RESEARCH ARTICLE JPAR 2021

Journal of Pharmaceutical Advanced Research

(An International Multidisciplinary Peer Review Open Access monthly Journal)

Available online at: www.jparonline.com

Therapeutic role of Zinc and Vitamin-C against Cadmium induced Oxidative stress in selected tissues of male albino rat: A study with reference to Metallothionein quantification

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Received: 04.04.2020 Revised: 28.04.2021 Accepted: 08.05.2021 Published: 30.05.2021

ABSTRACT: Background: 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. **Aim:** The present study is carried out to know the beneficial role of Zinc (Zn) and Vitamin-C supplementation against Cd induced alterations in liver, kidney and testis of Cd treated rats with special reference to metallothionein (MT) protein. **Method:** Wistar strain male albino rats were treated with Cadmium Chloride at a dose of 1/10th LD_{50} / 48 h 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 a third group supplemented with both Zn and Vitamin-C for again 7, 15 and 30 d long sojourn. **Results:** A significant elevation in LPO levels with decreased activity levels of GST and GPx were observed during Cd intoxication are reversed under Zn and Vitamin-C supplementation. MT protein levels were significantly elevated in the test tissues during Cd treatment and also after supplementation with Zn and / or Vit-C. **Conclusion:** Our study reveals that combination of Zn and Vitamin C supplementation is effective in detoxifying the Cd body burden from the selected test tissues of the rat. Zn and / or Vit-C envisage therapeutic role of trace elements in combating the heavy metal, Cd insult.

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Keywords: Cadmium, Oxidative stress, Liver, Kidney; Testis, Rat.

INTRODUCTION:

Heavy metals are recognized as the most toxic compounds with no physiological function in the organism. Arsenic, lead, mercury, cadmium and chromium are the most toxic heavy metals released into the environment by human activities. Among them Cadmium (Cd) is an internationally recognized toxic, heavy metal with no biological role in the human body. Increased industrialization and human activity caused the extensive use of Cd for various uses, including

electroplating, cosmetics, cadmium pigments, Cd fume inhalation, fertilizers and pesticides [1]. Cadmium is highly toxic to humans and animals upon its exposure by various modes (inhalation, ingestion and absorption) and is known to promote hepatotoxicity, nephrotoxicity and testicular damage [2-4]. Only 5 % of cadmium is absorbed gastro intestinally and retention of absorbed cadmium makes the organism more susceptible to its accumulation leading to pathogenicity. MT has a prominent role in Cd retention due to its long biological half-life of more than 20 years, it gets accumulated in the human body [5,6]. Cd mostly gets accumulated in the liver and kidney, when compared to other organs. Studies of [4] showed that at low concentrations Cd is known to damage liver, kidney and testis. Cd intoxication is known to promote the formation of collagen fibres, mononuclear cell infiltration, edema, heamorrhages, mitochondrial and nuclear changes, nuclear DNA fragmentation, apoptosis and necrosis in all the tissues including liver, kidney and testis.

Metallothionein (MT) is cysteine rich, metal-binding protein that occurs throughout the plant and animal kingdom. Metallothionein exists in four isoforms among mammals, MT-I, II, III and IV. MT-I and MT-II isoforms are found majorly in the liver and kidney. MT-I, II, and III isoforms are present in the central nervous system and testis. MT-III is found in the mouse and human brain. MT- IV is found in stratified epithelium, skin and upper G.I. Tract [7,8]. MT synthesis occurs in order to protect the organism from heavy metal toxicity. Induction of MT protein depends on the amount of Cd accumulated in tissues.

Exposure to divalent metal cations like Cadmium (Cd^{2+}) activates MT and thus protects organisms from its toxicity [9]. In addition, increased synthesis of MT has a protective role against oxidative stress [10,11]. Oxidative stress is developed in the organism due to imbalance between pro-oxidants produced and antioxidant capacity of the organism promoting tissue injuring [12]. MT is synthesized in different organs of the body including liver and kidney and its production is dependent on availability of minerals in our food like zinc, copper, and selenium.

Vitamin-C is a water-soluble antioxidant that gets synthesized in the liver and kidney and controls oxidative stress [13,6]. In addition, MT also plays a prominent role in Cd retention in the tissues under supplementation of vitamin-C. Under normal physiological conditions MT-I is known to bind with Vit C but the exact mechanism by which MT protein induction takes place under Vit C supplementation is not yet elucidated clearly. Vitamin-C neutralizes ROS species scavenging free radicals and induces MT either indirectly or by antioxidants [14].

Zinc has the capacity to induce synthesis of metallothionein and prevent the organism from oxidative stress [15,16]. MT (zinc-concentrating protein) involves homeostatic control of metal ions including Zinc metabolism [17,4]. MT protein synthesis increased upon supplementation of Zinc that is known to produce antioxidants [11]. Zinc inhibits absorption of cadmium from the gastrointestinal tract and causes its accumulation in the body. Cd is known to interrupt Zn-related functions in the body.

Lipid peroxidation results in the production of malondialdehyde, hydrogen peroxide and hydroxyl radicals under Cd stress. The enzymatic antioxidants, including catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GP_X), glutathione reductase (GR) and glutathione-S-transferase (GST) are reduced under Cd-intoxication. Nutrient supplementation has the capacity to restore the antioxidant status depleted under Cd-intoxication.

GST has a prominent role in cell survival and protects the organism from oxidative stress. Glutathione peroxidase is a hydrogen peroxide degrading enzyme, where decreased activity of GPx impairs GSH homeostasis in all the tissues. Therefore, the present study was designed to evaluate the role of Zn and vitamin C individually and in combination against Cd induced oxidative stress with reference to metallothionein quantification in liver, kidney and testis of male albino rats.

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 °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. 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 $1/10^{th}$ LD_{50} / 48 h i.e. 22.5 mg/Kg body weight of rat 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 body weight of rat), second group vitamin-C (200 mg/Kg body weight of rat) alone and a third group supplemented with both Zn and vitamin 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, kidney and testis were quickly isolated in ice cold conditions and weighed to their nearest mg using Shimadzu electronic balance. After weighing, tissues were immediately used for the assay of oxidative stress enzymes like GST and GPx the levels of LPO and MT protein quantification.

Assay of oxidative stress Enzymes:

***Lipid peroxidation (LPO)*:**

The LPO was determined by the thiobarbituric acid (TBA) method [18]. The tissues were homogenized in 1.5 % KCl (20 % w/v). To 1 ml of tissue homogenate, 2.5 ml of 20 % trichloro acetic acid (TCA) was added and the contents were centrifuged at 3,500g for 10 min and the precipitate was dissolved in 2.5 ml of 0.05M sulphuric acid. To this, 3 ml of TBA was added and the samples were kept in a hot water bath for 30 min. The samples were cooled and malonaldehyde (MDA) was extracted with 4 ml of n-butanol and the colour was read at 530 nm in a UV spectrophotometer (Hitachi U-2000) against the reagent blank. Trimethoxy pentane (TMP) was used as an external standard. Values are expressed in µ moles of MDA formed/ g tissue/ h.

***Glutathione – S – transferase (GST) (EC: 2.5.1.18)*:**

GST activity was measured with its conventional substrate 1-chloro 2,4-dinitro benzene (CDNB) at 340 nm as per the method mentioned in literature [19]. The tissues were homogenized in 50 mM Tris-Hcl buffer pH 7.4 containing 0.25 M sucrose and centrifuged at 4000 g for 15 min at 4 °C and the supernatant was again centrifuged at 16,000 g for 1 h at 4 °C. The pellet was discarded and the supernatant was used as the enzyme source. The reaction mixture in a volume of 3 ml contained 2.4 ml of 0.3 M potassium phosphate buffer pH 6.9, 0.1 ml of 30 mM CDNB, 0.1 ml of 30 mM glutathione and the appropriate enzyme source. The reaction was initiated by the addition of glutathione and the absorbance was read at 340 nm against reagent blank and the activity was expressed as µ moles of thioether formed/ mg protein/ min.

