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Original Article

PROTECTIVE EFFECT OF ZINC AND VITAMIN-C AGAINST CADMIUM INDUCED BIOACCUMULATION IN LIVER AND KIDNEY OF MALE ALBINO RAT

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ABSTRACT

Cadmium (Cd) is a toxic and non-essential heavy metal. The present study is carried out to know the protective effect of zinc (Zn) and vitamin C supplementation against Cd induced bioaccumulation in liver and kidney of Cd treated rats. Male wistar albino rats were treated with cadmium chloride($CdCl_2$) at a dose of 1/10th $LD_{50/}$ 48h i.e. 22.5 mg/Kg body weight for 7, 15 and 30 days (d) time intervals. Then 15 d Cd treated rats were divided into three groups. The first group received Zn (12 mg/Kg), second group received vitamin C (200 mg/Kg) alone and third group supplemented with both Zn and vitamin C again for 7, 15 and 30 d long sojourn. After specific time intervals, rats were decapitated and tested for Cd bioaccumulation by Atomic Absorption Spectrophotometer (AAS). There was a significant elevation in Cd levels in both the tested tissues with increased period of Cd treatment. Maximum Cd accumulation was found in 30 d Cd treated rat kidney (42.80 ± 0.30 µg /gm wet wt. of the tissue). Maximum decrease in Cd accumulation was found in 30 d treated rat kidney (5.04 ± 0.08µg / gm wet wt. of the tissue) supplemented with the combination of Zn and vitamin C. Our findings clearly indicates that combined supplementation of Zn and vitamin C is more effective in reducing the Cd body burden when compared to the other modes of supplementation i.e. Zn and vitamin C alone in the male albino rat.

Key words Cadmium, bioaccumulation, Zinc, Vitamin C

INTRODUCTION

Environmental pollution is a global problem and is common to both developed as well as developing countries. This pollution was caused by numerous chemicals, xenobiotics and heavy metals etc. Among the heavy metals, Cadmium (Cd) is one of the most toxic, non- essential heavy metal with many industrial uses that can contribute to a well-defined spectrum of diseases in animal models as well as in humans [1,2]. Cd is known for its corrosive nature and is widely used in paints and dyes, cement and phosphate fertilizers [3]. Cd is an environmental pollutant that has serious toxicity in humans and animals and causes Itai-Itai disease [4] and also induces the onset of anemia, decreases red blood cell count and hemoglobin concentration [5]. Cd has an extremely long half-life (20-30 Years) in the human body and is highly cumulative, especially in the liver and kidney [6,7]. The main sources of Cd are storage batteries, electroplating, pigments, plastics, fertilizer industries and cigarette smoking. The kidney is considered as the criticalorgan in long term low level exposure to Cd [8].

Cd has been shown to affect cells by multiple modes, making the elucidation of its mechanism of action a very complex task. It can cause damage to cell membrane and certain organelles alter signal transduction pathways and / or affect the intracellular enzymatic systems. Chronic Cd exposure has been involved in a variety of pathological conditions such as nephrotoxicity, neurotoxicity and carcinogenicity [9]. Metabolic, histological changes, membrane damage, altered gene expression and apoptosis [10]. Some of the toxic effects of Cd exposure are hepatic damage, renal dysfunction, hypertension, central nervous system injury and testicular atrophy [11].

It is a ubiquitous toxic metal and induce oxidative damage by disturbing the prooxidant – antioxidant balance in the tissues [12]. Cd like many other heavy metals is antagonistic to essential trace elements like Zn, Fe, Cu, Ca etc., [13] and competes with these trace elements for binding sites as transport and storage proteins, metalloenzymes and receptors.

Zn is a ubiquitous essential trace element with numerous functions in biological systems. Although Zn is an essential requirement for good health, excess Zn can be harmful. Excessive absorption of Zn suppresses Cu and Fe absorption. [14]. It occurs in all living cells as a constituent of metalloenzymes involved in major metabolic pathways. It plays a catalytic, inhibitory or accessory role in the regulatory enzymes such as kinases or phosphatases. It is a

critical part of Zn-finger domains that regulate scores of protein – protein or protein – nucleic acid interactions [15]. Zn controls several enzymes of intermediary metabolism, such as DNA and RNA synthesis, gene expression, and immunocompetence and plays a significant role in homeostasis of hormones [16]. Zn also takes part in the defense against excessive amounts and following damage of certain metals, and it does so through the interaction with metallothionein. It has been noted that Zn has a relationship with many enzymes in the body and can prevent cell damage through activation of the antioxidant defense system [17,18].

