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RESEARCH ARTICLE

ZINC AND IRON SUPPLEMENTATION: A POSSIBLE DEFENSE AGAINST CADMIUM INDUCED BIOACCUMULATION IN LIVER AND KIDNEY OF MALE ALBINO RAT

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Abstract

Cadmium (Cd) is a highly toxic, non-essential heavy metal with many industrial uses that can contribute to a well – defined spectrum of diseases in animals as well as in humans. The present study is carried out to know the possible role of trace elements like zinc (Zn) and iron (Fe) supplementation to combat cadmium induced bioaccumulation in liver and kidney of Cd treated rats. Wistar strain male albino rats were treated with cadmium chloride 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 Fe (40 mg/Kg) alone and third group supplemented with both Zn and Fe and observed for again 7, 15 and 30d long sojurn. After the specific time intervals, rats were decapitated and tested for bioaccumulation levels of Cd by Atomic Absorption Spectrophotometer (AAS – Schimadzu AA6300). There was a significant elevation in Cd concentrations in both the test tissues with increased period of Cd treatment. Maximum Cd accumulation was found in 30d Cd treated rat kidney (31.5782 ± 0.499µg / gm wet wt. of the tissue). However there was significant reduction in Cd bioaccumulation with Zn and Fe supplements both individually and in combination suggesting their vital role in heavy metal detoxification. Maximum decrease in Cd accumulation was found in 30d rat kidney (8.327±0.450µg / gm wet wt. of the tissue) supplemented with the combination of Zn and Fe. Our findings clearly envisages that combined supplementation of Zn and Fe is more effective in reducing the Cd body burden when compared to the other modes of supplementation i.e. Zn and Fe alone in the male albino rat

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INTRODUCTION:

Metal toxicities have received widespread attention in recent years because of increasing amounts of heavy metals that are released into the environment and their extended persistence and toxicity to a wide variety of organisms. Development of civilization is paralelly accompanied by an enhancement of environmental contamination by many heavy metals such as mercury, arsenic, aluminium, chromium, lead, cadmium etc.,

Among the heavy metals, Cd is one of the common toxic heavy metal and is widely distributed in the environment. The accumulation of Cd is consistently increased when a certain amount is ingested continuously (Shibutani *et al.,* 2001). 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 eg. nephrotoxicity, neurotoxicity and carcinogenicity (Sethi, *et al.,* 2006), metabolic, histological changes, membrane damage, altered gene expression and apoptosis (Usha Rani, 2000; Siraj Basha and Usha Rani, 2003; Haki *et al.,* 2005; Ognjanovic *et al.,* 2008).

Some of the toxic effects of Cd exposure are hepatic damage, renal dysfunction, hypertension, central nervous system injury and testicular atrophy (Jeyaprakash and Chinnaswamy, 2005; Ognjanovic *et al.*, 2008). Although Cd is widely distributed throughout the body, most of it accumulates in the liver and kidney and alters biochemical and functional changes in the organs. Cd can provoke homeostasis alteration of physiological trace elements such as zinc (Zn), copper (Cu), selenium (Se), calcium (Ca) and iron (Fe) (Huang, *et al.*, 2006), which are the constituents of several enzymes and proteins.

Zn is a ubiquitous essential trace element with numerous functions in biological systems. It occurs in all living cells as a constituent of metallo enzymes 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 (Moulis, 2010). Zn controls several enzymes of intermediary metabolism, DNA and RNA synthesis, gene expression, immunocompetence and plays a significant role in homeostasis of hormones (Brandao *et al.*, 1995).

Fe plays an essential role in biological processes and is important to maintain iron concentration within its narrow normal range (Park *et. al.*, 2002). It is also a vital component of several enzymes and proteins. Fe is essential for maintaining proper cell functions and is normally tightly controlled by transporters and storage proteins (Ram *et al.*, 2000). Fe has several vital functions in the body including transportation of oxygen to body tissues and as a transport medium for electrons within cells. It is an integrated part of many important reactions in various tissues.

Hence, an attempt is made in the present study on the interactions of Zn and / or Fe against Cd induced bioaccumulation in liver and kidney of a mammalian model, rat.

MATERIALS AND METHODS:

Chemicals: Cadmium as cadmium chloride ($CdCl_2$), zinc as zinc chloride ($ZnCl_2$), and iron as ferric chloride ($FeCl_3$) 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.