***Glutathione peroxidase (GPx) (EC: 1.11.1.9)*:**

GPx was determined by a modified method [20] at 37 °C. 5 % (w/v) of tissue homogenate were prepared in a 50 mM phosphate buffer (pH 7.0) containing 0.1 mM EDTA. The homogenates were centrifuged at 10,000 g for 10 min at 4 °C in cold centrifuge. The resulting supernatant was used as an enzyme source. The reaction mixture consisted of 500 µl of phosphate buffer, 100 µl of 0.01 M GSH (reduced form), 100 µl of 1.5 mM NADPH and 100 µL of GR (0.24 units). The 100 µl of tissue extract was added to the reaction mixture and incubated at 37 °C for 10 min. Then 50 µL of 12 mM t-butyl hydroperoxide was added to 450 µL of tissue reaction mixture and measured at 340 nm for 180 s. The molar extinction coefficient of 6.22×10^3 M cm^{-1} was used to determine the activity. The enzyme activity was expressed in µ moles of NADPH oxidized / mg protein / min.

***Metallothionein quantification*:**

The initial isolation of MT protein from liver, kidney and testis homogenates were carried out by following [21]. The clear supernatants thus obtained from liver, kidney and testis homogenates was again subjected to the purification process. Supernatant fractions of each tissue were applied to a column of Sephadex, G-75 (5 × 50 cm) equilibrated with 10 mM Tris-HCl buffer (pH 7.4). Further purification of MT protein was carried out by Ion

exchange chromatography using DEAE-32CELLULOSE by following the method as mentioned in literature [22]. Purified MT protein quantification was performed by using [23].

Estimation of protein content:

The protein content of the tissues was estimated by the method as mentioned in literature [23]. About 1 % (w/v) homogenates of the tissues were prepared in 0.25 M ice cold sucrose solution. To 0.5 ml homogenate, 1 ml 10 % TCA was added and the samples were centrifuged at 1000 g for 15 min. Supernatant was discarded and the residues were dissolved in 1ml of 1N sodium hydroxide. To this 4 ml of alkaline copper reagent was added, followed by 0.4 ml of Folin-phenol reagent (1:1 - folin: H_2O). The color was measured at 600 nm in a UV spectrophotometer (Hitachi U-2000) against reagent blank. The protein content of the tissues was calculated using a standard protein (BSA) graph.

Data analysis:

The data were 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:

The data on the alterations in the oxidative stress enzymes such as GST and GPx as well as LPO in Cd treated rat liver, kidney and testis before and after supplementation with Zn and / or Vitamin C are tabulated in (Fig 1 to 6). The decreased enzyme levels (GST and GPx) in the Cd treated rats were statistically significant ($P<0.05$). The supplementation of Zn and/ or Vit-C significantly reversed the Cd induced alterations in the oxidative stress enzymes.

Our results revealed that LPO levels were increased markedly in both liver, kidney and testis of Cd treated rats at all the test periods when compared to the controls. The MDA levels were increased with the time intervals of Cd treatment compared to controls and were maximum for 30 d rat kidney (40.40 ± 0.08 µ moles of MDA formed/g tissue/ h). After supplementation with Zn and/ or Vit C, the LPO levels were progressively decreased at all time periods in both the test tissues. Maximum depletion was observed in 30d Zn and Vit-C mixture supplemented rats' kidney (21.94 ±0.12 µ moles of MDA formed/g tissue/ h).

GST activity levels also showed a progressive decrement at all the time intervals of Cd treatment with a maximum depletion in 30d rat liver (3.87 ± 0.10 µ moles of thioether formed/ mg protein/ min). Further supplementation with both Zn and vitamin-C, the GST activity reached to normalcy in 30 d rat kidney (13.86 ± 0.18 µ moles of thioether formed/ mg protein/ min) suggesting the protective role of trace elements Zn and Vitamin C.

GPx activity levels also showed a significant decrease in all the time intervals of Cd treatment with a maximum decrease in 30 d testis (1.27 ± 0.07 µ moles of NAPDPH oxidized/ mg protein/ min). However the aforesaid Cd inhibited GPx activity levels were markedly elevated in both the test tissues after supplementation with the trace elements Zn and vitamin-C individually as well as in combination. The combination of Zn and vitamin-C at the time interval of 30d as a supplement was more effective in elevating the GPx activity levels in the testis tissue of rats (5.56 ± 0.21 µ moles of NAPDPH oxidized/ mg protein/ min).

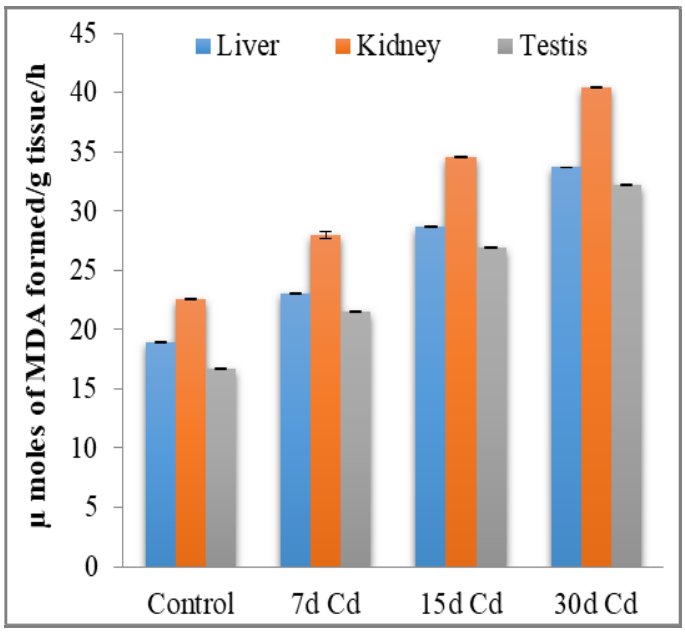

Fig 1. LPO levels (µ moles of MDA formed/ g tissue/ h) in liver, kidney and testis of Cd treated rats.
Each bar represents Mean + SD (n=6). All values indicate the level of significance P<0.05.

Quantification of MT protein content was carried out in liver, kidney and testis tissues of control rats. Cd treated as well as Zn and/ or Vit C supplementations to the 15 d Cd treated rats. Results revealed that MT levels were profoundly increased in liver, kidney and testis of Cd treated rats at all-time intervals when compared to the controls.

