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Study on hematological parameters of Snow Trout exposed to Zinc pyrithione (ZnPT) at sub-lethal concentration

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ABSTRACT
ZnPT is widely used as active ingredients in anti-dandruff shampoos or as additive in cosmetics and dermatitis treatment. It is discharged into the aquatic environment with civilian waste water. This study investigates the effect of zinc pyrithione on the hematology of fresh water snow trout known as *Schizothorax niger* at sub-lethal level. Investigation revealed the hematological alterations in fresh water fish. The Zinc pyrithione caused a mean decrease of Hematocrit, Haemoglobin, Mean Corpuscular Haemoglobin. Increase in Erythrocytes increased was recorded. Leucocytes showed elevated pattern unlike Erythrocytes. The MCV and MCH also varied significantly. On the basis of these findings, it may be concluded that the zinc pyrithione has strong influence on the hematological parameters of *Schizothorax niger.*

Keywords: *Schizothorax niger*, snow trout, ZnPT, Hematology

1. INTRODUCTION

Zinc pyrithione or pyrithione zinc is a coordination complex of zinc. It has fungi-static (inhibits the division of fungal cells) and bacterio-static (inhibits bacterial cell division) properties and is used in the treatment of scalp dermatitis. ZnPT enters aquatic ecosystem through human usages may have adverse effects on aquatic communities (Goka. 2018), which includes wide range of marine organisms, including algae, bivalves, sea urchins, polychaetes, crustaceans, and fish, typically at µg/L levels. ZnPT can be trans-chelated into other compounds in the presence of metal ions, and photodegrades when exposed to UV light. ZnPT is also reported to be biodegraded or hydrolyzed forming several metabolites of their own toxicity and stability. However, ZnPT accumulates in the water column or sediment, if it does not degrade at certain environmental conditions. ZPT contamination of aquatic ecosystems has long been recognized as a serious pollution problem. When fish are exposed to elevated levels of ZPT in a polluted aquatic ecosystem, they tend to take up from their direct environment (Mochida et al., 2006). Anti-fungal and anti-bacterial contamination may have devastating effects on the ecological balance of the recipient environment and a diversity of aquatic organisms (Nunes et al., 2015). Dallas (2018) noticed increased PCV and Hb values with a slightly decrease in number of lymphocytes on exposing flounders, *Platichthys flexus* to two different concentrations of ZPT. Fish living in water with heavy pollution of Anti-fungal and anti-bacterial display increased (Hb) and (PCV) values and decreased white blood cells count (Marcheselli et al., 2011). (Onduka et al.,2019) recorded an increase in (Hb) content of *Tilapia zilli* exposed to Anti-fungal and anti-bacterial for 14 days.

The study of different biochemical and cellular constituents in blood is of fundamental importance in the physiological and physio-pathological evaluation of animals, because morphological and quantitative variations in blood parameters can be induced by pollutants and other environmental factors (Thomas, 1999). According to (Madsen et al., 2000), the study of the hematological picture is frequently utilized for the detection of physio-pathological changes in different stress fungal and anti-bacterial have been observed by (Marcheselli et al., 2011), (Dallas et al., 2018). *Schizothorax niger* is commonly known as "Snow Trout" because it inhabits the snow fed streams and rivers where temperature ranges from 10-18°c. It is a delicate fish which loves cold, well oxygenated and pollution free waters. In order to investigate the toxicity of ZnPT, present study was undertaken to characterize the effect of ZnPT at sub-lethal concentration to evaluate hematological effects resulting from the exposure to the snow-trout.

2. MATERIALS AND METHODS

Toxicant preparation:

Stock solution of ZnPT (10g/L) was prepared by dissolving ZnPT (approx. 95%, RANKEM) in pure dimethyl sulfoxide (approx. 98%, HIMEDIA) and stored in amber glass bottle in the dark at room temperature. Working solution at designed nominal mixture concentration was obtained by diluting with sample tank's water in volumetric flasks before dosing.

