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Effects of Cadmium on Some Haematological Parameters of the Mozambique Tilapia, *Oreochromis mossambicus* (Peters 1852)

R.A.A.R. Ranatunge, M.R. Wijesinghe*, W.D. Ratnasooriya and R. Wijesekera

Department of Zoology, University of Colombo, Sri Lanka

✉ mayuri@zoology.cmb.ac.lk

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Abstract: Haematological profiles have been frequently used to assess the health status of fish subject to metallic stress. The present study examined potential alterations that may arise in the haematological profile of the Mozambique Tilapia (*Oreochromis mossambicus*) due to exposure to cadmium (Cd). Adult female fish collected from an aquarium were housed in glass tanks and exposed to three concentrations (0.2, 2 and 10 mg l^{-1}) of $CdCl_2$ for five days. At the end of the trial, fish were sacrificed to obtain blood to record five primary (erythrocyte count - RBC, lymphocyte count - WBC, haemoglobin concentration - Hb, packed cell volume - PCV and clotting time) and three secondary (mean cell haemoglobin - MCH, mean corpuscular haemoglobin concentration - MCHC and mean cell volume - MCV) haematological parameters. Results revealed that the observed patterns were erratic and significant changes were observed only at the low (0.2 mg l^{-1}) and high (10 mg l^{-1}) levels of exposure. Significant increases were noted in RBC and WBC counts, Hb, PCV, MCH, MCHC and MCV, while the clotting time was significantly reduced (2.2 minutes in the control and 0.8 at the highest concentration). These results are of particular concern since the lowest test concentration (0.2 mg l^{-1}), which was capable of inducing changes in some of the blood parameters, falls within the ranges recorded in some of the water bodies of Sri Lanka.

Key words: Blood parameters, cadmium, heavy metals, haematology, Tilapia.

Introduction

Heavy metal contamination of freshwater bodies in Sri Lanka, as elsewhere in the world, has recently become a potential hazard, threatening the survival of organisms inhabiting these ecosystems. Among the heavy metal contaminants in Sri Lanka, cadmium (Cd) is of particular importance because it is commonly found in relatively high proportions (0.02-0.2 mg l^{-1}) in many of the country's freshwater ecosystems (Bandara et al., 2008; Manage and Wijesinghe, 2009). Several anthropogenic activities such as the production of nickel-cadmium batteries, electroplating, phosphate mining and the use of phosphorus-fertilizers can contribute to Cd contamination of aquatic systems. Previous studies have revealed that Cd is directly responsible for causing mortality in fish (Irwin et al., 1997), and retarding growth, disrupting biochemical processes and causing alterations in haematological parameters (Shalaby, 2007) and histology (Randi et al., 1996). Although a large number of studies have attempted to assess toxicity of these harmful substances by monitoring survival, growth and development in the exposed organisms (Sarnowski and Witeska, 2008; Woodworth and Pascoe, 1981), only a few studies have focussed on their effects on blood parameters. Effects on haematological profiles of fish subject to metallic stress are particularly important as they indicate the health status of the organisms (Vinodhini

*Corresponding Author

and Narayanan, 2009). In the present study, authors investigate the effects of Cd on haematological parameters of the Mozambique Tilapia (*Oreochromis mossambicus*), an edible fish species cultivated on a commercial scale in many water bodies across Sri Lanka.

Methodology

Selection of Exposure Levels

The selection of the test concentrations was based on several factors. The field levels of Cd recorded in the water bodies of Sri Lanka was considered to be of primary importance since this would indicate the general levels that aquatic organisms are exposed to in the long run. However, when effluents are added periodically into water bodies, it results in a sudden influx of heavy metals exposing the aquatic organisms to relatively high concentrations, over a shorter duration since dilution would take place thereafter. The experiments conducted in the present study attempted to simulate both these field situations. Since the present study involved empirical trials, we also considered the levels and durations used in previous exposure trials (Al-Attar, 2005; Oner et al., 2008; Alwan et al., 2009). Accordingly, three concentrations, namely, 0.2 mg l^{-1} (low), 2 mg l^{-1} (mid) and 10 mg l^{-1} (high) were selected for the trials.

Cadmium chloride ($CdCl_2$) was used in the present study following previous exposure experiments by Garcia et al. (1999), Sarnowski and Witeska (2008), and Vinodhini and Narayanan (2008). Analytical grade, hydrated cadmium chloride with the formula of $CdCl_2.2.5H_2O$ = 228.34 (99% purity) (BDH laboratory reagents, Poole, England) was used to prepare a stock solution of 1000 mg l^{-1} using distilled water, from which a further stock solution of 100 mg l^{-1} was prepared. Appropriate volumes of the stock solution were used to prepare the test concentrations.

