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Role Of Selenium and Vitamins E And C In Combating Cadmium Bioaccumulation in The Selected Tissues of Rats: A Therapeutic Approach

Kutagolla Peera[1], Surekha Agraharam[2], Obaiah Jamakala[3]*

[1]Department of Zoology, Sri Venkateswara University, Tirupati-517502, Andhra Pradesh, India.
[2]Department of Botany, Sri Venkateswara Arts College, Tirupati-517502, Andhra Pradesh, India.
[3]Division of Environmental Toxicology and Animal Physiology, Department of Zoology, Sri Venkateswara College, University of Delhi, New Delhi-110021, India.

**Corresponding author's E-mail: obaiah@svc.ac.in*

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Abstract

The aim of the present investigation is to check the therapeutic role of selenium (Se) and vitaminE and C on cadmium (Cd) induced bioaccumulation in liver, kidney and testis of Cd treated rats. Wistar strain male albino rats were treated with cadmium chloride at a dose of 1/10th of 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 two groups. Group I supplemented with Se (1mg/kg body weight) and II group received combination of Se, vitamins E (300mg/kg) and C (200mg/kg) and observed for 7, 15 and 30days. After specific time intervals, rats were decapitated and tissues such as liver, kidney and testis were isolated and used for the estimation of Cd bioaccumulation levels by using Atomic Absorption Spectrophotometer (AAS – Schimadzu AA6300). There was a significant elevation in Cd concentrations in the test tissues with increased period of Cd treatment. Maximum Cd accumulation was found in 30d Cd treated rat kidney (42.80±0.30µg / gm). However, there was a significant reduction in Cd bioaccumulation with Se and vitamin E and C supplementation. Maximum decrease in Cd accumulation was found in 30d rat kidney (5.04 ±0.08µg / gm) supplemented with the combination of Se andvitamin E and C. Our findings clearly envisage that combined supplementation of Se and vitamin E and C is more effective in reducing the Cd body burden when compared to the individual mode of supplementation.

Keywords: *Cadmium, Bioaccumulation, Selenium, Vitamin E and C, Rat*

1. Introduction

Metal toxicity 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 (Honghua *et al.*, 2020; Cheng *et al.*, 2022). Development of civilization is parallelly accompanied by an enhancement of environmental contamination by several heavy metals such as mercury, arsenic, aluminium, chromium, lead, cadmium etc.,

Among the heavy metals, Cd is one of the most common toxic heavy metals 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; Genchi *et al.*, 2020). 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 (Sethi, *et al.*, 2006), metabolic, histological changes, membrane damage, altered gene expression and apoptosis (Usha Rani, 2000; Siraj Basha and Usha Rani, 2003; Ognjanovic*et al.*, 2008; Mehrdad *et al.*, 2017; Genchi *et al.*, 2020).

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; Satarug, 2018). 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 (Satarug *et al.*, 2020; Genchi *et al.*, 2020; Balali-Mood *et al*., 2021). 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) as well as small molecule antioxidants such as Vitamin E and Vitamin C, which are the constituents of several enzymes and proteins (Muszynska and Labudda, 2019; Unsal *et al.*, 2020; Jomova *et al.*, 2022).

She is an essential micro nutrient with numerous functions in biological systems. As a promising chemopreventive agent, its use requires consumption over the long turn. The physiological role of Se involves antioxidant protection because many Se-Cys containing proteins are antioxidant enzymes such as the glutathione peroxidases and non enzymaticantioxidant, glutathione (Schwizer*et al.*, 2004; Chen *et al.*, 2012). The biological activities of Se, as a nutrient, a cancer preventive agent or even a toxicant are dependent on the dose and the chemical form of Se. Seoccurs in all living cells as a constituent of GPx and GSH. It is involved in the metabolism of GSH which can reduce toxicity of Cd (Burk, 2002).

Se interacts with Cd in culture cells in relation to cellular uptake (Frisk *et al.*, 2002). It was also known that Se has a certain protective role from the toxic effects of Cd and other heavy metals (Bendich, 1990; Ognjanovic, 2008). This protection includes the capability of Se to alter the distribution of Cd in tissues and induces binding of the Cd-Se complexes to proteins, which are similar to metallothioneins (Jamba *et al.* 1997). Studies have demonstrated that Se-Cd by plasma selenoprotein P leading to the proposal that this protein may function to chelate Cd, reducing its toxicity. Several authors viz. Newairy*et al.*, (2007), reported that Se in the form of Se-Cys may enhance the production of MT and as an antioxidant it plays a major role in inhibition of ROS generated oxidative stress during Cd intoxication thereby mitigating the Cd toxicity and also it acts as a antioxidant in biological processes (Zhang *et al.*, 2020).