Animals: Three months old Wistar strain male albino rats weighing 180 ± 20g 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 ± 2^0 C, 50 ± 20% relative humidity, 12h light - dark cycle) with Standard rat chow (Sai Durga 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.10(ii) / a / CPCSCA / IAEC / SVU / AUR-JO dt 22-12-2008), 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 deionised water without Cd. The experimental rats were treated with Cd as $CdCl_2$ at a dose of $1/10^{th}$ LD_{50} /48h i.e. 22.5 mg / Kg body weight over a period of 7, 15 and 30 days (d) time intervals. Then the 15d Cd treated rats were divided into three groups. Group I received supplementation of Zn (12mg / Kg) for 7, 15 and 30d. Group II received Fe supplementation (40 mg / Kg) and Group III animals were supplemented with both Zn and Fe at the above said doses for 7, 15 and 30d 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 bioaccumulation studies.

BIO-ACCUMULATION STUDIES:

Cd concentrations in the test tissues were measured by the method of Kanno *et al.*, (1994). After the specified time intervals the tissues liver and kidney were isolated and immediately they were 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 5ml 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 and Analysis of variance (ANOVA) using standard statistical software, SPSS (version 16) software. All values are expressed as Mean ± SD of 6 individual samples. Significant differences were indicated at $P < 0.05$ level.

RESULTS:

Bio-accumulation of Cd concentration was analyzed in liver and kidney of control, Cd treated and Zn and / or Fe 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. 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 30d Cd (31.578 ± 0.499 µ g / g) than liver (28.4871± 0.125 µ g / g).

Table – 1: Cd accumulation (µg / g wet weight of the tissue) in the tissues of rats.

Tissue	Control	7d Cd	15d Cd	30d Cd
Liver	0.015^{a} ± 0.002	8.649^{b} ± 0.272	16.019^{c} ± 0.085	28.487^{d} ± 0.125
Kidney	0.023^{a} ± 0.0029	11.778^{b} ± 0.094	19.326^{c} ± 0.062	31.578^{d} ± 0.499

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

Means of the same row followed by different letter (s) differ significantly ($p<0.05$)

Zn and / or Fe supplementation significantly decreased the bioaccumulation levels of Cd in both the test tissues for all the time intervals. Maximum decrease in Cd bioaccumulation levels was observed in 30d rat kidney under the combination of both the supplements Zn and Fe (8.327 ± 0.450 µ g/g) than the other modes of supplementation at all the time intervals (Table- 2).

Table – 2: Cd concentrations (µg / g wet weight of the tissue) in the tissues of rats after supplementation with the combination of Zn and Fe.

Tissue	15d Cd	7d Zn and Fe	15d Zn and Fe	30d Zn and Fe
Liver	16.019^{a} ± 0.085	13.378^{b} ± 0.454	11.516^{c} ± 0.414	8.138^{d} ± 0.198
Kidney	19.326^{a} ± 0.062	15.319^{b} ± 0.362	13.451^{c} ± 0.435	8.327^{d} ± 0.450

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

Means of the same row followed by different letter (s) differ significantly ($p<0.05$).

However with Fe 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 30d liver tissue (7.047 ± 0.119 µg / g) than kidney tissue (9.512 ± 0.438 µg / g).

Table – 3: Cd concentrations (µg / g wet weight of the tissue) in the tissues of rats after supplementation with Fe.

Tissue	15d Cd	7d Fe	15d Fe	30d Fe
Liver	16.019^{a} ± 0.085	13.849^{b} ± 0.452	10.835^{c} ± 0.289	7.047^{d} ± 0.119
Kidney	19.326^{a} ± 0.062	15.331^{b} ± 0.408	13.560^{c} ± 0.493	9.512^{d} ± 0.438

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

Means of the same row followed by different letter (s) differ significantly ($p<0.05$)

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 (10.798 ± 0.323 µ g / g) than the liver tissue (10.578 ± 0.440µ g / g) at all the time intervals (Table – 4). From the results, it can clearly envisages that Zn and Fe supplementation for 30d duration showed a tremendous reduction in the Cd body burden for both the tissues and moreover the decreased rate of accumulation was highly significant.

Table – 4: Cd concentrations (µg / g wet weight of the tissue) in the tissues of rats after supplementation with Zn.

