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Original Article

MITIGATING ROLE OF ZINC AND IRON AGAINST CADMIUM INDUCED TOXICITY IN LIVER AND KIDNEY OF MALE ALBINO RAT: A STUDY WITH REFERENCE TO METALLOTHIONEIN QUANTIFICATION

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ABSTRACT

Objective: The present study is carried out to know the mitigating role of zinc (Zn) and / or iron (Fe) supplementation on cadmium (Cd) induced toxicity in rats with special reference to metallothionein (MT) protein.

Methods: Wistar strain male albino rats were treated orally with Cd at a dose of $1/10^{th}$ of LD_{50} / 48h (i. e. 22.5 mg/kg) for 7, 15 and 30 days (d) long sojourn. 15d Cd treated rats were then subjected to trace element supplementations of Zn (12mg/kg) and Fe (40mg/kg) individually and in combination for another 7, 15 and 30d time intervals. After specific time intervals, rats were decapitated and tissues like liver and kidney were isolated. The vital oxidative stress enzymes such as GST and GPx were assayed by using the standard methods in the test tissues. LPO levels were also measured by using the standard protocol. MTs, the metal binding proteins which are the first line of defense against Cd toxicity were quantified by using the standard methods in the test tissues.

Results: A significant ($P < 0.05$ level) elevation in LPO levels with decreased activity levels of GST and GPx were observed during Cd intoxication. With Zn and Fe supplementation, a significant reversal in the above said parameters were observed. MT protein levels were significantly elevated in the test tissues during Cd treatment and also after supplementation with Zn and / or Fe. Maximum MT protein synthesis was observed in 30d rat kidney under combined supplementation of both Zn and Fe.

Conclusion: The present study focuses on the mitigating role of trace elements Zn and Fe in reducing the Cd body burden from the selected tissues of rat. Supplementation with Zn and / or Fe envisages the therapeutic role of trace elements in combating the heavy metal, Cd insult.

Keywords: Cadmium, Oxidative stress enzymes, Zinc and iron supplementation, Metallothionein, Liver, Kidney, Rat.

INTRODUCTION

Environmental pollution is a global problem and is common to both developed as well as developing countries. This pollution was caused by numerous chemicals, xenobiotics, heavy metals etc. Among the heavy metals, Cadmium (Cd) is one of the most toxic, non- essential heavy metal with many industrial uses that can contribute to a well-defined spectrum of diseases in animal models as well as in humans [1, 2].

Over the past two centuries, anthropogenic and industrial activities have led to high emissions of Cd into the environment at concentrations significantly exceeding those originating from natural sources [3]. Cd has an estimated elimination half-life period of 20-30 years in the human body [4] and is highly cumulative, especially in the liver and kidney [5 - 8]. The main sources of Cd are storage batteries, electroplating, pigments, plastics, fertilizer industries and cigarette smoking.

It is an ubiquitous toxic metal and induces oxidative damage by disturbing the prooxidant – antioxidant balance in the tissues [9]. Cd is readily distributed in tissues after exposure and interferes with intracellular signaling network and gene regulation at multiple levels and induces lipid peroxidation (LPO). Lipid peroxides that accumulate due to LPO are known to be harmful to cells and tissues and inhibit the antioxidant system of the cells [10, 11]. As a result of this inhibition, the electron transport chain becomes highly reduced, electrons are transferred directly to available oxygen and lead to enhanced formation of reactive oxygen species (ROS) [12]. ROS may lead to increase oxidative stress in tissues, cellular damage, peroxidation of membrane lipids and loss of membrane bound enzymes [13, 14], which might result in histological changes and physiological damage to different organs [15, 8, 16, 3]. Intake of Cd results in consumption of glutathione (GSH) and protein binding sulfhydral groups and subsequently the levels of free radicals such as hydrogen peroxide, hydroxide and superoxide are increased [17].

