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ZINC AND VITAMIN-E IMPACT ON CADMIUM INDUCED BIOACCUMULATION IN LIVER AND KIDNEY OF MALE ALBINO RAT

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ABSTRACT

Cadmium (Cd) is a toxic, non-essential and industrial pollutant. The aim of the present work is to know the zinc (Zn) and vitamin-E impact on Cd induced bioaccumulation in liver and kidney of Cd treated rats. Wistar strain male albino rats were treated with cadmium chloride at a dose of 1/10th LD50 / 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 vitamin-E (300 mg/Kg) alone and third group supplemented with both Zn and vitamin-E for again 7, 15 and 30d long sojourn. After the specific time intervals, rats were decapitated and tested for Cd bioaccumulation levels 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 (32.78 ± 0.29µg / gm). However there was a significant reduction in Cd bioaccumulation with Zn and vitamin-E supplementation. Maximum decrease in Cd accumulation was found in 30d rat kidney (8.62±0.15µg / gm) supplemented with the combination of Zn and vitamin-E. Our findings clearly envisage that combined supplementation of Zn and vitamin-E is more effective in reducing the Cd body burden when compared to the other modes of supplementation.

KEYWORDS: Cadmium, Bioaccumulation, Zinc, Vitamin-E, Rat.

INTRODUCTION

Environmental pollution was caused by numerous chemicals, xenobiotics, heavy metals and so on. Among the different heavy metals, Cadmium (Cd) is one of the most toxic, non- essential industrial pollutant and can contribute to a well-defined spectrum of diseases in animal models as well as in humans.[1,2] Cd has an extremely long half-life (20-30 Years) and is highly accumulate mainly in the liver and kidney of an organism.[3-9] The main sources of Cd are storage batteries, electroplating, pigments, plastics, fertilizer industries and cigarette smoking. The kidney is considered as the critical organ in long term low level exposure to Cd.[10, 11]

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 hepatotoxicity, nephrotoxicity, neurotoxicity, carcinogenicity, metabolic activities, histological changes, membrane damage, gene expression and apoptosis.[12,13] Some of the toxic effects of Cd exposure are hepatic damage, renal dysfunction, hypertension, central nervous system injury and testicular atrophy.[14-16]

Cd like many other heavy metals is antagonistic to essential trace elements like zinc (Zn), selenium (Se), iron (Fe), copper (Cu), calcium (Ca) etc., and antioxidant vitamins. The heavy metals always compete with these trace elements and antioxidant vitamins for binding sites as transport and storage.[17]

Zn is an essential trace element for good health. It is a ubiquitous antioxidant 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. Zn controls several enzymes of intermediary metabolism, DNA and RNA synthesis, gene expression, immunocompetence and plays a significant role in homeostasis of hormones.[18-20] 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.[21,22]

Vitamin E (Alpha-tocopherol) is the major lipid-soluble, naturally occurring antioxidant in biological systems and is present in the cell membrane of various tissues, which protects cells against lipid peroxidation.[23,24] Vitamin E plays an important role in controlling the oxidative stress and recognized as essential nutrient for all species of animals. It prevents free radical-induced injury by blocking the free radical chain reaction. Its reaction with active radicals produces tocopheroxyl radicals, being further reduced to tocopherol via vitamin C or GSH.[25,26] In other words, vitamin E has been shown to have protective effect against metal induced toxicity.[27-29]

Therefore, the present study was designed to evaluate the impact of Zn and vitamin E separetely and in combination against cadmium chloride induced toxicity in rats.

MATERIALS AND METHODS

Chemicals

Cd as cadmium chloride ($CdCl_2$), Zn as zinc chloride ($ZnCl_2$) and vitamin-E 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, India. The chemicals used in this study were of the highest purity.

Animals

3 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 ± 2°C, 50 ± 20% relative humidity, 12-h 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 deionized water without Cd. The experimental rats were treated with Cd 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 15d Cd treated rats were divided into three groups. The first group received Zn (12 mg/Kg), second group vitamin-E (300 mg/Kg) alone and third group supplemented with both Zn and vitamin-E for again 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 analysis of bioaccumulation levels and were stored at −80°C for future use.

Bio-accumulation studies

Cd concentrations in the test tissues were measured by the method of Kanno *et al*.,[30] 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 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 (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 $P< 0.05$ level.