Vitamin E (α-tocopherol) is an important antioxidant, residing mainly in cell membranes. It is thought to interrupt the chain reactions involved in lipid peroxidation and to scavenge ROS generated during the univalent reduction of molecular oxygen. Both Vitamin C and E jointly protects lipid structures against Cd induced peroxidation (Frei, 1991).

Vitamin C (Ascorbic acid or ascorbate) is an important water-soluble antioxidant that reduces sulfhydryls, scavenges free radicals, and protects against endogenous oxidative DNA damage (Frei, 1991). Vitamin C, after being converted to dehydroascorbic acid by free radical reactions, is regenerated via the glutathione enzyme complex (Halliwell *et al.*, 1996). Vitamin C can prevent increased lipid peroxidation levels resulting from Cd toxicity (Rekha *et al.*, 2011).

Small molecule antioxidants such as Vitamin E and Vitamin C interact with oxidizing radicals (Gupta *et al.*, 2004). Vitamin E terminates the chain reaction of lipid peroxidation in the membranes and lipoproteins and Vitamin C scavenges aqueous ROS by rapid electron transfer and thus inhibits lipid peroxidation (Gupta *et al.*, 2004), as well as reducing the level of oxidized vitamin E (tocopheroxyl radicals). Ascorbate can regenerate tocopherol from the tocopheroxyl radical, which is formed when tocopherol reacts with a lipid peroxyl radical (Frei, 1991). Antioxidant compounds are known to be tightly linked in interlocking cycles of regeneration and recycling in which vitamin E and Vitamin C appears to play a major role (Gupta *et al.*, 2004; Rekha *et al.*, 2011).

2. Materials And Methods

Chemicals

Most of the chemicals and cadmium chloride were obtained from Sigma Chemical Co (St Louis, MO, USA) and other standard companies in India. 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 ± 20 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.

Animal Exposure

After acclimatization the animals were treated with Cd as $CdCl_2$ at the sub lethal dose 1/10 of LD_{50} (i.e.27.5mg/kgwt) which was chosen for the present study (Venkata Krishna Reddy, 1998) for 7, 15 and 30 days (d) period .Then the 15d Cd treated rats were divided into two groups. Group I supplemented

with Se (1mg/kg body weight) and II group received the combination of both Se and vitamins E (300mg/kg) and C (200mg/kg) and observed for 7, 15 and 30days.

Isolation of tissues

After specific time intervals, the control and experimental rats were decapitated and tissues such as liver, kidney and testis 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 such as liver, kidney and testis 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).

