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Ameliorative effect of zinc and vitamin-C against cadmium induced bioaccumulation in selected tissues of male albino rat

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Abstract
The present study was conducted to evaluate the toxic effects of cadmium on bioaccumulation and antioxidant enzyme status in Wistar strain male albino rats to determine the potential benefits of different antioxidant enzymes. Zinc (Zn) and Vitamin C (Vit C) are tested to know whether they have beneficial effects in ameliorating Cd induced toxic effects. To achieve this objective, albino rats ($n = 6$), weighing 180 ± 20 gm were treated with Cadmium Chloride ($CdCl_2$) at a dose of 1/10th LD50/48 h i.e. that is 22.5 mg/kg body weight for 7, 15 and 30 days (d). Later 15d Cd treated rats were divided into three groups. The 1st groups of rats were subjected to Zinc (12mg/kg), 2nd group to Vitamin-C (200 mg/kg) and the 3rd group to combination of Zinc + Vitamin-C supplementation for 7, 15 and 30d time periods. After the specific time intervals, rat tissues were obtained and evaluated Bioaccumulation, Glutathione (GSH), Glutathione Reductase (GR) levels in liver, kidney and testis. Bioaccumulation in Cd-intoxicated rats were significantly increased with concomitant decrease in Zinc and Vitamin C concentration ($P < 0.001$) in Cd treated group when compared to the control group. More significant and beneficial effects were observed with Cd + Zinc, Cd + Vitamin C, and Cd + Zinc + Vitamin C treated groups in reducing Cd-induced bioaccumulation. Hence our present findings suggest that Zinc and Vitamin C have protective role in reversing the Cd induced bioaccumulation and exert beneficial effects in adult male albino rats by restoring antioxidant capacity.

Keywords: cadmium, bioaccumulation, antioxidant enzymes, liver, kidney, rat, testis

Introduction

Cadmium (Cd^{+2}, atomic number 48, atomic mass number 112) is one of the most toxic, non-essential, non-biodegradable heavy metal with no biological role in the organisms present predominantly in +2 oxidation state (Branca *et al*., 2020) [4]. Cadmium is soft, ductile, bluish-white, odorless and tasteless heavy metal with potent toxic effects. Cadmium primarily damage lung, liver, kidney, and testis by induction of oxidative stress in the organism by formation of reactive oxygen species such as superoxide, hydroxyl radical, hydrogen peroxide and nitric oxide (Patra *et al*., 2011) [24]. Cd induced bioaccumulation and biomagnification are the characteristic features of heavy metals promoted toxic effects are demonstrated by previous studies of (Kesavulu & Usha Rani, 2016) [20]. Upon Cd bioaccumulation tissue peroxidation of lipid membranes is enhanced and known to promote apoptosis and necrosis within the tissue (Jahan *et al*., 2014) [17]. Glutathione is non-enzymatic, master antioxidant and the first line of defense against oxidative stress and involved in detoxification of H_2O_2 (Forman *et al*., 2009) [12]. GSH depletion enhances Cd-induced hepatotoxicity, disrupts thiol homeostasis and promotes ROS-mediated cell death (Branca *et al*., 2020) [4]. Binding of Cd to sulfhydryl groups (thiol group) promotes distortion of protein structure as sulfhydryl groups are protein-bound and lowers GSH levels (Gupta *et al*., 2004) [14]. Glutathione reductase (GR, EC 1.6.4.2), is an enzymatic antioxidant, NADPH-dependent oxidoreductase enzyme that catalyses decomposition of H_2O_2 and maintains the levels of GSH. GR reduces oxidized form of glutathione (GSSG) into reduced glutathione (GSH) and maintains optimal GSH/GSSG ratio (Hao *et al*., 2015) [15] and scavenge free radicals. Depletion of GSH and increase in GSSG inhibits GR activity that alters intracellular thiol oxidative stress and impairs the ability of cellular functions to scavenge ROS (Zhao *et al*., 2009) [31].