Sulfhydral – rich, metal binding protein, Metallothionein (MT) may function in a manner similar to GSH. Wherein MT provides an intracellular 'nucleophilic sink' to trap free radicals, electrophiles and alkylating agents. MT's is a class of low molecular weight (6-7kDa), cysteine rich and transition metal binding proteins. MT's occur throughout the animal kingdom and are also found in higher plants, eukaryotic microorganisms and in some prokaryotes [18 - 21]. MT's have the capacity to bind heavy metals through the thiol group of its cysteine residues. A large amount of subsequent work has shown MT to serve in many cell types in the management of essential divalent metal cations, to interfere with the toxic effects of xenobiotics, heavy metals, free radicals and to serve as a regulator of specific transcription factors. Cells that contain an excess amount of MT are resistant to Cd toxicity [22, 23].

The complex inter-relationships between Cd and some essential trace elements have not been elucidated. Several essential trace elements like zinc (Zn), iron (Fe), selenium (Se) and copper (Cu) participate in controlling various metabolic and signaling pathways. Among the trace elements Zn and Fe are essential for maintenance of life and health. Zn is an essential trace metal with numerous functions in biological systems. Zn controls several enzymes of intermediary metabolism, DNA and RNA synthesis, gene expression, immunocompetence and plays a significant role in homeostasis of hormones. 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 [24, 25]. Fe plays an essential role in many biological processes and it is important to maintain iron concentration within its narrow normal range. Fe supplementation reduces Cd retention and Cd induced anemia during fast growth in young rats [26]. *In vitro* studies suggests that there is a competition for transport mechanism between Cd and some essential trace elements like Zn

and Cu [27], Fe [28] in rats, Zn and Se in Japanese quails [29] and calcium in suckling rats [30]. Hence, in the present study Zn and Fe were chosen as trace element supplements.

MATERIALS AND METHODS

Chemicals

Cadmium as cadmium chloride ($CdCl_2$), zinc as zinc chloride ($ZnCl_2$), and iron as ferric chloride ($FeCl_3$) were purchased from Merck (Dormstadt, Germany). All other chemicals which were used in the present study were obtained from the standard chemical companies like Sigma Chemical Co. (St Louis, Mo, USA) and SD Fine Chemicals. The chemicals used in this study were of the highest purity.

Animals

Three months old Wistar strain male albino rats weighing 180 ± 20g were chosen for the present study. The animals were obtained from Sri Venkateswara Traders, Bangalore, Karnataka, India and were kept in stainless-steel mesh cages, housed under standard laboratory conditions (23 ± 2^0C, 50 ± 20% relative humidity, 12h light - dark cycle) with Standard rat chow (Sai Durga feeds and foods, Bangalore, India) and drinking water *ad libitum*. The rats were acclimatized to the laboratory conditions for 10 days. The protocol and animal use has been approved by the Institutional Animal Ethics Committee (Resol. No.10(ii) / a / CPCSCA / IAEC / SVU / AUR-JO dt 22-12-2008), Sri Venkateswara University, Tirupati, Andhra Pradesh, India.

Experimental design

After acclimatization, the rats were divided into two groups, namely control and experimental. Control rats received only de-ionised water without Cd. The experimental rats were treated with Cd as $CdCl_2$ at a dose of $1/10^{th}$ LD_{50} /48h i. e. 22.5 mg / Kg body weight over a period of 7, 15 and 30 days (d) time intervals. Then the 15d Cd treated rats were divided into three groups. Group I received supplementation of Zn (12mg / Kg) for 7, 15 and 30d. Group II received Fe supplementation (40 mg / Kg) and Group III animals were supplemented with both Zn and Fe at the above said doses for 7, 15 and 30d long sojourn.

Isolation of tissues

After specific time intervals, the control and experimental rats were decapitated and tissues such as liver and kidney were quickly isolated under ice cold conditions and weighed to their nearest mg using Shimadzu electronic balance. After weighing, tissues were immediately used for the assay of oxidative stress enzymes like GST, GPx, the levels of LPO and MT protein quantification.

Assay of oxidative stress enzymes

Lipid peroxidation (LPO)

The LPO was determined by the TBA method of Ohkawa *et al*., [31]. The tissues were homogenized in 1.5% KCl (20% W/V). To 1 ml of tissue homogenate 2.5 ml of 20% TCA was added and the contents were centrifuged at 3,500g for 10 minutes (min) and the precipitate was dissolved in 2.5 ml of 0.05M sulphuric acid. To this, 3 ml of thiobarbituric acid (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 the external standard. Values are expressed in µ moles of MDA formed / g tissue / hr.

