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Cadmium Effect on Histo-Biomarkers and Melano-Macrophage Centers in Liver and Kidney of *Cyprinus carpio*

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Abstract: The present study is aimed to observe the effect of cadmium on structural modulations of liver and kidney of carp fish, *Cyprinus carpio* exposed to 1/5 sub lethal concentration for 30 days. It is evident that necrotic and degenerative changes were observed on day 30 and also observed to increase the average number and size of Melano-macrophage centers (MMCs) in liver and kidney of carp fish compared with control. The results explained that the histopathological biomarkers gave a way for understanding the morphological modulations due to stress induced by a metabolic toxicant present in aquatic environment. It is also observed that the cell debris is accumulated in the haemopoietic tissues of liver and kidney which are engulfed by MMCs. We suggest that these cells are able to play a role in cleansing the circulation of foreign macromolecules and particles in carps, whereas they are mainly involved in the regular uptake of scavenger debris compounds in carp fishes.

Key words: *Cyprinus carpio* % Heavy Metals % Histopathological Changes % Melano-Macrophage Centers

INTRODUCTION

It is well known that the global fisheries is facing constant decline in fish stocks, both in coastal and Inland water resources on account of constantly increasing water pollution. Among the pollutants, the heavy metals cause various abnormalities in fish as well as human beings. Heavy metals from industries and through various resources disturb the aquatic environment and lead to environmental health hazards; ultimately disturb the balance of Nature [1, 2].

Among the heavy metals, cadmium is considered as a major aquatic pollutant in many parts of the world. Cadmium (Cd) is one of the non-essential heavy metals known for its non-corrosive nature is widely used in paints and dyes, cement and phosphate fertilizers. Cd occurs naturally in the environment in insignificant amounts but its release in the recent past is steadily increasing due to human activities causing pollution of soil and aquatic systems.

Indiscriminate discharge of heavy metals into aquatic media mostly affects non target organisms such as fishes, prawns and crabs which are of highly consumable and having great economic importance and exportable value. In recent years, there has been an increasing interest in the utilization of fishes as bioindicators of the integrity of aquatic environmental systems [3]. Several studies have indicate enhanced levels of both non-essential and essential heavy metal load in liver, gill, kidney and muscle tissues of fishes [4,2].

Accumulation of closely packed highly pigmented phagocytic cells, called “Melano-macrophages (MMO), present in lympho-haemopoietic tissues and liver of different teleosts. It is accepted that the melanin present in MMO is derived from melanocytes and melanophores by means of engulfment [9], though the process of melanin endocytosis has not directly been demonstrated. Finally, MMO have been considered to be functionally exhausted cells and demonstrations of their possible antibacterial phagocytic capacity are sparse [5].

In vertebrates, including fish, two types of immune responses existed to invade the xenobiotics enter in to the body, are innate and adaptive. The innate immunity is the first line of memory where as the adaptive immunity is second line of defence which shows memory. Phagocytosis, the cellular ingestion and digestion of a

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particulate matter a defence mechanism is present in all animals usually, phagocytes is mediated by macrophages and polymorphonuclear leucocytes. In fish haemopoietic tissues are considered as macrophages, containing greenish brown pigment representing breakdown products of haemoglobin from degenerated red blood cells [5,6]. Three types of pigments-melanin, haemosiderin and Lipofuscin, can be present in one and the same macrophage [6, 7].

Many studies suggest that the general function of the centres is the focalization of destruction, detoxification or recycling of endogenous and exogenous materials [8, 9]. Melano - macrophage centers also play an important role in the fish response of fish to foreign materials, including infection causing agents. The scavenging functions and migration of macrophages were observed in various species of fish by injected Indian ink [9, 10].

The role of macrophages and melano-macrophage centers and their pigments has been used as biomarkers for environmental pollution [11]. The present study is aimed to study the toxic effect of cadmium chloride on modulations in number and structure of melano-macrophage centers of liver and kidney of commercially important carp fish, *Cyprinus carpio.*

MATERIALS AND METHODS

The carp fish species *Cyprinus carpio*, (weight 115.34±2.52 g) were obtained from the Government fish breed and culture farm (Figure, 1). Immediately they were transferred to our laboratory and put in large aerated water containing tanks. The unchlorinated tap water supplied from reservoir was aerated and used through out the experimental period. Healthy and active fish were separated and maintained at least for ten days and fed on commercial and formulated dry pelleted feed. The physico-chemical parameters of used water, through out the experimental period, were as indicated in Table according to APHA, 1998 [12].

LC_{50} Determination: The 96 hours median lethal concentration (LC_{50}) of cadmium chloride to *Cyprinus carpio* was determined by Proibit Analysis [13].