Zinc is an essential trace element with many biological roles that shows defense against certain metals, and prevent cell damage (Kesavulu & Usha Rani, 2016) [20]. Lipid peroxidation is enhanced under Zn deficiency, but under normal conditions Zn prevents testicular tumors (Patra *et al*., 2011) [24] and cadmium treated rat group showed lower content of Zn. Zn maintains intracellular levels of Glutathione and protects cell membrane against oxidative injury (Celino *et al*., 2011) [8].

Vitamin C (Ascorbic acid) is powerful chain breaking antioxidant with protective effects that neutralizes free radicals like hydrogen peroxide, hydroxyl, singlet oxygen, superoxide, nitric oxide, hypochlorous acid radicals (Kesavulu & Usha Rani, 2021) [19]. Vitamin C reduces the Cd bioaccumulation, toxic effects and its absorption that declines an enhanced renal and hepatic cadmium burden (El-Refaiy & Eissa, 2013) [11].

Our study raises the idea that Zinc and Vitamin C precisely eliminates the toxic effects of cadmium imposed on living system. Biological impact of cadmium along with natural antioxidant enzymes status was assayed and re-examined to show their effects. The present study was aimed to determine whether Zinc and Vitamin C have protective effects against the harmful effects of cadmium intoxication in rats and their prominence in restoring antioxidant capacity.

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 Chemical Co. (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 ± 2^0C, 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 Cadmium Chloride 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-C (200 mg/Kg) alone and third group supplemented with both Zn and Vitamin C 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, 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 Bioaccumulation and the assay of oxidative stress enzymes like GSH, GR levels quantification.

Bio-accumulation studies

Cd concentrations in the test tissues were measured by the method of Kanno *et al*., (1994) [18].

Assay of Oxidative stress enzymes

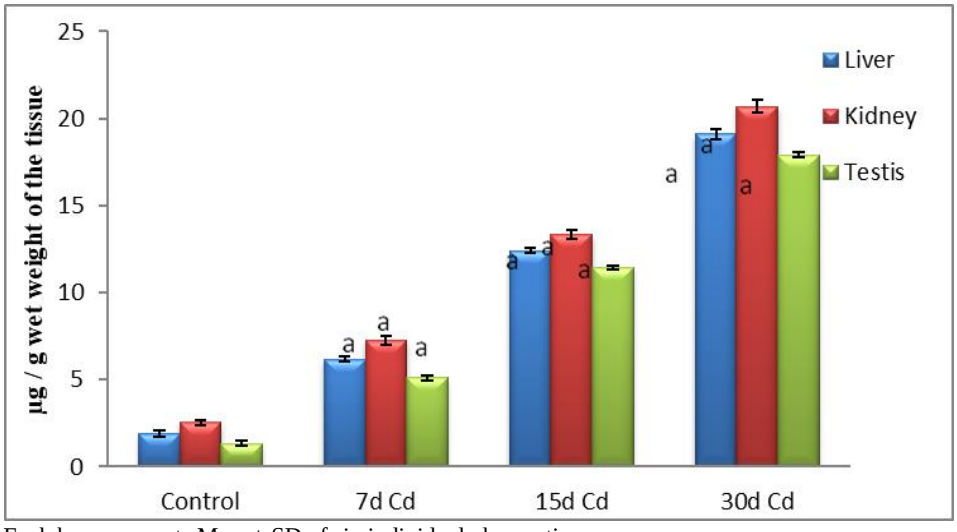
GR activity was determined by a slightly modified method of Carlberg and Mannervik (1985) [7] Glutathione (GSH) content was estimated according to the method of Theodorus *et al.*, (1981) [30].

Data analysis

The data were 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 a- P<0.001, b-P<0.01, c-P<0.05, d-Non Significant.