Tissue	15d Cd	7d Zn	15d Zn	30d Zn
Liver	16.019^{a} ± 0.085	14.283^{b} ± 0.443	12.842^{c} ± 0.356	10.578^{d} ± 0.440
Kidney	19.326^{a} ± 0.062	16.517^{b} ± 0.422	14.024^{c} ± 0.014	10.798^{d} ± 0.323

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

Means of the same row followed by different letter (s) differ significantly ($p<0.05$)

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 Fig – 1. The Cd accumulation levels were elevated in the test tissues with the increased time of Cd treatment (Shibutani *et al*., 2001). Similar trend was also observed in the present study. Bioaccumulation and bio-magnification are the characteristic features of heavy metals including Cd. 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 (Uleckiene and Zabulyte, 2002; Paltanaviciene *et al*., 2006; Zabulyte *et al*., 2007). 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 (Shibutani *et al*., 2001; Nad *et al.*, 2005). Cd accumulates mainly in the liver and kidney and has a long half life in an organism (Nordberg *et al.*, 2007; Tim *et al.*, 2008). In long term chronic occupational exposure to Cd, kidney is usually the most critically affected organ (Nad *et al.*, 2005; Zabulyte *et al.*, 2007). 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. 30d Cd treated rat kidney (32.578 ± 0.499 µg/g wet weight of the tissue) and liver (28.487 ± 0.125 µg/g wet weight of the tissue) showed greater accumulation of Cd concentration when compared to controls (Fig. 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 (Klaassen *et al*., 2009). 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 (Bhattacharyya, 2000; Yilmaz, 2005). 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 (Brzoska *et al*., 2000; Alhazza, 2008) and forms Cd-MT complex. Thus formed Cd-MT complex is further transported to kidney (Nad *et al*., 2005; Asagba, 2009) continuously and there it may accumulate more. Because kidney acts as a detoxifying organ (Massanyi *et al*., 2003; Linde *et al*., 2004) 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) and Linde *et al.*, (2004) in rats and also the same was reported by Usha Rani, (2000) in fresh water teleost, *Oreochromis mossambicus* exposed to Cd.

Cd not only bio-accumulates but also accumulation of Cd is known to disturb the trace elements distribution in the tissues of organisms (Turgut *et al*., 2007). In rats treated with Cd, there was a significant decrease in the levels of essential trace elements such as Fe, Cu, Zn and Se as compared to normal control (Jeyaprakash and Chinnaswamy, 2005). This may be due to interference of Cd on absorption and transport of these trace elements, 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 trace elements (Renata and Izabela, 2007). Several essential trace elements like Zn, Fe, Se, Ca and Cu participate in controlling various metabolic and signaling pathways (Peraza *et al.*, 1998; Flora *et al.*, 2008; Asagba, 2009). Among the essential trace elements Zn and Fe are required for maintenance of life and health (Turgut *et al*., 2007). 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 (Ozturk *et al*., 2003; Ozdemir and Inanc, 2005). The toxicity of Cd may result from disturbances in Zn metabolisms leading to the disruption of Cd as an antimetabolite of Zn.

Fe plays an essential role in many biological processes. It is absolutely vital to life (Weinberg, 2004; Zecca *et al*., 2004; Turgut *et al*., 2007). Fe is a central element of the heme molecule, which is a critical part of hemoglobin and essential for Oxygen transport. Heme is also a part of many essential enzymes such as the cytochrome series and catalase (Brewer, 2007). It is essential for maintaining proper cell functions and is normally tightly controlled by transporter and storage proteins (Ram *et al.*, 2000). One of the important findings of the present study is that

supplementation with Zn and / or Fe significantly reduces Cd burden in the liver and kidney of Cd treated rats (Table – 2, 3 and 4). The interactions between trace elements such as Zn and Fe with Cd is poorly understood, however, it is believed that Cd competes for Zn and Fe thereby displacing Zn and Fe in the vital organs (Li *et al.*, 2000; Gunnarsson *et al.*, 2004; Jeyaprakash and Chinnaswamy, 2005). Trace elements supplementation has shown protective effect against Cd accumulation and toxicity in rats fed with inorganic Cd salt (Matek *et al*., 2002; Piasek *et al*., 2004).

Supplementation of Zn either alone or in combination with Fe greatly reduced the Cd body burden in the tissues (Peraza *et al.*, 1998; Saric *et al.*, 2002; Piasek *et al.*, 2004; Renata and Izabela, 2007; Flora *et al.*, 2008). Zn functions as a complex antioxidant. It has the ability to form coordinating bonds with electronegative atoms (Prasad *et al.*, 2004). It regulates MT synthesis. Zn inhibited oxidative stress induced by Cd (Li *et al.*, 2000). 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 (Oishi *et al.*, 2000; Lonnerdall, 2000; Satarug *et al.*, 2001). 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 (Bonda *et al.*, 2004; Amara *et al*., 2008). 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 (Dilek and Cengiz, 2008) because Zn and Cd competes for a common transport mechanism in the organisms. Thus, Zn supplementation has showed beneficial effects on Cd toxicity (Gunnarsson *et al.*, 2004; Bashandy *et al.*, 2006). This may be the reason for the reduced Cd accumulation in the test tissues supplemented with Zn in the present study.

