CODEN [USA]: IAJPBB ISSN: 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

http://doi.org/10.5281/zenodo.1181460

Available online at: http://www.iajps.com Research Article

EFFECT OF HEAVY METAL, CADMIUM CHLORIDE ON PROTEIN AND AMINO ACID CONTENT CHANGES IN FRESHWATER EXOTIC FISH, *HYPOPTHALMICHTHYS MOLITRIX*

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Abstract:

The metal works industries release a good amount of heavy metals like mercury, cadmium, manganese, nickel and chromium which ultimately fall in the water bodies. Heavy metals are known to cause alterations in various tissues of fish at the biochemical level. Cadmium is an extremely toxic heavy metal which is widely used in mining, metallurgical operation, electroplating industries manufacturing vinyl plastics, electrical contacts, metallic and plastic pipes. Effluents from such plants are sources of cadmium into aquatic environments. Hence an attempt was made to study the impact of sublethal concentration of cadmium chloride on the protein and amino acid content changes in gill, liver and kidney of the freshwater exotic fish, Hypopthalmichthys molitrix. As the period of exposure to sublethal concentration of cadmium chloride has considerable influence on the metabolic changes the study was made at 7, 14 and 21 days. The protein and amino acid levels changes in gill, liver and kidney were estimated in control and experimental fishes. Significant elevation of amino acid whereas protein level decreased in gill, liver and kidney of fish treated with sublethal concentration of cadmium chloride when compared with control fish. But the maximum decrease of protein and elevation of amino acid contents were observed in all tissues at 21 days. These results suggest that the cadmium chloride causes deleterious effects on protein metabolism.

Key words: *Hypopthalmichthys molitrix, cadmium chloride, protein metabolism, gill, liver, kidney*

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Please cite this article in press as S. Kamaraju and K. Ramasamy, ***Effect of Heavy Metal, Cadmium Chloride on Protein and Amino Acid Content Changes in Freshwater Exotic Fish, Hypopthalmichthys Molitrix****, Indo Am. J. P. Sci, 2018; 05(02).*

INTRODUCTION:

Water pollution refers to any type of aquatic contamination rendering the water body poisoned by toxic chemicals, which affect living organisms and all forms of life. Heavy metals constitute an important group of environmentally hazardous substances. During this century, many lakes in India have received elevated inputs of heavy metals as a result of an increase in atmospheric deposition [1]. Pollution by heavy metals is an important problem due to the metals' persistence in the environment. Since the aquatic environment is the ultimate recipient of the pollutants produced by natural and anthropogenic sources, accumulation, and persistence of heavy metals in the aquatic environment constitute a formidable threat to biological life [2-5].The random use of different pesticides often causes lot of damage on non-target organism. Organophosphate pesticides constitute a large proportion of the total synthetic chemicals employed for the control of pests in the field of agriculture, veterinary practices and public health [44].

Fish is one of our most valuable sources of protein food. Worldwide, people obtain about 25% of their animal protein from fish and shellfish. The protein found in fish is of high biological value, which means that fish can be used as the sole source of protein in the diet [6]. It is known that physiological and biochemical parameters in fish blood and tissues could change when exposed to heavy metals and that these parameters are extremely sensitive to these elements [7]. Protein is the most important and abundant biochemical constituent present in the animal body, particularly in fish. Proteins are important in all biological systems, playing a wide variety of structural and functional roles. Thus, proteins play a fundamental role in the process of life. The special proteins have unique roles such as structural and functional elements of cells and tissues. The protein metabolism alters due to toxicant present in the aquatic medium. Thus, it was argued that several biochemical parameters in fish blood and tissues could be used as an indicator of heavy metal toxicity [8]. Because heavy metal contamination in an aquatic environment exerts an extra stress on fish, there must be several other changes in the fish metabolism when exposed to heavy metals [9].