Ascorbic acid (vitamin C) is a water-soluble dietary antioxidant that plays an important role in controlling the oxidative stress [19] In humans, vitamin C is essential to a healthy diet as well as being a highly effective antioxidant, acting to lessen Oxidative stress ; a substrate for ascorbate peroxidise in plants (APX is plant specific enzyme) [20]. It has been reported that ascorbic acid enhances Cd transport and decreases its uptake in rat intestinal segments [21]. It has also been demonstrated that vitamin C is one of the most effective factors reducing an enhanced renal and hepatic cadmium burden in pigs fed on a diet enriched with Cu [22]. Vitamin C is found in high concentrations in Immune system, and is consumed quickly during infections. It is not certain how vitamin C interacts with the immune system; it has been hypothesized to modulate the activities of Phagocyte, the production of Cytokine and Lymphocyte and the number of molecule in Monocyte [23]. There is a longstanding belief among the mainstream medical community that vitamin C causes kidney stones, which is based on little science [24]. Although recent studies have found a relationship[25] a clear link between excess Ascorbic acid" intake and Kidney stone formation has not been generally established[26]. Some case reports exist for a link between patients with oxalate deposits and a history of high-dose vitamin C usage [27]. Vitamin C is absorbed by the intestines using a sodium-ion dependent channel. It is transported through the intestine via both glucose-sensitive and glucose-insensitive mechanisms. The presence of large quantities of sugar either in the intestines or in the blood can slow absorption [28].

MATERIALS AND METHODS

Chemicals

Cd as cadmium chloride ($CdCl_2$), Zn as zinc chloride ($ZnCl_2$) and vitamin C 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 Aldrich (St Louis, MO, USA) and SD Fine Chemicals, India. The chemicals used in this study were of the highest purity.

Animals

Three months-old Wistar strain male albino rats weighing 180 ± 20 g were chosen for the present study. The animals were obtained from Sri Venkateswara Traders, Bangalore, Karnataka, India and were kept in stainless steel mesh cages, housed under standard laboratory conditions (23 2C, 50 20% relative humidity, 12h light-dark cycle) with standard rat chow (SaiDurga Feeds and Foods, Bangalore, India) and drinking water *ad libitum*. The rats were acclimatized to the laboratory conditions for 10 days. The protocol and animal use has been approved by the Institutional Animal Ethics Committee, (Resol. No. 58/2012/(i)/a/CPCSEA/IAEC/ SVU/AUR – VK), Sri Venkateswara University, Tirupati, Andhra Pradesh, India.

Experimental design

After acclimatization, the rats were divided into two groups, namely control and experimental. Control rats received only deionized water without Cd. The experimental rats were treated with $Cdcl_2$ at a dose of $1/10^{th}$ LD_{50} / 48h i.e. 22.5 mg/Kg body weight for 7, 15 and 30 days (d) time intervals. Then 15d Cd treated rats were divided into three groups. The first group received Zn (12 mg/Kg), second group Vit-C (200 mg/Kg) alone and third group supplemented with both Zn and Vit-C for again 7, 15 and 30 d long sojourn.

Isolation of tissues

After specific time intervals, the control and experimental rats were decapitated and tissues such as liver and kidney were quickly isolated under ice cold conditions and weighed to their nearest mg using Shimadzu electronic balance. After weighing, tissues were immediately used for the analysis of bioaccumulation levels and were stored at $80^{\circ}C$ for future use.

Bio-accumulation studies

Cd concentrations in the test tissues were measured by the method of Kanno *et al*., 1994[29]. After the specific time intervals the tissues like liver and kidney were isolated and immediately washed with saline (0.9%) and 50mg of each tissue was digested in 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 of double distilled water. From this 1 ml was

withdrawn and analyzed for Cd concentrations by using Atomic Absorption Spectrophotometer (Schimadzu AA 6300).

