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International Journal of Current Research
Vol. 8, Issue, 12, pp.43383-43389, December, 2016

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

CADMIUM INDUCED BIOACCUMULATION AND OXIDATIVE STRESS IN LIVER AND KIDNEY OF MALE ALBINO RAT

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ARTICLE INFO

Article History:
Received 12th September, 2016
Received in revised form
18th October, 2016
Accepted 25th November, 2016
Published online 30th December, 2016

Key words:
Cadmium,
Bioaccumulation,
Oxidative stress, Rat

ABSTRACT

The present study was carried out to know the cadmium (Cd) induced bioaccumulation and oxidative stress in liver and kidney of Cd treated rats. Wistar strain male albino rats were treated with cadmium chloride ($CdCl_2$) at a dose of 1/10th LD_{50} / 48h i.e. 22.5 mg/kg body weight for 7, 15 and 30 days (d) long sojourn. After the specific time intervals, rats were decapitated and tissues like liver and kidney were isolated for the analysis of Cd bioaccumulation and assay of oxidative stress enzymes such as catalase (CAT), superoxide dismutase (SOD), glutathione -S-transferase (GST) and glutathione peroxidase (GPx). Simultaneously lipid peroxidation (LPO) levels were also measured. There was a significant elevation in Cd bioaccumulation in both the test tissues with increased period of Cd treatment. Maximum Cd accumulation wasfound in 30d Cd treated rat kidney. A significant elevation in LPO levels with decreased activity levels of CAT, SOD, GPx, and GST were observed during Cd intoxication. Our study clearly reveals that Cd intoxication can disturb the antioxidant defense system and increase the body burden of Cd in the tissues.

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Citation: Obaiah Jamakala and Usha Rani, A., 2016. "Cadmium induced bioaccumulation and oxidative stress in liver and kidney of male albino rat", *International Journal of Current Research*, 8, (12), 43383-43389.

INTRODUCTION

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 (Akeem *et al.,* 2011; Nobuhiko *et al.,* 2012). Cd has an extremely long half-life (20-30 Years) in the human body and is highly cumulative, especially in the liver and kidney (Flora *et al.,* 2008; Hijova and Nistiar, 2005; Mahtap and Ethem, 2006; Nordberg *et al.,* 2007; Tim *et al.,* 2008). Kidney is considered as the critical organ in long term low level exposure to Cd (Asagba, 2009). The main sources of Cd are storage batteries, electroplating, pigments, plastics, fertilizer industries and cigarette smoking. Although Cd is widely distributed throughout the body, most of it accumulates in the liver and kidney and alters biochemical and functional changes in the organs. Cd can cause Itai - Itai disease in humans as well as in animals (Nad *et al.,* 2005) and also it induces the onset of anemia, decreases red blood cell count and hemoglobin concentration (Ognjanovic *et al.,* 2003). Some of the toxic effects of Cd exposure are hepatic damage, renal dysfunction, hypertension, central nervous system injury and testicular atrophy (Usha Rani, 2000; Siraj Basha and Usha Rani, 2003; Haki *et al.,* 2005; Jeyaprakash and Chinnaswamy, 2005; Ognjanovic *et al.,* 2008). It is a ubiquitous toxic metal and induces oxidative damage by disturbing the prooxidant – antioxidant balance in the tissues (Ognjanovic *et al.,* 2008). Hence, the present study focused on the bioaccumulation of Cd and perturbations in the antioxidant defense system.

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MATERIALS AND METHODS

Chemicals

Cd as cadmium chloride ($CdCl_2$) was 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. 10(ii)/a/CPCSCA/IAEC/SVU/AUR‑JO dt 22‑12‑2008), Sri Venkateswara University, Tirupati, Andhra Pradesh, India.

Experimental design

After acclimatization, the rats were divided into two groups, namely control and experimental. Control rats received only deionized water without Cd. The experimental rats were treated with Cd as $CdCl_2$ at a dose of 1/10th LD_{50}/48h i.e., 22.5 mg/kg body weight over a period of 7, 15, and 30 days (d) time intervals.

