethality toxicity was done to know the metabolic changes in fish. Hematological and biochemical parameters are used as an index to detect physiological changes and to assess structural and functional status of health during stress conditions in a number of fish species (Adhikari *et al.,* 2004; Suvetha *et al.,* 2010 and David *et al.,* 2010). The work related to the impact of zinc oxide and its deleterious effects on biochemical and haematological parameters in *Labeo rohita* is totally wanting. Hence the present study was carried out.

2. Materials and methods:

2.1. Experimental fish and laboratory conditions:

Healthy fingerlings of *Labeo rohita* (5±1cm) were purchased from Palani, Tamil Nadu, India and acclimatized to laboratory conditions for about 15 days before the commencement of the experiment. Feeding was stopped at least one hour prior to replacement of water. Water (one third) was changed frequently to remove the excretory wastes. Feeding was withheld for 24h before the commencement of the experiment to keep the experimental animals more or less in the same metabolic state. During acclimatization, the fish stock was maintained at natural photoperiod and normal temperature. During acclimation water pH 7.6 ± 0.2, temperature 28 ± 1ºC, dissolved oxygen 6.4 ± 0.06 mg/L and hardness 320 ± 0.21mg/L were measured. This ensures sufficient oxygen for the fish and the environment is devoid of any accumulated metabolic wastes.

2.2. Acute toxicity test:

Healthy fish were selected for the acute toxicity test and was conducted following the Organization for Economic Cooperation and Development guideline (OECD, No. 203) under static conditions. Five different concentrations of ZnO were selected for median lethal concentration (LC_{50}) viz., 0, 3, 6, 12 and 24mg/L and 0 served as control. Each treatment was conducted in triplicate and placed under the same conditions. Groups of seven *Labeo rohita* fishes were exposed to various concentrations of ZnO for 96 h. Values of mortalities were measured at 24, 48, 72 and 96 h, and dead fish were immediately removed to avoid possible deterioration of the water quality. The LC_{50} values were calculated by SPSS software Probit Analysis.

2.3. Sub – acute toxicity test:

In order to investigate the effect of zinc oxide on hematological and biochemical parameters of the fish, three sub acute concentrations of ZnO along with one control were selected. Based on the acute toxicity test, $1/100^{th}$ (T_1), $1/50^{th}$ (T_2) and $1/10^{th}$ (T_3) of LC_{50} value were selected as concentration for sub-acute toxicity test. The stock suspension was prepared as same as that of acute toxicity test and fish were exposed for a period of 14 days. This experiment was done with triplicate set up. A control (T_0) without test suspension was conducted under the same condition. During the exposure period behavioural observation

were made twice a day. At the end of 7th and 14th day of exposure, the blood samples were collected and the experimental fishes were sacrificed to dissect out the gill, muscle and liver sample for biochemical analysis.

2.4. Analysis of hematological parameters:

Blood samples collected from fish in each exposure group were subjected to complete blood profile analysis. The haematological parameters are Complete Blood Count (CBC), such as white blood cells (WBC) (cells/cumm), polymorph, neutrophils (%), lymphocytes (%), eosinophils (%), haemoglobin (gm/dl), Red blood cells (RBC) (millions/cmm), haematocrit (Hct) (%), Mean corpuscular hemoglobin (MCH) (pg), Mean corpuscular hemoglobin concentration (MCHC) (%) and platelets (lakhs/cumm). WBC and RBC are counted using hemocytometer method (Stevens, 1997) and haemoglobin (Hb) was analyzed by cyanomethemoglobin method (Richard Lee *et al.*, 1998).

2.5. Analysis of Biochemical Parameters

2.5.1. Total Protein and Carbohydrate:

Total protein and carbohydrate content was determined spectrometrically at 660 and 630nm using Lowey's (1951) and Anthrone method (Sadasivam *et al.*, 1991). For protein estimation 100mg of fish tissue samples (gill, muscle and liver) were homogenized with 5mL of Tris buffer. This homogenate was centrifuged and supernatant was used. 0.1mL of sample makeup to 1mL with water and 5mL of reagent (1% $CuSO_4$, 1% Sodium potassium tartrate and 2g Na_2CO_3 in 0.1N NaOH mixed in ratio of 100:1:1) was added. After ten minutes 0.5mL of Folin reagent was added and incubated it for 30min. in dark. Blue colour was developed and read at 660nm. For carbohydrate estimation, 100mg of fish organ samples was grinded with 5mL of 30% of KOH and centrifuged. 1mL of this supernatant was added to 5mL of 2.5N HCl and heated by using water bath for 3hrs. After cooling 0.1mL of sample is makeup to 1mL with water, 4mL of anthrone reagent was added and read at 630nm. Total protein and carbohydrate content was calculated by comparing with standard graph. Total Lipid content was determined by Folch method (1952). A weighed amount of muscle, gill and liver was mixed well and homogenized with chloroform-methanol mixture (2:1) and the filtrate was kept overnight undisturbed. The lower phase containing the lipid were pooled and evaporated under vacuum at room temperature. Then lipid extract was re-dissolved in 3ml of chloroform-methanol (2:1) and aliquots were taken for the estimation.