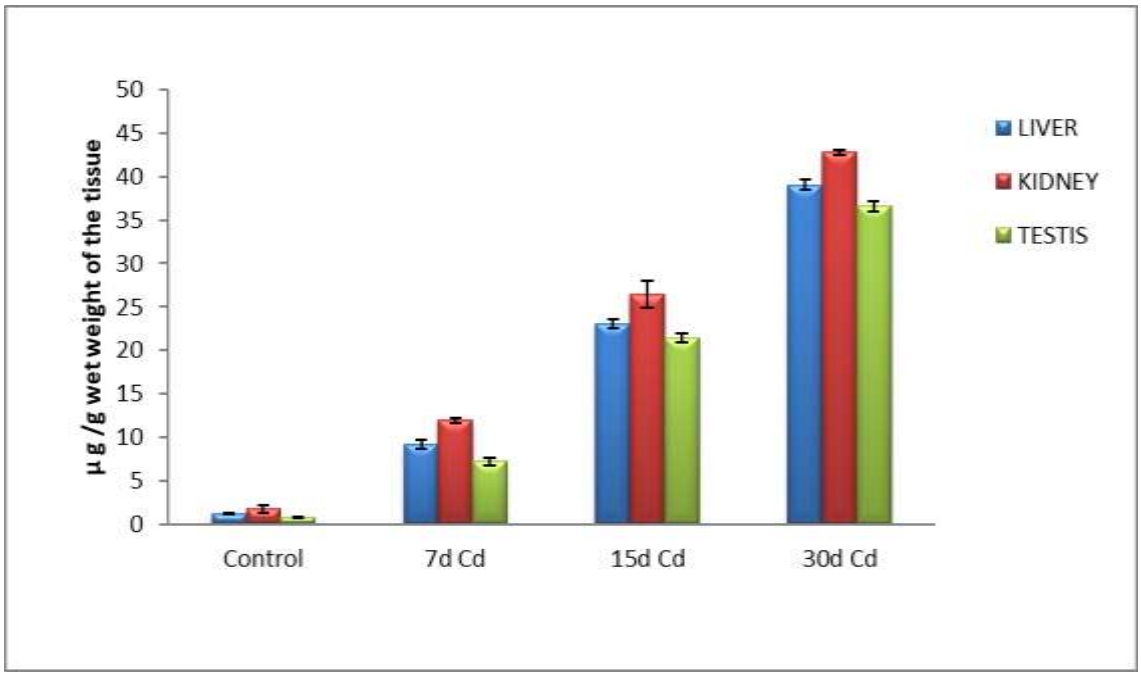
Data analysis

The data was subjected to statistical analysis such as mean, standard deviation (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.

3. Results and Discussion

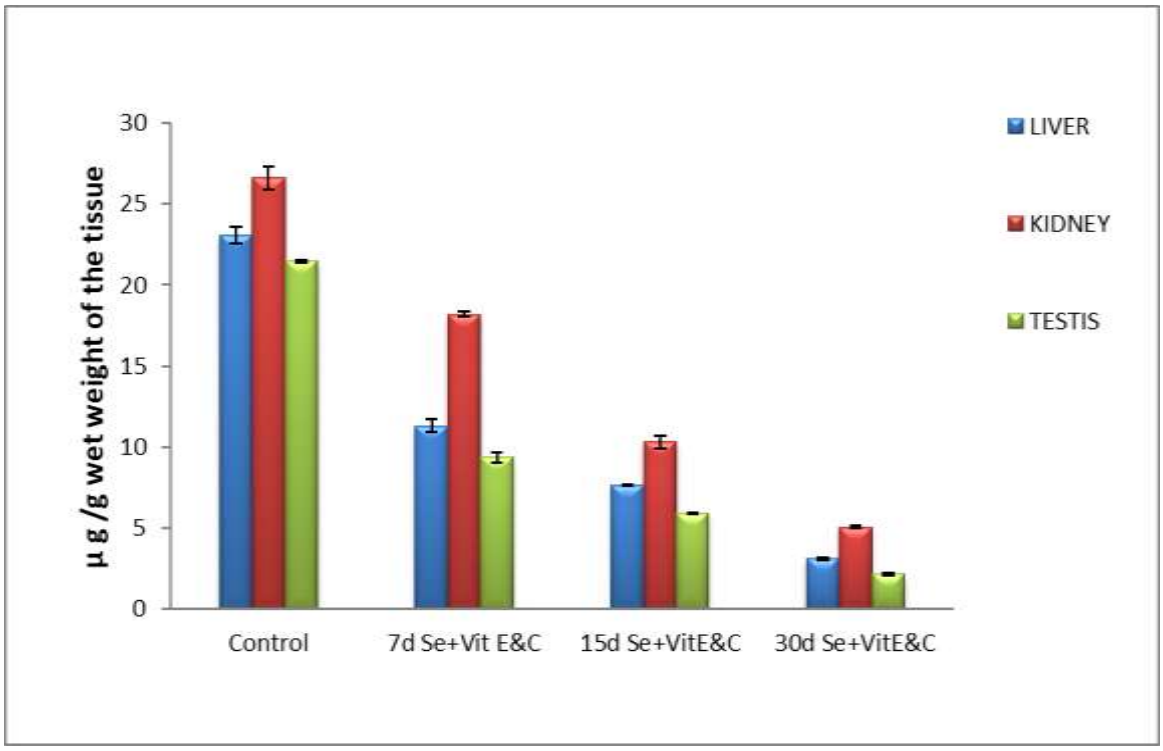
Bio-accumulation of Cd concentration was analyzed in liver, kidney and testis of control, Cd treated and Se and / or vitamins E and C supplemented male albino rats for the specified time intervals. The mean Cd levels were found to be significantly increased in the test tissuessuch as liver, kidney and testis of Cd treated rats when compared to the controls. The accumulation of Cd significantly increased with the increased duration of treatment (Fig. 1). Cd accumulation was high in the kidney of rats treated for 30d Cd (42.80±0.30µ g / g) than liver (39.08±0.64µ g / g) and testis (36.60±0.62µ g / g).