Isolation of tissues

After specific time intervals, the control and experimental rats were decapitated and tissues such as liver and kidney were quickly isolated under ice cold conditions and weighed to their nearest mg using Shimadzu electronic balance. After weighing, tissues were immediately used for the analysis of Cd bioaccumulation and assay of oxidative stress enzymes like catalase (CAT), superoxide dismutase (SOD), glutathione - S‑transferase (GST) and glutathione peroxidase (GPx). Simultaneously lipid peroxidation (LPO) levels were also measured.

Bio-accumulation studies

Cd concentrations in the test tissues were measured by the method of Kanno *et al.,* (1994). After the specific time intervals the tissues like liver and kidney were isolated and immediately washed with saline (0.9%) and 50mg of each tissue was digested in acid mixture of Nitric acid : Perchloric acid (3:2 v/v) for overnight. The acid mixture was then subjected to evaporation and the residue obtained was dissolved in 5ml of double distilled water. From this 1 ml was withdrawn and analyzed for Cd concentrations by using Atomic Absorption Spectrophotometer (Schimadzu AA 6300).

Assay of oxidative stress enzymes

LPO

The LPO was determined by the thiobarbituric acid (TBA) method of Ohkawa *et al.,* (1979). The tissues were homogenized in 1.5% KCl (20% W/V). To 1ml of tissue homogenate, 2.5 ml of 20% trichloroacetic acid (TCA) was added and the contents were centrifuged at 3,500 g for 10 min and the precipitate was dissolved in 2.5 ml of 0.05 M sulfuric 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 color was read at 530 nm in an ultraviolet (UV) spectrophotometer (Hitachi U‑2000) against the reagent blank. Trimethoxy pentane (TMP) was used as external standard. Values are expressed in micromoles of MDA formed/gram tissue/hour.

CAT (EC: 1.11.1.6)

CAT activity was measured by a slightly modified method of Aebi (1984) at room temperature. The tissue was homogenized in ice‑cold 50 mM phosphate buffer (pH 7.0) containing 0.1 m Methylene diamine tetra acetic acid (EDTA) to give 5% homogenate (W/V). The homogenates were centrifuged at 10,000 rpm for 10 min at 4°C in cold centrifuge. The resulting supernatant was used as enzyme source. A 10 μl of 100% ethyl alcohol (EtOH) was added to 100 μl of tissue extract and then placed in an ice bath for 30 min. After 30 min, the tubes were kept at room temperature followed by the addition of 10 μl of Triton X‑100 RS. In a cuvette containing 200 μl of phosphate buffer, 50 μl of tissue extract and 250 μl of 0.006 M H_2O_2 (in phosphate buffer) were added and the decrease in optical density was measured at 240 nm for 60s in a UV spectrophotometer (Hitachi U‑2000). The molar extinction coefficient of 43.6 $M\ cm^{-1}$ was used to determine CAT activity. One unit of activity is equal to the moles of H_2O_2 degraded/mg protein/min.

SOD (EC: 1.15.1.1)

SOD activity was determined according to the method of Misra and Fridovich (1972) at room temperature. The tissue was homogenized in ice‑cold 50 mM phosphate buffer (pH 7.0) containing 0.1 mM EDTA to give 5% homogenate (W/V). The homogenates were centrifuged at 10,000 rpm for 10 min at 4°C in cold centrifuge. The supernatant was separated and used for enzyme assay. A 100 μl of tissue extract was added to 880 μl (0.05 M, pH 10.2, containing 0.1 mM EDTA) carbonate buffer; 20 μl of 30 mM epinephrine (in 0.05% acetic acid) was added to the mixture, and the optical density values were measured at 480 nm for 4 min using UVspectrophotometer (Hitachi U‑2000). Values are expressed in superoxide anion reduced/milligram protein/minute.

GST (EC: 2.5.1.18)

GST activity was measured with its conventional substrate 1‑chloro, 2,4‑dinitrobenzene (CDNB) at 340 nm as per the method of Habig *et al.,* (1974). The tissues were homogenized in 50 mM Tris‑HCl buffer pH 7.4 containing 0.25 M sucrose and centrifuged at 4,000 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 micromoles of thioether formed/milligram protein/minute.