2.6. Data analysis:

The values are expressed as mean ± standard deviation (SD). The results were evaluated by using the SPSS (version 12.0) and Origin 8 software. One-Way Analysis of Variance (ANOVA) were analyzed using software package SPSS 20 version and significant value were set as $p<0.05$.

3. Results and Discussion:

The median lethal concentrations of *Labeo rohita* exposed to different concentration of ZnO are presented in table 1. No mortality was observed in control and 100% mortality was recorded at high concentration. The 96hrs LC_{50} of ZnO was determined as 3.96mg/L. This result indicates mortality rate depends on increasing concentration and time of exposure. Similarly, Zhu *et al.*, (2008), Lin-peng Yu *et al.*, (2011) and Charjan and Kulkarani, (2013) reported that the LC_{50} ZnO was 1.55mg/L and 2.25mg/L in zebra fish and 4.72mg/L in *Channa orientails* exposed to zinc sulphate. Behavioural changes include swimming with jerk movement, loss of equilibrium, hovering, comates behaviour, changes in body colour and hemorrhagic patches were observed during sublethal exposure. Galhardo *et al.*, (2008) and Suganthi *et al.*, (2015) reported similar behavioural changes in *Oreochromis mossambicus* exposed to ZnO NPs. The behavioral observations are important to detect the toxicity of metals in fishes because it is more sensitive than the others. Metabolism and maintenance behavior are the primary goal to remove particles from the

body surface of the fish while entering to the aquatic medium (Chen *et al.*, 2011, Wyman and Walters-Wyman, 1985).

3.1 Hematological parameters:

Hematological parameters are important and economical method for assessing the metal toxicity on fishes. Younus Ahmad *et al.*, (2015) reported that hematological parameters act as reflector of whole body status of the animal exposed to toxicants. The alterations in hematological parameter were listed in table (2) and values of MCH, MCV and MCHC was presented in Fig.1. In the present study, RBC and Hb count were significantly decreased ($p<0.05$) from 7^{th} day to 14^{th} day than control. Tripathi *et al.*, (2003) and Paria Akbary *et al.*, (2018) also reported similar decrease of Hb, RBC count in grey mullet exposed to heavy metal. At the same time, Hct count at T_1 and T_2 were increased in 7^{th} day with the decreasing at T_3 concentration and gradually decreased when compared to respective control. At the end of 7^{th} day exposure WBC and Platelets were increased than that of control and significantly decreased with time exposure to 14^{th} day compared to control. At the end of 7^{th} and 14^{th} day, MCV, MCH, MCHC were significantly increased and proves fish are in hemolytic anemic condition due to the heavy metals accumulation and Celik *et al.*, (2013) reported that the hemolytic anemia caused by the rupture of erythrocytes which leads to the death of fish.

Table 4: Probit Analysis (LC_{50}) of Bulk Zinc Oxide Exposed to *Labeo rohita*

PROBIT	95% Confidence Limits for Concentration			95% Confidence Limits for Log Concentration		
	Estimate	Lower Bound	Upper Bound	Estimate	Lower Bound	Upper Bound
LC_{01}	0.421	0.001	1.317	-0.375	-.2.974	0.120
LC_{05}	0.809	0.009	1.972	-0.092	-2.065	0.295
LC_{10}	1.146	0.026	2.457	0.059	-1.584	0.390
LC_{15}	1.449	0.55	2.861	0.161	-1.260	0.457
LC_{20}	1.746	0.099	3.240	0.242	-1.005	0.511
LC_{25}	2.049	0.163	3.618	0.312	-0.787	0.558
LC_{30}	2.366	0.255	4.010	0.374	-0.593	0.603
LC_{35}	2.703	0.384	4.432	0.432	-0.415	0.647
LC_{40}	3.067	0.563	4.902	0.487	-0.249	0.690
LC_{45}	3.466	0.808	5.449	0.540	-0.092	0.736
LC_{50}	**3.964**	1.141	6.115	0.592	0.057	0.786

Table 2: Hematological parameters of *Labeo rohita* exposed to ZnO

Hematological Parameter	Treatment	Experimental Periods (in days)	
		7	14
RBC (Millon/Cumm)	T_0 Control	1.72 ± 0.06	1.74 ± 0.02
	T_1	1.29 ± 0.15	1.26 ± 0.20
	T_2	0.79 ± 0.09	0.70 ± 0.04
	T_3	0.55 ± 0.05	0.40 ± 0.02
Hb (gm/dl)	T_0 Control	10.2 ± 0.32	10 ± 0.29