RESULTS

Bio-accumulation of Cd concentration was analyzed in liver and kidney of control, Cd treated and Zn and /or Vitamin E 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. Cd accumulation was high in the kidney of rats treated for 30d than liver (Fig. 1).

Zn and / or Vitamin E 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 Vitamin E than the other modes of supplementation at all the time intervals (Fig. 2).

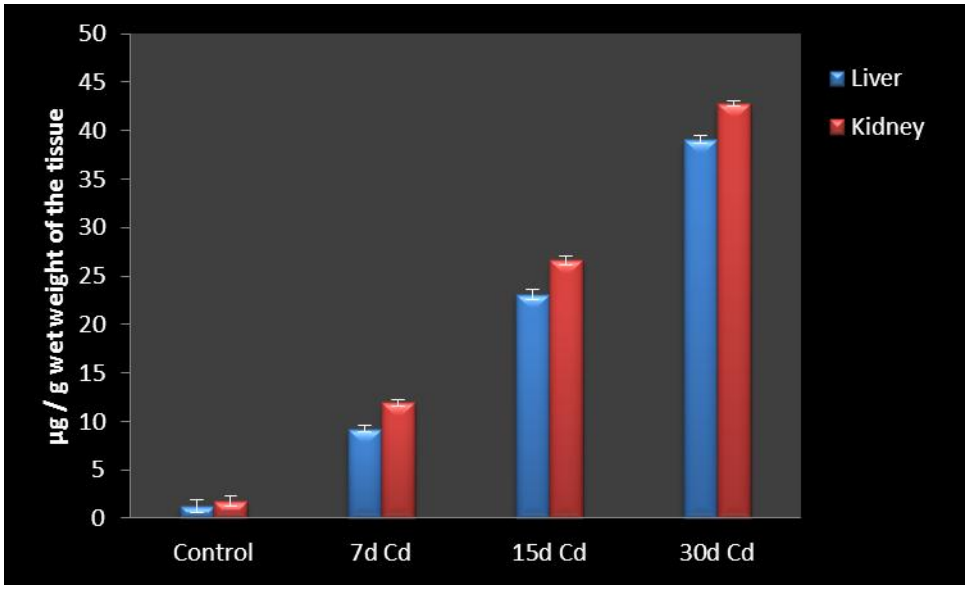

Fig.1: Cd Bio-accumulation levels in liver and kidney tissues of Cd treated rats. Values are expressed as mean ± SD (n = 6 rats in each group). Statistical significance was evaluated by one-way analysis of variance (ANOVA) and the Duncan's Multiple Range Test (DMRT). All experimental mean values are significant at P<0.05 level over control.

