International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 11, Issue 1, 2019

Original Article

SUPPLEMENTATION OF CALCIUM AND SELENIUM AGAINST CADMIUM INDUCED BIOACCUMULATION IN SELECTED TISSUES OF FRESH WATER FISH, *OREOCHROMIS MOSSAMBICUS*

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Received: 05 Oct 2018 Revised and Accepted: 28 Nov 2018

ABSTRACT

Objective: Cadmium (Cd) is one of the most hazardous heavy metals in aquatic environments and could threaten aquatic organisms including fish. The present study was carried out to know the protective effects of calcium (Ca) and selenium (Se) in reducing the Cd bioaccumulation in selected tissues of fresh water fish, *Oreochromis mossambicus*.

Methods: The fresh water fish, *Oreochromis mossambicus (Tilapia)* was brought from the local ponds and were allowed for acclimatization to the laboratory conditions. After acclimatization, fish were exposed to sublethal concentration of Cd ($1/10^{th}$ of LC_{50}/48h, i.e., 5 ppm) for 7, 15 and 30 d (d) period. 15d Cd-exposed fish were divided into three groups. The first group of fish were subjected to Ca (1 ppm) supplementation, second group received only Se (1 ppm) supplement and third group of fish were supplemented with the combination of both Ca and Se at the above said doses and observed for 7, 15 and 30d time periods. After specific time intervals, liver, kidney, gill and intestine tissues were isolated and used for Cd bioaccumulation studies.

Results: Cd concentration levels significantly ($P<0.05$) increased in the test tissues with increased period of exposure. Maximum Cd accumulation was found in 30d Cd-exposed fish kidney tissue (22.611±0.676 µg/gm wet wt. of the tissue). However, after supplementing with Ca and/or Se, there was a significant reversal in the levels of Cd concentration in all the test tissues. Maximum reduction was observed under Ca alone supplementation.

Conclusion: The present study clearly reveals that individual supplementation of Ca tends to detoxify the Cd body burden in the test tissues than the other modes of supplementation.

Keywords: Cadmium, Bioaccumulation, Supplementation, Fish

DOI: http://dx.doi.org/10.22159/ijpps.2019v11i1.30167

INTRODUCTION

Metal pollution is one of the most dangerous consequences of industrial activities to the aquatic environment [1]. Because of their persistent nature and slow elimination from environmental compartments, metals are the largest and most widespread groups of contaminants [2]. The diverse deleterious health effect upon exposure to toxic heavy metals such as cadmium (Cd), lead (Pb) and mercury (Hg), etc. in the environment is a matter of serious concern and a global issue. Heavy metals accumulate in the tissues of aquatic animals and may become toxic when accumulation reaches a substantially high level. Studies carried out with different fish species have revealed that non-essential metals can produce toxic effects in fish by disturbing physiological activities [3], biochemical processes [4], reproduction and growth [5].

Cd was readily bioaccumulated and bioconcentrates in aquatic organisms. Tissue Cd concentrations build up at the site of exposure, gills in a waterborne exposure or gastrointestinal tract in a dietborne exposure and are transferred via the circulation to other tissues. Cd accumulates in nearly all tissues and organs with liver, kidney, and gill reaching relatively high levels. Available reports indicate that the kidney, liver, and gills were the critical targets for Cd in fishes [6-8] in which they have been reported to cause significant metabolic, biochemical and physiological effects. The bioaccumulation of heavy metals, biochemical, pathological and antioxidant alterations induced by different heavy metals and organic compounds has been studied by several investigators in the different tissues of rats and fish [9-15].

Despite many years of research, we are still far from effective treatment of heavy metal poisoning. The main therapeutic option for metal poisoning relies in chelation therapy. Chelating agents are capable of linking together metal ions to form complex structures which can be easily excreted from the body. We have reported here on the interactions between Cd, Se and Ca in *Tilapia*. Cd can compete and interact metabolically with essential nutrients such as Se, Ca, Zn, Cu and Fe in the body [16-17]. Se is important in many biochemical and physiological processes. It plays a major role in the modification of the thiol and hydroxyl groups in the Cys and Tyr. It was reported that Cd may inhibit the Se metabolism at many stages by interfering their absorption, distribution to different tissues, transport into cells and/or transport into several intracellular structures and also indirectly affecting the synthesis of various Se dependent enzymes and proteins [18-22].