The body proteins are in a dynamic state, constantly being broken down and replaced. This is a rapid process in organs such as liver, kidney, intestinal mucosa and pancreas. Metabolism is a series of biochemical reactions occurring in the cells of living organisms to release energy for building up body tissues. Very few attempts have been made on the effect of heavy metal on protein metabolism in fish. Appreciable decrease in the protein level of liver, muscle intestine, brain, gill and blood of *Heteropenustes fossilis* is noticed after the fish exposed to nickel for 30, 60 and 90 days [10-11] have reported a decrease in total protein and increase amino acids in the muscle and gill tissues of *Lamellidens marginalis* exposed to chromium for 72 hrs. Even though many studies are available in heavy metal toxicity study of various fishes and environmental monitoring studies[13-19] for our best of knowledge there is no report available on *Hypopthalmichthys molitrix* exposed to cadmium chloride. Investigation on toxicity makes it possible to evaluate the effects of sublethal concentration on growth, behavior, physiology and biology of organisms, to determent their adaptation capabilities and to forecast possible consequences to toxic effect [43] Hence the present investigation has been carried out on changes in the protein and amino acid content in gill, liver and kidney of *Hypopthalmichthys molitrix* exposed to sublethal concentration of cadmium chloride.

MATERIALS AND METHODS:

The fish *Hypopthalmichthys molitrix* having mean weight 14 - 16 gm and length 12 – 14 cm were collected from PSP fish farm, at Puthur and acclimatized to laboratory conditions. They were given the treatment of 0.1% KMNO4 solution and then kept in plastic pools for acclimatization for a period of two weeks. They were fed twice daily i.e. morning and evening on rice bran and oil cake daily. The cadmium chloride was used in this study and stock solutions were prepared. Cadmium chloride, LC 50 was found out for 96 h (28 mg/L) [20] and $1/15^{th}$ (1.86 mg/L), $1/10^{th}$ (2.8 mg/L) and $1/5^{th}$ (5.6 mg/L) taken as sublethal concentrations for this study. Forty fish were selected and divided into 4 groups of 10 each. The first group was maintained in free from cadmium chloride and served as the control. The other 3 groups were exposed to sub lethal concentration of cadmium chloride, 10 litre capacity aquaria. The 2^{nd}, 3^{rd} and 4^{th} groups were exposed to cadmium chloride, for 7, 14 and 21 days respectively. At the end of each exposure period, the fish were sacrificed and the required tissues were collected for protein and amino acid estimation. The protein and amino acid content in gill, liver and kidney of *Hypopthalmichthys molitrix* were estimated by the method of [21-22] respectively. The data obtained were analyzed by applying analysis of variance DMRT one way ANOVA to test the level of significance [23].

RESULTS:

PROTEIN AND AMINO ACID

The present results revealed that cadmium chloride induced alterations are time dependent and tissue-specific. Cadmium chloride has been shown to elevate the amino acid and decrease the levels of protein in gill, liver and kidney of *Hypophthalmichthys molitrix* exposed to low, medium and high sublethal concentrations of cadmium chloride for a period of 7, 14 and 21 days (Table 1 and 2).

Table 1: Protein (mg/g) levels in the gill, liver and kidney of *Hypophthalmichthys molitrix* exposed to sublethal concentration of cadmium chloride

Treatments	7 days	14 days	21 days
Gill			
Control	106.27 ± 8.47^{c}	107.32 ± 8.55^{c}	109.44 ± 8.71^{c}
Low concentration	88.80 ± 7.12^{b}	82.66 ± 6.68^{b}	79.17 ± 6.41^{b}
% change over control	(-16.44)	(-22.97)	(-27.65)
Medium concentration	81.64 ± 6.59^{ab}	73.97 ± 6.02^{a}	68.56 ± 5.60^{a}
% change over control	(-23.17)	(-31.07)	(-37.35)
High Concentration	78.75 ± 6.38^{a}	69.83 ± 5.70^{a}	63.35 ± 5.21^{a}
% change over control	(-25.89)	(-34.93)	(-42.11)
Liver			
Control	120.34 ± 9.55^{c}	123.76 ± 9.80^{c}	129.81 ± 10.21^{c}
Low concentration	101.16 ± 8.08^{b}	104.52 ± 8.34^{b}	93.28 ± 7.48^{b}
% change over control	(-15.93)	(-15.54)	(-27.79)
Medium concentration	92.08 ± 7.39^{ab}	90.34 ± 7.26^{a}	74.33 ± 6.04^{a}
% change over control	(-23.48)	(-27.00)	(-42.46)
High Concentration	87.30 ± 7.03^{a}	88.68 ± 6.75^{a}	65.29 ± 5.35^{a}
% change over control	(-27.45)	(-28.34)	(-49.45)
Kidney			
Control	75.73 ± 6.15^{a}	76.59 ± 6.21^{c}	75.97 ± 6.17^{c}
Low concentration	73.63 ± 5.94^{a}	71.85 ± 5.86^{ac}	67.56 ± 5.52^{b}
% change over control	(-2.77)	(-6.18)	(-11.07)
Medium concentration	71.15 ± 5.72^{a}	66.68 ± 5.46^{b}	62.71 ± 5.16^{ab}
% change over control	(-6.04)	(-12.96)	(-17.45)
High Concentration	69.37 ± 5.66^{a}	60.23 ± 5.35^{a}	58.25 ± 4.74^{a}
% change over control	(-8.39)	(-21.36)	(-23.32)