Test organism:

Samples of *Schizothorax niger* were made available by local support. As *Schizothorax niger* prefers water temperature range of 10-18^{O} C therefore investigation was conducted during monsoon. Fishes were kept at room temperature with 12h:12h light:dark photoperiod and fed with wheat flour balls for 2 weeks before exposure.

Zinc pyrithione (ZnPT) was exposed for 8 weeks at sub-lethal concentration 175.53 µg/L (Bao, *et al.*, 2014). Test groups were set up in duplicates for each concentration, plus the control group. Blood samples were collected from four individuals bi-weekly for each concentration at 0, 2, 4, 6 and 8th weeks of exposure. Experimental fishes of *Schizothorax niger* (mean TL, 9.1 ± 0.39 cm; mean weight 141.71 ± 0.29 g) were kept in plastic holding tanks with ample water at the Laboratory. Holding tanks were cleaned at alternate day and water was changed every 48-hour.

After exposure, two fishes were caught individually with a dip net and stunned with a blow on the head. The caudal artery at the peduncle was punctured. Blood samples were collected by using a micro-capillary and sampling tubes treated with anticoagulant (disodium salt of EDTA). Blood samples were taken within 40 seconds after collecting the fish from the water tanks. Erythrocyte count (EC) was performed with haemocytometer grid on a microscope after dilution of the blood with Hendricks' solution. Similarly, leukocyte count (LC) was performed with haemocytometer grid on a microscope after dilution of the blood with Shaw's solutions A and B.

Hematocrit (Ht) was analyzed using capillary tubes filled with blood and centrifuged at 11000r/min for 3 minutes. Hematocrit was measured with a microcapillary reader. Hemoglobin (HB) was analyzed using the Sahli- Hellige determination method (Hesser, 1960; Blaxhall,1972). Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated using the following formulae:

$$MCV(\mu m^3) = \frac{Ht(\%) \times 10}{EC(cellsmm^{-3})};$$

$$MCH(Pg/cell) = \frac{Hb(g/100ml) \times 10}{EC(Cells/mm^3)}$$

$$MCHC(g/100ml) = \frac{Hb(g/100ml) \times 100}{Ht(\%)}$$

Statistical analysis was carried out using the SPSS. Statistical procedures used include summary statistics and analysis of variance.

3. RESULTS

Control and treated fishes fed normally throughout the sub-lethal exposure period. When food was introduced, these fish fed vigorously at the water surface and consumed almost all food supplied. No mortality was recorded in any group. Exposure of *Schizothorax niger* to ZPT for eight weeks caused a mean decrease of Hematocrit (26.35 ± 1.34 to 18.31±2.76), in

Haemoglobin (9.89±0.43 g/100ml to 8.26±1.34 g/100ml), in Mean Corpuscular Haemoglobin (205.24±17.11 µg/cell to 179.24±56.03 µg/cell) (Table 1). Erythrocytes increased from 1.35±62.33 to 1.19±62.33 x10^{6} cells/mm^{3} (Table 1). Leucocytes showed deviation from 52.30±19.31 x10^3 cells/mm^3 to 24.25±2.47 x10^3 cells/mm^3 (Table 1). The mean corpuscular volume and mean corpuscular hemoglobin concentration also varied (Table 1). The fish in the exposed tank had an erythrocyte peak (1.35±62.33 x 10^6 cells/mm^3) in week 4. The leucocytes peak (52.30±19.31 X 10^6 cells/mm^3) was also recorded in week 4, in fish from the highest concentration tank. The hemoglobin concentrations of the control tanks recorded between 10.98±0.47 to 11.74±0.67 (g/100ml) and remained higher than those of the exposure concentration tanks throughout the investigating period. There was no definite trend in mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration apart from the general decrease in MCV and MCH at the end of the 6th week. Therefore, it may be stated that, hematological speculations in exposed samples were time and dose-dependent.