	T_1	10.2 ± 0.36	09 ± 0.53
	T_2	7.2 ± 0.57	6.9 ± 0.60
	T_3	6.9 ± 0.12	5.9 ± 0.65
HCT (%)	T_0 Control	27.1 ± 1.62	27.12 ± 1.57
	T_1	31.1 ± 1.05	25.4 ± 0.44
	T_2	34.0 ± 0.57	22.9 ± 0.96
	T_3	25.4 ± 1.10	20.3 ± 1.69
WBC (Cells/Cumm)	T_0 Control	1660 ±12.4	1666.6 ± 12.4
	T_1	1833 ± 25.0	1269.6 ± 17.3
	T_2	4100 ± 12.5	1026 ±18.6
	T_3	3746 ± 11.0	883 ±0.9
Platelets (Lakhs/Cumm)	T_0 Control	73000 ± 0.0	73006 ± 0.0
	T_1	668666 ± 13.3	87000 ± 25.1
	T_2	76333.3 ± 59.3	118000 ± 26.4
	T_3	100666 ± 71.6	139000 ± 40.0

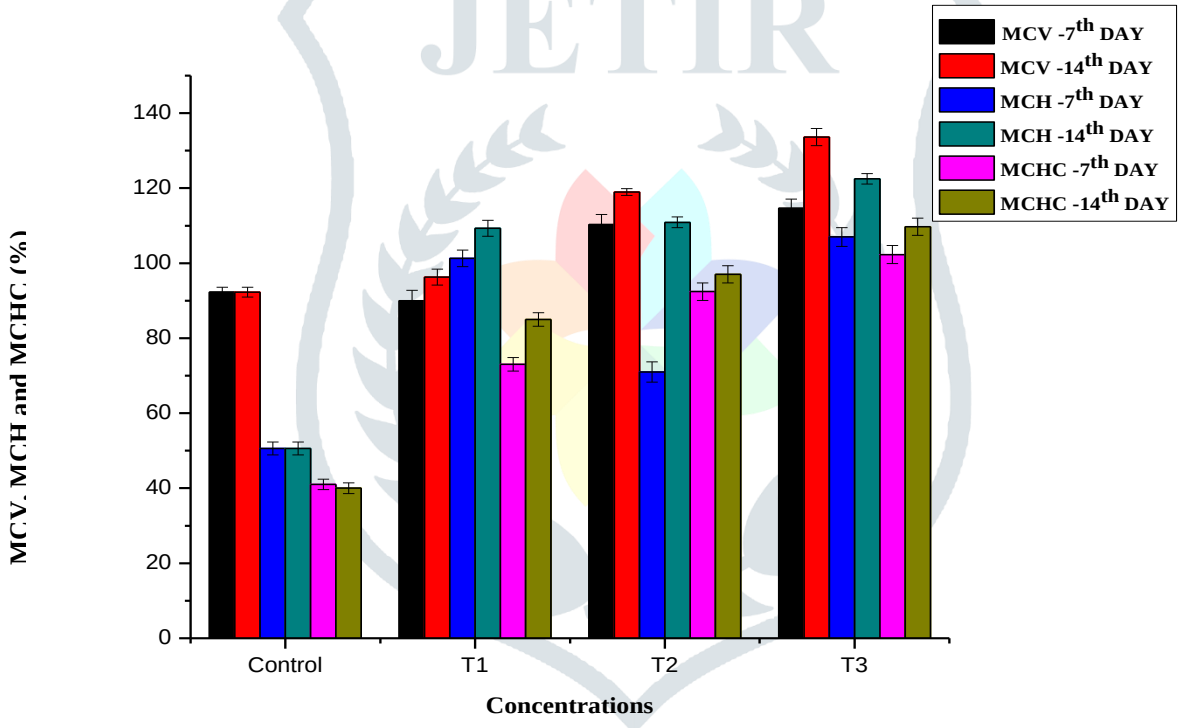
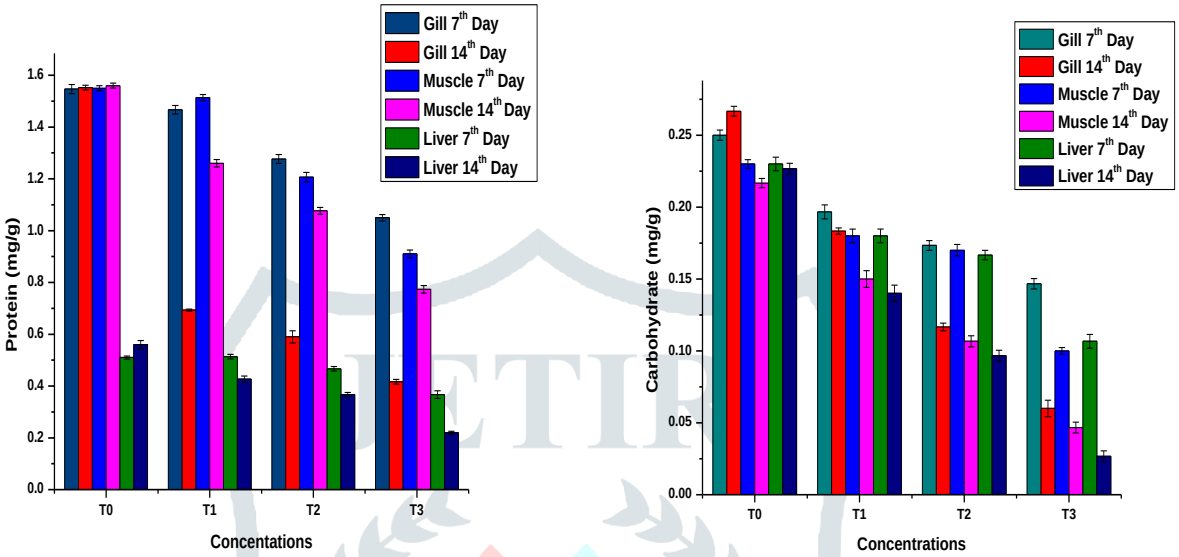