Experimental Design: The acclimatized experimental carp fish, *Cyprinus carpio*, were divided into two groups:

Table 1: Physico-chemical parameters of experimental water.

Parameter	Value
pH	7.1 ± 0.3
Temperature	27 ± 0.5°C
Dissolved oxygen (as $CaCO_3$)	289 ± 3 mg L^{-1}
Hardness of water	632±0.5 mg L^{-1}
Chlorides	265 ± 0.5 mg L^{-1}
Photoperiod	12:12± 0.5 hours (L:D)

Fig. 1: Cyprinus carpio

Group I: Used as control. They were fed on commercial feed containing no heavy metal.

Group II: Exposed to daily concentration of cadmium chloride ($CdCl_2$) for seven days with 1/5 LC_{50}.Each group was comprised of ten fish Triplicate to each group was also maintained simultaneously. At the end of experimental period the animals were sacrificed then liver and kidney tissues were dissected out for further use.

Histopathological Studies: Histopathological examinations of liver and kidney tissue of *Cyprinus carpio* were carried by the method of Humason [14]. The photographs at 200X magnification were taken with computer aided zoom microscope.

The pigments haemosiderin, Lipofuscin and melanin were identified by the method of Gurr [15], planimetric measurements of MMC and counting of MMCs in fish liver and kidney tissue. Histological examinations are carried out by the method of Kranz and Gercken [16]. Histological examinations and size of the MMCs are done under the Trinocular microscope and photographs are also taken.

Haemosideron: Sections were deparaffinised, hydrated, immersed in yellow ammonium solution for one hour. The sections washed in distilled water and immersed in 10% haematoxylin (alcoholic) for 1-4 hours. Then the sections were washed in 70% absolutely alcohol and followed by rinse in 50% alcohol and washed in distilled water. Counter stained in neutral red and mounted with DPX, the Haemosiderin appeared as dark blue.

Lipofuscins: The tissue sections were deparaffinised, hydrated, immersed in 1% Ferric chloride and washed in continuous flowing water, rinsed in 1% alcoholic potassium hydroxide followed by immediate rinse in 70% alcohol, then washed in distilled water and counter stained in neutral red. The slides were mounted with DPX. Lipofuscins appeared as dark blue.

Melanin: Deparaffinised, hydrated sections were immersed in 2.5% Ferrous sulphate solution for one hour, washed in distilled water for 20 minutes, immersed in 1% potassium ferricyanide for 30 minutes and washed in 1% acetic acid, mounted with DPX, melanin appeared as green spots.

Differentiation of Melanin from Lipofuscin: Nile blue method was followed for differentiation of melanin from Lipofuscin. Deparaffinised and hydrated tissue sections were stained in Nile blue solution A for 20 minutes, then washed in continuous flowing water for 10-20 minutes and mounted with DPX. Lipofuscins were appeared as blue spots and melanin colourless.

Statistical Analysis: The significance of deviations from control was calculated by using students "t" test [13].

RESULTS AND DISCUSSION

LC_{50} value of $CdCl_2$ to *Cyprinus carpio* was determined as 36.75 ± 2.23 m g LG^{1} and 1/5 of LC_{50} (7.35 mg LG^{1}) was taken as a sub lethal treatment concentration for a period of 30 days. Exposure to $CdCl_2$ caused significant modulations in structural integrity, number of MMCs and free macrophages of liver and kidney tissues of carp fish, *Cyprinus carpio*. Results are represented in Table 2, Figures: 2-6.

The morphological appearance, size and cellular contents of MMCs in teleosts differ between specimens, species and organs [6, 17 and 18]. The macrophages in teleosts, usually, take up scavenger molecules, worn out and damaged cells coagulated blood and damaged connective tissue fibrils, which cells uptake of various scavenger materials and act to clean up the tissue after infection and mechanical damage [6, 19, 20]. Such materials are usually degraded within the macrophage vacuoles and reused in the synthesis of new substances. Undegradable tissue debris may gradually accumulate in these cells [6, 21] appears as dense, grey or faintly yellow macrophage pigment, Lipofuscin, composed mainly of polyunsaturated fatty acids denaturated by strongly oxidative substances [6,20].

Table 2: Variations in the number and size (îm) of Melano-macrophage centers (MMCs) in liver and kidney of *Cyprinus carpio* treated with 1/5 sub lethal concentration of cadmium chloride.

	Number of MMC		Size of MMC	
Organs	Control	$CdCl_2$ treated	Control	$CdCl_2$ treated
Liver	8.43 ±1.16 ------	38.25 ± 2.34 (353.74)	2.63 ± 0.43 ------	18.70 ± 3.16 (616.47)
Kidney	15.33±1.82 -----	43.61±2.66 (184.47)	8.42±1.82 -----	46.14±2.42 (447.98)

Values are mean ± SD of six individual observations. Mean values are significant at P<0.05. Values are presented in parentheses indicate the percentage change over the control.