Discussion

ZnPT may induce a mean decrease in the Hematocrit, Hemoglobin and Mean Corpuscular Volume level of *Schizothorax niger* during this 8-week exposure period. Most of the investigated hematological parameters showed deviation from the control group. Hematocrit value of *S. niger* shown slightly lower values than control group. The change could be attributed to the fact that change in environment has direct effect on the hematology of the fish Montero *et al.* (1999). Thus, continuous discharge of the ZnPT into water bodies over time might lead the negative hematological speculations in aquatic life in nearby water bodies. The concentration of hemoglobin decreased in the blood of fish exposed to ZnPT, According to Pamila *et al*., (1991) the reduction in hemoglobin content in a fish exposed to pollutant could be due to the inhibitory effect of those substances on the enzyme system responsible for synthesis of hemoglobin. Also evidence has showed that ZPT influences the differential blood count (Thomas *et al*., 1999). The results are in good concurrence with earlier works (Mochida *et al*., 2006) that reported a significant decrease in RBC's hemoglobin and packed cell volume of fresh water fish exposed to ZnPT. The perturbation in these blood indices may be attributed to a defense reaction against toxicity through the stimulation of erythropoiesis. The related decreases in hematological indices implicate the toxic effect of the ZnPT that affects both metabolic and hematopoietic activities of *Schizothorax niger*.

The leucocytes count shows fluctuation with the change in external environment particularly pollution and infection. Ruane *et al.* (2002) who subjected common carp to net confinement observed decrease in white blood cells. *Cyprinus carpio* during physiological response (Ruane *et al.* (2002) reported increasing stocking density causes reduction in white blood cells. Sakthivel and Sampath (1989). Studied white blood cells and differential leucocyte count reduced significantly in C*yprinus carpio* during ZnPT exposure.

Pedro *et al.* (2005) observed that erythrocytes and leucocytes count showed significant fluctuations depending on season and environmental toxicant exposure.

The present haematological data provides valuable information in assessing the health of *Schizothorax niger* and in monitoring stress responses to the Zinc pyrithione.

Table 1. Hematological alterations (mean±SD) recorded in snow Trout

Parameter	1 st Week	2nd Week	4th Week	6th Week	8th week
Heamatocrit (%) (PCV)	26.35 ± 1.34	25.53±3.67	22.17±2.08	21.93±2.76	18.31±2.76
Heamoglobin (g/100ml)	9.89±0.43	9.82±0.93	9.46±1.34	8.26±1.34	9.13±1.29
Leucocytes (x10^3 cell/mm^3)	24.25±2.47	31.47±10.23	52.30±19.31	46.61±11.21	40.11±11.21
Erythrocytes (x10^6 cell/mm^3)	1.19±11.51	1.21±65.27	1.35±62.33	1.32±41.29	1.26±41.29
MCV (µm^3)	205.93±17.11	181.53±39.54	179.24±56.32	192.71±21.62	183.31±61.61
MCH (µ **g)**	63.22±7.65	51.23±14.67	47.61±19.54	33.88±17.43	24.81±17.43
MCHC (g/100 ml)	36.82±0.76	34.22±2.14	34.07±3.89	32.63±6.34	31.54±6.34