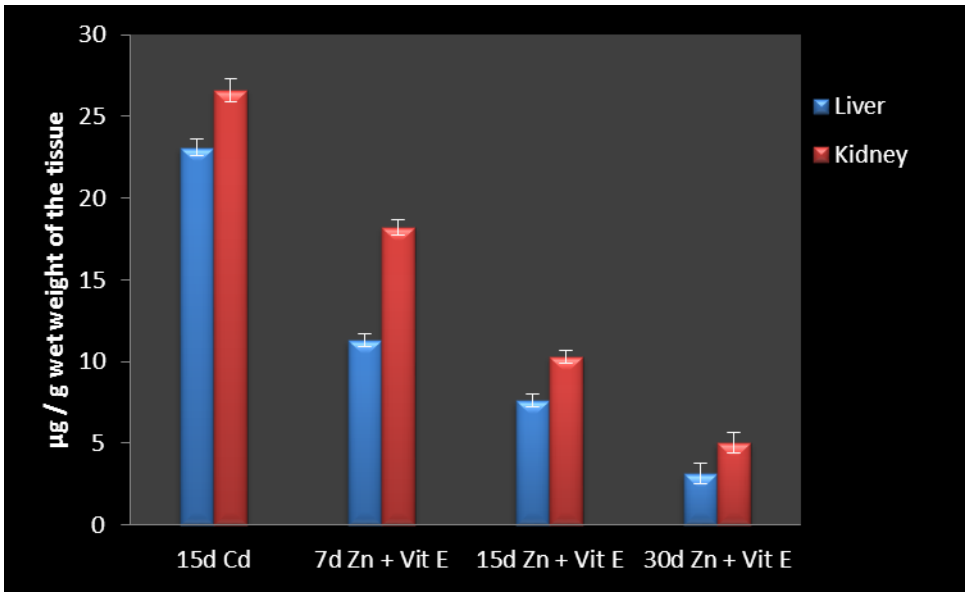

Fig.2: Cd Bio-accumulation levels in liver and kidney tissues of both Zn and vitamin E supplemented Cd treated rats. Values are expressed as mean ± SD (n = 6 rats in each group). Statistical significance was evaluated by one-way analysis of variance (ANOVA) and the Duncan's Multiple Range Test (DMRT). All experimental mean values are significant at P<0.05 level over control. 15d Cd treated rats considered as control for supplementation groups.

However with Vitamin E alone supplementation, we could find both kidney and liver tissues showing medium levels of decrease in Cd concentrations. Maximum reduction was found in 30d liver tissue than kidney tissue (Fig. 3). 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 than the liver tissue at all the time intervals (Fig. 4).

From the results, it can clearly envisages that Zn and Vitamin-E supplementation for 30d 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.

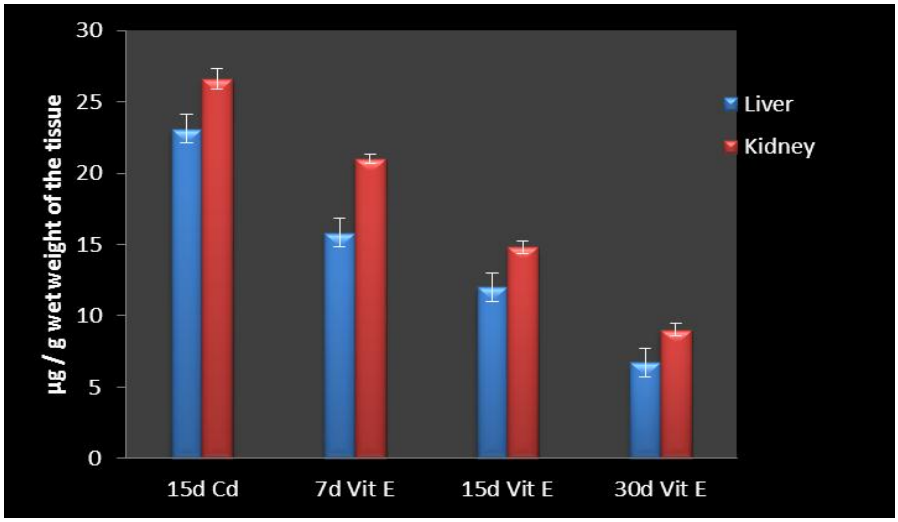

Fig.3: Cd Bio-accumulation levels in liver and kidney tissues of vitamin E supplemented Cd treated rats. Values are expressed as mean ± SD (n = 6 rats in each group). Statistical significance was evaluated by one-way analysis of variance (ANOVA) and the Duncan's Multiple Range Test (DMRT). All experimental mean values are significant at P<0.05 level over control. 15d Cd treated rats considered as control for supplementation groups.