Results

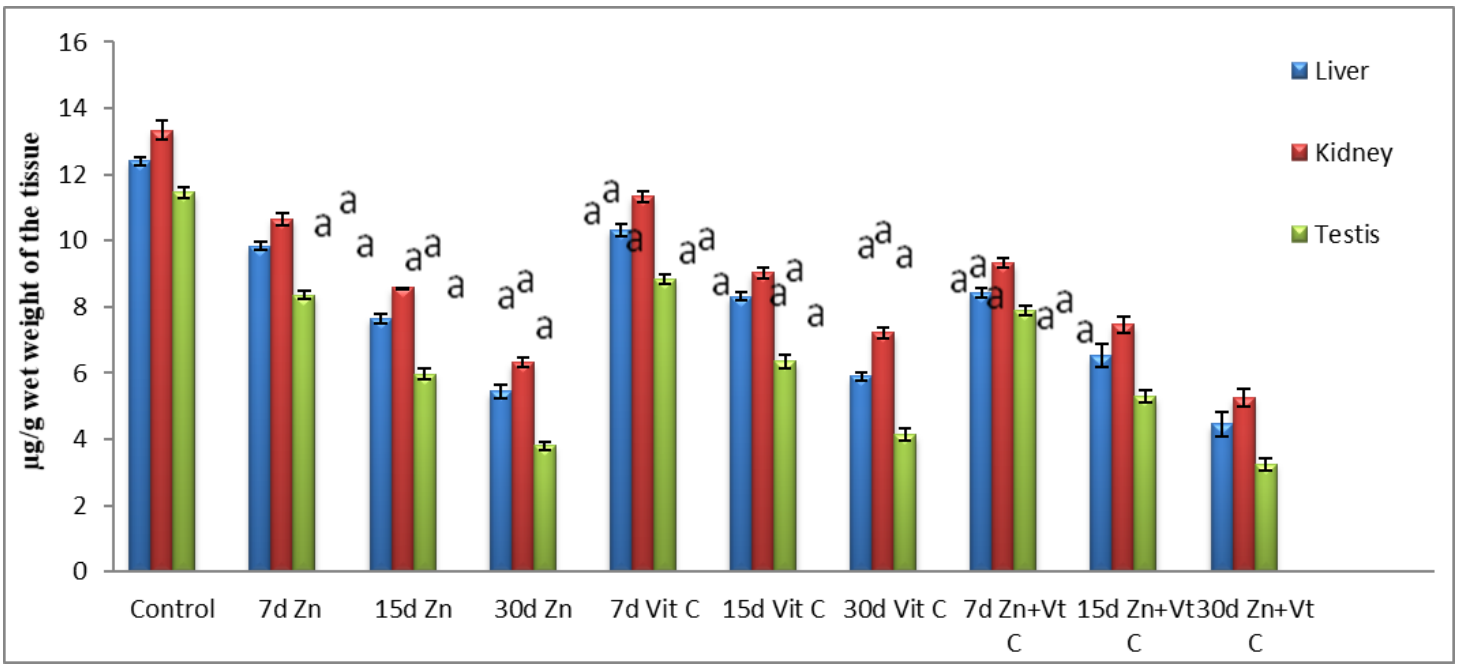
The amount of Cd bio-accumulated in the liver and kidney of male Albino rats under Cd treatment and Zn and Vit-C supplementation was analyzed. Cd bio-accumulation was significantly more in the kidney of 30d Cd treated rats (20.69±0.37µg/g) than the liver and testis of 30d Cd treated rats (19.09±0.28µg/g and 17.91±0.16µg/g).



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

Fig 1: Cd bio-accumulation (µ g /g wet weight of the tissue) in the liver, kidney and testis of rats under Cd intoxication.