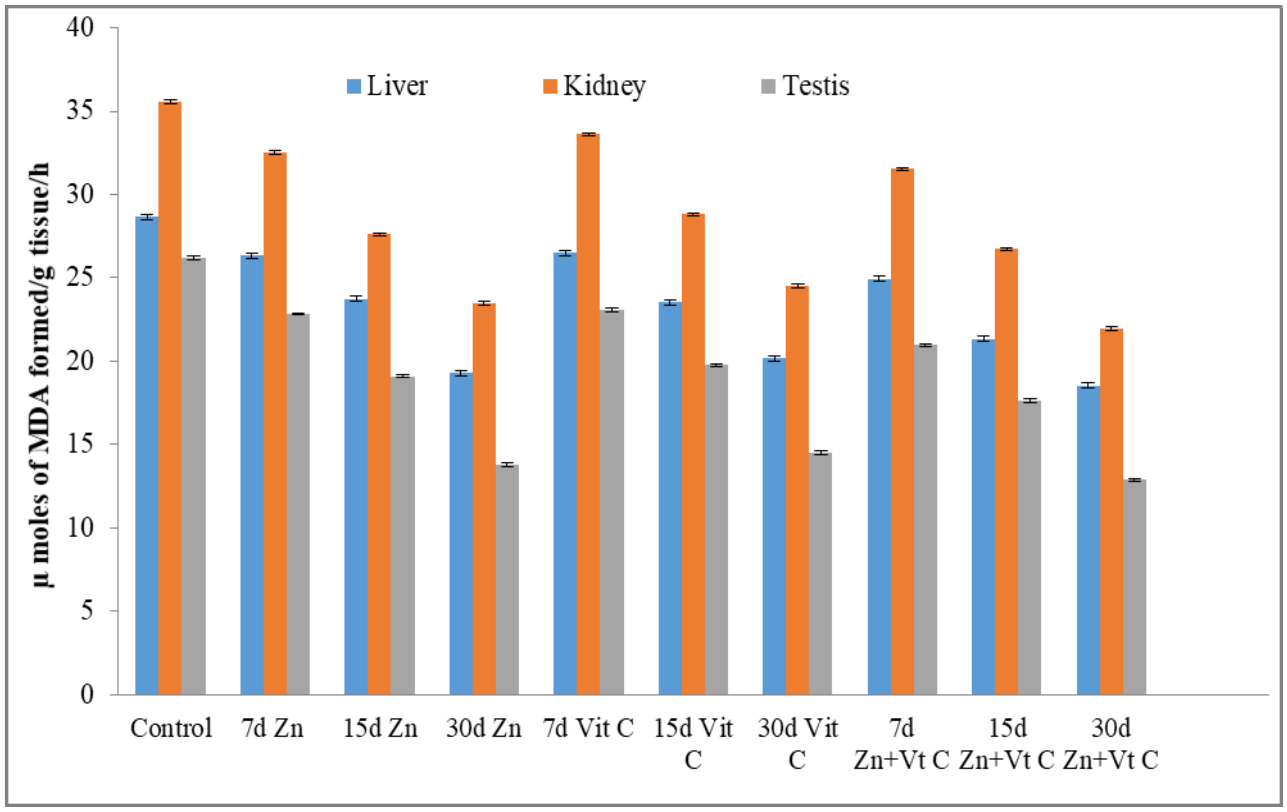

Fig 2. LPO levels (µ moles of MDA formed/ g tissue/ HR) in the liver and kidney of Cd treated rats after Zn, Vit-C and Zn+Vit C Supplementation.

Each bar represents Mean+ SD of six individual observations. All values indicate the level of significance P<0.05.

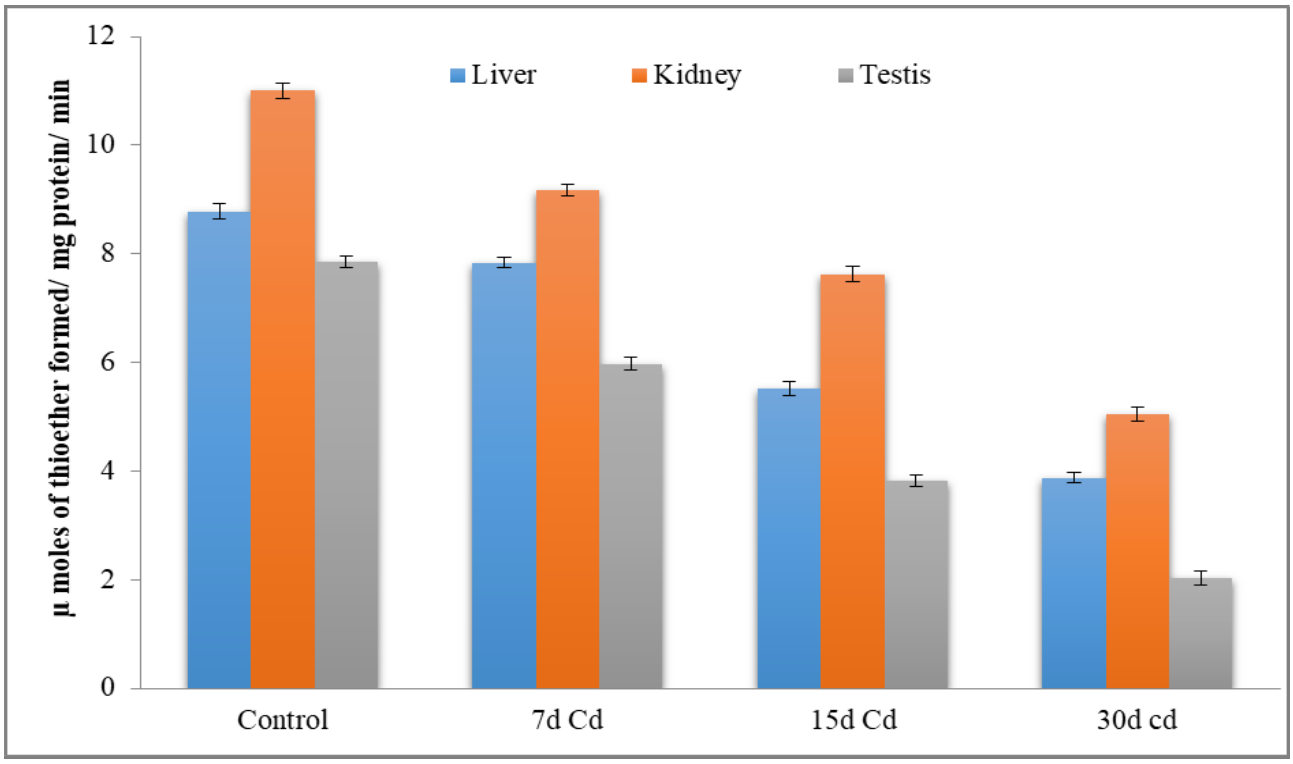

Fig 3. GST activity levels (µ moles of thioether formed /mg protein / min) in liver, kidney and testis of Cd treated rats

Each bar represents Mean+ SD of six individual observations. All values indicates the level of significance P<0.05.