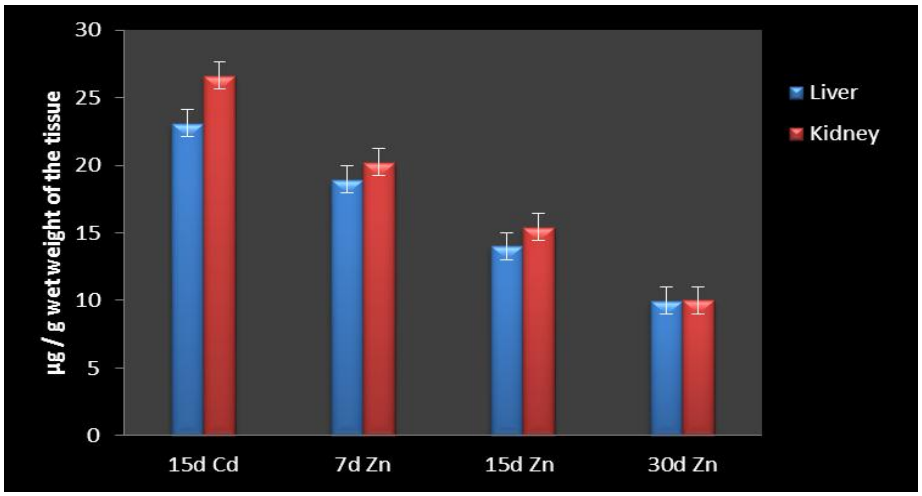

Fig.4: Cd Bio-accumulation levels in liver and kidney tissues of Zn supplemented Cd treated rats. Values are expressed as mean ± SD (n = 6 rats in each group). Statistical significance was evaluated by one-way analysis of variance (ANOVA) and the Duncan's Multiple Range Test (DMRT). All experimental mean values are significant at P<0.05 level over control. 15d Cd treated rats considered as control for supplementation groups.

DISCUSSION

Cadmium is a non-essential, very toxic metal and also an environmental and industrial pollutant which is present in soil, water, air and food. This metal causes variety of toxic effects on various body tissues of both human and animals. The present work mainly 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.[31-36]

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.[9,37-39] 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 of Shibutani *et al*.,[31, 40] Cd accumulates mainly in the liver and kidney and has a long half-life in an organism.[41,42] In long term chronic occupational exposure to Cd, kidney is usually the most critically affected organ.[39,40] 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 and liver showed greater accumulation of Cd concentration when compared to controls (Fig. 1). High levels of Cd accumulation found 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 [43]. 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.[44,45] 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 [46,47] and forms Cd-MT complex. Thus formed Cd-MT complex is further transported to kidney[40,48] continuously and there it may accumulate more. Because kidney acts as a detoxifying organ[49,50] 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.*,[49] and Linde *et al.*,[50] in rats and also the same was reported by Usha Rani[51] and Obaiah and Usha Rani[33,34,36,52] 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 dietary nutrients distribution in the tissues of organisms.[53] In rats treated with Cd, there was a significant decrease in the levels of essential nutrients as compared to normal control.[15] This may be due to interference of Cd on absorption and transport of these essential nutrients, which might have resulted in the depletion of these nutrients in Cd treated rats. One of the most important characteristics of Cd toxicity is its interaction with physiologically essential micronutrients and antioxidant vitamins.[54] Several essential micronutrients like Zn, Fe, Se, Ca, Cu and antioxidant vitamins participate in controlling various metabolic and signaling pathways.[5,48,55] Among the essential micronutrients Zn is required for maintenance of life and health.[53]

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.[21,22] 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 Vit-E significantly reduces Cd burden in the liver and kidney of Cd treated rats. The interactions between Zn and Vit-E with Cd is poorly understood, however, it is believed that Cd competes for Zn thereby displacing Zn in the vital organs.[15,56,57] Essential micronutrients and Vit-E supplementation has shown protective effect against Cd accumulation and toxicity in rats fed with inorganic Cd salt.[7-10,29,58,59,60]

Supplementation of Zn either alone or in combination with Vit-E greatly reduced the Cd body burden in the tissues.[5,54,55,59] Zn functions as a complex antioxidant. It has the ability to form coordinating bonds with electronegative atoms. It regulates MT synthesis. Zn inhibited oxidative stress induced by Cd.[56] and 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.[61-63] 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.[64,65] 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[66] because Zn and Cd competes for a common transport mechanism in the organisms. Thus, Zn supplementation has showed beneficial effects on Cd toxicity.[18,57] 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 Zn and Vit-E to the diet results in a significant protection against Cd accumulation and toxicity in rats fed with inorganic Cd salt.[67] It seems clear that Cd speciation and the mineral status of the diet have a considerable impact on the extent Cd uptake in rats.[67]

It is clear from the present investigation that the toxicity of Cd is affected by the supplementation of both Zn and Vit-E 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-E 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*.,[56] Martinez *et al*.,[68] Piasek *et al.*,[59] and Bashandy *et al.*,[18] in rats, Hollis *et al*.,[69] in rainbow trout and Ghosh and Adhikari[70] in *Cirrhinamrigala.* Combined supplementation with Zn and Vit-E is one of the strategies that can be used to improve the iron and Zn status of organisms.[71]

CONCLUSION

The mixture of Zn and Vit-E supplementation was more effective in reducing the Cd body burden than the individual trace elements and antioxidant vitamins supplementation thereby enhancing the elimination of Cd from the body and binding to target proteins.

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