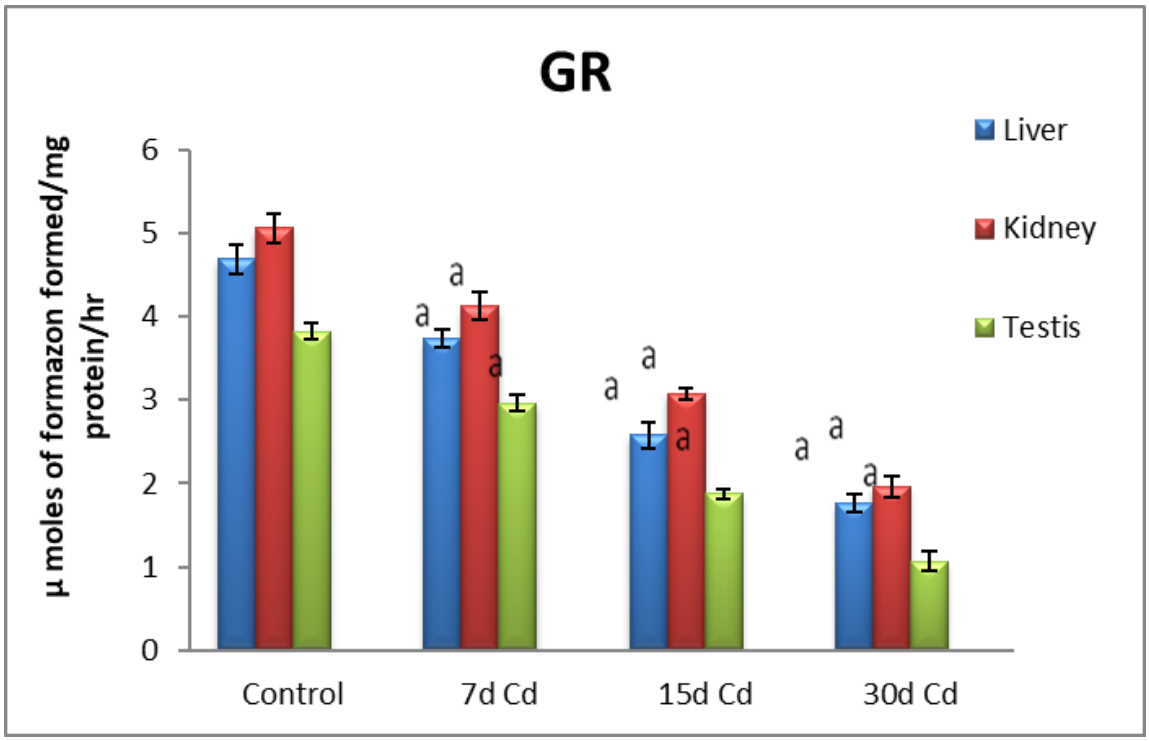
With the Supplementation of both Zn and Vit-C the tissues, liver, kidney and testis showed a slight decrement in 15d Cd treated rats at all the time intervals. The Maximum decrement in the accumulation of Cd was observed in 30d kidney tissue (6.32±0.15µg/g) than the liver tissue (5.44 ± 0.21µg/g) and the testis tissue (3.80±0.12µg/g) at all the time intervals (Fig–1). Maximum decline in the Cd bioaccumulation was observed in the kidney, of 30d rat under the combined supplementation of Zn and Vit-C (5.24±0.27µg/g) when compared with the individual supplementation of Zn and Vit-C at all the time intervals (Fig-2).



Each bar represents Mean+ SD of six individual observations. All values indicates the level of significance a- P<0.001 Control- 15d Cd treated rats