Exposure Trials

Adult female fish (112 ± 5.3 g and 3-5 months of age) were collected from the National Aquatic Research Agency (NARA), Colombo. The fish were housed in large tanks for acclimatization for over a week. The experimental tanks were glass tanks of 60 × 30 × 60 cm containing 30 L of water which had been allowed to age for 48 hours for chlorine levels to dissipate. A single fish was then randomly assigned to each tank and as sacrificing was required only four to five fish were used for each treatment and control. After acclimatization, fish were exposed to the three selected concentrations (0.2, 2 and 10 mg l^{-1}) of $CdCl_2$ for five days, with renewal of the medium after three days. Tanks were kept at room temperature and exposed to the 12 hr light: 12 hr dark photoperiod. The light intensity, pH, temperature and dissolved oxygen levels of the tanks were measured every other day. Fish were fed on commercial fish food pellets, the quantity determined to be 2% of the initial body weight of the fish. The method used for the empirical trials was in accordance with the OECD (2008).

At the end of the trial, the fish were euthanized using Tricaine methanesulphonate (MS-222) and blood was obtained by tail ablation (Al-Attar, 2005). The caudal peduncle was severed with a scalpel blade (Canada Department of Fisheries and Oceans, 2004). Blood was collected into an eppendorf tube containing EDTA. The initial five drops of blood was not collected. Multiple samples of blood were analyzed from an individual fish for determination of the five primary blood parameters and three secondary blood parameters. For red blood cell (RBC) counts, blood was diluted with Hayem's fluid containing sodium chloride (1.0-2.0 g), sodium sulphate (5 g), mercuric chloride (0.5 g) and distilled water (200 ml), while for white blood cell (WBC) counts, blood was diluted with Turk's fluid containing glacial acetic acid (1.0 ml) and distilled water (100 ml) (Vinobaba, 2009).

Counting was done using the Neubauer hemocytometer (BS 748, Weber, Middlesex, England). Pack Cell Volume (PCV) was determined by the microhematocrit technique (Hawksley micro-hematocrit reader, England) and the haemoglobin level (Hb) by the Sahli Hemoglobin meter (Tokyo, Japan). The standard secondary indices namely, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated using the primary parameters. Clotting time was determined using two drops of blood which was collected into a separate glass vial without an anticoagulant. For this the vial was turned upside down at 30 second intervals till blood flow ceased.

Results

The values obtained for the haematological parameters of the Cd-treated and control fish are shown in Table 1. The one-way ANOVA and post hoc Tukey's tests were performed separately for each haematological parameter to examine for significant deviations from the values recorded in the control fish. With respect to RBC counts, significant deviations from the control fish were recorded

Table 1: Mean values of haematological parameters (± S.E.) of *Oreochromis mossambicus* exposed to three concentrations of Cd for five days

Parameters	*Control 0 mg l^{-1} (n = 5)*	*Low 0.20 mg l^{-1} (n = 4)*	*Mid 2.0 mg l^{-1} (n = 4)*	*High 10.0 mg l^{-1} (n = 5)*
PCV (%)	20.40 ± 0.98	23.00 ± 1.0	23.50 ± 0.86	25.25* ± 1.18
RBC count ($\times 10^6/\mu$ l^{-1} blood)	1.51 ± 0.04	2.08* ± 0.13	1.55 ± 0.11	1.16 ± 0.17
WBC count ($\times 10^6$/g d l^{-1} blood)	1.54 ± 0.01	1.19 ± 0.10	1.27 ± 0.21	2.68* ± 0.34
Hb (g Hb/100 ml blood)	5.58 ± 0.11	7.38* ± 0.38	6.25 ± 0.32	7.00* ± 0.22
Clotting time (min)	2.2 ± 0.2	1.75 ± 0.25	2.25 ± 0.43	0.875* ± 0.12
MCH (µg)	36.98 ± 1.3	35.53 ± 2.12	41.0 ± 3.51	64.02* ± 8.87
MCHC (g dl^{-1})	27.62 ± 1.51	32.14* ± 1.52	26.61 ± 1.07	27.83 ± 0.99
MCV (µg)	135.56 ± 9.13	111.12 ± 7.63	153.57 ± 8.45	233.50* ± 37.6

* indicates where parameters were significantly different from the control ($p < 0.05$).