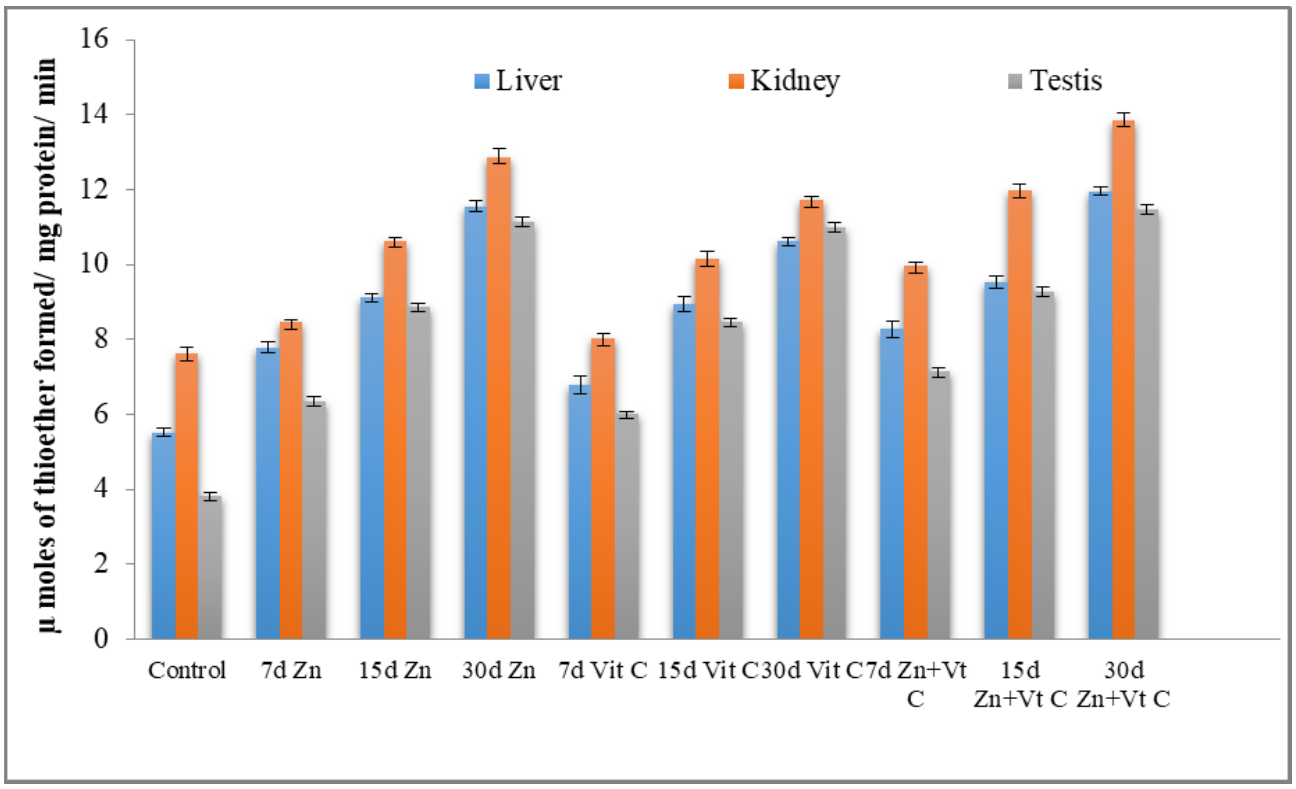

Fig 4. GST activity levels (µ moles of thioether formed /mg protein / min) in liver, kidney and testis of Cd treated rats after Zinc, Vit C, and Zinc+ Vit C Supplementation.

Each bar represents Mean+ SD of six individual observations. All values indicate the level of significance P<0.05.

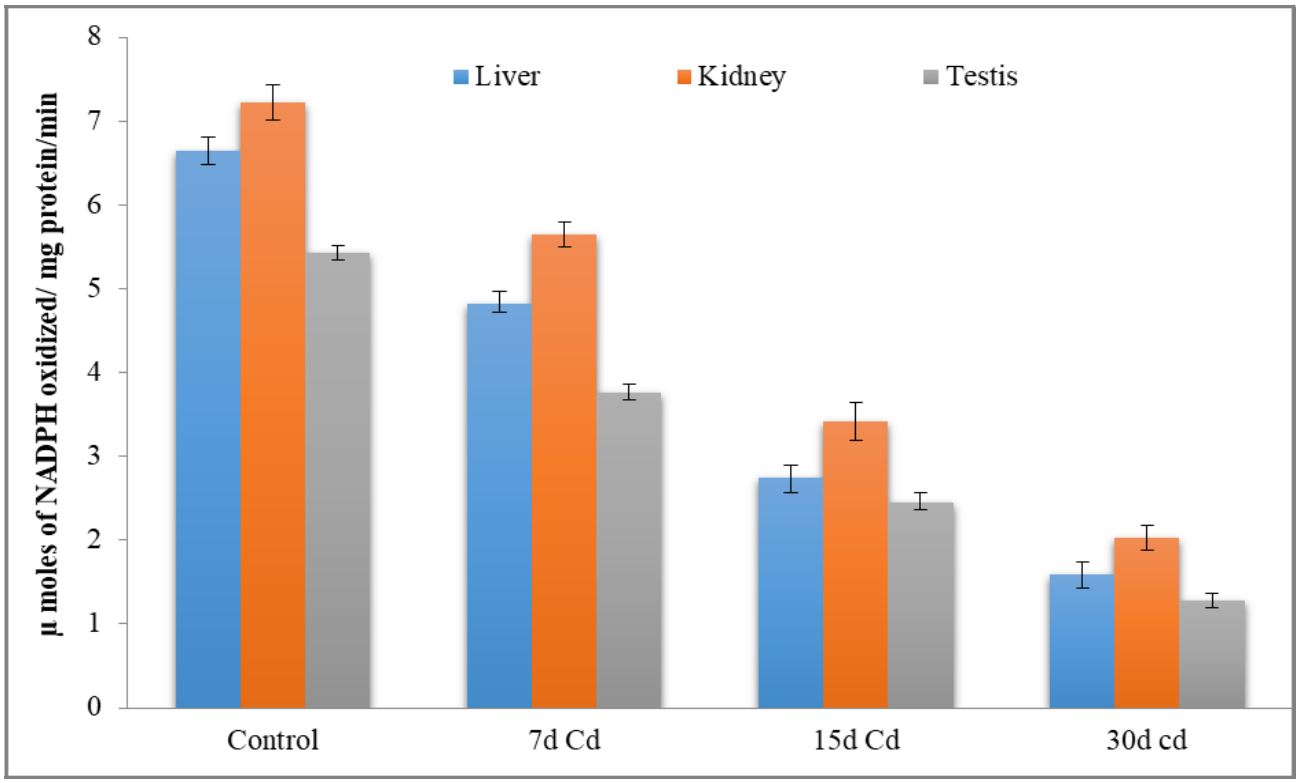

Fig 5. GPx activity levels (µmoles of NADPH oxidized /mg protein / min) in liver, kidney and testis of Cd treated rats.

Each bar represents Mean+ SD of six individual observations. All values indicates the level of significance P<0.05.

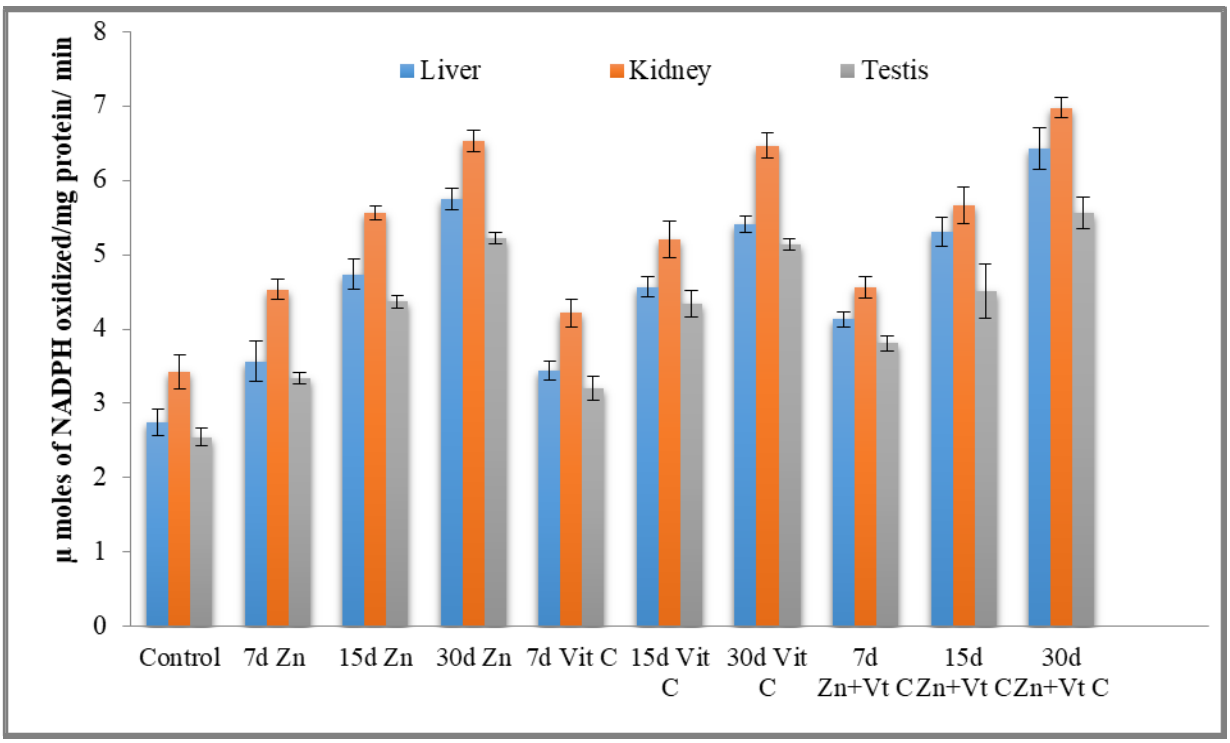

Fig 6. GPx activity levels (µmoles of NADPH oxidized /mg protein / min) in liver, kidney and testis of Cd treated rats Zinc, Vit C, and Zinc + Vit C Supplementation.