Fig.1: Cd bioaccumulation levels in the selected tissues of rats treated with Cd.



Se and/or vitamins E and C supplementation significantly decreased the bioaccumulation levels of Cd in all the test tissues for all the time intervals. Maximum decrease in Cd bioaccumulation levels was observed in 30d rat kidney under the combination of the supplements Se and vitamins E and C (5.04±0.08µ g/g) than the other modes of supplementation at all the time intervals (Fig.3).

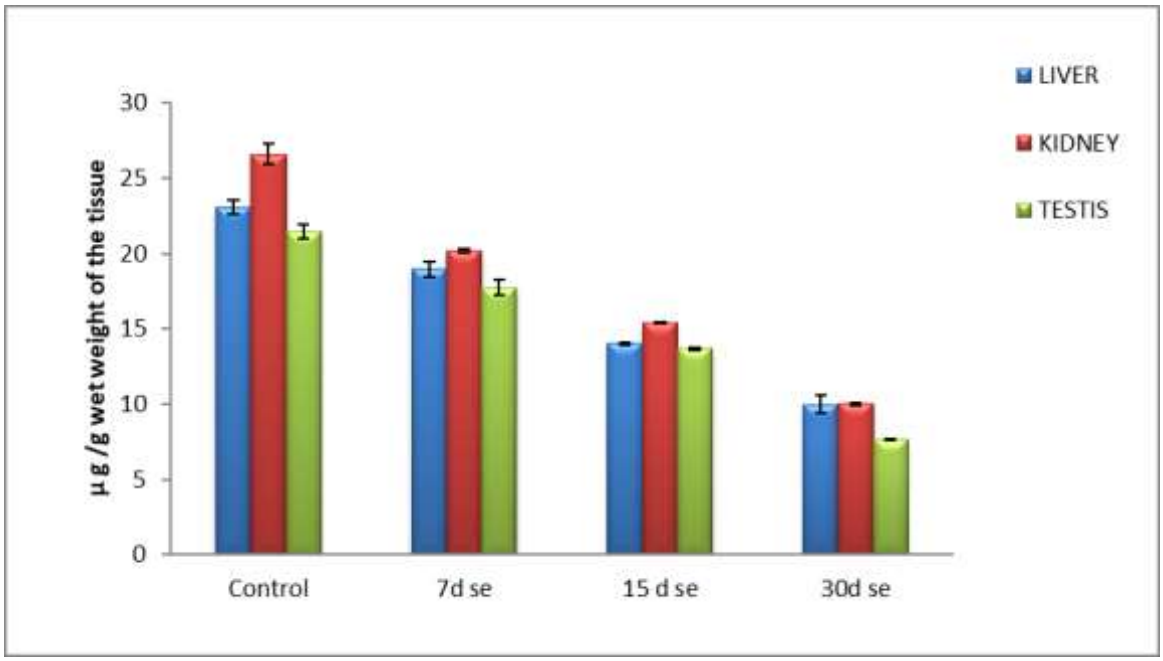
Fig. 3: Cd concentrations in the selected tissues of rats after supplementation with the combination of Se and Vit-E&C



- 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 mean values are significant at P<0.05 level over 15d Cd exposure

With Se alone supplementation, the test tissues showed low level of decrement than the combination of the supplements Se and vitamins E and C 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 (10.32±0.38µ g / g) than the liver tissue (7.62±0.05µ g / g) and testis (5.93±0.05µ g / g) at all the time intervals (Fig.2). From the results, it can clearly envisages that Se and/or vitamins E and C supplementation for 30d duration showed a tremendous reduction in the Cd body burden for all the tissues and moreover the decreased rate of accumulation was highly significant.

Fig-2: Cd concentrations in the selected tissues of rats after supplementation with Se.