only in fish exposed to the lowest level of Cd (0.2 mg l^{-1}); the RBC values were significantly higher (2.08×10^6 mm^{-3}) than that of control fish at the low exposure level. The impact of Cd on WBC counts was different, with significant increases (2.68×10^6 mm^{-3}) being recorded only at the high exposure level of 10 mg l^{-1}, whilst WBC counts at the low exposure level were similar to the control. Significant deviations were recorded for PCV values with the highest PCV being at the high exposure level of 10 mg l^{-1}. As expected, changes in Hb content were similar to that observed with RBC counts, with the highest Hb content (7.38×10^6 mm^{-3}) being noted at the low exposure level of 0.2 mg l^{-1}, although a significant increase was also noted at the high exposure level. Considering the secondary indices, both MCH and MCV had the highest values (MCV, 233.50 µg, MCH, 64.02 µg) at the high exposure level (10 mg l^{-1}). In sharp contrast the MCHC significantly increased at the low exposure level. With regard to blood clotting time, a significant reduction occurred at the high exposure level whereas there was no significant change from the control in the other two treatments. These results indicate that, although no consistent patterns can be recognized across the tested exposure concentrations, Cd has the potential to induce haematological changes.

Discussion

The present study demonstrates the fact that Cd has the potential to induce changes in some haematological parameters of the adult Tilapia, although the noted changes were erratic with no significant trends being evident from low to high exposure levels. The recorded values obtained for the haematological parameters of the control fish in the present investigation are within limits of ranges recorded in other Tilapia species elsewhere (McNulty, 2002; Ishikawa et al., 2007; Akinrotimi et al., 2012). As for the haematological parameters of the treated fish in the present study, Cd exposure significantly increased erythrocyte (RBC) counts, lymphocyte (WBC) counts and the haemoglobin (Hb) content, although such changes were not consistent across the tested concentrations. Changes in RBC counts and Hb concentration were noted even at the low exposure level of 0.20 mg l^{-1}, whilst changes in WBC were only at the high level. Increases in RBC, WBCs and Hb concentration with exposure to Cd have been recorded by others. For instance, in the Dog Fish (*Scyliochinus canicula*), the RBC counts increased with exposure to Cd within 96 hours (Tort and Hernández-Pascual, 2006), while similar trends were recorded for RBC counts, Hb concentrations and PCV in *Tilapia zillii*, with aluminium (Alwan et al., 2009). Erythrocytes generally increase in animals to compensate for poor oxygen uptake during stressful conditions. The increase in RBCs occurs via two mechanisms, i.e. Erythropoiesis (RBC production) and the release of a large number of mature RBCs from the spleen for circulation (Alwan et al., 2009). The second mechanism is most likely responsible for the observed results since the former would require a longer duration.

Some have also reported contradictory results to those observed here. Heath (1995) stated that Cd causes anemia or lowering of RBC counts, which results from the reduction of iron absorption in the gut of the fish. Shalaby (2007) noted that *O. niloticus* exposed to high levels of Cd caused a reduction in the RBC counts and Hb content, and has suggested that the destruction of mature RBCs and/or the inhibition of RBC production are responsible for these observed trends.

In the present study, Cd also triggered increases in the Pack Cell Volume (PCV), mean cell volume (MCV), mean cell haemoglobin concentration (MCHC) and mean cell haemoglobin (MCH), which could be expected to result from changes in RBC counts and Hb content induced by the contaminant. Alwan et al. (2009) specifically attribute such changes to the swelling of erythrocytes in stressed animals. The sharp reduction in clotting time at the high concentration observed in the present study is probably due to thrombocytopenia i.e. a reduction in platelets, which has been noted in fish exposed to heavy metals (Srivastava et al., 2004).

In the present study, at the high exposure level of 10 mg l^{-1}, there was a significant increase in WBCs, while no effects were evident at the lower concentrations. This is consistent with the patterns observed by others; in a study where Cd^{2+} was injected to *O. mossambicus*, those treated with relatively low doses (0.6 mg/kg) did not show alterations in WBC counts but significant increases were noted when fish were injected with higher doses (2 mg/kg) (Chang and Wu, 2003). Cd in fish species has been variously shown to cause inhibition or stimulation of the immune system (Heath, 1995; Chang and Wu, 2003). If immune suppression occurs in exposed animals, this would increase their susceptibility to infections which would in turn result in an increase in the WBC counts as was evident in the present study.

Overall, these results indicate that Cd exposure could result in erratic changes in some haematological parameters of the adults of the Mozambique Tilapia, *Oreochromis mossambicus*. What is more significant, however, is the fact that some of these alterations were evident at the Cd level of 0.2 mg l^{-1} which has been recorded in some of the freshwater bodies in Sri Lanka.

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