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 of Habig *et al*., [32]. 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 4^0C and the supernatant was again centrifuged at 16,000 g for 1 hour (hr) at 4^0C. 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 the 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 of Flohe and Gunzler [33] at 37^0C. 5% (W/V) of tissue homogenate was prepared in 50 mM phosphate buffer (pH 7.0) containing 0.1 mM EDTA. The homogenates were centrifuged at 10,000 g for 10 min at 4^0C in cold centrifuge. The resulting supernatant was used as enzyme source. The reaction mixture consisted of 500 µl of phosphate buffer, 100µl of 0.01 M GSH (reduced form), 100 µl of 1.5 mM NADPH and 100µl of GR (0.24 units). The 100µl of tissue extract was added to the reaction mixture and incubated at 37^0C 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 $X10^3$ 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 and kidney homogenates were carried out by following Fowler *et al.,* [34]. The clear supernatants thus obtained from liver and kidney homogenates was again subjected to the purification process. Supernatant fractions of each tissue was applied to a column of Sephadex, G-75 (5 x 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 of Overnell and Coombs [35]. Purified MT protein quantification was performed by using Lowry *et al.,* [36].

Estimation of protein content

Protein content of the tissues was estimated by the method of Lowry *et al.,* [36]. 1 % (W/V) homogenates of the tissues were prepared in 0.25 M ice cold sucrose solution. To 0.5 ml of homogenate, 1 ml 10% TCA was added and the samples were centrifuged at 1000g for 15 min. Supernatant was discarded and the residues were dissolved in 1 ml of 1N sodium hydroxide. To this 4 ml of alkaline copper reagent was added followed by 0.4 ml of the folin-phenol reagent (1:1folin: 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 protein (BSA) standard graph.

Data analysis

The data was subjected to statistical analysis such as mean, standard deviation and Analysis of variance (ANOVA) using standard statistical software, SPSS (version 16) software. All values are expressed as Mean ± SD of 6 individual samples. Significant differences were indicated at $P < 0.05$ level.

RESULTS

Results revealed that LPO levels were significantly ($P < 0.05$ level) increased in both liver and kidney of Cd treated rats over control (Fig. 1). The LPO levels were increased with the time intervals of Cd treatment compared to control and were maximum for 30d kidney (71.083 ± 2.113 µ moles of MDA formed /g tissue / hr). After supplementation with Zn and / or Fe, the LPO levels were progressively decreased at all time periods in both the test tissues. Maximum depletion was observed in Zn and Fe combined supplemented 30d rat kidney (38.791±1.278 µ moles of MDA formed /g tissue / hr).

GST activity levels also showed a progressive decrement at all time intervals of Cd treatment with a maximum depletion in 30d rat liver (15.146 ± 0.715 µ moles of thioether formed / mg protein / min). Further supplementation with both Zn and Fe, the GST activity reached to normalcy in 30d rat kidney (30.299 ± 0.288 µ moles of thioether formed / mg protein / min) suggesting the protective role of trace elements Zn and Fe (Fig. 2).

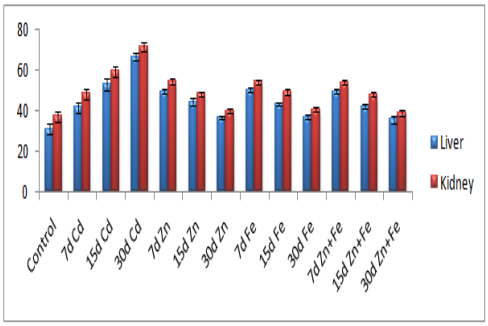

Fig. 1: The levels of LPO (µ moles of MDA formed / g tissue / hr) in liver and kidney of Cd treated rats before after supplementation with Zn and / or Fe.