In several studies dietary iron appeared to decrease Cd toxicity but the exact mechanism of this interaction is unknown (Matinez *et al.*, 2001). Fe supplementation reduces Cd retention and Cd induced anemia during fast growth in young rats (Schumann, 1996). It has been postulated that in addition to ascorbic acid, Fe has ability to inhibit the intestinal absorption of Cd (Martinez *et al.*, 2001). It has been suggested that Cd exposure reduces dietary iron absorption in experimental animals and thus Fe supplementation competes with Cd thereby interfering with Cd bio-accumulation (Oishi *et al.*, 2000; Piasek *et al.*, 2004). The concentration of Fe in the diet is known to have impact on the absorption of Cd and its disposition in organs and tissues. Oral Cd exposure (such as contaminated food stuffs or industrial wastes) may reduce bioavailability of dietary iron for absorption (Piasek *et al.*, 2004). Earlier investigators have shown that Fe absorption is strongly inhibited by Cd (Oishi *et al.*, 2000). Further, Fe and Cd most probably compete at the binding sites of the iron intestinal transfer system (IITS) (Bhattacharyya *et al.*, 2000; Tallkvist *et al.*, 2001; Park *et al.*, 2002).

Intake of dietary iron depends on biological bioavailability and physiological demands and loss. This may suggest that the supplementation of Fe in the present study may be helpful in the absorption of Fe at high level, which may involve in eliminating the Cd from the tissues. Absorption of Fe takes place in the same section of the intestine as Cd. They compete for the same proteins, ferritin and transferrin. In physiological conditions, both proteins are responsible for Fe intake and transfer within the body. Cd, through binding to ferritin in the intestinal mucosa, lowers iron intake and results in decreased iron levels in the tissues, especially in the liver. The protein transferrin which donates Fe to the heme moity in haemoglobin synthesis, can bind to a variety of metals including Cd (Renata and Izabela, 2007). Ferritin or transferrin might have been involved in the Cd-Fe interactions observed during Cd intoxication. Cd-Fe interactions have shown that ascorbic acid protects against anemia. Ascorbic acid doesn't have a direct effect on Cd, but the nutrient improves Fe absorption in the gastrointestinal tract. It has also been postulated that in addition to this effect ascorbic acid plus Fe might inhibit the intestinal absorption of Cd (Martinez *et al.*, 2001). Hence, it might be one of the reasons for the low levels of Cd accumulation in 30d Fe supplemented liver tissue (7.047 ± 0.119 µ g / g) (Fig. 3). Other plausible reason for reduced Cd concentrations in the selected tissues might be the tighter binding of Cd to the high molecular weight iron-citrate complexes than to citrate which may affect its absorption and thus provide a possible protection against oral Cd toxicity (Martinez *et al.*, 2001). Our results are in agreement with the earlier reports of Casalino *et al*., (1997), Jeyaprakash and Chinnaswamy, (2005) and Turgut et al., (2007) in rats under Cd stress. Supplementation of Fe alone showed maximum reduction of Cd tissue burden in liver than in kidney during experimental intervals of 7, 15 and 30days which is evidenced by the absorbed Fe eliminating Cd from the test tissues. Low iron in diet was associated with significant and dose related increase in Cd concentration in the liver. These findings indicate that administered Cd interacts with dietary iron and further on the Cd bio-accumulation in the liver. It has also been suggested that addition of extra Ca/P, Zn and Fe to the diet results in a significant protection against Cd accumulation and toxicity

in rats fed with inorganic Cd salt (Groten *et al.,* 1992; Park *et al.,* 2002). It is clear from the present investigation that the toxicity of Cd is affected by the supplementation of both Zn and Fe 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. These essential trace elements Zn and Fe 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), Martinez *et al*., (2001), Piasek *et al.,* (2004) and Bashandy *et al.,* (2006) in rats, Hollis *et al*., (2000) in rainbow trout and Ghosh and Adhikari (2006) in *Cirrhina mrigala.* Combined supplementation with Zn and Fe is one of the strategies that can be used to improve the iron and Zn status of organisms (Olivares *et al.,* 2007). The mixture of Zn and Fe supplementation was more effective in reducing the Cd body burden than the individual trace element supplementation thereby enhancing the elimination of Cd from the body and binding to target proteins.

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