Each bar represents Mean+ SD of six individual observations. All values indicate the level of significance P<0.05.

On 30 d Cd treated rat kidney showed maximum synthesis of MT protein (16.242 ± 0.454 µg/ g wet weight of the tissue) and 30 d Cd rat liver (13.124 ± µg/ g wet weight of the tissue) followed by 30 d Cd treated rat testis (11.826 ± at all the treatment time intervals (Fig 7).

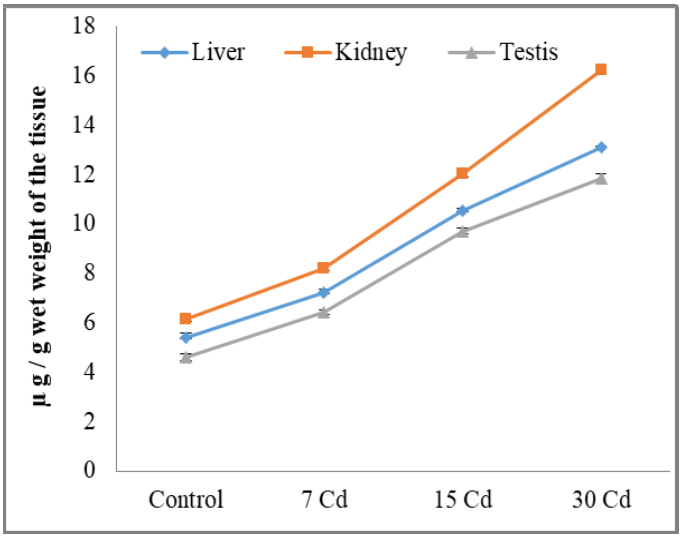

Fig 7. MT Protein (µ g / g wet weight of the tissue) levels in the selected tissues of Cd treated rats.

After supplementation with Zn and / or Vit C to 15d Cd treated rats, the MT levels were highly elevated in both liver, kidney and testis during all the time intervals. Maximum MT protein synthesis was found in 30 d rat kidney under combined supplementation of Zn and Vit C (17.74 ± 0.21 µg/ g wet weight of the tissue) (Fig 10). A Moderate increment in the synthesis of MT protein was found in 30 d Zn supplemented rat kidney, liver and testis (17.34 ± 0.26 µg/ g wet weight of the tissue, 15.05 ±

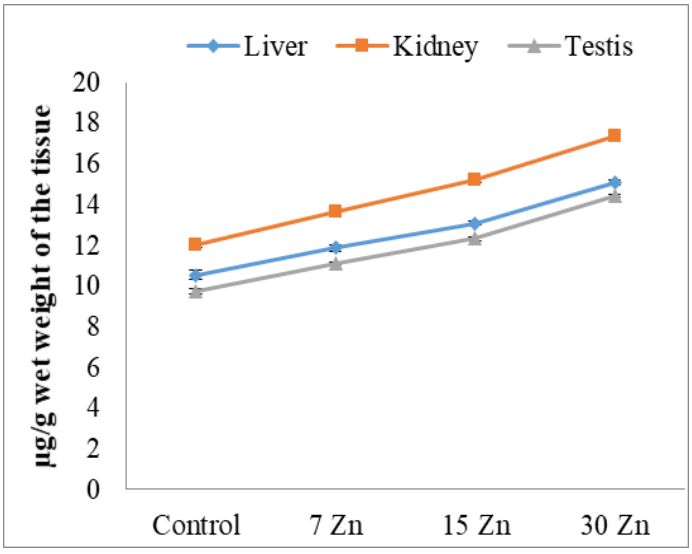

Fig 8. MT Protein (µg/g wet weight of the tissue) levels in the tissues of Cd treated rats after supplemented with Zn.

0.24 µg/ g wet weight of the tissue and 14.41 ± 0.17 respectively). While in the 30 d Vit C alone supplementation, both the test tissues showed low level of increment in MT protein content (16.995 ± 0.24 µg/ g wet weight of the tissue in kidney, 14.884 ± 0.22 µg/ g wet weight of the tissue in liver and 13.93 ± 0.14 µg/ g wet weight of the tissue in testis) than the other modes of supplementation (Fig 8 to 10).

From the present investigation, it is clear that the MT protein synthesis was high in the combined supplementation of Zn and Vit C than the individual supplementation of Zn and Vit C. The elevation in MT synthesis indicates its role in detoxification of heavy metal Cd and also in scavenging of ROS, which were generated by Cd burden in the liver, kidney and testis tissues.

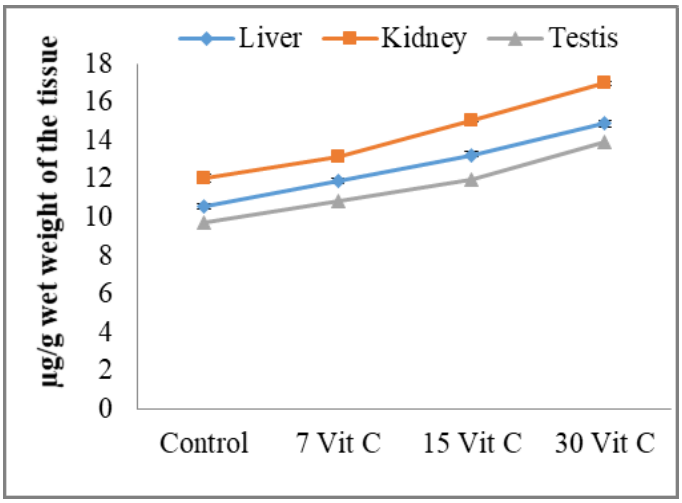

Fig 9. MT Protein (µg/g wet weight of tissue) levels in the tissues of Cd treated rats after supplementation with Vit C.

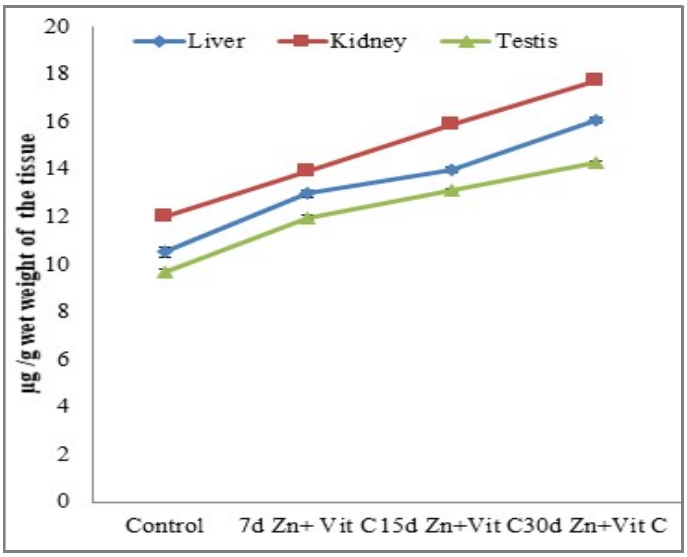

Fig 10. MT Protein (µg/g wet weight of tissue) levels in the tissues of Cd treated rats after supplementation with Zn and Vit C.