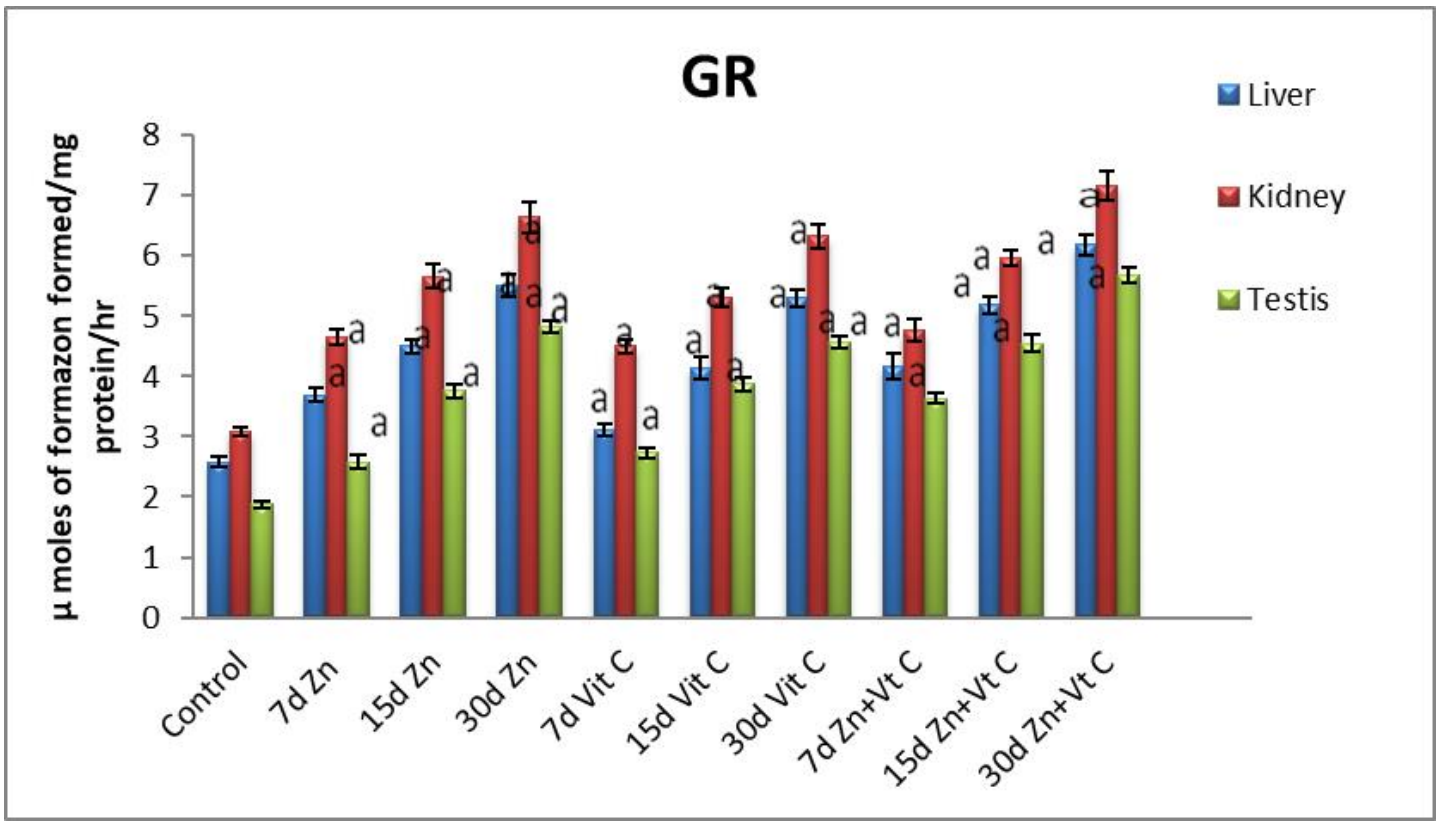
Fig 2: Cd bio-accumulation (µg /g wet weight of the tissue) in the liver, kidney and testis of rats under Zn, Vit C, and Zn+ Vit C supplementation

GR activity levels also showed a progressive decrement at all the time intervals of Cd treatment with a maximum depletion in 30d rat liver (3.06 ± 0.13µ moles of formazon formed/mg protein/hr). Further supplementation with both Zn and vitamin-C, the GR activity reached to normalcy in 30d rat kidney (7.93 ± 0.10 µ moles of thioether formed / mg protein / min) suggesting the protective role of trace elements Zn and Vitamin C.



