Journal of Aquaculture Science **October 2023 Vol 8 (2): 67-73**
DOI: https://doi.org/10.20473/joas.v8i2.48704

The Metrics Profiles of Melanomacrophages Centre on the Spleen of Carp (*Cyprinus carpio*) Exposed to Mercury Chloride

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Submitted: 25 May 2023 Revised: 13 June 2023 Accepted: 29 September 2023 Publish: 28 October 2023

ABSTRACT

Increasing the number of heavy metals in the aquatic environment result in the accumulation of contaminants in fish body tissue, making fish an ideal bio-indicator of environmental pollution. The spleen included in the hematopoietic tissue contains macrophages. Macrophages form aggregates that contain pigments referred to melanomacrophage centres (MMCs). This research aims to analyse the metric profiles of MMCs on the common carp (*Cyprinus carpio*) spleen as a non-specific cellular immune response against mercury chloride exposure. This study used a Completely Randomized Design method with four treatments at different concentrations of mercury chloride ($HgCl_2$) of 0 $mg.l^{-1}$, 0.01 $mg.l^{-1}$, 0.05 $mg.l^{-1}$, and 0.1 $mg.l^{-1}$. Each treatment replicated five times. Data on the number and size of MMCs were analyzed by Analysis of Variance (ANOVA) and continued by using Duncan's Multiple Range Test (DMRT) to determine the differences between treatments. The result showed that the concentration of mercury chloride in water affects the metrics profile of spleen MMCs of carp. The number and size of MMCs of carp spleen increased at the concentration of 0.01 $mg.l^{-1}$ and 0.05 $mg.l^{-1}$ but decreased at the level of 0.1 $mg.l^{-1}$. The results suggest that the profile of MMCs as a non-specific cellular immune response can function as bio-indicators of environmental pollution.

Keywords: *Environmental pollution, Melanomacrophage Centers, Mercury chloride, Spleen, Cyprinus carpio.*

INTRODUCTION

Heavy metals, such as mercury, cadmium, copper, lead, and zinc, are dangerous pollutants that affect the aquatic environment and fish. They are harmful for fish health (Radkhah et al., 2022). Most of this metal accumulates in fish tissues and causes fish poisoning, affects the vital operations and reproduction of fish, weakens the immune system, and encourages pathological changes (Authman et al., 2015). Therefore, fish are commonly used to assess the quality of aquatic environments and as a bio-indicator of environmental pollution (Ogundiran and Fawole, 2018; Radkhah et al., 2016). The spleen is an organ responsible for producing immune responses and eliminating foreign particles (Rezaee *et al*., 2013). The accumulation of mercury chloride in the spleen affects survival, the growth of fish, and the immune response (Aboud, 2010). It also can cause immunotoxic effects (Hedayati *et al*., 2010). Moreover, mercury chloride causes interference with cellular and humoral immune responses in tilapia (El-Boshy and Taha, 2011). Macrophage activity is part of cellular immune response mechanism in fish (Reddy, 2012).

Macrophages will form pigment-containing aggregates called melanomacrophages centres (Bols *et al*., 2001). Melanomacrophages centers (MMCs) are structures found in the spleen, kidneys, and sometimes in the liver (Agius and Roberts, 2003; Facey and Blazer, 2005). They function as

repositories for the remnant of damaged cells and are considered a primitive form of lymph nodes found in higher vertebrates (Agius and Roberts, 2003). The activity of macrophages that accumulate in the kidney, spleen, and liver of the carp fish due to exposure to mercury chloride illustrates the role of MMCs in capturing non-antigenic material, namely foreign material in the form of damaged cells due to mercury chloride exposure (Tjahjaningsih et al., 2017). The structure of MMCs is easily visualized histologically through three pigments: hemosiderin, melanin, and ceroid/lipofuscin (Fournie et al., 2001; Agius and Roberts, 2003).