DISCUSSION:

The results of the present study revealed that Cd induces significant alterations in the LPO levels and certain enzymatic and non – enzymatic antioxidant enzyme status in liver, kidney and testis of male albino rats at all specific time intervals. These activities were progressively reversed after using essential micronutrient supplements like Zn and / or Vit-C.

Congestion of blood vessels, destruction of mitochondrial cristae, nucleus fragmentation, sinusoidal dilation and collagenous fiber formation occurs under Cd-induced stress in liver tissue [24,25]. Shrinkage of the glomerular network, parenchymal infiltrations, glomerular atrophy and necrosis of proximal tubule cells are observed under Cd stress in the kidney [26,15]. Cd promotes testicular damage promoting edema, degenerative changes in the seminiferous tubules, emaciated leydig cells and sertoli cells hemorrhages and cell death in testis [27]. Such complications under Cd stress can be significantly reversed under supplementation of nutrients zinc and vitamin-C.

In the present study, bioactivation of cadmium under Cd-intoxication is known to inhibit total antioxidant capacity and increase the formation of reactive oxygen species (free radicals). Increased free radicals promote the formation of malondialdehyde, a sign of lipid peroxidation that increases stress in animals and causes tissue damage. Free radicals like superoxide, oxygen radical, hydroxyl, peroxyl radical and nitric oxide are formed during Cd stress. Free radicals tend to produce non-radical species like hydrogen peroxide, singlet oxygen, triplet oxygen, ozone, hypochlorous acid, nitroxyl anion and nitrosyl cation that easily promotes formation of free radicals [28,29]. Hence antioxidants like SOD, CAT, GST, GPx and GR are reduced under oxidative insult.

Cd induced oxidative stress and promoted membrane damage, DNA damage and altered antioxidant defense system of the cell [30]. From the results, it is clearly evident that LPO levels increased markedly in liver, kidney and testis of Cd treated rats when compared to the control. Increased MDA levels denote the oxidative stress in the organism and were more prominent in 30 d Cd treated rats when compared to other time intervals. With the increase in LPO levels, total antioxidant capacity of GST and GPx reduced significantly. These findings are in agreement with earlier reports in pigs, rats and in chicks under Cd stress [31-33].

The key role of GST is to detoxify xenobiotics and store them until they are converted to harmless or less toxic compounds. Reduced GST content may take place due to its over consumption to escape from the toxicity of peroxides under Cd insult. Under normal physiological conditions GST breaks down the chain of lipid peroxides and renders them non-toxic. Further action was carried out by GPx that catalyzes the reduction of H_2O_2 to water and converts reduced glutathione (GSH) to form glutathione disulfide (GSSG) [34,35]. Moreover, decreased activity of GST and GPx would induce excess free radical production thus injuring the corresponding tissues. Impairment in GSH homeostasis may be the reason for the decreased GPx activity in liver, kidney and testis thus promoting tissue damage under Cd insult. Due to less availability of GSH under Cd stress, a decrease in the GPx activity levels has been observed in experimental tissues.

The non-enzymatic antioxidants include vitamin C, vitamin E, reduced glutathione, bilirubin, carotenoids and flavonoids have a protective role against heavy metal toxicity [36,12]. Zinc and vitamin-C has a prominent role to detoxify the toxic effects of cadmium along with MT and hence has the ability to lower the cadmium retention in the body. The activity of GST and GPx are restored under the supplementation of Zinc and vitamin C either individually or in combination with the present study.

Supplementation with Zn and Vitamin C individually and in combination there was a significant alteration in the antioxidant status of liver, kidney and testis of rats. Zinc showed a protective role in preventing Cd-induced toxicity [37-38] and restored the organ damage under Cd toxicity. In addition Zn supplementation could compete with binding sites for Cd uptake and prevents binding of Cd at active sites rendering them inactive. Zn alters the activity of GST and GPx through scavenging of free radicals.

Vitamin C is an important dietary antioxidant that significantly decreases oxidative damage of macromolecules such as lipids, DNA and proteins [39] and prevents oxidative stress mediated diseases [40]. Vitamin C has the ability to reduce oxidative stress generated in the liver, kidney and testis [37]. Cd exposure depleted the levels of vitamin-C in all the test tissues under experimentation in a time-dependent manner. Supplementation of Vit C combats with Cd induced oxidative stress during long term exposure, i.e., 30 days' time intervals.

Therefore, in the study, supplementation with Zn and Vit C either individually or in combination to Cd treated rats, decreased oxidative stress in animals. The combination treatment of Zn and Vit C is more effective in reducing the Cd toxicity induced alterations when compared to individual supplementation. Hence our results suggest that Zn and Vit C combined treatment could help to maintain balance between pro-oxidants status and antioxidants status, thereby maintaining antioxidant homeostasis in the tissues of Cd treated rats.

MTs are cytoplasmic proteins, where its synthesis is essential to protect tissues from the toxic effects of Cd [41]. In the present study, MT protein quantification was carried out in liver, kidney and testis of the male albino rat. Cd treated rats showed more amounts of MT protein levels when compared to control rats in the present study. To increase at the time of Cd exposure increased synthesis of MT takes place in rats. In the present study, the supplementation of Zinc and/or Vit C influenced the MT expression in all the test tissues under experimentation. The present studies are in consonance with earlier reports [42-44]. Increased synthesis of MT produces more antioxidant effects against oxidative stress induced by Cd. At low doses of Cd, Metallothionein showed protection against Cd induced toxicity, but at higher doses of Cd the binding capacity of MT is decreased promoting oxidative stress in animal [45].

The Cd is absorbed into the body by inhalation (90 %), ingestion (5%) and absorption (0.5%) and gets transported to various parts through blood. Blood carries cadmium with the help of RBC and forms a Cd-Albumin (Cd-Alb) complex. The complex, thus formed enters into the liver and undergoes dissociation into Cd and Albumin. Further, Cd stimulates the synthesis of metallothionein in the liver and forms a Cd-MT complex. Cd-MT complex is a detoxified form and does not promote hepatotoxicity. Cd occupies the active sites of MT completely, and extra Cd leftover beyond the active sites promote hepatotoxicity [46,47,6].

Cd-MT further enters into the kidney and causes nephrotoxicity [38]. Cd-MT complex entered into the kidney is degraded by lysosomes and free Cd molecules promote nephrotoxicity. Kidney gets more affected under Cd intoxication when compared with liver, because Cd first gets accumulated in the kidney and further starts to accumulate in liver [38]. MT concentration is higher in testis when compared to the liver and kidney under Cd intoxication. But cadmium

tends to accumulate more in the liver (30 %) and kidney (30 %) when compared to testis. Cd binds to MT present in the testis and promotes testicular injury. Cd-MT complex is further eliminated from the body via urinary or faecal excretion. Cd-MT complex in the urine is an indication of tubular injury [48]. Urine and blood cadmium concentrations are an index of total body burden.

The mechanism of MT in the liver and kidney is different from the testis. But a similar trend was observed in liver, kidney and testis with an increase in MT, promoted increases in Cd accumulation [49]. MT synthesis is more in the kidney when compared to liver and testes. Cd detoxification occurs due to enhanced MT synthesis [50].