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

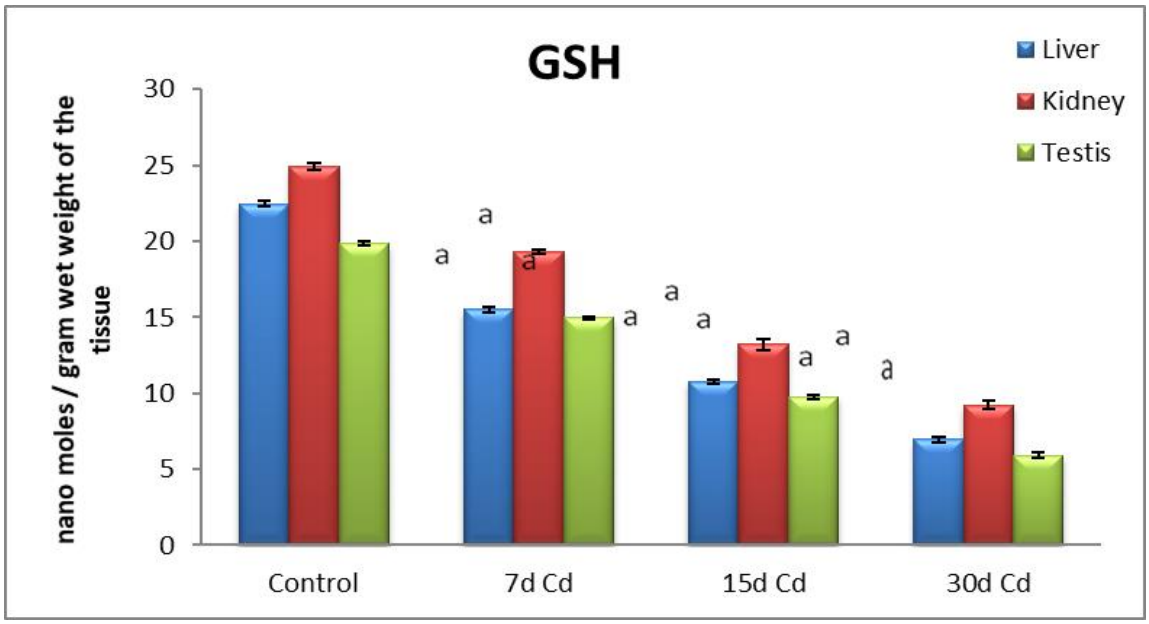
Fig 3: GR activity levels (µ moles of formazon formed/mg protein/hr) in liver, kidney and testis of Cd treated rats



Each bar represents Mean+ SD of six individual observations. All values indicates the level of significance a- P<0.001 Control- 15d Cd treated rats