Macrophage aggregates (MA) or MMC are used as non-specific cellular biomarkers of environmental exposure to contaminants or as bioindicators of environmental pollution (Facey and Blazer, 2005: Suresh, 2009; Balamurugan et al., 2012; Reddy, 2012). Furthermore, there were changes in the number of MMCs in gourami fish that stressed by exposure to the heavy metal mercury chloride ($HgCl_2$) (Mubarokah et al., 2018: Radkhah et al., 2022). Under conditions of environmental stress, MMCs can increase in size (Agius and Robert, 2003; Ribeiro *et al*., 2011). Suresh (2009) proves there is an increase in the size and number of MMCs in the spleen of *Tilapia mossambica* fish exposed to cadmium chloride.

Broeg *et al.* (2005) also proved that chromium exposure led to an increase in the number of MMCs without a concomitant increase in size but with a decrease in the size instead. Based on the background, this study investigated how the MMCs metrics profile in common carp (*C. carpio*) spleen exposed to mercury chloride ($HgCl_2$), including the number and size of MMCs. Carp is a freshwater fish approved by the Environmental Protection Agency (EPA) as a test fish for acute toxicity (EPA, 1996). According to Çoban *et al*. (2013), carp is commonly utilized due to their ability to adapt to polluted environments. Moreover, they classified as a demersal fish that are more exposed to heavy metals than pelagic fish.

MATERIALS AND METHODS

Preparation

The research materials included 100 carp fish measuring 18 - 20 cm and weighing 75 - 80 g, pellet feed, and mercury chloride ($HgCl_2$). Ingredients to make preparations histology of organs include the solution of fixative, distilled water, formaldehyde, liquid paraffin, alcohol 50%, 70%, 80%, 90%, 96%, absolute alcohol, xylol, acid alcohol, and dye hematoxylin-eosin (HE). The study used an aquarium with dimension 50 x 30 x 35 cm and each aquarium was filled with 25 liters of water. Before treatment, the mercury chloride content in the spleen organs of the carp and water in each aquarium was measured first.

Experimental design

This study used the Completely Randomized Design method with four treatments of mercury chloride namely Treatment A: 0 $mg.l^{-1}$, B: 0.01 $mg.l^{-1}$, C: 0.05 $mg.l^{-1}$, and D: 0.1 $mg.l^{-1}$. The number of carp used was five for each treatment with five replications of treatment. Feed pellets were given at satiation level in the morning and evening. Water media was replaced every three days with stock water containing mercury chloride concentration according to treatment.

Parameter measurement

The measurement of mercury chloride content using method cold vapor method and then read using

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Atomic Absorption Spectrophotometry (AAS) at the Centre of Environmental Health Engineering and Disease Control, Surabaya. The exposure of carp to mercury chloride concentrations of all doses was performed for 21 days (El-Boshy and Taha, 2011). After exposure to mercury chloride for 21 days, the fish were dissected, and their spleen organs removed. Samples of carp spleen organs were tested for mercury chloride content and histopathological preparations.

Histopathological slide preparations were made using paraffin and hematoxylin-eosin (HE) staining for the hemosiderin pigment (Suresh, 2009). Observation of the number and size parameters of MMCs from histopathologic preparations using a microscope with 400 x magnification. The number and size of MMCs are calculated using the motic image plus application that is on the computer.

Data analysis

Data on the number and size of MMCs were analyzed by Analysis of Variance and continued by using Duncan's multiple range test to determine the differences between treatments. Changes in carp behavior during the study and water quality i.e: Temperature, pH, and dissolved oxygen were used as supporting data. Changes in fish behavior observed during the study were related to changes in normal behavior caused by mercury chloride exposure compared with 0 $mg.l^{-1}$ treatment as a control group.

RESULTS AND DISCUSSIONS

The concentration of mercury chloride ($HgCl_2$) in the water media of each treatment was lower than the concentration of mercury chloride ($HgCl_2$) in the carp spleen (Table 1). Methyl mercury is first transferred to the blood, moves to the internal organs, and is slowly transported out of the organs to the muscle tissue (Smith, 2012). The distribution and accumulation of mercury vary depending on the concentration of heavy metals in water (Jezierska and Witeska, 2006).