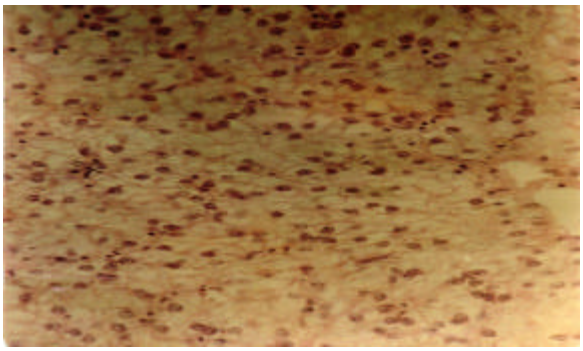

Fig 2: Section of the Liver of *Cyprinus carpio* (Control): Showing large number of hepatocytes and blood vessels

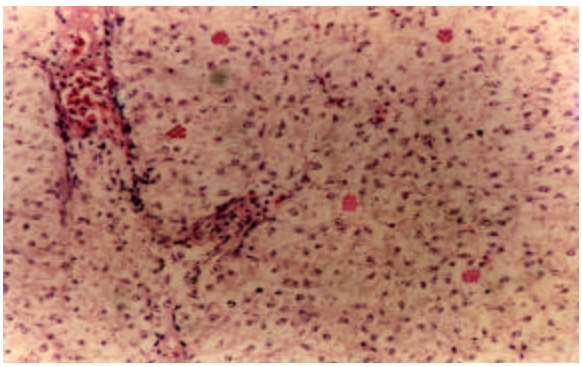

Fig 3: Section of the Liver of *Cyprinus carpio* (Experimental-15 days): Showing cytoplasmic degeneration, portal traid with lymphocytes, blood corpuscles and appearance of melano-macrophage centres.

Liver is the main storage and central metabolizing organ that plays a dual role of secretion of digestive enzymes and as storage organ for nutrients. The liver of control fish comprises of continuous mass of hepatic cells arranged in cords. The hepatocytes large in size and the nucleus are centrally situated. It is evidenced in the present study that the liver showed severe cytoplasmic

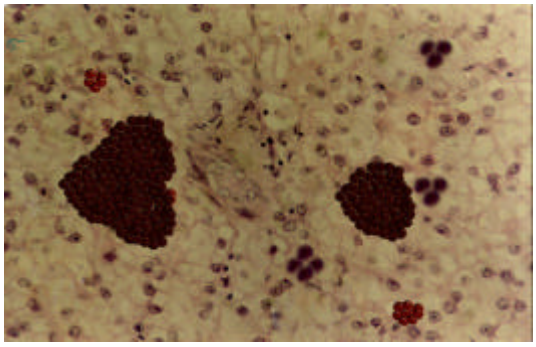

Fig 4: Section of the Liver of *Cyprinus carpio* (Experimental-30 days): Deposition of haemosiderin in the melano-macrophage centres of a plaice with Chronic cadmium chloride toxicity. The sequestered iron stains brown red and melanin/lipofuscin very dark brown red.

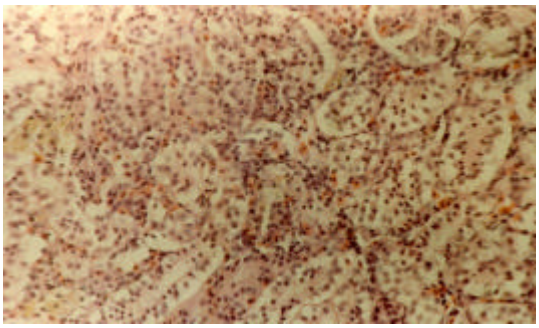

Fig 5: Section of the Kidney of *Cyprinus carpio* (Control): Showing glomeruli, renal tubules and haemopoietic tissue.

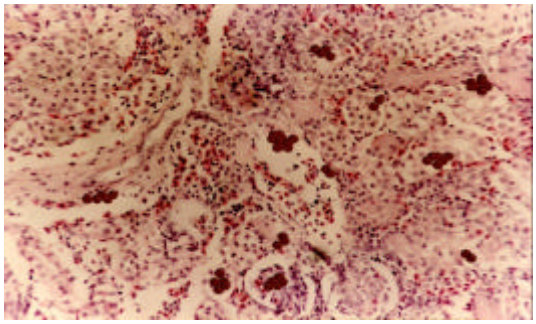

Fig 6: Section of the Kidney of *Cyprinus carpio* (Experimental -15 days): Appearance of melano-macrophage cells in haemopoietic tissue of kidney.

degenerations, central vein engrossed with RBC and vacuolization are observed on days 15 and 30 (Fig-2,3 and 4). This is the main reason why the liver is more often recommended as a n indicator of water pollution than any other organs of fish [2, 22, 23].