GPx (EC: 1.11.1.9)

GPx was determined by a modified method of Flohe and Gunzler (1984) at 37°C. A 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°C 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 100 μl of glutathione reductase (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 × 103 M cm−1 was used to determine the activity. The enzyme activity was expressed in micromoles of NADPH oxidized/milligram protein/minute.

Estimation of protein content

Protein content of the tissues was estimated by the method of Lowry *et al.,* (1951). One percent (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 1,000 g 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 Folin‑phenol reagent (1:1folin: H2O). 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 (bovine serum albumin (BSA)) graph.

Data analysis

The data was subjected to statistical analysis such as mean, standard deviation (SD), and analysis of variance (ANOVA) using standard statistical software, Statistical Package for Social Sciences (SPSS; version 16). All values are expressed as mean ± SD of six individual samples. Significant differences were indicated at P < 0.05 level.

RESULTS

Cd concentration (Bioaccumulation) was analyzed in liver and kidney of control and Cd treated male albino rats for the specified time intervals. The mean Cd levels were found to be significantly (P<0.05) increased in both liver and kidney tissues of Cd treated rats when compared to the controls (Fig.1). The accumulation of Cd significantly increased with the increased duration of treatment. Cd accumulation was high in the kidney (42.80 ± 0.30 µg) of rats treated with Cd for 30d time period than liver (39.08 ± 0.64 µg). LPO levels were increased markedly in both liver and kidney of Cd treated rats at all the time periods when compared to the controls (Fig.2). The MDA levels were elevated with the increased time intervals of Cd treatment compared to controls and maximum was observed in 30d rat kidney (64.08 ± 0.21 µ moles of MDA formed /g tissue / hr). CAT activity levels measured in control and Cd treated rats. The activity levels were progressively decreased during Cd treatment when compared with control (Fig.3). Maximum decrement was found in 30d Cd treated rat kidney (1.29 ± 0.52µ moles of H_2O_2 metabolized/mg protein/ min) than the other treatment periods. There was significant decrease in SOD activity during all time intervals of Cd treatment and it was maximum for 30d rat liver (1.38 ± 0.59 superoxide anion reduced / mg protein / min) than kidney (Fig.4). GST activity levels also showed a progressive decrement during Cd treatment at all the time periods when compared with control (Fig.5).

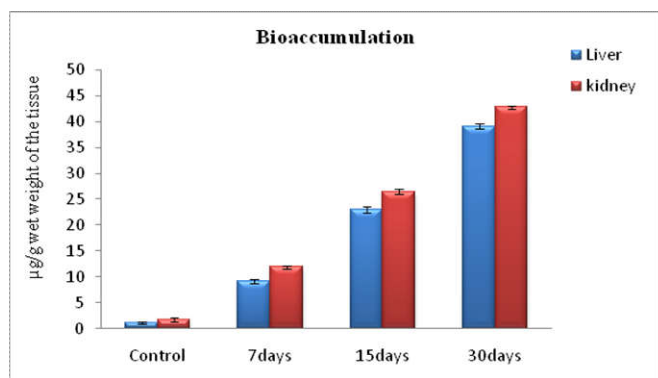

Fig.1: Bioaccumulation levels in liver and kidney of control and Cd‑treated rats. Values are expressed as mean±SD (n = 6 rats in each group), Statistical significance was evaluated by one-way analysis of variance (ANOVA) and the Duncan's Multiple Range Test (DMRT), All experimental mean values are significant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

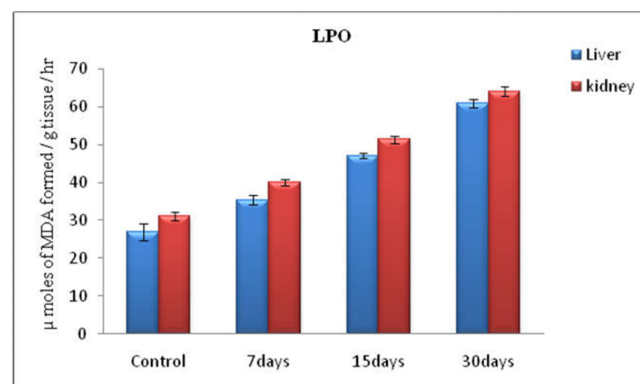

Fig.2 Alterations in the levels of LPO in liver and kidney of control and Cd‑treated rats. Values are expressed as mean±SD (n = 6 rats in each group), Statistical significance was evaluated by one-way analysis of variance (ANOVA) and the Duncan's Multiple Range Test (DMRT), All experimental mean values are significant at P<0.05 level over control