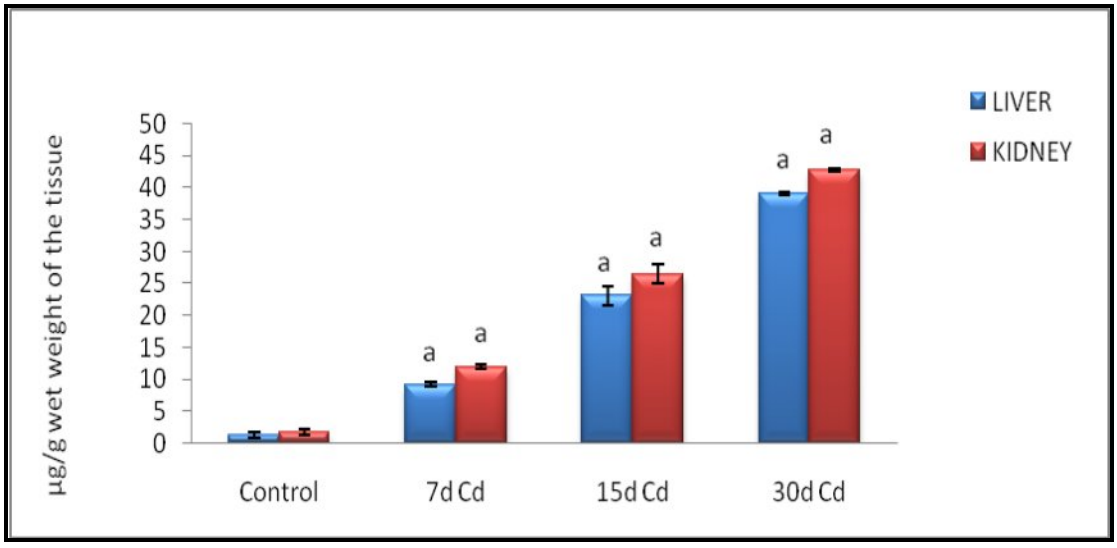
Data analysis

The data was subjected to statistical analysis such as mean, standard deviation (SD), and analysis of variance (ANOVA) using standard statistical software, Statistical Package for Social Sciences (SPSS; version 16). All values are expressed as mean SD of six individual samples. Significant differences were indicated at $P < 0.001$ level.

RESULTS

Bio-accumulation of Cd concentration was analyzed in liver and kidney of control, Cd treated and Zn and /or vitamin C supplemented male albino rats for the specified time intervals. The mean Cd levels were found to be significantly increased in both liver and kidney tissues of Cd treated rats when compared to the controls (Figure. 1). The accumulation of Cd significantly increased with the increased duration of treatment (Table. 1). Cd accumulation was high in the kidney of rats treated for 30 d Cd (42.80 ± 0.30) than liver (39.08 ± 0.64 µg/g).

Figure. 1 Cd accumulation (µg /g wet weight of the tissue) in the liver and kidney of rats treated with Cd



Each bar represents Mean+ SD of six individual observations.

a- indicates the level of significance P<0.001.

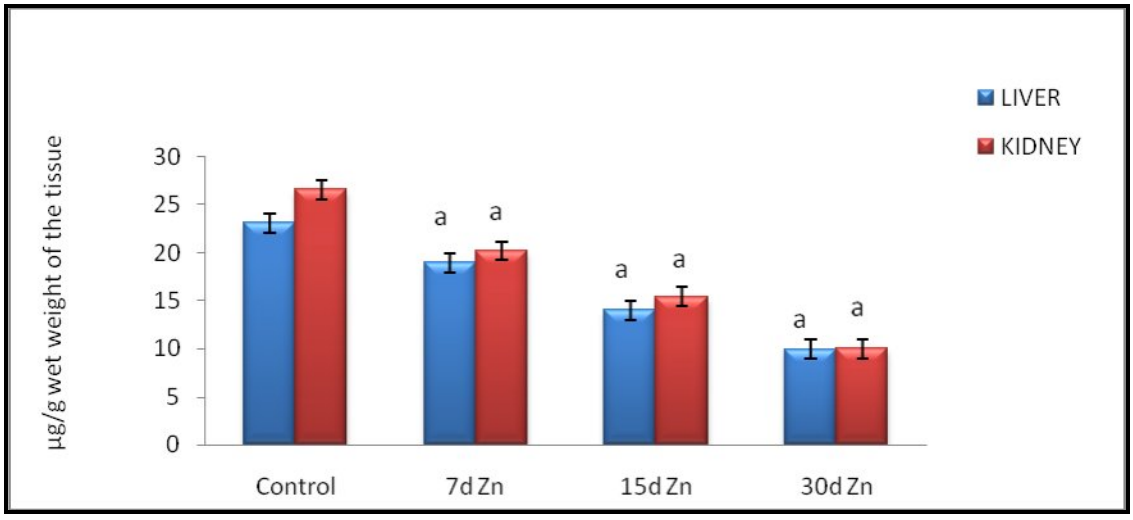
Table. 1 Cd accumulation (µg / g wet weight of the tissue) in the liver and kidney of rats