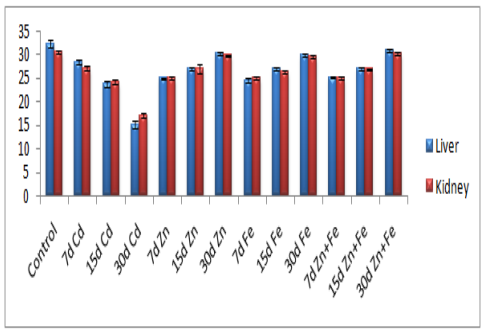

Fig. 2: GST activity levels (µ moles of thioether formed / mg protein / min) in liver and kidney of Cd treated rats before after supplementation with Zn and / or Fe.

GPx activity levels also showed a significant decrease at all time intervals of Cd treatment with a maximum decrease in 30d kidney (0.822 ± 0.097 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 Fe both individually as well as in combination (Fig. 3). The combination of Zn and Fe at 30d as supplement was more effective in elevating the GPx activity levels in the liver tissue of rats (1.266 ± 0.008 µ moles of NAPDPH oxidized / mg protein / min).

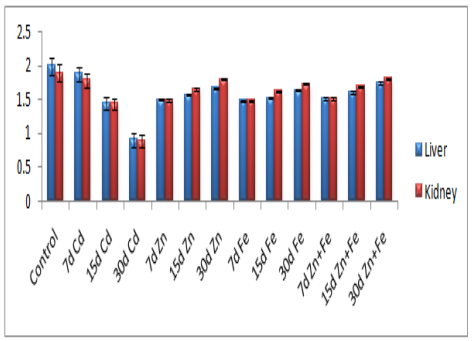

Fig. 3: GPx activity levels (µ moles of NADPH oxidized / mg protein / min) in liver and kidney of Cd treated rats before after supplementation with Zn and / or Fe.

Quantification of MT protein content was carried out in both liver and kidney tissues of control rats. Cd treated as well as Zn and / or Fe supplementations to the 15d Cd treated rats. Results revealed that MT levels were profoundly increased in both liver and kidney of Cd treated rats at all time intervals when compared to the controls. 30d Cd treated rat kidney showed maximum synthesis of MT protein (15.095 ± 0.454 µg / g wet weight of the tissue) followed by 30d Cd treated rat liver (12.013 ± 0.282 µg / g wet weight of the tissue) at all the treatment time intervals (Fig. 4).

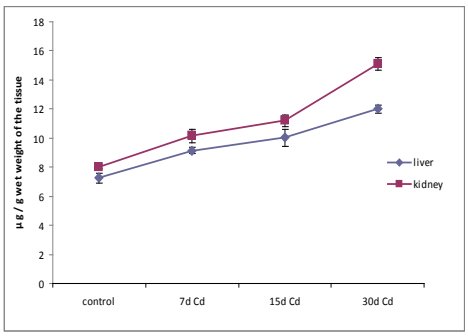

Fig. 4: MT Protein (µ g / g wet weight of the tissue) levels in the selected tissues of Cd treated rats.

After supplementation with Zn and / or Fe to 15d Cd treated rats, the MT levels were highly elevated in both liver and kidney during all the time intervals. Maximum MT protein synthesis was found in 30d rat kidney under combined supplementation of Zn and Fe (17.481 ± 0.313 µg / g wet weight of the tissue) (Fig. 7). Moderate increment in the synthesis of MT protein was found in 30d Zn supplemented rat kidney and liver (17.141 ± 0.363 µg / g wet weight of the tissue and 14.827 ± 0.313 µg / g wet weight of the tissue respectively). While in the 30d Fe alone supplementation, both the test tissues showed low level of increment in MT protein content (16.579 ± 0.342 µg / g wet weight of the tissue in kidney and 13.886 ± 0.324 µg / g wet weight of the tissue in liver) than the other modes of supplementation (Fig. 5 - 7).