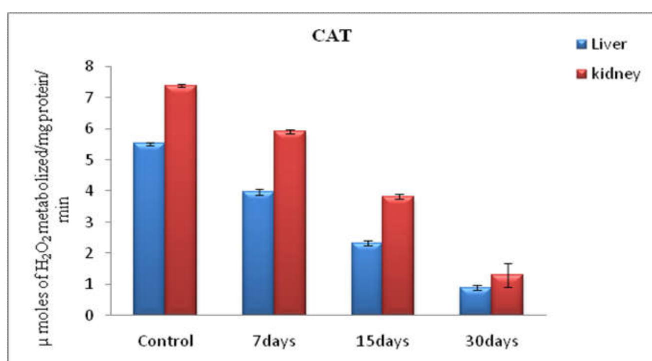

Fig. 3. Alterations in the activity levels of CAT in liver and kidney of control and Cd‑treated rats. Values are expressed as mean±SD (n = 6 rats in each group), Statistical significance was evaluated by one-way analysis of variance (ANOVA) and the Duncan's Multiple Range Test (DMRT), All experimental mean values are significant at P<0.05 level over control

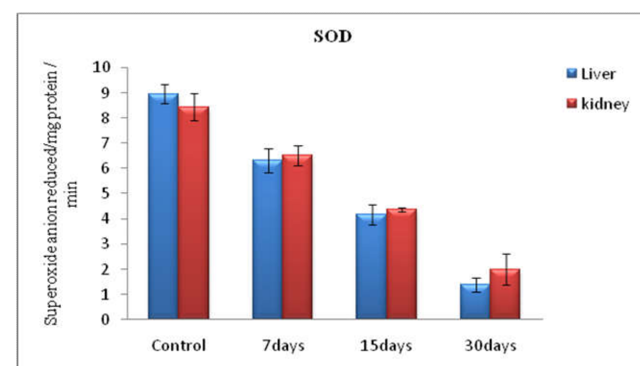

Fig.4: Alterations in the activity levels of SOD in liver and kidney of control and Cd‑treated rats. Values are expressed as mean±SD (n = 6 rats in each group), Statistical significance was evaluated by one-way analysis of variance (ANOVA) and the Duncan's Multiple Range Test (DMRT), All experimental mean values are significant at P<0.05 level over control

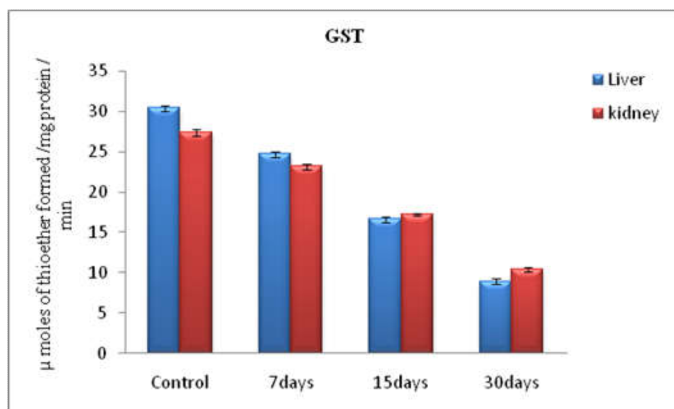

Fig. 5. Alterations in the activity levels of GST in liver and kidney of control and Cd‑treated rats. Values are expressed as mean±SD (n = 6 rats in each group), Statistical significance was evaluated by one-way analysis of variance (ANOVA) and the Duncan's Multiple Range Test (DMRT), All experimental mean values are significant at P<0.05 level over control