Tissue	Control	7d Cd	15d Cd	30d Cd
Liver	1.25 ± 0.09	9.23[a] ± 0.31	23.09[a] ± 0.50	39.08[a] ± 0.64
Kidney	1.74 ± 0.42	11.91[a] ± 0.35	26.61[a] ± 1.56	42.80[a] ± 0.30

Mean± SD of six individual observations.
+ and – percent increase and decrease respectively over control.
a- indicates the level of significance p<0.001.

With Zn alone supplementation, both the test tissues showed moderate decrement at all the time intervals when compared to the 15d Cd treated rats. Maximum reduction in Cd bio-accumulation was observed in 30d kidney tissue (10.01 ± 0.11µg/g) than the liver tissue (9.97 ± 0.59µg/g) at all the time intervals (Table. 2). From the results, it can clearly envisages (Figure. 2) that Zn and vitamin C supplementation for 30 d duration showed a tremendous reduction in the Cd body burden for both the tissues and more over the decreased rate of accumulation was highly significant.

Figure. 2 Cd concentrations (µg / g wet weight of the tissue) in the liver and kidney of rats after supplementation with Zn



Each bar represents Mean+ SD of six individual observations.
a- indicates the level of significance P<0.001.

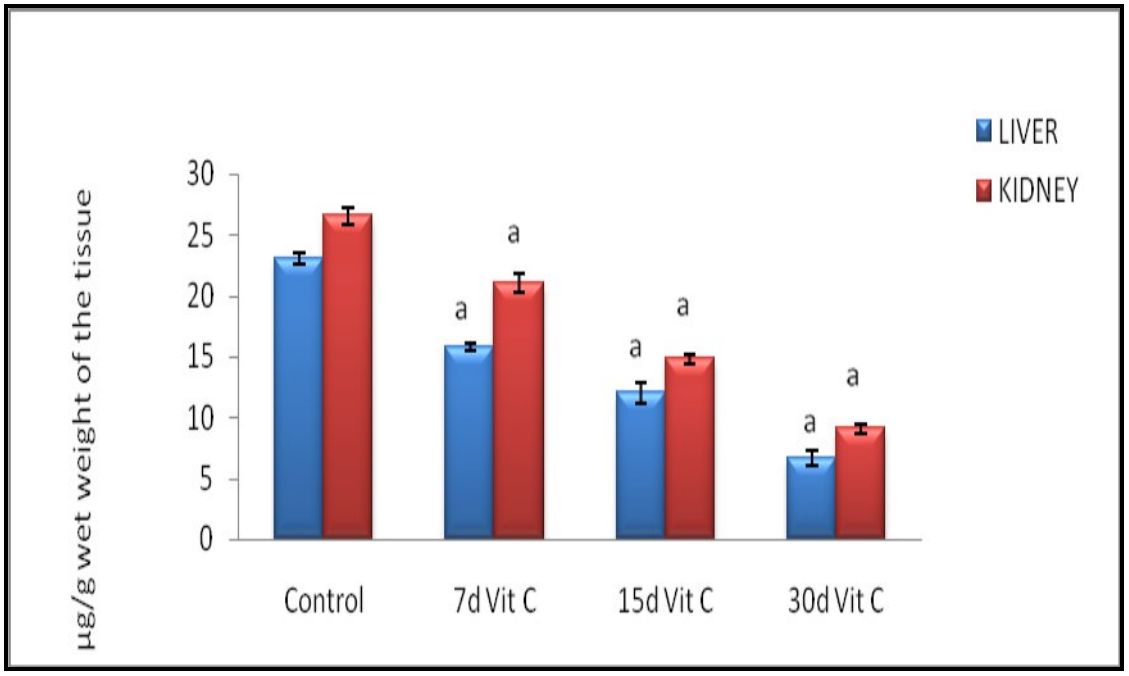
Table. 2 Cd concentrations (µg / g wet weight of the tissue) in the liver and kidney of rats after supplementation with Zn