Se is an essential nutrient for living organisms and is required for the synthesis of various selenoproteins (SeP). Se plays a vital role in the synthesis of a non-enzymatic antioxidant, GSH and also MT protein synthesis [23-25]. It can prevent cell damage through the activation of the antioxidant system [26-28]. Se is the most prevalent nutrient in the body and is involved in the structure and function of some enzymatic and nonenzymatic antioxidants, collectively representing all major biochemical categories and therefore is essential for normal cell function and metabolism.

Ca supplementation using the inorganic calcium chloride, protected against accumulation of Cd in kidney, liver and other selected tissues of fish [29]. Niyogi *et al.*, [30] reported that water hardness has an ameliorating effect against Cd uptake and toxicity in fish. As water hardness (defined as the sum of Ca and magnesium (Mg) concentrations) increases acute waterborne Cd toxicity decreases [31]. The major hardness cation, Ca changes gill permeability, competes with Cd for binding sites on the surface of gills and reduces toxicity.

Cd accumulation was reduced with the increase of waterborne and dietary Ca [32-34]. Higher water Ca levels reduce the amount of Cd binding to gills [17] and reduce branchial Cd uptake rates resulting

in lower accumulation in the kidney, liver and other tissues of fish. This approach is based on the fact that freshwater fish have two main uptake pathways for ions: the gills (waterborne ions) and the gastrointestinal tract (dietary ions) and can control the total uptake by changing the proportion of each kind of uptake according to the situation. If the fish are acquiring more ions via the gastrointestinal route, they may reduce ion uptake rates at gills thereby simultaneously reducing metal uptake via the same branchial transport pathway [29]. For example, Na and Cu [35] share the same transport pathway, and experiments showed that high dietary Na (as NaCl) decreased gill uptake rates of both Na and Cu and reduced internal accumulation of Cu in rainbow trout [36]. As Ca and Cd compete for the same transport pathway, this explains why elevation of Ca (as CaCl2) protected against Cd accumulation in several fish tissues [37-39]. Reduced plasma Ca levels and resulting hypocalcemia after Cd exposure have been proposed as the fundamental mechanism of Cd toxicity. However, Ca-rich water may have ameliorating effect in a Ca-deficient environment where fish may be vulnerable towards Cd toxicity. Hence the present study was carried out to know whether the supplementation of Se and Ca either individually or in combination would reduce the Cd bioaccumulation in the selected tissues of Cd-exposed *Oreochromis mossambicus*.

MATERIALS AND METHODS

Chemicals

Cadmium as cadmium chloride, calcium as calcium chloride and selenium as sodium selenite were purchased from Merck (Dormstadt, Germany). All other chemicals which were used in the present study were obtained from the standard chemical companies like Sigma Chemical Co. (St Louis, Mo, USA) and SD Fine Chemicals. The chemicals used in this study were of the highest purity.

Collection and exposure of animals

Fish *Oreochromis mossambicus (Tilapia)* weighing 10±2 gm were collected from the local freshwater ponds and acclimatized to laboratory conditions for a week in separate troughs. The experiment was carried out in the laboratory of Department of Zoology, Sri Venkateswara University, Tirupati. The laboratory temperature was maintained at 28±20C. The fish were fed *ad libitum* with groundnut cake and water was renewed for every 24 h with routine changing of troughs leaving no faecal matter. The protocol and animal use has been approved by the Institutional Animal Ethics Committee (Resol. No. 10(ii)/a/CPCSCA/IAEC/SVU/AUR-JO dt 22-12-2008), Sri Venkateswara University, Tirupati, Andhra Pradesh, India.

After acclimatization fish were divided into two groups, namely control and experimental, the experimental fish have exposed to sub lethal concentration of cadmium chloride ($CdCl_2$) i.e., 5 ppm ($1/10^{th}$ of LC_{50}/48 h) daily for 7, 15 and 30 d (d) time intervals. The LC_{50}/48hr for Cd has been determined in *Tilapia mossambica* from our laboratory [40], and these values are once again confirmed before proceeding to the present study. Then 15d Cd-exposed fish were divided into three groups. Group-I received only Ca (1 ppm) for 7, 15 and 30d periods. Group-II received Se supplementation (1 ppm) and Group-III was supplemented with both Ca and Se at the above said concentrations for 7, 15 and 30d long sojourn.

Isolation of tissues

After specific time intervals fish were sacrificed. Tissues like the liver, kidney, gill, and intestine were isolated and were immediately used for bioaccumulation studies.