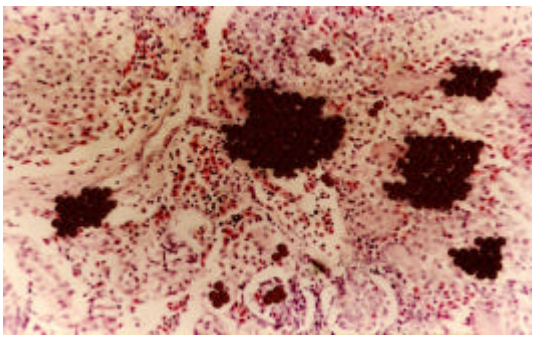

Fig 7: Section of the Kidney of *Cyprinus carpio* (Experimental -30 days): Showing bunches of melano-macrophage centres in haemopoietic centers of kidney of fish.

In control fish liver shows macrophages and few Melano macrophages centers (MMC) and also free macrophages. The MMC are smaller in size and appeared as aggregate clusters adjacent to blood vessels (Table 2, Fig 2, 3). In fish exposed to sub lethal concentration of $CdCl_2$, the size and number of MMC is significantly increased compared to the control indicating the toxic intensity of $CdCl_2$ in the aquatic media.

The teleostean kidney consists of head kidney and body which contains lymphoid tissue and many nephropores with intestinal lymphoid tissue respectively. The interstitial lymphoid tissue is the major haemopoietic tissue. The sub lethal concentration of $CdCl_2$ caused highly degenerative changes in haemopoietic tissue, swelling in renal tubules, cellular hypertrophy and also caused vacuolization of tubular epithelium and degenerative of kidney. The above results are supported by the findings of Janardana Reddy *et al*. [2]. The kidney of control fish also consists of free macrophages and MMC. They occurred mainly in the haemopoietic tissue of these organs, in close proximity to the vascular system, i.e. sinusoids and kidney (Fig-4). The size and number of MMC are observed to increase in kidney tissue of carp fish indicating the intensity of toxic action of heavy metal $CdCl_2$ in carp fish. In both control and $CdCl_2$ treated carp fish the MMC appeared as yellow and dark brown in colour. Haemosiderin and melanin contained MMC are observed in liver and kidney of carp fish, whereas Lipofuscin is not appeared in $CdCl_2$ treated fish (Fig 5, 6,7).

Pulsford *et al.* [24] were examined by x-ray microanalysis for the presence of heavy metals within the melanomacrophage centres. Ultrastructurally these centres consisted of groups of pigment-containing

macrophages in which melanin was the most prominent pigment, but lipofuscin and haemosiderin were also present. Some of the macrophages also contained irregular metal particles and minerals with associated metals. Numerous single macrophages associated with the kidney vascular system may play a significant role in the clearance of scavenger and foreign substances from the circulation in teleosts [25, 26].

Fourine *et al.* [27] have undertaken extensive and detailed field assessment of the utility of Melano-macrophage centers as general indicators of exposure of fish to degraded environments. The also stated that melano-macrophage centre densities of greater than 40/mm^2 correlated with exposure to either hypoxic conditions or chemical contamination of sediments. Within the macrophages, lipofuscin generally appears to be the most abundant pigment, melanin is often, but not always, the other major compact haemosiderin can be present in considerable quantities under certain conditions such as haemolytic anaemia [6].

In general, the macrophages in teleosts take up scavenger molecules, worn-out or damaged cells, coagulated blood and damaged connective tissue fibrils, i.e. these cells have a regular uptake of various scavenger materials, foreign substances and act to clean up the tissue after infection and mechanical damage [17, 25 and 26]. Such materials are usually degraded within the macrophage vacuoles and reused in the synthesis of new substances. MMCs maintain lysosomal enzymes, which act on much of the engulfed material and finally reach a definitively exhausted state. They are mainly involved in iron and haemoglobin metabolism and tissue catabolism [28]. The present study also concludes that the occurrence and changes in MMC and appearance of pigments in MMC of liver and kidney indicates that the MMC could be considered as a biomarker of stress induced by the heavy metal toxicity present in the aquatic medium.

The present study concludes that large numbers of macrophages appeared in liver and kidney are capable of taking up the debris in haemopoietic tissue in common carp fish. We suggest that the macrophages in kidney and liver in carps are able to play a role in the cleansing of the circulation of foreign macromolecules and particles.

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