Tissue	15d Cd	7d Zn	15d Zn	30d Zn
Liver	$23.09^{a} \pm 0.50$	$18.96^{a} \pm 0.50$	$14.01^{a} \pm 0.08$	$9.97^{a} \pm 0.59$
Kidney	$26.61^{a} \pm 0.69$	$20.21^{a} \pm 0.12$	$15.41^{a} \pm 0.07$	$10.01^{a} \pm 0.11$

Mean± SD of six individual observations.
+ and – percent increase and decrease respectively over control.
a- indicates the level of significance $p<0.001$.

However with vitamin C alone supplementation, we could find both kidney and liver tissues showing high levels of decrease in Cd concentrations (Table. 3). Maximum reduction was found in 30 d liver tissue (6.72 ± 0.63µg /g) than kidney tissue (9.02 ± 0.37µg /g) (Figure. 3).

Figure. 3 Cd concentrations (µg / g wet weight of the tissue) in the liver and kidney of rats after supplementation with vitamin C



Each bar represents Mean+ SD of six individual observations.
a- indicates the level of significance $P<0.001$.

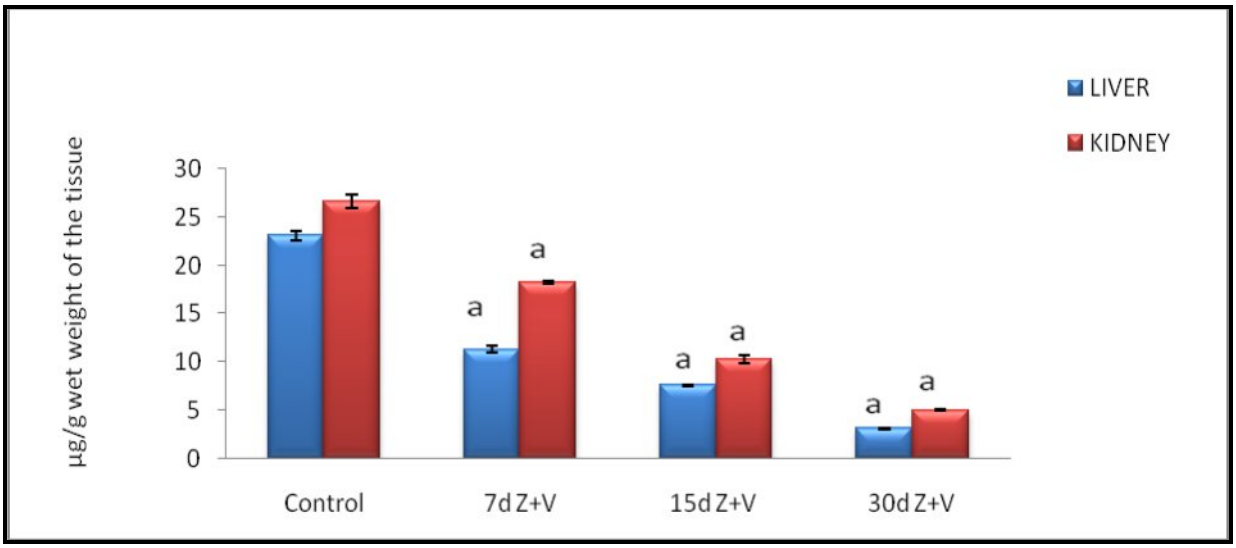
Table. 3 Cd concentrations (µg / g wet weight of the tissue) in liver and kidney of rats after supplementation with Vit – C.

Tissue	15d Cd	7d Vit c	15d Vit c	30d Vitc
Liver	$23.09^{a} \pm 0.50$	$15.81^{a} \pm 0.34$	$12.02^{a} \pm 0.08$	$6.72^{a} \pm 0.63$
Kidney	$26.61^{a} \pm 0.69$	$21.01^{a} \pm 0.07$	$14.80^{a} \pm 0.46$	$9.02^{a} \pm 0.37$

Mean± SD of six individual observations.
+ and – percent increase and decrease respectively over control.
a- indicates the level of significance $p<0.001$.