All the values mean ± SD of six observations
+/– indicates the % change over control
values which are not sharing common superscript differ significantly at 5% ($p < 0.05$)
Duncan multiple range test (DMRT)

Table 2: Amino acid (mg/g) levels in the gill, liver and kidney of *Hypophthalmichthys molitrix* exposed to sublethal concentration of cadmium chloride

Treatments	7 days	14 days	21 days
Gill			
Control	2.58 ± 0.24^{a}	2.66 ± 0.23^{a}	2.62 ± 0.22^{a}
Low concentration	3.74 ± 0.32^{b}	4.54 ± 0.38^{b}	5.85 ± 0.47^{b}
% change over control	(44.96)	(70.67)	(123.28)
Medium concentration	4.26 ± 0.36^{c}	5.38 ± 0.44^{c}	8.42 ± 0.67^{c}
% change over control	(65.11)	(102.25)	(221.37)
High Concentration	4.72 ± 0.40^{d}	6.92 ± 0.55^{d}	11.56 ± 0.91^{d}
% change over control	(82.94)	(160.15)	(341.22)
Liver			
Control	4.28 ± 0.36^{a}	4.31 ± 0.35^{c}	4.26 ± 0.35^{a}
Low concentration	5.34 ± 0.45^{b}	5.74 ± 0.46^{b}	7.95 ± 0.63^{b}
% change over control	(24.76)	(33.17)	(86.61)
Medium concentration	6.26 ± 0.52^{c}	8.05 ± 0.64^{c}	9.84 ± 0.78^{c}
% change over control	(46.26)	(88.74)	(130.98)
High Concentration	8.42 ± 0.68^{d}	11.86 ± 0.93^{d}	14.58 ± 1.14^{d}
% change over control	(96.72)	(175.17)	(242.25)
Kidney			
Control	2.04 ± 0.19^{a}	2.10 ± 0.19^{a}	2.08 ± 0.19^{a}
Low concentration	2.58 ± 0.24^{b}	3.19 ± 0.27^{b}	4.40 ± 0.36^{b}
% change over control	(26.47)	(51.90)	(111.53)
Medium concentration	3.22 ± 0.28^{c}	4.08 ± 0.34^{c}	5.15 ± 0.42^{c}
% change over control	(57.84)	(94.28)	(147.59)
High Concentration	3.75 ± 0.32^{d}	5.82 ± 0.47^{d}	7.44 ± 0.59^{d}
% change over control	(83.82)	(177.14)	(257.69)

All the values mean ± SD of six observations
+/– indicates the % change over control
values which are not sharing common superscript differ significantly at 5% ($p < 0.05$)
Duncan multiple range test (DMRT)

DISCUSSION:

The gills are directly in contact with water and widely known as susceptible organs for metal pollution [24]. The gills are not only the prime organs for gaseous exchange; they also perform several other physiological functions including osmoregulation and excretion. Changes in environmental parameters often damage this vital organ because of its delicate structure [25]. The gill epithelium provides an extensive surface of contact with the environment to facilitate ion transport, gaseous exchange[26], and ironically, exchange of aquatic toxicants and hazardous agents[27]. Among all tissues, liver showed higher protein content which might be due to greater concentration of enzyme. Liver is the site of metabolism [28]. The liver plays an important role in the synthesis of proteins. The kidney, which is an important organ of excretion and osmoregulation, is indirectly affected by pollution through blood circulation [29-30] have reported that the kidney is the metabolic site of the body and further they have stated that it may become susceptible to injuries caused by the intoxication of the effluent. [31] have reported that the kidney was the site of degradation and detoxification of toxic substances.