The toxic effect of heavy metal accumulation can be seen in the changes in carp fish behavior after being exposed to mercury for 21 days, as seen in the fish's movements, which tend to be hyperactive, and the accumulation of mucus on the surface of their bodies. In addition, respiratory problems were seen in the group of fish exposed to 0.05 $mg.l^{-1}$ and 0.1 $mg.l^{-1}$ mercury chloride, which resulted in mortality on days 5 (two fish from the 0.05 $mg.l^{-1}$ group) and 16 (one fish from the 0.1 $mg.l^{-1}$ group). The behaviour of catfish exposed to mercury with symptoms of hyperactivity and trying to jump out due to skin irritation, anxiety, respiratory problems, loss of balance, excessive mucus accumulation, and finally death (Guedenon et al., 2012).

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Table 1. The data on $HgCl_2$ content in maintenance water media at the beginning of treatment and spleen organ of carp after 21 days exposure to heavy metals mercury chloride

Treatment ($mg.l^{-1}$)	The content of mercury chloride in water media ($mg.l^{-1}$)	The content of mercury chloride in the spleen organ ($mg.l^{-1}$)
A (0)	0	0
B (0.01)	0.0164	0.4403
C (0.05)	0.0546	12.5573
D (0.1)	0.0700	11.9047

The average water quality conditions during the study were a temperature of 26 – 29°C, dissolved oxygen of 4 $mg.L^{-1}$, and a water pH of 7. The temperature and pH values were relatively stable due to changing the water's concentration of mercury chloride according to the treatment every three days. Giving aeration to the water of the carp environmental medium resulted in a relatively stable DO value. Exposure to mercury chloride ($HgCl_2$) at different concentrations affected the number and size of spleen MMCs (Figure 1). According to Wolke (1992) and Bols *et al.* (2001), alterations observed in MMCs due to pollutant responses include number, size, shape, and pigment. The metric profile (amount and size) of MMCs in the spleen of carp exposed to mercury chloride increased compared to the control and was highest in the treatment exposed to 0.05 $mg.l^{-1}$ $HgCl_2$. The 0.05 $mg.l^{-1}$ treatment showed an increase in MMCs phagocytosis activity, followed by an increase in the number and size of MMCs (Table 2). In the treatment of 0.1 $mg.l^{-1}$ mercury chloride exposure, the number and size of carp spleen MMCs decreased compared to the carp fish group exposed to 0.05 $mg.l^{-1}$ $HgCl_2$ (Table 2). The decrease in the number of MMCs is due to reduced chemotactic and phagocytic activity of macrophages at high levels of pollution (Suresh, 2009). Furthermore, the heavy metal concentrations in aquatic systems can be reduced immunity, immune dysfunction, or excessive activation in exposed carp fish (Rajeshkumar et al. 2017). The immune-suppressant effect enabled the phagocytic activity of carp macrophages at a 0.1 $mg.l^{-1}$ mercury chloride concentration to be inhibited.

Table 2. The metrics profile (number and size) MMCs in the spleen of carp after exposure to mercury chloride ($HgCl_2$) for 21 days

Treatment ($mg.l^{-1}$)	Average of Number (mm^{-2})	Average of Size (µm)
A (0)	$29.65^{c} \pm 7.09005$	$6.6780^{b} \pm 0.90566$
B (0.01)	$60.65^{b} \pm 7.90688$	$11.6580^{b} \pm 0.44483$
C (0.05)	$90.25^{a} \pm 8.52936$	$28.6740^{a} \pm 9.3432$
D (0.1)	$54.15^{b} \pm 1.74642$	$11.0640^{b} \pm 1.32096$