Zn and / or vitamin C supplementation significantly decreased the bioaccumulation levels of Cd in both the test tissues for all the time intervals. Figure. 4 showed that maximum decrease in Cd bioaccumulation levels was observed in 30d rat kidney under the combination of both the supplements Zn and vitamin C (5.04 ± 0.08 µg/g) than the other modes of supplementation at all the time intervals (Table. 4).

Figure. 4 Cd concentrations (µg / g wet weight of the tissue) in the liver and kidney of rats after supplementation with the combination of Zn and vitamin C



Each bar represents Mean+ SD of six individual observations.
a- indicates the level of significance $P<0.001$.

Table. 4 Cd concentrations (µg / g wet weight of the tissue) in the liver and kidney of rats after supplementation with the combination of Zn and Vit-C.

Tissue	15d Cd	7d Zn and Vit C	15d Zn and Vit C	30d Zn and Vit C
Liver	23.09 ± 0.50	11.32[a] ± 0.38	7.62[a] ± 0.05	3.12[a] ± 0.07
Kidney	26.61 ± 0.69	18.21[a] ± 0.15	10.32[a] ± 0.38	5.04[a] ±0.08

Mean± SD of six individual observations.
+ and – percent increase and decrease respectively over control.
a - indicates the level of significance $p<0.001$.

DISCUSSION

Cd is one of the most dangerous occupational and environmental toxicant and is mainly accumulated in the kidney and liver of animals. The present work focused on the pattern of Cd bio-accumulation in the liver and kidney of male albino rats. The Cd accumulation levels in test tissues in response to time dependent Cd burden are depicted in Figure. 1. The Cd accumulation levels were elevated in the test tissues with the increased time of Cd treatment [30]. Similar trend was also observed in the present study.

Bioaccumulation and bio-magnification are the characteristic features of heavy metals. Cd occurs in the air, water, plant and animal tissues. The inhalation or absorption of Cd from various sources may lead to its accumulation in the body [31,32]. The findings of the present study suggest that exposure to Cd leads to accumulation of Cd in the liver and kidney of rats over a period of 30 days. The results are in consonance with earlier reports [30,4]. Cd accumulates mainly in the liver and kidney and has a long half-life in an organism [6,7]. In long term chronic occupational exposure to Cd, kidney is usually the most critically affected organ [4,32]. Kidney is well known to be a major target organ of Cd in animals and humans. During chronic exposure the heavy metal Cd accumulates in renal cortex upto what appears to constitute a critical level at which the incidence of overt mal function in a human population at risk begins to increase. Cd absorption and accumulation in the tissues depends on many factors, chief among them being the

dose, route of administration, interaction with other substances and rate of elimination from the body.

30 d Cd treated rat kidney (42.80 ± 0.30 µg/g wet weight of the tissue) and liver (39.08 ± 0.64 µg/g wet weight of the tissue) showed greater accumulation of Cd concentration when compared to controls (Figure. 1). The high levels of Cd accumulation in both liver and kidney over time might be due to involvement of these organs in the detoxification and moreover being the major organs of metabolic activities [33]. Further, it might also be transported / routed into these organs from other tissues in the body for the purpose of subsequent elimination.

From the observed pattern of Cd accumulation in the tissues, it is obvious that the kidney showed high concentration of Cd load than liver [34,35]. It might be due to as and when the Cd enters into the body, it reaches the liver through circulation and induces the synthesis of MT in liver tissue [36,37] and forms Cd-MT complex. Thus formed Cd-MT complex is further transported to kidney [4,38] continuously and there it may accumulate more. Because kidney acts as a detoxifying organ [39,40] and also involved in the elimination of Cd. 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 is taken up by lysosomes and degraded by proteases and releases Cd, which may result in renal accumulation of the metal. Thus, these factors might have accounted for the raised level of Cd in the kidney during Cd treatment. Present observations are in agreement with the previous reports of Massanyi *et al.,* (2003) [39] and Linde *et al.,* (2004) [40] in rats and also the same was reported by Usha Rani, (2000) [10] and Obaiah and Usha Rani, (2014) [41] in fresh water teleost, *Oreochromis mossambicus exposed* to Cd.