Bioaccumulation studies

The Cd concentration levels in the selected tissues were measured by following the method of Kanno *et al.* [41]. After the specifice time intervals the test tissues like liver and kidney were isolated in ice cold conditions and then immediately they were washed with saline (0.9%) and 50 mg of each tissue was digested in an acid mixture of Nitric acid: Perchloric acid (3:2 V/V) for overnight. The acid mixture was then subjected to evaporation and the residue obtained was dissolved in 5 ml double distilled water. From this 1 ml was withdrawn and analyzed for Cd concentrations using Atomic Absorption Spectrophotometer (Schimadzu AA 6300). Cd concentrations were expressed as µg/gm wet. weight of the tissue.

Data analysis

The data was subjected to statistical measures such as mean, standard deviation and Analysis of variance (ANOVA) using standard statistical software, SPSS (version 16). All values are expressed as mean±SD of 6 individual samples. Significant differences were indicated at P<0.05 level.

RESULTS

The effect of sublethal concentrations of Cd accumulation has been studied in different tissues like liver, kidney, gill, and intestine of freshwater edible fish *Oreochromis mossambicus*. Cd bioaccumulation was increased significantly with the increased period of exposure (i.e. 7, 15 and 30 d) to the heavy metal Cd in all the test tissues (fig. 1).

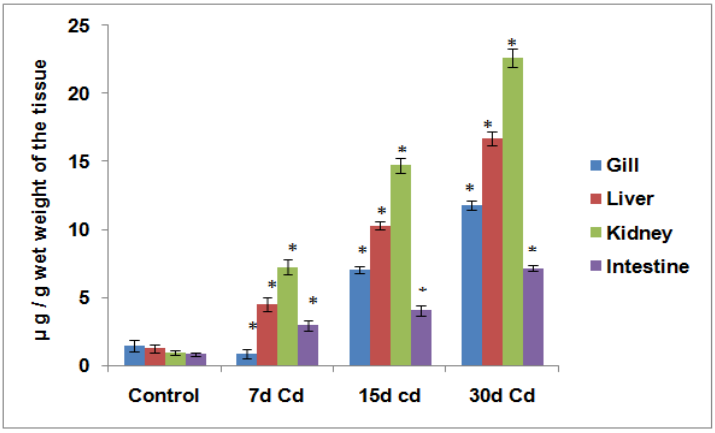

Fig. 1: Bioaccumulation (µg/g wet weight of the tissue) levels in different tissues of *O. mossambicus* exposed to Cd

❖ All values are expressed as mean±SD of 6 individual samples.

❖ Statistical significance was evaluated by one-way analysis of variance (ANOVA) and the Duncan's Multiple Range Test (DMRT).

❖ All mean values are significant at P<0.05 level over 15d Cd exposure.

❖ NS-Non significant.

From the results, it is clear that there is a significant increase in Cd accumulation in the selected tissues like gill, liver, kidney, and intestine of *Tilapia* exposed to Cd at different time intervals. Among these tissues, the maximum level of Cd bioaccumulation was observed in 30d fish kidney (22.611±0.676 µg/g wet weight) indicating that the kidney is the major site of Cd accumulation.

Further liver accumulated high Cd concentrations when compared to other tissues under study. Among all the selected tissues lowest concentration of Cd was observed in the intestine of 30d exposed fish (7.151±0.192 µg/g wet weight).

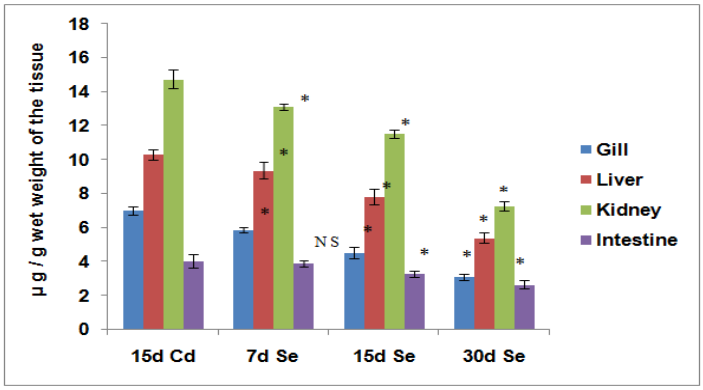

Fig. 2: Bioaccumulation (µg/g wet weight of the tissue) levels in different tissues of Cd-exposed *O. mossambicus* after supplementation with Se

- ❖ All values are expressed as mean±SD of 6 individual samples.
- ❖ Statistical significance was evaluated by one-way analysis of variance (ANOVA) and the Duncan's Multiple Range Test (DMRT).
- ❖ All mean values are significant at P<0.05 level over 15d Cd exposure.
- ❖ NS-Non significant.

