Haematological Response of *Rastrineobola argentea* Exposed To Subchronic Doses of Inorganic Cadmium (Cd), Methylmercury and Polychlorinated Biphenyl (PCB) via Dietary Exposure Pathway

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Abstract: ***Haematological indices provide information on various aspects of fish health when exposed to environmental contaminants. We evaluated the effects of cadmium, methyl mercury (MeHg), and Polychlorinated Biphenyl (PCB) on the haematological parameters of a tropical fish. One hundred and eighty (180) mature individuals of the freshwater fish Rastrienobola argentea were exposed to dietary subchronic doses of Cd (4 µg g g^{-1} $CaCl_2$), MeHg (0.1 CH_3HgCl µg g^{-1}) and PCBs (0.2 µg g^{-1}). After 45 days, blood was sampled from exposed and control groups to evaluate haematological effects of contaminants on erythrocytes, total leukocytes and differential leukocytes counts, haematocrit, heemoglobin concentration, red blood cell indices, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC). Results showed that red blood cells counts, haemoglobin concentration, haematocrit, leukocytes, neutrophils, and mononuclear cells counts, significantly ($P < 0.05$) decrease between control groups compared with the Cd, MeHg and PCB tested groups. The MCV, MCH and MHC showed significant ($P > 0.05$) increase between control groups with the Cd, MeHg and PCB tested groups. The present study shows that changes in haematological parameters were detectable at sub-chronic exposure to contaminants, but their application in field biomonitoring using R. argentea will need more detailed studies.***

Keywords: Biomarkers; MeHg, PCB, Haematology; Tropical fish; *Rastrineobola argentea*

1. Introduction

The release of industrial, domestic, and urban wastes generated through anthropogenic activities into aquatic ecosystems normally cause stress to the aquatic life. These include metals and their methylated forms and organic contaminants. Currently, the widespread uses of metals, the legacies of past contamination and new technologies, continue to increase the concentration of metal into the aquatic environment [1]. Cadmium has no known roles in the fish and will be detrimental even in low exposure doses, is considered an ubiquitous toxicant and poses significant health risk in many parts of the world. This metal is one of the most commonly used metals in industry and its toxicity is of concern to public health due to its persistence in the environment [2,3]. Industrial uses of Cd and agricultural uses of phosphate fertilizers have caused widespread dispersion of the metal at trace levels into the environment and human foodstuffs [4,5]. Methyl mercury (MeHg) is a highly lipophilic environmental contaminant which easily crosses the blood barrier, and the primary route of exposure is through ingestion of contaminated food [6]. Polychlorinated biphenyls (PCBs) were first manufactured commercially in 1929 and used widely as electric insulators in transformers, hydraulic fluids and paint additives [7]. Serious concerns about the distribution of PCBs were raised since they were found to be ubiquitous and persistent in the environment and biota samples such as soil, water, animal and human tissues [8]. Although the production of PCBs has been banned since the early 1970s [9], PCBs persist as legacy pollutants in which the chronic toxicity still represents a serious environmental risk. These contaminants have different modes of action on the aquatic organism by exuding stress response in aquatic organisms. Thus, the assessment of environmental disturbances requires the elucidation of stress effects throughout the hierarchy of biological organization.

The use of haematological endpoints is reasonable biomarkers of fish health [10,11]. Knowledge of the haematological characteristics is an important tool that can be used as an effective and sensitive index to monitor physiological and pathological changes in fishes [12]. Normal ranges for various blood parameters in fish have been established by different investigators in fish physiology and pathology [13,14]. In addition, haematological studies provide quite frequently and routinely accepted procedures in fish diagnosis to evaluate the interactions between dietary levels of nutrients [15]. Although fish blood parameters have been increasingly determined in environmental monitoring programs as valuable indicators of physiological changes in the presence of toxicants, the most important barrier to using these findings in environmental studies is the lack of basic information about the blood response to stressors for many tropical species [16]. The aim of this study was to determine the effects of subchronic dietary exposures to metals (Cd), its methylated form (MeHg) and organic contaminant (PCB) in a tropical fish *Rastrineobola argentea* by analysis of haematological biomarkers.