Figure 1: MCV, MCH and MCHC content of *Labeo rohita* exposed to ZnO

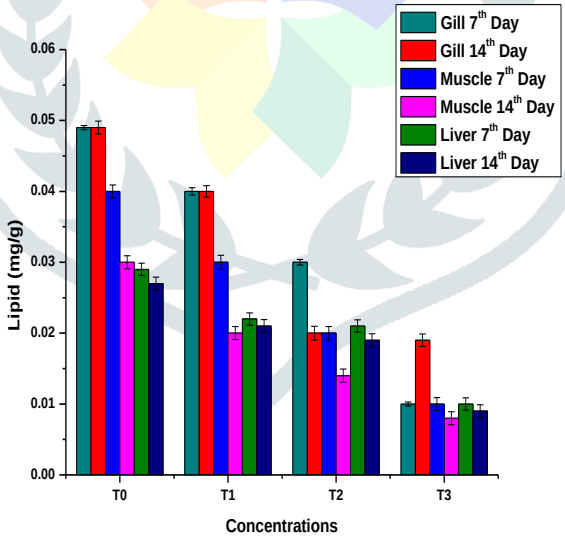
3.2. Biochemical analysis:

The total protein content in *Labeo rohita* exposed to ZnO was presented in Fig. 2. The protein content in gill, muscle and liver was significantly decreased ($p<0.05$) gradually when the concentration increased with increasing exposure time from 7th to 14th day compared to that of respective control (Fig. 2a). But Latifeh *et al.*, (2018) reported that the exposure of common carp (*Cyprinus carpio L.*) to ZnO nanoparticles did not show significant disturbance in biochemical parameters. The carbohydrate content in gill increased in control from 7th to 14th day whereas in treatment significantly decreased with increasing concentration in all the tissue samples (Fig. 2b). Mehjbeen Javed and Nazura Usmani, (2014) reported that fish exposed to heavy metals, the carbohydrate level in liver and muscle glycogen depleted significantly when compared to the control. During stress condition protein and carbohydrate acts as immediate source of

energy to overcome the problem which resulted to reduction of levels in the fish tissues (Mathan Ramesh *et al.*, 2014). Likewise total lipid content were also significantly decreased ($p<0.05$) compared to that of control (Fig. 2c). Similarly, Vinodhini and Narayanan, (2008) reported that the lipid content was decreased compared to control of *Cprinus carpio* exposed to heavy metals whereas Amr *et al.*, (2015) and Rajan *et al.*, (2016) also reported that the protein, lipid and carbohydrate level were decreased with increasing concentration of zinc oxide.



(a) Protein content in gill, muscle and liver **(b) Carbohydrate content in gill, muscle and liver**



(c) Lipid content in gill, muscle and liver

Figure 2: Biochemical changes of gill, muscle and liver of *Labeo rohita* exposed to ZnO

4. Conclusion:

In the present study, it is concluded that the *Labeo rohita* exposed to various concentrations zinc oxide causes deleterious changes in biochemical and hematological parameters. The changes of these parameters were act as biomarker to determine the toxicity of ZnO in aquatic biota. This study also suggests that awareness about zinc oxide to be needed before applications in various industrial sectors and then the

toxic potential of these particles must be assessed before discharge into the aquatic environment to protect aquatic biota as well as human lives.

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