When 15d Cd-exposed fish were supplemented with Se and/or Ca, Cd concentration levels were profoundly decreased in the test tissues during all the time periods of experimentation. Maximum decrement of Cd accumulation was found in 30d Ca supplemented fish gill (2.149±0.227 µg/g wet weight of the tissue) than the other tissues (fig. 2). However with the combined supplementation of Se and Ca, all the test tissues showed moderate levels of Cd uptake for all the time periods (fig. 4).

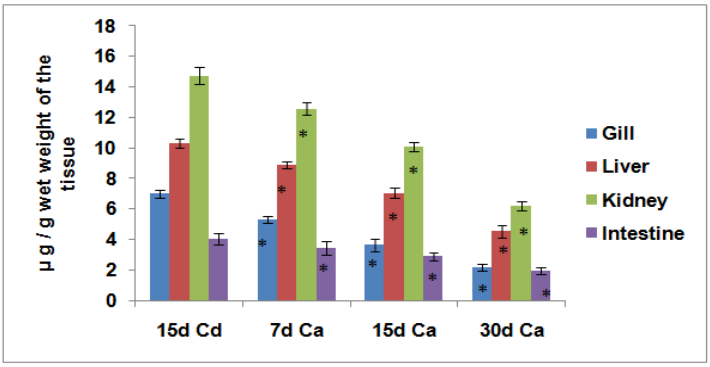

Fig. 3: Bioaccumulation (µg/g wet weight of the tissue) levels in different tissues of Cd-exposed *O. mossambicus* after supplementation with Ca

- ❖ All values are expressed as mean±SD of 6 individual samples.
- ❖ Statistical significance was evaluated by one-way analysis of variance (ANOVA) and the Duncan's Multiple Range Test (DMRT).
- ❖ All mean values are significant at P<0.05 level over 15d Cd exposure.
- ❖ NS-Non significant.

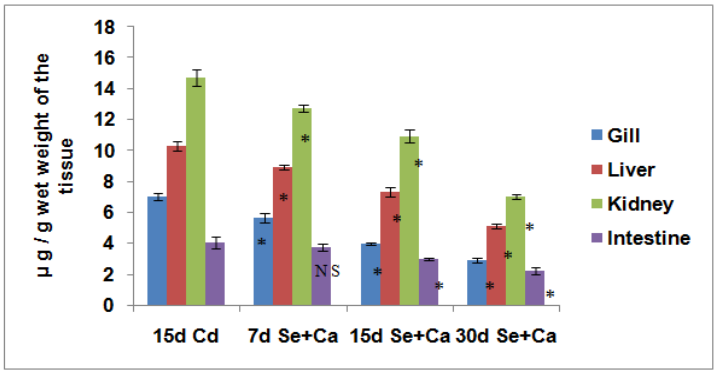

Fig. 4: Bioaccumulation (µg/g wet weight of the tissue) levels in different tissues of Cd-exposed *O. mossambicus* after supplementation with Se+Ca

- All values are expressed as mean±SD of 6 individual samples.
- Statistical significance was evaluated by one-way analysis of variance (ANOVA) and the Duncan's Multiple Range Test (DMRT).
- All mean values are significant at $P<0.05$ level over 15d Cd exposure.
- NS-Non significant.

Individual Se supplementation significantly reduced the Cd accumulation levels in the gill, liver, kidney and intestine tissues (3.105±0.189, 5.389±0.307, 7.254±0.274 and 2.650±0.251 µg/g wet weight of the tissue) during only 30d supplementation period (fig. 3). Hence, the low level of depletion in Cd concentration was found in the test tissues under individual supplementation of Se when compared to other modes of supplementation.

From the above results, it is clearly understood that the Ca alone as a supplement could tremendously reduce the Cd body burden than the other modes of supplementation in the selected tissues.

DISCUSSION

Bioaccumulation of trace metals in aquatic and terrestrial ecosystems are of special concern in the interest of human welfare. Toxic elements such as heavy metal are known to bioaccumulate in the tissues of freshwater animals like fish. A growing number of evidence has shown that several factors influence Cd accumulation in fish tissues. These factors include the environmental metal concentration and time of exposure. Several authors showed that animal tissues, contaminated in the laboratory, accumulate heavy metals in a concentration and contamination period dependent manner [42-44].