2. Materials and Methods

The experiments were approved by the animal welfare committee before starting the experiments. A total of about 180 *R. argentea* (mean weight = 1.50 ± 0.42 g) were collected between April and July 2010 off the coast of Lake Victoria, Kenya (0°12'40''S and 34°49'30''E) and transported to the Kenya Marine and Fisheries Research (KEMFRI) Laboratory Kisumu in Kenya and acclimated to the experimental condition for 28 days (mean weight: 23.50 ± 0.42 g). Fish were reared in race-way type water tanks supplied with filtered lake water (0.45-μm filtered water). The renewal rate was 24 L h^{-1}; salinity: 0.5‰; pH: 7.4 ± 0.4; dissolved oxygen: > 5.0 mg l^{-1}. Fish were fed commercial feed of protein level 32%.

After 28 days, feeds were laced with Cd (4 μg g g^{-1} $CaCl_2$), MeHg (0.1 μg g^{-1} CH_3HgCl, Sigma), and 2, 2'4, 4'5, 5' (PCB 153) (0.2 μg g^{-1}) before the feeding experiments. The feeding was done for 45 days for a period lasting for 30 min in the morning (0800 h) and evening (1700 h). 20 individuals were kept as control groups.

After 45 days of feeding exposure, the individuals from each group (tested and control) were anesthetized with 0.02% MS222 (ethyl-ester.3.aminobenzoic acid, Sigma). Blood samples (1.5 mL) were taken by caudal puncture with heparinized syringes containing 0.1 mL of anticoagulant (after filling up and expelling about 1.0 mL), and no additional heparin was added to the Eppendorf tubes.

Numbers of erythrocytes (red blood cells (RBC) count, 10^6 cells ml^{-1}) and leukocytes (Lk count 10^4 cells ml^{-1}) were determined by the haemocytometer; haematocrit (Ht v/v ratio or %) was determined by the microhaematocrit method; haemoglobin concentrations (Hb g dl^{-1}) were determined measurement by the cianometahaemoglobin method [17]; and the leukocyte differential count was made in peripheral blood smears stained by Giemsa [18], giving the Neutrophils value of differential neutrophlis × (100 leukocytes $count)^{-1}$ and the Mononuclear value of differential lymphocytes plus monoytes × (100 leukocytes $count)^{-1}$. The total number of thrombocytes was estimated in relation to the numbers of red blood cells and the proportion of each cell type observed in the blood smears [19]. The red cell indices, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) were calculated from RBC, Ht, and Hb using protocols of Lee et al. [17].

3. Results

The means of the haematological values with their respective standard errors (± SE) are presented in Table 1. Haematological parameters including: red blood cells counts, haemoglobin concentration, haematocrit, leukocytes, neutrophils, and mononuclear cells counts, showed significant ($P < 0.05$) decrease between the control groups when compared with the Cd, MeHg and PCB tested groups (Table 1). Fish exposed to these contaminants showed reduced haematological parameters with most of the reduced blood parameters being recorded in fish exposed to MeHg. On the contrary, MCV, MCH and MHC showed significant ($P > 0.05$) increase between control groups with the Cd, MeHg and PCB tested groups.

Table 1: Blood parameters for *Rastrineobola argentea* exposure to inorganic cadmium [4 μg g g^{-1} $CaCl_2$], methyl mercury [0.1 MeHg μg g^{-1} CH_3HgCl], and Polychlorinated biphenyls [0.1 PCB μg g^{-1}] for 45 days