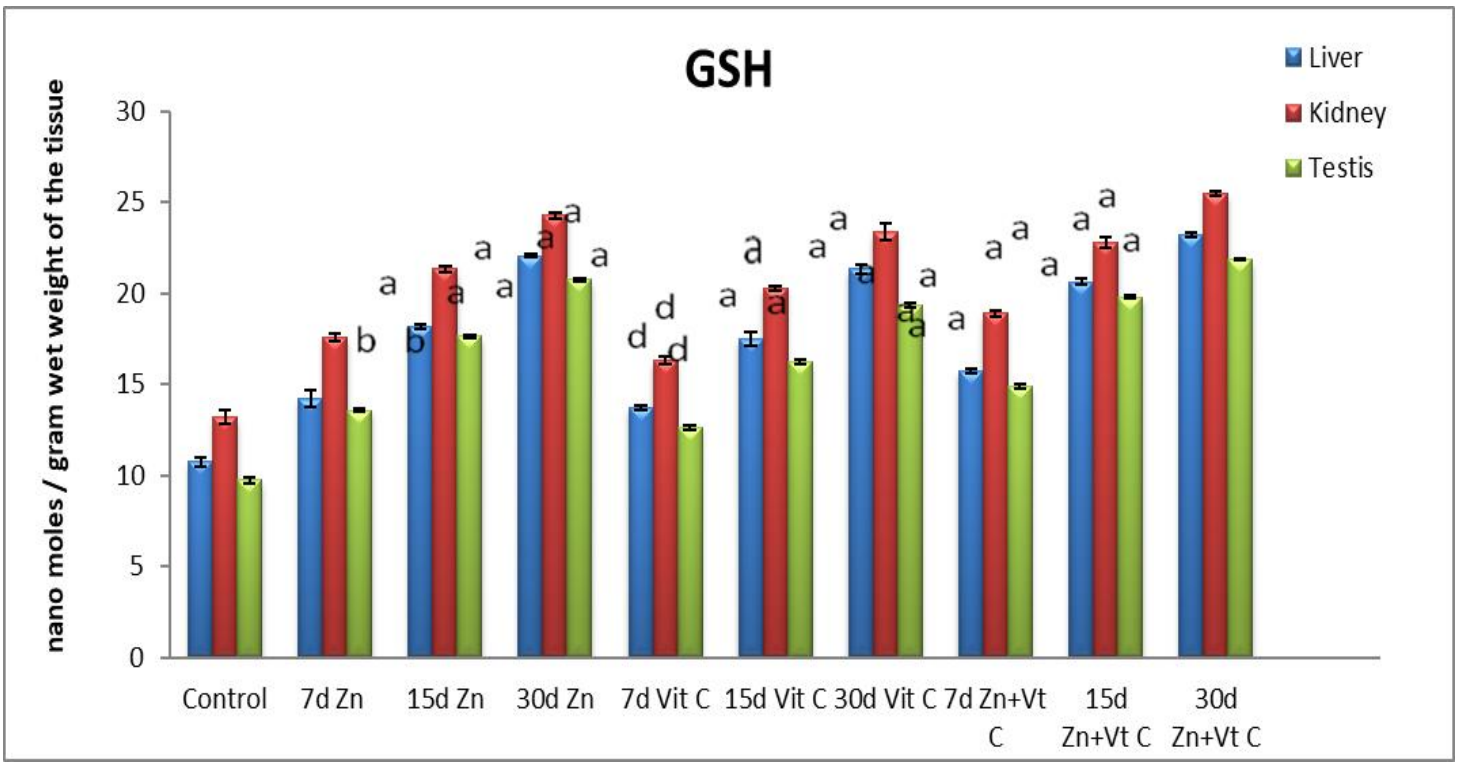
Fig 4: GR activity levels (µ moles of thioether formed /mg protein / min) in liver, kidney and testis of Cd treated rats after Zn, Vit C, and Zn+ Vit C Supplementation

GSH levels were also ameliorated in liver, kidney and testis in Cd treated rats supplemented with Zn and Vitamin C either alone or in combination over a period of 30 days. However, maximum increment in GSH content was found in kidney (25.47 ± 0.16 nano moles g wet weight of the tissue) under combined supplementation of Zn and Vitamin C than the liver and testis tissues in Cd treated rats during 30 day period than other modes of supplementation (Zn and Vitamin C individual treatment).



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

Fig 5: GSH (nano moles / gram wet weight of the tissue) levels in liver, kidney and testis of Cd treated rats.



Each bar represents Mean+ SD of six individual observations.

1. indicates the level of significance P<0.001
2. indicates the level of significance P<0.01
3. indicates the level of significance- Non significant Control- 15d Cd treated rats

Fig 6: GSH (nano moles / gram wet weight of the tissue) levels in liver, kidney and testis of Cd treated rats after Zn, Vit C, and Zn+ Vit C Supplementation.

Discussion

Cd triggers ROS generation and bioaccumulation more in liver when compared to other tissues due to presence of excess unsaturated lipids that induce oxidative damage (Sanjeev *et al*., 2019) [28]. Cadmium bioaccumulation is more in the liver when compared to kidneys, testis and bones (Sochon *et al*., 2018) [29]. Present study results showed maximum bioaccumulation in kidney when compared to liver and testis. Depletion of antioxidant enzymes and enhanced bioaccumulation under Cd induced oxidative stress in observed in kidney and testis (Dzobo & Naik, 2013) [9].

In the present study, Cd bioaccumulation enhanced in all the tissues (liver, kidney and testis), but maximum Cd bioaccumulation is observed in the kidney tissue when compared to liver and testis. To protect cell from toxic effects of Cd bioaccumulation an antioxidant system is well developed with various enzymes including Glutathione and Glutathione reductase.