Synthesis of MT protein was more in the kidney when compared to the liver and testis in all the modes of supplementation (individual and combined supplementation of Zn and Vit-C). Similar results are seen in rats treated with Cd. These results are supported by the studies of [51] suggesting that more synthesis of MT is seen in the kidney because it is a major "Critical Organ" of Cd toxicity. 30 d Cd exposed rat kidney showed more MT protein synthesis than other test tissues under experimentation.

Zinc homeostasis are under the control of MT, hence Zn induces the production of the metallothionein [52,49]. Zn supplementation could prevent Cd toxicity by increased synthesis of MT. Hence increased synthesis of MT is observed under the supplementation of zinc. Zinc and cadmium compete for the active sites on the metallothionein and thus decrease the absorption of cadmium, thus reducing lead toxicity.

Vit C induces MT and influences the expression of MT in all the test tissues of rats under experimentation. The exact mechanism by which MT protein induction occurs by Vit C is not elucidated. MT is considered as a ROS scavenger that reduces the adverse effects of cadmium [53]. Vit C has a prominent antioxidant capacity, hence along with MT it reduces the oxidative stress developed in the animal.

Our present results suggest that MT protein quantification indicated that the kidney tissue showed more MT expression when compared to liver and testis under Zn and/ or Vit C supplementation at all the time intervals of experimentation. Increased expression of MT protein was due to the ameliorative effects of Zn and/ or Vit C over Cd toxicity.

MT protein quantification studies showed that when Cd treated rats when supplemented with Zn and / or Vit C, the MT proteins provide protection against Cd induced oxidative stress and toxicity in the liver, kidney and testis. Based on the overall discussion, it may be concluded that Zn and Vit C supplementation either individually or in combination were more effective in the MT protein synthesis. The results are more supporting and reducing the stress in the organism by synthesis of more MT protein to combat Cd toxicity.

An overall study suggests that when compared to the individual supplementation of Zinc and Vitamin C combined supplementation showed more significant results. GST and GPx activities and MT protein synthesis were overall increased under Zn and/or Vit C supplementation which is essential to protect animals from Cd induced oxidative stress.

CONCLUSION:

From the present study results we conclude that significant elevation of LPO and significant decline in GST and GPx activities observed under Cd induced oxidative stress was reversed under supplementation of Zn and/or Vit C. The results are more significant and beneficial in combined supplementation of nutrients when compared to individual supplementation. MTs, the metal binding proteins are the first line of defence against Cd toxicity and reduces cadmium toxicity. Hence increased MT synthesis under supplementation of Zn and/or Vit C showed more beneficial effects against Cd induced oxidative stress.

ACKNOWLEDGEMENT:

The authors are highly thankful to the UGC, New Delhi for the financial support rendered with the award of Rajiv Gandhi National Fellowship and my research Supervisor, Dr. A. Usha Rani, Professor, Department of Zoology, Sri Venkateswara University, Tirupati.

REFERENCES:

1. Liu J, Goyer R, Waalkes MP. Toxic effects of metals. In: Klaassen CD, editor. Casarett and Doull's Toxicology. The Basic Science of Poisons. 9th ed. Ney York: McGraw-Hill Education; 2018. pp. 931-979.
2. Renugadevi J, Prabu SM. Cadmium-induced hepatotoxicity in rats and the protective effect of naringenin. Exp Toxicol Pathol, 2010; 62(2): 171-181.

3. Babaknejad N, Bahrami S, Moshtaghie AA, Nayeri H, Rajabi P, Iranpour FG. Cadmium Testicular Toxicity in Male Wistar Rats: Protective Roles of Zinc and Magnesium. Biol Trace Elem Res, 2018; 185: 106-115.
4. Genchi G, Stefania M, Graziantonio S, Carocci A, Catalano A. The Effects of Cadmium Toxicity. Int J Environ Res Public Health, 2020; 17: 37-82.
5. Klaassen CD, Liu J, Bhalchandra DA. Metallothionein Protection of Cadmium Toxicity. Toxicol Appl Pharmacol, 2009; 238: 215-220.
6. Kesavulu V, Usha Rani A. Biological activities of Selenium, Vitamin-E and Vitamin-C against Cadmium toxicity in liver and kidney of male albino rat. World J pharmacy Pharma Sci, 2021; 10 (2): 1264-1288.
7. Thirumoorthy N, Sunder AS, Kumar KTM, Kumar MS, Ganesh GNK, Chatterjee M. A Review of Metallothionein Isoforms and their Role in Pathophysiology. World J Surg Oncol, 2011; 9: 54-60.
8. Takahashi S. Molecular functions of metallothionein and its role in hematological malignancies. J Hematol Oncol, 2012; 5: 41-48.
9. Theocharis SE, Margeli AP, Koutselinis A. Metallothionein: A multifunctional protein from toxicity to cancer. Int J Biol Markers, 2003; 18:162-169.
10. Andrews GK. Regulation of Metallothionein gene expression by oxidative stress and metal ions. Biochem Pharmacol, 2000; 59: 95-104.
11. Min KS, Morishita F, Tetsuchikawahana N, Onasaka S. Induction of hepatic and renal metallothionein synthesis by ferric nitrilotriacetate in mice: Role of MT as an antioxidant. Toxicol Appl Pharm, 2005; 204: 9-17.
12. Venkataramanaiah P, Renuka M, Aparna Y, Babu C, Kesavulu V, Srinivasulu Reddy M. Impact of infertility activity of eugenol and *Ocimum sanctum* Linn. leaf extract on Antioxidant Enzymes study in female albino rats. Int J Biosci, 2020; 17 (6): 373-386.
13. Kini RD, Tripathi Y, Raghuveer CV, Pai SA, Ramaswamy C, Kamath P. Role of vitamin C as an antioxidant in cadmium chloride induced testicular damage. Int J Appl Biol Pharm Technol, 2011; 2(1): 97-101.
14. Wagner KR, Sharp FR, Ardizzone TD, Lu A, Clark JF. Heme and iron metabolism: Role in cerebral hemorrhage. J Cereb Blood Flow Metab, 2003; 23: 629-652.
15. Tizhe EV, Ibrahim ND, Fatihu MY, Onyebuchi II, Jonathan George BD, Ambali SF, *et al*. Influence of zinc supplementation on histopathological changes in the stomach, liver, kidney, brain, pancreas and spleen during subchronic exposure of Wistar rats to glyphosate. Comp Clin Pathol, 2014; 23: 1535-1543.
16. Zhai Q, Narbad A, Chen W. Dietary strategies for the treatment of Cadmium and Lead toxicity. Nutrients, 2015; 7: 552-571.
17. Waalkes MP. Cadmium carcinogenesis. Mutat Res, 2003; 533(1-2): 107-120
18. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem, 1979; 95: 351-358.
19. Habig WH, Pabst MJ, Jacoby WB. Glutathione–S-transferases: the first enzymatic step in mercapturic acid formation. J Biol Chem, 1974; 249: 7130-7139.
20. Flohe L, Gunzler WA. Assays of glutathione peroxidase. Methods Enzymol, 1984; 105: 114-121.
21. Fowler BA, Engel DW, Brouwee M. Purification and characterization studies of cadmium binding proteins from the *American oyster*, *Crassostrea virginica*. Environ Health Perspect, 1986; 65: 163-169.
22. Overnell J, Coombs TL. Purification and properties of plaice metallothionein, a cadmium binding protein from the liver of the Plaice (*Pleuronectes platessa*). Biochem J, 1979; 183: 277-283.
23. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with Folin phenol reagent. J Biol Chem, 1951; 193: 265-275.
24. Salinska A, Wlostowski T, Zambrzycka E. Effect of dietary cadmium and/or lead on histopathological changes in the kidneys and liver of bank voles Myodesglareolus kept in different group densities. Ecotoxicology, 2012; 21 (8): 2235-2243.
25. Ramesh G, Madhuri D, Lakshman M, Gopal Reddy A. Histopathological and ultrastructural changes of liver and kidney induced by lead and cadmium alone and combined exposure in male wistar rats. The Pharma Innovation J, 2019; 8(2): 407-413.
26. Brzoska MM, Moniuszko-jakoniuk J, Piłat-marcinkiewicz B, Sawicki B. Liver and kidney function and histology in rats exposed to cadmium and ethanol. Alcohol Alcoholism, 2003; 38(1): 2-10.
27. Hisayoshi OHTA, Yonggang QI, Kenichi OHBA, Tatsushi TOYOOKA, Rui-Sheng W. Role