	Control	Cd	MeHg	(PCB 153)
RBC (×10^6 μl)	3.47 ± 0.62	2.54 ± 0.43	2.42 ± 0.51	2.83 ± 0.52
Leukocyte counts (×10^4 cells ml^{-1})	4.22 ± 0.75	3.45 ± 0.89	2.98 ± 0.87	3.12 ± 0.67
Haemoglobin (%)	7.23 ± 0.64	6.02 ± 0.94	5.32 ± 0.89	5.41 ± 0.55
Haematocrit (%)	33.22 ± 2.88	27.88 ± 1.11	26.03 ± 1.01	26.33 ± 0.97
Neutrophils counts (100 leukocytes $count)^{-1}$	45.94 ± 2.88	33.23 ± 4.11	24.52 ± 3.77	26.78 ± 2.33
Mononuclear cell counts (100 leukocytes $count)^{-1}$	73.23 ± 6.22	60.12 ± 4.55	59.89 ± 4.66	64.78 ± 3.89
MCV (mm^3 $cell^{-1}$)	123.42 ± 5.99	154.34 ± 8.78	172.22 ± 7.98	165.33 ± 10.54
MCH ((pg $cell^{-1}$)	17.12 ± 2.44	21.33 ± 4.22	25.33 ± 5.92	24.55 ± 3.32
MCHC (g dl^{-1})	13.89 ± 3.11	18.21 ± 3.88	18.89 ± 4.11	18.44 ± 1.11

MCV - Mean Corpuscular Volume; MCH - Mean Corpuscular Haemoglobin; MCHC - Mean Corpuscular Haemoglobin Concentration

4. Discussion

There is consistent lack of information related to haematological analysis of fish chronically exposed to food contaminated by metals, and organic substances. Therefore the aim of the current study was to determine the sub-chronic dietary exposures to metals (Cd), its methylated form (MeHg) and organic contaminant (PCB) in a tropical fish *R. argentea* by analysis of haematological biomarkers. We recorded no mortality of fish during experiment suggesting that the levels used were indeed low to cause toxic effects in fish. The reduction in RBC and leucocyte counts suggests a reduction in the blood O_2^- carrying capacity to the pollutant pointing to the cytoxic effects of the pollutants as reported for *Onchorynchus mykiss* after an acute exposure to aluminum [20] and in *Labeo rohita* exposed to sublethal levels of cypermethrin and carbofuran [12]. Nevertheless, other mechanisms of toxicity may be associated with the O_2^- carrying capacity such as the inhibition of iron absorption and defective iron metabolism shortening the life span in erythrocytes [21]. The reduced leukocyte counts could also be related to the presence of tecidual damages such as necrosis [22]. The values observed for haematocrit and Hb in *R. argentea* from control groups are relatively close to those of other tropical and nontropical species of fish such as *Ictalurus punctatus* (23.9%) [15] and *Colossoma macropomum* (20–23%). After exposure to pollutants, the haematocrit values of fish have been reported to reduce [10, 23]. Therefore the large reduction in haemotocrit values when fish was exposed to MeHg after trophic subchronic exposure, indicate that MeHg may be more toxic to the cells. These results do not agree with those of Chowdhury et al. [24] and Oliveira Ribeiro et al. [25] who noted an increase of blood haematocrit and haemoglobin during environmental hypoxia and chronic or acute exposure to waterborne metals (Cd, Zn, Cu, Al, and Ni) to increase blood oxygen carrying capacity when impairment of gas exchange occurs. It is known that changes in leukocyte counts after exposure to pollutants may be associated to a decrease in nonspecific immunity of the fish. In the current work the effects of MeHg on the leukocytes counts were among the most evident compared with those of other tested metals and control groups.

The increased MCV, MCH and MCHC observed in individuals of *R. argentea* exposed to MeHg may be explained by the presence of a larger amount of older or larger red blood cells as described by Hardig and Hoglund [26]. In addition, the exposure to mainly MeHg could also affect the mechanism of red blood cell turnover, increasing the number of circulating older cells and inducing an anemic state [12]. Some studies have pointed out a chronic effect of MeHg in head kidney of *Hoplias malabaricus*, indicating severe damage to this haematopoietic tissue [25] and supporting the hypothesis of a failure in the red blood cells turnover.

5. Conclusion

Haematological parameters could be useful to evaluate the effects of contaminants. The present results showed that under experimental conditions blood parameters were sensitive to different aspects of contaminant exposure. Also, its ability to adapt to experimental conditions, its voracious behavior and its food chain position make *R. argentea* an interesting model to be used in experiments testing dietary exposure to contaminants but the application of these findings to preparation of environmental diagnoses will need a more investigation and must be validated in situ before establishing them as biomarkers.

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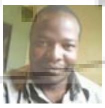

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