- 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 mean values are significant at P<0.05 level over 15d Cd exposure

Cd is one of the most dangerous occupational and environmental toxicants 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, kidney and testis 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, kidney and testis of rats over a period of 30 days. The results are in consonance with earlier reports (Shibutani *et al.,* 2001; Nad *et al.,* 2005; Obaiah and Usha Rani, 2012,2014, 2015, 2016, 2017). Cd accumulates mainly in the liver and kidney and has a long half-life in an organism (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 (42.80±0.30 µg/g wet weight of the tissue) and liver (39.08 ± 0.64µg/g wet weight of the tissue) showed greater accumulation of Cd concentration than testis (36.60 ± 0.62µg/g wet weight of the tissue) when compared to control (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; Biswas and Mano, 2021). 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 (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 (Se, Zn, Cu, Fe, Ca etc.) and small molecule antioxidants (Vitamin E and Vitamin C) distribution in the tissues of organisms (Turgut *et al.,* 2007; Branca *et al*., 2018). 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 and Obaiah *et al*., 2009). 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 Seis required for maintenance of life and health (Turgut *et al.,* 2007). Se 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. She 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 Se 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 Se metabolisms leading to the disruption of Cd as an antimetabolite of Se.

Small molecule antioxidants such as Vitamin E and Vitamin C plays an essential role in many biological processes. They are absolutely vital to life (Turgut *et al.,* 2007). They are essential for maintaining proper cell functions. One of the important findings of the present study is that supplementation with Se and / or vitamin E and C significantly reduces Cd burden in the liver, kidney and testis of Cd treated rats. The interactions between trace elements (Se, Zn and Fe) and small molecule antioxidants (Vitamin E and Vitamin C) with Cd is poorly understood, however, it is believed that Cd competes for trace elements and small molecule antioxidants thereby displacing Se and small molecule antioxidants in the vital organs (Li *et al.,* 2000; Gunnarsson *et al.,* 2004; Jeyaprakash and Chinnaswamy, 2005). Trace elements and small molecule antioxidants 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).

Vit C and E play a beneficial role in reducing the toxicity and absorption of Cd (Grosicki, 2004; Amal El-Refaiyand FawzyEissa, 2013). Earlier studies on the beneficial role of Vit- C and E on Cd-induced organ toxicity have reported that antioxidant supplements with Vit-C and E play prophylactic effect in those pathophysiological situations (Koyuturk *et al.,* 2007: Acharya *et al.,* 2008; Amal El-Refaiy and FawzyEissa, 2013). The antioxidant properties of ascorbic acid and vit. E could be an important factor in the protection against Cd-induced DNA damages and tissue toxicity (Das *et al.,* 2002) and ascorbic acid has been reported as an anti-sister chromatid exchanges induced by cyclophosphamide in mice (Vijayalaxmi and Venu 1999). Generally, these results indicated that ascorbic acid and vit E have protective effects against hepatic injury caused by cyclophosphamide and it plays a role in increasing the antioxidant status as well as lowering the oxidative damage (Manda and Bhatia 2003; Senthilkumar *et al.,* 2006; Sugumar *et al.,*2007; Abdel-Fattah *et al.,* 2010; Jacob *et al.,* 2002; Nancy, 2013).

Vit- C and E areimportant dietary antioxidants and significantly decrease the adverse effects of Cd induced bioaccumulation and reactive species such as reactive oxygen that can cause oxidative damage to macromolecules such as lipids, DNA and proteins which are associated in several diseases (EL-Gazzar *et al.,* 2014). Vit- C and E protects DNA against damage induced by reactive oxygen species (Amal El-Refaiy and FawzyEissa, 2013). The combined effect of Se and Vit- C & E were stronger than the sum of the effects of the compounds applied separately.

4. Conclusion

Depletion of tissue reserves of endogenous antioxidants, ascorbic acid, vit. E and glutathione on exposure to Cd is the expected biologic response since cadmium is known to induce oxidative stress through generation of oxy- radicals (Flora, 2009). That Exposure to cadmium induces depletion of ascorbic acid in the liver and kidney is well documented by Patra *et al.,* 2011. Vit- C and E plays a beneficial role in reducing the toxicity and absorption of Cd. Hence, the supplementation of Se either alone or in combination with Vit- C and E greatly reduces the Cd body burden in the tissues. The mixture of Se and Vit- C and E supplementation was more effective in reducing the Cd body burden than the individual supplementation thereby enhancing the elimination of Cd from the body and binding to target proteins.

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