of metallothionein-like cadmium-binding protein (MTLCdBP) in the protective mechanism against cadmium toxicity in the testis. Ind Health, 2019; 57(5): 570-579.
28. Cabre M, Comps J, Paternain JL, Ferre N, Joven J. Time course of changes in hepatic lipid peroxidation and glutathione metabolism in rats with carbon tetrachloride induced cirrhosis. Clin Exp Pharmacol Physiol, 2000; 27 (9): 694-699.
29. Mitra P , Ghosh T , Mitra PK. Isolation of a compound from Amorphophallus paeoniifolius Tuber and studies on its *in vitro* antioxidant activity. J Pharm Adv Res, 2019; 2(6): 561-564.
30. Hisar O, Yıldırım S, Sonmez AY, Aras HN, Gultepe N. Changes in liver and kidney antioxidant enzyme activities in the rainbow trout (*Oncorhynchus mykiss*) exposed cadmium. Asian J Chem, 2009; 21: 3133-3137.
31. Han XY, Xu ZR, Wang YZ, Huang QC. Effect of cadmium on lipid peroxidation and activities of antioxidant enzymes in growing pigs. Biol Trace Elem Res, 2006; 110(3): 251-263.
32. Roopha PD, Padmalatha C. Effect of Herbal Preparation on Heavy Metal (Cadmium) Induced Antioxidant System in Female Wistar Rats. J Med Toxicol, 2012; 8(2): 101-107.
33. Meena Bai M, Haseena Bhanu SK, Thyagaraju K. Determination of cadmium and zinc levels and oxidative status in cadmium treated developing chick embryonic liver. IOSR J Pharmacy, 2014; 4(5): 4-13.
34. Ognjanovic BI, Markovic SD, Pavlovic SZ, Zikic RV, Stajn AS, Saicic ZS. Effect of chronic cadmium exposure on antioxidant defense system in some tissues of rats: Protective effect of Selenium. Physiol Res, 2008; 57: 403-411.
35. Jovanovic MJ, Nikolic RS, Kocic GM, Krstic SN, Krsmanovic, MM. Glutathione protects liver and kidney tissue from cadmium and lead provoked lipid peroxidation. J Serb Chem Soc, 2012; 77: 1-7.
36. Masella R, Di Benedetto R, Varì R, Filesi C, Giovannini C. Novel mechanisms of natural antioxidant compounds in biological systems: Involvement of glutathione and glutathione related enzymes. J Nutr Biochem, 2005; 16: 577-586.
37. Patra R, Amiya C, Rautray K, Swarup D. Oxidative Stress in Lead and Cadmium Toxicity and Its Amelioration. Vet Med Int, 2011; 5: 457327: 1-9.
38. Mahran AA, HusamEldien HO, Abd El-Mawla AMA, Attia AM. Protective Effect of Zinc (Zn) on the Histology and Histo chemistry of Liver and Kidney of Albino Rat Treated with Cadmium. J Cytol Histol, 2011; 2: 2157-7099.
39. You WC, Zhang L, Gail MH, Chang YS, Liu WD, Ma JL, *et al*. Gastric dysplasia and gastric cancer: *Helicobacter pylori*, serum vitamin C, and other risk factors. J Natl Cancer Inst, 2000; 4: 92(19): 1607-1612.
40. Bhavana R, Baby M, Thomas B. Comparative assessment of Antioxidants Vitamin C, Total Phenolics and Flavonoids of selected commercial and underutilized fruits. J Pharm Adv Res, 2018; 1(7): 335-340.
41. Ozturk A, Baltaci AK, Mogulkoc R, Oztekin E, Sivrikaya A, Kurtogh E, *et al*. Effects of zinc deficiency and supplementation on malondialdehyde and glutathione levels in blood and tissues of rats performing swimming exercise. Biol Trace Elem Res, 2003; 94: 157-166.
42. Lu J, Jin T, Nordberg GF, Nordberg M. Metallothionein gene expression in peripheral lymphocytes and renal dysfunction in a population environmentally exposed to cadmium. Toxicol Appl Pharmacol, 2005; 206: 150-156.
43. Chaumont SB, Maupoil V, Berthelot A. Metallothionein induction in the liver, kidney, heart and aorta of cadmium and and isoproterenol treated rats. J Appl Toxicol, 2006; 26: 47-55.
44. Kukner A, Colakoglu N, Kara H, Oner H, Ozogul C, Ozan E. Ultrastructural changes in the kidney of rats with acute exposure to cadmium and effects of exogenous metallothionein. Biol Trace Elem Res, 2007; 119: 137-146.
45. Xu LC, Sun H, Wang SY, Song L, Chang HC, Wang XR. The roles of metallothionein on cadmium-induced testes damages in Sprague–Dawley rats. Environ Toxicol Pharmacol, 2005; 20: 83-87.
46. Leslie EM, Liu J, Klaassen CD, Waalkes MP. Acquired cadmium resistance n metallothionein – I/II (-/-) Knock out cells: Role of the T-type calcium channel cacnα_{1G} in cadmium uptake. Mol Pharmacol, 2006; 69(2): 629-639.
47. Roy HK, Nayak BS. Evaluation of antilipid peroxidation activity of *Hibiscus sabdariffa linn,* International Journal Pharmaceutical Research and Technology, 2010, 2(1), 23-25.

48. Sabolic I, Breljak D, Skarica M, Carol M, Kramberger H. Role of metallothionein in cadmium traffic and toxicity in kidneys and other mammalian organs. BioMetals, 2010; 23: 897-926.
49. Bonda E, Włostowski T, Krasowska A. Testicular toxicity induced by dietary cadmium is associated with decreased testicular zinc and increased hepatic and renal metallothionein and zinc in the bank vole (*Clethrionomys glareolus*). Biometals, 2004; 17: 615-624.
50. Goering, PL, Fisher BR, and Kish CL. Stress protein synthesis induced in rat liver by cadmium precedes hepatotoxicity. Toxicol Appl Pharmacol, 1993; 122: 139-148.
51. Hashem FA, Dallak M, Bashir N, Abbas M, Elessa R, Khalil M, *et al*. Camel's milk protects against cadmium chloride induced toxicity in white albino rats. Am J Pharmacol Toxicol, 2009; 4 (3): 107-117.
52. Suzuki CA, Ohta H, Albores A, Koropatnick J, Cherian MG. Induction of metallothionein synthesis by zinc in cadmium pretreated rats. Toxicol, 1990; 63: 273-284.
53. Bolkent S, Sacan O, Yanardag R. Effects of Vitamin E, Vitamin C, and Selenium on Gastric Fundus in Cadmium Toxicity in Male Rats. Int J Toxicol, 2008; 27: 217-222.

Conflict of Interest: None
Source of Funding: Nil
Paper Citation: Kesavulu. V, Usha Rani. A*. Therapeutic role of Zinc and Vitamin-C against Cadmium induced Oxidative stress in selected tissues of male albino rat: A study with reference to Metallothionein quantification. J Pharm Adv Res, 2021; 4(5): 1238-1250.