Cd not only bio-accumulates but also accumulation of Cd is known to disturb the essential micronutrients distribution in the tissues of organisms [42]. In rats treated with Cd, there was a significant decrease in the levels of essential micronutrients such as Fe, Cu, Zn and Se as compared to normal control [43]. This may be due to interference of Cd on absorption and transport of these essential micronutrients, which might have resulted in the depletion of these metals in Cd treated rats. One of the most important characteristics of Cd toxicity is its interaction with physiologically essential micronutrients [44]. Several essential micronutrients like Zn, Fe, Se, Ca and Cu participate in controlling various metabolic and signaling pathways [5,38]. Among the essential micronutrients Zn is required for maintenance of life and health [42].

Zn is an essential trace metal with numerous functions in biological systems. It controls several enzymes of intermediary metabolism, DNA and RNA synthesis, gene expression, immunocompetence and plays a significant role in homeostasis of hormones. Zn takes part in the defense against excessive amounts and following damage of certain metals, and it does so through the interaction with MT. It has been noted that Zn has a relationship with many enzymes in the body and can prevent cell damage through activation of the antioxidant defense system [18,17]. The toxicity of Cd may result from disturbances in Zn metabolisms leading to the disruption of Cd as an antimetabolite of Zn.

One of the important findings of the present study is that supplementation with Zn and / or vitamin C significantly reduces Cd burden in the liver and kidney of Cd treated rats. The interactions between Zn and Vit-C with Cd is poorly understood, however, it is believed that Cd competes for Zn thereby displacing Zn in the vital organs[45,46,43]. Essential micronutrients and Vit-C supplementation has shown protective effect against Cd accumulation and toxicity in rats fed with inorganic Cd salt [47, 48, 41].

Supplementation of Zn either alone or in combination with Fe greatly reduced the Cd body burden in the tissues [49,48, 44, 5]. Zn functions as a complex antioxidant. It has the ability to form coordinating bonds with electronegative atoms [50,]. It regulates MT synthesis. Zn inhibited oxidative stress induced by Cd [45]. Zn prevented damage to the tissues from Cd exposure. This suggests Cd interference with Zn related metabolic functions. The competitive mechanism of interaction is a plausible mechanism of Zn in relation to Cd toxicity. Interactions between Cd and Zn occurs as early as in an intestine during absorption, but more intensive interactions take place during accumulation in the tissues.

Interactions of Cd and Zn have been widely studied in experimental animals under condition of oral ingestion of Cd. It has been shown that Cd may inhibit Zn activities at many stages interfering with its absorption, distribution to different tissues, transport into cells and / or transport into several intracellular structures [51,52,53]. The most compelling reason for the protective effects of Zn against Cd toxicity is that Zn induces the synthesis of the metal binding protein, MT in the tissues [54,55]. Interaction of Zn with Cd results in an increase in the excretion of Cd. This has been proposed as a mechanism by which Zn protects against Cd toxicity [56] because Zn and Cd competes for a common transport mechanism in the organisms. Thus, Zn supplementation has showed beneficial effects on Cd toxicity [46,57]. This may be the

reason for the reduced Cd accumulation in the test tissues supplemented with Zn in the present study.

It has also been suggested that addition of extra Ca/P, Zn and Vit-C to the diet results in a significant protection against Cd accumulation and toxicity in rats fed with inorganic Cd salt [58]. It seems clear that Cd speciation and the mineral status of the diet have a considerable impact on the extent Cd uptake in rats [59].

It is clear from the present investigation that the toxicity of Cd is affected by the supplementation of both Zn and vitamin C which in turn reduces the accumulation of Cd through competitive inhibition either at the metal binding sites of the enzymatic and non-enzymatic antioxidants and also displacement of Cd at MT protein in the test tissues. The essential micronutrient Zn and Vit-C compete with the Cd for the same binding sites. Increased bioavailability of these supplements in the body may result in reduction of Cd accumulation in liver and kidney. Similar findings were also reported by Li *et al*., 2000 [45], Martinez *et al*., 2001 [60], Piasek *et al.,* 2004 [48] and Bashandy *et al.,*2006 [57] in rats, Hollis *et al*., 2000 [61] in rainbow trout and Ghosh and Adhikari 2006 [62] in *Cirrhinamrigala.* Combined supplementation with Zn and vitamin C is one of the strategies that can be used to improve the iron and Zn status of organisms [63].

The mixture of Zn and vitamin C supplementation was more effective in reducing the Cd body burden than the individual traceelement supplementation there by enhancing the elimination of Cd from the body and binding to target proteins.

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