Fish have the ability to accumulate heavy metal in their tissues by the absorption along the gill surface and gut tract wall to higher levels than the toxic concentration in their environment [8]. In recent years Cd occupies a unique place among metals because of its diverse toxic effects: its long biological half-life (20–30 y in humans), low rate of excretion from the body (1–2 µg/day) and its predominant long-term storage in soft tissues. Cd accumulates and proves to be very toxic in many organs, such as kidney, liver, brain, bone, blood system and lung [8]. Cd has been recognized as one of the most toxic environmental and industrial pollutant due to its ability to induce severe alterations in various organs and tissues in species of animals and humans following either acute or chronic exposure.

Several past studies investigated Cd accumulation and its distribution in organs [2, 13-15, 45-50]. Entry of heavy metals into the organs of a fish mainly takes place by the adsorption and absorption, and the rate of accumulation is a function of uptake and depuration rates. Reynders *et al.*, [7] reported that on the tissue level, highest Cd concentrations were observed in kidneys of carp (*Cyprinus carpio*) and roach (*Rutilus Rutilus*), followed by gills, intestine, and liver. Increased Cd concentrations in gills and intestine probably reflected the source of metals uptake from water and food, since gills generally accumulate much higher metal concentrations than the intestine during waterborne exposure.

The Cd accumulation levels in test tissues in response to time-dependent Cd burden were depicted in fig. 1. Data from the present study indicated that Cd exposure produces a significant accumulation of Cd in the test tissues such as gill, liver, kidney, and intestine. Similar observations are found in Persian sturgeon, *Acipenser persicus* reported by Zahedi *et al.*, [44] exposed to sublethal concentration of Cd. Pollutants rarely distribute uniformly in animal body tissues, but they can accumulate to varying degrees in particular target organs, such as liver, gill or kidney. In our, study Cd has accumulated significantly in the kidney followed by liver, gill, and intestine. These results are similar to those reported by Pretto *et al.*, [51] in *Rhamdia quelen*.

Our study is also proven that fish kidney and liver accumulate a higher proportion (22.611±0.676 µg/g and 16.671±0.504 µg/g) of the body burden of Cd. The increased accumulation of Cd in the kidney and liver over time could be due to the involvement of these organs in the detoxification and removal of toxic substances circulating in the stream. Moreover, since these organs are the major organs of metabolic activities including detoxification of Cd [2, 46, 52]. Cd might also be transported into these organs from other tissues like the gills and intestine, for the purpose of subsequent elimination. In an attempt to detoxify Cd, liver tissue produces MT. It was assumed that Cd-MT was released from the liver cells and then gradually redistributed to the kidney, which is the main target organ for chronic Cd toxicity [53]. The kidney is thus the final destination of all the Cd from various tissues as it has also been shown that Cd-MT is filtered through the glomerulus and is reabsorbed by the proximal tubular cells, possibly by endocytosis. Within these cells, the complex was taken up by lysosomes and degraded by proteases to release Cd, which may result in renal accumulation of the metal. Thus, these factors might have accounted for the raised level of the heavy metal in the kidney during the exposure periods.

Gills were known as a temporary target organ for Cd accumulation after which the Cd is transferred to organs, such as liver, kidney, and intestine by way of the circulatory system or the enterohepatic circulation [33]. A Similar level of Cd accumulation was observed in gills when silver catfish were exposed to this metal for 7 and 14 d. These results indicated that Cd accumulation occurs in the liver, kidney and gills of fish and it appears that a long period of time is required for its elimination. In the present study gill also accumulates a higher proportion of Cd (11.785±0.342 µg/g). In the aqueous medium in which the Cd salt was dissolved, there were two major routes through which Cd can be taken up by the fish. The principle one was the oral route with subsequent intestinal absorption, while the other is the gill. Several reasons have been proposed to justify the gills as the primary site for Cd uptake, such as proximity to toxicants due to its external position, it's highly branched structural and vascular nature with the resultant highly increased surface area through which large volumes of water pass through the gill surface amongst other tissues [8, 54]. The greater Cd accumulation of gills found in this study could be explained by the nonspecific binding of Cd ions with glycoproteins present on the gill surface, which can depress Cd uptake.

The intestine of fish accumulated the lowest level of Cd (7.151±0.192 µg/g), even after 30 d of exposure. During waterborne exposure of Cd, metal levels in the digestive tract were usually low. In the case of dietary exposure of metals, their concentrations in the digestive tract increase and remain high until the end of exposure [55]. In accordance with the absence of lesions in the digestive tract well known to be impacted in fish exposed to the metal via food items, even though changes to intestine epithelia have also been found in fish exposed to increased concentrations of waterborne Cd with exposure period [45, 56].