In the gill, liver and kidney protein content had decreased, whereas total free amino acids content had increased at all periods of exposure when *Hypopthalmichthys molitrix* was exposed with sublethal concentration of cadmium chloride. The decreased protein levels in the gill, liver and kidney tissues at sublethal concentration of cadmium chloride may be due to the enhanced proteolysis leads to amino acid contents were increased. The decreased trend of the protein content as observed in the present study in most of the fish tissues is due to metabolic utilization of ketoacids to gluconeogenesis pathway for the synthesis of glucose or due to directing free amino acid for the synthesis of necessary proteins.

The liver is affected considerably when there is a disturbance in protein metabolism. The accumulation of toxic substance in liver may alter its function [32]. A reduction in the protein content in the kidney could possibly be due to protein breakdown leading to increased amino acid pool of tissue [33].

The protein content declined gradually in gill, liver and muscle tissues of *O. mossambicus* when exposed to deltamethrin and it was reported that it might be due to the utilization of protein controls to counteract the toxicant stress caused by pesticide [34]. Appreciable decrease in the protein level of liver, muscle intestine, brain, gill and blood of *Heteropenustes fossilis* is noticed after the fish exposed to nickel for 30, 60 and 90 days [35]. Similarly fish, *Oreochromis mossambicus* treated with pesticide, Lannate showed the protein content of gill, liver, kidney, brain and muscle were greatly reduced[36].

[37] have reported that the protein content of liver decreased and amino acid level increased in an air-breathing fish, *Channa punctatus* when exposed to sublethal concentration of dairy effluent. The decrease in liver and muscle protein has been reported in the sugar mill effluent treated *Channa punctatus* after 96 hr exposure[38]. The protein contents in liver and kidney of *Catla catla* are depleted under the sublethal stress of chromium [39-40] has reported the decrease in protein content of liver, muscle and kidney in *Channa punctatus* when exposed to sublethal concentration of phenyl mercuric acetate.

[41] have observed a decrease in carbohydrate, protein and lipid contents of gill, liver and kidney in *Cyprinus carpio* communis exposed to sublethal concentration of tannery effluent. [42] has reported that depletion in protein level was due to diversification of energy to meet the impending energy demand when the animals were under toxic stress.

Studies of [43] have also revealed the increase in the amino acid level at similar situations. The elevated amino acid levels in the kidney of sublethal treated *Hypopthalmichthys molitrix* during effluent intoxication indicate a high turnover of amino acids, which should normally lead to increased deamination and oxidation of amino acids. Similar increase in kidney amino acid level also was reported in *T. mossambica* exposed to sublethal concentration of heptachlor for 15 days[44-45] have attributed the decrease in protein content and an increase in amino acid content in the liver, kidney and muscle of *O. mossambicus* exposed to phenol. [46] have reported a decrease in total protein and increase amino acids in the muscle and gill tissues of *Lamellidens marginalis* exposed to chromium.

CONCLUSION:

A reduction in the protein content in the present investigation in *Hypopthalmichthys molitrix* suggests that the tissue protein undergoes proteolysis, which results in an increase in the production of amino acids. These amino acids are utilized for energy production during stressful situation in the intoxicated fishes. It is evident that proteins are degraded to meet the energy requirements during cadmium chloride exposure. It can be concluded that in *Hypopthalmichthys molitrix* exposed to cadmium chloride at sublethal concentration causes energy crisis and alter protein metabolism.

ACKNOWLEDGEMENT:

The authors are thankful to authorities of Manonmaniam Sundaranar University and Head of the Department of Zoology, Annamalai University for providing facilities.

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