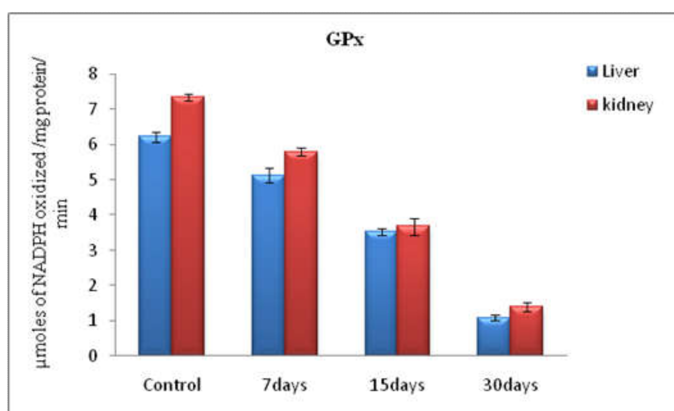

Fig. 6. Alterations in the activity levels of GPx in liver and kidney of control and Cd‑treated rats. Values are expressed as mean±SD (n = 6 rats in each group), Statistical significance was evaluated by one-way analysis of variance (ANOVA) and the Duncan's Multiple Range Test (DMRT), All experimental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

Maximum depletion of GST activity was found in 30d rat liver (8.95 ± 0.15 µ moles of thioether formed / mg protein / min). GPx activity levels also significantly decreased at all the time intervals of Cd treatment when compared with control (Fig.6). 30d rat kidney (1.39 ± 0.27 µ moles of NAPDPH oxidized / mg protein / min) showed maximum level of depletion in the activity of GPx. The decreased enzyme activity levels (CAT, SOD, GST and GPx) in the Cd treated rats were statistically significant (P<0.05).

DISCUSSION

Cd is one of the most dangerous occupational and environmental toxicant and is mainly accumulated in the kidney and liver of animals. The present work focused on the pattern of Cd bio-accumulation and alterations in the antioxidant defense system in the liver and kidney of male albino rats. The Cd accumulation levels were elevated in the test tissues with the increased time of Cd treatment (Shibutani *et al*., 2001). Bioaccumulation and bio-magnification are the characteristic features of heavy metals including Cd. Cd occurs in the air, water, plant and animal tissues. The inhalation or absorption of Cd from various sources may lead to its accumulation in the body (Uleckiene and Zabulyte, 2002; Paltanaviciene *et al*., 2006; Zabulyte *et al*., 2007). The findings of the present study suggest that exposure of Cd leads to accumulation in the liver and kidney of rats in time dependent manner. The results are in consonance with earlier reports (Shibutani *et al*., 2001; Nad *et al.,* 2005; Bhavani *et al*., 2014; Obaiah and Usha Rani, 2012; 2013; 2014; 2015; 2016). Cd accumulates mainly in the liver and kidney and has a long half-life in an organism (Nordberg *et al.,* 2007; Tim *et al.,* 2008). In long term chronic occupational exposure to Cd, kidney is usually the most critically affected organ (Nad *et al.,* 2005; Zabulyte *et al.,* 2007). Kidney is well known to be a major target organ of Cd in animals and humans. During chronic exposure the heavy metal Cd accumulates in renal cortex. Cd absorption and accumulation in the tissues depends on many factors, chief among them being the dose, route of administration, interaction with other substances and rate of elimination from the body.