Cd bioaccumulation is known to disturb the trace elements distribution in the tissues of organisms [57]. One of the most important characteristics of Cd toxicity was its interaction with physiologically nutrient elements [58]. Several nutrient elements like Ca, Se, Zn, Fe and Cu participate in controlling various metabolic and signaling pathways [15, 47, 59-61]. Among these elements, Se and Ca are required for maintenance of life and health [34, 62]. In the present study significant reduction in Cd bioaccumulation was observed in the selected tissues of fish supplemented with Se and/or Ca.

The interaction between Se, Ca and Cd has not been fully elucidated. However, it was believed that Cd competes for Se and Ca in several metabolic pathways and thereby reduces the levels of Se and Ca in the vital organs [63]. One of the important findings of the present study is that supplementation with Se and/or Ca significantly reduces Cd burden in the test tissues of *Tilapia*.

In our study, Se supplementation showed depletion of Cd accumulation in the selected tissues of Cd-exposed fish. This might be due to the depression of nonprotein sulfhydryl (-SH) groups in fish tissues. It suggests that Se protects cells from toxic effects of Cd by maintaining the availability of antioxidant non protein-SH groups and further because of Cd affinity towards-SH groups may in turn reduce free Cd level in test tissues, thus attenuating the toxic effect of Cd. Tawwab and Wafeek [64] reported that Se prevents acute Cd toxicity through a mechanism that does involve the induction of metallothionein. In the present study, the bioavailability of Se

increases with the supplementation, which inturn is responsible in reducing the Cd body burden. The principle role of Se is associated with the control of LPO, because Se was a component of selenoenzymes contributing to the antioxidant system. Recently Cogun *et al.*, [65] also reported the protective effect of Se against heavy metal Hg toxicity in *Oreochromis niloticus.* Lazarus *et al.*, [23] also evaluated the influence of Se on Cd retention in suckling rats. Results of the present study revealed a significant decrease in Cd body burden in fish supplemented with Se, which was in agreement with earlier studies on zebrafish, *Danio rerio* exposed to Cd [66]. Hence, the bioavailability of Se might be helpful in the reduction of Cd accumulation.

Ca is an important element needed for the maintenance of membrane integrity and ion regulation. Cd can damage gills and decreases the activity of gill Ca-ATPase, which leads to fish hypocalcaemia and can result in skeletal deformities and disturbance in Ca balance [33]. The most sensitive cellular targets of Cd seem to be ion transport and signal transduction. These include intracellular mobilization of second messenger's inositol triphosphate and Ca, inhibition of plasma membrane Ca channels and inhibition of Ca-ATP ases of the sarcoplasmic reticulum [67].

The present study revealed interesting interactions between Ca supplementation and the response to Cd exposure. Among all the exposure periods, 30 d Ca supplementation showed a reduction in tissue Cd accumulation. It was clear from the present study that the toxicity of metal was affected by Ca which in turn reduces the toxic effect of metal through competitive inhibition at the gill surface. The nontoxic Ca competes with the toxic metals for the same binding sites [15,29,68]. Baldisserotto *et al.*, [29] also demonstrated that trout fed for 7d with 60 mg/g Ca showed reductions in waterborne Cd uptake comparable with the present study.

The efficiency of Ca supplementation in reducing waterborne Cd accumulation was highest at the site of uptake, the gill and this lead to a lower accumulation of Cd in internal tissues. Upon uptake from the water, Cd like other metals bind to transport proteins in the plasma and was distributed via the arterial system to internal organs, where it may be stored or excreted [69]. Increased Ca levels in the medium resulted in a slower transfer of Cd from the gills to the blood and the rate of Cd accumulation was lowered in the liver, kidney and other selected tissues. Similar findings were reported in rainbow trout by Hollis *et al.*, [70] and in *Cirrhina mrigala* by Ghosh and Adhikari [71]. In the absence of elevated Ca, test tissues accumulated Cd continuously over the duration of the experiment.

CONCLUSION

It could be therefore concluded that either Se and/or Ca supplementation might play a vital role in reducing the Cd tissue burden of freshwater fish. However, Ca alone supplementation has a pronounced effect in depleting Cd concentrations when compared to other modes of supplementation, thereby mitigating the risk of potential hazards to human health. Such dietary manipulations may further provide the best defense against environmental exposures to heavy metals and needs more attention.

AUTHORS CONTRIBUTIONS

All the author have contributed equally

CONFLICT OF INTERESTS

Declared none

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