Zinc supplementation decreased Cd bioaccumulation and prevented liver damage (Rogalska *et al*., 2011)[25], preventing hepatotoxicity and nephrotoxicity (Sochon *et al*., 2018)[29], accumulation of testicular cadmium is prevented (Bashandy *et al*., 2016) [3].

Vitamin C supplementation showed prominent effects against Cd bioaccumulation in liver (Sahiti *et al*., 2020) [26], kidney (Ali *et al*., 2019) [2] and testis (Zhu *et al*., 2020) [32]. Vitamin C reduced Cd burden in all the above tissues including liver, kidney and testis was shown by the studies of Grosicki, (2004) [13].

In the present study, Cd induced bioaccumulation progressively declined GSH and GR activity levels in the test tissues due to generation of H_2O_2 radicals that promotes lipid peroxidation which is toxic to cell. Hence increased levels of bioaccumulation were observed in rats under Cd intoxication and reduced antioxidant enzyme capacity.

Decrease in GR and GSH activity levels are indicators of pathogenesis that induce oxidative stress in the organism. Decline in GR, GSH content and NADPH content under toxic intoxication promotes cellular and tissue damage (Hao *et al*., 2015) [15]. Glutathione (GSH), first line of defense against Cd induced hepatotoxicity decreased when Cd binds to thiol group. If GSH levels depleted inhibits the glutathione reductase activity that is responsible for conversion of GSSG to GSH.

Metal intoxication decreased GSH levels in liver are restored under Vitamin C supplementation, with decline of LPO in liver (El-Gazzar *et al*., 2014) [10]. Kidney damage under Cd-induced toxic effects reduced GSH in kidney were under vitamin C preventing renal toxicity (Bulan *et al*., 2008) [6]. GSH has the ability to alter Cd toxicity, and showed much significant effects in preventing testicular damage by lowering MDA levels under supplementation of vitamin C (Kini *et al*., 2011) [21].

Zinc supplementation also showed similar protective effects against Cd toxicity by enhancing GSH activity in chick embryonic liver and reversed the decrease in GSH/GSSG in Cd exposed rats (Meenabai *et al*., 2014) [23]. Decreased GSH levels and promoted testicular injury was prevented by zinc and recorded significant increase in testis GSH levels (Ibrahim, 2017)

[16]. Complexation of Cd to glutathione in kidney reduced GSH content is restored under zinc was shown by our reports.

Reduced GR activity under Cd induced toxic insult was restored due to Zinc and Vitamin C. Vitamin C prevented alcohol-induced hepatotoxicity and also enhanced GR activity in liver (Abhilash *et al*., 2012) [1]. Zinc deficiency *in vitro* and *in vivo* enhanced the MDA levels in tissues can be significantly restored upon Zinc supplementation to Cd treated rats (Bruno *et al*., 2007) [5]. Enhanced GR activity in liver was observed under Zn supplementation (Meenabai *et al*., 2014) [23].

Zinc deficiency is found in chronic kidney disease conditions that reduced GR content under diseased conditions was restored upon Calcium and Zinc supplementation and prevented Cd-induced nephrotoxicity (Marreiro *et al*., 2017) [22]. Cd^{2+} showed structural similarity to zinc and caused protein misfolding may mediate cell death, hence Zinc supplementation may prevent such effects in testis (Sandbichler and Höckner, 2016) [27].

Hence in the present study supplementation of Zinc and Vitamin C altered the changes caused by cadmium bioaccumulation. However, protective effect was observed in the combined supplementation when compared to individual supplementation indicating the ameliorative effect of Zinc and Vitamin C in the test tissues under study. Hence Zinc and Vitamin C supplementation in the present study showed a decline in Cd bioaccumulation levels with progressive enhancement of GSH and GR levels.

Conclusion

Overall conclusion, of our present results showed nutrient supplementation reduced Cd bioaccumulation by restoring GSH and GR antioxidant enzyme status. Our findings are essential to disclose the exact role of Cd toxicity and nutrients supplementation in alteration of antioxidant enzyme capacity in liver, kidney and testis. This study may pave a way for future research in ameliorating Cd toxicity and control of diseases occurred under its toxic effects.

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