In the present study, 30d Cd treated rat kidney (42.80 ± 0.30 µg/g wet weight of the tissue) and liver (39.08 ± 0.64 µg/g wet weight of the tissue) showed greater accumulation of Cd concentration when compared to control. The high levels of Cd accumulation in both liver and kidney over time might be due to involvement of these organs in the detoxification and moreover being the major organs of metabolic activities (Klaassen *et al*., 2009). Further, it might also be transported / routed into these organs from other tissues in the body for the purpose of subsequent elimination. From the observed pattern of Cd accumulation in the tissues, it is obvious that the kidney showed high concentration of Cd load than liver (Bhattacharyya, 2000; Yilmaz, 2005). It might be due to as and when the Cd enters into the body, it reaches the liver through circulation and induces the synthesis of MT in liver tissue (Brzoska *et al*., 2000; Alhazza, 2008) and forms Cd-MT complex. Thus formed Cd-MT complex is further transported to kidney (Nad *et al*., 2005; Asagba, 2009) continuously and there it may accumulate more. Because kidney acts as a detoxifying organ (Massanyi *et al*., 2003; Linde *et al*., 2004) and also involved in the elimination of Cd. The kidney is thus the final destination of all the Cd from various tissues as it has also been shown that Cd-MT is filtered through the glomerulus and is reabsorbed by the proximal tubular cells, possibly by endocytosis. Within these cells, the complex is taken up by lysosomes and degraded by proteases and releases Cd, which may result in renal accumulation of the metal. Thus, these factors might have accounted for the raised level of Cd in the kidney during Cd treatment. Present observations are in agreement with the previous reports of Massanyi *et al.,* (2003) and Linde *et al.,* (2004) in rats and also the same was reported by Usha Rani, (2000) and Obaiah and Usha Rani, (2014) in fresh water teleost, *Oreochromis mossambicus* exposed to Cd.
Cd not only bio-accumulates but also accumulation of Cd is known to disturb the prooxidant – antioxidant balance in the tissues of organisms (Turgut*et al*., 2007). The results of the present study revealed that Cd induces significant alterations in the levels of LPO and certain antioxidant enzymes status in liver and kidney of male albino rat. Several mechanisms have been proposed for Cd induced various abnormalities, but none have yet been defined explicitly. Recently, oxidative stress has been reported as one of the important mechanisms of toxic effects of Cd. Cd induced oxidative stress shows significant impact on membrane, DNA and antioxidant defense system of the cell (Hisar *et al.,* 2009). From the results, it is evident that LPO increased markedly in both liver and kidney of Cd treated rats when compared to the control. MDA levels in the tissues

are used as an index of oxidative stress. In our study, elevated MDA levels were observed in Cd treated rats as an indication of increased oxidative stress. The increased MDA levels were more prominent in 30d Cd treated rats when compared to that of other scheduled time intervals. Cd may induce oxidative damage in different tissues by enhancing peroxidation of membrane lipids and altering the antioxidant defense system of the cells. As liver contains more amounts of unsaturated lipids, it undergoes oxidative damage by reactive oxygen species (ROS) than the kidney. Cd triggers the generation of ROS in the organism (Jovanovic *et al.,* 2012). In the present study, Cd treated rats showed not only a significant increase in LPO but also significant decrease in the activity levels of antioxidant enzymes such as CAT, SOD, GST and GPx. These findings are in agreement with earlier reports of Stohs *et al.,* (2001) and Kim *et al.,* (2011) in rats and Carp respectively under Cd stress.

During oxidative stress the CAT activity levels progressively decreased due to high accumulation of H_2O_2 in the tissues and thereby more peroxidation of lipids is favored. This could be the reason for increased LPO levels observed in Cd intoxicated rats. The significant decrease in CAT activity may be due to its inactivation by superoxide radical or due to decrease in the rate of the reaction as a result of the excess production of H_2O_2 to water and oxygen. The decrement in the CAT activity levels with Cd treatment indicates inefficient scavenging of hydrogen peroxide due to oxidative inactivation of enzyme. SOD is an important antioxidant enzyme that inhibits oxyradical formation and is usually used as a biomarker to indicate oxidative stress (Zhang *et al.,* 2004). The decrease in SOD activity may be due to its inhibition by the excess production of ROS as evidenced by LPO in the present study. Excessive production of ROS may result in alterations in the biological activity of cellular macromolecules. Therefore, the reduction in the activity of SOD may result in a number of deleterious effects due to the accumulation of superoxide radicals. Administration of Cd into rats in the present study may lead to generation of peroxy radical, O2•– which is associated with inactivation of SOD and CAT. This may be the reason for the significant reduction in the activities of CAT and SOD in liver and kidney of Cd treated rats when compared to control. It is in accordance with results from earlier studies in which there was a decrease in SOD activity in rats (Nagaraj *et al.,* 2000) and fish (Obaiah and Usha Rani, 2013) subjected to heavy metal, Cd.

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 (Han *et al.*, 2006). 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, (2000) 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 the 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 Waisberg *et al.,* (2003), Ognjanovic *et al.,* (2008) and Messaoudi *et al.,* (2009) 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 (Mahendran and Shyamala Devi, 2001). Therefore, it can be concluded Cd induced bioaccumulation and oxidative stress in a time-dependent manner disturb the prooxidant – antioxidant balance in the tissues of organisms.

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