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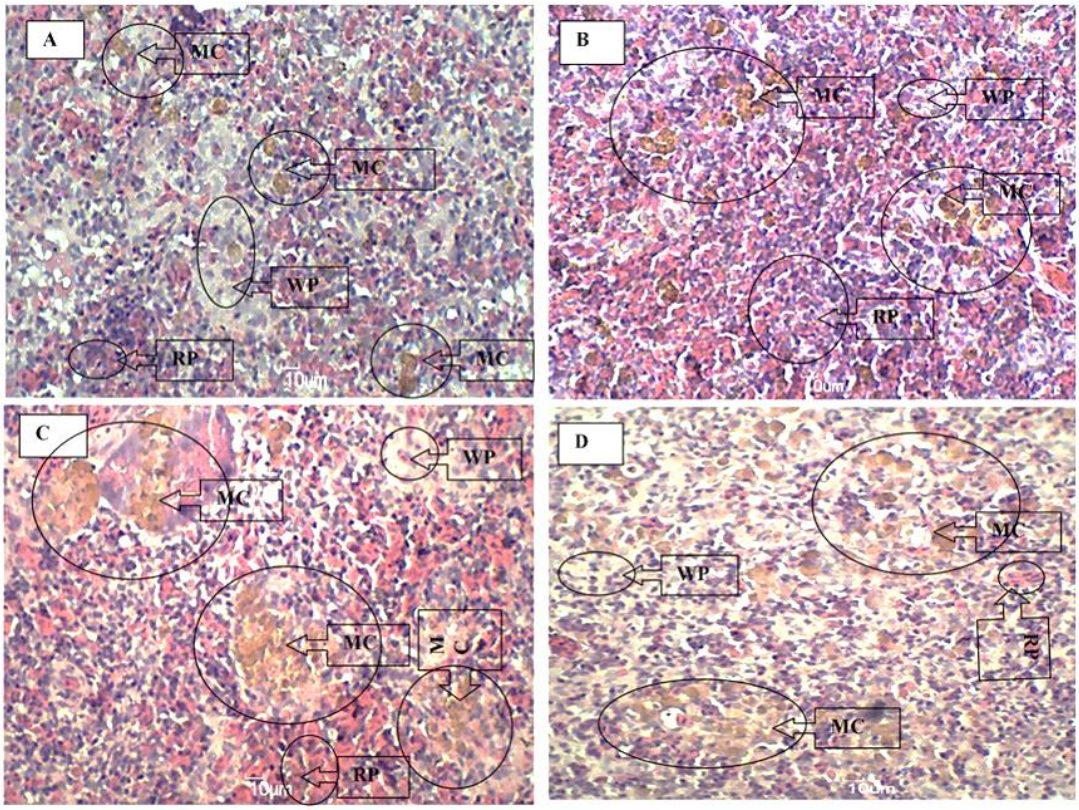

Figure 1. Histopathological features of the carp spleen with magnification 400x. A (0 mg.l^{-1}), B: mercury chloride concentration (0.01 mg.l^{-1}), C: mercury chloride concentration (0.05 mg.l^{-1}), and D: mercury chloride concentration (0.1 mg.l^{-1}). The spleen consists of white pulp (WP), red pulp (RP) and melanomacrophage centers (MC).

The accumulation of mercury in the carp spleen also causes spleen cells to be damaged or lysed so that they become targets for phagocytosis by macrophages. According to Manrique et al (2014), the function of MMCs is to destroy, detoxify, or recycle endogenous and exogenous substances such as foreign bodies and cell metabolic activities. In addition, MMCs also clean and remove foreign particles (Balamurugan et al., 2012). The relationship between MMCs and mercury was demonstrated by increased parenchymal cell degradation or additional accumulation by products of lipid metabolism, which was inhibited by mercury. These cell fragments and metabolites are phagocytosed by MMCs (Meinelt et al., 1997), so that macrophage aggregates meet the anatomical and cytological biomarker requirements of fish health and environmental degradation (Wolke, 1992).

CONCLUSION

Mercury chloride ($HgCl_2$) in water affects the metric profile of carp spleen MMCs. The number and size of exposed carp fish spleen MMCs increased at concentrations of 0.01 mg.l^{-1} and 0.05 mg.l^{-1} but decreased at concentrations of 0.1 mg.l^{-1}. In environmental conditions polluted by heavy metals, the metric profile of carp MMCs can be proposed as a biomarker

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