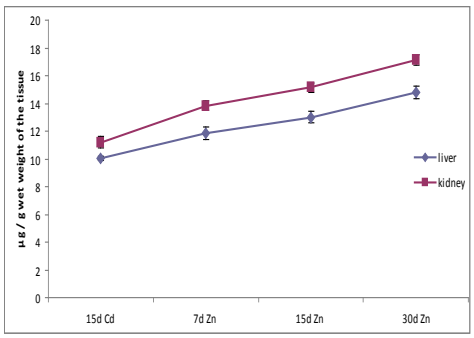

Fig. 5: MT Protein (µ g / g wet weight of the tissue) levels in the tissues of Cd treated rats after supplemented with Zn.

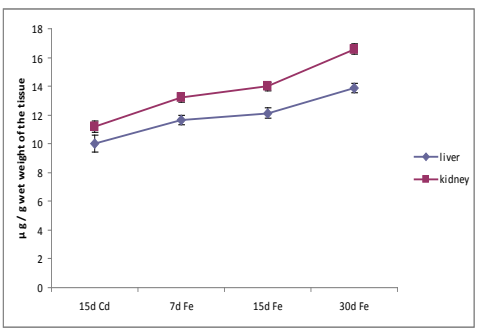

Fig. 6: MT Protein (µ g / g wet weight of tissue) levels in the tissues of Cd treated rats after supplementation with Fe.

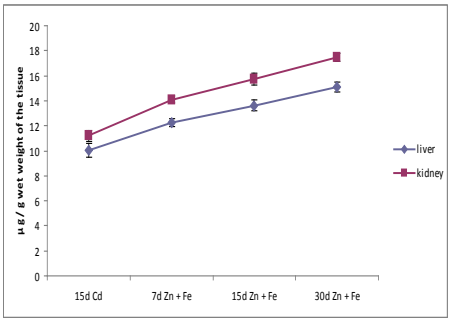

Fig. 7: MT Protein (µ g / g wet weight of tissue) levels in the tissues of Cd treated rats after supplementation with Zn and Fe.

From the present investigation, it is clear that the MT protein synthesis was high in the combined supplementation of Zn and Fe than the individual supplementation of Zn and Fe. 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 and kidney tissues.

DISCUSSION

The results of the present study revealed that Cd induces significant alterations in the levels of LPO and certain oxidative stress enzymes status in liver and kidney of male albino rat at all specific time intervals. These activities were progressively reversed after using trace element supplements like Zn and / or Fe.

Several mechanisms have been proposed for Cd induced various abnormalities, but none have yet been defined explicitly. Disruption of a variety of biochemical processes have been proposed rather than a single mechanism responsible for Cd toxicity. Recently, oxidative stress has been reported as one of the important mechanisms of toxic effects of Cd. Cd induced oxidative stress shows the significant impact on membrane, DNA and an antioxidant defense system of the cell [37, 38].

In our study, the levels of LPO were significantly increased in the liver and kidney of Cd treated rats in a time-dependent manner, suggesting that long-term exposure to Cd profoundly causes LPO thereby resulting in oxidative damage. Several studies also demonstrated an increased LPO levels in the liver and kidney of test animals during Cd treatment [39 - 44]. LPO has been reported to enhance tissue water content, permeability and several fatty acids and lysophospholipids are released, leading to changes in biomembrane microviscosity and kinetic properties. This changes the ultrastructure and integrity of membrane causing loss of membrane bound enzymes [10, 45, 11]. Further, it has been suggested that during Cd treatment, potentially harmful byproducts are generated including ROS in the organisms [43]. ROS thus generated by Cd intoxication reacts with membrane lipids and causes LPO and finally it may lead to cell death [46, 47].