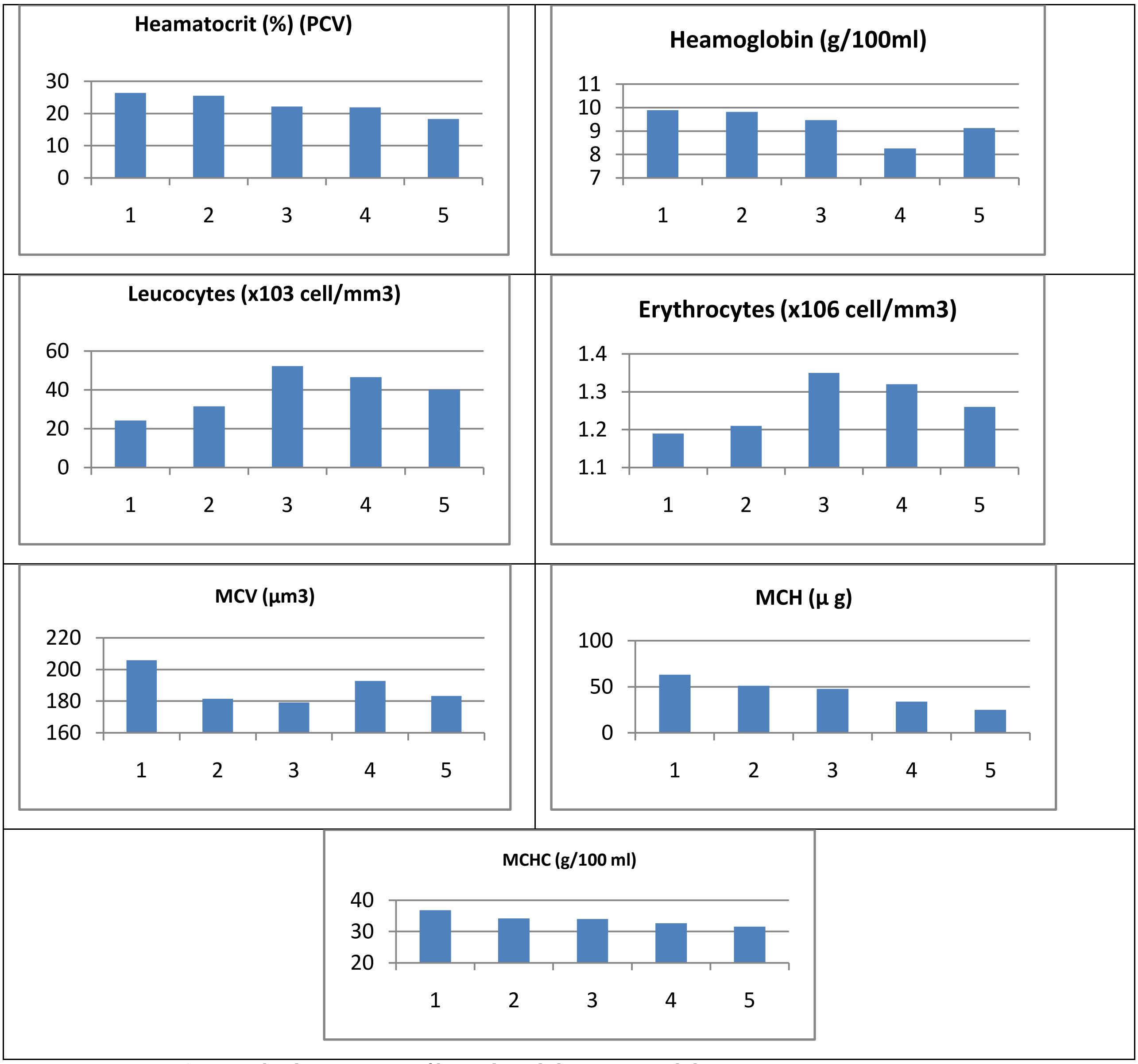

Fig 1: Graphical representation of hematological alterations recorded in snow Trout

4. CONCLUSIONS

Sub-lethal concentration of Zinc pyrithione (ZnPT), released in aquatic environment by human use (antidandruff shampoos) is an additional source of stress for aquatic organisms. Sub-lethal concentrations of ZnPT in the fresh water will not necessarily result in mortality but in-fact may result in several physiological dysfunctions in fish which could induce changes in blood parameters. This investigation suggests that use of any antidandruff shampoos containing ZnPT should be avoided near natural water bodies.

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