The reduced GST activity in the tissues may be due to over consumption of the enzyme GST to escape from the toxicity of peroxides under Cd insult. GST catalyzes the reaction of the thiol (-SH) group of GSH with electrophilic reagents such as those generated by microsomal metabolism of xenobiotics, thereby neutralizing their electrophilic sites and rendering the products more water soluble [48]. The decrease in GST activity might have resulted with Cd effect on GSH because of its high affinity to this molecule where a sulfhydryl acid, an amino acid and two carboxylic acid groups, as well as two peptide linkages represent reactive sites for metals. Reactions of metals with glutathione might lead to either the formation of complexes or the oxidation of glutathione. The decreased GST activity in the test tissues is in agreement with El-Missiry and Shalaby [49] in Cd treated rat brain and testis. Moreover, the decrease in the activity of each of them would induce increased free radicals thus injuring the corresponding tissues. GPx is a hydrogen peroxide degrading enzyme. Its activity was significantly decreased in both liver and kidney under Cd body burden at all time intervals. The decreased GPx activity in the current study may be due to impairment in GSH homeostasis in liver and kidney tissues. As a result of this, liver and kidney tissue damage might have occurred under Cd insult. Recently Ognjanovic *et al*., [9], Messaoudi *et al*., [50] and Obaiah and Usha Rani [51] also reported decreased GPx activity in the liver and kidney tissues of rats under Cd stress. It may be due to either free radical dependent inactivation of enzyme or depletion of its co-substrate i. e., GSH and NADPH in the Cd treated rat liver and kidney. Cd administered rat tissues showed decreased GSH content due to over utilization by the cells in the tissues. Due to non-bioavailability of GSH under Cd burden, decrement in the activity levels of GPx has also been observed in experimental tissues. Depletion of GSH may render in GPx inactivation and / or less activity [52].

Studies by Fariss [53] have shown that the free radical scavengers and antioxidants are useful in protecting the tissues against Cd toxicity. Zn and Fe are the two important free radical scavengers [54] which play an important role in many biological functions including pro-oxidant and antioxidant status. In the present study, rats treated to Cd for 15 days were challenged with Zn and / or Fe to know whether these antioxidants have ability to mitigate Cd-induced oxidative stress or not. The time point, 15 days were selected because, a complete deterioration of antioxidant status was observed in the liver and kidney of these rats treated to Cd. Surprisingly, the results of the present study indicates that supplementation of Zn or Fe or a combination of both Zn and Fe reverses the Cd induced oxidative stress in the liver and kidney of rats. Supplementation of Zn and Fe either individually or in combination, inhibited the formation of MDA in the liver and kidney of experimental animals. A significant reduction in the MDA was observed in the liver tissue of Zn supplemented Cd treated rats during 15 and 30 days time intervals (Table - VI). Based on the available literature it is clearly evident that most of the antioxidant enzymes become inactive by Cd exposure due to the direct binding of the Cd to enzyme active sites if they contains –SH group or by displacement of metal co-factors from their active sites [55, 17]. Further, it has been suggested that Zn and Fe supplements sustain the bioavailability of the essential trace elements thereby playing a role in displacement of Cd from the metal binding sites of enzymes, which in turn may help in bringing down the enzymatic antioxidants to normal and functional [56 - 59, 50, 60]. Recently, it has also been reported that Zn and Fe provide protection against Cd induced alteration through the induction of MT either directly or indirectly [61, 62] and activation of antioxidant defense system and in turn decrease the ROS generation [63 - 65]. These findings are confirmed that the trace elements (Zn and Fe) may reduce the Cd mediated tissue damage by blocking the oxidative chain reaction and suppressing the formation of LPO products such as MDA. Our results in the present investigation are in consonance with earlier studies [66 - 68, 59].

In recent years, Cd has been recognized as one of the most toxic environmental and industrial pollutant due to its ability to induce severe alterations in various organs and tissues. One of tissue protection mechanisms against these toxic effects of Cd is MT synthesis [69, 21]. MTs are cytoplasmic proteins that sequester certain divalent metal cations and are considered as primary cellular defense against the toxic transition metal Cd. In the present investigation, MT protein quantification was carried out in both liver and kidney tissues of the male albino rat. MT protein levels were significantly increased in Cd treated rats over controls in the present study which indicates that Cd exposure induces MT synthesis in liver and kidney of rats. Our results are in consonance with the earlier reports of Haki Kara *et al*., [70] and Kukner *et al*., [69]. Previous studies of Lu *et al*., [71, 72], Chaumont *et al*., [73], Chen *et al*., [74] and Kukner *et al*., [69] revealed that the MT synthesis was high in Cd exposed workers. In another study, Kang [22] also reported that the cells contain an excess amount of MT that are resistant to Cd toxicity.

To protect from heavy metal toxicity, organisms synthesizes more MT protein as it involves in the homeostasis of essential metal ions (Zn and Fe), detoxification of heavy metals (Hg and Cd), protection against oxidative damage through scavenging of ROS, cell proliferation and apoptosis [75 - 79]. Our results revealed that high

levels of MT protein synthesis were found in liver and kidney of rats during supplementation with essential trace elements like Zn and Fe over Cd treated as well as control rats. However, the occurrence of MT protein in the control rats in the present study indicates the ubiquitous nature in the organism of non-polluted environments [80 - 83, 23]. Increased synthesis of MT has been thought to produce antioxidant effect against ROS in Cd and other heavy metal intoxications [84].

More synthesis of MT protein was found in kidney tissue than liver in all the modes of supplementation with Zn and / or Fe as well as Cd treatment. A notable induction of MT protein under Cd over load in the kidney of the present study suggests that kidney might serve as a "Critical Organ" to Cd toxicity. The present data shows the low level of hepatic MT concentration than renal MT concentrations. Many reports suggested that ingestion of Cd is absorbed and transported to plasma where it binds with albumin to form Cd-albumin complex [85] via pulmonary or gastro intestinal route. Cd-albumin is absorbed predominantly by the liver and Cd is released from the albumin in the liver tissue. The released Cd induces synthesis of MT in the liver and most of the Cd is bound to MT [86, 87]. As the liver is the first site of Cd bioaccumulation, where Cd binds with MT [88], the Cd-MT complex transported to kidney tissue might have caused MT increase in the renal tissue. Several authors also reported that MT protein is induced in liver under Cd intoxication, as it is the first site of accumulation of Cd and plays a vital role in the formation of Cd-MT complex and from there the complex is transported to kidney [85, 74, 89, 90].

It is believed that MT plays an important role in Zn metabolism and is popularly known as reservoir of Zn. Zn-MT rescues the function of Cd - substituted tramtrack, a zinc finger transcription factor [91, 92]. When Cd displaces Zn in tramtrack, this protein loses DNA binding activity. Incubating Zn - MT with Cd tramtrack *in vitro* allows the exchange of Cd and Zn, with the transcription factor regaining its DNA binding activity. Hence, Zn-MT may rescue zinc finger proteins from inactivation by other metals. Fe is another essential trace element that plays an important role in MT and hemoglobin synthesis and also in redox reactions. It is an essential nutrient to almost all organisms [93, 94] and plays an essential role in biological processes. Fe induces MT either indirectly or by way of antioxidant response elements [28, 95]. In the present study, the supplemented Fe influenced the expression of MT in both the test tissues of rat under experimentation. The mechanism of MT protein induction by Fe is not elucidated.

Quantification of MT protein revealed that the kidney tissue showed more expression of MT than the liver tissue under Zn and / or Fe supplementation at all the time intervals of experimentation. Yasutake and Hirayama [96] reported that the supplementary Zn and / or Fe enhances MT turnover in the kidney than liver, although the mechanisms of such processes are not understood at present. However, our studies suggest that the increase of MT protein level in both the test tissues is probably mediated by the differential expression of MT gene. Interestingly, supplemented Zn and / or Fe was found to elevate the MT protein expression in the liver and kidney tissues of Cd treated rats, providing further evidence of the ameliorative effects of Zn and / or Fe supplementation against Cd induced stress response in Wistar strain male albino rats. It is well known that Zn and / or Fe provides protection against Cd induced alterations through the induction of MT either directly or indirectly [97, 62, 98], activation of antioxidant defense system and decreases the ROS generation [63, 64, 65].

From the MT studies, it is clear that when Cd treated rats were subjected to Zn and / or Fe supplementation, the MT proteins provides protection against Cd induced oxidative stress and toxicity in the liver and kidney tissues. Based on the overall discussion it may be concluded that the mixture of Zn and Fe supplementation was more effective in the MT protein synthesis as well as in reducing the Cd body burden from the tissues than individual supplementation of Zn and Fe.

CONFLICT OF INTERESTS

Declared None

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